Research Note

Effects of Monochromatic Light on Immune Response of Broilers

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ABSTRACT A total of 260 one-day-old Arbor Acres male broilers were exposed to red light (RL), green light (GL), blue light (BL), and white light (WL), respectively, by using a light-emitting diode system for 7 wk. There were 5 replicate pens for each light treatment and 13 birds per pen. The effects of monochromatic light on the immune response were studied. The results indicated that proliferation of peripheral blood T lymphocytes in the GL group was significantly increased (by 80.8 and 54.8%) compared with those in the RL and BL groups, respectively, at 21 d of age ($P < 0.05$). At 49 d of age, however, the proliferation response was significantly increased in the BL group compared with the RL group (26.9%, $P < 0.05$). Moreover, the GL group showed a significant elevation in the serum anti-Newcastle disease virus level as compared with that of the RL group at 28 d of age (32.9%, $P < 0.05$). In contrast, no significant difference in serum anti-Newcastle disease virus level was observed among the BL, RL, and WL groups at this age ($P > 0.05$). By 49 d of age, the antibody titer was higher in the BL group than in the RL group (62.8%, $P < 0.05$). However, no significant difference in antibody titer was seen among the BL, GL, and WL groups at this age. Interestingly, the BL group showed a 44.0% reduction in the level of serum interleukin-1β as compared with that in the RL group at 49 d of age ($P < 0.05$). These results suggest that GL and BL enhance the immune response better than RL, and that BL may play a role in alleviating the stress response in broilers.

Key words: monochromatic light, immune response, stress, broiler

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INTRODUCTION

Light is an important exogenous factor for controlling many physiological and behavioral processes in birds. Recently, many reports have been published regarding the effect of light spectra on the growth performance in broilers. For example, broilers reared under blue or green fluorescent lamps gained more weight than those exposed to red or white light (Wabeck and Skoglund, 1974; Prayitno et al., 1997; Rozenboim et al., 1998). Green light accelerates muscle growth at an early age, whereas blue light stimulates growth in older birds (Halevy et al., 1998; Rozenboim et al., 1999, 2004). There are, however, some conflicting reports concerning the effect of monochromatic light on bird growth. Mature female Japanese quail had lower weights when reared under green light and blue light compared with those reared under red or white light (Woodard et al., 1969). Lewis et al. (2007) revealed that the use of green light had no benefit for the immature pullet on either its maturation or its subsequent productivity in the laying house during a rearing period up to 71 wk.

On the other hand, the effect of light information on the immune response is poorly understood. In mammals, a short photoperiod could enhance both cellular and humoral responses of the immune system compared with a long photoperiod (Nelson and Blom, 1994; Demas and Nelson, 1996; Demas et al., 1996). In birds, Bentley et al. (1998) suggested that immune function was suppressed in adult starlings photostimulated with long days (18L:6D). Both the cellular and humoral immune responses were greater when birds were placed in daily light-dark cycle treatments as compared with constant light (Moore and Siopes, 2000). Onbaşılar et al. (2007) reported that broilers housed in intermittent lighting had higher antibody titers of anti-Newcastle disease virus (**NDV**) compared with continuous lighting. These results suggested that photostimulation plays an important role in affecting the immune response. However, little information has been published to date regarding the effect of light color on the immune response for avian species.

In this study, we addressed the effects of various monochromatic lights from a light-emitting diode (**LED**) system on immune response, including antibody production, peripheral blood T-lymphocyte proliferation, and serum interleukin-1β (**IL-1β**) level in broilers. Our experiments

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demonstrate that GL and BL enhance the immune response better than RL. Our results suggest that BL may play a role in alleviating the stress response in broilers.

MATERIALS AND METHODS

Light Treatments and Animal Management

A total of 260 one-day-old Arbor Acre male broilers from Beijing Huadu Breeding Co. Ltd. (Beijing, China) were used in this study. All broilers were randomly cared for in 4 light-controlled rooms (n = 65) and were exposed to blue light (480 nm, **BL**), green light (560 nm, **GL**), red light (660 nm, **RL**), and white light (400 to 700 nm, **WL**), respectively, with an LED system (Rozenboim et al., 1999; Er et al., 2007) for 7 wk. Each room contained 5 replicate pens (13 birds per pen) at a density of 11.5 birds/m^2. The LED lamps were placed 10 cm above the heads of broilers by using plastic crosses attached to the ceiling of the room. All light sources were equalized at the intensity of 15 lx, with a light period of 23 h daily (23L:1D; lights off at 2300 h). Chicks had ad libitum access to feed and water, and diets were formulated to meet the nutrient recommendations for poultry (NRC, 1994). The temperature in the chicken house was set at 33°C for the first 7 d and was reduced by 3°C each consecutive week until it reached to 24°C. All procedures were approved by the Animal Care and Use Committee of China Agricultural University.

Blood Sampling and Analyses

On d 21 and 49, 3 birds from each pen were randomly selected and anesthetized with Nembutal (45 mg/kg of BW, i.p.; Shanghai Chemical Factory, Shanghai, China). Blood samples were collected into 5-mL heparinized vacuum tubes, and the serum harvested from the blood samples was stored at –20°C until analysis. A second 5-mL sample was taken from the same bird for the lymphocyte proliferation study. All blood responses were analyzed in triplicate.

Detection of Serum IL-1β

Serum IL-1β was measured by using a commercially available broiler ELISA kit (BioSource International Inc., Beijing, China). Minimum detectability of broiler IL-1β was 15 pg/mL, and the intraassay CV was 10%.

T-Lymphocyte Proliferation Assay of Peripheral Blood

Proliferation of peripheral blood T lymphocytes was determined by using a previously described method (Mosmann, 1983) with some modifications. Briefly, lymphocytes were isolated from peripheral blood by using a lymphocyte density-gradient centrifugation medium (Tianjin Blood Research Center, Tianjin, China) and were separated by density-gradient centrifugation at 150 × g for 30 min at 4°C. Lymphocytes were collected at the interface and washed 3 times with Hanks balanced salt solution without Ca^{2+} and Mg^{2+}, after which the lymphocytes were suspended in 2 mL of RPMI 1640 complete media (Gibco BRL, Grand Island, NY) supplemented with 10% (vol/vol) heat-inactivated fetal calf serum, 100 IU/mL of penicillin, 100 mg/mL of streptomycin, and 25 m*M* HEPES buffer (Sigma Chemical Inc., St. Louis, MO). The live cells were detected by trypan blue dye exclusion, and the cell suspensions were diluted to a final concentration of 1×10^7 cells/mL in RPMI 1640 medium.

A 190-μL quantity of cell suspension was coincubated with concanavalin A (Sigma; final concentration, 45 μg/mL) in a 96-well plate (Costar 3599, Corning Inc., Corning, NY) with a total culture volume of 200 μL, and the plates were then incubated at 41°C in a 5% CO_2 incubator for 72 h. Subsequently, 10 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (Sigma) solution was added to the cell culture to make a final concentration of 5 mg/mL (in 1:15 *M* PBS, pH 7.4). The cells were incubated for a further 4 h at 41°C, and 100 μL of a 10% SDS (Shanghai Chemical Factory) in 0.04 *M* HCl solution was added to lyse the cells and solubilize the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium crystals. The absorbance of each sample was read at 570 nm via an automated ELISA reader (Model 550, Bio-Rad, St. Louis, MO).

Antibody Response to NDV

Newcastle disease IV strain vaccine (Intervet Inc., Millsbro, DE) was administered at d 3, and the second vaccination was injected at d 20. Antibody response was measured at d 14, 28, 42, and 49 by the hemagglutination inhibition technique. Briefly, 25 μL of serum containing antibody was serially diluted into a 96-well plate with PBS (pH 7.4, 4°C). The same volume of virus antigen was added to react and bind with the antibody. Addition of 2% red blood cell solution in each well will show the ability of NDV left to agglutinate with red blood cells. If enough antibodies are bound to virus during the incubation period, hemagglutination will be inhibited completely. The titers were expressed as $\log_2$ of the reciprocal of the last serum dilution showing hemagglutation inhibition.

Statistical Analysis

Data were reported as means ± SD and analyzed by one-way ANOVA with SPSS 10.0 (SPSS Inc., Chicago, IL). The significance of difference among the different groups was evaluated by a least significant difference post hoc multiple comparisons test. The significance level was set at $P < 0.05$.

RESULTS AND DISCUSSION

Effects of Monochromatic Light on the Cellular Immune Response in Broilers

Using a cell proliferation assay, we observed that the peripheral blood T-lymphocyte proliferation response to

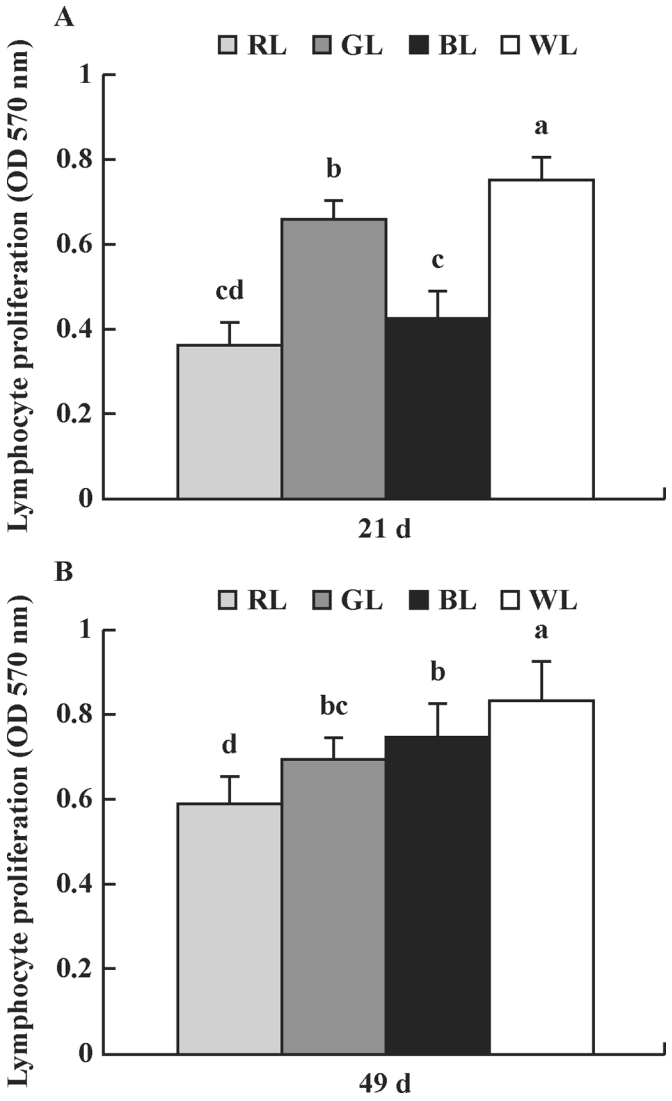

Figure 1. Histograms demonstrating peripheral blood T-lymphocyte proliferation in response to concanavalin A of male broilers reared under red light (RL), green light (GL), blue light (BL) and white light (WL) at 21 and 49 d of age. Peripheral blood lymphocyte proliferation was measured by Mossman's colorimetric method and expressed as the optical density (OD) value at 570 nm. Values are expressed as mean ± SD from 15 broilers (3 broilers per pen) in each treatment on the day indicated. [a–d]Bars marked with no common letters are significantly different ($P < 0.05$).

mitogen concanavalin A stimulation was poorer in the RL group than in the other light groups, but it was the best in the WL group during the entire experimental period (Figure 1). However, at 21 d of age (after 3 wk of photostimulation), the lymphocyte proliferation was significantly increased (by 80.8 and 54.8%) in the GL group as compared with the RL and BL group, respectively ($P < 0.05$). No significant difference was found between the RL and BL groups ($P > 0.05$). By 49 d of age, the lymphocyte proliferation response of the BL group was greater by 26.9% than that of the RL group ($P < 0.05$), but no significance was detected between the BL and GL groups ($P > 0.05$). These findings indicated that treatments of both BL and GL could promote T-lymphocyte proliferation to enhance the cellular immune function in broilers as compared with treatment of RL. However, the onset of cellular immunity enhancement occurred at various phases of photostimulation according to different monochromatic lights. As shown in Figure 1A and 1B, the enhancement with GL occurred at the early growth stage (21 d), and the enhancement with BL occurred at the later growth stage (49 d).

In contrast, some previous studies revealed that the phytohemagglutinin-induced dermal response (cutaneous basophil hypersensitivity) of turkey breeder hens was significantly greater in a red light group than in green and blue light groups after 15 wk of photostimulation (Scott and Siopes, 1994). The different results between the study of Scott and Siopes (1994) and our studies were probably due to the different methods used in the experiments, because Scott and Siopes used a longer photostimulation time (15 wk) and fluorescent lamps as the light source. We used LED lamps, which are currently available commercially. The major benefits of using LED lamps are their good efficiency, long operating life, moisture resistance, and availability in different peak wavelengths. Another plausible explanation may be that different animals were used in these experiments.

Effect of Monochromatic Light on the Humoral Immune Response in Broilers

It has been proposed that the factors that affect antibody production in broilers include the cage floor and density conditions (Onbaşılar and Aksoy, 2005), taurine supplementation (Lee et al., 2004), and the light schedule (Kirby and Froman, 1991; Moore and Siopes, 2000; Onbaşılar et al., 2007). To better understand the effect of monochromatic light on antibody production in broilers, we investigated anti-NDV antibody production in light-treated broilers. We found that the anti-NDV antibody titers were greater in the GL and BL groups than in the RL group during the entire experimental period (Table 1). At d 14 after the first vaccination, the GL group had greater antibody titers as compared with all other groups, although this difference was not significant ($P > 0.05$). The peak in antibody titers was observed in all treated groups at d 28 after the second vaccination. The antibody titer of the GL group was the greatest and was significantly increased (by 32.9%) compared with that of the RL group ($P < 0.05$), but no significant difference was detected among the WL, RL, and BL groups ($P > 0.05$). At 42 d of age (after 6 wk of photostimulation), the antibody titer of the BL group was the greatest and was significantly increased (by 38.3 and 31.7%) as compared with the RL and WL groups ($P < 0.05$). However, no significant difference was seen between the GL and BL groups at 42 d of age ($P > 0.05$). By 49 d of age, the antibody titer of the BL group was 62.8% greater than that of the RL group ($P < 0.05$). These data suggest that BL and GL could maintain longer antibody effective times and promote greater antibody production and humoral immune function in broilers as compared with RL.

Table 1. Effects of various monochromatic lights on serum antibody production after vaccination for Newcastle disease virus at various intervals, as determined by hemagglutination inhibition assay[1,2]

	Group[3]			
Age	RL	GL	BL	WL
14 d	5.3 ± 1.2	6.7 ± 0.6	6.0 ± 1.4	5.7 ± 0.6
28 d	7.0 ± 0.8^{bc}	.3 ± 1.5^{a}	.0 ± 1.0^{ab}	.3 ± 0.6^{abc}
42 d	6.0 ± 0.0^{cd}	.5 ± 0.7^{abc}	.3 ± 1.5^{a}	.3 ± 0.6^{c}
49 d	4.3 ± 0.6^{c}	.3 ± 0.6^{ab}	.0 ± 0.8^{a}	.7 ± 1.5^{abc}

$^{a–d}$Means within a row lacking a common superscript differ ($P < 0.05$).

[1]Vaccine was administered at d 3, and the second vaccination was injected at d 20. Antibody response was measured at d 14, 28, 42, and 49 by a hemagglutination inhibition technique.

[2]Values are expressed as mean ± SD of hemagglutination titers ($\log_2$) from 15 broilers (3 broilers per pen) in each treatment on the day indicated.

[3]RL = red light (660 nm); GL = green light (560 nm); BL = blue light (480 nm); WL = white light (400 to 700 nm).

Effect of Monochromatic Light on the Stress Response in Broilers

During broiler development, a stress response can occur because of a variety of factors, such as rearing density (Heckert et al., 2002) and light duration (Buckland et al., 1976; Campo and Davila, 2002; but see Freeman et al., 1981). In addition, it has been reported that the proinflammatory cytokines, such as IL-1β, play an important role in regulation during the stress response (Klasing, 1988). Interleukin-1β can stimulate neurons in the hypothalamus to secrete corticotropin-releasing hormone, which stimulates the adrenal cortex to produce corticosterone in birds (Berkenbosch et al., 1987). In birds, corticosterone is the major stress hormone (Thaxton and Puvadolpirod, 2000). These data suggest that IL-1β is one accepted indicator of the stress response in birds. In the present study, we investigated the alteration of IL-1β concentration in serum to address the effect of monochromatic light on the stress response in broilers.

When broilers were exposed to monochromatic light, their IL-1β level in serum was greatest in the WL group but lowest in the BL group at 21 d of age (Table 2). However, there were no significant differences in IL-1β level among the RL, GL, and BL groups ($P > 0.05$). The IL-1β level of the BL group remained the lowest until 49 d of age. The amount of IL-1β in the BL group was 44.0 and 59.4% less than those of the RL and WL groups ($P < 0.05$) (Table 2). No significant difference in IL-1β level was detected between the BL and GL groups ($P > 0.05$). As reported previously, the concentration of IL-1β in serum can be considered as a criterion reflecting stress intensity. An increase in IL-1β concentration would result in activation of the stress response in animals (Spurlock, 1997). Therefore, our results suggest that BL may be helpful to prevent excessive IL-1β expression compared with RL and WL. In addition, our results were in agreement with the previous report of Xie et al. (2008), who suggested that the spleen weights in the RL group were significantly decreased compared with those of the BL group in older broilers, because the weights of the secondary lymphoid organs decreased during the stress response (Donker and Beuving, 1989). We can infer from the present study that BL would alleviate the negative effects induced by the stress response, subsequently leading to a well-balanced immune response status, especially in older broilers (49 d).

Table 2. Effects of various monochromatic lights on level of serum interleukin-1β (pg/mL) in broilers at 21 and 49 d old[1]

	Group[2]			
Age	RL	GL	BL	WL
21 d	42.9 ± 4.1^{ab}	7.8 ± 1.1^{b}	3.4 ± 1.2^{b}	8.6 ± 6.5^{a}
49 d	103.2 ± 11.6^{a}	0.6 ± 2.4^{b}	1.7 ± 6.3^{b}	14.3 ± 9.2^{a}

^{a,b}Means within a row lacking a common superscript differ ($P < 0.05$).

[1]Values are expressed as mean ± SD from 15 broilers (3 broilers per pen) in each treatment on the day indicated.

[2]RL = red light (660 nm); GL = green light (560 nm); BL = blue light (480 nm); WL = white light (400 to 700 nm).

In summary, the present study showed that anti-NDV titers and peripheral blood T-lymphocyte proliferation were significantly increased by GL at the early growth stage (before 28 d) and by BL at the later growth stage (after 28 d) as compared with RL. We further found that the level of serum IL-1β was significantly decreased in the BL group as compared with serum IL-1β levels of the other light-treated groups. These findings suggest that GL (560 nm) and BL (480 nm) better enhance cellular and humoral immune responses, and that BL may play a role in alleviating the stress response in broilers.

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