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Intermittent Photoperiod Schedule does not Influence Brain and Serum Melatonin and Selected Serum Antioxidant Enzymes Activity in Broiler Chickens

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Abstract

This study evaluated the effect of the intermittent light program on serum and brain melatonin concentrations, antioxidant enzyme activities, and homocysteine concentration in broiler chickens. A total of 60 one-day-old broiler chickens (Cobb 500) were distributed in three light-proof controlled rooms (20 chicks per room). All birds were reared in continuous light until 3 days of age. Then, chicks were treated as follows: 1) intermittent lighting program (1L: 3D cycles), 2) continuous lighting program (24L), and 3) nonintermittent restricted lighting program (8L: 16D). At day 42 of age, sera and brains were collected from all chicks of each group (at the end of the dark phase for groups 1 and 3). Serum and brain melatonin concentrations were not affected by the different photoperiod schedules. Serum glutathione reductase, glucose-6-phosphate dehydrogenase, superoxide dismutase, and glutathione peroxidase activity as well as homocysteine concentration were also similar between different treatment groups. In conclusion, intermittent lighting program during the rearing period does not affect serum and brain melatonin levels as well as antioxidant status at the end of the dark phase in broilers.

Introduction

In traditional poultry production systems, chickens are usually faced with different stressor which may lead to free radical production (Surai, 2002). Therefore, chickens are constantly exposed to free radical attack which may damage different molecules such as DNA, proteins, lipids, and carbohydrates (Halliwell *et al*., 1992). Both enzymatic and non-enzymatic antioxidants are recognized as the first line of defense against damage by free radicals and related reactants. The enzymatic defense mechanisms include superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), and glucose-6-phosphate dehydrogenase (G6PD) (Singh and Pathak, 1990) whereas direct free radical scavengers such as melatonin (Diplock, 1995) are classified as non-enzymatic defense system (Pablos *et al*., 1998).

Melatonin is synthesized mainly in the pineal gland and extra-pineal areas and is known to have powerful antioxidant properties (Hardeland, 2005) among several other important biological actions (Apeldoorn *et al*., 1999; Kliger *et al*., 2000; Zeman *et al*., 2001; Brennan *et al*., 2002; Gharib *et al*., 2008). Melatonin is mainly produced during a long period of darkness (Zeman and Herichová, 2011). Photoperiodic variations in melatonin synthesis have previously been investigated in

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poultry (Pablos *et al*., 1998; Zawilska *et al*., 2007). The length of dark phase influences the duration of elevated melatonin in turkeys' pineal gland and retina (Zawilska *et al*., 2007) whereas Liou *et al*. (1987) reported that the amplitude of melatonin concentrations was greater when hens were held under a short dark period.

Lighting regimes are now routinely used in broiler production to improve growth performance as well as reduce the incidence of metabolic disorders such as leg problems, sudden death syndrome and ascites. Light programs include a period of darkness each day that may have positive influence on melatonin production (Lessons and Summers, 2005). Similar to mammals, its secretion increases soon after the onset of darkness, peaks approximately in the middle of the dark phase, and then declines to basal levels (Pablos *et al*., 1998). Previous reports indicated that peak level of melatonin in chicks coincides with the peak antioxidant capacity of plasma and SOD activity in different tissues of chicks (Albarrán *et al*., 2001) and that photoperiodic-dependent variation in melatonin synthesis influences the rhythmic activity of antioxidative enzymes (Barlow-Walden *et al*., 1995; Pablos *et al*., 1995; Reiter, 1995; Pablos *et al*., 1998; Albarrán *et al*., 2001). This illustrates that changes in physiological levels of melatonin are adequate to alter the antioxidative defense system. In contrast, continuous light exposure caused a significant reduction in both serum melatonin concentration and antioxidant levels in mammals (Benot *et al*., 1998; 1999) and chickens (Pablos *et al*., 1998; Albarrán *et al*., 2001). In addition, melatonin decreased the homocysteine level in brain homogenates (Osuna *et al*., 2002) and blood of rats (Murawska-Cialowicz *et al*., 2008) and therefore may have a role in protecting cells from oxidative damage (Osuna *et al*., 2002; Yuce *et al*., 2007).

As far as we know, previous studies (Albarrán *et al*., 2001; Zawilska *et al*., 2007) have evaluated effects of lighting programs with long, dark phases (nonintermittent photoperiods) and continuous light exposure on melatonin levels in turkeys and chickens. Since intermittent lighting program (1L:3D) is a well-established and globally used program in rearing of broilers, we investigated its effects during the rearing period on serum and brain melatonin level and antioxidant status of broilers at the end of dark phase in comparison with continuous and non-intermittent (8L:16D) lighting programs.

Materials and Methods

Experimental design

60 one-day-old commercial broiler chicks (Cobb 500) of both sexes were purchased and kept in separate rooms with food and water *ad libitum*. The nutritional requirements were balanced according to Cobb 500 manual recommendations. All birds were reared in continuous light until 3 days of age before being placed in one of three light programs: 1) intermittent lighting (IL) (1L:3D cycles), 2) continuous lighting (CL) (24L), or 3) non-intermittent, restricted lighting (NIL) (8L:16D). The light intensity at the level of birds' heads of each group was approximately the same.

At day 42 of age, sera were collected from all chicks of each group at 8:00 am. Blood collection was done under dim red light at the end of the dark phase for groups 1 and 3 and under the normal room light for group 2. Thereafter, chicks were humanely killed and brains were dissected and kept frozen (-70ºC) until use. Serum and brain melatonin levels as well as selected serum antioxidant enzyme activity or concentration were assayed. Furthermore, feed intake, body weight, and FCR were measured at the end of the experiment. The experiment was carried out in accordance with the guidelines for use of animals in research approved by School of Veterinary Medicine, Shiraz University.

Melatonin determination

Melatonin levels in sera and brain homogenates were measured by enzyme-linked immunosorbent assay (ELISA), using a commercial kit (Chicken melatonin (MT) ELISA Kit, Shanghai Crystal Day Biotech, China) according to manufacturer's protocol.

To prepare brain homogenates, brains were rinsed in ice-cold PBS (0.02 mol/L, pH=7.0-7.2) to remove excess blood, minced into small pieces, then homogenized on ice. The resulting suspension was subjected to ultra-sonication for further breakage of the cell membranes (3 times with 30 sec intervals). After that, the homogenates were centrifuged for 5 minutes at 1500 × *g*. The supernatant was removed and assayed immediately.

Enzymatic activity

Superoxide dismutase (SOD)

Blood SOD activity was evaluated with SOD detection Biorex kit (Fars, Iran), based on Woolliams *et al*. (1983) method, according to the manufacturer's instructions. The role of SOD is to accelerate the dismutation of toxic superoxide produced during oxidative energy processes to hydrogen peroxide and molecular oxygen. This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. SOD activity is quantified as the degree of inhibition of this reaction where one unit of SOD causes 50% inhibition of the rate of reduction of INT under the conditions of the assay. SOD levels were recorded at 505 nm and expressed as unit per liter (U/L).

Glutathione reductase (GR)

The activity of GR was determined with Biorex kit (Fars, Iran) in serum based on methods from Paglia and Valentine (1967). One enzyme unit was defined as the oxidation of 1 µmol NADPH per minute at 340 nm and expressed as unit per liter of serum.

Glutathione peroxidase (GPx)

The blood activity of GPx was evaluated with GPx detection Biorex kit (Fars, Iran) based on Paglia and Valentine's (1967) method, according to the manufacturer's instructions. GPx catalyzes the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm against a blank was measured spectrophotometrically. One unit (U) of GPx activity was defined as the amount of enzyme that converts 1 µmol of NADPH to $NADP^+$ per minute. The GPx activity was expressed as unit per liter (U/L).

Glucose-6-phosphate dehydrogenase (G6PD)

G6PD activity was assayed according to methods described by Burtis & Ashwood (1994). The potency of the enzyme in reducing $NADP^+$ was quantified by measuring the rate of change in absorbance at 340 nm and expressed as unit per milliliter (U/mL).

Serum homocysteine determination

The measurement of serum total homocysteine (tHcy) was performed using the enzymatic assay kit (Diazyme Com, USA) and measured as micromoles per liter of serum.

Statistical analysis

Data were analyzed by SPSS software, version 11.5 (SPSS Inc, Chicago, Illinois). For comparison of different parameters among three experimental groups, one-way ANOVA test and Tukey's multiple comparison tests were used. A *P*-value less than 0.05 was considered statistically significant.

Results

No significant differences were observed in serum and brain melatonin concentrations between chicks from different lighting programs (Figure 1A and B, respectively). Serum SOD, GR, GPx and G6PD activity of birds were also similar (Figure 1C to F, respectively) as was serum homocysteine concentration (Fig. 1G). Final body weight and feed intake were not significantly affected by the different lighting programs ($P > 0.05$). FCR in chickens reared under IL program were slightly lower than the other groups though the difference was insignificant ($P > 0.05$) (data not shown).

Discussion

The intermittent lighting program has previously been shown to have beneficial effects on numerous parameters of broiler health, including survival, culling rate, immunity and leg health. Body weight gain, feed conversion ratio, carcass quality and fat deposition in broilers have also been shown to improve (Rahimi *et al*., 2005; Onbaşılar *et al*., 2007; Mahmud *et al*., 2011). With reason, intermittent lighting program is commonly used in commercial broiler chicken production. Effects of lighting programs on melatonin levels in turkeys and chickens have mostly been investigated in programs with long dark phases (non-intermittent photoperiods) or continuous light exposure. Data about the possible effects of intermittent lighting program are scarce, which motivated our investigation on the effects of this lighting program (1L:3D) during rearing period on serum and brain melatonin levels as well as antioxidant status of broilers in comparison to continuous and non-intermittent (8L:16D) lighting programs.

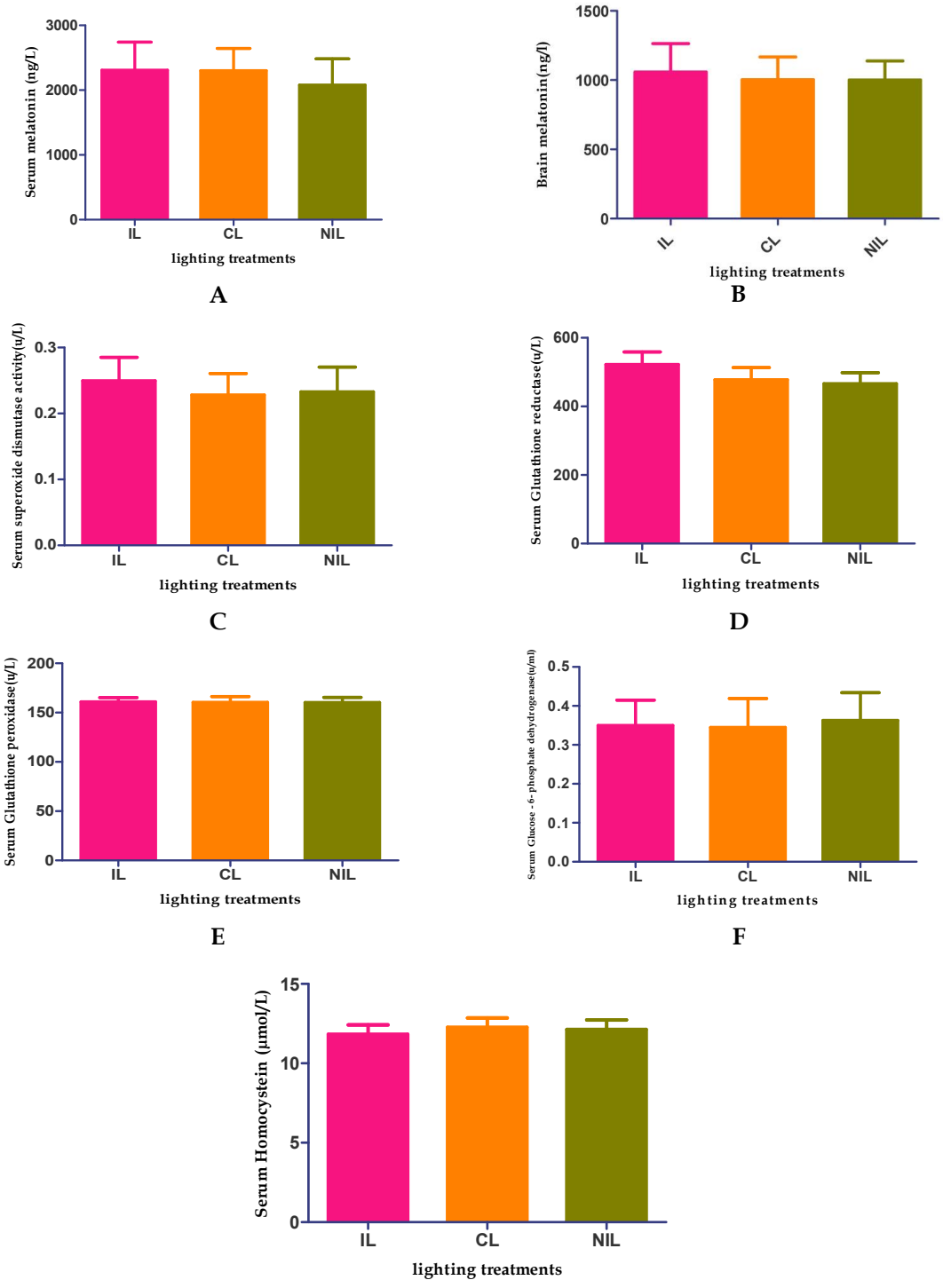

Figure 1. A) Serum melatonin concentration; B) Brain melatonin concentration; C) Serum superoxide dismutase activity; D) Serum glutathione reductase activity; E) Serum glutathione peroxidase activity; F) Serum glucose-6-phosphate dehydrogenase activity; and G) Serum homocysteine concentration in all groups of the experiment (mean±SD).
Treatments: IL: Intermittent lighting program (1L:3D); CL: Continuous lighting program (24L); NIL: non intermittent restricted lighting program (8L:16D); No significant difference was observed among groups ($P > 0.05$).

Zawilska *et al*. (2007) evaluated the effect of photoperiod on melatonin content of the pineal gland, serum and retina of turkeys in 3 different lighting conditions including 16L:8D (long photoperiod), 12L:12D (regular photoperiod), and 8L:16D (short photoperiod). They reported that during the light phase, mean melatonin concentrations in the pineal gland and retina of turkeys subjected to the long photoperiod were significantly higher compared with those from birds maintained under the regular and short photoperiods. In addition, mean circulating melatonin levels were lowest in the short photoperiod. In contrast, we observed that intermittent lighting programs did not affect melatonin levels and antioxidant status in birds compared to continuous and non-intermittent programs. The reason for this discrepancy may be related to the fact that chicken's melatonin secretion increases soon after the onset of darkness and peaks in the middle of the dark phase, approximately. For example, Pablos *et al.* (1998) found that serum melatonin levels peaked in chickens after 4.5 hrs of a 10-hr dark period before declining. Moreover, melatonin levels rapidly fluctuate in response to light. For instance, Vakkuri *et al*. (1985) showed that light exposure for 80 min in pigeons reduced plasma melatonin by 85% and more importantly, melatonin concentration fell to 50% of the original value over 12 min. It is noteworthy that in contrast to Zawilska *et al*. (2007), we did not assay melatonin concentrations at different times during light and dark phases since we were interested only in the long-term effects of intermittent photoperiod on melatonin levels. Therefore, although there may be a fast rise and fall in melatonin secretion during short dark phase and an appreciable change in secretion pattern of melatonin during dark phase (Zawilska *et al*., 2007), we observed no significant changes in melatonin levels of serum and brain immediately before the beginning of light period. This may be due to a fast return of melatonin to basal level and shows that long-term exposure of intermittent lighting program does not affect the overall concentration of melatonin at the end of dark phase.

The antioxidant defense system is a crucial defense mechanism of the body against free radicals produced mainly in stressful conditions (Surai, 2002). The antioxidant properties of melatonin, along with its diverse functions, have been investigated in several previous studies. Melatonin is a powerful free-radical scavenger and a wide-spectrum antioxidant (Pieri *et al*., 1994; Hardeland, 2005). The amplitude of circadian melatonin rhythm is inversely related to the length of photoperiod in turkeys (Zawilska *et al*., 2007). Continuous exposure to light can abolish nocturnal melatonin rises accompanied by a reduction in night-time increases in GPx and SOD activities in several tissues of chicks (Pablos *et al*., 1998; Albarrán *et al*., 2001) and rodents (Tomas-Zapico *et al*., 2003; Túnez *et al*., 2003). Similarly, GPx activity decreased in several tissues of rats following pinealectomy (Baydas *et al*. 2002). These findings are consistent with our results as there were no significant differences in melatonin content in parallel with a lack of significant differences in antioxidant activities in chickens under different lighting programs.

In conclusion, intermittent lighting program during the rearing period of broilers does not affect serum and brain melatonin levels as well as antioxidant status at the end of the dark phase.

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