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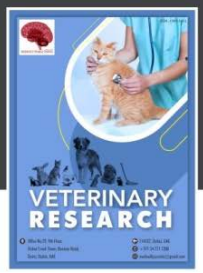

The Effect of Various Light Programs on Heterophil to Lymphocyte Ratio and on Antibodytiter Against Newcastle Disease

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Key words: Light program, Newcastle antibody titer, Heterophil to lymphocyte ratio, broiler, t-test method

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Abstract: The light is an important environmental factor in industrial poultry husbandry. The light programs play an important role in appropriate management and an infrastructure for desired function of broilers. In this study, the effect of various light programs on heterophil to lymphocyte ratio and on antibody titer against Newcastle disease with hemaglutination inhibition test was evaluated. In addition, 800 one-day ROSS 308 broiler chicken were prepared and randomly divided into four groups, each group contained 200 chicken, nominated as A-D. The groups kept at controlled isolated locations, containing two different light programs. Group A was adjusted on 23 h lighting and one hour darkness and group B was designed as recommended program for Ross breed (2-3 kg end period body weight). Although, group C and D were considered as control groups and did not receive Newcastle vaccine. During husbandry period the groups were in same conditions and just were different in lighting programs. The groups A and B on 8, 18 and 29 days were vaccinated against Newcastle disease and on 1, 7, 14, 21, 35, 28 and 42 day old the blood samples were taken for blood smears for heterophil to lymphocyte ratio count and antibody titer assessment with hemaglutination inhibition test. The obtained data were analyzed with t-test method. The results showed differences of heterophil to lymphocyte ratio between A and B groups on 7, 14 and 42 day old which the ratio was significantly higher in group A than group B ($p<0.05$). On 21 and 28 days the significant difference in this ratio was not observed ($p>0.05$). On 35 day-old the heterophil to lymphocyte ratio was significantly

higher in group B than group A ($p<0.05$). Furthermore, the antibody titer of Newcastle vaccine showed a relative priority in group B on 7, 14, 21, 28, 35 and 42 days than group A whereas no significant difference were observed in any days ($p>0.05$). The results indicated stress condition in group A, containing lighting program for Ross husbandry (2-3 kg end body weight) than group B.

INTRODUCTION

The light is an important environmental factor in poultry husbandry and is effective in several poultry behavioral and physiological aspects. Duration, intensity of lighting and light wave length are of various light aspects which affect poultry behavior. The duration of lighting in layers influence on age of puberty and egg production rate and growth rate of broilers (Ali and Cheng, 1985; Spies *et al*., 2000). The light intensity impacts on poultry behavioral and reproduction affair (Gill and Leighton, 1984). Generally, there are two indices for poultry stress assessment. First, corticosteroid level assessment and the second is heterophil to lymphocyte ratio which increases in parallel with stress. It is found that anti-Newcastle antibody titer in broilers withintermittent lighting was more than those with permanent lighting (Gill and Leighton, 1988). The effect of light intensity change on growth and behavior of broilers was detected (Morris, 1967). Hester, etc. demonstrated that heterophil to lymphocyte ratio increased in stress condition and is beneficial as a stress index. Furthermore, in latter study the effect of temperature on heterophil and lymphocyte was evaluated which indicated that with the rate rises with temperature increase. Furtheremor, Zabudeskii and Skutar (1993) found that in cold stress in domestic chicken, the white blood cells migration decreases but the heterophil to lymphocyte increases as well as hunger and thirst.

Fitco *et al*. (1993) indicated that metabolic activity of blood heterophils increases upon stress in chicken. Grass and Sigel (1983) demonstrated that hetrophil to lymphocyte ratio is a functional index in chicken stress assessment. Various breeds recommend different lighting programs which little information is available regarding homoral immunity and heterophil to lymphocyte influences. Stress in poultry probably is a risk factor for decreasing gain weight and appropriate immunity, thus may be increase the risk of disease occurrence, one of the major problems in poultry husbandry, due to immunity suppression. In addition, the probable stress effects due to immunity suppression, several pathogen emergence and gain weight trend in our country broiler husbandry system were not evaluated. Therefore, the present study was carried out to assess various lighting programson heterophil to lymphocyte ratio and anti-Newcatle antibody titer level which are of benefit for poultry industry as an effective tools in immunity improvement against respiratory complexes and other related diseases.

MATERIALS AND METHODS

In this study, 800 one-day-old Ross 308 breed chickens from 45 week-old parents, containing antibody against Newcastle, influenza, infectious bursal diseases and infectious bronchitis diseases. The chickens were free of transmissible maternal mycoplasma and salmonellas and they averagely weighed 41 g on the first day of the study. The chickens were randomly divided into four groups, each group containing 200 chickens, nominated as groups A,-D. Each group transferred to a location which selected for husbandry and all conditions were the same among all groups and the only deference was the light program.

The group A had 23 h lighting and 1 h darkness and group B was undergone light program of Ross recommended husbandry (Table 1 end weight 2-3 kg). The group C dedicated as control for group A and group D as control for group B which did not received Newcastle vaccine. Therefore, the light program for group C was as 23 h lightning and 1 h darkness, whereas in group D the light program was as Ross husbandry recommended light program. The group A and B were vaccinated against Newcastle on 8, 18 and 29 days and on 1, 7, 14, 21, 28, 35 and 42 days the effect of these two light program on antibody response upon Newcastle vaccination were evaluated. In addition, 10 chickens were randomly selected and the blood samples for blood smears taken on 1, 7, 14, 21, 28, 35 and 42 to assess heterophil to lymphocyte ratio and antibody titration by

Table 1: Ross 308 light program (3-2 end weight)

Difference (h)	Darkness (h)	Age (days)
0	0	0
1	1	1
8	9	100-160 g
1	8	22
1	7	23
1	6	24
1	5	5 day before slaughter
1	4	4 day before slaughter
1	3	3 day before slaughter
1	1	2 day before slaughter
1	1	1 day before slaughter

hemaglutination inhibition. Subsequently, after 3 min the smear were fixed in methanol, dried and prepared for heterophil to lymphocyte ratio counting, the blood samples were taken by 2cc-syringe without anticoagulant. The prepared serums underwent HI test by 8 unit hemaglutinating antigen against Newcastle virus.

RESULTS

In this study, the effect of different light programs on heterophil to lymphocyte ratio and antibody response of Newcastle vaccine in broilers were evaluated. The heterophil to lymphocyte between A and B groups was analyzed by t-test method which indicated significant difference, somehow on 7, 14 and 42 days in group A was more than group B, indicating better environment for group B. On 1, 21 and 28 days the heterophil to lymphocyte was the same in two groups and no significant difference was observed ($p>0.05$). However, on 35th day the heterophil to lymphocyte in groups A and B showed significant difference with group B was >A ($p<0.05$) (Table 1-6). Subsequently, the data was analyzed by t-test which indicated difference between A and B groups, representing group B developed lesser stress than A. Regarding anti-Newcastle antibody titer determined by HI test, the results showed priority in group B on 7, 14, 21, 28, 35 and 42 days than group A which showed no significant difference in any samples and groups C and B enjoyed downward trend for maternal Newcastle antibody ($p>0.05$).

DISCUSSION

Husbandry under different light programs will exert diverse effects on broilers performance. According to Moor and Siopes findings in 2000 the humoral and cellular immunity in birds which were kept in frequent periods of darkness and lightning was more than that birds which were under permanent light. In mammalian, a lighting period with short photoperiod days indicated more stimulatory effects on humoral and cellular immunity in comparison with long photoperiod days. Furthermore, the birds with 16 h photoperiod and 8 h darkness encompassed more weight and better bioavailability and anti-Newcastle antibody titers than chicks with 23 h photoperiod and 1 h darkness (Moraes *et al*., 2008). In addition, in an study in northern Carolina university in 2000 the effect of prepared in humoral immunity in Japanese quail was evaluated which in this study the effect of 3 short photoperiod days programs (8 h lightning and 16 h darkness) and long photoperiod day (8 h darkness and 16 h lighting) and constant light program (23-lighting and 1 h darkness) were assessed on immunity response. The short and long photoperiod programs induced similar immunity response than constant light program. In birds which reared in long photoperiod program indicated better humoral and cellular immunity than birds with constant light programs. In a study by Abbas *et al*. (2007) in Egypt national research in 2007 the effect of light programs on heat stress conditions of broilers chick immunity response was assessed. In this study, 2 permanent light programs (23 h lighting and 1 h darkness) and intermittent light program (3 h lighting and 1 h darkness) in 4-6 week old at 35 and 24°C temperatures in 4 groups were carried out. The broilers in heat stress (35°C) and intermittent photoperiod showed lower body temperature and pro-infflamatory cytokinase as well as corticosteron in

Table 2: The normal value of heterophils and lymphocytes in each microliter of chicken sample

Mean	Range	Measured parameters
-	Rare	Band heterophil
4500	3000-6000	Mature heterophil
14000	7000- 17500	Lymphocyte

Table 3: Heterophil and lymphocyte percentage in all stages of blood sampling in group A

Lymph (%)	Het (%)	Age (days)
66.5	33.5	1
60.5	39.5	7
61.5	38.5	14
62.5	37.5	21
67	33.0	28
67	33.5	35
60	40.0	42

Table 4: Heterophil and lymphocyte percentage in all stages of blood sampling in group B

Lymph (%)	Het (%)	Age (day)
66.5	33.5	1
72	28.0	7
68	32.0	14
62	39.0	21
67	33.0	28
64	40.0	35
67	33.0	42

Table 5: Heterophil and lymphocyte percentage distribution in chicken blood samples

Mean (%)	Range	Measured parameter
-	Infrequent (%)	Band heterophil
28	15-40	Mature heterophil
60	45-70	Lymphocyte

Table 6: Anti-Newcastle virus antibody titer mean by hemaglutination inhibition in two vaccinated groups

Days	Group A	Group B	Group C	Group D
1	7	7	7	7
7	6	6.3	6.2	6.2
14	3.9	4	4.1	4
21	4.9	5	3	3
28	3.4	4.4	1.5	1.7
35	3.6	3.7	1	1
42	3.1	3.2	1	1

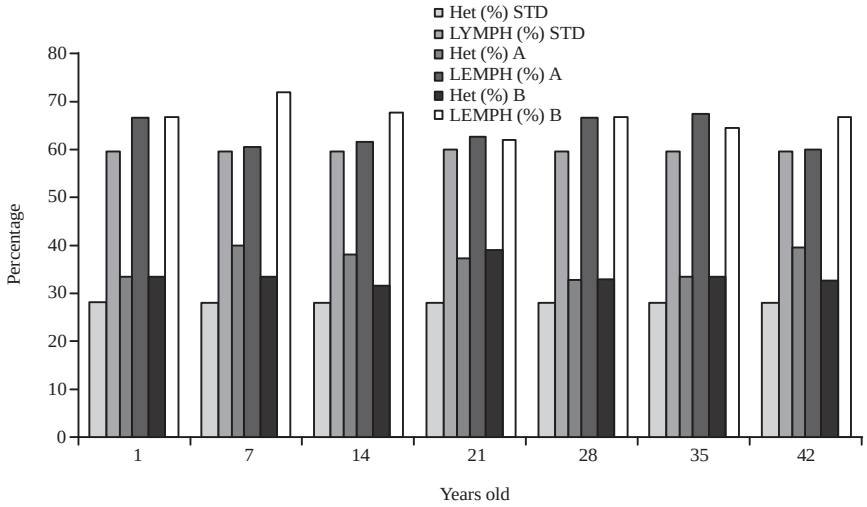

Fig. 1: Heterophil and lymphocyte percentage mean in A and B groups at all sampling stages in comparison with Standard value (STD)

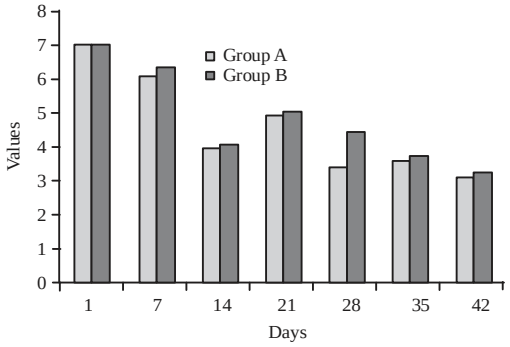

Fig. 2: Anti-Newcastle virus antibody titer mean by hemaglutination inhibition test in two vaccinated groups

plasma than chicks in heat stress (35°C) and permanent light program. However, in intermittent lighting program in heat stress (35°C) indicated more response in cutaneous basophil resulted from hypersensitivity and T cell in comparison with permanent lighting program with 35°C and 24°C temperatures the increase and decrease of mentioned factors were similar to 35°C temperature (Abbas *et al*., 2007) (Fig. 1 and 2).

CONCLUSION

The present study showed that in broilers lighting program with more darkness setting, the heterophil to lymphocyte increase which had more stress marker in birds was lesser and such difference for group B was significant ($p<0.05$) and humoral immunity antibody production against Newcastle disease was more in comparison with groups without long darkness period but no significant difference was observed ($p>0.05$). The laboratory results of such samples for heterophil and lymphocyte percentage in A and B groups are presented in this study.

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