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Full Length Research Paper

Cellular immune response of infectious bursal disease and Newcastle disease vaccinations in broilers exposed to monochromatic lights

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To investigate the effects of various monochromatic lights on T lymphocytes proliferation and serum nitric oxide production in chicken vaccinated with infectious bursal disease and newcastle disease vaccines, a total of 60 one-day-old broilers were exposed to red, green, blue, white and yellow light by using a light-emitting diode system for 6 weeks. The results indicated that the proliferation of peripheral blood T lymphocytes in the chickens exposed to white and green lights significantly increased when compared with other groups at 37 days of age ($P < 0.05$). In the early days, the results were different. The enhancement of T lymphocytes proliferation with green and yellow lights occurred at 18 days, whereas the enhancement with green light was less than other lights at 30 days. Red light promoted NO (nitrix oxide) production at maximum level in the chickens, while green light suppressed it at minimum level after 37 days. These results suggested that green and white lights had strong effects on immunity, especially at the last days of rearing.

Key words: Monochromatic lights, vaccination, nitric oxide, broiler chicken.

INTRODUCTION

In modern commercial poultry houses, birds are usually exposed to artificial light in the closed and controlled environment. Thus, photoperiod, intensity, spectra, and sources of light are major factors influencing current poultry management (Andrews and Zimmerman, 1990). Light has long been proposed to have a stimulatory effect on a range of biological functions such as synchronization of the circadian system, suppression of melatonin, regulation of sleep as well as improvements of alertness and cognition (Brainard et al., 2001; Vandewalle et al., 2007).

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The chicken eye, similarly to the human eye, is capable of seeing in a narrow part of the light spectrum (380 to 760 nm). Apart from the eyes, birds are equipped with active extra-retinal photoreceptors, located in several parts of the brain, which are involved in transduction of photostimulation (Rozenboim et al., 1998). In commercial layers, during the first and second season, total egg production was significantly influenced by light color, with the greatest number of eggs produced in the group treated with red light. Eggs laid under blue or green light were consistently larger than those laid under red light. The egg quality in G light was found to be the best (Rozenboimet al., 1998).

Early studies in broilers, revealed a significant interaction between light treatments and body growth in broilers. It has been reported that high intensity of light

reduced growth rate in broilers, while light intensities beyond 10.8 lux depresses growth. Repeated short cycles of illumination and darkness improved growth significantly, and broilers reared under intermittent light were heavier than controls (Halevy et al., 1998). Spectra of light are also important factors affecting growth in broilers (Rozenboim et al., 2004). It has been confirmed that enhanced skeletal muscle growth associated with many monochromatic light treatments in chickens is due to increased satellite cell proliferation, which leads to establishment of additional fibers in the growing muscle (Halevyet al., 1998; Halevy et al., 2006). On the other hand, it has been revealed that photostimulation plays an important role in affecting the immune response. In mammals, a short photoperiod could enhance both cellular and humoral responses of the immune system when compared with a long photoperiod (Demas and Nelson, 1996; Nelson and Blom, 1994; Xie et al., 2008b). In birds, it has been shown 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). Among spectra of light, it has been suggested that green and blue lights enhance the immune response better than others (Xieet al., 2008b).

In this study, we addressed the effects of five color lights (green, blue, red, yellow and white) on cellular and immune responses of infectious bursal disease and newcastle disease vaccinations in the broilers exposed to the aforementioned color lights. We also assessed serum nitric oxide production as signaling molecule in the light-treated groups.

MATERIALS AND METHODS

Animal management, light treatments and blood sampling

Sixty (60), one-day-old chickens, commercial fast-growing broiler (Ross 308) strains were divided into five equal groups (3 replicates in each group). Chicks were reared for 42 days in floor pens on wood shaving litter at standard condition and provided *ad libitum* access to water and a standard ratio (starter: 12.2 MJ metabolisable energy (ME)/kg diet and 210 g/kg crude protein (CP); grower: 12.5 MJ ME/kg and 190 g/kg CP; finisher: 13 MJ ME/kg and 175 g/kg CP) formulated to meet requirements for broilers (NRC, 1994). Each group of chickens (n = 8) were kept in 5 light-controlled rooms and were exposed to blue light (480 nm, BL), green light (560 nm, GL), yellow light (590 nm, YL), red light (660 nm, RL), and white light (400 to 700 nm, WL) with an LED (light-emitting diode) system (Er et al., 2007; Rozenboim et al., 1999). The LED lamps were placed 15 cm above the heads of broilers. All light sources were equalized at the intensity of 25 lx, with a light period of 23 h daily (23L:1D).

Infectious bursal disease vaccine (LIBDV intermediate strain) was administered at 16 and 22 days of age. Newcastle disease vaccine was also administered at 7 (B1 Hitchner strain), 15 (Avi new strain) and 25 (La Sota strain) days of age in all groups.

On days 8, 18, 23, 30 and 47, 6 birds from each group were randomly selected and blood samples were collected into 5-ml heparinized vacuum tubes for the lymphocyte proliferation study. The serum from the blood samples was also harvested and stored at -20°C until analysis.

T -Lymphocyte proliferative assay (MTT test)

The proliferative response of blood T lymphocytes stimulated by a mitogen (concanavalin A) was performed by colorimetric method based on salt of tetrazolium MTT according to Mosmann (1983) with some minor modifications. Samples of blood were diluted in a ratio of 1:1 with RPMI 1640 medium (Sigma, Germany). Lymphocytes were isolated from peripheral blood by Ficoll gradient centrifugation technique (Sigma Chemical Co., St. Louis, USA). Isolated lymphocytes (5×10^6) were suspended in RPMI 1640 with 10% fetal calf serum (FCS, Sigma, Chemical Co., St. Louis, USA) and poured into 96-well plates at amount of 80µl/well. Each well was filled with 100 µl of mitogen: concanavalin A (Con A, Sigma) at a concentration of 50 µg/ml or RPMI 1640. Plates were incubated for 68 h at 37°C (5% CO2). After incubation, 80 µl of MTT dilution (3-[4.5-dimethylazol- 2-yl]-5.2diphenyl tetrazolium bromide, Sigma) at concentration of 5 mg/ml PBS was added to each well and once more incubated for 4 h at 37°C (5% CO_2). Then, the plates were centrifuged for 5 min at 800 xg, the supernatant was removed, and 100 µl of isopropanol acid (0.04N HCL) (Sigma Chemical Co., St. Louis, USA) were added to the wells. After 10 min, reading of absorbance was performed in microreader MRX 1.1 (Dynex, Great Britain) at a wavelength of 570 nm.

Measurement of serum nitrite/nitrate as nitric oxide index

Serum samples of 8 birds in each group at 42 days of age were used for this test. Measurement of nitrate was based on the reduction of nitrate to nitrite by cadmium, then nitrite levels were quantified by a previously described Griess reagent procedure (Navarro-Gonzalvez et al., 1998). A standard curve of optical densities was generated using various concentrations of sodium nitrite (Sigma Chemical Co., St. Louis, USA) dissolved in RPMI 1640 medium. The amount of colored products was determined by spectrophotometry at 550 nm. The amount of nitrite was estimated according to the standard curve generated using known concentration of sodium nitrite.

Statistical analysis

All results were represented as mean ± SEM. The statistical analysis was carried out using SPSS 14.0 software (SPSS Inc., New York, USA). Comparisons were made among light-treated groups at the same age using analysis of variance (ANOVA). Differences were considered significant at $P < 0.05$.

RESULTS

Effects of monochromatic light on the cellular immune response

After 8 days of photo-stimulation

There was no significant ($P > 0.05$) difference in T-lymphocyte proliferation among groups exposed to

different monochromatic lights.

After 18 days of photo-stimulation

The T-lymphocyte proliferation was significantly increased in the YL and RL groups as compared to the GL, BL and WL groups ($P < 0.05$).

After 23 days of photo-stimulation

T-lymphocyte proliferation of BL and YL groups was significantly ($P < 0.05$) greater than WL, RL and GL groups.

After 30 days of photo-stimulation

T-lymphocyte proliferation of BL group was significantly ($P < 0.05$) lower than WL, RL, YL and GL groups. Among the four groups (WL, RL, YL and GL), maximum T-lymphocyte proliferation appeared in YL group that was not significant.

After 37 days of photo-stimulation

There was significant ($P < 0.05$) increase of T-lymphocyte proliferation in GL and WL groups as compared to other groups.

Effects of monochromatic light on serum nitric oxide

Serum nitrite and nitrate as nitric oxide (NO) metabolites (NO index) were measured by Griess reagent procedure. After 42 days, RL increased significantly ($P < 0.05$) NO production by 340.3, 209.8, 146.8 and 146.1% when compared with GL, YL, BL and WL, respectively (Figure 2). In fact, RL promoted NO production at maximum level, while GL suppressed it at minimum level as compared to other lights.

DISCUSSION

There is a close relationship between the environmental factors (such as light and temperature) and immune responses. Scott and Siopes (1994) reported that the phytohemagglutinin induced dermal response (cutaneous basophil hypersensitivity) in turkey breeder hens of RL group was significantly greater than that of GL and BL groups after 15 weeks photo-stimulation, whereas, by 23 weeks photo-stimulation, the RL group had the smallest dermal response. Using a cell proliferation assay, we observed that the peripheral blood T-lymphocyte proliferation response to mitogen concanavalin A stimulation was poorer in the YL group than in the other light groups, but it was the best in the WL group after 37 days. In the previous days, the results were different, the onset of cellular immunity enhancement occurred at various phases of photo-stimulation according to different monochromatic lights. As shown in Figure 1, the enhancement with GL and YL occurred at the early growth stage at 18 days, whereas the enhancement with GL was less than other lights at 30 days. Xie et al. (2008a) reported that BL increased significantly the diameter of splenic nodule and area of periarterial lymphatic sheath at 49 days. At 21 days of age, GL enhanced spleen lymphocytes proliferation in response to concanavalin A when compared with RL. Xie et al. (2008b) also showed that the proliferation of peripheral blood T lymphocytes in the GL group was significantly increased when compared with those in the RL and BL groups at 21 days of age, while at 49 days of age, the proliferation response was significantly increased in the BL group when compared with the RL group.

The different results between our study and previous studies were probably due to effects of vaccination on immunity. This depressive effect on T proliferation was predominant in 18 days of age (Figure 1). However, different animals and breeders, and different methods used in the experiments could affect the results. It must be noticed that in addition to previous used lights, we also used yellow light which showed new results as compared to other lights.

In this study, serum NO concentration was obviously larger in the RL group than in the other groups at 37 days of ages. Xie et al. (2008a) measured the splenocyte NO and reported that until 49 days, the NO concentration of RL group became the highest and was significantly increased as compared to that of WL, GL and BL groups. They suggested that suppressed NO production in WL, GL and BL groups, was favorable to splenocyte proliferation. In contrast, the RL promoted NO production, which was harmful to splenocyte proliferation. However, it should be noted that there are several factors involved in the gene transcription of nitric oxide producing enzymes and NO production, and some of these factors include cell proliferation, shear stress, growth factors (TGF-b, FGF, PDGF,VEGF, IL-2) and hormones (angiotensin II, oestrogens, insulin) (Li et al., 2002; Mills, 1991). It has also been confirmed that NO production changed is associated with immune interactions (Coleman, 2001).

It is concluded that T-lymphocyte proliferation response is best in the WL group and weak in the YL group after 37 days in chickens vaccinated with infectious bursal disease and newcastle disease vaccines, while RL group showed more NO production.

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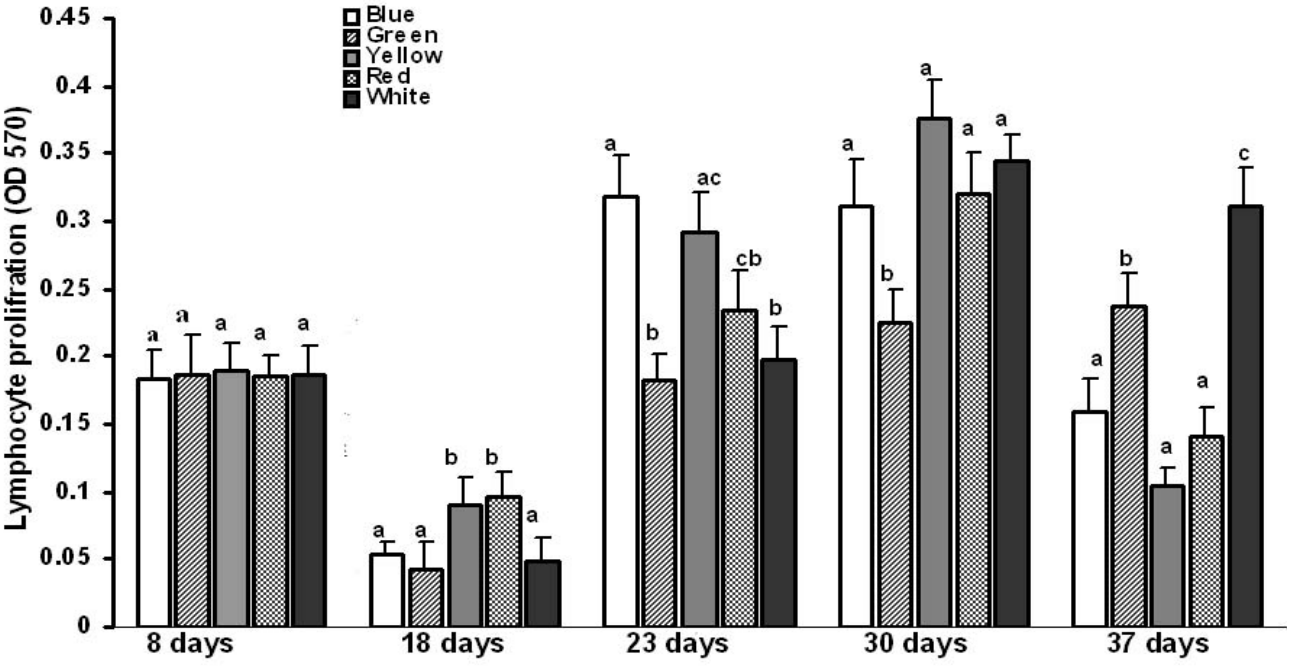

Figure 1. T-lymphocyte proliferation in response to concanavalin A of broilers reared under blue light (BL), green light (GL), yellow light (YL), red light (RL) and white light (WL) at different ages. Values are expressed as mean ± SEM from 9 broilers in each treatment on the day indicated. [abc]Bars that are not marked with common letters are significantly different ($P < 0.05$).

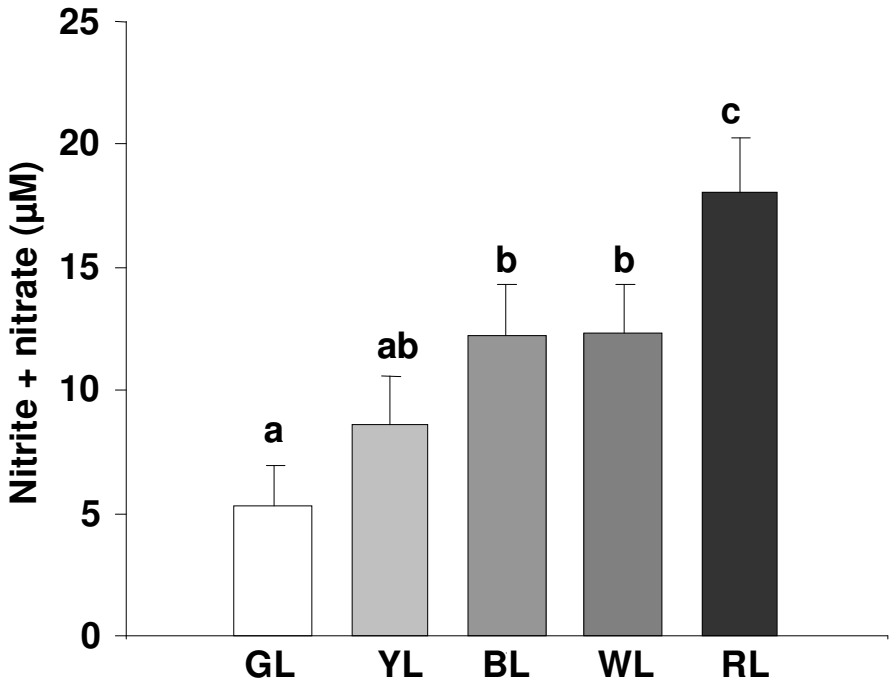

Figure 2. Comparison of serum nitrite + nitrate as nitric oxide index in broilers reared under blue light (BL), green light (GL), yellow light (YL), red light (RL) and white light (WL) at 37 days of age. Values are expressed as mean ± SEM from 9 broilers in each treatment. [abc]Bars that are not marked with common letters are significantly different ($P < 0.05$).

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