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Original Research Paper

An Investigation on the Effect of Light Color and Stocking Density on Some Blood Parameters of Broilers and Layers

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The study was designated to investigate the effect of light color and stocking density on some blood parameters of broilers and layers. A total of 675 Ross 308 one-day-old broiler chicks were used in this study. The birds were exposed to white light (WL) as a control, red light (RL), blue light (BL), green light (GL), and Blue – Green mix light (BGL) by a light-emitting diode system (LED) applied for 24 hours daily in separated rooms. The birds were randomly divided and housed into 9 wooden sealed pens of 1m^2 in three replicates for each density 12, 15 and 18 birds/m^2 in the room. In the second treatment, 180 Isa Brown layers were divided into 5 treatments with an average of 36 birds for each of five color light rooms (16 hours light- 8 hours dark) in three replicates for each density 5 and 7 birds/m^2 in the room. The results showed a significant interaction (P< 0.05) of light color and stocking density on Red blood cells (RBC) and White blood cells (WBC) under BGL at 12 birds/m^2 and Platelets (PLT) under 15 birds/m^2, whereas hemoglobin concentration (Hb) and packed cell volume (PCV) showed a significant difference under BGL treatment. No differences were revealed in all blood traits of layers under different light colors and bird densities except for WBC in layers reared under WL.

Keywords: Blood parameters, Light color, Stocking density.

INTRODUCTION

Lighting is a powerful exogenous factor in the control of many physiological and behavioral processes. Light may be the most critical of all environmental factors to birds and consist of photoperiod, lighting color and light intensity (Manser, 1996). Color is an important aspect of light that has been considered at one time as a management tool in poultry production (Prayitno *et al.,* 1997). The associated colors are Blue B (435-500 nm), Green G (500-565 nm), Yellow Y (500-600nm), Orange O (600-630 nm) and Red R (630-700 nm).

Wavelengths have different effects on broiler performance (Hakan and Ali, 2005). The eye of the chicken appears to be more sensitive to a broader spectrum than humans, in addition, chickens can see ultraviolet and infrared as well (Rierson, 2011). Light signals are perceived by the avian brain either through eyes (retina) or direct penetration of skull tissue. In the retina, light signals are transduced to electric impulses, directed towards the brain through various neural channels (Gunturkun, 2000).

Blood parameters are of diagnostic significance and have been shown to be major indices of the physiological, pathological, and nutritional status of an organism and could be used to interpret the effects of therapeutic or nutritional managements in human and veterinary medicine(Toghyani *et al.,* 2012). Effects of monochromatic light on the cellular immune response with light sources were equalized at the intensity of 25 lux, with a light period of 23 hours daily and administer at 25 La Sota strain in all groups. After 8 days of photo-stimulation there was no significant (P > 0.05) difference in lymphocyte proliferation among groups exposed to different monochromatic lights WL, RL, BL, YL and GL (Sadrzadeh *et al.,* 2011). Heshmatollah (2007) found that broilers showed no preference when given different light intensities, but did show a preference for green light compared with red, orange, or yellow. According to Kim *et al.* (2013), the WBC count and hemoglobin were similar among different LED color groups (*P* >0.05).

The higher numerical values of the rest of the blood parameters (RBC, Hct, and platelets) were found with the YL source. The WL treatment showed numerically lower values of RBC and Hct, whereas numerically lower platelet count was noticed under GL source. Stocking density is a much-discussed topic in animal science. Increasing stocking density generally leads to a decrease in welfare in many farm animal species (Petherick and Phillips, 2009). Stocking density is calculated by different ways; sometimes stocking density is reported using the number of birds per unit area or the amount of area per bird. Currently, many companies calculate stocking density by the pound. Instead of being expressed as the

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number of birds per unit area, density is calculated as bird weight per unit area (Fairchild, 2005).There was a consistent trend to elevate the heterophil to lymphocyte ratio when bird density was increased, mean value in the highest density (20 birds/m2) differing significantly from those in the two lowest densities (4 and 8 birds/m2) (Campo *et al.,* 2005).Stocking density had significantly important influence on PCV, also lighting program x stocking density x litter amount were found to have influence on total white blood cell (P<0.05), total red blood cell and H/L ratio (P<0.05, P<0.001) respectively (Petek *et al.,* 2010).

MATERIALS AND METHODS

Birds and husbandry

A total of 675 Ross 308 one-day-old broiler chicks were used in the first experiment. The chicks were reared in the poultry farm at the College of Veterinary Medicine, Basra University for 7 weeks. All broilers were cared for in 5 light-controlled rooms (n = 135) and were exposed to white light as control (WL), red light (RL), blue light (BL), green light (GL), and Blue – Green mixed light (BGL), at birds eye level with ~~an~~ a light-emitting diode system (LED) for 7 weeks applied for 24 hours daily in separated rooms (3 x 3 x 4 meters) with light intensity 5 watt/m^2.

The birds were randomly housed into 9 wooden sealed pens of 1m^2 in three replicates for each density 12, 15 and 18 birds/m^2. Room temperature was initially 34°C and was subsequently reduced by 2°C/week to 26°C at 35 day. In the second treatment, a total of 180 Isa Brown layers were raised under control condition from 25 week until 36 week of age. They divided into 5 treatments with an average of 36 birds for each of five color light rooms (16 hours light- 8 hours dark) in three replicates for each density 5 and 7 birds/m^2 in the room. Half cylinder plastic feeders were placed in each pen.

The birds were supplied with feed and water *ad* libitum, and Pellet diets were formulated to meet the nutrient recommendations for poultry according to NRC (1994). In broilers, total dietary metabolic energy for the starter, grower and finisher were 2925, 3111 and 3171 kcal/kg respectively, while the values of crude protein were 22.21, 20.14 and 18.08 % respectively. In layers, total dietary metabolic energy was 2759 kcal/kg and 17.75% crude protein according to Isa Brown programs. A nipple water drinking system was set up in each pen and was manually adjusted as birds grew to ensure the watering system was kept at a proper level.

Hematological traits

At the end of 5 week age for broilers and 36 week for layers, 1 bird of average weight from each replicate was selected and blood was collected from the wing vein. Blood samples were taken in EDTA anticoagulant-treated syringes (Odunsi *et al.,* 1999), and labeled according to each replicate and treatment and then stored at +4^{0}C until analyzed. The hematological parameters include red blood cells (RBC), white blood cells (WBC), hemoglobin concentration (Hb), packed cell volume (PCV), platelet count (PLT) and heterophil/lymphocyte ratio (H/L) ratio were determined by routine methods as described by Campbell (1988).

RESULTS AND DISCUSSION

Blood parameters of broilers

The determination of blood component values using laboratory exams is an important procedure to aid the diagnosis of several diseases and dysfunctions, as they provide reliable results, and may also give inputs for research studies on nutrition, physiology, and pathology (Bounous *et al.,* 2000). The physiological importance of erythrocytes in the domestic livestock has prompted studies that led to the establishment of some indices with which the health and performance of the animals can be monitored (Awoniyi *et al.,* 2004). The blood parameters of broilers presented in Tables 1 showed a significant effect (P <0.05) for the color light used in the number of RBC in broilers reared under the effect of BGL 3.67 x10^9/ml.

The significance may reflect the high production performance of broilers of this group, and this improvement reflected positively on the parameters of blood according to Zongo and Petitean (1990). According to Kim *et al.* (2013), the higher numerical values of RBC were found with the yellow light source. The table indicated the existence of a significant effect (P <0.05) of stocking density in the number of RBC as it recorded the highest value 3.32 x10^9/ml in the treatment of broilers at the level of density 18 birds / m^2. The significant effect of stocking density was in consistent with Petek *et al.* (2010), who found that the number of RBC of broilers under 19 and 23 birds /m^2 was 3.05 and 3.30 million cells/ml respectively.

The result disagreed with that of Sekeroglu *et al.* (2011), who pointed to the lack of significant impact of the density (9, 13 and 17 birds /m^2) in the number of RBCof broiler chickens. The analysis of variance recorded a significant interaction (P <0.05) between the effect of light color and stocking in the number of RBC3.73 x10^9/ml in the treatment of birds reared under the influence of BGL at level of density 12 birds/m^2.

As shown in Table 1, a significant effect (P <0.05) of color light in the number of WBC 23.46x 10^6/ml in birds reared under the influence of BGL. The results of WBC of the current study are consistent with Xie *et al.* (2008), who reported a significant increase in the number of T lymphocytes in chickens reared under the influence of green light at 21 days and light blue at 49 days, while disagreed with Kim *et al.* (2013), who referred that the number of WBC did not significantly affected by lighting colors (white, blue, red, green, yellow and incandescent lamp) at 35 days.

Sadrzadeh *et al.* (2011) reported that broilers after 37 days of photo-stimulation showed a significant (P < 0.05) increase of T-lymphocyte proliferation in GL and WL groups as compared to other groups. Xie *et al.* (2008) suggest that proliferation of peripheral blood T lymphocytes in GL and BL enhance the immune response better than RL, and that BL may play a role in alleviating the stress response in broilers. For stocking density, the table also showed a significant effect (P <0.05) for the density on the number of WBC 22.58 x 10^6 /ml in broilers reared at the level of 15 birds/m^2.

This result was consistent with Petek *et al.* (2010), who revealed an increase in the number of WBC at high stocking density 15.19 and 23 birds/m^2. The analysis of variance recorded that the interaction between light color and stocking density was significantly higher (P <0.05) in the number of WBC 24.57x10^6/ml in birds reared under the influence of BGL at the level of density 15 birds/m^2.

Table 1. Effect of color light and stocking density on some blood parameters of broilers at 35th day of age (M±SE)

Blood parameters	Color light / Stocking density	WL	RL	BL	GL	BGL	Effect of stocking density
RBC (ml) x10^9	12 bird/m^2	2.95±0.00	3.29± 0.01	3.04 ± 0.01	3.50± 0.05	**3.73x^A 0.0	3.30^{ab}±0.02
	15 bird/m^2	2.89^B±0.01	3.38± 0.04	2.99 ±0.01	3.50 ±0.02	3.68±0.00	3.28^b±0.02
	18 bird/m^2	3.03±0.01	3.37 ±0.02	3.06 ±0.01	3.52 ±0.00	3.62±0.02	3.32^a±0.01
	Effect of color light *	2.95 ^{e}±0.01	3.34 ^{c}±0.02	3.03^d±0.01	3.50^b±0.03	3.67^a±0.01	*
WBC (ml) x10^6	12 bird/m^2	21.76 ±0.03	22.21± 0.01	21.87±0.04	22.16±0.04	23.06±0.01	22.21^c±0.02
	15 bird/m^2	21.60^B±0.02	22.80±0.04	21.61±0.01	22.35±0.00	**24.57^A±0.0	22.58^a±0.02
	18 bird/m^2	22.44±0.02	22.88± 0.05	21.81±0.02	22.32±0.01	22.75± 0.02	22.44^b±0.02
	Effect of color light *	21.93^d±0.02	22.63^b±0.03	21.76^e±0.02	22.27^c±0.0	23.46^a±0.02	*
PLT (ml) x10^6	12 bird/m^2	17 ± 1.00	11^B±0.28	14±0.43	18±0.11	29±1.15	17^c±0.59
	15 bird/m^2	22 ±0.14	26 ±0.50	18 ±0.20	13 ±0.60	** 36^A±0.40	23^a±0.36
	18 bird/m^2	36^A ±0.45	14±0.25	19±0.13	12±0.10	25+0.67	21^b±0.32
	Effect of color light *	25 ^{b}±0.53	17 ^{c}±0.34	17 ^{c}±0.25	14^d±0.27	30 ^{a}±0.74	*
Hb (g/100 ml)	12 bird/m^2	12.5±0.25	12.8±0.11	12.7±0.46	14.5±0.11	16.0±0.37	13.6±0.26
	15 bird/m^2	12.6±0.37	13.1±0.30	12.4±0.32	14.5±0.30	15.6±0.26	13.6±0.31
	18 bird/m^2	13.1±0.17	13.0±0.28	12.8±0.25	14.6±0.25	15.6±0.47	13.8±0.28
	Effect of color light *	12.5 ^{c}±0.26	12.9 ^{c}±0.23	12.6 ^{c}±0.34	14.5^b±0.22	15.7 ^{a}±0.36	N.S.
PCV (%)	12 bird/m^2	40.6±0.23	38.1 ±0.50	40.2±0.75	47.3±0.90	52.3±1.15	43.7±0.70
	15 bird/m^2	39.9±0.61	40.3±0.20	39.3±0.36	47.0±0.25	51.1±0.56	43.5±0.39
	18 bird/m^2	41.8±0.10	39.5±0.32	39.6±0.32	47.6±0.51	51.1±0.97	43.9±0.44
	Effect of color light *	40.7 ^{c}±0.31	39.3 ^{c}±0.34	47.3 ^{b}±0.47	51.5^a±0.55	51.5^a±0.89	N.S.

***a, b, c Means in horizontal rows with different superscripts were significantly different of light colour and in vertical rows of stocking density at (p<0.05). SE: standard error.N.S. not significant.

****A, B, C: Means with different superscripts were significantly different of interaction between light color and stocking density at (p<0.05).

Table 1 showed a significant effect (P <0.05) of color light used in the number of platelets at different experimental treatments. The high value recorded was 30 x10^6/ml in broilers reared under the influence of BGL. As for stocking density, the high number of platelets was found 23.00 x10^6/ml in broilers at level of density 15 birds/m^2. The analysis of variance recorded that the number of platelets was36 x10^{6}inbroilers reared under the influence of BGL at the level of density 15 birds/m^2. This value referred to a significant interaction (P <0.05) between the two factors of the present study.

The other two parameters Hb and PCV were significantly higher (P <0.05) in broilers reared under the influence of BGL which recorded 15.7 g/100 ml and 51.5% respectively, while stocking density showed no significant effect on the values of these parameters under different densities. The significant results of Hb and PCV in broilers reared under the influence of BGL may reflect the increase in RBC numbers which leads to an increase in PCV rate due to close relationship between these values (Sturkie, 1986). The results of this work reported that the total percentage of blood volume composed of cellular elements possibly attributed the increase in these parameters due to a preference of broilers to the combination of green and blue light because it improves feed conversion, increases the proportion of homogeneity in the herd, increased body weight, the low percentage of mortality rate and reduce stress (Poultec, 2012).

Blood parameters of layers

Due to lack of reference values for avian blood profiles, it is not widely used in poultry. There is a close relationship between the environmental factors such as light and health condition. More important among such health conditions is blood parameters which can be monitored using many indices such as of the present study. Blood parameters as shown in Table 2 revealed the effect of light color and stocking density in layers at 36 weeks of age, as the table shows the lack of a significant effect of the color light used in the number of RBC in various treatments. The result referred to the lack of a significant effect of the color light in the number of RBC in various treatments was consistent with Scott and Siopes (1994), who recorded the absence of significant differences in the number of RBC for chicken turkeys at 30 weeks of age under color lighting (Blue, Red, Green and incandescent lamp).

Table 2. Effect of color light and stocking density on some blood parameters of layers at 36th week of age (M±SE)

Blood parameter	Colorlight / Stocking density	WL	RL	BL	GL	BGL	Effect of stocking density
RBC (ml) x10^9	5 bird/m^2	2.84 ± 0.17	2.62 ± 0.02	2.53 ± 0.11	2.51 ± 0.09	2.55 ± 0.03	2.61±0.08
	7 bird/m^2	2.62 ± 0.17	2.47 ± 0.08	2.58 ± 0.05	2.71 ± 0.17	2.46 ± 0.09	2.57 ± 0.11
	Effect of color light N. S.	2.73± 0.17	2.54 ±0.05	2.56 ±0.08	2.61± 0.13	2.51± 0.06	N. S.
WBC (ml) x10^6	5 bird/m^2	18.9 ± 0.6	17.6 ± 0.0	16.9 ± 0.5	17.1 + 0.4	17.4 ± 0.1	17.6± 0.3
	7 bird/m^2	17.8±0.9	17.1 ± 0.6	17.7 ± 0.0	18.4 ± 0.6	16.8 ± 0.3	17.6+0.4
	Effect of color light *	18.4^a±0.8	17.4 ^{b}±0.3	17.3 ^{ab} ±0.2	17.7^{ab}±0.5	17.1^b +0.2	N. S.
PLT (ml) x10^6	5 bird/m^2	16.33 + 2.3	14.00 ± 1.0	16.66± 1.7	15.0 + 1.0	19.33± 1.7	16.26 ±1.5
	7 bird/m^2	16.50 + 0.2	18.00 + 2.3	17.66+ 2.6	18.33 + 0.8	10.00+ 3.0	16.10 ±1.8
	Effect of color light N. S.	16.41 ±1.3	16.00±1.6	17.16 ±2.1	16.66±0.9	14.66±2.4	N. S.
Hb (g/100 ml)	5 bird/m^2	12.23 ±0.78	11.36 ±0.51	10.86±0.66	10.73±0.12	11.33±0.33	11.36±0.48
	7 bird/m^2	11.50 ±0.57	10.73 ±0.52	11.63±0.39	11.90±0.81	11.03±0.53	11.30±0.56
	Effect of color light N. S.	11.86 ±0.67	11.05 ±0.51	11.25±0.52	11.31±0.46	11.18±0.43	N. S.
PCV (%)	5 bird/m^2	35.83 ±2.18	32.60 ±0.70	31.70±1.26	31.70±0.20	32.66 ±0.37	33.37±0.94
	7 bird/m^2	33.50± 1.27	31.86 ±1.36	33.46±0.39	34.93±2.25	33.10 ±1.41	32.90±1.33
	Effect of color light N. S.	34.66 ±1.72	32.23± 1.03	32.58±0.82	33.31±1.22	32.88 ±0.89	N. S.
H/L ratio	5 bird/m^2	0.23 ±0.02	0.23 ±0.17	0.23 ± 0.03	0.24 ±0.06	0.23 ±0.11	0.23 ±0.07
	7 bird/m^2	0.24 ±0.19	0.23 ±0.02	0.22 ±0.18	0.23 ±0.09	0.22 ±0.01	0.22 ±0.09
	Effect of color light N. S.	0.23±0.10	0.23 ±0.09	0.22 ±0.10	0.23 ±0.07	0.22 ±0.06	N. S.

*****a, b, c Means in horizontal rows with different superscripts were significantly different of light colour and in vertical rows of stocking density at ($p<0.05$). SE: standard error.N.S. not significant.

The table recorded a significant effect ($P<0.05$) of color light used in the number of WBC in layers in various groups and recorded the highest rate 18.42x10^6/ ml in the blood of chickens reared under the influence of WL. The result of the current do not agree with Scott and Siopes (1994), who referred to the absence of significant differences in the number of WBC of chicken turkeys.

The other parameters of blood (PLT, Hb and PCV) showed no significant differences even for heterophil / lymphocyte ratio (H/L ratio); the result was same in various groups. The table indicated that there are no significant differences of stocking density in the values of all blood parameters of layers at various treatments. The non-significant result of H/L ratio agreed with that obtained by Hassan *et al.* (2013), which revealed that the use of color lighting (red light program 12 hours and then the green two hours and then the blue two hours, the red light program 14 hours and then the green two hours, white light, blue light, light green, red light) had no significant effect on the rate of H/L ratio in the blood of chickens.

As well as, the finding is concurrent with the findings of Lien *et al.* (2007) who emphasized that H/L ratios were not affected by light intensity or photoperiod. The results showed no significant differences of stocking density in the values of all blood parameters of layers at various treatments. Although there is a clear positive correlation between stocking density and economic return (Buijs *et al.,* 2009; Petek *et al.,* 2010), studies have shown that higher stocking densities compromise the welfare of animals involved (Meluzzi and Sirri, 2009). The result of the present study is probably due to the decrease of stress factors.

CONCLUSION

This topic is of importance to explain on which light color and which density should be considered optimal. It was concluded that monochromatic light is a potential light source that might provide a beneficial effect on blood parameters. The findings suggest that GL (560 nm) and BL (480 nm) better enhance blood parameters in broilers at the level of density 12birds/m^2 but no differences were observed in most of blood traits of layers under different light colors and bird densities.

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