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The impacts of distinct light spectra on the growth properties and maternal productivity of rats

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The impacts of distinct light spectra on the growth properties and maternal productivity of rats [*]

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ABSTRACT: The aim of this study is to determine how the complete visible light spectrum and white light affect the growth characteristics of rat puppies, oxidative stress measures, and maternal productivity. In the study, a total of 56 female and 28 male breeding rats (*Sprague Dawley*) were mated, with 8 female and 4 male rats in each group. Their growth characteristics were followed until the 63rd day. At the end of the study, 4 female and 4 male rats from each group were euthanized under anesthesia. Oxidative stress parameters were determined in their blood samples. The green lighting group had the highest puppy yield and weaning rate. The blue lighting group had the highest live weight and live weight gain during the suckling period. The red and green lighting groups had the highest pubertal weights. The highest feed consumption rate was obtained in the green lighting group. Feed utilization and water consumption were similar among the lighting groups. The white lighting group had the highest level of total antioxidant status (TAS), while the red lighting group had the lowest TAS level. The highest levels of total oxidant status (TOS) and oxidative stress index (OSI) were found in the red lighting group. These results suggested that the rats were affected differently by the light spectrum at different physiological periods.

Keywords: Rat; visible spectrum; lighting; fertility; growth

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INTRODUCTION

Electromagnetic spectrum is a scale on which electromagnetic waves are arrayed by their wavelengths. This spectrum is visible only in radiation with wavelengths of 380 to 780 nanometer (nm) and is referred to as light (visible light). Since the wavelength varies infinitely from 380 nm to 780 nm, there are an infinite number of colors, from violet to red, but we can mention six primary colors; purple (380-450 nm), blue (450-495 nm), green (495-570 nm), yellow (570-590 nm), orange (590-620 nm), and red (620-780 nm). On the other hand, white is the light in which all waves are visible together (Ozkaya and Tufekci, 2011).

Light is an important macro-environmental factor that affects many metabolic and physiological parameters of living organisms (Miho and Takatoshi, 2012; Gutiérrez-Pérez et al., 2023; Ma et al., 2024). The studies have focused on the effects of light on the physiology and welfare of rats, especially on photoperiod (Khizhkin et al., 2019; Liu et al. 2022), light intensity (Kaidzu et al., 2021), and light source (Benedetto et al., 2019; Niklaus et al., 2020). Recent studies on the rat eye have indicated that the spectral quality of light also has important effects on the physiology and welfare of rats (Solly, 2018; Nie et al., 2023). While humans are trichromats with cone cells sensitive to green, red, and blue light, rats and mice are dichromats with cone cells sensitive to ultraviolet and green light (Westö et al., 2022). Rats lack cone cells sensitive to red light; however, despite the common assumption that red light is invisible to rodents, rats can detect red light and even the entire light spectrum (between 380 and 780 nm). In rats, physiologically photosensitive retinal ganglion cells (different from rod and cone cells) are also important in regulating neuroendocrine, circadian, and neurobehavioral processes (Niklaus et al., 2020; Nikbakht and Diamond, 2021). These cells can respond to different wavelengths of light compared to other photoreceptors. Considering these effects, few studies have investigated the effects of various color lighting treatments (Wang et al. 2011; Dedeke et al., 2017), colorful rat cages (Wren et al., 2014; LaFollettea et al., 2019), and colorful objects used in the cages (Wren-Dail et al., 2016) on several performance parameters of rats and their physiological and metabolic properties. However, since many factors such as pigmentation, body temperature, hormonal state, age, species, and sex are effective in meeting the light needs of rats, further studies are required to identify their needs. All these factors should be taken into consideration when setting the lighting level for the care room of rats (Anonymous, 2011).

The aim of this study is to assess the effects of the entire visible light spectrum and white light on the fertility properties of rats for the first time as well as examining the parameters affecting growth performance between the birth-weaning and weaning-puberty periods and the effects of colors on oxidative stress parameters in rats during the growth period.

MATERIALS AND METHODS

Animal Care

Approval was obtained from the Local Ethics Committee for Animal Experiments at Adiyaman University with decision no 2021/002 on 25/02/2021.

Experimental Design

The study was conducted at the Experimental Animal Breeding Practice and Research Centre of Adiyaman University. There were seven experimental groups formed by considering the electromagnetic spectrum; purple (445 nm), blue (460 nm), green (530 nm), yellow (570 nm), orange (595 nm), red (720 nm), and white (6500 Kelvin) lighting groups (Anonymous, 2024). Compact fluorescent lamps were used for lighting (60 cm, 18 W). They were fitted to the shelves to accommodate two lamps in each group. Sizes of these shelves were 165 cm in length, 44 cm in width, and 32 cm in height. The sizes of the cages had a 37-cm length, a 21-cm width, and an 18-cm height. Rat cages were fixed on the shelves to allow light to shine on them. Each lighting group was organized to avoid any effect of the light emitted from the lamps on each other. The light intensity was set to about 50 lux in each group (Extech Instruments Lt505, England). The duration of the light/dark cycle was set at 12/12 hours. The study was planned as four replicates for each color group. A total of 56 female and 28 male breeding rats (*Sprague Dawley*, 12-14 weeks old) including 8 female and 4 male rats for each group were used in the study [Taking into account type I error (alpha) of 0.05, power (1-beta) of 0.8, and effect size of 0.56, the minimum sample size needed to detect a significant difference with this test should be at least 8 in each group, or totaling 56 (Arslan et al., 2018)]. *Sprague Dawley* rats were outbred and albino. The breeding female and male rats were weighed at the beginning of the experiment and distributed to the experimental groups so that the groups had similar initial live weights (Table 1). The breed-

ing rats were weighed on a Neckufe/JCS-B, Türkiye (30kg/1g) balance.

Males and females were housed in separate cages for five days to adapt to the environment with different color lighting. Then, female rats were distributed into groups and allowed to mat for 1 week. 2 female rats and 1 male rat were housed in each of the cages. At the end of 12 days, male rats were taken from the cages and excluded from the study. The pregnancies of female rats were followed for 21 days, and the fertility and survival ability of the rats giving birth were calculated using the following formulas;

Birth rate: (Number of females giving birth /total female) x100

Infertility rate: (Infertile females/total females) x 100

Puppy yield: (Puppies born/total females) x 100

Weaned puppy ratio: (Weaned puppies /total females) x100

Viability at weaning: (Number of live puppies at weaning /total number of puppies at born) x 100

The birth weights and sexes of the puppies were determined. During the 21-day lactation period, all male and female puppies were weighed weekly and their live weight gains were determined. At the end of the lactation period, all puppies were weighed again, and 8 male and 8 female rats having equal initial live weights were selected, and their development was followed until the puberty period. Thus, the growth parameters from the lactation period to puberty period were evaluated independently.

The live weight of the puppies was recorded weekly, starting from their birth. They were weighed using Radwag/PS 750.R2, Poland (0.001g) brand/model scales. During the whole study period, they were raised in transparent-colored conventional cages without environmental enrichment. Wood shavings were placed as bedding material in the cages. The material were changed weekly. The rats were kept in climate-controlled rooms with a temperature of 25 °C and a humidity level of 50-55%. They were fed pellet feed which was supplied by a commercial company and its composition is presented in Table 2. The rats consumed feed and water *ad libitum* daily after weighing, and the remaining feed and water were weighed and taken away. Feed and water consumption of the groups was calculated weekly.

At the end of the experiment (d 63), randomly 4 male rats and 4 female rats from each group were euthanized under anesthesia. Their blood samples were taken during this procedure and were centrifuged at 4000 rpm. Serum was extracted from them. The samples were assessed for levels of total antioxidant status (TAS) and total oxidant status (TOS) with colorimetric kits manufactured by RelAssay using the Thermo/3001 brand/model Microplate Spectrophotometer. The oxidative stress index (OSI) was calculated by using the formula; OSI=TOS/TAS*100.

Statistical Analysis

The live weight and live weight gain of the puppies during the suckling period were affected by birth weight, sex, and litter size. Therefore, the Least Squares method in the MINITAB software was used after the normality analysis of the data to determine the overall impact of the light spectrum and other main effects (birth weight, sex, and litter size) on these parameters. In order to determine the adjusted live weights and live weight gains of the puppies according to the main effects for the periods examined, the following equation was first utilized to calculate the birth weight of any puppies:

Table 1. Balanced live weights of male and female breeders at the beginning of the experiment		
Experimental groups (g)	Female	Male
Purple	228.750	327.250
Blue	229.250	325.000
Green	229.625	326.750
Yellow	229.500	325.750
Orange	228.750	328.500
Red	229.125	327.250
White	228.500	328.750
P	*1.000*	*1.000*
SEM	1.770	4.258

Table 2. Composition of the commercial feed

Ingredient, %	
Corn, 3100	17.00
Barley	7.55
Wheat Bran, Coarse	13.00
Soybean Meal, Solvent 48 HP	27.00
Cotton Seed Meal, Press, 28 HP	28.50
Corn Germ Meal, Press	1.00
Dicalcium Phosphate	0.90
Marble Dust	4.00
Salt	0.80
Vitamin-Mineral Mix *	0.25
Nutrients, (%)	
Dry Matter	89.67
Crude Protein	25.00
Crude Fat	2.04
Crude Ash	10.00
Crude Fiber	9.24
Starch	19.00
Calcium	1.89
Available Phosphorus	0.82
Sodium	0.35
ME, kcal/kg**	2400

* Supplied per kilogram of diet= Retinyl acetate: 12.000 IU; cholecalciferol: 2400 IU; dl-α-tocopheryl acetate: 30 mg; menadione sodium bisulfite: 2.5 mg; thiamine-hydrochloride: 3 mg; riboflavin: 7 mg; niacin: 40 mg; d-pantothenic acid: 8 mg; pyridoxine hydrochloride: 4 mg; vitamin B12: 0.015 mg; vitamin C: 50 mg; folic acid: 1 mg; D-biotin: 0.045 mg; choline chloride: 125 mg; Mn: 80 mg; Fe: 30 mg; Zn: 60 mg; Cu: 5 mg; Co: 0.1 mg; I: 0.4 mg; Se: 0.15 mg.

** Obtained by calculation

$$Yijkl = \mu + ai + ai + bj + bj + ck + eijkl$$

The formula, $Yijkl = U + ai + ai + bj + ck + dZ-ijkl + eijkl$, was used to calculate the live weights on days seven, fourteen, and twenty-one, the live weight gains, and the effect sizes of environmental factors affecting these characteristics (Simsek and Bayraktar, 2006; Waiz et al., 2018).

μ = Expected mean; ai = Effect of light spectrum (i= Purple, blue, green, yellow, orange, red, white); bj = Effect of litter size (j = 1-5, 6-10, >10); ck= Effect of sex (k= Male, female); eijkl = Effect of factors other than the factors examined (error term); U = Used in the calculation of the expected mean (μ=U+dZijkl); d = Birth weight; Z = Partial regression of live weight on birth weight in the period examined.

The SPSS software was used to calculate the statistics related to performance and stress parameters between weaning and puberty (d 63), and the statistical analysis was carried out by the GLM procedure. The Tukey HSD test was run for further analysis. The means of fertility and survival ability of the color groups were compared by Chi-square analysis. The data were expressed as means ± standard error and differences were considered as significant at the level of P≤0.05.

RESULTS

At the beginning of the experiment, the live weights of male and female individuals of all groups were balanced, as shown in Table 1 (P>0.05).

The litter size decreased significantly in the blue and white lighting groups (P<0.05). The fertility rate was 1050% in the green color group. The highest rate of weaned puppies was found in the green lighting group, followed by the purple and yellow lighting groups. The survival rate increased in the purple and yellow lighting groups (P<0.05) (Table 3)

Table 4 shows the adjusted live weight and live weight gains of the puppies during the suckling period (days 1-21). The birth weight was higher in the green lighting group, those with litter size 6-10, and male ones (P<0.001). The highest weaning weight and weight gains were observed in the blue lighting group and groups with litter size 1-5 (P<0.001).

Table 3. Effect of light spectrum on some fertility and viability traits in rats

	Birth rate		Infertility rate		Puppy yield		Weaning rate		Survival rate	
	N	%	N	%	N	%	N	%	N	%
Purple	8	100.0	0	0.0	48(F)+31(M)=79	987.5^a	48(F)+31(M)=79	987.5^b	79	100.0^a
Blue	5	62.5	3	37.5	28(F)+25(M)=53	662.5^b	27(F)+23(M)=50	625.0^c	50	94.3^b
Green	5	62.5	3	37.5	39(F)+45(M)=84	1050.0^a	39(F)+41(M)=80	1000.0^a	80	95.2^b
Yellow	7	87.5	1	12.5	42(F)+37(M)=79	987.5^a	42(F)+37(M)=79	987.5^b	79	100.0^a
Orange	7	87.5	1	12.5	32(F)+36(M)=68	850.0^a	28(F)+35(M)=63	787.5^c	63	92.6^b
Red	5	62.5	3	37.5	26(F)+41(M)=67	837.5^a	26(F)+40(M)=66	825.0^c	66	98.5^b
White	6	75.0	2	25.0	29(F)+24(M)=53	662.5^b	28(F)+23(M)=51	637.5^c	51	96.2^b
P-Values	P=0.420		P=0.420		P=0.014		P=0.007		P=0.027	

N= Number, F= Female, M= Male, a,b: Show the differences in terms of the means of the rats examined in different color groups (P<0.05)

Table 4. Adjusted live weights and daily live weight gains of the experimental groups during the suckling periods according to the Least Squares Method

Days	Birth	7	14	21	1-7	8-14	15-21	1-21
Experimental groups (g)								
Purple	6.502^b	12.692^b	21.107^{cd}	30.538^c	0.884^b	1.202^c	1.347^{bc}	1.145^c
Blue	6.520^b	13.747^a	23.413^b	34.426^a	1.032^a	1.381^b	1.573^a	1.329^a
Green	6.781^a	12.423^b	20.570^d	29.598^d	0.806^b	1.164^d	1.290^c	1.087^d
Yellow	6.642^{ab}	12.754^b	21.759^c	31.308^c	0.873^b	1.129^d	1.364^b	1.175^c
Orange	6.315^c	13.009^b	21.640^c	32.855^{bc}	0.956^b	1.233^c	1.602^a	1.264^b
Red	6.243^c	13.101^b	21.297^c	29.722^d	0.980^b	1.171^{cd}	1.204^d	1.118^d
White	6.001^d	13.939^a	24.375^a	33.187^{ab}	1.134^a	1.491^a	1.259^c	1.295^{ab}
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Litter size								
1-5	6.448^b	14.836^a	24.305^a	37.047^a	1.198^a	1.353^a	1.820^a	1.457^a
6-10	6.654^a	12.276^b	21.494^b	30.201^b	0.803^b	1.317^b	1.244^b	1.121^b
>10	6.118^b	12.173^b	20.270^c	27.738^c	0.865^b	1.157^c	1.067^c	1.030^c
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Gender								
Male	6.660	13.138	22.218	31.750	0.925	1.297	1.362	1.195
Female	6.200	13.052	21.828	31.574	0.979	1.254	1.392	1.208
P	<0.001	0.396	0.083	0.646	0.394	0.143	0.459	0.544
SEM	0.017	0.058	0.088	0.150	0.008	0.006	0.010	0.006

a, b, c, d: For the same feature in the same column, there is a significant difference (P<0.05) between the means shown with different letters.

At the end of the study, the highest live weight was found in the red and green lighting groups and male rats (P<0.001). According to interactions, the highest live weight in male rats was recorded in the red lighting group; whereas, the highest live weight in female rats was recorded in the green lighting group (P<0.05). When examining the live weight gain between days 28 and 63, the red and green lighting groups had higher live weight gain (P<0.001) (Table 5).

The feed consumption increased in the green lighting group and decreased in the blue lighting group between days 28-63 (P<0.001) (Table 6). There was no significant difference among color groups on these days in terms of feed conversion rate (P>0.05).

During the whole study period, males consumed more feed than female ones (P<0.001).

Water consumption rate was similar in all experimental groups between days 28-63 (P>0.05). While the plasma TAS value was highest in the white lighting group, the lowest was observed in the yellow and red lighting groups (P<0.001). The opposite was measured in TOS and OSI values (P<0.001). These parameters were significantly higher in females than males (P<0.001) (Table 7).

DISCUSSION

The study revealed that different colors of lighting in the cages affected the performance parameters of

Table 5. Effects of the experimental groups on live weights and live weight gains between weaning and puberty periods

Days		28	35	42	49	56	63	28-35	36-42	43-49	50-56	57-63	28-63
Experimental groups (g)													
Purple		47.418	73.500^a	100.562^{ab}	130.687^{abc}	160.562^{ab}	184.812^{ab}	3.725	3.866^{bc}	4.303^{ab}	4.267^a	3.464^{ab}	3.925^{ab}
Blue		47.325	64.625^b	96.687^b	124.750^{bc}	153.312^{ab}	174.625^b	2.471	4.580^a	4.008^{ab}	4.080^{ab}	3.044^b	3.637^b
Green		47.356	72.937^{ab}	103.^{187ab}	138.500^a	166.375^a	191.812^a	3.654	4.321^{ab}	5.044^a	3.982^{abc}	3.633^{ab}	4.127^a
Yellow		47.412	69.250^{ab}	97.125^b	127.625^{abc}	153.125^{ab}	181.812^{ab}	3.119	3.982^{abc}	4.357^{ab}	3.642^{abc}	4.098^{ab}	3.840^{ab}
Orange		47.293	72.812^{ab}	98.312^{ab}	130.812^{abc}	155.437^{ab}	188.125^{ab}	3.645	3.642^c	4.642^{ab}	3.517^{bc}	4.669^a	4.023^{ab}
Red		47.412	73.750^a	105.937^a	135.875^{ab}	159.250^{ab}	192.750^a	3.762	4.598^a	4.276^{ab}	3.339^c	4.785^a	4.152^a
White		47.412	70.000^{ab}	95.750^b	123.125^c	147.812^b	174.625^b	3.226	3.678^{bc}	3.910^b	3.526^{bc}	3.830^{ab}	3.634^b
P		1.000	0.008	0.040	<0.001	<0.001	<0.001	0.058	<0.001	<0.001	0.002	<0.001	<0.001
Male		49.560	74.125	103.357	133.500	163.357	196.910	3.509	4.176	4.306	4.265	4.793	4.210
Female		45.191	67.839	95.946	126.892	149.750	171.250	3.235	4.015	4.420	3.265	3.071	3.601
P		<0.001	<0.001	<0.001	0.003	<0.001	<0.001	0.265	0.277	0.394	<0.001	<0.001	<0.001
Male	Purple	49.675	75.375	100.625	133.000	170.125	200.125^{ab}	3.671	3.607^b	4.625^{ab}	5.303^a	4.285	4.298^{ab}
	Blue	49.512	69.625	99.500	123.625	153.875	177.500^c	2.873	4.267^{ab}	3.446^b	4.321^b	3.375	3.656^b
	Green	49.487	76.250	105.125	140.250	169.750	202.125^{ab}	3.823	4.125^{ab}	5.017^a	4.214^b	6.625	4.361^{ab}
	Yellow	49.612	71.875	105.000	133.500	165.125	201.375^{ab}	3.180	4.732^{ab}	4.071^{ab}	4.517^b	5.178	4.336^{ab}
	Orange	49.425	75.500	100.250	135.625	163.125	200.625^{ab}	3.725	3.535^b	5.053^a	3.928^{bc}	5.357	4.320^{ab}
	Red	49.600	75.875	111.000	142.375	166.750	210.875^a	3.753	5.017^a	4.482^{ab}	3.482^c	6.303	4.607^a
	White	49.612	74.375	102.000	126.125	154.750	185.750^{bc}	3.537	3.946^{ab}	3.446^b	4.089^{bc}	4.428	3.889^{ab}
Female	Purple	45.162	71.625	100.500	128.375	151.000	169.500^{AB}	3.780	4.125^{ABC}	3.982^B	3.232^{AB}	2.642	3.552^{AB}
	Blue	45.137	59.625	93.875	125.875	152.750	171.750^{AB}	2.069	4.892^A	4.571^{AB}	3.839^A	2.714	3.617^{AB}
	Green	45.225	69.625	101.250	136.750	163.000	181.500^A	3.485	4.517^{AB}	5.071^A	3.750^A	2.642	3.893^A
	Yellow	45.212	66.624	89.250	121.750	141.125	162.250^B	3.058	3.232^C	4.642^{AB}	2.767^B	3.017	3.343^B
	Orange	45.162	70.125	96.375	126.000	147.750	175.625^{AB}	3.566	3.750^{BC}	4.232^{AB}	3.107^{AB}	3.982	3.727^{AB}
	Red	45.225	71.625	100.875	129.375	151.750	174.625^{AB}	3.771	4.178^{ABC}	4.071^B	3.196^{AB}	3.267	3.697^{AB}
	White	45.212	65.325	89.500	120.125	140.875	163.500^B	2.916	3.410^C	4.375^{AB}	2.964^{AB}	3.232	3.379^B
P		1.000	0.890	0.288	0.508	0.153	0.020	0.950	<0.001	<0.001	0.003	0.062	0.034
SEM		0.440	0.794	1.032	1.182	1.395	1.911	0.123	0.085	0.076	0.090	0.140	0.052

a,b,c: Show the differences in terms of the means of the rats examined in different color groups (P<0.05). A, B, C: Show the differences in the means of the female rats examined in different color groups (P<0.05).

Table 6. Effects of the experimental groups on feed consumption (g) and feed conversion traits between weaning and puberty periods

Experimental groups		28-35	36-42	43-49	50-56	57-63	28-63	28-35	36-42	43-49	50-56	57-63	28-63
Purple		10.089^b	14.794	18.428^b	21.142^{ab}	22.785^{ab}	17.448^{ab}	2.762^b	3.900	4.340^{ab}	5.202	7.119	4.467
Blue		7.294^c	14.196	17.875^b	20.169^b	22.669^{ab}	16.441^b	4.940^a	3.416	4.706^{ab}	5.076	7.891	4.617
Green		10.375^{ab}	14.937	19.732^a	22.696^a	24.401^a	18.428^a	3.863^{ab}	3.712	3.932^b	5.847	11.266	4.515
Yellow		9.839^b	14.562	17.723^b	20.616^b	22.928^{ab}	17.133^{ab}	3.452^{ab}	3.827	4.284^{ab}	6.424	5.883	4.508
Orange		10.008^b	14.464	18.473^b	20.508^b	22.053^b	17.101^{ab}	2.939^b	4.156	4.084^b	5.967	9.135	4.314
Red		11.017^a	14.955	18.526^b	20.267^b	24.089^a	17.771^{ab}	3.182^{ab}	3.311	4.433^{ab}	6.367	5.643	4.341
White		10.107^b	14.214	18.098^b	20.687^b	23.196^{ab}	17.260^{ab}	3.393^{ab}	3.994	4.909^a	13.871	7.357	4.778
P		*<0.001*	*0.077*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.040*	*0.173*	*0.028*	*0.424*	*0.707*	*0.166*
Male		10.364	15.216	19.109	22.091	25.051	18.366	3.695	3.907	4.691	5.374	6.702	4.430
Female		9.273	13.961	17.706	19.648	21.270	16.371	3.314	3.611	4.078	8.556	8.811	4.582
P		*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.296*	*0.114*	*<0.001*	*0.174*	*0.353*	*0.239*
Male	Purple	10.553	15.339^{ab}	19.160^{bc}	22.964^{ab}	25.125^{bc}	18.628^{ab}	2.929	4.320	4.186^{ab}	5.982	4.343	4.343
	Blue	8.089	14.589^b	18.071^{cd}	20.392^d	24.464^c	17.121^d	5.133	3.945	5.489^{ab}	7.368	4.849	4.849
	Green	10.928	15.178^{ab}	20.267^a	23.767^a	26.375^a	19.303^a	4.586	4.130	4.075^{ab}	5.816	4.482	4.482
	Yellow	10.482	16.000^a	19.321^{bc}	22.696^{ab}	25.785^{ab}	18.857^{ab}	3.725	3.473	5.070^{ab}	5.051	4.395	4.395
	Orange	10.196	14.500^b	18.785^{bcd}	21.821^{bc}	22.267^d	17.514^{cd}	2.980	4.334	3.804^b	12.632	4.106	4.106
	Red	11.464	15.839^a	19.660^{ab}	21.250^{cd}	26.464^a	18.935^{ab}	3.259	3.198	4.556^{ab}	4.306	4.146	4.146
	White	10.839	15.071^{ab}	18.500^{cd}	21.750^{bc}	24.875^{bc}	18.207^{bc}	3.253	3.951	5.656^a	5.757	4.689	4.689
Female	Purple	9.625	14.250^{ABC}	17.696^{BC}	19.321^B	20.446^{CD}	16.267^{BC}	2.595	3.479	4.493^A	8.256	4.591	4.591
	Blue	6.500	13.803^{ABC}	17.678^{BC}	19.946^B	20.875^{BCD}	15.760^{CD}	4.747	2.887	3.924^{AB}	8.414	4.385	4.385
	Green	9.821	14.696^A	19.196^A	21.625^A	22.428^A	17.553^A	3.141	3.290	3.790^{AB}	16.716	4.549	4.549
	Yellow	9.196	13.125^C	16.125^D	18.535^C	20.071^D	15.410^D	3.178	4.181	3.498^B	6.715	4.622	4.622
	Orange	9.821	14.428^{AB}	18.160^B	19.196^{BC}	21.839^{AB}	16.689^B	2.899	3.978	4.364^A	5.637	4.522	4.522
	Red	10.571	14.071^{ABC}	17.392^C	19.285^{BC}	21.714^{AB}	16.607^B	3.105	3.423	4.311^A	6.980	4.535	4.535
	White	9.375	13.357^{BC}	17.696^{BC}	19.625^B	21.517^{ABC}	16.314^{BC}	3.533	4.036	4.163^{AB}	8.957	4.867	4.867
P		*0.327*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.927*	*0.109*	*<0.001*	*0.174*	*0.259*	*0.239*
SEM		1.348	0.099	0.109	0.066	0.221	0.127	0.184	0.096	0.095	1.167	0.923	0.049

a, b, c, d: Show the differences in terms of the means of the rats examined in different color groups (P<0.05). A, B, C, D: Show the differences in the the means of the female rats examined in different color groups (P<0.05

Table 7. Effects of light spectrum on water consumption values (mL) between the weaning and puberty periods of the rats and oxidative stress parameters

Experimental groups		28-35	36-42	43-49	50-56	57-63	28-63	TAS	TOS	OSİ
Purple		26.598^{ab}	34.205^a	41.035^{ab}	46.607^b	48.580^c	39.412	2.332^{bc}	0.353^b	0.015^b
Blue		21.214^d	33.366^{ab}	42.696^a	47.857^a	50.178^a	39.060	2.607^{ab}	0.287^{bc}	0.011^b
Green		25.553^{bc}	31.875^b	41.580^{ab}	47.196^{ab}	48.937^c	39.027	2.350^{bc}	0.255^{bc}	0.011^b
Yellow		24.366^c	34.178^a	40.089^b	45.812^c	48.812^c	38.652	2.007^c	0.240^{bc}	0.012^b
Orange		27.071^{ab}	34.875^a	41.375^{ab}	46.660^b	48.392^c	39.672	2.063^{bc}	0.213^c	0.011^b
Red		28.098^a	32.589^b	40.616^{ab}	47.250^a	49.232^b	39.557	1.888^c	0.515^a	0.032^a
White		26.589^{ab}	31.910^b	40.000^b	46.303^{ab}	49.616^b	38.882	2.965^a	0.075^d	0.003^c
P		<0.001	<0.001	<0.001	<0.001	0.026	0.484	<0.001	<0.001	<0.001
Male		26.568	34.410	42.426	49.153	52.928	41.096	2.150	0.230	0.010
Female		24.714	32.160	39.686	44.471	45.285	37.266	2.482	0.324	0.016
P		0.143	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	<0.001
Male	Purple	27.303^a	35.446^a	42.250^b	49.553^a	53.214^a	41.555	2.413^a	0.250^{bc}	0.010^{ab}
	Blue	23.303^b	35.178^a	42.857^a	47.767^b	53.214^a	40.460	2.430^a	0.313^{ab}	0.013^{ab}
	Green	26.607^{ab}	32.535^b	42.500^{ab}	49.571^a	53.178^a	40.875	2.270^{ab}	0.247^{bc}	0.011^{ab}
	Yellow	25.446^{ab}	35.178^a	42.678^{ab}	49.303^a	53.214^a	41.165	1.627^b	0.163^{bc}	0.010^{ab}
	Orange	27.714^a	35.178^a	42.857^a	49.553^a	50.535^b	41.165	1.820^{ab}	0.107^{bc}	0.007^b
	Red	28.785^a	32.678^b	41.517^c	49.053^a	53.571^a	41.120	2.407^a	0.473^a	0.019^a
	White	26.821^a	34.678^a	42.321^b	49.267^a	53.571^a	41.330	2.080^{ab}	0.053^c	0.003^b
Female	Purple	25.892^{AB}	32.964^{AB}	39.821^B	43.660^{BC}	43.946^C	37.270	2.250^B	0.457^A	0.020^B
	Blue	19.125^D	31.553^{BC}	42.535^A	47.946^A	47.142^A	37.660	2.783^B	0.260^B	0.009^B
	Green	24.500^{BC}	31.214^{BC}	40.660^B	44.821^B	44.696^{BC}	37.180	2.430^B	0.263^B	0.011^B
	Yellow	23.285^C	33.178^{AB}	37.500^C	42.321^A	44.410^{BC}	36.140	2.387^B	0.317^B	0.013^B
	Orange	26.428^{AB}	34.571^A	39.892^B	43.767^{BC}	46.250^{AB}	38.180	2.307^B	0.320^B	0.014^B
	Red	27.410^A	32.500^{AB}	39.714^B	45.446^B	44.892^{BC}	37.995	1.370^C	0.557^A	0.045^A
	White	26.357^{AB}	29.142^C	37.678^C	43.339^{BC}	45.660^{ABC}	36.435	3.850^A	0.097^C	0.003^B
P		<0.001	<0.001	<0.001	<0.001	<0.001	0.240	<0.001	0.020	0.002
SEM		0.277	0.216	0.200	0.274	0.400	0.298	0.095	0.024	0.017

a, b, c, d: Show the differences in terms of the means of the rats examined in different color groups (P<0.05). A, B, C, D: Show the differences in the means of the female rats examined in different color groups (P<0.05), TAS : Total antioxidant (mmol Trolox Equiv/L), TOS: Total oxidant (µmol H2O2 Equiv/L), OSI value: TOS/TAS*100

rats differently during different physiological periods (gestation-birth, birth-weaning, and weaning-puberty). Although the highest birth rate was detected in the purple lighting group, the highest fertility rate was found in the green lighting group (1050%). The fertility rate was significantly lower in the white and blue lighting groups. These findings suggested that purple and green colors increased the ovulation rate in rats compared to the other colors. Red light, which has a long wavelength, is known to have a strong ability to penetrate the brains of chickens, yet no research has been conducted on how the light spectrum affects fertility rate of rats. Red light, which is more effective on the hypothalamus and pineal gland, increases the ovulation rate in laying hens and thus affects egg production (Gongruttananun, 2011). Rats with more cone cells susceptible to green light are considered to have a better perception of this wavelength of light and an improved ovulation (Niklaus et al., 2020). The weaning rate was also significantly higher in the green lighting group (1000%). Survival ability in suckling periods was the highest in the purple and yellow lighting groups (100%). Nazari et al., (2020) found that purple color alleviated anxiety in humans, while Lee et al., (2021) reported that yellow color made humans feel comfortable and stable. In terms of similar effects, purple and yellow colors were considered to be positively effective on the survival ability of the puppy rats (Table 3).

While birth weight was high in the green lighting group, weaning weight was high in the blue and white lighting groups (Table 4). The high weaning weight in the blue and white lighting groups was correlated with the low fertility rate (662.5%) in these groups. The low weaning weight in the green lighting group was caused by the high fertility rate in this group (1050%); therefore, the mothers had more pups to feed. Likewise, the highest birth weight was found in the group with litter size 6-10, and the highest weaning weight was found in the group with litter size 1-5.

This group was followed by 6-10 and >10 groups, respectively. Daily live weight gain also was low in the groups with a high litter size. It is known that birth and weaning weight are significantly associated with litter size (Rödela et al., 2008; Haipeng et al., 2021). A high litter size increases the number of pups to be fed by the mother. In the study, it was found that the birth weight of male puppies was higher than that of their female counterparts. Likewise, Yildiz et al., (2007) reported that male rats had higher body weight and organ weights when compared to female ones.

On the twenty-eighth day of the study following the suckling period, the experiment was initiated again by equalizing the live weights for males and females in each lighting group to determine the effect of the light spectra on their growth until puberty (Table 5). The weight on the 63rd day was the highest in the red and green lighting groups and the lowest in the blue and white lighting groups. Likewise, the live weight gain between the 28th and 63rd days was the highest in the red and green lighting groups and the lowest in the blue and white lighting groups. The highest weight on the 63rd day and the highest live weight gain between the 28th and 63rd days were observed in the red lighting group in males and the green lighting group in females. Therefore, lighting affected live weight differently in males and females. Some studies on avian species (Bayraktar et al., 2019) reported that green light had a significant ameliorative effect on live weight, which is compatible with the results of this study. Simsek et al., (2020) revealed that green lighting in partridge cages improved the absorption of intestinal villi and significantly increased the live weight of partridges. Zhang et al., (2014) reported that green lighting increased mRNA expression of MyoD, myogenin, and myostatin genes in broiler chickens at late stages of incubation and in newly hatched chicks and thus had a positive effect on live weight. It was suggested that green lighting had a positive effect on live weight in rat puppies through similar mechanisms. Londe et al., (2018) reported that red lighting significantly elevated blood testosterone levels of males and may affect muscle performance. Similarly, it was considered that high live weight on the 63rd day and high live weight gain in males were attributed to the testosterone hormone. Indeed, Davidyan et al., (2021) found that the testosterone hormone was very important for the development of muscle mass in rats, especially during puberty, and its critical importance disappeared with advancing age. In their study, Wren-Dail et al., (2016) augmented standard rat cages with amber, transparent, red, and opaque

igloos and found that rats preferred red, amber, and opaque tunnels more than transparent color tunnels. Feed consumption, water consumption, live weight gain, and plasma melatonin levels were higher in the red group. Unlike these studies, Dedeke et al., (2017) examined ambient (control), blue, red, yellow, and white lamps. They investigated the weight gain and some blood parameters of albino rats (*Rattus Norvegicus*), which were raised under fluorescent lamps with 300 lux light intensity and 15-watt energy, from birth to the age of 63 days. It was found that the lighting colors were not effective on the live weight gain and hematological parameters of the rats. Wren et al., (2014) found that plasma melatonin levels elevated by 29%, 74%, and 48% in rats raised in amber, blue, and red-colored cages, compared to a transparent-colored cage. They found that lactic acid, corticosterone, insulin, and leptin levels were affected differently in colored cages. However, the color of the cage used in the study was ineffective for feed consumption and live weight gain. Wang et al., (2011) found that the melatonin level of the pineal gland of guinea pigs raised under green, blue, and white lighting showed a green>white>blue pattern. Melatonin hormone is known to play an important role in the production of growth hormones. In another study (Erhui et al., 2011), body weight was substantially correlated with elevated levels of growth hormones.

When the feed consumption was examined (Table 6), the highest feed consumption rate between the 28th and 63rd days was found in the green lighting group and the lowest value was observed in the blue lighting group. Based on these findings, it was considered that green lighting improved feed consumption. Likewise, the highest feed consumption in male and female rats was found in the green lighting group. In general, another reason for the high live weight in the green lighting group was considered to be high feed consumption. The feed consumption rate among male rats was higher than that of females in this study. Despite that, the males also had higher live weights, meaning that their feed conversion values were similar to those of females. It is known that the food consumption habits of males are different from those of females and males consume more food and have a higher tendency to consume sugary, fatty, and distinctive flavors (Grzymisławska et al., 2020). Except for the first week, males drank more water compared to their female counterparts during the whole experiment. The higher water consumption in male rats was associated with the physiology, high live weight, and high feed consumption of males (Golher et al., 2021).

In the study, it was found that in general (days 28-63) different color lighting did not affect water consumption of the rats (Table 7).

Oxidative stress refers to the disruption of the balance between biological defense systems and free radical production in the body. Most of the studies have examined the effects of light on eye health through oxidative stress parameters. Ratnayake et al., (2020) reported that blue light triggered oxidative stress in the retina, increased the destruction of polyunsaturated fatty acids, and led to DNA damage. In their study, Kaidzu et al., (2022) investigated the effects of seven wavelengths ranging from 420 nm to 620 nm on the eyes of rats and showed that light below 440 nm was partially dangerous for their eye, damage intensified as the exposure time prolonged, and wavelengths above 500 nm were not harmful to the eye. In this study, it was also found that plasma TOS and OSI values were higher in the red lighting group. The elevation in plasma TOS and OSI levels may be due to the increase in the amount of oxidant substances in the body (Akdag et al., 2010). These findings may indicate that red lighting induced stress in rats. Red is a powerful color that has attention-enhancing, brain-activating, movement-increasing, and energizing effects (Kutlu, 2018). Despite these positive effects, the perceived weight of the red is high. It increases the effect of environmental stimuli, especially those close to the red band of the light spectrum. An extended period spent in the presence of heavy colors causes stresses for the body (Sağocak, 2005; Kutlu, 2018). The perceived weight of the red is likely to cause the elevated TOS and OSI levels in the rats in that color group. LaFollette et al., (2019) determined the effect of housing rats in different cage colors (red or clear) and light intensities (25 or 200 lx) on USV production during tickling [Heterospecific play, or "rat tickling," is a technique that mimics aspects of rat rough-and-tumble play and elicits 50-kHz ultrasonic vocalizations (USVs)]. They found that red cage resulted in the most negative effect at low intensity. Conversely, clear cage resulted in the most positive effect. Likewise, rats in this study were adversely affected and stressed by 50 lux red lights (low dose). In another study, Sherwin and Glen (2003) kept the mice in red, black, green, or white cages for five weeks. They determined that mice in red cages tended to spend more time in closed arms suggesting that they were more anxious. Choi et al., (2018) examined the oxidative stress parameters in the liver of fish (*Paralichthys olivaceus*) that were exposed to starvation stress for nine days by growing them in units with blue, green, red, and white (control) LED lamps at two intensities (0.3 and 0.6 W/m^2). They found that green and blue lighting, especially green lighting, attenuated the effects of oxidative stress caused by starvation when compared to white light, while red lighting caused more stress. More effective results were achieved as the light intensity rose. The plasma TAS level was significantly higher in the white lighting group in the present study. These findings may indicate that antioxidant metabolism is activated in rats raised under white light. Furthermore, low TOS and OSI levels in this group may suggest that rats raised under white light significantly overcome environmental factors. When male and female rats were evaluated separately, it was determined that the red lighting induced stress in both groups, and antioxidant metabolism was activated more in males for adaptation.

When the effects of the light spectrum on the fertility and growth properties of rats were evaluated in general, it was possible to conclude that green lighting promoted maternal productivity and the growth of the rat puppies until puberty in more than the other groups. Although red lighting had a positive effect on live weight, the induction of stress in rats was considered a restrictive factor for the use of this light for lighting purposes. On the other hand, although white lighting had a positive effect on their antioxidant metabolism, it did not affect their live weight gain, and the lowest puberty weight was found in the white and blue lighting groups. Puberty weight was higher in male rats compared to female ones and they adapted to the study conditions more than females.

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