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Effect of a combination of green and blue monochromatic light on broiler immune response

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ABSTRACT

Our previous study suggested that green light or blue light would enhance the broiler immune response; this study was conducted to evaluate whether a combination of green and blue monochromatic light would result in improved immune response. A total of 192 Arbor Acre male broilers were exposed to white light, red light, green light, and blue light from 0 to 26 days. From 27 to 49 days, half of the broilers in green light and blue light were switched to blue light (G–B) and green light (B–G), respectively. The levels of anti-Newcastle disease virus (NDV) and anti-bovine serum albumin (BSA) IgG in G–B group were elevated by 11.9–40.3% and 17.4–48.7%, respectively, compared to single monochromatic lights ($P < 0.05$). Moreover, the proliferation of peripheral blood T and B lymphocytes and the IL-2 concentration in the G–B groups increased by 10.4–36.2%, 10.0–50.0% and 24.7–60.3% ($P < 0.05$), respectively, compared with the single monochromatic light groups. However, the serum TNF-α concentration in the G–B group was reduced by 3.64–40.5% compared to other groups, and no significant difference was found between the G–B and B–G groups in any type of detection index at the end of the experiment. These results suggested that the combination of G–B and B–G monochromatic light could effectively enhance the antibody titer, the proliferation index of lymphocytes and alleviate the stress response in broilers. Therefore, the combination of green and blue monochromatic light can improve the immune function of broilers.

1. Introduction

Chickens have a special visual system and can detect a broader spectrum of colors than humans. Thus, chickens are extremely sensitive to subtle differences in light color. It is reported that different light wavelengths have varying stimulatory effects on the retina and pineal cells of birds and can result in behavioral changes that affect growth, development and productivity [1–3]. For instance, blue light has a calming effect on birds, while red will enhance feather pecking and cannibalism. Blue-green light stimulates growth in chickens, while orange-red stimulates reproduction [2,4]. Previous studies in our laboratory also indicated that green light enhanced muscle growth in chickens in the early stages, whereas blue light promoted this growth at a later age [5]. Interestingly, the growth and productivity were improved when broilers were switched from green to blue (G–B) or switched from blue to green (B–G) [6]. A similar result was also shown in Anak [7] and Ross broilers [8].

On the other hand, light was also found to play an important role in the immune response. A study on photoperiod influence on birds showed that a short-day photoperiod induced an increase in the immune response in comparison with a long-day photoperiod [9–11]. In addition, both the cellular and humoral immune responses were greater when birds were placed in daily light–dark cycle treatments compared with constant light [12]. Although many studies have confirmed the role of light intensity and photoperiod in immune response, very little is known about the effects of light color on the immune response in birds. Our previous studies discovered that green light promoted cellular and humoral immune responses during the early stages and that blue light enhanced the immune responses during the later stages in broilers [13,14]. However, it remains to be confirmed whether a combination of green and blue monochromatic light will result in improved immune response. Therefore, this study aims to investigate this issue.

2. Materials and methods

2.1. Animals and lighting systems

A total of 192 Arbor Acre male broilers from Beijing Huadu Breeding Co. Ltd. (Beijing, China) were used in this study. All

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broilers were randomly housed in 4 light-controlled rooms (n = 48), and each room contained 6 separate cells (8 birds per cell) at a density of 11.5 birds/m^2, and each cell had a set of independent light sources. The birds were exposed to white light (400 to 700 nm, WL), red light (660 nm, RL), green light (560 nm, GL) or blue light (480 nm, BL), which was powered by an LED system [6]. When the chicks were 26 days of age, 3 cells from the GL group and 3 cells from the BL group were transferred to blue or green light, respectively, at 2300 h. The remaining 3 cells were maintained under the original light color. The LED lamps were placed 10 cm above the heads of the broilers using plastic crosses attached to the ceiling. All of the light sources were equalized at an intensity of 15 ± 0.2 lx, with a light schedule of 23 h daily (23 L: 1 D; lights off at 2300 h). The chicks had ad libitum access to feed and water, and the diets were formulated to meet the nutrient recommendations for poultry (NRC, 1994). The temperature in the chicken house was set at 32 °C for the first 7 days, and it was reduced by 1 °C every 2 days until it reached 24 °C. This condition was maintained by an electric heater thermostat until the end of the experiment. During the experiment, the temperature of the chicken house was measured three times a day. All of the chicks were immunized with a Newcastle disease strain IV vaccine (Intervet Inc., Millsbro, DE) at 0, 9 and 20 days of age. Bovine serum albumin (Sigma, USA) was administered to all of the birds in each cell at 19 days of age, and a second vaccination was injected at 33 days of age. All experimental procedures were approved by the Animal Welfare Committee of the Agricultural Research Organization, China Agricultural University.

2.2. Blood sampling

On days 26, 35 and 49, 3 birds from each group were randomly selected and anesthetized with Nembutal. A total of 5 mL blood sample was obtained from each bird. Of them, 3 mL bloods were used for the lymphocyte proliferative assay, and another 2 mL bloods were made serum for ELISA assay.

2.3. Lymphocyte proliferative assay

Proliferation of peripheral blood T and B lymphocytes was measured using a previously described method [15] with some minor modifications. The blood was mixed 1:1 with RPMI 1640 medium (Gibco, USA) supplemented with 25 mM HEPES buffer, 10% fetal calf serum, 100 U/mL of penicillin, and 100 μg/mL of streptomycin. The lymphocytes were isolated from the peripheral blood by density-gradient centrifugation. Live cells were detected by trypan blue dye exclusion, and the leukocyte concentrations were adjusted to 1×10^7 cells/mL in RPMI 1640 with 10% fetal bovine serum. A 190-μL cell suspension was co-incubated with Con A (Sigma, final concentration of 35 μg/mL) and lipopolysaccharide (Sigma, final concentration of 20 μg/mL) in 96-well plates at a total culture volume of 200 μL. The cells were then incubated at 41 °C in a humidified atmosphere of 5% CO_2 for 68 h. Subsequently, 10 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) diluted to a concentration of 5 mg/mL in PBS was added to each well, and it was incubated for 4 h at 37 °C (5% CO_2). Following incubation, 100 μL of 10% SDS in a 0.04 M HCl solution was added to each well to lyse the cells and solubilize the MTT crystals. After 10 min, the absorbance of each sample was detected at 570 nm with an automated microplate reader (Bio-Rad Inc., St Louis, MO, USA). The results are expressed as the stimulation index (SI), which was calculated for each sample as the OD value (570 nm) for cells with stimulation divided by the OD value (570 nm) for cells without stimulation.

2.4. Antibody response to NDV and BSA

The NDV antibody response was measured at 26, 35, and 49 days with a commercially available broiler ELISA kit according to the manufacturer's guidelines (CUSABIO Inc., Wuhan, China). The serum BSA concentration was measured at 26, 35, and 49 days with a commercially available ELISA kit (Uscn Life Science Inc., Wuhan, China). The minimum detectability of the broiler BSA was 51 pg/mL, and the intra-assay Coefficiency of Variation was 10%.

2.5. Cytokine determination

Serum IL-2 (Uscn Life Science Inc., Wuhan, China) and TNF-α (CUSABIO Inc., Wuhan, China) concentrations were measured using commercially available broiler ELISA kits, and the cytokines from 26-, 35-, and 49-day samples were measured according to the manufacturer's guidelines. The minimum detectability of broiler IL-2 was 6.5 pg/mL, and the intra-assay CV was 10%. The minimum detectability of broiler TNF-α was 0.27 pg/mL, and the intra-assay CV was 8%.

2.6. Statistical analysis

The data were analyzed by one-way ANOVA using SPSS 16.0 software (SPSS Inc., Chicago, IL). The differences between the means were determined using Duncan's multiple range tests. The significance level was set at $P < 0.05$. The data were presented as the mean ± SEM.

3. Results

3.1. Effects of light color switching on the humoral immune response

In broilers reared with single monochromatic lights in the initial stage (0 to 26 d), the anti-NDV antibody titers, $F(3,8) = 11.803$, $P = 0.003$ and anti-BSA IgG concentration, $F(3,8) = 10.69$, $P = 0.004$ showed significant light color-dependant variation. Duncan's multiple range tests indicated that the anti-NDV antibody titers (2.84 ± 0.14) in the GL group were the highest. The titers in the GL group were significantly increased (by 18.62% and 14.34%) over the birds reared with RL or WL ($P < 0.05$). However, there was no significant difference between the GL and BL groups ($P > 0.05$; Fig. 1). Anti-BSA IgG concentration (93.76 ± 4.96 pg/mL) was significantly increased (by 14.99–24.64%) in birds that were treated with GL when compared to the groups of birds receiving other colors of light ($P < 0.05$; Fig. 2).

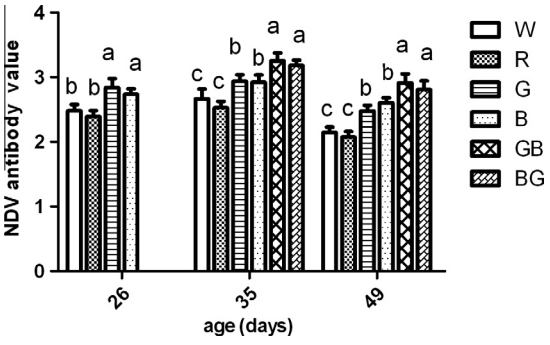

Fig. 1. The serum NDV (Newcastle disease virus) antibody levels of broilers reared with white light (W), red light (R), green light (G), blue light (B), combined green light and blue light (G–B), and combined blue light and green light (B–G) at various intervals (26 d, 35 d, 49 d). The bars marked with different letters are significantly different from each other ($P < 0.05$).