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The Effects of Photoperiod Programs on Broiler Chicken Performance and Immune Response

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Abstract: Understanding the role of photoperiod regimens in regulating broiler performance and the immune response is becoming highly important. The objectives of the current investigation are to analyze the effects of photoperiod regimens on humoral and cell-mediated immune response as well as production characteristics in broiler chicken. A total number of 300 one day old male broiler chicks were divided randomly into three equal groups. For 6 weeks, the first group received continuous light (23L:1D) and the second and Third group received non-intermittent restricted light (12L:12D) and intermittent light (2L:2D) respectively. Intermittent light regimen induced activation of both peripheral T and B lymphocyte proliferation and energized antibody production significantly compared to the other two regimens. Furthermore, in the non-intermittent restricted light group, plasma Corticosterone concentration and H/L ratio were significantly higher compared to continuous and intermittent light groups. In addition, intermittent light treatment caused elevation of total white blood cells (WBC) and plasma T_3 concentration significantly compared to control. Moreover, intermittent light regimen reduced mortality rate 3 times and improved body weight by 10% compared to control. On the other hand, non-intermittent restricted light regimen had no effect on mortality rate, and suppressed body weight by 10% compared to control. In general, both intermittent and non-intermittent restricted light regimen improved feed conversion significantly compared to continuous light regimen. Even though the intermittent and non-intermittent restricted light regimens have the same period of darkness, the intermittent light regimen would be more beneficial than non-intermittent restricted light program.

Key words: Light restriction, broilers, performance, immunity

Introduction

For many years, broiler chickens have usually been reared under continuous or near continuous (23L:1D) photoperiods to maximize feed consumption and growth rate. However, several investigations showed that using continuous light programs induces sleep deprivation and causes sever physiological stress responses (Campo and Davila, 2002; Kliger *et al*., 2000). Therefore, Most of the recent researches have focused on restricting light regimens to improve productivity of broiler chickens because the physical activity is very low during darkness and energy expenditure of activity is considerable (Rahimi *et al*., 2005). Aperdoorn *et al.* (1999) reported that chickens under intermittent light (1L: 3D) had lower total heat production during the second and third hour of the dark period than the chickens under continuous light program, although the means of heat production and values at the first hour of the dark period in intermittent light chickens were almost equal to those of the chickens exposed to continuous light. Melatonin stimulates lymphocytes proliferation (Kliger *et al.,* 2000), antibody production (Ahmed Abbas *et al.,* 2007) and the Interleukin 2 (IL-2) and interferon-((IFN-() (Garcia-Maurino *et al.,* 1997). Recent approaches in understanding the mechanism of pineal gland regulation of immune function have focused on either photoperiod or day length. Light-dark stimuli provide adequate environmental information necessary for physiological and behavioral adaptive changes. Increasing the scoot-period in poultry light program was reported to have positive immunomodulatory effect. Kliger *et al.* (2000) reported that using an intermittent instead of a non-intermittent restricted light program can have an immune enhancing effect on broiler chickens. Moore and Siopes (2000) reported improved cellular and humoral immune responses for Japanese quail raised under decreasing photoperiods compared to quail raised under continuous light condition. The current investigation was conducted to address the effects of different photoperiod regimens on the immune responses pattern and production performance of broiler chickens.

Materials and Methods

Animals: One day old male broiler chicks (cobb×cobb) were used in the current study and were kept for 6 weeks. The chicks were housed on the floor, with food and water available *ad libitum.*

Experimental design: Three hundred broiler chicks were divided randomly into three equal groups. Within each

group, chicks were housed in 10 pens of 10 chicks in each pen. All groups received 24-hr lighting for the first 3 days. On the fourth day, the first group was exposed to continuous light (23L:1D) and served as control. The second group received a non-intermittent restricted light (12L:12D), while the third group received intermittent light (2L:2D) during the same period till the end of experiment. A brooding temperature of 33^{o}C was maintained for 3 days and then it was reduced to 30^{o}C for the rest of the first week. The temperature was decreased 3^{o}C a week until the third week.

Body weight, feed consumption and feed conversion: Individual body weight was determined at 0, 21 and 42 days old chicks and similarly, feed consumption was recorded for the corresponding periods. Feed conversion was calculated according to following formula: feed conversion = feed intake / body weight.

Mortality rate: Mortality was recorded daily and post mortem examination was conducted.

Blood samples: At 6 weeks old, blood samples were taken from the brachial vein with a syringe rinsed with a heparin solution. Half ml of the blood samples were used instantly to measure total leukocytes and Hetrophil to lymphocytes ratio, 2.5 ml each, were centrifuged to harvest the plasma, which was stored at 20^{o}C until assayed.

Total white blood cells (WBC) counts: Total W.B.C. was counted as described previously (Haddad and Mashaly, 1990). Briefly, 490µ / of brilliant cresly blue dye was mixed with 10µ / whole blood sample and total leukocytes were counted using a hemocytometer.

Hormonal assay: Corticosterone and thyroid hormones were measured using radioimmunoassay (R.I.A.) kits (Gehad *et al*., 2002).

Heterophil to lymphocytes ratio (H/L): One drop blood was smeared on the glass slide. The smears were fixed and stained using Hema-3 (Fisher Science). One hundred leukocytes were counted on one slide for each bird and heterophil to lymphocyte ratio was calculated.

Antibodies titer: At 5 weeks of age, 10 broiler chickens from each light treatment were injected with 0.2ml of 5% sheep red blood cells (S.R.B.C.). One week later, the blood samples were collected and the antibody titer against S.R.B.C. was determined using microhemagylutination technique (Kirby and Froman, 1991).

Cell mediated immune responses

Proliferation assay for T and B lymphocytes: Briefly, 10 blood samples were collected from each treatment and mixed 1:1 with wash medium, RPMI 1640 with L-glutamine supplemented with penicillin (100 units/ml) / streptomycin (100 µg/ml). The cell suspensions were then layered onto histopaque 1077 and were centrifuged at 220 x g for 30 min to separate the leukocytes. The white blood cell layer was removed and washed twice. Leulocytes were adjusted to 1 x 10^{7} viable cells/ml in RPMI 1640 with 10% heat-activated fetal bovine serum (gbs). By using trypan blue exclusion, cell viability was mined to be = 95% for suspension of white blood cells. Leukocytes were plated in triplicate culture at 5 x 10^{5} lymphocytes / well in 96-well, round-bottom plates. Each well contained leukocytes in 50 µL of medium. Fifty µL of either Concanavalin-A (Con-A) or Pokeweed mitogen (PWM) (50 µg/ml) was added to selected wells, while control wells received 50 µL of RPMI 1640 10% FBS. The cultures were incubated for 48 h at 42^{o}C in 5% CO_2. Following incubation, 50 µL of ^{3}H-thymidine (2 µci/well) was added to each well. Eighteen hours later, the cultures were harvested onto glass fiber filter paper. ^{3}H-thymidine uptake was measured as counts per min. (cpm) using a scintillation counter to determine T-cell proliferation. The net cpm was obtained by subtracting the mean cpm of the control wells from the mean cpm of its corresponding mitogen wells.

Statistical analysis: The general linear models procedure of S.A.S. software was used to analyze data with one-way analysis of variance (when the effect of photoperiod on production and physiological parameters was the main effect) (S.A.S. Institute, 1996). Means were separated using Duncan's multiple-range test with significance set at $P<0.05$.

Results and Discussion

Cell-mediated immune response: Exposure to intermittent light regimen significantly induced stimulation of both T-lymphocyte cell proliferation in response to con-A mitogen and B-lymphocyte cell proliferation in response to P.W.M. mitogen compared to the other light schedule groups (Fig. 1). On the other hand, a non-intermittent restricted light schedule had no significant effects on T-cell or B-cell proliferation compared to control. In the present study, intermittent lighting was found to enhance mitogen-lymphocyte proliferation at 6 wks of age, when compared to continuous light. These results are supported by Kliger *et al*. (2000), who reported that increasing the dark period stimulated mitogen-induced splenocyte proliferation. This could be due to enhanced melatonin secretion during dark period (Maestroni, 1998ab and Abbas *et al*., 2007). Kliger *et al*. (2000) found that melatonin *in vitro* enhanced peripheral blood and splenic lymphocyte proliferation after stimulation with Con A and P.W.M. Champney *et al*. (1997) suggested that melatonin can disproportionately alter the number of blood and splenic T and B-lymphocytes, or it can modify

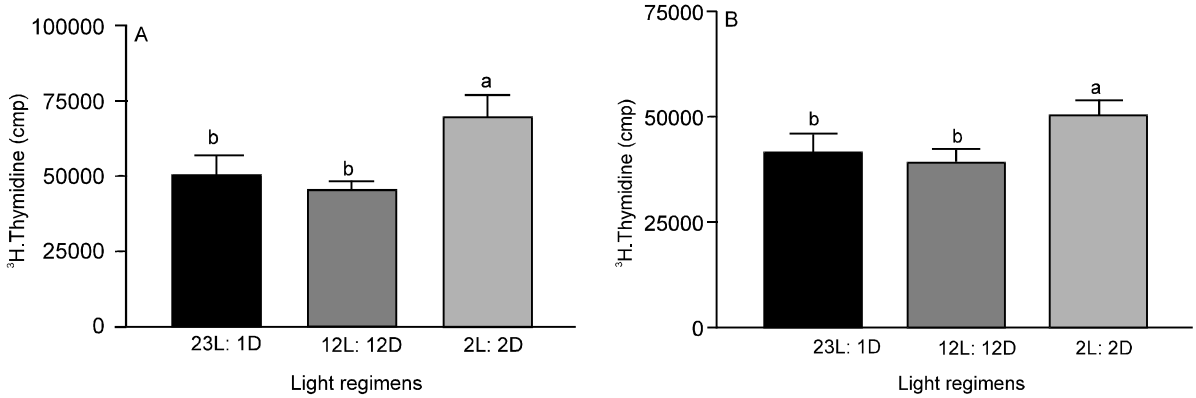

Fig. 1: The effects of light programs on Lymphocytes proliferation in response to (A) Con-A mitogen and (B) PWM mitogen in male broiler chickens at 6 weeks of age. Bars are means±SE. Bars with common letters are not significantly different ($p<0.05$) ($n = 10$)

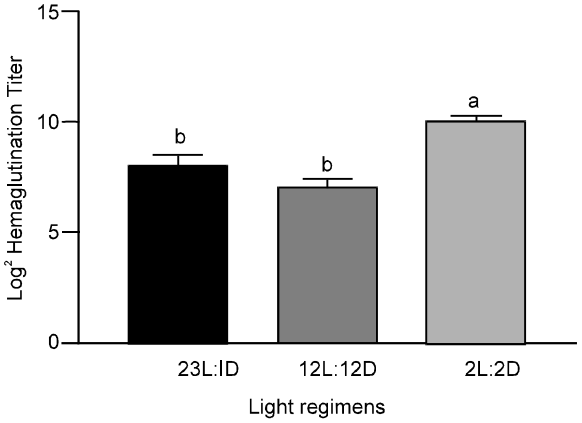

Fig. 2: The effect of light programs on antibody production against sheep red blood cells in male broiler chickens at 6 weeks of age. Bars are means±SE. Bars with common letters are not significantly different ($p<0.05$) ($n = 10$)

the intrinsic mitogen activity of each lymphocyte. Furthermore, melatonin can possibly increase lymphocyte proliferation by enhancing synthesis and secretion of IL-2 and IFN((Garcia-Mauriño *et al*., 1997) by activating T helper-1 cells (Maestroni, 1998b). Champney *et al*. (1998) found increased serum IFN(levels in Syrian hamsters after melatonin injection. Also, Maestroni *et al*. (1987) suggested that melatonin influences the immune response through opiatergic mechanisms. Another explanation is offered by Kuhlwein and Irwin (2001). They suggest that the increase in lymphocyte proliferation in response to the administration of physiological doses of melatonin can possibly be due to decreases in the production of the inhibitory cytokine IL-10.

Antibody titer and total white blood cells: The differences of the antibody titer in a non-intermittent restricted light group or continuous light group were not significant. However, the highest value of antibody titer was obtained significantly in the group that received intermittent light program compared to the other two groups that received a non-intermittent restricted light or continuous light program (Fig. 2). In addition, chickens exposed to a non-intermittent restricted light program had significantly lower total W.B.C. compared to intermittent light group or continuous light group. Furthermore, total W.B.C. was significantly higher in intermittent light group compared to control (Fig. 3). Intermittent light program, in the present study, enhanced antibody production and induced induction of total W.B.C. counts significantly compared to control. This could be due to increased melatonin levels in darkness as reported by Abbas *et al*. (2007). The effect of melatonin on immune function may be direct via melatonin receptors located on immune tissues, including W.B.C. (Calvo *et al*., 1995), or may be indirect by acting through other endocrine hormones, most notably thyroid hormone (Poon *et al.,* 1994). Furthermore, Mahmoud *et al*. (1994) reported that intermittent light program causes hypertrophy and increased cell ularity of the thymus. Moreover, Raghavendra *et al*. (2001) found that chronic administration of melatonin activates T helper-2 response. T helper-2 cytokines enhance B cell activation and increase the production of antibody (Kuby, 2000). The negative effects of a non-intermittent restricted light regimen on the immune response could be due to corticosterone. Our data showed that a non-intermittent restricted light treatment induced the elevation of plasma corticosterone (Fig. 4b). The mechanisms through which corticosterone suppresses immune activities could be due to initiating programmed lymphocyte death and inducing fragmentation of the cellular D.N.A. leading to cell death (Cohen and Duke, 1984). Trout *et al.* (1996) showed that both A.C.T.H. injection and heat stress

Table 1: The Effect of Different lighting programs on Economical Parameters in Broilers at 6 wks of age

	Lighting programs		
Production parameters	12L:1D	12L:12D	2L:2D
Body weight (Kg)	2.230±0.182[b]	2.014±0.131[c]	2.460±0.152[a]
Feed consumption (Kg)	4.386±0.227[a]	3.629±0.112[b]	4.243±0.160[a]
Feed conversion	1.967±0.256[a]	1.802±0.290[b]	1.725±0.312[c]
Mortality Rate	6/100	5/100	2/100

Values are means±SE. Values within each row with common letters are not significantly different (p<0.05) n=100

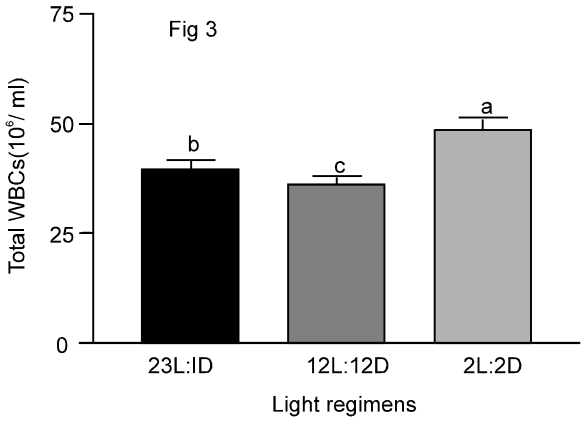

Fig. 3: The effect of light programs on total white blood cells in male broiler chickens at 6 weeks of age. Bars are means±SE. Bars with common letters are not significantly different (p<0.05) (n = 10)

caused significant decreases in the percentages of CD3, CD4 and CD8 lymphocytes in the blood. Indeed, experiments with mammals have indicated that both B and T lymphocytes are decreased in the circulation following glucocorticoid treatment (Fauci, 1975). Glucocorticoids have been reported to cause a re-distribution of lymphocytes from the circulation to the secondary lymphoid tissues (Fauci, 1975).

Heterophil to lymphocyte ratio (H/L): The results of the current study showed that a non-intermittent restricted light regimen induced elevation of H/L ratio, plasma corticosterone concentration, while intermittent light regimen had no significant effect on those parameters compared to control (Fig. 4). Siegel (1980) found that stress usually causes elevation of H/L ratio due to an induction of heterophil and reduction in lymphocytes. Therefore, H/L ratio can be used as a good indicator of stress. The higher H/L ratio, the more stress the birds are under (Kassab *et al*., 1992). Exposing broilers to 12 hr darkness in the a non-intermittent restricted light regimen seemed to be a stressful program causing elevation of corticosterone. McFarlane and Curtis (1989) reported that A.C.T.H. increases plasma corticosterone concentration. In general, H.P.A. axis involves perception in the brain with release of hypothalamic C.R.F. and vasopressin, which stimulates the anterior pituitary to secrete A.C.T.H. Circulating A.C.T.H. causes the adrenal cortex to produce glucocorticoids (Dohms and Metz, 1991). On the other hand, intermittent light regimen had no significant effect on H/L ratio and plasma corticosterone concentration. Renden *et al*. (1994) confirmed these results by reporting that corticosterone concentrations were the same at the continuous light (23L:1D) and intermittent light treatments (1L: 3D).

Thyroid: Both a non-intermittent restricted and intermittent light programs caused an increase in plasma T_3 compared to the continuous light group. The intermittent light group recorded the most significant and the highest value compared to control group (Fig. 5). This result could be due to both direct and indirect effects of melatonin on leptin hormone concentration. Abbas *et al.* (2007) showed that increasing dark period stimulates melatonin production. Furthermore, Mustonen *et al.* (2000) reported that melatonin increases the concentration of plasma leptin. In addition, Legradi *et al*. (1997) found that leptin raises the level of serum thyroxin (T_4) and prothyrotropin releasing hormone mR.N.A.

Body weight: At 3 weeks of age, there was no significant difference in the body weight between all treatments (data not shown). At 6 weeks of age, exposure to a non-intermittent restricted light reduced the body weight significantly compared to the other two light programs. Long dark period in the a non-intermittent restricted program, 12 hrs, could be a stress and a main factor inducing elevation in corticosterone level. Corticosterone is a key player in increasing pro-inflammatory cytokines. Johnson (1997) reported that pro-inflammatory cytokines, Il-1, Il-6 and TNF", inhibit growth by modulating the intermediary metabolism of carbohydrate, fat and protein substrates. Moreover, body weight was significantly heavier by an average of 230g/bird in the group that received the intermittent program compared to control. This result could be due to plasma T_3 concentration, which was significantly higher in the intermittent light group compared to the continuous light group. Even though intermittent light program (1L:3D) and non-intermittent restricted light

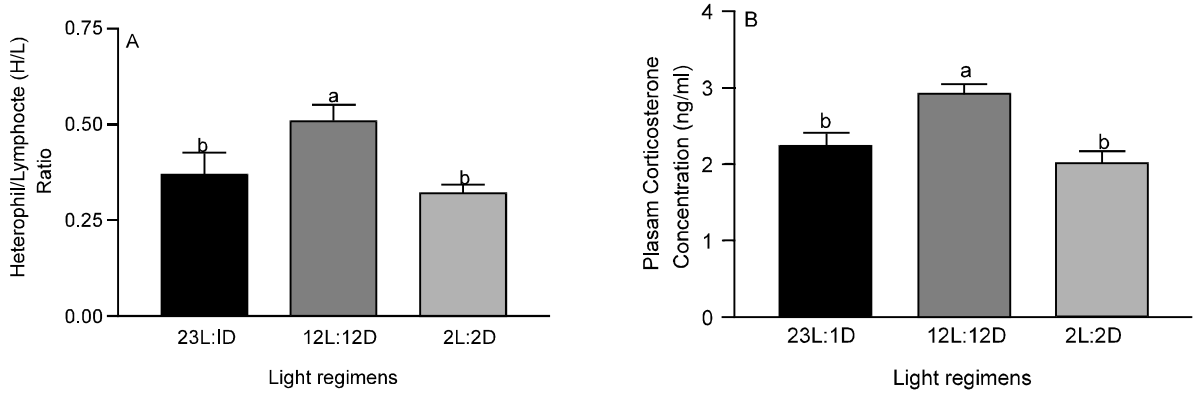

Fig. 4: The effect of light programs on (A) the H/L ratio in male broiler chickens and (B) the plasma corticosterone hormone concentration in broiler chickens at 6 weeks of age. Bars are means±SE. Bars with common letters are not significantly different ($p<0.05$) (n = 10)

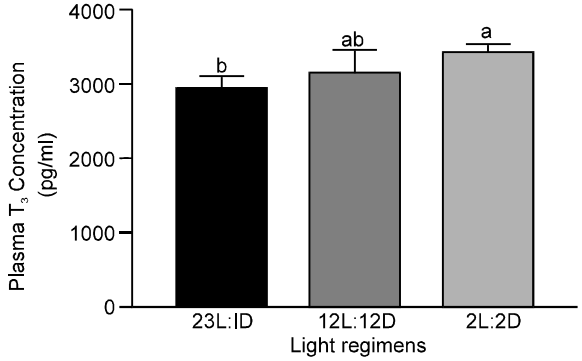

Fig. 5: The effect of light programs on the plasma thyroid hormone concentration in male broiler chickens at 6 weeks of age. Bars are means±SE. Bars with common letters are not significantly different ($p<0.05$) (n = 10)

program (12L:12D) have the same period of darkness, the body weigh was heavier of the chickens reared under intermittent light program. In the intermittent light program, the broilers eat satiation in the light period and then do not expand much energy during dark period, causing greater body weight gain (Ingram and Hatten, 2000).

Feed consumption and feed conversion: The lowest value of feed intake was obtained significantly in the a non-intermittent restricted light group compared to the continuous or intermittent light groups. In addition, there was no significant difference of feed consumption between the continuous light group and the intermittent light group. However, within all light treatments, the intermittent light group had the best feed conversion value followed by the a non-intermittent restricted light group. This result could be due to low physical activity and energy expenditure of the chickens raised under restricted light programs. The reduction of activity during darkness may result in lower heat production and higher feed efficiency (Rahimi *et al.,* 2005).

Mortality: Even though a non-intermittent restricted light had no significant impact on mortality rate compared to the continuous light group, the intermittent light regimen reduced mortality by 3 fold. This result may be due to the effect of the intermittent light program on slowing growth rate during early life. Rozenboim *et al*. (1999) and Gordon and Tucker (1995) reported that mortality rate and the incidence of sudden death Were significantly less in broilers receiving short or moderate photoperiod compared to broilers receiving a continuous light schedule. In addition, the data presented in the current investigation showed that the intermittent light program improved the immune performance by enhancing both humoral and cell-mediated response, which was a key factor in reducing mortality rate.

Conclusion: The results from the current investigation indicate that the intermittent photoperiod regimen enhances immune functions and the production performance of broiler chickens when compared with continuous or a non-intermittent restricted light regimens. These results point to the important role that photoperiod plays in affecting the immune response and production performance. However, a non-intermittent restricted light regimen seems to be a stressful program that induces elevation of H/L ratio and plasma Corticosterone concentration.

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