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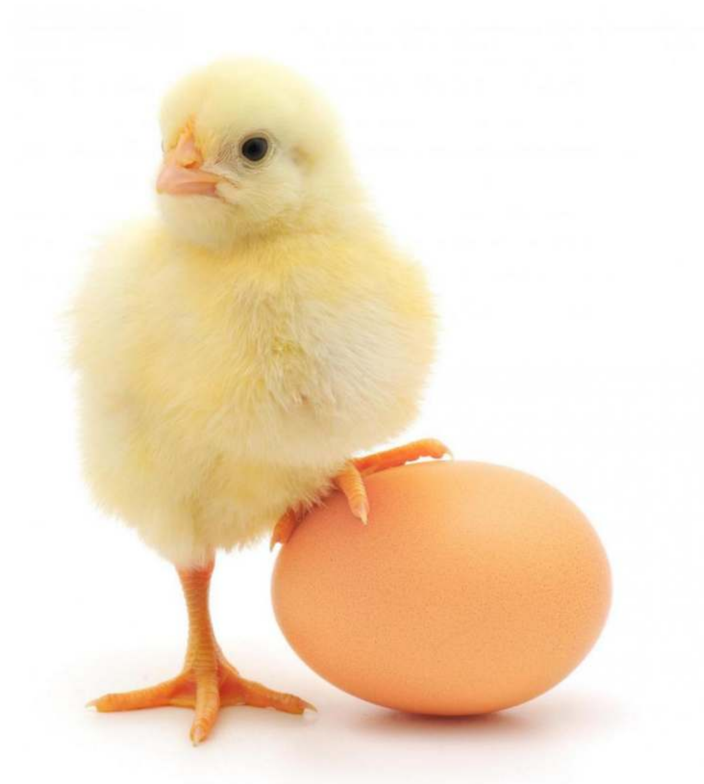

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The Role of Photoperiod and Melatonin on Alleviation of the Negative Impact of Heat Stress on Broilers

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Abstract: Two experiments were conducted to test that broilers, reared under intermittent light program or fed a diet containing melatonin, would dampen the deleterious effects of heat stress on their performance and immune response. In the first experiment, two groups of one-day-old male broiler chicks received 23h Light: 1h Dark (CL), while another two groups received intermittent light (IL) (1hL: 3hD) programs. From 4-6 weeks of age, a group from each lighting program was exposed to heat stress (35°C). The other two groups were kept under 24°C. At 35°C, birds under IL program had 5 times less mortality and 41% higher body weight at six weeks of age compared to the CL birds. Moreover, heat-stressed birds under IL program had 11% better feed conversion and higher T_3 concentrations and antibody production, compared to the CL group. In the second experiment, two groups of one-day-old male broiler chicks were fed a diet containing 40 ppm melatonin (MEL) while another two groups received a MEL-free diet, from 4-6 weeks of age. Concurrently, a group from each MEL treatment was reared under 35°C. The other two groups were kept under 24°C. Under 35°C, birds receiving MEL had less mortality, better feed conversion and higher antibody production, compared to the MEL-free diet group. There were no significant differences in body weight, feed consumption and T_3 concentrations between these two groups. These results indicate that IL program may be used effectively to improve broiler's ability to alleviate the negative effects of heat stress more than MEL administration does.

Key words: Light restriction, melatonin, heat stress, broilers performance, immunity

Introduction

The negative effects of heat stress on broiler performance at the economical and physiological levels are well documented. Cooper and Washburn (1998) observed that high ambient temperature (32°C) reduced the growth of broilers in the last three weeks and body weight at marketing age. In addition, Yalcin *et al.* (1997) and Yahav and Plavnik (1999) reported a decrease in body weight and feed consumption of broilers reared under high temperature (32°C), from 3-6 weeks of age, when compared to counterparts reared under optimum temperature (19°C). Heat stress is associated with high mortality rate (Naughton *et al.*, 2002) and low plasma triiodothyronine (T_3) hormone concentrations (Yahav and McMurty, 2001). In addition, heat stress increase heterophil/lymphocyte (H/L) ratio (Gharib *et al.*, 2005) and suppresses humoral immune response (Trout *et al.*, 1996; Gharib *et al.*, 2005).

Lighting is a powerful exogenous factor in control of many physiological and behavioral processes (Olanrewaju *et al.*, 2006). The change from continuous light (CL) to intermittent light (IL) at a young age initially results in a depression of growth (Buyse *et al.*, 1996; Classen *et al.*, 2004; Rahimi *et al.*, 2005; Bölükbasi and Emsen, 2006). However, the final body weight of broilers raised under IL is similar to, or even higher than, that of broilers reared in CL schedule. It is obvious that the initial reduction in body weight of birds under IL conditions is followed by compensatory growth. Significant improvement in feed conversion (Rahimi *et al.*, 2005; Bölükbasi and Emsen, 2006) and lower mortality (Gordon and Tucker, 1995; Rozenboim *et al.*, 1999) has also been recorded in broilers reared under IL schedule compared to birds receiving long photoperiod (23hL: 1hD).

Kuhn *et al.* (1996) observed that thyroid hormones concentrations in broilers were not affected by lighting schedule from 1 to 6 weeks of age. Extending the dark period increased total white blood cells and decreased H/L ratio (Moore and Siopes, 2000). In recent years, researchers have found an association between the duration of environmental photoperiod and some immune parameters. Most of this research has indicated that short photoperiod enhances both cellular and humoral immune response (Moore and Siopes, 2000; Kliger *et al.*, 2000; Raghavendra *et al.*, 2000).

Melatonin is a hormone released from the pineal gland, during the dark hours of the day, which is involved in establishing circadian rhythms of body temperature, several essential metabolic functions that influence feed/water intake patterns, digestion and secretion of several lymphokines that are integral to normal immune function (Aperdoorn *et al.*, 1999). It was reported that, MEL addition to the diet depressed feed intake and improved feed conversion (Phetteplace and Nockels, 1985), decrease the occurrence of sudden death syndrome and mortality (Clark and Classen, 1995). Clark and Classen (1995) reported that there was a linear decrease in weight gain for the first two weeks of age due to increased levels of MEL in feed. After 14 days, this effect was no longer obvious. Moreover, MEL addition to the diet was reported to ameliorate the detrimental effects of heat stress on performance traits in Japanese quail (Sahin *et al.*, 2003). The effects of MEL on thyroid function are contradictory. Melatonin administration *in vivo* inhibited thyroid cell proliferation (Wright *et al.*, 2000) and decreased T_3 concentrations (Ozturk *et al.*, 2000). While, Mustonen *et al.* (2000) showed that thyroxin concentrations increased with MEL administration. Melatonin administration increased white blood cells count in the Japanese quail (Moore and Siopes, 2000), enhanced chicken splenic lymphocyte proliferation (Kliger *et al.*, 2000) and murine antibody production (Maestroni *et al.*, 1987). Also, MEL administration reduced H/L ratio in the Japanese quail (Moore and Siopes, 2000) and in broiler chicken (Brennan *et al.*, 2002).

The effect of intermittent light and melatonin administration under stressful conditions is not well investigated. Therefore, the following study was conducted to investigate the possibility of enhancing broiler performance and immune response under heat stress by using intermittent light regimen and melatonin supplementation.

Materials and Methods

Experimental protocol: This study consisted of two experiments carried out at the Poultry Education Center of the Pennsylvania State University. Two different lighting programs (first experiment) or melatonin addition to the diet (second experiment) was tested to alleviate the detrimental effects of heat stress on broiler performance and immune response. Both experiments were conducted using male broiler chicks.

Experiment 1

Experimental design: Three hundred and twenty one-day-old male broiler chicks (Cobb × Cobb) were randomly divided into four equal groups and raised in four environmental chambers. Chicks were housed in broiler cages with *ad libitum* access to water and feed that met National Research Council (NRC, 1994) recommendations. In each group, 80 chicks were housed in 8 cages. All groups received 24 h lighting for the first three days. On the fourth day, the first and second groups were exposed to nearly continuous light (CL) (23hL: 1hD) versus intermittent light (IL) (1hL: 3hD) for the third and fourth groups. A brooding temperature of 33°C was maintained for the first 3 days then decreased to 30°C for the rest of the first week. The temperature was reduced 2°C a week until the third week of age. From the beginning of the 4th to the end of the 6th weeks of age, the second and fourth groups were exposed to 35°C versus 24°C in the first and third groups. Relative humidity was set at 50% in the 4 chambers.

Experiment 2

Experimental design: One hundred and sixty (one-day-old) male broiler chicks were divided randomly into four groups and raised in four environmental chambers. Chicks were housed in broiler cages with *ad libitum* access to water and feed that met NRC (1994) recommendations. In each group, 40 chicks were housed in 4 cages. All groups received 24 h light for the first 3 days, then 23-h light and one-hr dark until the end of experiment. The brooding temperature was 33°C for the first three days and then it was decreased to 30°C for the rest of the first week. The temperature was reduced 2°C per week. At the beginning of the 4th to the end of the 6th weeks of age, the third and fourth groups were exposed to 35°C versus 24°C for the first and second groups. Relative humidity was set at 50% in the four chambers. The second and fourth groups received 40 ppm MEL in the feed versus 0 ppm MEL for the first and third groups.

Measurements

Production parameters: Individual body weights were obtained on days 21 and 42 of age. Feed intake, on cage basis, was recorded for the corresponding periods and feed conversion was calculated by dividing feed consumption over total body weight gain. The total body weights included dead birds. Mortality was recorded daily and post mortem examination was conducted.

Physiological parameters

Triiodothyronine (T_3): At 6 weeks of age, blood samples were collected, from both experiments and plasma separation was conducted to measure T_3 concentrations. Radioimmunoassay (RIA) kits (ICN Biomedical, Costa Mesa, CA) were used to measure the T_3 hormone.

Heterophil/Lymphocyte (H/L) ratio: Generally, Heterophil/Lymphocyte (H/L) ratio increases by stress and can be used as an indicator of stress (Hester *et al.*, 1996; Gharib *et al.*, 2005). To measure H/L ratio, 8 blood smears, from each treatment group, were prepared and stained using Hema-3 (Fisher Science). A total of 100 white cells were counted and heterophil/lymphocyte ratios were calculated (Gross and Siegel, 1983).

Antibodies against sheep red blood cells (SRBC): At 5 weeks of age, 8 broilers, from each treatment group, were injected, intramuscularly, with 0.2 mL of 5% SRBC suspension prepared in 0.9% physiological saline. One week following the injection, the antibody production against SRBC was measured using micro hemagglutination technique (van der Zijpp and Leenstra, 1980). Blood samples were collected from each treatment group and sera were separated and inactivated at 56°C for 30 min. Using 96-well plates, 50 µL of 0.9% saline was added to all wells, followed with 25 µL of the sera samples. Serial dilutions were made from the first through the eleventh well, while the twelfth well was kept as the control. Next, 50 µL of 5% SRBC suspension was added to each well. The wells were mixed and incubated at 42°C for 45 min. Antibody values were expressed as log2 of the reciprocal dilution where the last agglutination was observed.

Statistical analysis: The general liner model procedure (SAS Institute, 1999) was used to analyze data with two-way analysis of variance with temperature and photoperiod as the main effects in the first experiment and temperature and MEL administration as the main effects in the second experiment. When differences among means were found, means were separated using Duncan's multiple-range test with significance set at $p<0.05$ (Steel and Torrie, 1980).

Results

Experiment 1

Mortality: The effects of lighting programs and heat stress on mortality rates are shown in Table 1. During the first 3 weeks, before heat exposure, there were no differences in total mortality between intermittent light (IL) (3/80) and continuous light (CL) (3/80) groups. During the heat exposure period, under the CL program, the mortality rate of the heat stressed group was almost twice as high as in the non-stressed group. While, under the IL program, the mortality was less in heat stressed birds compared to non-heat stressed ones, during the same time period. In the non heat stressed birds, the mortality rate was 2.5 folds higher in the CL group than the IL group, from 4-6 weeks of age. On the other hand, under heat stress conditions, mortality rate was five folds higher in the CL group compared to the IL one.

Body weight: Under normal temperature, there were no significant effects of lighting program on body weight, at 3 or 6 weeks of age (Table 1). Exposure to heat stress, from 4-6 weeks of age, significantly decreased the body weight at 6 weeks of age, regardless of the lighting program compared to non-heat stressed birds (Table 1). However, body weight of heat stressed chicken reared under IL program was significantly higher than the CL group, at 6 weeks of age.

Feed consumption: Chickens grown under the IL program consumed significantly less feed, from 0-3 weeks of age, compared to those reared under CL program (Table 1). Generally, heat stress exposure significantly decreased feed consumption regardless of the lighting program, compared to chicks raised under control temperature. Nevertheless, feed consumption was significantly higher in the IL group compared to the CL group in the heat stressed birds. In the non-stressed birds, there was no significant difference in feed consumption between IL and CL groups.

Feed conversion: Chicks reared under IL program had better feed conversion from 0-3 weeks of age than those raised under CL program (Table 1). In general, chronic heat stress had a significant adverse effect on feed conversion from 0-6 weeks of age, regardless of the lighting program, compared to non-heat stressed chicks. Whereas, under heat stress conditions, feed conversion of chickens reared under IL program was significantly better than the CL birds, from 4-6 and from 0-6 weeks of age.

Triiodothyronine (T_3): At the middle of light period, there was no significant effect of heat stress or light program on plasma T_3 concentrations (Fig. 1). While, In the

Table 1: Effects of light program and heat-stress (from 4-6 weeks of age) on some broiler economical parameters

	Treatments			
Parameters	CL + CT	CL + HS	IL + CT	IL + HS
Mortality 0-3 weeks	0/80	3/80	1/80	2/80
Mortality 4-6 weeks	9/80	19/80	4/80	2/80
Body weight at 3 weeks (Kg)	$0.971 \pm 0.11^{a*}$	0.968 ± 0.07^{a}	0.967 ± 0.10^{a}	0.902 ± 0.11^{a}
Body weight at 6weeks (Kg)	2.736 ± 0.170^{a}	1.476 ± 0.030^{c}	2.723 ± 0.139^{a}	2.088 ± 0.095^{b}
Feed consumption 0- 3weeks (Kg/bird)	1.232 ± 0.042^{a}	1.221 ± 0.025^{a}	1.151 ± 0.042^{b}	1.172 ± 0.056^{b}
Feed consumption 4- 6 weeks (Kg/bird)	3.243 ± 0.245^{a}	1.656 ± 0.129^{c}	3.338 ± 0.152^{a}	2.422 ± 0.172^{b}
Feed conversion 0- 3weeks	1.322 ± 0.059^{ab}	1.310 ± 0.083^{b}	1.237 ± 0.022^{c}	1.358 ± 0.048^{a}
Feed conversion 4- 6 weeks	1.836 ± 0.288^{b}	3.427 ± 0.324^{a}	1.905 ± 0.175^{b}	2.040 ± 0.104^{b}
Feed conversion 0-6 weeks	1.611 ± 0.087^{c}	1.948 ± 0.078^{a}	1.601 ± 0.056^{c}	1.733 ± 0.045^{b}

Values are means ± SE. Values within each row with no common letters are significantly different ($p<0.05$), CL: continuous light program (23hL: 1hD). IL: Intermittent light program. (1hL: 3hD), CT: 24°C temperatures. HS: Heat stress (35°C) (4-6 weeks of age)

Table 2: Some broiler economical parameters before (at 3weeks of age) and after (at 6 weeks of age) Melatonin and heat stress treatments

	Treatments			
Parameters	No MEL + CT	No MEL + HS	MEL + CT	MEL + HS
Mortality 0-3 weeks	0/40	2/40	2/40	0/40
Mortality 4-6 weeks	4/40	8/40	1/40	1/40
Body weight at 3 weeks (Kg)	0.956 ± 0.05 ^{a*}	0.951 ± 0.12^{a}	0.976 ± 0.07^{a}	0.960 ± 0.11^{a}
Body weight at 6 weeks (Kg)	2.780 ± 0.182^{a}	1.364 ± 0.131^{b}	2.791 ± 0.210^{a}	1.460 ± 0.152^{b}
Feed consumption 0- 3 weeks (Kg/bird)	1.198 ± 0.011^{a}	1.265 ± 0.048^{a}	1.237 ± 0.070^{a}	1.225 ± 0.048^{a}
Feed consumption 4- 6 weeks (Kg/bird)	3.397 ± 0.227^{a}	1.537 ± 0.112^{b}	3.470 ± 0.311^{a}	1.502 ± 0.160^{b}
Feed conversion 0- 3 weeks	1.310 ± 0.033^{a}	1.350 ± 0.060^{a}	1.360 ± 0.069^{a}	1.330 ± 0.060^{a}
Feed conversion 4-6 weeks	1.860 ± 0.069^{c}	4.050 ± 0.832^{a}	1.880 ± 0.150^{c}	3.060 ± 0.600^{b}
Feed conversion 0- 6 weeks	1.677 ± 0.056^{c}	2.122 ± 0.410^{a}	1.710 ± 0.090^{c}	1.925 ± 0.212^{b}

Values are means ± SE. Values within each row with no common letters are significantly different ($p<0.05$), No MEL: 0 ppm melatonin-fed. MEL: melatonin-fed (40 ppm). CT: 24°C temperatures. HS: Heat stress (35°C) (4-6 weeks of age)

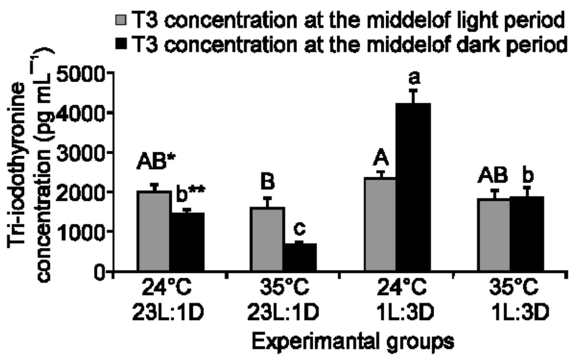

Fig. 1: Effects of light program and heat stress on Tri-iodothyronine (T3) concentration in broiler chickens at 6 weeks of age, *Means with different, upper case, letters differ significantly ($p<0.05$), (n = 8). **Means with different, lower case, letters differ significantly ($p<0.05$), (n = 8)

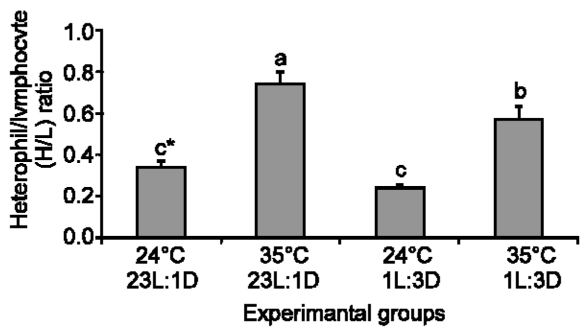

Fig. 2: Effects of light program and heat stress on heterophil/lymphocyte (H/L) ratio in broiler chickens at 6 weeks of age. *Means with different letters differ significantly ($p<0.05$), (n = 8)

middle of the dark period, plasma T_3 concentration was significantly lower in the heat stressed groups compared to the non-stressed groups, regardless of light treatment. Within each heat treatment, the plasma T_3 concentration was significantly higher in the IL group compared to the CL group.

Heterophils/Lymphocytes (H/L) ratio: Under control temperature (24°C), there was no significant difference in the H/L ratio, at six weeks of age, between IL and CL groups (Fig. 2). Chronic heat stress significantly increased H/L ratio as compared to the 24°C temperature groups, regardless of the light program applied. Chickens reared under the IL light program and exposed to chronic heat stress had significantly lower H/L ratio than those reared under CL and heat stress program.

Humoral Immune response: Under 24°C temperature condition, there was no significant effect of the lighting program on antibody production against SRBC (Fig. 3). In addition, heat stress had no significant effect on the antibody production in the chickens reared under IL program. However, within CL groups, exposure to heat stress decreased antibody titers, compared to the non-heat stressed chickens.

Experiment 2

Mortality: Before heat treatment, during the first 3 weeks, there were no differences in total mortality between the no MEL treated groups (2/80) and the MEL fed groups (2/80) (Table 2). Within the MEL treated groups, there was no difference in mortality, from 4-6 weeks of age, between heat stressed and non-heat stressed birds. Under 24°C, the mortality in the MEL fed group was four times lowers than the no MEL group, through the same time period. Likewise, in the heat-stressed birds, the mortality was much less (1/40) in the MEL treated group than the no MEL group (8/40).

Body weight: There were no significant effects of MEL administration on body weight at 3 or 6 weeks of age, regardless of the heat stress treatment (Table 2). Exposure to heat stress, from 4-6 weeks of age, significantly decreased the body weight at 6 weeks of age, regardless of the MEL addition, compared to non-heat stressed birds.

Feed consumption: From 4-6 weeks of age, heat stress exposure significantly decreased feed consumption

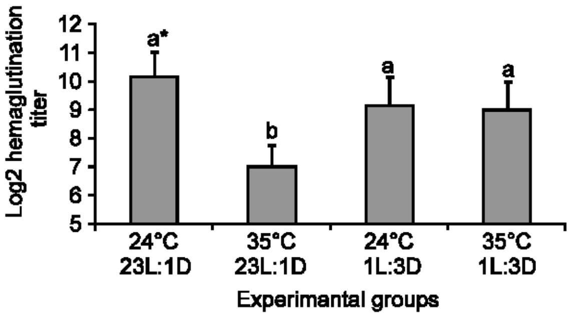

Fig. 3: Effects of light program and heat stress on antibody production against sheep red blood cells (SRBC) in broiler chickens at 6 weeks of age, birds were injected with SRBC suspension at 5 weeks of age. *Means with different letters differ significantly (p<0.05), (n = 8)

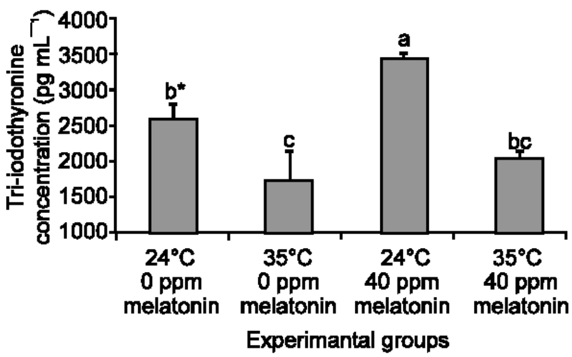

Fig. 4: Effects of melatonin addition and heat stress on Tri-iodothyronine (T3) concentration in broiler chickens at 6 weeks of age, *Means with different letters differ significantly (p<0.05), (n = 8)

regardless of the MEL addition, compared to chicks raised under 24°C temperature (Table 2). In general, there was no significant effect of MEL treatment on feed consumption, regardless of the heat treatment.

Feed conversion: In general, Inferior feed conversion efficiency from 0-6 weeks of age was observed due to the heat stress, regardless of the MEL addition (Table 2), compared to non- heat stressed birds. However, under heat stress conditions, feed conversion of chickens fed with 40 ppm MEL-diet was significantly better than the no MEL-fed birds, from 4-6 and from 0-6 weeks of age.

Triiodothyronine (T_3): Plasma concentration of T_3 was significantly lower in the heat stressed groups compared to the non-stressed ones, regardless of MEL treatment (Fig. 4). In the non-heat stressed groups, MEL addition significantly increased the plasma T_3 concentration as compared to the no MEL treatment. Under heat stress conditions, no significant effect was observed due to MEL treatment.

Heterophils/Lymphocytes (H/L) ratio: Under 24°C, the H/L ratio, at six weeks of age, was not affected by MEL treatment (Fig. 5). Chronic heat stress from 4-6 week of age significantly increased H/L ratio as compared to the 24°C temperature groups, regardless of the MEL treatment. However, H/L ratio was significantly less in the 40 ppm MEL-fed group compared to the no MEL-fed group, in the heat-stressed birds.

Humoral immune response: Under non-heat stress condition, no significant effect of MEL treatment on antibody production against SRBC was observed (Fig. 6). In addition, there was no significant effect due to the chronic heat stress on the antibody production in the MEL fed groups. Whereas, heat stress exposure decreased antibody titer in the no MEL-fed group, compared to their non-heat stressed control birds.

Discussion

In the present study, the results showed that chickens, under CL program or non-MEL fed, that were subjected to heat stress had higher mortality, as compared to non-heat stressed ones. Similar results were reported by Naughton *et al.* (2002). The mortality of the chickens reared under the IL program was two and ten times less in the non-heat stressed and stressed birds, respectively, as compared to the CL program groups. Most of the mortality in CL group was due to sudden death syndrome. Similar results were observed by Bölükbasi and Emsen (2006). Better livability could be due to slower growth rate during early life. Maintaining birds on a short photoperiod resulted in slow early weight gain (Walker, 1996; Gordon and Tucker, 1995).

In MEL-fed groups, the mortality did not increase during heat stress period. These results may be due to a variety of reasons. Clark and Classen (1995) reported that, in broilers, MEL addition in the diet (40 ppm) resulted in a 36% decrease in the occurrence of sudden death syndrome. Melatonin enhances the activity of membrane calcium pumps (Chen *et al.*, 1993), decreases the intracellular calcium concentration (Vanecek and Klein, 1995) and regulates calmodulin (Anton-Tay *et al.*, 1998).

In the current study, the body weights of the heat-stressed birds were significantly heavier in the IL group, by about 600 g/bird, than the CL group. This could be due to higher plasma T_3 concentrations in the IL group than the CL group during the dark period. Johnson (1997) reported that pro-inflammatory cytokines (IL-1, IL-6 and TNFα) inhibit growth by modulating the intermediary metabolism of carbohydrate, fat and protein substrates and act on the brain to reduce feed consumption. Abbas *et al.* (2007) showed that IL program decreased the production of those pro-inflammatory cytokines; therefore body weight is expected to be higher.

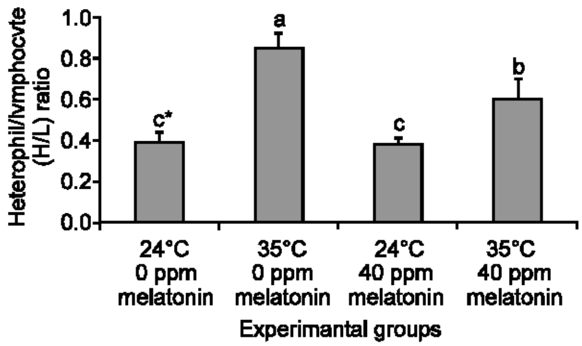

Fig. 5: Effects of melatonin addition and heat stress on heterophil/lymphocyte (H/L) ratio in broiler chickens at 6 weeks of age. *Means with different letters differ significantly ($p<0.05$), ($n = 8$)

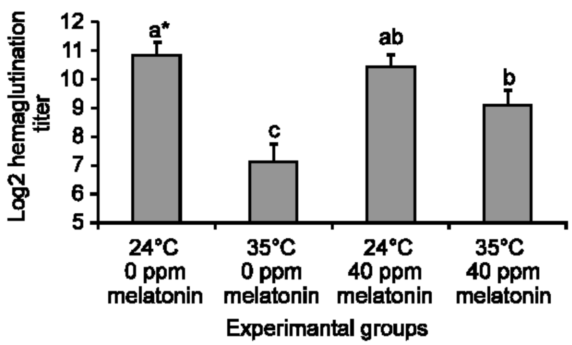

Fig. 6: Effects of melatonin addition and heat stress on antibody production against sheep red blood cells(SRBC)in broiler chickens at 6 weeks of age, birds were injected with SRBC suspension at 5 weeks of age. *Means with different letters differ significantly ($p<0.05$), ($n = 8$)

Feed consumption, from 0-3 weeks of age, in the IL groups was significantly lower than the CL groups. Similar results were observed by Bölükbasi and Emsen (2006). These results could be due to the fact that access to feed was lower by 75% in 1hL: 3hD groups compared to 23hL: 1hD groups. Whereas, there was no significant effect of lighting program on the feed consumption from 4-6 weeks of age, under non-heat stress conditions. These results agree with those stated by Rahimi *et al.* (2005).

Chickens exposed to chronic heat stress under IL program consumed significantly more feed, as compared to those raised under CL program. This could be due to the fact that body temperature was less by 0.6°C in the IL group, as observed on the same birds in our previous work (Abbas *et al.*, 2007).

Overall, no significant effect of MEL treatment on body weight or feed consumption was observed, at all studied ages or under both heat treatments. These results agree with those of Aperdoorn *et al.* (1999) and Brennan *et al.* (2002).

Feed conversion, in the heat-stressed groups, was significantly improved in the IL program and 40 ppm-MEL-fed groups, as compared to CL program and 0 ppm- MEL-fed groups. Our results are in agreement with those reported by Phetteplace and Nockels (1985), Clark and Classen (1995) and Bölükbasi and Emsen (2006). These results could be due to suppressing pro-inflammatory cytokine production (Johnson, 1997). Abbas *et al.* (2007) reported that IL program and MEL addition decreased the production of these pro-inflammatory cytokines.

In the non-heat stressed birds, our data showed that IL program or MEL administration increased plasma T_3 concentrations at the middle of the dark period. These results could be due to both direct and indirect effects of MEL on leptin hormone concentrations. Mustonen *et al.* (2000) reported that MEL increases the concentrations of plasma leptin. In addition, Ahima *et al.* (1996) and Legradi *et al.* (1997) found that leptin raises the levels of serum thyroxin (T_4) and prothyrotropin releasing hormone mRNA in the para ventricular cells.

In this study, the results showed that exposure to heat stress increased H/L ratio, while IL regimen and MEL administration suppressed this elevation. Similar results were observed by Moore and Siopes (2000), in Japanese quail. Brennan *et al.* (2002) confirmed these results by reporting that MEL may reduce stress, as indicated by the reduction in H/L ratio, during the dark period.

Our results indicated that high environmental temperature in the CL group or in 0 ppm MEL fed group decreased the humoral immune response against SRBC of broiler chickens. Environmentally stressed poultry generally have a depressed humoral immune response (Atta, 1990; Gharib *et al.*, 2005). Heat stress increases the incorporation of endogenously produced corticosterone into lymphoid cells (Siegel and Gould, 1982), which in turn causes the suppression of interleukin 2 production (Farrar *et al.*, 1980).

Intermittent light program or MEL administration, in the present study, enhanced humoral immune response and increased antibody production against SRBC under heat stress conditions. These results agree with those reported by Kirby and Froman (1991) and Moore and Siopes (2000). The pineal hormone melatonin (MEL) may be the mediator of these photoperiod-immune effects due to its direct and indirect effects on immune function (Kliger *et al.*, 2000; Brennan *et al.*, 2002).

Conclusion: The present study showed that heat stress caused high mortality rate, suppressed production parameters, increased H/L ratio and inhibited plasma T_3 hormone secretion and antibody production against SRBC. The IL program had a greater impact on alleviating the negative effects of heat stress than did MEL administration. These results mean that there are other factors in addition to MEL administration that can alleviate the negative effects of heat stress. Those factors may include lowering the physical activity and energy expenditure during darkness. From the practical point of view, it could be concluded that, in addition of lowering the cost of electricity by about 75%, the IL (1hL: 3hD) program may be used as an effective management practice to improve broiler's ability to dampen the deleterious effects of heat stress on their performance and immune response. In the open house system; IL program can be used only during the night.

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