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Different light programs affect the titer of vaccination of Newcastle in broiler chicken

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A B S T R A C T

Light is an important factor in the functioning of the body of birds, especially the immune system. Management of the lighting program in closed systems of poultry production, mainly by affecting the concentration of melatonin, can be operational on the effectiveness of vaccination. Newcastle disease can cause extensive damage to the poultry industry. Therefore, its timely diagnosis and assessment of the success of vaccination with methods such as the hemagglutination inhibition test are of particular importance. This study investigated the effect of two photoperiods on antibody titer against Newcastle virus following vaccination. Four groups of Ross broilers were examined for six weeks. Groups A and B received the live Newcastle vaccine twice, and groups C and D were not vaccinated against Newcastle. Groups A and C received a lighting program from the management handbook of Ross Broiler, and groups B and D received the continuous lighting program (23L:1D). During the breeding period, blood samples were taken six times from each group, and the HI test measured the antibody titer. The results showed that despite the higher antibody titer in group B compared to group A, this difference was not statistically significant (P>0.05). The feed conversion rate was higher in the groups that received the continuous photoperiod, and their mortality rate was lower than that of the other two groups. Comparing the results of the present study with those of various studies and articles shows the importance of the lighting regime in the success of vaccination.

Keywords: *Broiler, Photoperiod, Newcastle disease, Lighting program.*

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1 Introduction

Light is an important environmental factor in controlling many biological processes (1). Compared to humans, a more comprehensive range of light waves can be detected by the unique visual system of avian species (2). How light is received and processed and how it affects the biology of the bird's body is explained in detail by Davide M. Dominoni (3).

The lighting program or regime comprises three main components: wavelength, light intensity, and photoperiod. The wavelength determines the color of the light, and the photoperiod expresses the length and distribution of light and dark hours during the day and night (1, 4). In general, photoperiod is the most prominent factor in light regime management in industrial poultry farming (5). Currently, a great variety of light programs and tools are available to poultry producers, which allows them to modify light management in closed houses based on the physiological requirements of birds and breeding objectives (1, 6). For an extended period, broilers were raised under continuous photoperiods to maximize feed consumption until research showed that this light schedule caused sleep deprivation and many other problems.

In contrast, different light and dark cycles evoke the circadian rhythm of birds (5, 7). preferable functioning of the immune system, well-being of birds and reduction of aggressive behaviors, coping with stress, proper development and growth of the reproductive, skeletal, and muscular system, and obtaining more favorable production indicators are the advantages that can be achieved with the correct management of the lighting regime in industrial poultry production (1, 6, 8-10). The lighting program is important not only after hatching but also during incubation. Chickens that receive suitable light and darkness periods during the embryonic stage have better biological factors and performance during life and production (11-13).

The importance of light and the lighting program in birds' immune systems and the response to stresses has been demonstrated (4, 5). This effect is mainly attributed to the pineal gland's function in melatonin secretion (14). This hormone, primarily secreted in the dark, regulates the circadian rhythm and is generally considered an immune stimulant(15, 16). Also, in conditions of light stress, immunity can be suppressed (17). It is known that the lymphatic tissues and immune cells of birds have melatonin receptors, and their function is affected by the concentration of this hormone. The importance of this concentration in the development of general immunity and the immunity of the respiratory system of birds (which has an undeniable role in dealing with essential diseases such as Newcastle disease and Avian Influenza) has been proven (18).

The seasonal immune change pattern can prove melatonin's immunoregulatory role in birds. In colder seasons, short day length and long darkness cause the concentration of melatonin to increase. As a result, cell proliferation and weighting in lymphatic organs such as the spleen occur. Also, in such a situation, the total number of leukocytes increases. In contrast, during warmer seasons with longer daylight hours, decreased melatonin and increased gonadal/adrenal steroid levels are responsible for decreased immune competence. Such a pattern protects the bird from the environmental stress of cold seasons and their consequences and prepares it to breed in warmer seasons (18, 19). Adequate light regimes can stimulate the expression of immune receptors (such as Toll-like receptors) (20). This positive effect on synthesizing interleukins and interferons, which are immune mediators, has also been proven (7). Appropriate photoperiod increases the mitogenic and proliferative responses of T and B lymphocytes, serum IgG level, and the percentage of CD3+, CD4+, and CD8+ cells in broilers. It has been shown that melatonin can effectively restore the impaired activity of T-helper cells and increase the activity of natural killer cells (7, 21, 22). Based on these findings, various researchers have suggested increasing the length of the dark period as an effective solution to strengthen the poultry's immune system (18, 23).

The effect of light pollution and the change of circadian rhythm on the state of the immune system of wild birds has also been investigated, which indicates a decrease in the level of immunity in these birds due to the increase in the duration of lighting (3, 24).

The results of research on the effect of the light regime and its mechanism on the immune system have been presented in more detail by other researchers (3).

Newcastle disease (ND) is caused by Avian paramyxovirus 1 (APMV-1) in many birds (including commercial poultry) (25). APMV1 infection, based on the virulence of the virus pathotype, causes a wide range of clinical manifestations, from asymptomatic viral replication to severe neurological, respiratory, and gastrointestinal symptoms and 100% mortality through sudden death (26). Due to the potential of this disease to cause significant economic losses to the poultry industry, its occurrence is reportable to the Office International des Epizooties (OIE) (27). Vaccination and biosecurity principles are

recommended as the most effective disease control strategy. Although virus isolation is considered the standard diagnostic strategy for ND, serological tests such as hemagglutination inhibition (HI) and Enzyme-Linked Immunosorbent Assay (ELISA) are the industry's first choice for diagnosing this disease (in addition to an assessment of vaccination success) (25, 28). The basis of these tests is binding the known antigen to the antibody in the serum, which evaluates the humoral immune response to the virus. Depending on the virulence of the virus, the environmental condition, and internal factors of the body, the patterns of immune response to the pathogen change (28, 29).

The present study aims to investigate the effect of a lighting program with different hours of darkness compared to a continuous lighting program in producing antibody titers against the Newcastle virus following vaccination.

2 Methods and Materials

2.1 Experimental design and husbandry conditions

Four hundred one-day-old Ross308 broiler chickens were obtained from a mother flock at 48 weeks. The levels of maternal antibodies against Newcastle disease, Avian influenza, Infectious bursal disease, and Infectious bronchitis were assessed as desirable, and the chickens were free of infection with Mycoplasma and Salmonella species. The chickens were randomly divided into four groups of 100 chickens. Each group was kept in a house that consisted of 4 parts of 2 x 5 meters with ad libitum access to feed and water for 42 days and a slaughter weight of 2-3 kg. The feed formulation was balanced according to the requirements of the Ross308 broiler and was provided to the birds in the form of pellets in three formulas: Starter (0 to 12 days old), Grower (13 to 25 days old), and Finisher (26 to 42 days old). Other breeding conditions were managed similarly for all groups based on the Ross broiler management handbook (30), and only the lighting program and receipt and non-receipt of Newcastle vaccine were different among the groups.

All four groups, at the age of 1 day and 24 days, received the H120 live vaccine against infectious bronchitis agents by eye drops and drinking water, respectively. Also, the D78 Live vaccine was administrated to each group in drinking water at 15 and 22 days to protect the chickens against Infectious bursal disease. Groups A and B received the Newcastle Hitchner. B1 eye drop vaccine at the age of 8 days and the Newcastle COLON30 vaccine in drinking water on the 18th day of rearing. Groups C and D were not vaccinated for Newcastle disease as control groups.

For groups A and C, the Ross308 breeding management light program (Table 1) was applied, and for groups B and D, a continuous light program (23L:1D) was applied.

2.1.1 Sampling and information record

On six occasions, on days 1, 7, 16, 24, 31, and 42, ten chickens were randomly selected from each group, and blood samples were taken. This procedure was performed with a 2-cc syringe without any anticoagulant agent. The collected sample was placed at room temperature for 1 hour until the serum was initially separated from the clot, and, in the next step, the serum was wholly obtained by centrifugation.

Information on the amount of feed consumed was recorded weekly, and 10% of the chickens in each group were randomly selected and carefully weighed every week. Each group's mortality and feed conversion rate (FCR) were recorded until the end of the breeding period.

2.2 Serological assessment

The sera were kept at 56°C for 30 minutes to remove the effect of non-specific inhibitors. First, 25 microliters of PBS solution were poured into all plate wells. Then, 25 microliters of the test serum were loaded in the first well, which was repeated up to the 12th well. In the next step, 25 microliters of Newcastle antigen solution with 8 HA units were added to each well. After 30 minutes, 25 microliters of red blood cell suspension of non-vaccinated chickens without maternal antibodies were added to all wells. The results were observed and recorded after 30 minutes.

2.3 Statistical analysis

Statistics were analyzed using SPSS software version 26. A chi-square test was also conducted to determine significance.

3 Results

On each occasion of serological examination, the mean, minimum, and maximum antibody titer of Newcastle disease virus in group B was higher than in group A, although statistically, this difference was not significant ($P>0.05$) (Table 1). The maternal Newcastle antibody titer was reduced without infection in groups C and D.

On days 14, 21, 28, 35, and 42, an increasing trend was observed in the feed consumption of groups A and C. The results of weighing the chickens did not show any significant difference between the groups, but on days 14, 21, 28, and 35, a trend of weight gain was observed in groups B and D, which continued until the 42nd day.

The comparison of feed conversion rates showed that at the end of the experiment, the FCR of groups A and C was higher than groups B and D (1.85, 1.77, 1.81, and 1.75 for groups A to B, respectively). Also, the mortality rates of groups B and D were less than those of groups A and C.

Table 1. The titer of ND using HI test in Groups A and B. No significant differences between groups ($p \leq 0.05$)

Age (Day)	Group A HI Titers			Group B HI Titers		
	Min.	Mean	Max.	Min.	Mean	Max.
1	7	7	8	7	7	8
7	5	6	7	5	6.3	7
16	2	3.9	5	3	4	5
24	2	4.9	7	3	5	7
31	2	3.4	6	2	4.4	8
37	2	3.6	5	2	3.7	6
42	2	3.1	4	2	3.2	5

4 Discussion

Changes in different components of the light program in broiler breeding affect the performance of different systems of the bird's body and production results. The findings of our study revealed that rearing Ross 308 broiler chickens in a light program with more hours of light deprivation did not create a significant difference in the production of humoral immune antibodies against the Newcastle disease virus. However, the titer was numerically higher in this group.

In research conducted at the University of North Carolina in 2000, the effect of lighting conditions as an immune modulator and melatonin supplementation on the humoral and cellular immune response in Japanese quail was investigated. In the first experiment, three light treatments of short days (8:16LD), long days (16:8LD), and constant light (LL) were considered, and in the second experiment, the LL group received melatonin at different doses. Cutaneous basophil hypersensitivity reaction to phytohemagglutinin (PHA-P) as an indicator of cellular immune reaction, and primary antibody titers were evaluated seven days post-intravenous injection of Chukar red blood cell suspension were measured as an indicator of humoral immune reaction. Both 8:16LD and 16:8LD treatments had significantly better cellular and humoral immune responses than the LL group. In the second experiment, melatonin could improve the immune function of the LL group (31).

Onbaşılar et al. (2007) investigated the effect of two different photoperiods on production performance, occurrence of skeletal system disorders, some blood factors, and antibody production of 200 one-day-old male broiler chicks (Ross PM3). One group received continuous lighting (24L:0D) for 42 days, and the other group experienced intermittent lighting (IL) photoperiod (1L:3D) during the same period. The results indicated that the intermittent lighting group had a higher antibody titer against NDV (32).

The study conducted by Moraes et al. in 2008 expressed the effect of 4 light programs of 23L:1E (23L), increasing (INC), 16L:8E (16L), and natural light (NAT) on the production and immunological performance of male Cobb-500 broiler chickens. The experiment was conducted with four groups, including 35 birds, and six repetitions. No significant difference was observed between the groups regarding antibody titer against Newcastle disease virus (33).

Gharib et al. 2008 evaluated the role of photoperiod and melatonin in reducing the negative impact of heat stress on broilers. In the first experiment, four groups of male Cobb × Cobb broiler chickens were raised under a continuous (23h Light: 1h Dark) and intermittent (1hL: 3hD) light program, and then one group from each light program, at a temperature of 35°C and one group was placed at a temperature of 24°C. The group that received the intermittent light program was less affected by heat stress and its adverse effects. In the second experiment, two groups were kept at 35°C, two at 24°C, and one receiving melatonin at each temperature. The group receiving melatonin showed more excellent heat stress resistance (34).

In the study conducted by Xie et al. in 2008, the effect of monochromatic light on the immune response of birds was investigated. Two hundred sixty-one-day-old Arbor Acres male broilers in four groups were exposed to monochromatic

red, green, blue, or white light for seven weeks. At 28 days, the group that received the green light showed a higher serum level of antibodies against the Newcastle disease virus than the group that received the red light. The level of this antibody on day 49 was higher in the group that received blue light than in the red light group. The proliferation of peripheral blood T lymphocytes increased in 21 days in green light compared to blue and red light and in 42 days in blue light compared to red light (35).

2008 Abbas et al. divided 300 one-day-old male broiler chicks (cobb × cobb) into three random groups. They exposed them to 3 photoperiods of continuous light (23L:1D), non-intermittent restricted light (12L:12D), and intermittent light (2L:2D) for six weeks. In chickens exposed to intermittent light, peripheral B and T lymphocyte proliferation and antibody production were significantly induced. Also, factors indicating stress, including plasma corticosterone concentration and heterophil/lymphocyte ratio, were significantly higher in the treatment 12L:12D than in the other two groups. Compared to the control group, total white blood cells (WBC) and plasma T concentration increased significantly in the intermittent light group. The results of this experiment show that intermittent photoperiods, compared to continuous or non-intermittent restricted photoperiods, improve the cellular and humoral immune function of birds and reduce stress (7).

In a study conducted by Abbas et al. in 2014, 150 broilers were divided into five treatments, and each treatment received a different light intensity over 42 days. This study showed that the antibody titer against the infectious bursal disease virus at six weeks of age showed a significant increase in the birds reared with the lowest light intensity compared to other groups. However, on the contrary, the antibody titer against the NDV was not significantly different between these groups at this time. Also, the Bursa of Fabricius in this group had more weight than other groups in 2 to 6 weeks (16).

In the study by Firozi et al. (2014), 4000 chickens were randomly placed in 4 halls and kept for 42 days with green, sunny yellow, blue, and red light. The results showed that the reduction of maternal antibodies was the lowest in the green light group. At the end of the breeding period, the birds exposed to green and blue light had the highest ND antibody titer among all groups, but this difference was not statistically significant. Birds in the yellow-light house significantly increased total serum protein compared to other groups (36).

In a study by Sharideh and Zaghari 2017, three hundred and sixty 308-day-old male chicks were reared for 42 days. Chickens were randomly divided into three groups, for which neutral white light, warm white light, and incandescent light were considered. The test results showed that the group that received warm white light had significantly higher indices of humoral immunity and antibody titers against the Newcastle disease virus than the other two groups. Although this difference was also present in the antibody titer against the Avian influenza virus, it was not statistically significant (8).

In a study conducted by Assam and Hassan in 2019, 9 different photoperiods (including 23:1, 18:6 continuous, and 18:6 intermittent photoperiods, each with three colors: red, blue, and white) for 252 Ross broiler chicken, were applied in 9 random groups during 40 days. This study showed that the 23:1 continuous photoperiod group with each light color significantly produced more antibody titers against the Newcastle disease virus than other photoperiods with the same light color (37).

In a study conducted by Tarek Mahmoud Mousa-Balabel and Karima Mohamed Abdo Abofarag in 2022, three groups of 60 commercial Indian River (IR) broiler chicks, two days before and two days after vaccination with NDV- Lasota, respectively, were treated with white light color (WLC), blue light color (BLC) and white light color and supplemented with vita E and Se in the drinking water (WES). The results showed that the body weight gain was significantly higher in broilers raised under BLC and WEC than in the WLC group. Also, these two groups had higher feed conversion ratio (FCR) and Newcastle disease virus antibody titer and lower heterophil/lymphocyte ratio compared to WLC treatment (38).

Regarding photoperiod, most of the results of other investigations were consistent with the present study's findings. However, a significant difference was observed in the antibody titer against the Newcastle virus in the experiments, which made a difference in light color. Comparing the results of various studies and articles shows the importance of the optical program and attention to all three components in the success of vaccination.

Conflict of Interest

The authors declared no conflicts of interest.

Author Contributions

H. HN. supervised the project. All authors contributed to the study conception and design Material preparation, data collection and analysis were performed by A. D., A, S.D, M.S.B and N.S. The first draft of the manuscript was written by A. SM and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability Statement

Data are available from the first author upon reasonable request.

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Ethical Consideration

The faculty of veterinary medicine, Garmsar Branch, Islamic Azad University, approved the study (code: GBIAU. VET.2020.06).

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