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Effects of Various LED Light Colors on Growth and Immune Response in Broilers

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We evaluated the effects of different light-emitting diode (LED) colors between blue and green on growth performance and the immune response in broilers. A total of 1,200 1-day-old Ross broilers were divided randomly into six groups and exposed to pure blue (PB), bright blue (BB), sky blue (SB), greenish blue (GB), pure green (PG), or white (W) using LEDs for 6 weeks. Consequently, body weights were higher in chickens reared under PB and GB on day (d) 7 and SB on d 21 than the other groups. Chickens in the PB group on d 42 were the heaviest among the groups, followed by the BB group and were significantly heavier than the W group. Splenocyte proliferation was significantly enhanced in chickens reared under PB followed by BB on d 42 and proliferation of peripheral blood mononuclear cells was significantly enhanced in chickens reared under BB on d 42. In addition, chickens in the BB group showed significantly elevated nitric oxide production on d 42, indicating activation of macrophages. These results suggest that immune function and growth of broilers can be improved at the later stage by rearing under shorter wavelength LEDs such as PB and BB.

Key words: broiler, growth, immune, light-emitting diode light

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Introduction

Artificial light sources as an external environment factor are an important part of growth and the immunity in broilers. It is well known that lighting factors, such as light intensity, exposure time, and color, affect the physiology and immune-competence of chickens (Foss *et al*., 1972; Rozenboim *et al*., 1999; Xie *et al*., 2008a; Blatchford *et al*., 2009).

Monochromatic light-emitting diode (LED) sources have been considered recently to manage poultry, as they reduce electricity consumption and are environmentally friendly. Therefore, many countries including Europe and the USA have been replacing incandescent lamps with LEDs. The Korean government also adopted a new energy-saving target of achieving 100% LED lighting by public utilities in 2020, and colored LEDs have received considerable attention as alternatives to conventional lighting in the poultry industry (Hassan *et al*., 2013).

The color of monochromatic and mixed LEDs affects growth, development, and behavior in chickens (Rozenboim *et al*., 2004; Cao *et al*., 2008; Sultana *et al*., 2013). Changing the lighting regime to an LED system may impact not only growth and development but also immune function in broilers. Xie *et al.* (2008b) evaluated humoral and cellular immunity in broilers reared under blue, green, and red light by comparing antibody titers after vaccination with Newcastle disease virus and T-cell proliferative response for 7 weeks. They suggested that green (560 nm) and blue light (480 nm) promote greater antibody production and increase proliferation of peripheral blood mononuclear cells (PBMCs) compared with those of broilers reared under red light (660 nm). Green and blue light reportedly enhance broiler immune function; however, little is known about the immune response of broilers reared under different blue to green LED colors, which could enable applying more diverse LED colors (Xie *et al*., 2008a; Xie *et al*., 2008b; Zhang Z *et al*., 2014).

The objective of this study was to investigate the effects of various blue to green LEDs on growth and the immune response, including lymphocyte proliferation and nitric oxide (NO) production in broilers.

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Materials and Methods

Animal Management and Light Treatments

A total of 1,200 Ross broiler chicks were used in this study. One-day-old chicks were allocated randomly (irrespective of the sex) into six light treatment groups (n=200). Each group was divided to four replicates pens (n=50). The birds were housed in a free-run room at a density of 50 chicks/5 m^2. The 50 birds in each room were exposed for 6 weeks to pure blue (PB, 450−460 nm), bright blue (BB, 470−480 nm), sky blue (SB, 490−500 nm), greenish-blue (GB, 510−520 nm), and pure green (PG, 530−540 nm) using LEDs. White (W, fluorescent light) served as negative control. The LED lamps (Good i-Tech, Iksan, South Korea) were placed 2.2 m above the birds using plastic crosses attached to the ceiling. The lighting program was recommended by primary breeders. Light intensity was equalized at the bird head level, so birds received the same amount of light. Illumination was changed to 15 lx on d 0−3, 10 lx on d 4−10, and 5 lx on d 11−35. Each room was assigned a lighting regimen of 24L:0D (L=light, D=dark) on d 0−3, 22L:2D on d 4−10, 20L:4D on d 11−17, 18L:6D on d 18−24, and 16L:8D on d 25−42. The broilers had free access to feed (1−7 days of age, pre-starter pellet with 20.5% crude protein (CP) and 3,000 kcal ME/kg was fed, followed by a starter ration with 18.5% CP and 3,050 kcal ME/kg, and a grower ration with 17.5% CP and 3,100 kcal ME/kg to the end of the experiment) and water at all times. The temperature was set to 33℃ for the first week and decreased by 2−3℃ each week until reaching 22℃. Relative humidity was set to 60−65%, and an air circulating system was employed during the entire study period.

All experimental and animal management procedures were undertaken in accordance with the requirements of the Animal Care and Ethics Committee of Chonbuk National University. The animal facility at Chonbuk National University is fully accredited by the National Association of Laboratory Animal Care (approval number: CBU 2014-00058).

Sampling and Tissue Culture for Analysis

All birds in six groups were weighted individually on d 7, 21, and 42. Daily feed consumption was measured, and feed efficiency was calculated for 200 birds in each group. Feed intake was expressed on a bird basis with total feed consumed per pen divided by bird count per pen for that period. The feed efficiency was also expressed as total pen weight gain divided by total feed consumed per pen. Blood and spleen samples were collected and pooled from 16 birds in each group (n=4/pen) on d 21 and 42 for lymphocyte proliferation and NO production assays. PBMCs were isolated using Ficoll−Hypaque (GE Healthcare, Little Chalfont, UK) according to the manufacturer's recommendations, centrifuged, and suspended in RPMI 1640 (GIBCO, Grand Island, NY, USA) medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), penicillin, streptomycin, and sodium bicarbonate. The spleens were aseptically resected and mechanically dissociated with a 60-mesh stainless steel screen (Sigma-Aldrich, St. Louis, MO, USA). Red blood cells were lysed in a hypotonic saline solution (17 mM Tris and 0.14 mM NH_4Cl, pH 7.65) for 5 min at room temperature. The lymphocytes were counted using trypan blue dye exclusion and suspended in RPMI 1640 supplemented with 10% heat-inactivated FBS. The PBMCs and splenocytes were seeded in 96-well flat-bottomed microtiter plates and tested in triplicate. The plates for the proliferation assay were incubated in a humidified atmosphere at 37℃ with 5% CO_2.

Lymphocyte Proliferation Assay of Splenocytes and PBMCs

Lymphocyte proliferation was assessed using a MTT method described previously with some modifications (Mosmann, 1983). Briefly, PBMCs and splenocytes were diluted in RPMI 1640 medium to a final concentration of 5×10^6 cells/mL and 2×10^6 cells/mL, respectively. A 50 μL aliquot of cell suspension was dispersed to each well of a 96-well flat-bottomed microtiter plate and cultured alone or with concanavalin A (Con A; Sigma-Aldrich) (final concentration, 10 μg/mL) or lipopolysaccharide (LPS; Sigma-Aldrich) (final concentration, 1 μg/mL). After 48 h incubation at 37℃ in a 5% CO_2 incubator, 10 μL of MTT (Sigma-Aldrich) (final concentration, 0.5 mg/mL) was added to each well, and the incubation was continued for another 2 h before measuring optical density at 490 nm using an enzyme-linked immunosorbent assay reader (Perkin-Elmer, Wakthanm, MA, USA).

NO Production Assay

NO production was quantified using a NO Detection Kit (iNtRON Biotechnology, Seongnam, Korea) based on the Griess method, according to the manufacturer's instructions (Green *et al*., 1982). Splenocytes were prepared with Con A under the same conditions described above and incubated for 24 h. A 50 μL aliquot of the supernatant was used for the assay. The quantity of nitrite was estimated according to the standard curve generated, using a known concentration of sodium nitrite dissolved in RPMI 1640 medium. Absorbance was measured by spectrophotometry using the microplate reader mentioned above at 550 nm.

Statistical Analysis

All data were analyzed using one-way analysis of variance and SPSS 11.0 software (SPSS Inc., Chicago, IL, USA). Significant differences between the groups were further analyzed using the Scheffe's test and a P-value <0.05 was considered significant.

Results

Growth

Body weights, total feed intake, and feed efficiency of the broilers reared under different LEDs are presented in Table 1. No significant differences in body weights were observed on d 7 and d 21 in any of the groups. However, the PB groups were significantly heavier than that in the W group on d 42. No significant differences in feed intake were observed among the groups on d 7, 21, or 42, although intake tended to be higher in the BB, SB, and PB groups, respectively. Feed efficiency tended to be higher in the BB group on d 7, the PB group on d 21, and the W group on d 42.

Table 1. **Effects of various light emitting diodes on body weight, feed intake, and feed efficiency in broilers at 7, 21, and 49 days of age**

Item	Age (day)	Light treatment					
		W	PB	BB	SB	GB	PG
Body weight (g)	7	115.66±1.74	116.78±2.26	114.49±2.88	114.39±3.00	116.78±2.65	116.46±1.00
	21	689.88±11.24	689.84±12.09	699.83±15.19	721.23±19.58	704.92±10.09	696.07±14.42
	42	1628.96±103.61[b]	1819.80±177.90[a]	1746.71±105.54[ab]	1697.51±105.02[ab]	1690.70±146.63[ab]	1703.81±113.78[ab]
Feed intake (g)	7	118.61±3.26	118.50±4.26	118.97±6.63	116.15±3.91	118.84±4.85	117.63±5.56
	21	1050.30±15.83	1050.67±14.74	1050.70±14.97	1092.04±48.13	1061.39±26.16	1049.40±16.12
	42	2970.08±157.79	3233.52±70.57	3122.42±177.13	3047.42±120.78	3031.89±108.86	3098.30±148.28
Feed efficiency	7	1.03±0.0081	1.02±0.0048	1.04±0.0105	1.02±0.0048	1.02±0.0059	1.02±0.0017
	21	1.52±0.0010	1.52±0.0101	1.51±0.0011	1.51±0.0023	1.51±0.0097	1.51±0.0036
	42	1.83±0.0038	1.78±0.0547	1.79±0.0135	1.80±0.0161	1.80±0.0145	1.82±0.0169

[a–b] Values with no common letters are significantly different ($P<0.05$).
Values are mean±SD ($n=200$/group). W, white light; PB, pure blue; BB, bright blue; SB, sky blue; GB, greenish blue; PG, pure green.

Splenocyte and PBMC Proliferation

Fig. 1 shows splenocyte proliferation in broilers stimulated with Con A or LPS on d 21 and 42. No significant differences in the Con A or LPS-induced splenocyte proliferative response among the PB, BB, SB, GB, and PG groups were observed on d 21 (Fig. 1a). However, significant increases were found in the PB group for the Con A and LPS-induced splenocyte proliferative responses on d 42 (Fig. 1b). The PB group showed significantly higher stimulation with Con A and LPS followed by the BB group.

The GB group had the highest Con A-stimulated PBMC proliferation on d 21 but no significant differences were observed in the W and SB groups (Fig. 2a). The Con A-induced proliferative responses were significantly higher on d 42 in the BB and PG groups than those in the other groups (Fig. 2b). The LED color did not affect any of the immune parameters examined on d 21; however, there was a trend for greater cell proliferation responses in broilers reared under PB or BB than under the other regimens on d 42.

Splenocyte NO Production

Data on splenocyte NO production in broilers reared under the different LEDs are presented in Fig. 3. Splenocyte NO level on d 21 was significantly higher in the W group than that in the other groups. The PG group also tended to show the highest level of NO production compared to that of the other groups (Fig. 3a). NO concentration in the BB group was significantly higher than that in the other groups on d 42 (Fig. 3b) followed by the PB group.

Discussion

This study was conducted to investigate the effects of blue to green LEDs on growth performance and the immune response of broilers. Although body weight, splenocyte and PBMC proliferation, and NO production were unaffected by the LED color at the early stage, chickens in the PB group were the heaviest among the groups and were significantly heavier than the W group at the later stage. Splenocyte proliferation in the PB group, PBMC proliferation in the BB group, and NO production in the BB group were significantly higher than those in the other groups at the later stage. These data are consistent with a previous report that rearing broilers under blue light (480 nm) accelerates growth and enhances the immune response in older birds (Rozenboim *et al*., 2004; Xie *et al*., 2008a, b; Karakaya *et al*., 2009; Hassan *et al*., 2014). Rozenboim *et al.* (1999) reported that green (560 nm) and blue light (480 nm) enhance growth rate significantly more than white or red light at different times and suggested that green light enhances growth at a younger age, whereas blue light enhances growth at an older age. They also reported that switching the environmental light spectra from green to blue on d 10 or 20 accelerates growth in male broiler chicks (Rozenboim *et al*., 2004). Moreover, weight gain is significantly higher in blue light (460 nm) and mixed green×blue light treatments during the growing period (4−5 weeks) than that in the other groups in which white (fluorescent light), red (660 nm), yellow (600 nm), green (550 nm), and mixed red×yellow and yellow×green light colors were provided (Hassan *et al*., 2014).

It is important to maintain immune function in broilers because poor immune status can decrease disease resistance leading to reduced productivity. We observed lymphocyte proliferation by splenocytes, which induced T and B lymphocyte proliferation. The spleen is the largest lymphoid organ and a major site of the immune response to blood-borne antigens. In this study, T lymphocyte proliferation in the PB group increased significantly after stimulation with Con A, and B lymphocytes were stimulated by LPS on d 42. The spleen consists of abundant T lymphocytes in the peri-arteriolar lymphoid sheaths of the white pulp and the sinuses of the red pulp, as well as B lymphocyte-rich lymphoid follicles in the peri-ellipsoidal white pulp of the spleen. (Davison *et al*., 2008; Abbas *et al*., 2010). Xie *et al*. (2008a) demonstrated that blue light (480 nm) enhances splenocyte proliferation in broilers compared with red light (660 nm) and suggested that the immune response of broilers to blue light was stronger at the later growth stage.

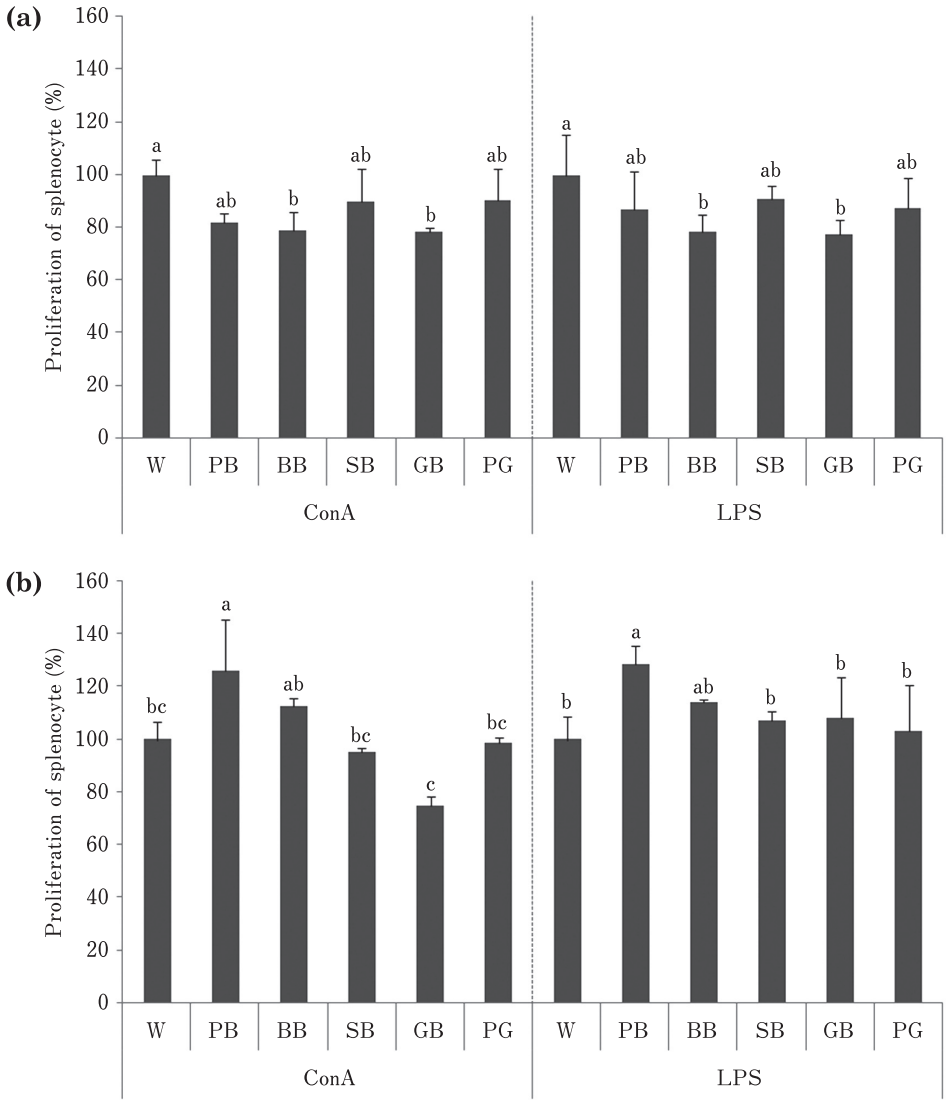

Fig. 1. **Effects of various light emitting diode (LED) colors on splenocyte proliferation in broilers.** Mitogen-stimulated splenocytes on days 21 (a) and 42 (b). Values are mean±SD from 16 broilers in each group. Values with no common letters are significantly different (P $<$0.05). Con A, concanavalin A; LPS, lipopolysaccharide; W, white light; PB, pure blue; BB, bright blue; SB, sky blue; GB, greenish blue; PG, pure green.

The BB group showed better PBMC proliferation compared to that by the other groups on d 42, indicating that shorter wavelengths, such as PB and BB, improve the immune function of broilers compared to longer wavelengths such as PG. These findings are in agreement with a previous study of immune reaction in broilers reared under four different monochromatic lights (Xie *et al*., 2008b). Those authors suggested that blue light (480 nm) enhances the broiler immune response at a later stage (d 49) by increasing Con A-induced peripheral blood T-lymphocyte proliferation. In addition, broilers switched from green (560 nm) to blue (480 nm) light on d 26 showed a significant increase in PBMC proliferation on d 49 compared with the white (400 to 700 nm), red (660 nm), and green groups (Zhang *et al*., 2014).

NO can be used as a quantitative index of macrophage activation because it performs phagocytosis and produces antimicrobial compounds, such as reactive oxygen intermediates and NO (Ding *et al*., 1988; Karaca *et al*., 1995; Davison *et al*., 2008). We confirmed that NO production in the BB group of broilers on d 42 was higher than that of the other groups, indicating the immune stimulating response of

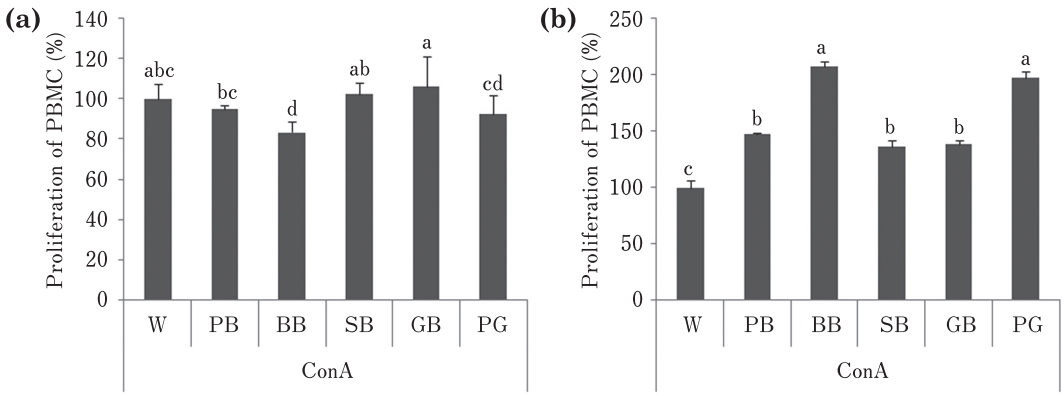

Fig. 2. **Effects of various light emitting diode (LED) colors on peripheral blood mononuclear cell (PBMC) proliferation in broilers.** Mitogen-stimulated PBMCs on days 21 (a) and 42 (b). Values are mean ±SD from 16 broilers in each group. Values with no common letters are significantly different ($P<0.05$). Con A, concanavalin A; W, white light; PB, pure blue; BB, bright blue; SB, sky blue; GB, greenish blue; PG, pure green.

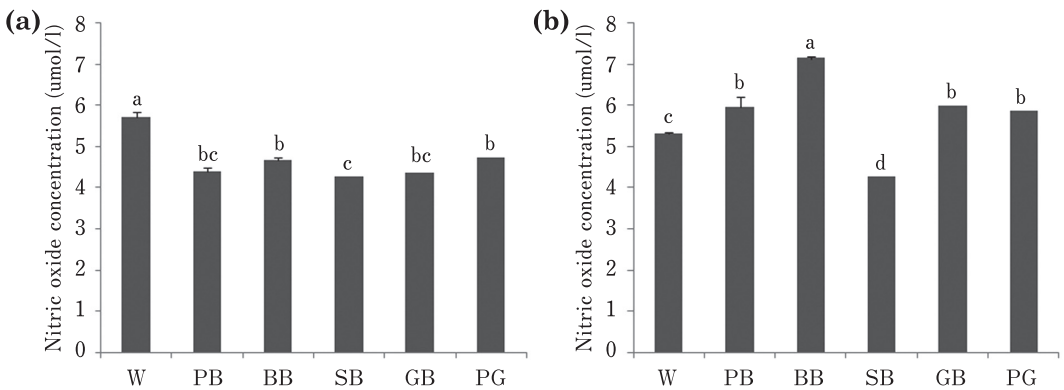

Fig. 3. **Effects of various light emitting diode (LED) colors on nitric oxide (NO) production by splenocytes in broilers on days 21 (a) and 42 (b).** NO production was measured using Griess reagent and expressed as μmol/L. Values are mean±SD from 16 broilers in each group. Values with no common letters are significantly different ($P<0.05$). W, white light; PB, pure blue; BB, bright blue; SB, sky blue; GB, greenish blue; PG, pure green.

macrophages in the spleen. These NO production results are similar to those of Con A-induced cell proliferation on d 42 in this study, which was higher in splenocytes of the PB and BB groups and in PBMCs of the BB group than those in the other groups, suggesting that macrophages in the red pulp are activated by lymphocytes in the spleen.

None of the groups showed consistent increases in splenocyte and PBMC proliferation on d 21, but the PB and BB groups showed enhanced splenocyte and PBMC proliferation and NO production on d 42. These results may have resulted from the immune insufficiency that occurs in young chickens. Previous studies have reported that immunosuppression observed in chickens during the first 1–2 weeks of life is due, at least in part, to a lack of T cell function (Kline and Sanders, 1980; Schaefer *et al*., 1985; Lowenthal *et al*., 1994). Therefore, the inconsistency on d 21 may have been a result of chicken age and a developing immune system.

In conclusion, we showed that broilers reared under PB light regimens were heavier than those reared under other LEDs during the late growth stage. In addition, the splenocyte and PBMC proliferation responses increased in broilers reared under PB and BB light at the later stage, and NO production under BB light at the later stage. These results

suggest that shorter wavelength light, such as blue, rather than relatively longer wavelengths, such as green, may enhance growth performance and immune status of broilers at a later stage. Therefore, applying PB or BB light at this stage may be useful to promote chicken growth and immunity.

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