International Journal of Poultry Science 7 (2): 193-201, 2008
ISSN 1682-8356

The Role of Light Program and Melatonin on Alleviation of Inflammation Induced by Lipopolysaccharide Injection in Broiler Chickens

Ahmed E. Gehad[1], Gamal M. Mehaisen[2], Ahmed O. Abbas[2] and Magdi M. Mashaly[3,4]
[1]Department of Animal and Poultry Production, National Research Centre, Cairo, Egypt
[2]Department of Animal Production, Faculty of Agriculture, Cairo University, Egypt
[3]Department of Poultry Science, the Pennsylvania State University, University Park, PA, USA
[4]Kuwait Institute for Scientific Research, Safat, Kuwait, 13109

Abstract: Two experiments were conducted with male broiler chickens to study the effect of light program and melatonin injection on enhancing the immune response and reducing the inflammatory response induced by lipopolysaccharide (LPS) injection. In the first experiment, one-day-old broiler chicks were divided into two groups: The first group was exposed to continuous light (CL) (23L: 1D), whereas the other group was exposed to intermittent light (IL) (1L: 3D). At 6 wks of age, within each light program, chickens were injected intravenously (*iv.*) with either 3 mg/kg BW LPS, or with sterile saline. Blood samples were collected at 0, 3, 12 and 24 h post-injection. Total white blood cells (WBC), at 3, 12 and 24 h, were significantly higher in the IL-LPS group compared to the CL-LPS group. Body temperature and plasma corticosterone concentration at 3 and 12 h, were significantly lower in the IL-LPS group, compared to the CL-LPS group. Interleukin-1-like activity and IL-6 concentration were significantly lower in the IL-LPS group compared to the CL-LPS group at 3, 12 and 24 h. In the second experiment, 6 wk-old broiler chicks raised under CL, were divided into three groups. The first group was injected *i.v.* with 40 mg/kg BW melatonin, followed by 3 mg/Kg BW LPS 1 hour later. The second group was injected with saline followed by LPS 1 hour later. The third group received two saline injections 1 hour apart. Blood samples were collected at 0, 3, 12 and 24 h after the second injection. T lymphocyte proliferation were significantly higher in the melatonin-LPS group compared to the saline-LPS group. Plasma corticosterone concentration and body temperature were significantly lower in the melatonin-LPS group compared to the saline-LPS group at 3 h. IL-1-like activity and IL-6 concentration were significantly higher in the saline-LPS group compared to the other two groups at 3 and 12 h post injection. Total WBC was significantly higher in the melatonin-LPS group compared to the saline-LPS group at 12 and 24 hr. Our results indicate that intermittent light and melatonin injection can enhance the immune response and reduce the inflammation induced by LPS injection.

Key words: Light, melatonin, LPS, broilers

Introduction

Bacterial lipopolysaccharide (LPS, endotoxin) is an integral component of the cell wall of gram-negative bacteria, including *E. coli* and a number of other pathogens such as *Salmonella*, *Bordetella* and *Campylobacter*. LPS is also the major antigen of gram negative bacteria recognized by the immune system of higher organisms, many aspects of tissue injury caused by infection of gram-negative bacteria can be mimicked by LPS administration (Abbas *et al.,* 2000). The biological effects of LPS have been extensively studied in mammals (Berczi, 1998; Alexander and Rietschel, 2001) Evidence from the literature confirm that birds and mammals respond similarly to LPS (Xie *et al.,* 2000; Gehad *et al.,* 2002a,b; Wang *et al.,* 2003), although it was reported that tolerance to LPS is greater in birds than in mammals (Adler and DaMassa, 1979; Roeder *et al.,* 1989). Intravenous injection of LPS to chickens will induce a significant increase of acute phase proteins and proinflammatory cytokines levels in the circulation (Xie *et al.,* 2000; Gehad *et al.,* 2002a) and will alter the proportion of different lymphocyte subsets in the circulation (Gehad *et al.,* 2002b). Furthermore, LPS administration to chickens will either directly or indirectly activate the hypothalamo-pituitary-adrenal axis resulting in a significant increase in the level of corticosterone in the circulation (Gehad *et al.,* 2002a). Exposure of chickens to LPS will induce fever (Xie *et al.,* 2000), diarrhea, lethargy, and withdrawal from feed and water (Wang *et al.,* 2003) which will often lead to a deterioration in the production parameters. Melatonin is a hormone secreted in the dark from the pineal gland and sets the internal biological clock that governs different daily and seasonal cycles or rhythms in various physiological systems in birds (Pang *et al.,* 1996). Melatonin is a potent immunomodulator, that counteracts immunosuppression observed with ageing (Caroleo *et al.,* 1994) exposure to virus (Bonilla *et al.,* 2001) and acute stress (Bruck *et al.,* 2004). In japanese quail, pinealectomy significantly decreased humoral and

cellular immune responses, and melatonin administration to pinealectomised birds restored humoral and cellular immune responses to the levels of control birds (Moore *at al.,* 2002). The subcutaneous injection of melatonin to chickens enhanced mitogenic activity for T and B cells as well as increased total leukocyte counts (Brennan *et al.,* 2002). Increasing the dark period in light programs for poultry was shown to have positive immunomodulatory effect. Kliger *et al.* (2000) reported that using an intermittent instead of a constant light program can have an immune enhancing effect on broiler chickens. Moore and Siopes (2000) also reported improved cellular and humoral immune responses for japanese quail raised under decreasing photoperiods compared to quail raised under constant light condition. In mammals there is a plethora of literature on the roles of melatonin and photoperiod during bacterial LPS (endotoxin) infection (Nava *et al.,* 1997; Bilbo *et al.,* 2002; D'Emmanuele di Villa Bianca *et al.*, 2004; Carrillo-Vico *et al.,* 2005). However, their roles during LPS infection in poultry have not been evaluated. This study was undertaken to examine the effects of different light program (constant *vs.* intermittent) and exogenous melatonin on the immune response of broiler chickens following exposure to bacterial LPS from *E.coli*.

Materials and Methods

Experimental protocol: The study consisted of two experiments. In the first experiment, the effect of lighting program on the immune and febrile response of 6 wk-old male broiler chickens injected with either LPS from *E. coli* serotype (055:B5) (Sigma chemical Co., St. Louis, MO) or sterile saline was investigated. In the second experiment, the effect of exogenous melatonin injection on the immune and febrile response of 6 wk-old male broiler chickens injected with either LPS from *E. coli* serotype (055:B5) or sterile saline was investigated. Preliminary sets of experiments were conducted to determine the best dose of LPS to use in this study.

I- Experiment 1

Experimental design: One hundred and sixty one-day-old male broiler chicks (Cobb×Cobb) obtained from a local hatchery were used in this study. The chicks were housed in battery cages with *ad libitum* access to water and feed that met NRC (1994) recommendations. Chicks were randomly assigned into two groups. Both groups received 24-h light for the first three days. On the fourth day, one group was exposed to continuous light (23L:1D) (CL) and the other to intermittent light (1L:3D) (IL). At 6 wks of age, within each group, 15 chicks were injected intravenously in the brachial wing vein with 3 mg/Kg BW LPS in 0.5 ml volume of sterile saline, whereas the other 15 chicks were injected with 0.5 ml of sterile saline (0.9% NaCl).

II- Experiment 2

Experimental Design: One hundred and sixty one-day-old male broiler chicks (Cobb×Cobb) obtained from a local hatchery were used in this study. The chicks were housed in battery cages with *ad libitum* access to water and feed that met NRC (1994) recommendations. Chicks were raised under 24h light for the first three days, then CL thereafter. At 6 wks of age, 45 broiler chicks were randomly assigned into three groups. The first group was injected intravenously in the brachial wing vein with 40 mg/Kg BW melatonin in 0.5 ml ethanol, followed by 3 mg/Kg BW LPS injection in 0.5 ml of sterile saline after one hr. The second group was injected with 0.5 ml sterile saline, followed by 3 mg/Kg BW LPS injection in 0.5 ml of sterile saline one hr later. The third group received two 0.5 ml sterile saline injections one hr apart and used as a control for the first two groups.

Blood collection: Five ml. of heparinized blood were collected from the brachial vein at 0, 3, 12 and 24 h after the first injection in experiment 1. For experiment 2, the blood samples were collected at 0, 3, 12 and 24 h after the second injection. Three ml of blood were centrifuged to collect plasma. Plasma was divided into multiple aliquots and stored in -20^{o}C until assayed. Plasma was used for hormonal and cytokine assays. The remaining whole blood volume was used to asses total white blood cells in the circulation and T-lymphocyte proliferation in response to Concanavalin-A mitogen. T-lymphocyte proliferation was examined in both experiments at the 3 h time point only.

Measurements

Body temperature: Body temperature for birds in the different groups in both experiments were measured using a thermocouple rectal thermometer with a 3-cm insertion probe at 6 wk of age. Time of measurement was 3 h after the first injection in experiment 1 and 3 hr after the second injection in experiment 2.

Hormonal assay: Corticosterone was measured using radioimmunoassay (RIA) kits (Gehad *et al.*, 2002a).

Cytokine assays

IL-1 like activity in the plasma: IL-1-like activity in the plasma was measured using the thymocyte comitogensis assay as previously described by Korver and Klasing (1997) and Gehad *et al.* (2002a). This assay is based on measuring the ability of IL-1 to enhance thymocytes proliferation. Briefly, thymocytes, at a concentration of 40 x 10^6/ml live cells, were prepared using thymuses of 6 wk-old broiler chickens from a different hatch. Plasma samples were diluted 1:1 using 24% polyethylene glycol (PEG) to yield a total volume of 600 µL per tube. One hundred µL of diluted plasma samples were added to each well of a 96-well round

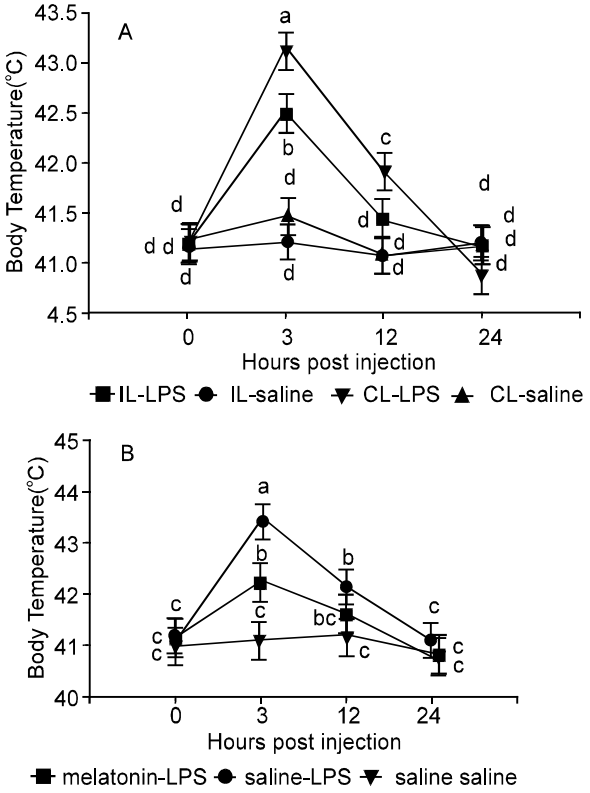

Fig. 1: The effect of light program (A) and melatonin (B) on the body temperature of 6 wk old broiler male chickens injected with LPS or saline. Values are means±SE. Values with common letters are not significantly different (p>0.05) (n=8).

bottom plate, then the samples were serially diluted until the concentration of PEG was 1.5% in the sample using RPMI 1640 medium plus 5% fetal bovine serum (FBS). Fifty µL of PHA-P (20 mg/ml) and another 50 µL of the thymocyte suspension were added to the wells. Control wells received 100 µL of RPMI 1640 5% FBS, 1% L-glutamine and 3% PEG instead of diluted plasma. The cells were incubated for 48 h at 41°C in 5% CO_2, then 50 µl of ^{3}H-thymidine was added to all the wells to give an activity of 1 µci/well. Cells were incubated for an additional 18 h, harvested onto glass fiber filters and ^{3}H-thymidine uptake was measured as counts per min. (cpm) using a scintillation counter .

Interleukin-1 activity was reported as a stimulation index, which is the ratio of the cpm of the thymocytes incubated with the plasma 3% PEG (IL-1 source) to the thymocytes incubated in the medium 3% PEG (no IL-1).

IL-6 Bioassay: The IL-6 levels of plasma samples were measured using an IL-6 dependent murine hybridoma 7TD1 cells (Shuster *et al.,* 1993). Plasma samples were diluted 1:10 with RPMI 1640 medium. One hundred µL of diluted plasma were added to 7TD1 cells (1x10^6 cells/100 µL/well), whereas control wells received 100 µL of RPMI 1640 5% FBS and L-glutamine. The culture plates were incubated for 44h at 37°C in 5% CO_2. Following incubation, 25 uL of MTT dye (4mg/ml in PBS), was added to all wells and the plates were incubated for an additional 4h. Following MTT incubation, 100 µL of medium were removed and another 100 µL of 2-propanol / 0.4N HCl were mixed into each well. The absorbance was determined at a wave length of at 550 nm, using an automated plate reader and software. The concentrations of IL-6 in plasma samples were calculated from a standard curve generated in the same assay using recombinant human IL-6.

Cellular immune responses

Total white blood cells: Four hundred and ninety µL of brilliant cresyl blue dye was mixed with 10 µL whole blood sample and the total leukocytes were counted using a hemocytometer slide.

Peripheral blood T-cells proliferation response to Concanavalin-A mitogen: Briefly, 100 µL whole blood was diluted thirty times with RPMI 1640 medium 10% FBS. One hundred µL of diluted blood was plated in triplicate wells in 96-well round-bottom plates. Fifty µL of Concanavalin-A (Con-A) (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°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 model (SAS Institute, 1996) was used to analyze data with three-way analysis of variance (repeated measures) with time after injection, light program and antigen as the main effects in the first experiment, while T-lymphocyte proliferation was analyzed with a two-way analysis of variance with light program and antigen as the main effects. In the second experiment, the same model was used to analyze data with three-way analysis of variance (repeated measures) with time after injection, melatonin, and antigen as the main effects, while T-lymphocyte proliferation analyzed with two-way analysis of variance with melatonin and antigen as the main effect. Means were separated using Duncan's multiple-range test with significance set at p<0.05.

Results

Body temperature: The change in body temperature over time (24h) following LPS and saline injections in 6 wk old male broiler chickens subjected to different light programs is shown in Fig. 1a. There were no significant differences between any of the groups at 0 and 24h post

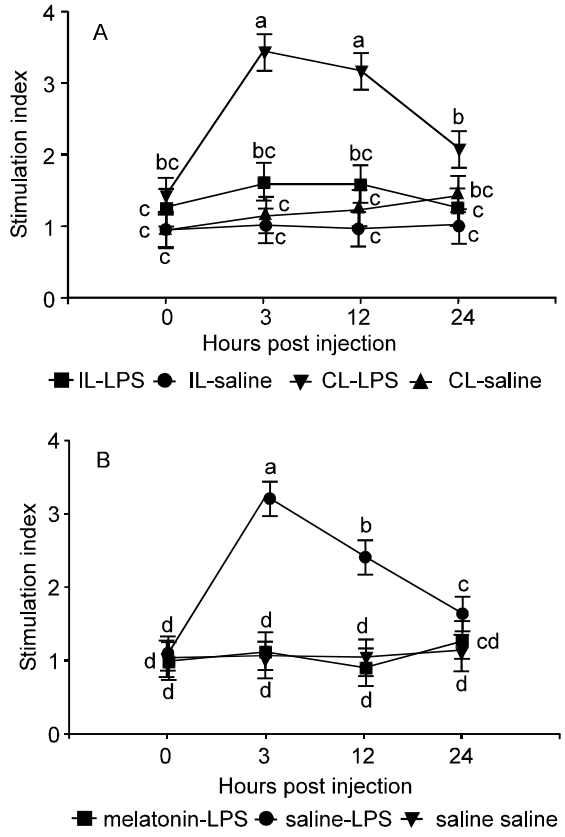

Fig. 2: The effect of light program (A) and melatonin (B) on the plasma levels of IL-1 like activity of 6 wk old broiler male chickens injected with LPS or saline. Values are means±SE. Values with common letters are not significantly different (p>0.05) (n=8).

injection. At 3h, body temperature was significantly higher in the LPS-injected groups compared to their controls. However, at the same time point body temperature was significantly lower in the IL-LPS group compared to CL-LPS group. At 12h, the CL-LPS group was still significantly higher than the other groups. The change in body temperature over time following LPS and melatonin injections in 6 wk old male broiler chickens is shown in Fig. 1b. At 0 and 24h post-injection, there were no significant differences in body temperature between any of the groups. However, at 3h, the body temperature of the LPS-groups was significantly higher than the control groups. Meanwhile, body temperature was significantly lower in the melatonin-LPS group compared to the saline-LPS group. Furthermore, at 12h, there were no significant differences in body temperature between the melatonin-LPS group and the control group or between melatonin-LPS group and the saline-LPS group. However, the body temperature was still significantly higher in the saline-LPS group compared to the control group.

Interleukin-1: The change in plasma IL-1-like activity overtime following LPS and saline injections in boiler chickens subjected to different light programs is shown in Fig. 2a. There were no significant differences between any of the groups at 0h. At 3 and 12h post-injection, there were no significant differences between any of the groups except for the CL-LPS group, which was significantly higher than the other groups. At 24h the CL-LPS was significantly different only from IL-LPS and IL-saline groups. The change in IL-1-like activity overtime following LPS and melatonin injections is shown in Fig. 2b. At 0h, there was no significant difference in IL-1-like activity between any of the groups. However, at 3 and 12h, IL-1-like activity was significantly higher in the saline-LPS group compared to the melatonin-LPS and control groups. At 24h, the differences between the saline-LPS group and the melatonin-LPS group were not significant, whereas IL-1 activity was still significantly higher in the saline-LPS group compared to the control.

Interleukin-6: The change in plasma IL-6 concentration overtime following LPS and saline injections in broiler chickens subjected to different light programs is shown in Fig. 3a. At 0h, there was no significant difference between any of the groups. However, at 3, 12 and 24h, IL-6 concentrations in the LPS groups were significantly higher compared to the saline groups. Interleukin-6 concentration was significantly lower in the IL-LPS group compared to the CL-LPS group from 3h up to 24h. The change in plasma IL-6 concentration overtime following LPS and melatonin injections is shown in Fig. 3b. At 0h, there was no significant difference between any of the groups. At 3, 12 and 24h, IL-6 concentrations in LPS groups were significantly higher compared to their controls. Interleukin-6 concentration was significantly lower in the melatonin-LPS group compared to the saline-LPS group at 3 and 12h, while there was no significant difference between the same two groups at 24h.

Total white blood cells: The change in total WBC count overtime following LPS and saline injections in broiler chickens subjected to different light programs in Fig. 4a. At 0h, there was no significant difference within the CL-groups or the IL-groups. However, total WBC count was numerically higher in IL-LPS group and was significantly higher in the saline-IL group compared to the CL-groups. At 3, 12 and 24h, within light treatment, LPS injection reduced total WBC count significantly compared to the saline-injected groups, except at 24h, where the differences were not significant within the IL-groups. In addition, within the LPS or saline groups, the total WBC count was always significantly higher in the IL-groups compared to the CL-groups at 3, 12 and 24h. The change in total WBC count overtime following LPS and melatonin injections is shown in Fig. 4b. There were

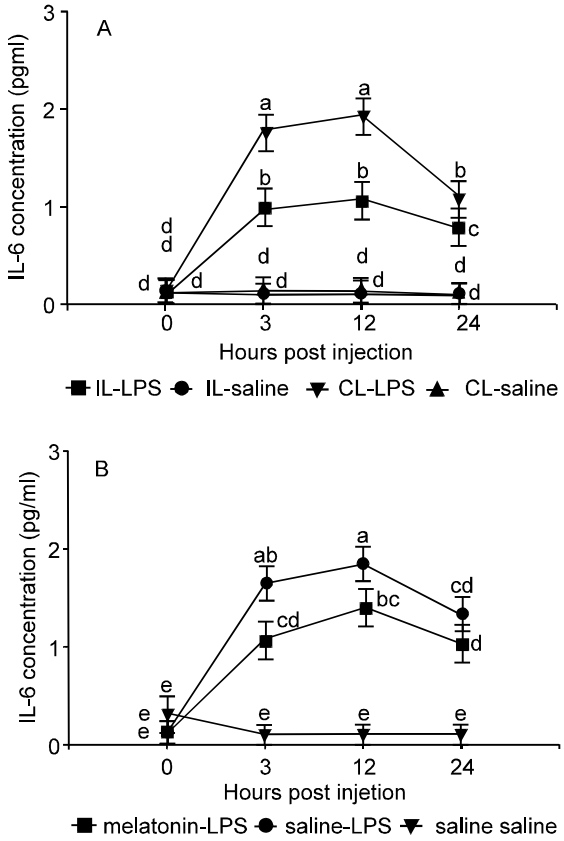

Fig. 3: The effect of light program (A) and melatonin (B) on the plasma levels of IL-6 of 6 wk old broiler male chickens injected with LPS or saline. Values are means±SE. Values with common letters are not significantly different (p>0.05) (n=8).

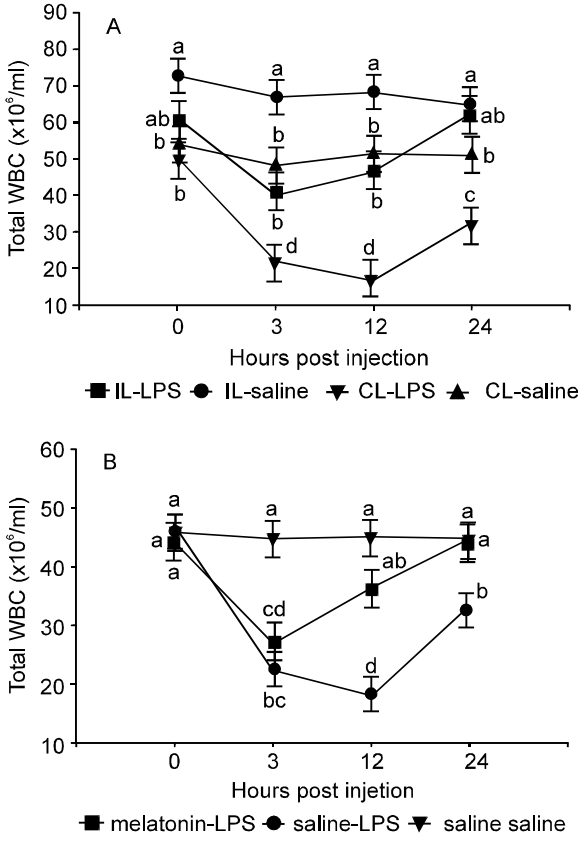

Fig. 4: The effect of light program (A) and melatonin (B) on the numbers of Total WBC in the circulation of 6 wk old broiler male chickens injected with LPS or saline. Values are means±SE. Values with common letters are not significantly different (p>0.05) (n=8).

no significant differences in total WBC count between any of the groups at 0 h. At 3h, there was no significant difference in total WBC count between melatonin-LPS group and saline-LPS group, but both were significantly lower compared to their control. At 12 and 24h, there were no significant differences between melatonin-LPS group and the control, but both of them were significantly higher than the saline-LPS group.

T-Lymphocyte Proliferation: The effect of LPS injection and different light programs on T lymphocyte proliferation is shown in Fig. 5a. Within each light treatment, LPS injection significantly reduced T lymphocyte proliferation compared to the saline-injected groups. T lymphocyte proliferation was numerically higher in the IL-LPS group compared to the CL-LPS group. In addition, within saline-injected groups, T lymphocyte proliferation was significantly higher in the IL group compared to the CL group. The effect of LPS and melatonin injections in male broiler chickens on T lymphocyte proliferation is shown in Fig. 5b. T lymphocyte proliferation was significantly lower in the LPS-injected groups compared to their control. However, T lymphocyte proliferation was significantly higher in the melatonin-LPS group compared to the saline-LPS group.

Plasma corticosterone concentration: The change in plasma corticosterone concentration overtime following LPS and saline injections in broiler chickens subjected to different light program is shown in Fig. 6a. At 0hr, there were no significant differences among the different groups. At 3, 12 and 24h, within light treatment, LPS injection significantly increased the corticosterone concentration compared to the saline injected groups. However, within the LPS-injected group, corticosterone concentration was significantly lower in the IL-group compared to the CL-group at 3 and 12h. The change in plasma corticosterone concentration overtime following LPS and melatonin injections is shown in Fig. 6b. At 0h,

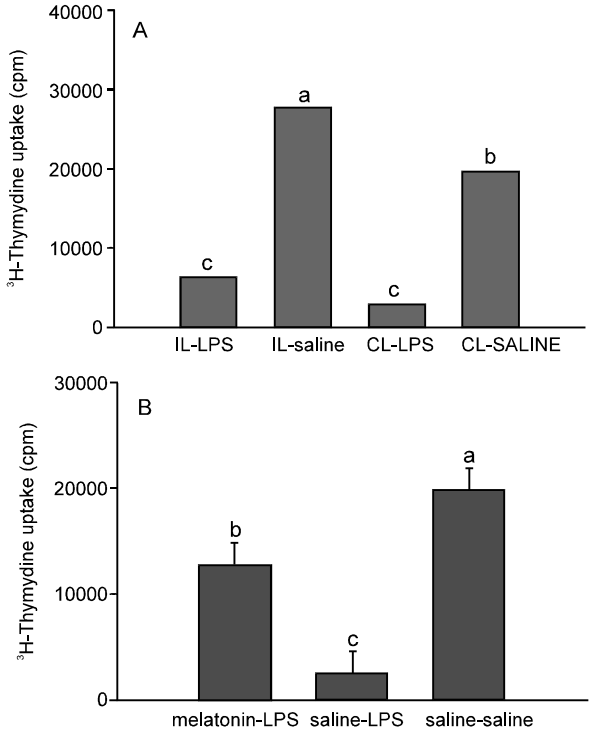

Fig. 5: The effect of light program (A) and melatonin (B) on the proliferation of peripheral blood T-cells in response to Con-A mitogen of 6 wk old broiler male chickens injected with LPS or saline. Bars are means±SE. Bars with common letters are not significantly different (p>0.05) (n=8).

there were no significant differences between any of the groups. At 3h, the corticosterone concentration was significantly lower in the melatonin-LPS group compared to the saline-LPS group and both of them were significantly higher than the control group. At 12 and 24h, there were no significant differences in corticosterone concentration within the LPS-injected groups; however, both groups had significantly higher corticosterone concentrations than the control group.

Discussion

Intravenous injection of *E.coli*. LPS induced a significant rise in body temperature as early as 3 h post injection in broiler chickens housed under different light programs or melatonin injected. Earlier Xie *et al.* (2000) found similar results upon the injection of LPS from *S. typhimurium* to broiler chickens. Fever following LPS exposure is triggered by the release of endogenous pyrogens from various immune cells. Endogenous mediators (pyrogens) of fever include cytokines among which IL-1 and IL-6 are considered the most important (Dinarello, 1997). Prostaglandin (PGE_2) is also considered a key mediator of fever acting downstream of IL-1 and IL-6 and responsible for the upward resetting of the set point of thermoregulation (Blatteis and Sehic, 1997). In this study, broiler chickens housed under IL or melatonin injected exhibited a significantly lower rise in body temperature 3h post LPS injection than either chickens housed in CL or not melatonin injected. The significantly lower levels of the endogenous pyrogens; IL-1 and IL-6 following LPS injection in IL and melatonin injected chickens than either chickens housed in CL or not melatonin injected can be behind this difference in the febrile response between the different groups. Intermittent light and melatonin can be also inducing this antipyretic response on another level. Bilbo *et al.* (2002) reported reduced expression of inducible cyclooxygenase (COX-2) in the brains of hamsters housed in short day lengths compared to hamsters housed in long day lengths following LPS injection. Inducible cyclooxygenase is a rate limiting enzyme in the synthesis of the key pyrogen PGE_2 (Blatteis and Sehic, 1997). Melatonin was suggested as the likely candidate for the reduction of central COX-2 expression in the hamster study. Day length is transduced into physiological signals by the duration of the nightly secretion of melatonin from the pineal gland (Reiter, 1991). Hamsters housed in short day environments or broilers in intermittent light as in this study, will experience longer durations of melatonin secretion than hamsters in long days or broilers in constant light. Melatonin was shown earlier to attenuate the LPS induced production of cytokines in rats (Nava *et al.,* 1997). Exogenous melatonin treatment also inhibits the production of COX-2 *in vivo* in animals with acute inflammation (Cuzzocrea *et al.,* 1999). Dampening the LPS induced febrile response through the application of IL programs or melatonin addition can help chickens recover faster from the state of lethargy and feed avoidance associated with LPS exposure and can in turn improve the production efficiency of those chickens. The *i.v.* injection of LPS significantly raised the levels of IL-1 and IL-6 in the circulation. Gehad *et al.* (2002a) and Xie *et al.* (2000) reported similar results following LPS injection to chickens. However, chickens housed under IL or melatonin injected had significantly lower levels of both IL-1 and 6 in the periphery following LPS injection than either chickens housed under CL or not melatonin injected respectively. In hamsters housed under short day conditions IL-1 and IL-6 concentration in the periphery were significantly lower than their levels in long day hamsters following LPS injection (Bilbo *et al.,* 2002). In rats, melatonin administration before or following LPS injection significantly decreased the peripheral concentrations of IL-1 and IL-6 compared to rats that did not receive melatonin (Nava *et al.,* 1997). The mechanisms underlying this anti-inflammatory effect for melatonin are still being discovered. However, Li *et al.* (2005) reported that the nuclear translocation of the nuclear factor - kappa $, a transcription factor that

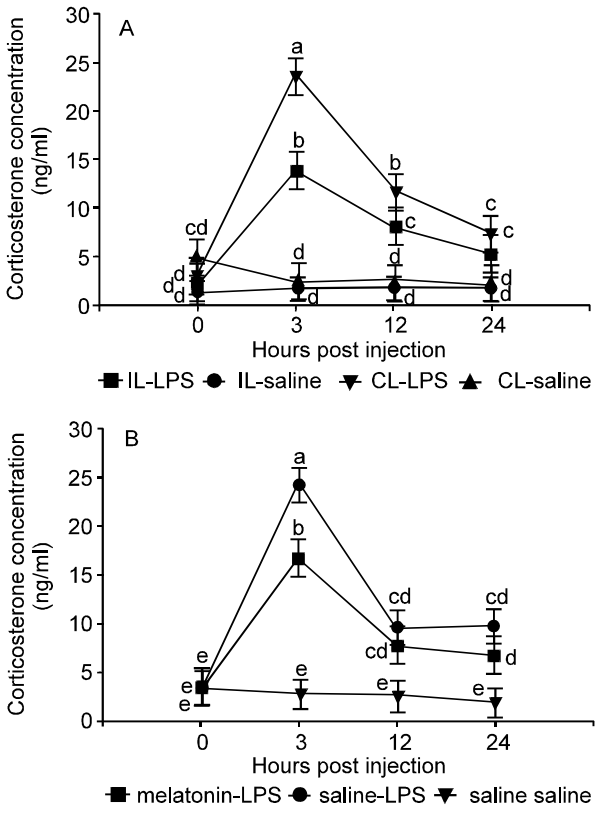

Fig. 6: The effect of light program (A) and melatonin (B) on the plasma corticosterone levels of 6 wk old broiler male chickens injected with LPS or saline. Values are means±SE. Values with common letters are not significantly different ($p>0.05$) ($n=5$).

regulates the transcription of a wide number of genes involved in immune and inflammatory responses was inhibited by melatonin. Proinflammatory cytokines such as IL-1, IL-6 and TNF are the principal mediators of the sickness behavior associated with LPS exposure (Abbas *et al.,* 2000). Therefore, the blunted inflammatory response of broiler chickens housed under IL or melatonin injected may represent an adaptation advantage for those birds against infection with LPS from gram negative bacteria. Total white blood cell (WBC) counts decreased significantly following LPS injection. Earlier we have found that WBC decreased significantly in the circulation following *B. abortus* antigen that also contains LPS (Trout *et al.,* 1988). Gehad *et al.* (2002b) also found that the percentage different subtypes of T-cells decreased significantly post LPS injection in chickens. However, chickens housed under IL or injected with melatonin experienced a lesser decrease in WBC counts following LPS injection and a faster recovery to baseline counts compared CL or no melatonin chickens, respectively. In those two groups (CL and no melatonin) there was a significantly higher corticosterone response following LPS injection compared to the IL and melatonin injectd groups, respectively. Glucocorticoids will induce the apoptosis of T-cells (Kirsch *et al.,* 1999). Melatonin treatment has been shown to attenuate adrenocortical secretory responses to acute and chronic stress (Konakchieva *et al.,* 1997). Furthermore, melatonin was also shown to prevent apoptosis in the spleens of mice undergoing septic shock (Carillo-Vico *et al.,* 2005). This antiapoptotic effect of melatonin is probably mediated through its ability to reduce mitochondrial reactive oxygen species generation (Jou *et al.,* 2004). At 3h post LPS injection, T-cell proliferation *in vitro* in response to Concanavalin-A was significantly inhibited in broilers regardless of light program or melatonin injection. Levels of the T-cell growth factor; IL-2 was significantly inhibited 3h post injection with LPS in chickens (Gehad *et al.,* 2002a). This can be also due to the effect of corticosterone, significantly high levels of corticosterone were observed in the circulation of the different groups following LPS injection. Corticosterone was found to inhibit the *in vitro* mitogenic proliferation of T-cells in chickens (Trout and Mashaly, 1995). In chickens injected with LPS, melatonin injection significantly increased T-cell proliferation but not to the level of the control chickens. Brennan *et al.* (2002) reported enhanced mitogenic activity for T-cells following the subcutaneous injection of melatonin to chickens. Melatonin activates the production of IL-2 and IFN-gamma by T helper cells and monocytes (Gracia-Maurino *et al.,* 1997) as well as counteracts the inhibitory effect of prostaglandin ($PG)E_2$ on IL-2 production in human lymphocytes (Carillo-Vico *et al.,* 2003). Interestingly, birds under IL with no LPS injected where presumably the melatonin exposure frequency was the highest, had the highest proliferation of T-cells. However, when LPS was injected to IL birds T-cell proliferation was significantly inhibited to the levels of CL birds. This raises an important question regarding the frequency of exposure versus concentration of melatonin in modulating different immune responses.

In summary, the application of an intermittent light program and melatonin injection to broiler chickens were successful in alleviating the immunosuppression associated with LPS (endotoxin) injection. Both methods can be used to redress the sickness behavior associated with LPS infection, which will ultimately reflect on better broiler performance. However, intermittent light programs can be even more attracting considering they will not only enhance immune functions but also reduce the overall production costs by decreasing funds allocated to illumination. To our knowledge this is the first report that either intermittent light or melatonin were used to alleviate the immunosuppression associated with infection with LPS in broiler chickens.

References

Abbas, A.K., A.H. Lichtman and J.S. Pober, 2000. Physiologic and pathologic responses to bacterial lipopolysaccharide. Cellular and Molecular Immunology. 4th Edn. W.B. Saunders, Philadelphia, page 227.

Adler, H.E. and A.J. DaMassa, 1979. Toxicity of endotoxin to chicks. Avian Dis., 23: 174-178.

Alexander, C. and E.T. Rietschel, 2001. Bacterial lipopolysaccharides and innate immunity. J. Endotoxin Res., 7: 167-202.

Berczi, I., 1998. Neurohormonal host defense in endotoxin shock. Ann. NY. Acad. Sci., 840: 787-802.

Bilbo, S.D., D.L. Drazen1, N. Quan, L. He and R.J. Nelson, 2002. Short day lengths attenuate the symptoms of infection in Siberian hamsters. Proc. R. Soc. Lond. Ser. B. Biol. Sci., 269: 447-454.

Blatteis, C.M. and E. Sehic, 1997. Circulating pyrogen signaling of the brain. A new working hypothesis. Ann. NY Acad. Sci., 15: 445-447.

Bonilla, E., C. Rodon, N. Valero, H. Pons, L. Chacin-Bonilla, J. Garcia Tamayo, Z. Rodriguez, S. Medina-Leendertz and F. Anez, 2001. Melatonin prolongs survival of immunodepressed mice infected with the Venezuelan equine encephalomyelitis virus. Trans. R. Soc. Trop. Med. Hyg., 95: 207-210.

Brennan, C.P., G.L. Hendricks III, T.M. El-Sheikh and M.M. Mashaly, 2002. Melatonin and the enhancement of immune responses in immature male chickens. Poult. Sci., 81: 371-375.

Bruck, R., H. Aeed, Y. Avni, H. Shirin, Z. Matas, M. Shahmurov, I. Avinoach, G. Zozulya, N. Weizman and A. Hochman, 2004. Melatonin inhibits nuclear factor kappa B activation and oxidative stress and protects against thioacetamide induced liver damage in rats. J. Hepatology., 40: 86-93.

Carillo-Vico, A., S. Garcia-Maurino, J.R. Calvo and J.M. Guerrero, 2003. Melatonin counteracts the inhibitory effect of PGE2 on IL-2 production in human lymphocytes via its mt1 membrane receptor. FASEB J., 17: 755-777.

Carillo-Vico, A., P.J. Lardone, L. Naji, J.M. Fernandez-Santos, I. MartinLacave, J.M. Guerrero and J.R. Calvo, 2005. Beneficial pleiotropic actions of melatonin in an experimental model of septic shock in mice: Regulation of pro-/anti-inflammatory cytokine network, protection against oxidative damage and anti-apoptotic effects J. Pineal Res., 39: 400-408.

Caroleo, M.C., G. Doria and G. Nistico, 1994. Melatonin restores immunodepression in aged and cyclophosphamide-treated mice. Ann. NY. Acad. Sci., 31,719: 343-352.

Cuzzocrea, S., G. Costantino, E. Mazzon and A.P. Caputi, 1999. Regulation of prostaglandin production in carrageenan-induced pleurisy by melatonin. J. Pineal Res., 27: 9-14.

D'Emmanuele di Villa Bianca, R., S. Marzocco, R. Di-Paola, G. Autore, A. Pinto, S. Cuzzocrea and R. Sorrentino, 2004. Melatonin prevents lipopolysaccharide - induced hyporeactivity in rat. J. Pineal. Res., 36: 146-154.

Dinarello, C.A., 1997. Proinflammatory and anti-inflammatory cytokines as mediators in the pathogenesis of septic shock. Chest., 112 (6 Suppl): 321S-329S.

Garcia-Maurino, S., M.G. Gonzalez-Haba, J.R. Calvo, M.R. El-Idrissi, V. Sanchez-Margalet, R. Goberna and J.M. Guerrero, 1997. Melatonin enhances IL-2, IL-6 and INF-gamma production by human circulating CD4+ cells. J. Immunol., 159: 574-581.

Gehad, A.E., H.S. Lillehoj, G.L. Hendricks III and M.M. Mashaly, 2002a. Initiation of humoral immunity. I. The role of cytokines and hormones in the initiation of humoral immunity using T-independent and T-dependent antigens. Dev. Comp. Immun., 26: 751-759.

Gehad, A.E., H.S. Lillehoj, G.L. Hendricks III and M.M. Mashaly, 2002b. Initiation of humoral immunity.II. The effects of T-independent and T-dependent antigens on the distribution of lymphocyte populations. Dev. Comp. Immun., 26: 761-771.

Jou, M.J., T.I. Peng, R.J. Reiter, S.B. Jou, H.Y. Wu and S.T. Wen, 2004. Visualization of the antioxidative effects of melatonin at the mitochondrial level during oxidative stress-induced apoptosis of rat brain astrocytes. J. Pineal Res., 37: 55-70.

Kirsch, A.H., A.A. Mahmood, J. Enders, L. Bohra, B. Bonish, K. Weber and D.A. Fox, 1999. Apoptosis of human T-cells: Induction by glucocorticoids or surface receptor ligation in vitro and ex vivo. J. Biol. Reg. Homeost. Agents., 13: 80-89.

Kliger, C.A., A.E. Gehad, R.M. Hulet, W.B. Roush, H.S. Lillehoj and M.M. Mashaly, 2000. Effect of photoperiod and melatonin on lymphocyte activities in male broiler chickens. Poult. Sci., 79: 18-25.

Konakchieva, R., Y. Mitev, O.F. Almeida and V.K. Patchev, 1997. Chronic melatonin treatment and the hypothalamo - pituitary - adrenal axis in the rat: Attenuation of the secretory response to stress and effects on hypothalamic neuropeptide content and release. Biol. Cell., 89: 587-596.

Korver, D.R. and K.C. Klasing, 1997. Dietary fish oil alters specific and inflammatory immune responses in chicks. J. Nutr., 127: 2039-2046.

Li, J.H., J.P. Yu., H.G. Yu, X.M. Xu, L.L. Yu, J. Liu and H.S. Luo, 2005. Melatonin reduces inflammatory injury through inhibiting NF-kappaB activation in rats with colitis. Mediators of Inflamm., 31: 185-193.

Moore, C.B. and T.D. Siopes, 2000. Effects of light conditions and melatonin supplementation on the cellular and humoral immune responses in Japanese quail (Coturnix coturnix japonica). Gen. Comp. Endocrinol., 119: 95-104.

Moore, C.B., T.D. Siopes, C.T. Steele and H. Underwood, 2002. Pineal melatonin secretion, but not ocular melatonin secretion, is sufficient to maintain normal immune responses in Japanese quail (*Coturnix coturnix* japonica). Gen. Comp. Endocrinol., 126: 352-358.

National Research Council, 1994. Nutrient Requirements of Poultry. 9th Rev. Ed. National Academy Press, Washington, DC.

Nava, F., G. Calapai, G. Facciola, S. Cuzzocrea, G. Giuliani, A. DeSarro and A.P. Captui, 1997. Melatonin effects on inhibition of thirst and fever induced by lipopolysaccharide in rat. Eur. J. Pharmacol., 331: 267-274.

Pang, S.F., C.S. Pang, A.M.S. Poon, Q. Wan, Y. Song and G.M. Brown, 1996. An overview of melatonin and melatonin receptors in birds. Poult. Avian Biol. Rev., 7: 217-228.

Reiter, R.J., 1991. Pineal melatonin: Cell biology of its synthesis and of its physiological interactions. Endocr. Rev., 12: 151-180.

Roeder, D.J., M.G. Lei and D.C. Morrison, 1989. Endotoxic lipopolysaccharide- specific binding proteins on lymphoid cells of various animal species: Association with endotoxin susceptibility. Infect. Immun., 57: 1054-1058.

SAS Institute, 1996. SAS User's Guide: Statistics Version 6 Edition. SAS Institute, Inc. Cary, NC.

Shuster, D.E., M.E. Kehrli Jr. and M.G. Stevens, 1993. Cytokine production during endotoxin-induced mastitis in lactating dairy cows. Am. J. Vet. Res., 54: 80-85.

Trout, J.M. and M.M. Mashaly, 1995. Effects of in vitro corticosterone on chicken T- and B-lymphocyte proliferation. Br. Poult. Sci., 36: 813-820.

Trout, J.M., M.M. Mashaly and H.S. Siegel, 1988. Changes in the profiles of circulating white blood cells, corticosterone, T3 and T4 during the initiation of humoral immunity in immature male chickens. Dev. Comp. Immunol., 12: 331-346.

Wang, W., R.F. Wideman, Jr., M.E. Chapman, T.K. Bersi and G.F. Erf, 2003. Effect of Intravenous Endotoxin on Blood Cell Profiles of Broilers housed in Cages and Floor Litter Environments. Poult. Sci., 81: 1224-1230.

Xie, H., N.C. Rath., G.R. Huff, W.E. Huff and J.M. Balog, 2000. Effects of Salmonella typhimurium lipopolysaccharide on broiler chickens. Poult. Sci., 79: 33-40.