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Effect of dietary L-tryptophan supplementation and light-emitting diodes on growth and immune response of broilers

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Abstract

Light-emitting diodes (LEDs) lights are more energy-efficient and provide adequate illumination compared to compact fluorescent (CFL) lamps and incandescent light (ICD) bulbs. However, as new light sources, the LED lights may have a stress effect on broiler chickens. Thus, this study aimed to determine the effects of dietary L-tryptophan (Trp), as an anti-stress agent and different color temperatures of light-emitting diodes on immune responses and growth performance of male broiler chickens. Four hundred and eighty day-old Ross 308 male chicks were used from day 1 to 42. The chicks were randomly distributed into six treatment groups in a 2 × 3 factorial arrangement [0 or 1 g Trp per kg diet along with neutral-white (4286 K), warm-white (2990 K), and incandescent (2790 K) light bulbs] with four replicates of 20 chicks each. Results showed that dietary Trp and Trp×light interaction did not affect growth performance, immune responses, a total number of leukocytes, and different leukocytes count (heterophil, eosinophil, monocyte, and lymphocyte) of male broiler chickens. However, LEDs' different color temperatures significantly affected the feed conversion ratio (FCR) and primary antibody of sheep red blood cell (SRBC). The FCR was the lowest in the warm-white light, and primary SRBC antibody titers of the chicks were the highest. In conclusion, although adding Trp to male broiler diets did not affect the growth performance and immune responses of chickens, the warm-white light improved the FCR and primary SRBC.

Introduction

Many years ago, poultry industry has relied on incandescent (ICD) light bulbs to provide illumination in poultry houses. However, these bulbs are currently being phased out due to their relatively high power consumption. The light-emitting diodes (LEDs) lights are much more energy-saving and provide adequate illumination than ICD light bulbs.[1] Recently, LEDs emitting white light with a range of color temperatures have been developed. Color temperatures, a way to describe the light appearance, is measured in Kelvin's (K) degrees. Previous works on broilers showed that using monochromatic blue and green light may be beneficial to welfare,[2] performance,[3,4] and immune responses of chickens.[5] Different color lights have been found to have an alleviating effect on broiler stress responses due to a reduction in serum IL-1β, corticosterone, and heterophil: lymphocyte (H:L) ratio in the blood.[5,6] Huth and Archer showed that LED treatments improved feed conversion compared to CFL treatment.[6] They suggested that this might be a result of decreased stress levels found in the LED treatments.

The LED lights with different color temperatures, as new light sources, may have a stress effect on broiler chickens, which adversely affect growth performance and immune responses. On the other hand, it has been shown that under stressed conditions (under high stocking density), dietary supplementation of L-tryptophan (Trp) improves the growth performance and meat quality of ducks.[7] Also, aggression, feather-pecking behavior, and stress were decreased by adding Trp to the poultry diet.[8] In a study performed on an interaction between light and deity Trp in broiler chickens. It was shown that Trp in combination with discontinuous light enhanced immune responses of broilers.[9] Thus, this study's objective was to evaluate the interaction effect between dietary L-tryptophan and the different color temperatures of the

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LEDs (neutral-white and warm-white) in male broiler chickens on growth performance and immune responses.

Materials and Methods

Light and dietary treatments. A total number of 480 male Ross 308 broiler chicks was used to evaluate the effects of dietary L-tryptophan (Evonik Degussa GmbH, Hanau-Wolfgang, Germany), and the different color temperatures of the LEDs on broiler chicken health and performance were compared. From one to 42 days of age, this experiment involved six treatments in a 2 × 3 factorial arrangement: 0 or 1.00 g Trp per kg diet along with neutral-white (4286 K), warm-white (2990 K; both from Ava Noor Gostaran Alborz Co., Karaj, Iran), and ICD (2790 K) light bulbs. During the experimental period, the birds received a mash diet in a 3-phase feeding program (Table 1) and had free access to water and feed. Comparing the spectra between these bulbs was shown in our previous report under the same experimental condition.[10] Briefly, each treatment consisted of four pens (1.20 × 2.00 m) containing 20 male broiler chicks, each in a light-tight room equipped with one of the light sources. Light intensity was kept at 50.00 Gallilux of light using a Hato Galli-Luxmeter (Hato Lighting Solutions BV., Handelsstraat, The Netherlands) in the adjacent three rooms. All methods used were approved by the University of Tehran Department of Animal Science (2016-5-31).

Broiler chicken performance. Experimental units were checked daily for mortality. The mortality rate was calculated as the number of broiler chickens that died throughout the study compared to the initial number placed in the pen. Daily feed intake and body weight were recorded weekly to calculate feed conversion ratio (FCR). The feed conversion ratio was calculated by dividing the total feed intake per experimental unit by the total body weight gain per experimental unit and corrected for mortality and day 0 weight.

Anti-SRBC antibody assay. Total anti-sheep red blood cells (SRBC) antibody, IgG, and IgM titers were assayed using methods described previously.[10] Briefly, a phosphate-buffered suspension of SRBC (5.00%) was injected (1.00 mL) into the breast muscle of 12 chicks per treatment at 26 days of age. Seven days after injection, the birds were bled from the brachial vein (2.00 mL) into the heparinized tubes to evaluate the antibody titers. The blood samples were centrifuged for 12 min (1800 *g* at 18.00 ˚C), and the plasma was decanted and kept at – 20.00 ˚C for later analysis.

CBH response Details on the cutaneous basophil hypersensitivity (CBH) response to phytohemagglutinin-M (PHA-M; Gibco, Grand Island, USA) is reported elsewhere.[10] Briefly, at 41 days of age, 12 birds per treatment received 0.10 mL of PHA-M intradermally between the second and third digits of the right foot. The left foot was injected with 0.10 mL of PBS (pH = 7.50) as a control.

Table 1. Ingredients and chemical composition of diets fed to male broiler chicks (as-fed basis) in days.

Ingredient (g kg^{-1})	Starter	Grower	Finisher
Corn (7.50% CP)	566.60	580.30	637.90
Soybean meal (44.00% CP)	382.60	366.50	312.10
Fatty Acid (blend)	8.50	20.20	18.60
Dicalcium phosphate	16.40	15.30	14.00
Calcium carbonate	13.10	6.30	6.50
Common salt	3.20	3.20	3.20
Mineral premixes[1]	2.50	2.50	2.50
Vitamin premix[2]	2.50	2.50	2.50
DL-Methionine (99.00%)	2.10	2.50	2.10
L-Lysine hydrochloride (78.00%)	2.10	0.70	0.60
L-Threonine	0.40	0.00	0.00
Tryptophan	0.00	0.00	0.00
Composition			
AME (kcal kg^{-1})	2806	2913	2953
CP (%)	21.43	20.70	18.70
Calcium (%)	1.00	0.70	0.68
Available phosphorus (%)	0.43	0.40	0.37
Sodium (%)	0.14	0.14	0.14
Lysine (%)	1.39	1.24	1.09
Methionine (%)	0.53	0.57	0.50
Methionine + Cysteine (%)	0.90	0.92	0.83
Threonine (%)	0.90	0.84	0.75
Tryptophan (%)	0.27	0.25	0.22

CP = Crude protein.

[1] Provides (per kg of diet): 12,000 IU vitamin A (retinyl acetate); 3,500 IU cholecalciferol; 100 IU vitamin E (DL-α-tocopheryl acetate); 5.00 mg vitamin K; 3.00 mg thiamin; 12.00 mg riboflavin; 13.00 mg D-pantothenic acid; 50.00 mg niacin; 6.00 mg pyridoxine; 0.66 mg biotin; 2.00 mg folic acid; and 0.03 mg vitamin B12.

[2] Provides (per kg of diet): 10.00 mg copper ($CuSO_4 \cdot 5H_2O$); 2.00 mg iodine (KI); 50.00 mg iron ($FeSO_4 \cdot 7H_2O$); 120 mg manganese ($MnSO_4 \cdot H_2O$); 0.30 mg selenium (Na_2SeO_3); and 110 mg Zn (ZnO).

Immunization against Newcastle disease virus and avian influenza virus. All the birds were subcutaneously vaccinated against the Newcastle disease (ND) virus and avian influenza (AI) virus on the 9th day of age. To evaluate the antibody response to ND and AI, blood samples from 8 birds at 42 days of age were collected for subjecting their sera to hemagglutination inhibition (HI) tests to determine the antibody response titers of ND and AI vaccines.

Total and differential leukocyte enumeration. Total and differential leukocyte enumeration were assayed using methods described previously.[10] Briefly, at 42 days of age, blood samples (0.50 mL) were collected into EDTA-coated tubes from the brachial vein (eight birds per treatment) to enumerate the total leukocytes, using Natt-Herrick's solution and a hemocytometer.

Statistical analysis. The data were analyzed by general linear model (GLM) procedure of the SAS (version 9.1; SAS Institute, Cary, USA). The results were presented as LS mean ± SEM where they were compared by the Tukey's test (honestly significant difference; HSD) at $p \leq 0.05$. The GLM model used in the present study was as follows:

$$Y_{ijl}=\mu+T_i+L_j+TL_{ij}+e_{ijl}$$

where, Y_{ijl} is the observed dependent variable (including growth performance and immune responses data), μ is the mean of the population, T_i is the effect of i^{th} level of Trp (i = 1 and 2), L_j is the effect of j^{th} of a light source (i = 1, 2 and 3), TL_{ij} is the interaction between Trp levels and light sources and e_{ijl} is a random residual error.

Results

The effect of dietary Trp and light treatments on body weight, feed consumption, FCR, and mortality rate during the entire experimental period (1 to 42 days of age) is presented in Table 2. Dietary Trp and Trp×light interaction did not affect growth performance and mortality rate of male broiler chickens. The LEDs, and ICD light bulbs' different color temperatures had no significant effect ($p > 0.05$; Table 2) on body weight, feed consumption, and mortality rate. However, the different color temperatures of LEDs had a significant effect on FCR ($p \leq 0.05$). At warm-white light treatment and FCR of the chicks were the lowest compared to neutral-white and ICD light bulbs.

The lighting sources and dietary Trp did not affect secondary titers of SRBC, AI antibody responses, CBH ($p >$ 0.05; Table 3), and also leukocyte numbers and total leukocyte ($p \geq 0.05$; Table 4). However, LEDs' different color temperatures had a significant effect on primary SRBC antibody titers ($p \leq 0.05$; Table 3). The highest primary SRBC antibody titers were observed at warm-white light compared to neutral-white and ICD light bulbs.

Discussion

Dietary Trp did not affect the growth performance and immune responses of male broiler chickens. In agreement with the current results, Blair *et al.* reported that although the broiler's growth performance was affected by lighting pattern, the addition of Trp (0.27% to 0.60%) to the diet did not significantly influence broiler performance and H:L ratio.[9] However, Bowman and Nockels showed that Trp

Table 2. Effect of dietary L-tryptophan (Trp) and light treatments on male broiler chicken performance (whole period).

Variable	Body weight (g)	Body weight gain (g per chicken per day)	Feed intake (g per chicken per day)	Feed conversion ratio (g feed per g weight gain)	Mortality (%)
Light					
Warm-white	2663.62^{a}	61.26^{a}	107.96^{a}	1.77^{c}	2.81^{a}
Incandescent	2553.33^{a}	58.49^{a}	104.34^{a}	1.78^{bc}	0.62^{a}
Neutral-white	2602.48^{a}	60.67^{a}	110.98^{a}	1.83^{ab}	0.62^{a}
SEM	38.04	1.26	2.20	0.01	1.26
Trp levels (g kg^{-1})					
0.00	2594.59^{a}	60.15^{a}	108.72^{a}	1.81^{a}	1.66^{a}
1.00	2618.37^{a}	60.13^{a}	106.81^{a}	1.78^{a}	1.04^{a}
SEM	31.06	1.03	1.80	0.01	1.03
Effect (p-value)					
Light	0.15	0.29	0.13	0.02	0.38
Trp	0.59	0.98	0.46	0.15	0.67
Light×Trp	0.40	0.14	0.09	0.15	0.83

^{abc} Least Squares Means within each column without common superscript are significantly different ($p \leq 0.05$).

Table 3. Effect of dietary L-tryptophan (Trp) and light treatments on the primary and secondary antibody of sheep red blood cell (SRBC; on 33 and 40 days of age, respectively), Newcastle disease (ND) virus (on 42 days of age), and avian influenza (AI) virus antibody (on 42 days of age), and cutaneous basophilic hypersensitivity (CBH) response (on 41 days of age) of male broiler chicken performance. Data for antibody response of SRBC, ND, and AI are as log_2 of the complementary of the last dilution in which there was agglutination.

Variable	Primary SRBC	Secondary			ND	AI	CBH (mm)
		SRBC	IgG	IgM			
Light							
Warm-white	1.10^{a}	3.75^{a}	1.29^{a}	2.45^{a}	4.81^{a}	2.12^{a}	0.77^{a}
Incandescent	0.76^{b}	4.00^{a}	1.50^{a}	2.50^{a}	4.75^{a}	1.50^{a}	0.88^{a}
Neutral-white	0.66^{bc}	3.91^{a}	1.81^{a}	2.10^{a}	4.37^{a}	2.18^{a}	0.92^{a}
SEM	0.10	0.32	0.18	0.23	0.25	0.36	0.08
Trp levels (g kg^{-1})							
0.00	0.92^{a}	3.83^{a}	1.44^{a}	2.38^{a}	4.50^{a}	1.75^{a}	0.87^{a}
1.00	0.76^{a}	3.94^{a}	1.62^{a}	2.31^{a}	4.79^{a}	2.12^{a}	0.85^{a}
SEM	0.08	0.26	0.15	0.18	0.20	0.29	0.06
Effect (p-value)							
Light	0.01	0.85	0.15	0.42	0.49	0.34	0.42
Trp	0.16	0.76	0.40	0.79	0.33	0.37	0.86
Light×Trp	0.34	0.70	0.29	0.21	0.14	0.97	0.39

^{abc} Least Squares Means within each column without common superscript are significantly different ($p \leq 0.05$).

Table 4. Effect of dietary L-tryptophan (Trp) and light treatments on total and differential leukocyte count and heterophil to lymphocyte (H:L) ratio of male broiler chicken (at 42 days of age).

Variable	Total leukocyte per mm³	Heterophil (%)	Eosinophil (%)	Monocyte (%)	Lymphocyte (%)	H:L ratio
Light						
Warm-white	13120	27.50	5.18	7.93	59.37	0.51
Incandescent	13500	26.18	4.92	7.62	61.62	0.43
Neutral-white	12280	23.06	3.87	6.46	64.62	0.36
SEM	1080	1.80	0.58	0.84	2.16	0.05
Trp levels (g kg^{-1})						
0.00	12729	25.66	4.95	6.39	62.66	0.43
1.00	13200	25.50	4.37	8.29	61.08	0.43
SEM	880	1.47	0.48	0.64	61.08	0.04
Effect (p-value)						
Light	0.72	0.21	0.25	0.31	0.23	0.15
Trp	0.70	0.93	0.40	0.14	0.53	0.98
Light×Trp	0.24	0.91	0.23	0.13	0.79	0.90

No significant differences were observed in each column ($p > 0.05$).

combined with discontinuous light improved immunity in broiler chicks.[11] Tryptophan is considered as a precursor of serotonin (a neurotransmitter) and melatonin (a pineal gland hormone). Serotonin has many central nervous system roles such as induction of sleep, inhibit aggression, and modulate the stress response.[12] It was reported that adding Trp to the diet increased brain and plasma concentrations of serotonin and 5-hydroxyindole acetic acid.[13] Melatonin is involved in anti-inflammatory action and lymphocyte activation.[14] In studies conducted on broiler chicks suggested that melatonin could stimulate the cellular and humoral immune response.[15-17] By National Research Council, the Trp requirement for broiler chickens was estimated at 0.20% the diet.[18] A study on the Trp requirement of chickens, Rosa and Pesti found that at least 0.25% (of the diet) Trp was necessary to maximize body weight gain and feed efficiency. Hence, in this study, 0.22 to 0.27% (of the diet) Trp was used in control groups.[19] However, under stressed conditions, adding Trp to the diet has been shown to improve growth performance and meat quality effectively.[7] In a study on broiler chickens under experimental aflatoxicosis, it was shown that no significant effects were found on growth performance and immune responses between the control groups and treatment groups supplemented with L-Trp (250 mg kg^{-1} of diet); however, adding L-Trp to the diet, improved growth performance and immune responses compared to aflatoxin groups.[20]

In the present study, H:L ratio was about 0.40, inducted a normal environmental condition or an absence of physiological stress.[21,22] Also, the effect of different color temperatures of the LEDs and ICDs on differential leukocyte numbers and H:L ratio were not significant. Hence, under normal conditions, adding more Trp to the diet may not have a beneficial effect on growth performance and immune responses. Therefore, Trp×light interaction did not affect the growth performance and immune responses of male broiler chickens.

In this trial, although the effect of neutral-white on growth performance was not significant compared to ICD light bulbs, however, FCR was significantly lower in warm-white light compared to neutral-white. To assess cell-mediated immune and humoral antibody responses, the CBH response and SRBC were evaluated, respectively. The highest primary SRBC was observed in warm-white light compared to other light treatments; however, The CBH response, AI, and ND were not significant. In a study performed by Li *et al.*, it was suggested that green LED illumination could improve the antioxidative activity and secretion of melatonin to promote B lymphocyte proliferation of bursa of Fabricius in young broilers.[23] Melatonin biosynthesis and secretion could be modulated by environmental light.[23] Moore and Siopes showed that melatonin stimulates the cellular and humoral immune responses in Japanese quail.[24] Also, melatonin is involved in growth by influencing hormone growth.[25] It has been reported that melatonin improves feed efficiency.[26] In the present study, the warm-white light might increase the melatonin secretion, thereby improving humoral immune responses and FCR.

According to the results, although adding Trp to male broiler diets did not have interaction effects with light treatment on chickens' growth performance and immune responses. However, the warm-white light treatment improved the FCR and primary SRBC compared to neutral-white and ICD light bulbs.

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Conflict of interest

The authors do not have any particular conflicts of interest to declare.

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