

# A study of the employment of melatonin supplementation and darkness regime on reducing the negative effects of acute heat stress and mortality in broiler chickens

Hassanzadeh, M.<sup>1\*</sup>, Moghimi Niaki, A.A.<sup>1</sup>, Babapour, V.<sup>2</sup>, Mohit, A.<sup>3</sup>, Mirzaie, S.<sup>4</sup>

<sup>1</sup>Department of Avian Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

<sup>2</sup>Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

<sup>3</sup>Department of Animal Sciences, Faculty of Agriculture, University of Guilan, Rasht, Iran

<sup>4</sup>Institute for Agricultural Research of Iranian Organization for Sciences and Technology, Tehran, Iran

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## Correspondence

Hassanzadeh, M.  
Department of Avian Diseases,  
Faculty of Veterinary Medicine,  
University of Tehran, Iran  
Tel: +98(21) 61117150  
Fax: +98(21) 66933222  
Email: mhzadeh@ut.ac.ir

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## Abstract:

**BACKGROUND:** Heat stress is one of the most important environmental stressors challenging poultry production worldwide. Understanding and controlling environmental stressors is crucial for successful poultry production. **OBJECTIVES:** The aim of the present study was to investigate the effects of melatonin supplementation and darkness regime on reducing the negative effects of heat stress in broiler chickens. **METHODS:** A total of 400 broiler chickens (Arian) were obtained and subjected to four different groups. The control chickens were grown in normal conditions without receiving any treatment. The positive control birds were grown similar to the control but exposed to an acute heat stress for 6 h/d from days 35 to 40. The dark group chickens were reared under a dark schedule and exposed to heat stress. The birds of the fourth group were reared similar to the positive control but received 40 ppm melatonin in the diet from days 30 to 40 of age. Dead birds were autopsied. The weekly growth performance of chickens was determined and blood samples were taken for hormonal analysis. **RESULTS:** The number of dead birds due to heat stress was significantly decreased in chickens subjected to the dark program and/or those which received melatonin in the diet compared with those of positive control chickens. Such differences were accompanied with the significantly lower levels of plasma T3, T3/T4 and corticosterone in these groups of chickens. Additionally, plasma thiobarbituric acid reacting substances level was significantly higher in positive control broilers compared to their counterparts. **CONCLUSIONS:** Our findings indicate a reduction of metabolic rate and heat production in dark regime and melatonin supplementation that can reduce the side effects of heat stress and, therefore, reduce the mortality rate.

## Introduction

Genetic selection for a faster growth rate in

broiler chickens has predisposed them to physiological and metabolic disorders resulting in more sensitivity to harsh environmental con-

ditions including heat stress. Within the thermoneutral zone, the body temperature of birds is maintained by thermal equation. Heat stress occurs when ambient temperature goes above the thermoneutral zone which is between 18 to 22°C for grown broilers (Charles, 2002).

As a consequence of environmental temperature increment, the extra heat could be lost by evaporating the moisture of the respiratory tract through increased respiration rate or panting. In contrast, carbon dioxide would be lost during panting and the bicarbonate buffer system would lower the concentration of hydrogen ions, developing a condition known as respiratory alkalosis (Calder and Schmidt-Neilsen, 1967). During the respiratory alkalosis, the shift in blood pH increasingly depresses the feed intake and adversely affects the overall performance and also health of the birds (Ahmad and Sarwar, 2006). The excessive levels of reactive oxygen forms can be stimulated by stressful situations, e.g., heat stress and clinical conditions (Lara and Ros-tagno, 2013; Dröge, 2002). These result in the disturbance of balance between the oxidation and antioxidant defense systems, causing lipid peroxidation and oxidative damages to cell membranes, proteins, and DNA (Halliwell and Gutteridge, 1989). This could lead to higher mortality in chickens (Naughton et al., 2002; Gharib et al., 2008).

Hormonal changes occur in broilers under heat stress conditions and the stress responses in poultry are excited mainly by the activation of hypothalamic-pituitary-adrenal axis and of the nervous system (Ben Nathan et al., 1976). Previous studies have indicated that plasma triiodothyronine (T3) concentration of heat-stressed chickens may decrease or remain unchanged (Yahav and McMurtry, 2001; May et al., 1986). One of the mechanisms involved in the improved thermotolerance is modulation of heat production through changes in circulating T3 (Bowen et al., 1984; Yahav and Hurwitz, 1996). Lin et al. (2004) pointed a clear

positive relationship between circulating corticosterone levels and plasma lipid peroxidation, which is a direct indication of oxidative cellular damage in broiler chickens (Iqbal et al., 1990).

Melatonin is a neural hormone which is secreted mainly by the pineal gland during the darkness in chickens as in other animals. This hormone is involved in many biological functions including thermoregulation mechanism, reproductive and cardiopulmonary systems, and behaviors of birds (Apeldoorn et al., 1999; Ambriz-Tututi et al., 2009).

Several studies have shown that discontinuous lighting or darkness regime would improve the feed conversion rate and decrease the mortality rate of metabolic disorders such as leg problems, ascites, and sudden death syndrome (Blair et al., 1993; Özkan et al., 2000; Stub and Vestegaard, 2001; Sanotra et al., 2002; Hassanzadeh et al., 2012, 2014).

While most researchers have studied the physiological and/or metabolic changes in heat-stressed birds, only a few of them have implemented management activities to control heat-stressed mortality by taking the darkness regime. Based on these, the aim of the present study was to investigate the effects of melatonin supplementation and darkness regime on reducing the negative consequences of heat stress and its related mortality rate in broiler chickens.

## **Materials and Methods**

A total of 400-day-old fast growing broiler chickens (GGP stock of Pure Sir Broiler Line, Arian Co., Babolkenar, Iran) were reared under a nearly continuous lighting schedule (23 L: 1D) until day 9 of age. Thereafter, they were randomly divided over four equal-sized rooms (6×3 m<sup>2</sup>), each containing four floor pens (25 chicks per pen) and housed in a low altitude farm 50m above the sea level in the north of Iran. The chickens were reared for six weeks

on wood shavings under standard conditions and provided ad libitum access to water and a standard ration (Starter: 2850 kcal/kg metabolizable energy and 210 g/kg crude protein, Grower: 2950 kcal/kg metabolizable energy and 200 g/kg crude protein, Finisher: 3050 kcal/kg metabolizable energy and 190 g/kg crude protein formulated) to meet the requirements for broilers (National Research Council, 1994). In the first room, chickens of the negative control (ConN) group received 23L:1D lighting regime up to the end of the study. The positive control (ConP) chickens were grown similar to the ConN birds up to 35 days of age, but from days 35 to 40, these chickens were exposed to an acute heat stress for 6 h/d. Each day, the temperature was gradually raised up to  $38\pm 1^{\circ}\text{C}$  during the first hour and the degree was kept for the next 5 hours. In the third room (Dark), lighting schedules were provided as presented in Fig 1. Briefly, the dark-length schedules in this group consisted of: 1 to 9 days, 23L:1D; 10 to 11 days, 20L:4D; 12 to 13 days, 19L:5D; 14 to 31 days, 18L:6D; 32 to 33 days, 19L:5D; 34 to 35 days, 20L:4D; 36 to 41 days, 21L:3D and 41 to 42 days, 23L:1D. The birds of the fourth group (Melat) were reared similar to the ConN up to the 30<sup>th</sup> day, but they received 40 ppm melatonin (Vitane pharmaceutical) in the diet (Apeldoorn et al., 1999) from days 30 to 40 of age. In this study, chickens of the Dark and Melat groups were also exposed to an acute heat stress simultaneously in similar conditions, as already explained for ConP birds. The body weight, feed intake, and feed conversion ratios (FCR) were measured weekly and the mortality rate was recorded daily for each group separately. Blood samples of 10 birds from each group of chickens were randomly taken on days 10, 20, 30, 35, 38 and 42 of age in heparinized tubes. The plasma was separated and stored in aliquots at  $-20^{\circ}\text{C}$  for further analysis.

The plasma samples were sent to the Department of Biosystems, Catholic University

Leuven, Belgium, for determining the levels of thyroid hormones (T3, T4), corticosterone, and thiobarbituric acid reacting substances (TBARS). Thyroid hormones and corticosterone levels were measured by radioimmunoassay according to the method described earlier (Decuyper et al., 1983, 1994; Hassanzadeh et al. 2004).

Plasma lipid peroxidation was estimated by the spectrophotometric determination of TBARS as reported by Lin et al. (2006). TBARS were expressed as nanomoles of malondialdehyde (MDA) per milliliter of plasma.

Statistical analysis was performed using the "General linear model procedure" (SAS Institute Inc., 2008). If a significant overall effect ( $p<0.05$ ) was found, treatment means were compared using the Scheffe test.

## Results

The total number of dead birds due to acute heat stress, mean body weight, the feed intake, and FCR of the different groups of treated birds are summarized in Table 1.

During the 6 weeks of the experiment, 28 (7%) of 400 chickens died (Table 1). Of these, 19 (4.8%) died due to acute heat stress from days 35 to 41 of age. While the other 9 (2.3%) dead birds observed over the first 5 weeks of the growing period were due to neonatal infections (3 birds), arthritis (2 birds), sudden death syndrome (1 bird) and ascites (3 birds). Between days 35 to 41 of the study, 10 of 100 birds (10%) which have been reared under ConP program died due to heat stress, while the rate of heat-stressed mortality declined under the dark condition (5/100 birds) and also when 40 mg melatonin was supplied in the diet (4/100 birds).

Imposing birds to the dark conditions reduced the absolute body weight of chickens compared to the other three groups of birds at days 14 onwards but the difference was only

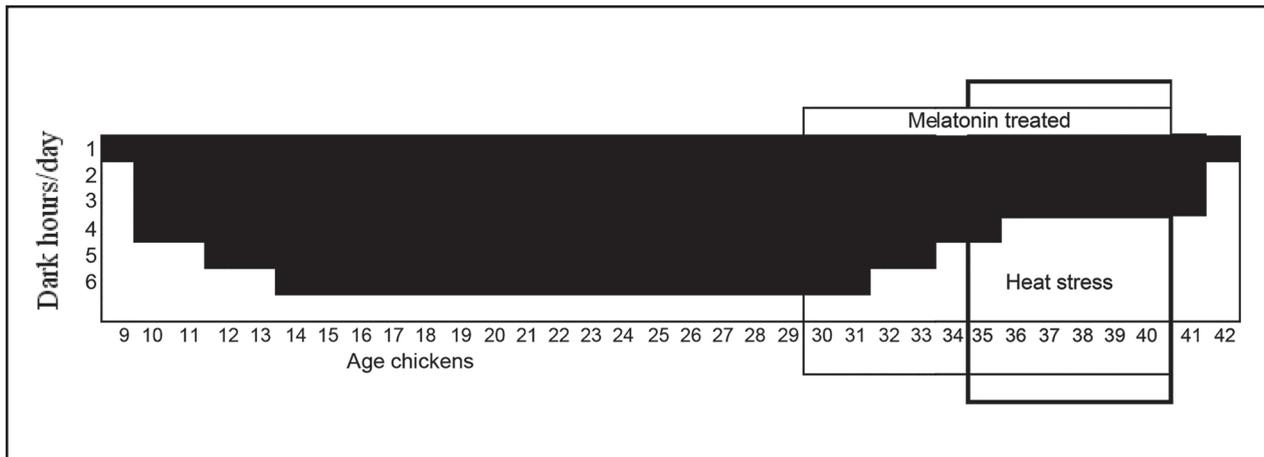


Figure 1. The duration of darkness regime, melatonin and heart stress treatments in experiment.

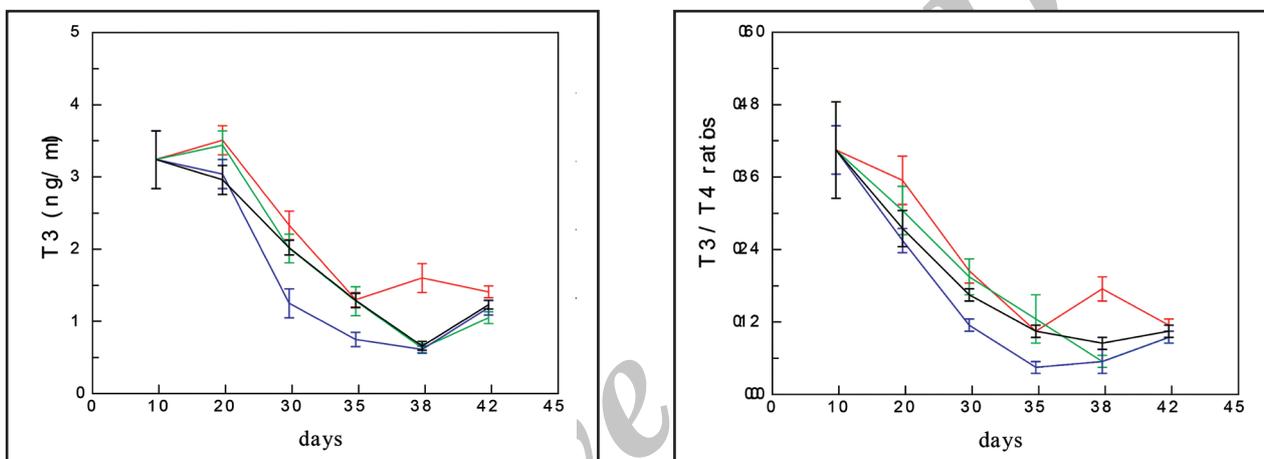


Figure 2. Effects of melatonin supplementation, darkness regime and acute heat stress on plasma  $T_3$  (ng/ml) concentrations and  $T_3/T_4$  ratios. ConN — ConP — Dark — Melat —

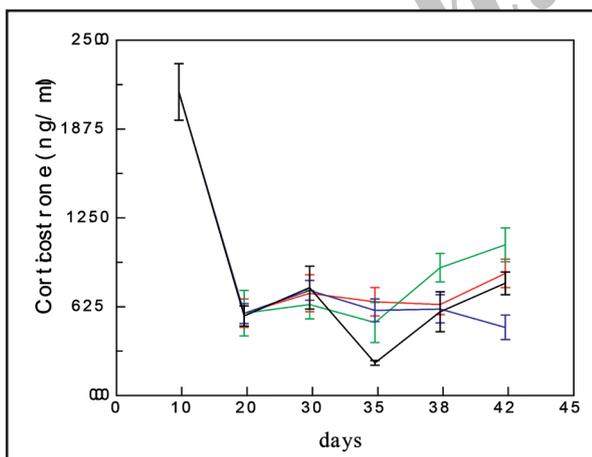


Figure 3. Effects of melatonin supplementation, darkness regime and acute heat stress on plasma corticosterone (ng/ml) concentration. ConN — ConP — Dark — Melat —

significant at day 35 ( $p < 0.001$ ) of age. Meanwhile, melatonin and short period exposure to heating stress had no significant effects on the

body weight of chickens (Table 1). Restricting lighting programs and melatonin had also no significant effects on feed intake or feed conversion ratios in all ages. However, feed consumption of dark-reared group chickens tended to be lower from day 14 on, but no significant influence was seen.

At days 30 and 35 of age, mean plasma  $T_3$  levels of Dark-reared birds were significantly ( $p < 0.0001$ ) lower than the ConN, ConP, and Melat groups. From day 35 onward, plasma  $T_3$  concentrations of ConP and Melat groups declined. Therefore, the  $T_3$  levels of these two groups of chickens were the same as the darkness regime birds and were significantly ( $p < 0.0001$ ) lower compared with the ConN at day 38 of age. Also on day 42, no significant difference was observed between the  $T_3$  levels

Table 1. Effects of melatonin supplementation and darkness regime on body weight (BW), feed intake (FI) and FCR of broiler chickens and the number of dead birds due to acute heat stress. (\*) number of dead birds to 100 birds. Within rows in each age, means with no common superscripts are significantly different ( $p < 0.05$ ).

Parameters/ age	ConN	ConP	Dark	Melat	p- Values
Dead /total birds*	0/100	10/100	5/100	4/100	-
<b>BW (g)</b> Day 14	388 ± 4	368 ± 8	362 ± 9	383 ± 6	NS
Day 28	1184 ± 13	1210 ± 34	1167 ± 28	1226 ± 23	NS
Day 35	1853 ± 16 <sup>a</sup>	1842 ± 15 <sup>a</sup>	1765 ± 17 <sup>b</sup>	1859 ± 17 <sup>a</sup>	0.001
Day 42	2275 ± 43	2269 ± 35	2208 ± 37	2264 ± 39	NS
<b>FI (g)</b> Day 1-14	495 ± 3	494 ± 4	497 ± 3	493 ± 2	NS
Day 14-28	1527 ± 44	1505 ± 29	1478 ± 80	1549 ± 31	NS
Day 28-35	1372 ± 20	1381 ± 88	1291 ± 16	1372 ± 32	NS
Day 35-42	1194 ± 56	1217 ± 69	1177 ± 41	1182 ± 55	NS
Day 1-42	4588 ± 66	4597 ± 46	4443 ± 49	4593 ± 67	NS
<b>FCR</b> Day 1-14	1.48 ± 0.05	1.61 ± 0.09	1.60 ± 0.08	1.49 ± 0.12	NS
Day 14-28	1.91 ± 0.11	1.77 ± 0.07	1.83 ± 0.05	1.84 ± 0.06	NS
Day 28-35	2.04 ± 0.05	2.18 ± 0.04	2.16 ± 0.03	2.14 ± 0.07	NS
Day 35-42	2.64 ± 0.21	2.74 ± 0.18	2.65 ± 0.15	2.97 ± 0.18	NS
Day 1-42	2.06 ± 0.11	2.07 ± 0.13	2.05 ± 0.08	2.07 ± 0.14	NS

Table 2. Effects of melatonin supplementation, darkness regime and acute heat stress on plasma TBARS levels. Within rows in each age, means with no common superscripts are significantly different ( $p < 0.05$ ).

Age	ConN	ConP	Dark	Melat	p-Values
<b>TBARS (nm/L)</b>					
Day 10	2.14 ± 0.26	2.17 ± 0.20	2.10 ± 0.19	2.11 ± 0.25	NS
Day 20	2.19 ± 0.38	1.99 ± 0.29	1.85 ± 0.12	1.95 ± 0.26	NS
Day 30	1.53 ± 0.16	1.39 ± 0.11	1.26 ± 0.12	1.46 ± 0.11	NS
Day 35	1.26 ± 0.11	1.43 ± 0.17	1.52 ± 0.21	1.44 ± 0.13	NS
Day 38	1.22 ± 0.05 <sup>b</sup>	1.51 ± 0.09 <sup>a</sup>	1.02 ± 0.06 <sup>b</sup>	1.03 ± 0.08 <sup>b</sup>	0.05
Day 42	1.25 ± 0.09	1.20 ± 0.10	1.36 ± 0.15	1.27 ± 0.14	NS

of the four groups (Fig. 2).

The results of T3/T4 ratios were almost similar to the T3 levels. Dark-reared birds showed significantly lower T3/T4 ratios than the other three groups on days 30 ( $p < 0.01$ ) and 35 ( $p < 0.001$ ) of age. On day 38, acute heat stress, dark exposure, and melatonin supplementation significantly ( $p < 0.0001$ ) decreased T3/T4 ratios compared to the control birds. No significant difference was pronounced among the T3/T4 ratios of the different groups at the end of the study (Fig. 2).

As shown in Fig. 3, no significant differences were found between the corticosterone values of chickens between the four groups in the first 30 days of the growing period, but melatonin-treated birds had significantly

( $p < 0.0001$ ) lower plasma corticosterone levels at day 35 of age. At day 42 of age, Dark-reared chickens showed significantly ( $p < 0.005$ ) lower plasma corticosterone levels compared to the other three group chickens,

Darkness and melatonin had no significant effects on plasma TBARS in the current study. However, exposure to heat stress had a significant ( $p < 0.05$ ) influence on plasma TBARS levels, only at day 38 of age. At this age, the plasma TBARS level was significantly ( $p < 0.05$ ) higher in ConP broilers compared to their counterparts (Table 2).

## Discussion

Heat stress causes a series of physiologi-

cal and metabolic changes in broiler chickens such as elevated body temperature, panting, and respiratory alkalosis (Deyhim and Teeter, 1991). This results in significant direct economic losses to the broiler industry due to the ruined feed efficiency of the flock and higher mortality rate at farm, especially at marketing ages (Cooper and Washburn, 1998; Naughton et al., 2002; Gharib et al., 2008; Kusandi and Djulardi, 2011). Data in the current study demonstrated that rearing fast growing broiler chickens under darkness regime or feeding with melatonin-supplemented diets leads to a reduction of the mortality rate of broiler chickens when being exposed to acute heat stress. It could be explained by different mechanisms.

Imposing dark schedules reduced the absolute body weight of chickens which coincided with a temporary lower tendency in feed intake (Table 1). This difference in growth rate results in a more concave growth pattern which decreased the heat production of the birds (Buyse et al., 1994; Buys et al., 1998; Julian, 2005) and, consequently, heat-induced mortality (Al-Masri, 2010). Such a claim has already been made in our previous works on ascites syndrome (Buys et al., 1998; Hassanzadeh et al., 2000, 2003, 2005, 2012, 2014), namely that limiting the number of hours of daily light would slightly slow the growth rate, especially at an earlier age. It also reduces the activity of birds that requires additional oxygen. Hence, it would diminish metabolic demands and extra heat production which predisposes chickens to develop metabolic disorders (Julian, 2005; Hassanzadeh et al., 2012, 2014).

It is well known that thyroid hormones play crucial roles in thermoregulation in avian species, and plasma T3 levels are positively correlated with heat production (Buyse et al., 1992; Decuyper and Kühn, 1984, 1988). It is an indicator for the metabolic demand acceleration of the basal metabolic rate by causing an increase in the mitochondria mass, mitochondria cytochrome content, and respiratory rate

(Decuyper and Kühn, 1984; Hassanzadeh et al., 2014). In the current study, the decrease of acute heat-induced mortality was accompanied with the diminished T3 and T3/T4 levels at days 30, 35, and 38 of age, in chickens reared under the dark regime (Fig. 1). These are again strengthening the view that a lower metabolic rate had a positive correlation on the side effects of heat-stressed birds. Such an idea is supported by the earlier hypothesis (Apeldoorn et al., 1999; Abbas et al., 2007; Gharib et al., 2008) which stated that restricting light schedules is triggered for heat tolerance, thermoregulation, and hence, health of chickens.

Many biochemical reactions increase with high temperature; it is, thus, very likely that elevated body temperature, in heat stress conditions, would enhance the generation of free radicals via accelerated metabolic reactions in cells and tissues. Lin et al. (2000) suggested that the increased generation of oxygen-free radicals and cytotoxicity of oxidants may mediate in part, heat-induced cellular damage through the lipid peroxidation. In contrast, melatonin, a pineal gland-derived hormone, plays an important role in the antioxidant defense system (Pieri et al., 1994; Barlow-Walden et al., 1995) and has an effective free-radical-scavenging activity (Ozturk et al., 2002). An additional mechanism that could be claimed to be effective in controlling the disadvantages of heat stress is the melatonin treatment of chickens.

Siegel (1995) reported that elevation of plasma corticosterone levels is recognized as an indicator of stress in avian species. Ben Nathan et al. (1976) have argued that exposure to high environmental temperatures will activate the hypothalamo-pituitary-adrenal-axis in chickens that culminates in increased plasma corticosterone levels. Our data showed that the broiler chickens exposed to acute heat stress but fed with a melatonin-supplemented diet (35 d) or reared under the dark program (42 d) had significantly lower plasma corticosterone levels than the ConP group chickens (Fig. 3).

Based on this phenomenon, it is reasonable to hypothesize that these two groups of birds had less difficulty to cope with high environmental temperature and had better tolerance, resulting in numerically reduced dead birds. There is also evidence that melatonin is able to modulate stress at both central and peripheral levels by exerting its inhibitory role in the hypothalamo-pituitary-adrenal axis of chickens and by suppressing hypothalamic gene expression of proopiomelanocortin that encodes ACTH (Decuyper et al., 1989; Rasmussen et al., 2003). The mechanisms might be involved in the current study, since in both Dark and Melat groups heat-induced mortality decrement was accompanied with a significant decrease of plasma corticosterone levels.

A significant elevation of plasma TBARS concentrations observed in ConP but not in the other chicken groups on day 38 of age (Table 2), is included in the studies of Lin et al. (2000) and Mahmoud and Edens (2003), since plasma lipid peroxidation is estimated by determining plasma TBARS (Lin et al., 2004ab). These results proved a disturbance of the equilibrium between the generation and reduction of oxidants that was led to increase lipid peroxidation in ConP birds compared to their counterparts. In contrast, it also means that a positive correlation might exist between dark and melatonin treatments with lipid peroxidation in heat-stressed chickens.

Plasma T3 levels of ConP chickens was significantly lower than the ConN chickens on day 38 of age (Fig. 2), and this is supported by previous studies which have reported that T3 concentrations consistently decrease in high temperature conditions (Geraert et al., 1996; Star et al., 2008). It appears that high temperature conditions improve thermotolerance by enhancing the ability to reduce T3 concentration followed by reducing the heat production of birds (Bowen et al., 1984; Yahav and Hurwitz, 1996).

Overall, no significant effect of melatonin

on absolute body weight, feed intake, or feed consumption ratios of chickens was observed throughout the study which is confirmed by Apeldoorn et al. (1999) and Brennan et al. (2002). However, it is inconsistent with the previous reports (Osei et al., 1989; Clark and Classen, 1995) and such differences could be due to the age of birds and/or the duration of melatonin use.

Significant negative effects of high ambient temperature on the growth performance of poultry have been widely reported (Cooper and Washburn, 1998; Sahin et al., 2003; Abbas et al., 2007). However, such results were not observed in our study. Thus, differences were most probably due to the method and the duration of heat exposure to the chickens. In the current study, acute heat stress has been applied for a few hours in the last days of the growing period. Therefore, we observed more acute stress responses, e.g., mortality and hormonal changes when compared with the chronic heat stress response, e.g., the ruined feed efficiency of birds.

Many authors have reported that the change of continuous to restricted lighting schedule is followed by initial growth depression. This depression is, however, followed by a period of compensatory growth in a way that the birds reared in alternative light reached almost the same final body weight at the marketing age, as those reared in continuous light (Classen et al., 1991; Buyse et al., 1994; Buys et al., 1998; Hassanzadeh et al., 2000, 2003, 2012, 2014). In the present study, although full compensatory growth was not achieved by chickens reared under dark conditions compared to their counterparts on 42 days of age, the difference (60 g) was not statistically significant. It seems that occasionally the management activities which are usually implemented to control metabolic disorders, e.g., heat stress at the farm level, are necessary to keep the profitability of broiler chicken farming, even by not being able to achieve the maximum growth potential

of broiler chickens at the marketing age. Additionally, our data showed that exposing broiler chickens to acute heat stress but feeding them with a melatonin-supplemented diet or rearing them under the dark program has significantly beneficial effects on the hormonal activities that are involved in many biological functions and the mortality rate of birds. Whether the positive effect of the dark regime seen here can be attributed to itself or to the secretion of melatonin will need further studying.

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## بررسی استفاده از ملاتونین خوراکی و تاریکی بر کاهش تلفات و عوارض ناشی از استرس حاد گرمایی در جوجه های گوشتی

محمد حسن زاده<sup>۱\*</sup> امیر احمد مقیمی نیکی<sup>۱</sup> وهاب باباپور<sup>۲</sup> اردشیر محیط<sup>۳</sup> سارا میرزایی<sup>۴</sup>

(۱) گروه بیماریهای پرندگان، دانشکده دامپزشکی دانشگاه تهران، تهران، ایران

(۲) گروه علوم پایه، دانشکده دامپزشکی دانشگاه تهران، تهران، ایران

(۳) گروه علوم دامی، دانشکده کشاورزی دانشگاه گیلان، رشت، ایران

(۴) مرکز تحقیقات کشاورزی سازمان پژوهش های علمی و تکنولوژی ایران، تهران، ایران

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### چکیده

**زمینه مطالعه:** استرس گرمایی از مهمترین استرس های محیطی است که خسارت تأثیرگذاری در صنعت طیور ایجاد می کند. شناخت این استرس و کنترل آن تأثیر بسیار مهمی در تولید این صنعت خواهد داشت. **هدف:** تأثیر استفاده از ملاتونین خوراکی و رژیم تاریکی بر عوارض منفی ناشی از استرس گرمایی مورد بررسی قرار می گیرد. **روش کار:** ۴۰۰ قطعه جوجه گوشتی نژاد آرین تهیه و به چهار گروه تقسیم و تا ۴۲ روزگی نگهداری شدند. جوجه های گروه کنترل در کل دوره از لحاظ دما و نور در شرایط عادی نگهداری شدند. در گروه کنترل مثبت، جوجه های آن تا ۳۵ روزگی مانند گروه اول پرورش یافتند و بین ۳۵ الی ۴۰ روزگی، روزانه ۶ ساعت در شرایط استرس حاد گرمایی قرار گرفتند. در گروه تاریکی، جوجه ها ضمن تحمل استرس گرمایی مانند سایر گروه ها، از یک مدل تاریکی استفاده کردند. در گروه ملاتونین، جوجه ها همانند گروه کنترل مثبت پرورش یافته و بین ۳۰ الی ۴۰ روزگی ملاتونین خوراکی به میزان ۴۰ ppm در دان مصرف نمودند. جوجه ها و غذای مصرفی آنها هفتگی توزین و تلفات روزانه کالبدگشایی و گزارش شد. از جوجه ها خونگیری و غلظت هورمون های خون آنها اندازه گیری شد. **نتایج:** استفاده از برنامه تاریکی و ملاتونین در دان سبب کاهش تعداد تلفات ناشی از استرس گرمایی گردید که این کاهش تلفات به شکل معنی داری با کاهش غلظت T<sub>3</sub>، نسبت T<sub>3</sub>/T<sub>4</sub> و کورتیکوسترون خون جوجه ها همراه شد. ضمناً میزان TBARS خون جوجه های گروه کنترل مثبت هم به شکل معنی داری نسبت به گروه های دیگر بالاتر بود. نتیجه گیری نهایی: این آزمایش نشان داد که استفاده از ملاتونین خوراکی و تاریکی در پرورش جوجه سبب کاهش فعالیت متابولیک و تولید گرما در جوجه های گوشتی شده که این مسئله سبب کاهش تلفات و عوارض ناشی از استرس حاد گرمایی خواهد شد.

**واژه های کلیدی:** جوجه گوشتی، کورتیکوسترون، استرس گرمایی، ملاتونین، T<sub>3</sub>/T<sub>4</sub>

\* نویسنده مسؤول: تلفن: ۶۱۱۷۱۵۰ (۲۱) ۰۹۸ + شماره: ۶۶۹۳۳۲۲۲ (۲۱) ۰۹۸ + Email: mhzadeh@ut.ac.ir

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