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RESEARCH ARTICLE

EFFECT OF LIGHT COLOR AND SOCKING DENSITY ON INTESTINAL MORPHOLOGY OF BROILER CHICKENS

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ABSTRACT

Many studies have been conducted to determine the effect of light on broiler performance, and many others on the effect of stocking density. However, there has been a very few investigation on the influence of light color on intestinal morphology of broilers. This study intended to evaluate the effects of color lights and bird density as it relates to small intestine (villi length and crypt depth) of broilers.A total of 675Ross 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 1m2 in three replicates for each density 12, 15 and 18 birds/m2 in the room.

INTRODUCTION

Many studies have been shown the effects of light and stocking density on broilers performance in experimental assay, but we decided to evaluate the effects of different color lights and densities on intestinal morphology. The small intestine is the main site of feed digestion, nutrients absorption and secretion of digestive fluids (Yamauchi, 2002). The mucus layer varies in thickness and composition throughout the gut and protects the underlying enterocytes lining the villus from mechanical, enzymatic, and chemical challenges in addition to being a source of intestinal lubrication (Sharma and Schumacher, 1995). Long villi are usually equated with excellent gut health, high absorptive efficiency and healthier intestinal tract of chickens (Alfaro *et al.,* 2007). Under magnification, intestinal villi and crypts are seen. In general, intestinal villi are 0.5-1.5 mm long and they are projecting out of the lamina and epithelium into the lumen of the small intestine. The base of the intestinal villi is covered by young epithelial cells which are known as intestinal crypts (Yamauchi, 2002). Lighting is a powerful exogenous factor in control of many physiological and behavioral processes (Leven, 2000). Light may be the most critical of all environmental factors to birds (Manser, 1996) and consist of three different aspects: quantitative aspect (photoperiod) as well as qualitative aspects include lighting color (wavelength) and light intensity (Heshmatollah, 2007). Day light has relatively wavelengths between 400 and 700 nm. Birds sense light through their eyes (retinal photoreceptors) and through photosensitive cells in the brain (extra-retinal photoreceptors) (El-Fiky*et al.,* 2008). 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) (Hakanand Ali, 2005). Affection of light on activity and reproduction of chickens had been known from many years ago and it was also shown by many researcher. A few studies have explored on color light preferences of broiler chicks. Taylor *et al.* (1969) observed that one day-old chicks prefer red and yellow lights in comparison with blue light. Rozenboim *et al.* (2004) referred that broilers reared under blue or green light were significantly heavier than those reared under red or white light. Heshmatollah, (2007) reported when chickens had the ability to choose among red, orange, yellow, or green lights, they spent significantly more time under green light. Their second preference was yellow light. Cao *et al.*(2008) described that, monochromatic light could significantly affect broiler growth and development. The GL promoted broiler growth better at early stage, and the BL promoted broiler growth better at a later stage under an illumination intensity of 15 lx. 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

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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 number of birds per unit area, density is calculated as bird weight per unit area (Fairchild, 2005). In broilers, high densities have been associated with a decline in body weight (BW), feed intake (FI) and feed conversion rate (FCR) (Sanotra*et al.,* 2001).

MATERIALS AND METHODS

Animals and animal husbandry

The research was done at the poultry farm, College of Veterinary Medicine, Basra University, Basra, Iraq. A total of 675 Ross 308 one-day-old broiler chicks were used in this study. The chicks were raised under control condition from day one until 35 days of age. Broiler chicks were reared into five light groups in separated rooms 3 x 3 x 4 meters with an average 135 chicks in each room under LED color lights include : White light as a control, Red light (660 nm), Blue light (480 nm), Green light (560 nm) and Blue – Green mix light. Stocking density (12, 15 and 18 birds/m2) were randomly housed into 9 wooden sealed pens of 1m2 in three replicates in the room. Light sources were equalized on the intensity of 5 watt/ m2 (20 lux) at bird head level and light period of 24 hours daily. Room temperature was initially 34°C which was subsequently reduced by 2°C/week to 22°C by 35 day. Three- dietary pellet rations were used to feed the chicks, which consist of starter, grower, and finisher diets. 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. Half cylinder plastic feeders were placed in each pen. The birds were supplied with feed and water ad libitum, and diets were formulated to meet the nutrient recommendations for poultry according to NRC, (1994).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.

Intestinal mucosal morphology

On days 35, three chickens were randomly selected from each replicate and slaughtered through a section in the jugular vein. The carcass was eviscerated manually by cutting around the vent to remove all of the viscera including the small intestine (Toghyani *et al.,* 2012).Pieces of 1 cm small intestine specimens were fixed in 10% buffered formalin. Each piece of tissue was trimmed at the thickness of 5 microns (µ) in size, fixed and dehydrated in a series of increasing alcohol concentration, and embedded in paraffin wax using rotary tissue processor. The sections were stained with Mayer's Hematoxylin and eosin for observation with a light microscope (Luna, 1968). Villus height was measured as the length between the tip of the villus and the villous-crypt axis. Measurements for crypt depth were taken from the valley between individual villi to the baso- lateral membrane as described by Baurhoo *et al.* (2007). Values are means from 10 different villi and only vertically oriented villi and crypts were measured (Uni *et al.,* 2003) and the ratio of villus length to crypt depth (V:C ratio) was calculated.

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:

$Yijk = M + Li + Dj + eijk$

Where: Yijk = Observation on the ij individual

M = Overall mean
Li = light effect
Dj = density effect
Eijk = Random error

RESULTS AND DISCUSSION

Over the last decades, there has been increased interest in intestinal growth/health as poultry nutritionists have attempted to adopt new approaches to deal with the broader changes in the overall agricultural landscape. Color is an important aspect of light that has been considered at one time as a management tool in poultry production (Prayitno, 1997).The results of this study revealed a significant increase ($P< 0.05$) of villus height 786 µm in broilers reared under WL but no significant effect ($P > 0.05$) was recorded under different levels of stocking density as shown in Table 1. The significant effect of WL on feed intake was confirmed by Rierson, (2011) who found that birds during weeks 1 to 3, prefer white light. The chicks demonstrated a preference for pelleted feed and white light ($P <0.01$) and they chose not to feed under green or blue light. A possible explanation as to why broilers prefer to consume feed under white light could be due to it helps them to identify texture differences they cannot see under different colors. Adopting a strategy allowing broiler chicks to feed under white light and rest under blue or green light. Prayitno *et al.* (1997) conducted a study to compare broilers behavior under red, white, blue, or green lights. It was concluded that broilers raised under red and white lights were more active than those raised under blue and green lights as expressed by greater walking activity in the white light treatment. It has been suggested that feed intake outstrips the increase in size of the gastro intestinal tract(GIT) and therefore rate of passage of feed through the intestine is faster (Sibbald, 1979). Ferket and Gernat (2003); Forbes (2005); Scott, (2005) agreed that voluntary feed intake is linked to growth rate.Two excellent reviews on the biological relationship between intestinal development and growth in avian species can be found in Konarzewski *et al.* (1989; 1990). Sklan *et al.* (1975) ; Sklan, and HuIwitz, (1980) referred to the increases in length, internal diameter, and villus volume of the small intestine together with the five to eight-fold increase in feed. Overall, the increased feed intake with age is accompanied by rapid development of the GIT, especially in the first week after hatch (Noy and Sklan, 1997). Karakaya *et al.* (2009) found that, feed consumption, body weight and total muscle weight values of the muscles from GL-BL and GL-GBL mix lighting groups were significantly higher than those of incandescent (control) lighting groups. Cao *et al.* (2008) indicated that broilers reared under BL were larger in the weights of carcass, breast muscle, thigh, crus, and net chamber at d 49 as compared with other light groups.

Table 1. Effect of light color and stocking density on Villus height (µm)of broiler intestine at 35 day (M ± SE)

Light color	WL	RL	BL	GL	BGL	Effect of stocking density
Stocking density						
12 birds/m^2	^{ab}685.8±17.63	^{a}777.6±15.74	^{a}763.2±42.53	^{b}651.6±25.55	^{c}473.4±11.24	670.3±24.51
15 birds/m^2	^{a}837.0±24.48	^{b}565.2±32.37	^{a}723.6±46.47	^{a}819.0±9.85	^{a}808.2±15.16	750.6±23.64
18 birds/m^2	^{a}835.2±7.74	^{b}626.4±30.99	^{b}572.4±34.22	^{a}754.8±12.67	^{a}743.4±31.64	706.44±21.99
Effect of light color	^{a}786.0±21.23	^{b}656.4±28.00	^{ab}686.4±31.18	^{ab}741.8±20.64	^{ab}675.0±40.38	
*						N.S.

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

Table 2. Effect of light color and stocking density on Crypt depth (µm)of broiler intestine at 35 day (M ± SE)

Light color	WL	RL	BL	GL	BGL	Effect of stocking density
Stocking density						
12birds/m^2	^{ab}147.6±14.4	^{a}172.8±10.41	^{a}176.4±12.91	^{ab}138.6±8.34	^{b}106.2±3.36	^{b}148.32±6.77
15birds/m^2	^{a}217.8±8.72	^{b}131.4±8.34	^{a}210.6±10.10	^{a}205.2±7.20	^{a}203.4±9.26	^{a}193.68±7.36
18birds/m^2	^{a}214.2±6.61	^{bc}158.4±8.34	^{c}147.6±14.40	^{ab}194.4±8.34	^{ab}187.2±5.24	^{a}180.36±6.20
Effect of light color	193.2±10.27	154.2±6.68	178.2±9.62	179.4±8.89	165.6±11.87	
N.S.						*

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

Table 3. Effect of light color and stocking density on V:C ratioof broiler intestine at 35 day (M ± SE)

Light color	WL	RL	BL	GL	BGL	Effect of stocking density
Stocking density						
12 birds/m^2	4.01±0.88	4.54±0.20	4.34±0.12	4.75±0.27	4.47±0.18	^{a}4.58±0.11
15 birds/m^2	^{ab}3.85±0.09	^{a}4.31±0.16	^{b}3.43±0.16	^{ab}4.00±0.12	^{ab}3.99±0.13	^{b}3.92±0.08
18 birds/m^2	3.90 ±	3.96 ±	3.94±0.23	3.90±0.12	3.97±0.13	^{b}3.93±0.06
Effect of light color	0.09	0.12	3.91±0.13	4.21±0.14	4.14±0.10	
N.S.	4.19±0.18	4.27±0.10				*

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

According to Xie *et al.* (2009), who studied Arbor Acres male broilers were exposed to red, green, blue and white light from LED lamps. The study found that small intestine mucosal structure is improved to an extent in broilers when illuminated with green light at the early growth stage (0-7 days) under 15 Lux light intensity, accordingly improving the absorption function of the small intestine and accelerating growth. Xie *et al.* (2011), found that villus height of small intestine was increased in broilers exposed to GL and BL than those in the WL group. The suggestion that both mucosal mechanical and immunological barriers of the small intestine might be improved by rearing broilers under GL at an early age and under BL at an older age. The results of Firouzi *et al.* (2014) indicated that the birds reared under yellow and blue light had the best and weakest performance, respectively. According to results in Table 2 and 3, no significant difference (P > 0.05) of color light was observed between treatments considering the crypt depth (µm) and V:C ratio of broiler intestine, while a significant effect (P < 0.05) of treatments was detected for the stocking density which recorded193.68 9(µm) in the treatment of 15 birds/m^2 for crypt depth and 4.58 in the treatment of 12 birds/m^2 for V:C ratio. Increased stocking densities are frequently reported to depress chicken growth performance, but the mechanisms behind this are not fully understood (Guardia, 2011). Riyad *et al.* (2014) revealed that The small intestine length (cm) was differed (P< 0.05) in broilers reared under GL and 18 birds/m2, while small intestine weight (gm) was higher (P< 0.05) under BL and 12 birds/m2 in compare with other groups at 35th day. Alaeldein *et al.* (2013) revealed that heavier breasts were obtained from birds which had subjected to the low stocking density (p<0.001). Total small intestine lengths and weights from birds which were subjected to the low density were the longest and the heaviest as compared to the other two groups.

Burkholder *et al.* (2008) reported that stressful conditions had a negative impact on intestinal morphology. A negative effect (Dozier *et al.,* 2006), or a positive effect in the first 3 week (Moreira *et al.,* 2004). These discrepancies between studies concerning the effect of stocking density on growth performance of the chicken during the first 3 week of life may be explained by the different experimental conditions, such as bird strain, the presence or absence of antibiotics, or litter type (Guardia, 2011).

Conclusion

Based on the results of the present study, it can be stated that light color light and stocking density affected broiler intestinal morphology. However, results from previous studies are not completely consistent, especially with respect to the use of colored light. These conflicts were probably due to differences of light source, light schedule, animal species, and age of experimental animals used in different investigations as well as different experimental conditions of stocking density.

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