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Blue Light Color Reduces the Newcastle Disease Post-Vaccinal Reactions of Indian River Broilers under Egyptian Conditions

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TMM-B presented the concept and design of the study. Material preparation, data collection and analysis was performed by both authors. The first draft of the manuscript was written by TMM-B, and both authors crrected the manuscript. Both authors read and approved the final manuscript.

Key words
Blue light, IR broilers, Immune response, Post-vaccine reactions, Vitamin E supplementation

ABSTRACT

Poultry producers accustomed to use strong prophylactic measures such as routine vaccination to control Newcastle Disease (ND) as a trial to increase their productivity. Despite ND vaccinations, several post vaccine reactions of ND are being reported globally, which are major hindrances to the development of the poultry industry in the developing countries. This experiment was planned to study the effects of blue light colour and vitamin E and Selenium (Se) supplementation before and after Newcastle disease vaccination (NDV-LaSota) on post vaccine reactions (immune response) of a recently imported Indian River (IR) broilers to Egypt. In this study, 180 one-day old IR broiler chicks were used. The chicks were randomly assigned to three treatment groups of 60 each. The chicks in the first group were reared under white light colour (WLC) and kept as a control group. The chicks in the second group were kept under blue light colour (BLC) from an incandescent bulb two days before and after vaccination. Chicks in the third group were kept under white light color and supplemented with vita E and Se in the drinking water (WES) for 2 days before and after vaccination at the rate of 5 g per liter drinking water. The chicks were reared on a deep litter system and housed into three well controlled pens of 5.46 m^2 with three replicates of 20 each using a density of 15 birds/m^2 in the room. The results showed that the broilers reared under BLC and WES had a significant ($p > 0.05$) higher body weight gain, with obviously, economic feed conversion ratio (FCR), Newcastle disease virus antibody titer and low heterophil/lymphocyte ratio in comparing with WLC. Poultry producers can use blue light color or vitamin E and Se supplementation shortly before and after the vaccination to reduce the stress of vaccination and the post vaccinal reactions in IR broilers.

INTRODUCTION

The broilers are characterized by rapid growth which has an important position in Egyptian economy (Farghly *et al*., 2019). Despite the fast growth, there are many factors that pull down the growth of broilers, causing economic losses, such as disease outbreaks (Chung *et al*., 2019). Under intensive productions, there is a higher risk for transmission of diseases, like Newcastle Disease (ND) which being responsible for most economic losses (Aini, 2006). Vaccination may have negative side effects, which may act against the benefits (Landman, 2012).

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ND vaccination is often routinely performed as an integral part of management (Corbanie *et al*., 2008), to provide some degree of protection against it by stimulating the bird's immune system to respond more effectively to limit losses if a disease occurs in unvaccinated birds. The common losses from ND are decreased body weights, and poor feed conversion. Poor performance is also associated with increased medication costs, and consequently increased production costs to produce a pound of broiler meat (Butcher *et al*., 2002).

After vaccination, the vaccine virus infects the target cells and replicates to stimulate the immune system, resulting in a variable normal vaccine reaction (nervous symptoms) such as a wing or leg paralysis and torticollis (Jackwood, 2012). Due to replication in the respiratory tract ND vaccine provoke respiratory distress and sometimes suffocation, but most importantly, the ND vaccines significantly increase the susceptibility of broilers to colibacillosis (Matthijs *et al*., 2003). A good basic rule is that a mild respiratory reaction should be detected 2 to 3 days after vaccination and should last for 5 to 7 days (Rohollahzadeh *et al*., 2018).

In many broiler flocks, high mortality was recorded following ND vaccination due to the severity and/or prolonged reactions (Luckert and Saif, 2003). The losses resulting from excessive vaccine reactions are more costly than the actual field challenge to these diseases. Some common factors associated with the excessive vaccine reaction: poorly designeed vaccine, inappropriate age for vaccination and use of aggressive route of vaccination, lack of antibiotic therapy to control the vaccine response, and uneven vaccination in which some birds receive multiple doses and others receive none. However, in the following days, vaccinated birds will horizontally shed the vaccine to pen mates. The horizontal transmission will be variable and there will be additional changes in the maternal antibody levels, further complicating the flock's reaction. Sanda (2015) proved that three weeks post vaccination, the mean NDV antibody titer was significantly ($P < 0.05$) higher (1.87±0.18) in chicks given multivitamins before and after the vaccination than (1.39±0.12) of the control.

Proper management of brooding temperature, litter management and drinker sanitation has been important to preventing stress and reducing pathogen load, which is commonly associated with excessive vaccination reactions. It is believed that vitamins and minerals have beneficial effects in improving the productive performance of poultry (Monoura *et al*., 2008). Like vitamins, importance of certain trace minerals in immune function has been increasingly evident, such as Se which has been found to affect various components of the immune system (Suttle and Jones, 1989). Poultry producer used the dim blue light on their farms to increase their body weight with economic feed conversion ratio (FCR). In addition, it's lowering the bird activity and increasing the heterophil/lymphocyte (H/L) ratio (Mousa-Balabel *et al*., 2021).

Considering the above factors, the present study was undertaken to evaluate the immune response of broiler chicks kept under blue light and vaccinated with Newcastle (LaSota) vaccine, and the drinking water of the bird is fortified with a multivitamin-mineral supplement before and after vaccination.

MATERIALS AND METHODS

Experimental birds, design and husbandry

This study was conducted under the temperate climatic conditions at Kafrelsheik Governorate, Egypt, during the months of October and November, 2020. In this study, 180 unsexed day-old commercial Indian River (IR) broiler chicks obtained from a local commercial hatchery in El-Gharbyia Governorate, Egypt. Their average body weight (BW) was 42 ±2.1g and brooded under standard brooding conditions. All birds were received at 33°C which decreased 3°C every week until 21°C at the fifth week on a deep litter system and kept under a light intensity of 40lux and 24h light length from 1 to 7 days of age (Mousa-Balabel *et al*., 2017). After 7 days, the light intensity was reduced to 15 lux, and the light-dark cycle was 23 h: 1 h. From d 8 to d 35, the chicks were randomly distributed between 3 equal separated environmental light proof rooms (2.6X2.1m each; 60 chicks) with three replicates of 20 chicks each with a trial end stocking density of 34 kg/m^2 (equivalent to 15 chicks per square meter) based on chick placement numbers (Rozenboim *et al*., 2004).

The birds were exposed to three different treatments following their identification with wing rings according to Senaratna *et al*. (2016). The chicks in the first pen were reared under white light color (WLC) from an incandescent bulb and kept as a control group. While, the chicks in the second pen were kept under blue light color (BLC) from an incandescent bulb two days before and after vaccination and the chicks in the third pen were kept under white light color (WES) and supplemented with vit E and Se (Zoosol Sel-E 200, DSM Nutritional Products Co. Italy) in their drinking water for 2 days before and after vaccination at the rate of 10 g to 2 liters according to Sanda (2015). These treatments were used to assess the effect of BLC and vit E and Se supplementation on IR broiler post vaccinal reactions under the Egyptian conditions. Throughout the duration of the study, all birds in the different treatments were given identical care and management (Xie *et al*., 2008). The chicks were reared on a deep litter system with water and feeding on commercial feeds (Alnour and Albaraka Company, El-Gharbyia Governorate, Tanta city, Egypt), *ad libitum*; broiler starter (metabolizable energy [ME] = 3,000 kcal/kg, crude protein [CP] = 23%); broiler finisher (ME = 3,100 kcal/kg, CP = 20%). The starter ration was used for feeding all broiler chicks from day 1 to day 21 of age and the finisher ration was used for feeding all broiler chicks from day 22 to day 35 of age. On 7 and 17 days of age, broilers were vaccinated with Servac Newcastle vaccine (Vet. Ser and Vaccine, Res. Inst., Abbassia, Cairo, Egypt) in the drinking water.

Initially, blood samples were collected from five chickens that were selected randomly before vaccination. Subsequently, blood sample collection was done on day 11 post second Lasota vaccination (11 days after the second vaccine administration) (Xie *et al*., 2008). The blood samples were drawn from wing vein (4-5ml) into sterile microtubes (one contained anticoagulant EDTA). The coagulated blood samples were centrifuged at 3000 rpm to harvest sera, which were transferred into clean and sterile Microtubes and stored at -20°C until its using for the estimation of NDV antibody titer (Xie *et al*., 2008). The uncoagulated blood samples were examined for CBCs and differential white blood cell analysis (Chung *et al*., 2020). Heterophil/ lymphocyte ratios were determined as described by Kaab *et al*. (2018).

Assessment of NDV antibody titers

The NDV antibody titer in isolated serum samples was measured using haemagglutination-inhibition (HI) test. The HI titer was expressed as the $\log_2$ reciprocal of the highest serum dilution producing 100% inhibition of HA activity (Rahimi and Khaksefidi, 2006).

The lymphoid (immune) organs

On day 28, after collecting blood samples, the same birds were weighed and slaughtered. Birds were opened to examine the abdominal cavity and to collect the immune organs (bursa of Fabricius, liver**,** thymus and spleen) to record their weight according to Cheng *et al*. (2017).

Performance characteristics

All broilers were individually weighed at the start of the experiment, 7, 14, 21, 28 and 35 days of age and body weight gain (BWG) between them was calculated. The total feed intake (TFI) was weekly calculated. Also, feed conversion ratio (FCR) was determined for relevant time periods. Birds were inspected twice daily (8 a.m. and 8 p.m.) for signs of disease to assess post vaccinal reaction and mortality. Total mortality records throughout the study period were calculated as a percentage of live birds at the start of each treatment according to El-Husseiny *et al*. (2000). The post vaccinal reaction examination was performed on dead and surviving birds of all groups. Relative weight of internal organs was also measured by following equation:

Relative weight % = organ weight x 100/ body weight

Ethical issues

All experimental procedures performed in the study followed the Guidelines of the Institutional Animal Care and Use Committee of Research Policy on Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt, at which the studies were conducted.

Statistical analysis

Data were reported as means ± SEM and analyzed by one-way ANOVA using Graph Pad prism 5. The significance of difference among the different groups was evaluated by Tukey's post hoc multiple comparison test. The significance level was set at $P < 0.05$.

RESULTS

Table I shows the mean output values of broiler performance that were held under various treatments of light and supplemented with vitamin E and Se. Results showed that the BWG at 35 days of age was higher in the birds kept under BLC and WES groups (2192.9±83.22 and 2140.86±62.23g, respectively) compared to those kept in WLC group (1927.48±79.02g).

Regarding the feed intake and mortality percent, Table I reveals that the overall feed intake or consumption was lower in the birds kept under BLC and WES groups (3980±95.03 and 3995±54.58 g, respectively) compared to those kept in WLC group (4115±124.5 g). However, the lowest percentage of livability rate was reported in broilers held under WLC group (93.4 %) compared to BLC and WES groups (100 and 100%, respectively).

The most economic FCR was recorded in the birds kept under BLC and WES groups (1.814±0.002 and 1.866±0.010, respectively) compared to those kept in WLC group (2.134±0.011).

Hematological and biochemical parameters were affected significantly ($P<0.05$) by blue light and supplemented with vitamin E and Se before and after NDV vaccination. Birds reared in the BLC and WES groups showed higher PCV (26.59±0.012 and 27.65±0.024%, respectively), hemoglobin (8.92±0.032 and 9.43±0.015 g/dl, respectively) and RBCs (2.39±0.045 and 2.17±0.051$\times 10^{12}$/l, respectively) compared to those kept in WLC group (PVC was 22.60±0.036, HB was 7.86±0.045 and RBCs was 1.81±0.032$\times 10^{12}$/l, respectively). While, H/L ratio was decreased (0.466±0.021 and 0.529±0.009, for BLC and WES groups, respectively, in comparison to 0.640±0.017 for WLC group). On the other hand, AST, ALT and the total protein (Albumin and Globulin) were didn't affect by the treatments (Table II).

Concerning the weight of lymphoid organs as a percent of the final body weight, the data in Table III shows that the weight of lymphoid organs (liver and Bursa of Fabricius) was significantly ($P<0.05$) increased by rearing the birds in BLC and WES groups. Meanwhile, the spleen and thymus weight were not affected.

The Newcastle disease antibody titers after vaccination, were significantly ($P<0.05$) increased obviously in the birds reared in the groups of BLC (4.02±0.024) and WES (2.98±0.031) in comparison to WLC (2.12±0.016) group (Table IV).

DISCUSSION

The mechanism of action of blue light increases plasma androgens (Rozenboim *et al*., 1999) which improve protein synthesis leading to muscle build-up (Crowley and Matt, 1996). The means ± standard errors of growth performance of IR chickens post-vaccination is presented in Table I. The highest (P<0.01) body weight gain was observed in BLC and WES groups in comparison to control (WLC) groups. In conformity with the present study increased body weight was noticed by Dalia *et al*. (2018) on supplementation with vitamin E and Se in the broiler diet. Similar findings were also reported by Maini *et al*. (2007); Bobade *et al*. (2009) in broilers. The total feed intake per broiler during the experimental trial was higher (P<0.05) under blue light and antioxidant (vitamin E and Se) supplemented groups than the control group. These results explain the improved body weight of the birds under the blue light color and antioxidant supplementation in the present study. Also, it confirms the findings of Liu *et al*. (2008) who observed that there was an increased breast muscle weight in birds reared under blue light at market age. This observation can be explained by the blue light stimulates the myofiber growth of the birds (Velo and Ceular, 2016). Also, the improvement in body weight gain of the antioxidant supplemented group might be due to an excellent chain breaking ability of antioxidant that protects cells and tissues from lipoperoxidative damage (Lin and Chang, 2006). Vitamin E interacts with Se containing enzyme glutathione peroxidase to prevent the oxidative breakdown of cells (Surai, 2000).

The difference in livability percentage of birds under treatments (blue light group and vita E supplementation) and the control group were similar to the results obtained by Bharat *et al*. (2013) in chicken and by Chung *et al*. (2005) in breeder hens which supplemented with vitamin E and C. This livability percentage may be related to better immune status as, vitamin E reduces the secretion of immunosuppressive factors and inhibits protein kinase C in cells of monocytes and lymphocytes thereby improve immunological system (Erf *et al*., 1998).

The birds under BL had an approximate FCR, which was comparable to that of vita E but significantly higher than those of the control group as shown in Table I. These results may be attributed to blue light enhanced the metabolic hormones and productive performance, including carcass weight compared to conventional white lights (Soliman and Hassan, 2019). In addition, Xie *et al*. (2011) proved that blue light enhanced the intestinal villi growth. Moreover, Kim *et al*. (2013) observed that blue light color is effective for feed utilization.

Table II shows the relationship between blue light color and vitamin E supplementation and the hematological parameters of broiler chicken. Packed cell volume, hemoglobin and differential leucytic counts were increased under blue light color and vitamin E supplementation. Moreover, red blood cells of birds in the BL group were significantly higher than those of the birds in the control group but were comparable to those of Vita E group. The increased level of the total erythrocyte count, hemoglobin content and packed cell volume might be due to effects on hematopoietic organs. This finding differs from the earlier study of Trans *et al*. (2000) who observed no significant effect of vitamin E supplementation on any of the hematological parameters (PCV and HB).

Heterophil to lymphocyte (H/L) ratio is a reliable stress indicator. It increases in a stressful condition (Kang *et al*., 2011). The H/L ratio of the birds kept under blue light and supplemented by vita E was significantly lower than those of the birds in the control group as shown in Table II. The lower heterophil to lymphocyte ratio of the birds under blue and vita E supplementation in the present study is in consensus with the earlier findings of Mohamed *et al*. (2014) who reported that the H/L ratio of chickens under white light was higher than that of blue light. The findings of Ke *et al*. (2011) also indicated blue light reduced oxidative enzymes. The poor performance of the birds raised under white light in the present study can be explained by a high heterophil/lymphocyte ratio is negatively correlated with body weight (Al-Murrani *et al*., 2006). The calming effect of blue light has been ascribed to be responsible for modulating stress impact on the birds (Xie *et al*., 2008; Firouzi *et al*., 2014).

The aspartate aminotransferase (AST), alanine aminotransferase (ALT) and the total protein (albumin and globulin) values of the birds across all treatments were similar. These results were explained by good health conditions with less muscle damage (Olanrewaju *et al*., 2008).

The effect of blue light color and vitamin E supplementation on lymphoid organs of broiler chickens was presented in Table III. The blue light color and vitamin E supplementation did not influence the spleen and thymus weight. On the other side, the liver and Bursa of Fabricius weight of the chickens under BL and vitamin E supplementation were similar but higher than those of the control group (P < 0.05). The improved relative weights of the bursa of Fabricius in the present study corroborate the findings of the previous study that light influences the immune response of birds (Blatchford *et al*., 2009). It has been shown that that blue light could improve immune function (Xie *et al*., 2008). These results are contrary to the findings of Dalia *et al*. (2018) who stated that supplementation of vitamin E and Se did not affect the lymphoid organ weight in broiler.

Singh and Haldar (2005) reported that the enlargement of lymphoid organs could be attributed to three possible reasons such as increase in cell proliferation, decrease in cell death and decline in lymphocytes trafficking to the periphery.

Broiler chickens experience an acute-phase response through vaccination, which reflects the innate immunity and stress related to immunization. Table III also shows antibody titers against the vaccine in the vaccinated three (n = 60 per group) groups. The humoral immune response to the treatment was compared 10 days after the second vaccinations (day 28). HI titer in blue light color and vitamin E supplementation groups was significantly (p < 0.5) higher than those of the control group**.** This means blue light and vitamin E supplementation introduced a stronger immune response (4.02±0.024 and 2.98±0.031, respectively) while the white light had the lowest response (2.12±0.016).

Table I. Effect of blue light and supplemented with vitamin E and Se before and after NDV (Lasota) vaccination on performance of IR broilers (Mean ± SE).

Item	Control	Blue light	Vitamin E	P value
Initial weight (g)	42.52±0.37[a]	42.10±0.21[a]	42.14±0.17[a]	0.5549
Final body weight (g)	1970±59.17[a]	2235±53.28[b]	2183±42.28[b]	0.0030
Body weight gain (g)	1927.48±79.02[a]	2192.9±83.22[b]	2140.86±62.23[b]	0.0023
Feed intake (g)	4115±124.5[a]	3980±95.03[b]	3995±54.58[b]	0.0036
Feed Conversion Ratio (FCR)	2.13±0.01[a]	1.81±0.002[c]	1.86±0.01[b]	0.0021
Livability rate (%)	93.4	100	100	

Means within each raw having different superscript letters differ significantly at P<0.05.

Table II. Effect of blue light and supplemented with vitamin E and Se before and after NDV (Lasota) vaccination on hematological and biochemical parameters of IR broiler.

Item	Control	Blue light	Vitamin E	P-value
PCV (%)	22.60±0.036[b]	26.59±0.012[a]	27.65±0.024[a]	0.0010
HB (g/dl)	7.86±0.045[b]	8.92±0.032[a]	9.43±0.015[a]	0.0041
RBCs ($\times 10^{12/l}$)	1.81±0.032[c]	2.39±0.045 [a]	2.17±0.051[b]	0.0042
WBCs ($\times 10^{12/l}$)	13.78±0.046[a]	11.44±0.052[b]	12.98±0.053[b]	0.0034
Heterophil (%)	37.45±0.044[a]	30.32±0.021[b]	31.86±0.031[b]	0.0022
Lymphocyte (%)	58.46±0.047[c]	65.02±0.046[a]	60.15±0.022 [b]	0.0011
H/L ratio	0.64±0.017[a]	0.466±0.021[c]	0.52±0.009[b]	0.0020
AST (IU/L)	64.01±0.025[a]	64.66±0.014[a]	64.99±0.032[a]	0.1615
ALT (IU/L)	26.42±0.078[a]	26.99±0.074[a]	26.82±0.036[a]	0.1226
Total protein (g/dl)	2.73±0.029[a]	2.78±0.004[a]	2.78±0.021[a]	0.2324
Albumin (g/dl)	1.31±0.009[a]	1.33±0.010[a]	1.31±0.013[a]	0.3412
Globulin (g/dl)	1.42±0.026[a]	1.45±0.031[a]	1.47±0.026[a]	0.1752

Means within each raw having different superscript letters differ significantly at P <0.05. RBCs, Red blood cells; PCV, packed cell volume; HB, Hemoglobin; WBCs, White blood cells; H/L, Heterophil/lymphocyte ratio; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase.

Clinical signs of various groups and different days

None of the groups showed any clinical and necropsy signs before vaccination. Three days after the vaccination, three cases of paralysis were noticed in the control group, but no clinical cases of the disease were observed in the treating groups. Since on the fourth day after vaccination, the mortality onset was noticed with some signs, including green and watery diarrhea, respiratory signs, head tremors, and torticollis.

Clinical signs of disease were not observed in any of the groups until day 3 post vaccination, control group showed disease signs thereafter. In the other groups from day 15 onwards moderate to severe depression, lameness and/or breathing with open beaks were observed in one to four birds per group. Some of these birds died, the others showed signs of disease up to the end of the experiment.

The group given multi-vitamin supplements (E and Se) before and after LaSota vaccination maintained high antibody titer throughout the period of the study. Rao *et al.* (2004) reported that vitamins and minerals are important in developing immunity.

In conclusion, the results of this study demonstrated that the BL and vitamin E supplementation are essential for rapid muscle development and improve the post vaccine reactions in IR broilers.

Table III. Effect of blue light and supplemented with vitamin E and Se before and after NDV (Lasota) vaccination on weight of lymphoid organs as a percent of the final body weight and on Newcastle disease antibody titers (log[10] transformed) of IR broiler.

Item	Control	Blue light	Vitamin E	P-value
Weights of lymphoid organs				
Liver %	0.96±0.052^c	1.34±0.027^b	1.45±0.024^a	0.0008
Spleen %	0.12±0.015^a	0.12±0.021^a	0.12±0.011^a	0.5919
Thymus %	0.69±0.023^a	0.70±0.034^a	0.70±0.052^a	0.1516
Bursa %	0.23±0.025^b	0.42±0.041^a	0.41±0.032^a	0.0061
ND antibody tita				
Before	1.69±0.016^b	1.99±0.021^a	2.05±0.029^a	0.0052
After	2.12±0.016^c	4.02±0.024^a	2.98±0.031^b	0.0031

Means within each raw having different superscript letters differ significantly at P <0.05.

Statement of conflict of interest

The authors have declared no conflict of interest.

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