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# The Effect of Color light and Stocking Density on Some Enzymes and Hormones of Broilers and Layers

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**Abstract**

***The study*** was designed to investigate the effect of color light and stocking density on serum Glutamic Oxaloacetic Transaminase (GOT) , Glutamic Pyruvic Transaminase (GPT), Triiodothyronine (T3), Thyroxine (T4) and Cortisol level in broilers and serum follicle stimulating hormone (FSH) and luteinizing hormone (LH) in layers. The experiment was conducted at the University of Basra/ College of veterinary medicine/ poultry farm. A total of 675 Ross 308 one day old broiler chicks reared for 5 weeks and 180 Isa Brown layers were raised from 25 weeks until 36 week of age, were used in this study. The birds were divided into experimental groups and 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 (LED) applied for 24 hours daily for broilers and 16 hours light- 8 hours dark for layers with light intensity 5 watt/m2 . The broilers randomly housed into 9 wooden sealed pens of 1m2 in three replicates for each density 12, 15 and 18 broilers/m2 and three replicates for each density 5 and 7 layers/m2. The results of this study showed a significant effect of BL on serum GOT enzyme level but no significant of GPT. The results also showed higher concentration of serum T3under RL and T4 concentration under BGL but no significance of Cortisol level in broiler serum between the experimental groups. In addition, significant increase of serum FSH hormone during dark period in layers reared under BL was also seen, while high concentration of serum LH hormone was observed during light period in layers reared under RL, and values of both of them were not significant under different densities.

In conclusion, this study revealed that values of GOT, T3 and T4 parameters of broilers were affected by color light, while the results of layers revealed a significant increase of serum FSH hormone during dark period under BL, but serum LH hormone was high during light period under RL. The values of density not differed significantly within chickens of same age during husbandry period.

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**Keywords:** broilers, color light, enzyme, hormone, layers, stocking density.

## Introduction

Artificial lighting consists of 3 aspects: photoperiod, wavelength, and light intensity. 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) and Yellow Y (500-600nm), Orange O (600-630 nm) and Red R (630-700 nm) (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 (Thiele, 2009). One of the characteristics of large commercial broiler operations is the stocking density of birds per 1 $m^2$ area and the choice of appropriate genetic material to ensure rapid attainment of required weight gains and the best feed conversion possible (Skomorucha *et al.,* 2004). Stocking density is inherently confounded with either the number of animals in a group, or with the total amount of space available to this group (Buijs *et al.,* 2012). GOT (Aspartate amino transferase AST) is not considered to be organ-specific because its activity can be found in many tissues. In general, normal plasma AST activity for birds and reptiles is less than 275 IU/L increased plasma AST activity suggests hepatic or muscle injury. However, generalized diseases, such as septicemia or toxemia may damage these tissues resulting in increased plasma AST activity. Like AST, plasma GPT (Alanine amino transferase ALT) is not considered to be organ specific in lower vertebrates. Normal plasma ALT activity less than 50 IU/L in birds. Plasma ALT activity may be more useful for the detection of liver disease in carnivorous birds than the non-carnivores (Campbell, 2004). El-Fiky *et al.,* (2008) showed that the Plasma GOT, GPT and alkaline phosphatase activities were not significantly affected by color of light, nor there were significant differences due to the interaction between light color and sex of turkeys.

Thyroid hormones play an important role in regulating the fat metabolism, and plasma concentrations of these hormones could be potential indicators of metabolic activity and physiological responses of birds at commercial poultry farming (Melesse *et al.,* 2011). Plasma T3 level of broilers reared under (short day 16 Light: 8 Dark) was significantly higher than (continuous 24 light) at 21 day of age but did not differ at 42 day. T4 levels did not change by treatments at both ages (Ozkan *et al.,* 2005). El-Husseiny *et al.,* (2000) found a significant positive influence of green color also on T3 and T4 concentrations in plasma. Rozenboim *et al.,* (2004) revealed that chicks at 35 day of age, T3 was higher in the birds exposed to WL, GL, and BG10 in comparison with those exposed to BL. T4 was significantly higher in BG10 compared with BL treated chicks. At 46 d of age, the highest T3 was monitored in chickens exposed to BG20, whereas T4 was the highest in the BG10 treated chickens.

Plasma Cortisol or corticosterone is the primary glucocorticoid used as a measure of endocrine response to stress in the HPA axis (hypothalamus-pituitary gland-adrenal gland) (Charmandari *et al.,* 2005). Corticosterone has marked effects on carbohydrate, lipid and protein metabolism (Nakagawa-Mizuyachi *et al.,* 2009). Olanrewaju *et al.,* (2010) found that Plasma corticosterone was not affected by light intensity. The results indicate that continuous exposure of high light intensity markedly affects various blood variables without inducing stress in broilers. 1-day old Ross 308 chicks reared in different light intensity (25, 10, 5, 2.5, and 0.2 lux), plasma corticosterone concentrations were not statistically affected by light intensity, suggesting an absence of stress (Olanrewaju *et al.,* 2011). Onbasilar and Aksoy, (2005) reported that Increasing cage density, from one to five hens per cage, resulted in a significant increase of the plasma corticosterone. Therefore, higher corticosterone levels in the 5 hens/cage groups could reflect higher stress conditions. During stress conditions, neural impulses come to the hypothalamus

and are converted to neuro-humoral factors. Corticotrophin-releasing factor stimulates the anterior hypophysis to secrete ACTH (adrenocortical trophic hormone), which, in turn, stimulated the adrenal for corticosterone secretion (Siegel, 1985).
FSH and LH are gonadotropins, thus the releasing hormone is called gonadotropin-releasing hormone. Gonadotropins from the pituitary gland then travel (via systemic circulation) to the gonads, testis in males and ovaries in females, where they affect gametogenic and steroidogenic function (Senger, 2003). The secreting of LH and FSH was enhanced by blue light from 19-36 week and the secreting of LH and FSH was enhanced by white and red lights from 37-53 week. The peak values of LH and FSH in All lights were appeared at morning within 24 hours (except FSH in white light) (Erdemtu, 2007). In Iraq, review of literature revealed only scarce research regarding the effects of color light on serum enzymes. So, this study intend to investigate the effect of color light and stocking density on serum Glutamic Oxaloacetic Transaminase (GOT) , Glutamic Pyruvic Transaminase (GPT), Triiodothyronine (T3) , Thyroxine (T4) and Cortisol level in broilers and serum follicle stimulating hormone (FSH) and luteinizing hormone (LH) in layers.

## 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 5 weeks. All broilers were kept 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 light-emitting diode system (LED) applied for 24 hours daily in separated rooms (3 × 3 × 4 meters) with light intensity 5 watt/$m^2$.The birds were randomly housed into 9 wooden sealed pens of 1$m^2$ 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 experiment, 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 (Isa Brown, 2010). 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.

## Biochemical Traits

At the end of 5 week age for broilers, 1 birds of average weight from each replicate were selected and blood was collected from the wing vein. For layers, 2 ml of blood were

collected twice in the light and in the dark period from 1 bird in each replicate at the end of the 36 week. Blood samples were taken in test tubes without anticoagulant and labeled according to each replicate then centrifuged and the collected serum stored at -20°C until analyzed (Al-Daraji *et al.,* 2008). The GOT and GPT enzymes were measured by used a specific kit of Egyptian Company for Biotechnology according to the manufacturer recommendations by spectrophotometer at the wavelength of 505 nanometer (nm) (Reitman and Frankel, 1957). T3 and T4 hormones measured according to the method of work on the Kit manufactured by the American company Adlitteram, as described by Iqbal, (2009) using Enzyme Linked Immuno Sorbent Assay (ELISA) type (Mindray MR-96 A) at a wavelength of 450 nm. Cortisol hormone was measured using Kit (Cortisol ELISA) of the company (DRG, Germany) and according to the manufacturer's instructions as described by Tietz, (1999). For FSH and LH test, FSH ELISA Kit from Human Company/ Germany was used according to Odell *et al.,* (1968) and LH according to Kosasa, (1981) using Elisa reader type (Mindray MR- 96 A) as it has been read absorbance at a wavelength of 450 nanometers during the 30 minutes of stopping the reaction and extracted concentrations of FSH and LH using a standard curve prepared for this purpose.

## Models of analysis

Data was analyzed using completely randomized design (CRD) according to SPSS (2009). The significant tests for the differences between each two means for any studied trait were done according to Duncan's multiple rang test.

**The model was:** $\mathbf{Y_{ijk} = \mathit{M} + L_i + D_j + (LD)_{ij} + e_{ijk}}$

Where: $Y_{ijk}$ = Observation on the ij individual; $M$ = Overall mean; $L_i$ = light effect; $D_j$ = density effect; $(LD)_{ij}$= Interaction between light and density; $e_{ijk}$ = Random error

## Results and discussion

## GOT and GPT enzymes

The appearance of abnormal amounts of certain enzymes of intercellular origin in the blood reflect damage to an organ or tissue. The liver is rich in some enzymes as ALT, AST and it's damage often results in releasing these enzymes to the blood (Kaplan *et al.,* 2003). The effect of color light and stocking density on GOT and GPT levels in the serum of broilers aged 35 days at different experimental treatments appear in Table 1. The results appear in this table shows significant effect ($P < 0.05$) for the color light used in the concentration of GOT enzyme in various treatments. The higher rate was 316.7 IU / L in the serum of broilers reared under the influence of BL, while recorded lower rate 179.6 IU / L in the serum of broilers reared under the influence of GL, and possibly attributed the reason for the high level of GOT enzyme to the high activity of the enzyme in birds under decline in white blood cells recorded in BL group (Stress leukeogram). As described by Al-Daraji *et al.,* (2008), GOT related with Stress - induced increased serum AST activity, where accompanied this case with the drop in the blood's white cells rate stress. The result of this study disagreed with that of El-Fiky *et al.,* (2008), who showed that the plasma GOT was not significantly affected by color of light (ultra violet, infra-

red, Incandescent light and fluorescent Light). The table also referred to the lack of significant effect (P>0.05) of color light in the concentration of GPT enzyme in serum of broilers at different groups at 35 day. The results of the current study agreed with Saad, (1995) and El-Fiky *et al*., (2008), who showed that the plasma GPT and alkaline phosphatase activities were not significantly affected by color of light, nor there were significant differences due to the interaction between light color and sex of turkeys. This indicated that color light had no harmful effect on liver function. As for stocking density, the result revealed no significant effect (P>0.05) in the concentration of GOT and GPT enzymes. This study confirmed with Results obtained by El- Deek and Al-Harthi, (2004), who reported that the concentration of GOT and GPT enzymes in the serum of chickens reared in three densities 10,14 and 18 birds/$m^2$ were not significantly affected due to the absence of stress factors. The results are also consistent with those obtained by Szabo *et al*., (2005), who mentioned about the effect of stocking density in the concentration of serum GPT enzyme of turkeys. The analysis of variance referred to the absence of significant interaction (P>0.05) between color light and stocking density of birds in the level of GOT and GPT enzymes in various treatments.

## T3 and T4 hormones

Circulating thyroid hormones T3 and T4 are important growth promoters and play a relatively important role in growth inhibition as well as compensatory growth acceleration in broilers (Yahav, 1999). Table 1 indicated a significant effect (P <0.05) of color light in the level of T3 hormone .The highest rate recorded was 1.72 ng / ml in the serum of broilers reared under the influence of RL, while the lower rate 1.30 ng/ml in serum of broilers reared under the influence of BGL. The result of the current study was inconsistent with the results obtained by Rozenboim *et al.,* (2004), who investigated the effect of switching green and blue monochromatic light at different ages on growth of male broiler birds. Rozenboim *et al*., (2004), didn't observed the association among light treatment, performance, and plasma T3 concentration. Cao *et al.,* (2008) showed that absolute weight and follicle area of thyroid were lower in the red light group than in other light groups (4.67%-41.47% and 22.16%-50.32%, P<0.05). The study indicates that the red light (660 nm) stimulated the growth of epithelial cells in the thyroid of broilers. The level of T4 was significantly differed (P <0.05) as shown in Table. 1, which recorded the highest rate 6.53 ng / ml in the serum of broilers reared under the influence of BGL, while the lowest rate of 3.96 ng / ml in broilers reared under WL.

The results of this study are compatible with the results obtained by Rozenboim *et al.,* (2004), that pointed to the existence of significant differences in the concentration of T4 hormone in the serum of broilers reared in the treatment of switching from blue to green light at 10 day, reaching 7.4 ng / ml compared to those reared under the effect of other color lights (blue, white and green). The result of this study is also in agreement with El-Husseiny *et al.,* (2000), which referred to the influence of the green light on the concentration of T4 in the blood serum. The results of this test possibly attributed the rise in the level of T4 hormone to its role in stimulating the metabolism, which is reflected in the increase on feed intake and body weight of broilers of this group. T3 hormone is known to be closely associated with feeding which is also a key factor influencing conversion of T4 to T3 with a diurnal pattern as reviewed by McNabb (2000). As showed

in Table 1, there is no significant effect (P>0.05) of stocking density in the concentration of T3 hormone in the blood serum of broilers reared at different experimental treatments. This result agreed with that of Davis *et al.,* (2000), who revealed that there is no effect of the density in the level of T3 and T4 hormones on his study about Hyline W-36 and DeKalb XL, housed in layer cages at two densities (361 and 482 cm2 per bird) with two replications each per strain and density combination. The results indicated that strain did not affect. The analysis of variance referred to the absence of significant interaction (P>0.05) of the effect of light color and density on hormonal level of T3 and T4 in blood serum of the various groups.

## Cortisol hormone

In birds, Corticosterone is the major stress hormone (Thaxton and Puvadolpirod ,2000). Thus, blood corticosterone concentration has been widely used as a measure of environmental stress in broilers (Zulkifli *et al.,* 2003). Table 1 reported that broilers had been exposed to five color lights showed an absence of significant effect (P>0.05) of light color on serum Cortisol concentration. The results of present study agreed with findings of Olanrewaju and Branton, (2011), who found that corticosterone concentration did not differ between various treatments, indicating that light schedule did not cause severe stress. The result of this work is disagreed with Xie *et al.,* (2007), who studied the effects of various monochromatic lights on serum Cortisol levels of broilers. The study recorded a significant increase of the hormone in the serum of broilers reared under the influence of WL at 21 and 49 day (139.382 $\pm$ 14.751 and 142.279 $\pm$ 5.107 ng/ml respectively). On the other hand, stocking density had no significant effect (P>0.05) on serum cortisol; result of the current study may be due to the absence of stress. This finding is in agreement with the results reported by Buijs *et al.,* (2009), who showed that corticosteroid levels, bursa weight, final body weight, and mortality were not significantly affected by stocking density.

The results of this study is compatible also with previous study of Davis *et al.,* (2000) ,who found that the increasing in stocking density did not affect corticosterone levels in hens. Similar results were obtained by Thaxton *et al.,* (2006). who failed to detect any effects of density on corticosterone, glucose, and cholesterol levels in broilers reared between 0.14 and 0.052 $m^2$ space allowance per bird (20 and 55 $kg/m^2$). However, mean corticosterone concentration according to Turkyilmaz *et al.,* (2008) on day 42 indicated an increase from 3.81 to 4.39 ng/ml with increased stocking density. Furthermore, there was no interaction among blood corticosterone concentration at 4, 5, and 6 weeks of age. Siegel, (1985) revealed that the ratio of heterophils to lymphocytes of the group having 5 hens/cage was higher than those of groups having 1 or 3 hens/cage. The researcher suggested that this condition could be explained by the elevated concentration of corticosterone in blood circulation, which causes an increase in heterophil count and a decline in lymphocyte count.

**Table 1:** Effect of color light and stocking density on some enzymes and hormones of broilers serum at $35^{th}$ day of age (M$\pm$SE)

| Biochemical test | Color light / Stocking density | WL | RL | BL | GL | BGL | Effect of stocking density |
|---|---|---|---|---|---|---|---|
| GOT (IU/L) | 12 bird/m² | 226.6 ±25.4 | 308.0 ±52.0 | 309.6 ±116.3 | 146.3 ± 31.9 | 292.0 ±120.0 | 256.5 ±69.1 |
| | 15 bird/m² | 282.6 ±32.8 | 332.3 ±42.2 | 339.0 ±145.5 | 169.0 ± 39.5 | 280.0 ± 96.8 | 280.6 ±71.3 |
| | 18 bird/m² | 267.0 ±76.0 | 286.6 ±50.8 | 301.6 ± 66.3 | 223.6 ± 64.6 | 236.6 ±126.6 | 263.1 ±76.9 |
| | Effect of color light * | 258.7 b ±44.7 | 309.0 ab ±48.3 | 316.7 a ±109.4 | 179.6 b ±45.3 | 269.5 ab ±14.4 | N. S |
| GPT (IU/L) | 12 bird/m² | 7.50± 0.75 | 6.76 ± 1.87 | 5.46 ± 0.53 | 6.03 ±1.40 | 5.23 ± 1.41 | 6.20 ± 1.19 |
| | 15 bird/m² | 7.13±1.22 | 5.10 ± 1.24 | 5.76 ± 0.63 | 5.63 ±1.94 | 5.00 ± 0.00 | 5.72 ± 1.00 |
| | 18 bird/m² | 5.60± 0.91 | 5.73 ± 1.63 | 6.33 ±0.88 | 6.83 ±1.54 | 4.30 ± 0.15 | 5.76 ± 1.02 |
| | Effect of color light N. S | 6.74± 0.96 | 5.86 ± 1.58 | 5.85 ± 0.68 | 6.16 ±1.62 | 4.84 ± 0.52 | N. S |
| T3 ng / ml | 12 bird/m² | 1.21± 0.17 | 1.20 ± 0.16 | 1.47 ± 0.32 | 1.22 ±0.00 | 1.36 ± 0.41 | 1.29 ± 0.21 |
| | 15 bird/m² | 1.73± 0.06 | 1.87 ± 0.18 | 1.09 ± 0.07 | 1.37 ±0.38 | 1.16 ±0.24 | 1.44 ± 0.18 |
| | 18 bird/m² | 1.52± 0.14 | 2.09 ± 0.17 | 1.24 ± 0.28 | 1.42 ±0.38 | 1.40 ± 0.08 | 1.53 ± 0.21 |
| | Effect of color light * | 1.49 ab ±0.1 | 1.72 a ±0.17 | 1.27 b ±0.2 | 1.33 ab ±0.2 | 1.30 b ±0.24 | N. S |
| T4 ng / ml | 12 bird/m² | 4.0 ± 0.05 | 6.0 ± 0.12 | 2.4 ± 0.03 | 6.2 ± 0.10 | 7.4 ± 0.12 | 5.20 ± 0.08 |
| | 15 bird/m² | 4.1 ± 0.00 | 4.0 ± 0.00 | 9.6 ± 0.39 | 4.3 ± 0.13 | 7.0 ± 0.37 | 5.80 ± 0.17 |
| | 18 bird/m² | 3.8 ± 0.00 | 5.3 ± 0.02 | 3.8 ± 0.11 | 4.6 ± 0.12 | 5.2 ± 0.18 | 4.54 ± 0.08 |
| | Effect of color light * | 3.96 c ±0.01 | 5.10 b ±0.04 | 5.26 b ±0.17 | 5.03 b ±0.1 | 6.53 a ±0.22 | N. S |
| Cortisol nmol/L | 12 bird/m² | 37.70 ±15.61 | 18.33 ± 0.88 | 23.00 ± 1.73 | 45.00 ±16.77 | 31.33± 16.42 | 31.07 ±10.2 |
| | 15 bird/m² | 27.40 ±11.52 | 29.33 ± 6.98 | 38.33 ± 6.83 | 38.83 ±29.14 | 34.00 ± 22.00 | 33.58 ±15.2 |
| | 18 bird/m² | 36.00 ±14.64 | 42.33 ± 3.75 | 23.33 ± 4.91 | 22.66 ± 1.66 | 40.80 ± 27.60 | 33.02 ±10.5 |
| | Effect of color light N. S | 33.70 ±13.92 | 30.00 ± 3.87 | 28.22 ± 4.49 | 35.50 ±15.85 | 35.37 ± 22.00 | N. S |

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

## FSH hormone

FSH and LH hormones act on the ovaries or testes to stimulate follicle and sperm production, respectively. In the ovary, the small follicles produce androgens and estrogens that stimulate development of secondary sexual characteristics, oviduct development and enlargement to secrete albumen and mobilization of calcium from bone are some of the other responses (Robinson and Renema, 1999). Table 2 referred to the effect of color light and stocking density on FSH level of layers aged 36 weeks. A significant effect (P <0.05) was recorded for the color light used in the concentration of FSH. In the dark period, the highest rate recorded in chicken serum reared under the influence of BL 0.116 IU / L, while the lowest rate 0.056 IU /L recorded in chicken serum reared under the influence of BGL but no significant difference (P>0.05) was recorded during the light period between different treatments. These findings are consistent with Wang *et al.,* (2011), who found the effect of color light on laying hens aged 8 months under the intensity of illumination 15 lux. The researcher noted an increase in the production of gonadotropin- releasing hormone (GnRH) in chickens reared under the influence of blue light compared to those reared under the influence of red, green and white. This result may be due to the direct effect of BL in the area of the hypothalamus through the skull receptors (Robinson *et al.,* 2003), which acted to activate the production of GnRH hormone and works to stimulate the anterior pituitary gland to manufacture the FSH and LH (Bedecarrats *et al.,* 2006). The Table 2 shows no significant effect (P>0.05) of birds density in the concentration of serum FSH in chickens either in the light or dark period. This result is agreed with Guo *et al.,* (2012), who pointed to the absence of

significant differences in the level of FSH in laying hens serum levels under different density. The analysis of variance refers to the existence of a significant interaction (P <0.05) between the color light and stocking density of birds in the level of FSH at dark period as it recorded the highest rate 0.135 IU / L in chicken reared under the influence of GL at the level of density 7 birds / $m^2$, while the low average recorded was 0.056 IU / L in chicken serum reared under the influence BGL at the level of density 7birds/$m^2$.

**Table 2:** Effect of color light and stocking density on serum FSH and LH of layers at 36 week of age (M±SE)

| Biochemical test | Color light / Stocking density | WL | RL | BL | GL | BGL | Effect of stocking density |
|---|---|---|---|---|---|---|---|
| FSH IU/L Dark period | 5 bird/m² | 0.120 ± 0.01 | 0.093 ± 0.01 | 0.128 ± 0.03 | 0.059 ± 0.00 | 0.058 ± 0.00 | 0.091 ± 0.01 |
| | 7 bird/m² | 0.084 ±0.01 | 0.079 ± 0.00 | 0.104 ± 0.01 | 0.135 A** ± 0.01 | 0.056 B ± 0.00 | 0.091 ± 0.00 |
| | Effect of color light * | 0.101 a ± 0.01 | 0.086 ab ±0.0 | 0.116 a ± 0.02 | 0.096 a ± 0.00 | 0.056 b ± 0.0 | N. S. |
| FSH IU/L Light period | 5 bird/m² | 0.064 ± 0.00 | 0.092 ± 0.01 | 0.086 ± 0.00 | 0.110 ± 0.00 | 0.130 ± 0.01 | 0.096 ±0.00 |
| | 7 bird/m² | 0.098 ± 0.00 | 0.097 ± 0.00 | 0.439 ± 0.00 | 0.164 ± 0.02 | 0.060 ± 0.02 | 0.171 ± 0.00 |
| | Effect of color light N. S. | 0.080 ± 0.00 | 0.094 ±0.00 | 0.262 ±0.00 | 0.137 ±0.01 | 0.094 ± 0.01 | N. S. |
| LH IU/L Dark period | 5 bird/m² | 0.606 ± 0.15 | 0.366 ± 0.07 | 0.398 ± 0.10 | 0.522 ± 0.01 | 0.488 ± 0.08 | 0.476 ± 0.08 |
| | 7 bird/m² | 0.403 ± 0.04 | 0.393 ± 0.06 | 0.495 ± 0.11 | 0.339 ± 0.05 | 0.311 ± 0.02 | 0.388 ± 0.05 |
| | Effect of color light N. S. | 0.504 ± 0.09 | 0.379 ± 0.06 | 0.446 ±0.10 | 0.430 ±0.03 | 0.399 ± 0.05 | N. S. |
| LH IU/L Light period | 5 bird/m² | 0.495 ± 0.11 | 0.720 ± 0.10 | 0.304 ± 0.03 | 0.421 ± 0.03 | 0.452 ± 0.17 | 0.478 ±0.08 |
| | 7 bird/m² | 0.566 ± 0.05 | 0.406 ± 0.02 | 0.449 ± 0.04 | 0.394 ± 0.11 | 0.159 ± 0.10 | 0.394 ± 0.06 |
| | Effect of color light * | 0.530 a ±0.0 | 0.562 a ±0.0 | 0.376 ab ± 0.0 | 0.407 ab ±0.0 | 0.305 b ± 0.1 | N. S. |

***a, b, c Means in horizontal rows with different superscripts were significantly different of light color 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).**

## LH hormone

As in Table 2, there is no significant effect (P>0.05) of color light on the level of LH hormone of layer serum in the dark period. In lighting period the table refers to the existence of a significant effect (P <0.05) for the color light used on serum LH level of layers reared under the influence of RL, which recorded the highest rate 0.562 IU /L, while recorded less value 0.305 IU / L in layers reared under the influence of BGL. These results do not agree with the results obtained by El-Fiky *et al.*, (2008), who found that the hormone LH concentration in turkeys serum in the period before 25 week of age increased significantly when using incandescent light compared with fluorescent or UV illumination. After the age of 40 weeks, the differences diminished and color effect became weaker. Erdemtu, (2007) revealed that the rise length of LH and FSH in blue light was longest and the peak value of LH and FSH in blue light was appeared at the last time from 25-34 week. The LH in white light was highest and significantly higher than those in other light groups from 37-49 weeks and the rise of LH in red light was persistent from 40-49 weeks. As for the effect of stocking density on the LH hormone level, the Table 2 displayed insignificant results (P>0.05) in the both periods of light and dark. This

result is probably due to the density of birds that do not have a clear effect on the physiological indicators of birds Nicol *et al.,* (2006). The analysis of variance showed no significant interaction (P>0.05) between the color light and density of birds on serum LH level of layers either in dark or light period.

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