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TONIC IMMOBILITY AND VIGILANCE BEHAVIOURAL RESPONSES OF BROILER CHICKENS TO DIFFERENT LIGHTING REGIMENS DURING THE HOT-DRY SEASON, AND THE BENEFICIAL EFFECT OF MELATONIN

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ABSTRACT

Experiments were conducted with the aim of determining the influence of melatonin administration on vigilance and tonic immobility (TI) responses of Marshall Broiler chickens, reared on different lighting regimens and subjected to heat stress during the hot-dry season in the Northern Guinea Savannah zone of Nigeria. Simple random sampling was used to assign 300 broiler chicks into three groups, comprising 100 chicks each. Group I (12D:12L cycle) was raised under natural photoperiod of 12 h light and 12 h darkness, without melatonin supplementation. Group II (LL) was kept under 24-h continuous lighting, without melatonin administration. Group III (LL + MEL) was raised under 24-h continuous lighting; with melatonin supplementation at 0.5 mg/kg per os, via drinking water using a syringe. Beginning from day-old, birds in group III were individually administered with melatonin once daily for 56 consecutive days at 17:00 h. TI was induced by manual restraint, and vigilance at self-righting graded for three days, two weeks apart in 15 labeled broiler chickens from each of the three groups at 06:00 h, 13:00 h and 18:00 h starting from week 4 up to week 8. Each bird was laid on its back in a U-shaped cradle, covered with cloth. Thermal microenvironment parameters of dry-bulb temperature (DBT) and relative humidity (RH) were recorded at the experimental site and concurrently during the vigilance and TI tests. The weekly mean temperature-humidity index (THI), calculated inside the poultry house, was lowest at week 4 of the study, with the value 48.60 ± 0.08 °C. At day 28, the relationship between the THI and TI induction attempts was stronger in 12D:12L cycle (r = 0.589, P < 0.001) than LL (r = 0.264, P > 0.05) and LL + MEL (r = 0.096, P > 0.05) broilers, indicating that the broiler chickens on 12D:12L cycle were more active compared to their melatonin-treated counterparts, apparently due to the adverse effects of high DBT and RH on the broilers during the hot-dry season. The results showed that the number of TI induction trial attempts fluctuated insignificantly as the hours of the day increased; especially in 12D:12L cycle birds, where the relationship was negatively correlated (r = -0.130, P > 0.05). The highest numbers of TI induction trial attempts (2.13 ± 0.34 and 2.15 ± 0.22) were recorded at 13:00 h in 12D:12L cycle and LL groups, respectively, when the birds were 56 days' old. The overall mean values of induction trial attempts differed significantly (P < 0.0001) between the groups; with the lowest mean values of 1.22 ± 0.4 recorded in Group III birds, administered with melatonin. At d 42, the lowest mean TI duration of 101.87 **±** *10.24 s in the LL group recorded at 06:00 h rose (P < 0.001) to 184.07 ± 23.69 s at 13:00 h. Again, the overall mean values of TI duration differed significantly (P < 0.0001) between the groups, with the highest mean duration of 167.82 ± 8.35 s, recorded in group III broiler chickens administered with melatonin. Unlike the result obtained on TI duration, but similar to that recorded in the number of induction attempts, no significant difference was found in the values of mean vigilance behaviour rankings at the different hours of the day. The overall mean vigilance behavioural ranking values of 1.85 ± 0.07 and 1.70 ± 0.08, obtained in 12D:12L cycle and LL broiler chickens, respectively were higher (Kruskal-Wallis test = 20.87; P < 0.0001) than the value of 1.44 ± 0.05 recorded in melatonin-treated birds. The results indicated that the broiler chickens belonging to both 12D:12L cycle and LL groups were more emotional, fearful or anxious, compared to their counterparts in group III that were treated with exogenous melatonin. It is concluded that melatonin elicits boldness and confidence by suppressing freezing behaviour in broiler chickens.*

Keywords: *Tonic immobility; lighting regimens; hot-dry season; broiler chickens; vigilance; melatonin*

1. INTRODUCTION

It has been established that multiple meteorological factors adversely affect the health and productivity of chickens and they constitute a major economic threat to poultry farmers in tropical zones of the world (Ayo *et al*., 2005; Sinkalu *et al.,* 2008; Piestun *et al*., 2011). High AT and high RH are the most important thermal environmental parameters, causing heat stress in the tropics (Oluyemi and Adetowun, 1979; Togo *et al*., 2005; Sinkalu and Ayo, 2009; Nardone *et al.,* 2010). Tonic immobility (TI) and vigilance in birds are anti-predator behaviours (Oden *et al*., 2005), which are of value in the measurement of the level of fear or stress. They are also important in the evaluation of

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adaptability of poultry to environmental stress factors, including climate variability (Forkman *et al.,* 2007; Saint-Dizier *et al.,* 2008). Fear reactions are elicited in several stressful situations, including those that are related to predator defence (Oden *et al*., 2005; Wang *et al*., 2014). Predator-prey interactions are regulated by the ability of individuals to detect, and then approach or avoid one another (Fernandez-Juricic *et al*., 2011). TI is the last in a series of defensive behaviours displayed in response to attack by a predator, and is thought to function by reducing stimuli leading to further attack (Gallup, 1979). Fear and anxiety are two closely related emotions, which increase with age in chickens (Nordquist *et al*., 2011). Fear is generally defined as a reaction to the perception of actual danger, whereas anxiety is defined as the reaction to a potential danger that threatens the integrity of the individual (Forkman *et al.,* 2007). Fear and anxiety are damaging stressors, resulting in impaired animal welfare (Spinka, 2012), poor production (Goerlich *et al*., 2012), regulation of the hypothalamic-pituitary-adrenal axis (Verbrugghe *et al*., 2012) and predispose to depressive-like behaviour (Zagron and Weinstock, 2006; Salmeto *et al*., 2011). TI is characterised as a fear response because it is attenuated by procedures that reduce fear and enhanced by those that increase it (Heiblum *et al.,* 1998). Some characteristics of TI described by Gallup (1979) include temporary suppression of the righting response, reduced vocalisation, intermittent eye closure, rigidity, Parkinsonian-like muscle tremors in the extremities, altered electroencephalographic patterns and changes in heart rate, respiration and core body temperature. For many years, broiler chickens have been reared under continuous or near continuous (23L:1D) photoperiods in order to maximize feed consumption and growth rate (Abbas *et al*., 2008). However, several investigations showed that using continuous lighting (LL) programmes induce sleep deprivation and severe stress responses (Kliger *et al*., 2000; Campo and Davila, 2002). The resultant effect of raising chickens under unfavourable conditions is oxidative stress, involving excessive free-radical generation and propagation, resulting in cell destruction and the associated production losses (Altan *et al*., 2003; Ayo *et al*., 2005; Schwean-Lardner *et al*., 2012). Hence, Ayo and Sinkalu (2003) showed for the first time that the administration of the antioxidant, ascorbic acid (vitamin C) to chickens ameliorated the heat-stress induced hyperthermia during the hot-dry season. Melatonin, the vitamin of life, is a potent antioxidant (Tan *et al*., 2003; Korkmaz *et al*., 2011; Sinkalu and Ayo, 2011), responsible for synchronising the circadian rhythms of animals (Deep *et al*., 2012; Vielma *et al*., 2014). Besides its antioxidant action, it has been shown repeatedly to potentiate the effects of other antioxidants such as ascorbate, trolox (a tocopherol analogue), reduced glutathione, or NADH (Gitto *et al*., 2001; Tan *et al*., 2003). Unlike other antioxidants, melatonin does not undergo redox cycling, the ability of a molecule to undergo reduction and oxidation repeatedly. Redox cycling may allow other antioxidants (such as vitamins C and E) to regain their antioxidant properties. On the other hand, melatonin, once oxidized, cannot be reduced to its former state because it forms several stable end-products upon reacting with free radicals (Tan *et al*., 2000b). Therefore, it has been referred to as a terminal (or suicidal) antioxidant (Tan *et al*., 2000a; Zisapel and Cardinali, 2008). In a behavioural study during the early rainy season in the Northern Guinea Savannah zone of Nigeria, Sinkalu and Ayo (2011) reported, for the first time, that melatonin administration induced hypoactivity in broiler chickens. Data on the modulating effects of exogenous melatonin administration on TI and vigilance behavioural responses of broilers, reared on different lighting regimens during the hot-dry season in the hot-humid zones of the world are currently lacking in the available literature.

The aim of the present study was to evaluate the impact of different lighting regimens and heat stress on TI and vigilance behavioural responses of broiler chickens, administered with melatonin during the hot-dry season.

2. MATERIALS AND METHODS

2.1 EXPERIMENTAL SITES AND LOCATIONS

The experiment was performed in the poultry house of the Department of Veterinary Physiology, Ahmadu Bello University, Zaria (11^o $10^/$ N, 07^o $38^/$ E), Kaduna State, located in the Northern Guinea Savannah zone of Nigeria.

2.2 EXPERIMENTAL PERIOD AND FLOCK MANAGEMENT

The research was carried out between 2^{nd} April and 29^{th} May, 2010 during the hot-dry season, prevailing in the Northern Guinea Savannah zone of Nigeria. Three hundred (300) broiler chicks, belonging to the Marshall breed were obtained at day-old from Fidan Farms, a branch of Fidan Investments Nigeria Limited, Ibadan, Oyo State, Nigeria on 2^{nd} April, 2010. The Indian Poultry Equipment Manufacturer's Association (IPEMA, 2010) reported that the Marshall breed was developed in Nashik, India for high performance. The breed was launched in 2009 as a break-through in the Indian and, by extension, global broiler industry. Meticulous screening for feed conversion and meat yield along with severe selection pressures on growth rates have ensured that Marshall breeds have an edge over other breeds of broiler chickens. They are ultra-converters and have shown best performance efficiencies across multiple metrics, including feed conversion, liveability, and growth rate. The breed is fast emerging as a leading broiler breed with sales in over 15 countries and a joint venture in Africa (IPEMA, 2010).

Complete routine vaccinations and appropriate medications were administered to the birds according to standard veterinary procedures, from day-old up to the end of the experiment. The chickens were vaccinated against infectious bursal disease at weeks 2 and 4; and against Newcastle disease at day old, and weeks 1 and 3 of age. They were given prophylactic treatment at week 5 against avian coccidiosis using Amprolium 250® (1 g/litre of drinking water). Deworming was carried out using piperazine solution, dissolved in drinking water at a single oral dose of 160 mg/kg. The broilers were raised on deep litter system and maintained on commercial feeds obtained from Vital Feeds, Grand Cereals and Oil Mills Limited, Jos, Plateau State, Nigeria (Table 1.0). Starter diets were fed to the chicks from day-old to 21 d of life, after which they were placed on finisher diet up to the end of the experiment. They were given access to feeds and water *ad libitum*. Birds belonging to groups I, II and III had overall mortality percent of 11, 14 and 3 %, respectively.

2.3 EXPERIMENTAL DESIGN

Simple random sampling was used to select and assign 300 broiler chicks into three groups, comprising hundred (100) chicks each. Group I was raised under natural photoperiod of about 12-h light and 12-h darkness cycle, without melatonin supplementation. Group II was kept under 24-h continuous lighting, without melatonin supplementation. Group III was raised under 24-h continuous lighting and administered daily and individually with melatonin (ISI Brands, Inc. Utah, USA) at 0.5 mg/kg *per os* (Nisbet *et al*., 2008), via drinking water. Beginning from day-old till end of the experiment, melatonin was administered once daily at 17:00 h (Deacon and Arendt, 1995).

Table 1: Nutritive contents of the ration fed to the broiler chickens

Nutrient Contents	Amount in % by Weight	
	Pelletised Starter Feed (1 – 21 d)	Pelletised Finisher Feed (22 – 56 d)
Calcium (%)	01.20	01.00
Available Phosphorous (%)	00.45	00.40
Metabolisable Energy (Kcal/kg)	2,800	2,900
*Proximate Analysis (%):		
a. Dry Matter	93.72	95.32
b. Crude Protein	22.00	18.56
c. Crude Fibre	03.94	04.27
d. Oil	05.09	04.23
e. Ash	10.47	07.91
f. Nitrogen Free Extract	58.50	65.03

*Analyzed in the Biochemical Laboratory, Department of Animal Science, Ahmadu Bello University, Zaria, Nigeria

2.4 EXPERIMENTAL PROCEDURES

2.4.1 Meteorological Data

The dry- and wet-bulb temperatures were taken concurrently with the hourly TI and vigilance behaviour tests for each treatment group, using dry- and wet-bulb thermometer (Brannan, UK). From the data, RH and temperature-humidity index (THI) at each hour of measurement were calculated. The THI was calculated using the following slightly modified equation of Avendano-Reyes *et al.* (2006):

$$THI = (0.41 \times AT) + RH\,(AT - 14.4) + 23.2$$

2.4.2 Measurement of Tonic Immobility Responses

Induction of TI was performed at 4th, 6th and 8th weeks as described by Oden *et al*. (2005) in 15 birds from each group at 06:00 h, 13:00 h and 18:00 h by placing a bird at each hour of measurement on its back onto an improvised cradle, and gently pressing its breast for 15 s. Each cradle was constructed from Dunlop foams measuring 50 x 40 x 25 cm and covered with cloth, similar to those used by Heiblum *et al*. (1998) and Saint-Dizier *et al*. (2008). The centre of the cradle was scooped to accommodate each bird on dorsal recumbence. If after releasing the bird, it righted itself within 2 s, the procedure was repeated. This was done up to 5 times. The resulting TI duration was recorded; and if no induction occurred, this was recorded as “0”. If the bird did not resume a standing position after 600 s, the TI was interrupted.

2.4.3 Vigilance Behaviour Ranking

The vigilance behaviour at self-righting of each of the TI tests observed in the birds was ranked or scored as described by Oden *et al*. (2005) with a slight modification on a scale of 1 to 3; with 1 representing fearlessness and 3 fearfulness. Briefly, vigilance behaviour of each of the birds at self-righting was ranked as follows: 1, if the process of self-righting was quick and spontaneous, 2, if the process was slow and with a short vigilance period (peeping and vocalisation); and 3, if the process was very slow and with a prolonged period of vigilance.

All data obtained, excluding those on vigilance behaviour, analyzed using the Kruskal-Wallis test, were subjected to repeated-measures analysis of variance and Pearson’s correlation analysis. Multiple means were compared by Duncan’s (1955) multiple range test. Values of $P < 0.05$ were considered significant. Data were expressed as mean ± standard error of the mean (mean ± S.E.M.). Analyses were performed using SPSS statistical software package (Version 16.0; New York, USA).

3. RESULTS

3.1 METEOROLOGICAL DATA INSIDE THE POULTRY HOUSE DURING THE STUDY PERIOD

The results of the meteorological data during the study period are presented in Tables 2 – 3. Table 2 shows that the weekly mean THI recorded inside the poultry house was lowest at 4th week of the study, with the value of 48.00 ± 0.08 °C, but highest at 8th week with the value of 51.57 ± 0.98 °C ($P < 0.001$). Inside the poultry house where the birds were reared, the RH fluctuated significantly ($P < 0.001$) between 67.76 ± 2.16 % recorded at 19:00 h, and 77.95 ± 1.45 % obtained at 06:00 h, with an overall mean of 72.70 ± 1.32 % (Table 3). The mean AT values fluctuated significantly ($P < 0.0001$) as the hours of the day increased, with peak value of 37.86 ± 0.63 °C recorded at 13:00 h (Table 3).

3.2 TONIC IMMOBILITY RESPONSES

The number of induction trial attempts fluctuated insignificantly ($P > 0.05$) as the hours of the day increased (Table 4). The highest numbers of induction trial attempts were 2.13 ± 0.34 and 2.15 ± 0.22 in 12D:12L cycle and CL groups, respectively, and they were recorded at 13:00 h, when the birds were 56 days' old. The overall mean values of induction trial attempts differed significantly ($P < 0.0001$) between the groups, with the lowest mean values of 1.22 ± 0.4 recorded in group III birds administered with melatonin (Table 7).

Table 5 shows that the TI duration fluctuated as the hours of the day increased during the study period. At d 42, the lowest mean TI duration of 101.87 **±** 10.24 s obtained in the CL group was recorded at 06:00 h, and the value rose ($P < 0.001$) to 184.07 ± 23.69 s at 13:00 h. The overall mean values of TI duration differed significantly ($P < 0.0001$) between the groups, with the highest mean duration of 167.82 ± 8.35 s being recorded in group III broiler chickens administered with melatonin (Table 7).

The results of the vigilance behavioural rankings of the broilers are shown in Table 6. Unlike the results obtained on TI duration, but similar to that recorded in the number of induction attempts, no significant difference was found in the values of mean vigilance behaviour rankings at the different hours of the day. The overall mean vigilance behaviour ranking values of 1.83 ± 0.07 and 1.70 ± 0.08, obtained in 12D:12L cycle and CL broiler chickens, respectively were higher ($P < 0.05$) than the value of 1.44 ± 0.05 recorded in melatonin-treated birds (Table 7).

Tables 8 – 10 shows that at day 28, the relationship between the THI and the TI induction attempts was stronger in 12D:12L cycle ($r = 0.589$, $P < 0.001$) than LL ($r = 0.264$, $P > 0.05$) and LL + MEL ($r = 0.096$, $P > 0.05$)

Table 2: Weekly thermal environment data inside the poultry house during the study period (Mean ± SEM)

Week	Relative humidity, %	Temperature-humidity index, °C	Dry-bulb temperature, °C
4	74.62 ± 2.34	48.00 ± 0.80^{a}	31.48 ± 0.84^{a}
6	69.05 ± 1.97	50.33 ± 1.06^{ab}	34.29 ± 0.93^{b}
8	74.43 ± 2.42	51.57 ± 0.98^{b}	34.48 ± 1.06^{b}
Overall Mean ± SEM	72.70 ± 1.32	49.97 ± 0.64	33.42 ± 0.57

$^{a,\,b}$: Means for the same column having different superscript letters are significantly ($P < 0.05$) different.

Table 3: Diurnal fluctuations in dry-bulb temperature, temperature-humidity index and relative humidity inside the poultry house during the study period (Mean ± SEM)

Hour of the day, h	Relative humidity, %	Temperature-humidity index, °C	Dry-bulb temperature, °C
06:00	77.95 ± 1.45^{b}	45.98 ± 0.32^{a}	28.62 ± 0.31^{a}
13:00	72.38 ± 2.61^{ab}	55.77 ± 1.04^{c}	37.86 ± 0.63^{c}
19:00	67.76 ± 2.16^{a}	49.94 ± 0.40^{b}	33.76 ± 0.60^{b}
Overall Mean ± SEM	72.70 ± 1.32	50.56 ± 0.64	33.41 ± 0.57

$^{a,\,b,\,c}$: Means for the same column having different superscript letters are significantly ($P < 0.05$) different.

Table 4: Effects of lighting regimens and melatonin administration on diurnal variations in the number of induction attempts of Marshall broiler chickens during the hot-dry season (Mean $\pm$ SEM; n = 15)

Age, Week	Hour of the day, h	Group		
		12D:12L	CL	CL+MEL
4	06:00	1.87 ± 0.19	1.27 ± 0.12	1.07 ± 0.07
	13:00	1.93 ± 0.32	1.40 ± 0.16	1.23 ± 0.09
	19:00	1.67 ± 0.19	1.74 ± 0.25	1.13 ± 0.09
6	06:00	1.73 ± 0.18	1.18 ± 0.09	1.20 ± 0.11
	13:00	1.89 ± 0.24	1.40 ± 0.16	1.49 ± 0.19
	19:00	1.67 ± 0.19	1.47 ± 0.17	1.33 ± 0.16
8	06:00	2.07 ± 0.3	1.67 ± 0.19	1.24 ± 0.11
	13:00	2.13 ± 0.34	2.15 ± 0.22	1. 27 ± 0.12
	19:00	1.75 ± 0.21	2.00 ± 0.22	1.21 ± 0.22

12D:12L cycle = 12 h darkness and 12 h light cycle; CL = Continuous lighting; CL+MEL = Continuous lighting with melatonin administration.

Table 5: Effects of light regimens and melatonin administration on diurnal variations in tonic immobility duration in Marshall Broiler chickens during the hot-dry season

Age, Week	Hour of the day, h	Group (s; Mean $\pm$ SEM; n = 15)		
		12D:12L	CL	CL+ MEL
4	06:00	43.33 ± 9.5	83.73 ± 8.25	117.40 ± 19.93
	13:00	73.60 ± 15. 99	88.27 ± 9.08	162.93 ± 26.79
	19:00	53.27 ± 11.03	95.20 ± 9.53	112.07 ± 14.80
6	06:00	76.27 ± 11.37	101.87 ± 10.24[a]	120.27 ± 10.40
	13:00	98.2 ± 13.21	184.07 ± 23.69[b]	135.47 ± 21.22
	19:00	92.6 ± 14.78	118.07 ± 14.21[a]	115.73 ± 27.06
8	06:00	54.33 ± 8.42	192.80 ± 29.34	226.0 ± 20.63
	13:00	68.53 ± 10.87	154. 67 ± 27.55	254.27 ± 20.07
	19:00	61.53 ± 9.4	151.53 ± 30.09	266.27 ± 15.61

[a, b:] Means for the same column having different superscript letters are significantly ($P < 0.05$) different. 12D:12L cycle = 12 h darkness and 12 h light cycle; CL = Continuous lighting; CL+MEL = Continuous lighting with melatonin administration.

Table 6: Effects of age, light regimen and melatonin administration on diurnal fluctuations in vigilance behaviour of Marshall Broiler chickens during the hot-dry season (Mean $\pm$ SEM; n = 15)

Age, Week	Hour of the day, h	Group		
		12D:12L	CL	CL+ MEL
4	06:00	1.73 ± 0.21	1.60 ± 0.19	1.53 ± 0.19
	13:00	1.83 ± 0.24	1.67 ± 0.18	1.35 ± 0.13
	19:00	1.72 ± 0.18	1.66 ± 0.16	1.47 ± 0.13
6	06:00	2.07 ± 0.18	1.40 ± 1.13	1.48 ± 1.2
	13:00	1.93 ± 0.23	1.47 ± 0.17	1.20 ± 0.15
	19:00	2.21 ± 0.23	1.93 ± 0.23	1.33 ± 0.13
8	06:00	1.65 ± 0.24	1.86 ± 0.19	1.55 ± 0.19
	13:00	1.67 ± 0.21	2.27 ± 0.52	1.60 ± 0.21
	19:00	1.78 ± 0.18	1.49 ± 0.13	1.53 ± 0.19

12D:12L cycle = 12 h darkness and 12 h light cycle; CL = Continuous lighting; CL+MEL = Continuous lighting with melatonin administration.

Table 7: Effects of age, light regimens and melatonin administration on overall mean tonic immobility responses in Marshall Broiler chickens during the hot-dry season (Mean $\pm$ SEM; n = 15).

		Group		
Parameter	Age, week	12D:12L	CL	CL + MEL
TI induction attempts	4	1.82 ± 0.14	1.47 ± 0.11	1.11 ± 0.05
	6	1.78 ± 0.12	1.33 ± 0.08	1.33 ± 0.09
	8	1.98 ± 0.16	1.93 ± 0.12	1.22 ± 0.06
	Overall mean	1.85 ± 0.80[c]	1.58 ± 0.06[b]	1.22 ± 0.4[a]
TI duration, s	4	56.73 ± 7.29	89.07 ± 5.11	130.8 ± 12.38
	6	89.02 ± 7.57	134.67 ± 10.99	123.82 ± 11.77
	8	61.47 ± 5.50	166.33 ± 16.61	248.84 ± 10.96
	Overall mean	69.07 ± 4.11[a]	130.02 ± 7.33[b]	167.82 ± 8.35[c]
Vigilance behaviour	4	1.76 ± 0.11	162.0 ± 0.10	1.44 ± 0.09
	6	2.09 ± 0.12	1.60 ± 0.11	1.31 ± 0.08
	8	1.71 ± 0.11	1.87 ± 0.19	1.56 ± 0.11
	Overall mean	1.83 ± 0.07[b]	1.70 ± 0.08[b]	1.44 ± 0.05[a]

[a, b, c:] Means for the same row having different superscript letters are significantly (P < 0.05) different. TI = Tonic Immobility; 12D:12L cycle = 12 h darkness and 12 h light cycle; CL = Continuous lighting; CL+MEL = Continuous lighting with melatonin administration.

Table 8: The relationships between thermal environment parameters and tonic immobility (TI) induction attempts of Marshall broiler chickens administered with melatonin during the hot-dry season at day 28

Correlated parameters	Group		
	12D:12L	CL	CL+MEL
TI induction attempts and relative humidity	-0.234[NS]	-0.078[NS]	-0.314[NS]
TI induction attempts and dry-bulb temperature	0.572***	0.283[NS]	0.260[NS]
TI induction attempts and temperature-humidity index	0.589***	0.264[NS]	0.096[NS]
TI induction attempts and hour of the day	-0.130[NS]	0.000[NS]	0.204[NS]

[NS] = Non-significant correlation (P > 0.05), *** = P < 0.001

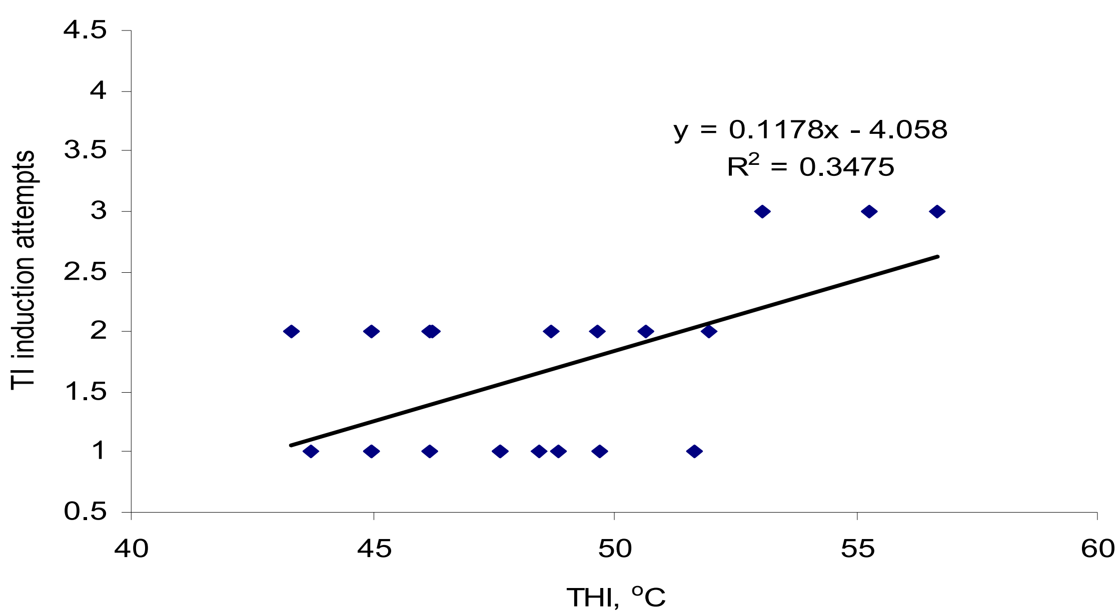

Figure 2:The relationship between temperature-humidity index and tonic immobility (TI)induction attempts of Marshall Broiler chickens administered with melatonin during the hot-dry season at day 28

Table 9: The relationship between meteorological parameters and tonic immobility (TI) induction attempts of Marshall broiler chickens administered with melatonin during the hot-dry season at day 42

Correlated parameters	Group		
	12D:12L	CL	CL+MEL
TI induction attempts and relative humidity	-0.065^{NS}	0.066^{NS}	-0.347^{NS}
TI induction attempts and dry-bulb temperature	0.376^{NS}	0.037^{NS}	0.142^{NS}
TI induction attempts and temperature-humidity index	0.351^{NS}	0.213^{NS}	-0.004^{NS}
TI induction attempts and hour of the day	0.023^{NS}	0.266^{NS}	0.145^{NS}

^{NS} = Non-significant correlation (P > 0.05)

Table 10: The relationship between thermal environment parameters and tonic immobility (TI) induction attempts of Marshall broiler chickens administered with melatonin during the hot-dry season at day 56

Correlated parameters	Group		
	12D:12L	CL	CL+MEL
TI induction attempts and relative humidity	0.219^{NS}	-0.153^{NS}	0.009^{NS}
TI induction attempts and dry-bulb temperature	-0.325^{NS}	-0.201^{NS}	0.015^{NS}
TI induction attempts and temperature-humidity index	-0.246^{NS}	-0.264^{NS}	-0.013^{NS}
TI induction attempts and hour of the day	-0.197^{NS}	0.219^{NS}	0.140^{NS}

^{NS} = Non-significant correlation (P > 0.05)

4. DISCUSSION

4.1 THERMAL ENVIRONMENT PARAMETERS DURING THE STUDY PERIOD

The thermal environment data obtained inside the poultry house were characterized by high THI, AT and RH, established for the hot-dry season in the Northern Guinea Savannah zone of Nigeria (Igono *et al*., 1983; Ayo *et al*., 1998). The AT recorded during the study period were outside the established thermoneutral zones of 12 – 24 ^{o}C (Selyansky, 1975; Plyaschenko and Sidorov, 1987) and 20.9 – 28.5 ^{o}C (Donkoh, 1989; Prinzinger *et al*., 1991) in poultry species reared in the temperate and tropical regions of the world, respectively. The finding agreed with the results obtained by Ryder *et al*. (2004) and Nardone *et al*. (2010) that mortality and poor performance of broiler chickens adversely affect the economy of broiler producers in regions in which ambient temperatures approach or exceed 40 ^{o}C. Similarly, the extremely high RH recorded in this study was about two times higher than the values of 30 to 40 %, established for chickens raised in the tropics (Oluyemi and Roberts, 2000). This factor may be predominantly responsible for the undesired heat stress the birds were subjected to, as demonstrated by the high THI obtained in the study. The high THI showed that the thermal environmental conditions, prevailing during the study period were uncomfortable for the raising of broiler chickens in the zone. This finding agrees with the report of Marai *et al.* (2002) that animals raised under conditions with THI higher than 29 °C suffer heat stress. Therefore, measures aimed at alleviating the high THI recorded in the present study are of paramount importance in order to reduce economic losses due to the adverse effects of heat stress on broilers. This is because during high environmental temperatures and high relative humidity, heat dissipation by evaporative cooling is impeded (Yahav *et al*., 1995; Keim *et al.,* 2002). Brown-Brandl *et al.* (1997) demonstrated in a study on physiological responses of male (tom) turkeys to temperature and humidity change with age that RH had a stronger effect than temperature. The harsh weather conditions of high AT reported in this work triggered panting, but elevated RH made evaporative cooling practically impossible (Keim *et al*., 2002). The meteorological conditions prevailing during the hot-dry season were unfavourable for the rearing of the broilers in the Northern Guinea Savannah zone of Nigeria. Hence, measures aimed at decreasing the adverse effects of the conditions on birds may enhance their performance and productivity in the zone.

Increased evaporation of water from the drinkers, body and faecal droppings of the broilers may be responsible for the extremely high RH recorded inside the poultry house during the present study, which prevented effective evaporative cooling. The finding agrees with the report of Keim *et al.* (2002) that meteorological parameters affecting heat transfer are a combination of air temperature, wind speed, relative humidity and radiation. The deleterious effects of these meteorological parameters and heat transfer from the environment to the body of the animal, has been reported to increase body temperature and respiratory rate in broilers (Kassim and Norziha, 1995; Nardone *et al.,* 2010). In addition, the meteorological effects include alteration in physiological characteristics of various circadian rhythms (Cable *et al*., 2007), impairment in performance, metabolic and health status, and immune response (Ayo *et al.,* 1996; Nardone *et al.,* 2010; Ondruska *et al*., 2011).

The lower number of TI induction attempts and longer TI duration recorded in the melatonin-treated group demonstrated that melatonin induced hypoactivity in the treated broiler chickens. This finding agrees with the result of Zhdanova (2005) that melatonin promotes sleep in healthy humans and other diurnal animals, if administered during habitual hours of wakefulness. Sleep is influenced by both ambient and body temperatures (Kumar *et al.,* 2009). Based on behavioural observations, Mujahid and Furuse (2009) and Moller (2010) reported that low-temperature exposure decreased distress, vocalisations and spontaneous activity, and induced sleep-like behaviour in neonatal chicks. In the present study, active wakefulness decreased, while standing or sitting motionless with eyes closed or open, and sleeping posture rose significantly. It has been shown that behavioural effects of melatonin differ from those of common hypnotics. Zhdanova (2005) reported that in contrast to common hypnotics, which have been repeatedly underscored, melatonin induces a behavioural state that resembles quiet wakefulness, which normally predisposes to normal sleep initiation, rather than sleepiness or drowsiness. Thus, melatonin-induced calming effect, hypo- and inactivity, demonstrated to enhance performance and productivity in broiler chickens (Marin *et al*., 1997a; Murakami *et al*., 2001; Zhdanova, 2005). Indeed, it has been established that inadequate rest is stressful to birds and drastically decreases feed intake, feed conversion ratio and, consequently, the overall weight gain of the birds (Blokhuis, 1984; Gordon, 1994; Melnychuk *et al*., 2004). The findings of the present study are consistent with the result of Campo and Davila (2002) that CL programmes inhibit sleep, which may increase physiological stress and, consequently, reduce performance.

The results of the present study showed that the melatonin-treated broilers exhibited less vigilance behaviour, compared to those raised on CL and 12D:12L cycle that were not administered with melatonin. This finding showed, for the first time, that melatonin markedly reduced fear in the broiler chickens. Furthermore, the results of the present study agree with the reports of Brooks *et al*. (2011) and Wang *et al.* (2014) that TI is an inherently stressful and fearful experience, which magnifies the degree of perturbation. The findings also agree with the result obtained by Oden *et al.* (2005) that female chickens in groups mixed with males were fearless because they had less vigilance behaviour, compared to their counterpart females in all-female groups. The findings of the present study show that the administration of melatonin in broiler chickens elicited boldness and confidence via freezing behaviour suppression. Overall, melatonin administration, by alleviating behavioural stress responses, may reduce the production losses incurred during the inevitable stressful management practices, involving rigorous feeding, continuous lighting and subjecting the broiler chickens to thermally stressful meteorological conditions of the hot-dry season in the hot-humid regions of the world.

5. REFERENCES

Abbas, A. O., Alm El-Dein, A. K., Desoky, A. A. and Galal, M. A. A. 2008: The effects of photoperiod programs on broiler chicken performance and immune response. *Intern. J. Poult. Sci.*, 7, 665 – 671.

Akinwande, A. I. and Adebule, A. O. A. 2003: Ascorbic acid and beta-carotene alleviate oxidative effect of London king size® cigarette smoke on tissue lipids. *Nig. J. Health Biochem Sci.,* 2 (1), 12 – 15.

Altan, O., Pabuccuoglu, A., Konyalioglu, S. and Bayracktar, H. 2003: Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. *Bri. Poult. Sci.*, 44, 545 – 550.

Axelrod, J. and Wurtman, R. J. 1968: Photic and neural control of indoleamine metabolism in the rat pineal gland. *Adv. Pharmacol.*, 6, 157 – 166.

Ayo, J. O. and Sinkalu, V. O. 2003: Effects of ascorbic acid on diurnal variations in rectal temperature of Shaver Brown pullets during the hot-dry season. *In*: Taiwo, A, A., Raji, A. M., Ogbonna, J. U. and Adebowale, E. A. (Editors). *Proc. 28th Ann. Conf. Nig. Soc. Anim. Prod.,* Ibadan, Oyo State, 28, 45 – 48.

Ayo, J. O., Minka, N. S. and Fayomi, A. 2005: Effects of ascorbic acid on rectal temperature of pullets transported by road during the hot-dry season. *Trop. J. Anim. Sci.*, 8, 43 – 48.

Bhimaraj, A. and Tang, W. H. W. 2012: Role of oxidative stress in disease progression in stage B, a pre-cursor of heart failure. *Heart Failure Clin.,* 8, 101 – 111.

Cable, N. T., Drust, B. and Gregson, W. A. 2007: The impact of altered climatic conditions and altitude on circadian physiology. *Physiol. Behav.,* 90, 267 – 273.

Campo, J. L. and Davila, S. G. 2002: Effect of photoperiod on heterophil to lymphocyte ratio and tonic immobility duration of chickens. *Poult. Sci.,* 81, 1637 – 1639.

Cardinali, D. P. and Pevet, P. 1998: Basic aspects of melatonin action. *Sleep Med. Rev.*, 2, 175 – 190.

Deacon, S. and Arendt, J. 1995: Melatonin-induced temperature suppression and its acute phase-shifting effects correlate in a dose-dependent manner in humans. *Brain Res.,* 688, 77 – 85.

Deep, A., Schwean-Lardner, K., Crowe, T. G., Fancher, B. I. and Classen, H. L. 2012: Effect of light intensity on broiler behaviour and diurnal rhythms. *Appl. Anim. Behav. Sci.,* 136, 50 – 56.

Duncan, D. B. 1955: Multiple range and multiple F test. *Biometrics,* 11, 1 – 42.

Elstner, E. F. 1991: Oxygen radicals-biochemical basis for their efficacy. *Klinische Wochenchrift,* 69, 949 – 956.

Fernandez-Juricic, E., Gall, M. D., Dolan, T., O' Rourke, C., Thomas, S. and Lynch, J. R. 2011: Visual systems and vigilance behaviour of two ground-foraging avian prey species: white-crowned sparrows and California towhees. *Anim. Behav.,* 81, 705 – 713.

Forkman, B., Boissy, A., Meunier-Salaun, M. C., Canali, E. and Jones, R. B. 2007: A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. *Physiol. Behav.,* 92, 340 – 374.

Fraser, P. A. 2011: The role of free radical generation in increasing cerebrovascular permeability. *Free Radical Biol. Med.,* 51, 967 – 977.

Gallup, G. G. 1979: Tonic immobility as a measure of fear in domestic fowl. *Anim Behav.,* 27, 316 – 317.

Gitto, E., Tan, D. X., Reiter, R. J., Karbownik, M., Manchester, L. C., Cuzzocrea, S., Fulia, F. and Berberi, I. 2001: Individual and synergistic antioxidative actions of melatonin: studies with vitamin E, vitamin C, glutathione and desferrioxamine (desferoxamine) in rat liver homogenates. *J. Pharm Pharmacol.*, 53, 1393 – 1401.

Goerlich, V. C., Natt, D., Elfwing, M., Macdonald, B. and Jensen, P. 2012: Transgenerational effects of early experience on behavioural, hormonal and gene expression responses to acute stress in the precocial chicken. *Hormones Behav.,* 61, 711 – 718.

Halliwell, B. and Whiteman, M. 2004: Measuring reactive species and oxidative damage *in vivo* and in cell culture: how should you do it and what do the results mean? *Br. J. Pharmacol.,* 142, 231 – 255.

Hardeland, R. and Pandi-Perumal, S. R. 2005: Melatonin, a potent antioxidative defense: Actions as a natural food constituent, gastrointestinal factor, drug and prodrug. *Nutri Metab.*, 2, 22 – 36.

Hardeland, R., Coto-Montes, A. and Poeggeler, B. 2003: Circadian rhythms, oxidative stress and antioxidative defense mechanisms. *Chronobiol. Intern.*, 20, 921 – 962.

Heiblum, R., Aizenstein, O., Gvaryahu, G., Voet, H., Robinzon, B. and Snapir, N. 1998: Tonic immobility and open field responses in domestic fowl chicks during the first week of life. *Appl. Anim. Behav. Sci.,* 60, 347 – 357.

Igono, M. O., Molokwu, E. C. I. and Aliu, Y. O. 1983: Seasonal variations in rectal temperature of Yankasa sheep. *Vet. Res. Comm.,* 6, 223 – 226.

Indian Poultry Equipment Manufacturers' Association (IPEMA, 2010). Our industry show. *Poultry India,* 2010.http://poultryindia.co.in/pi10/ExhiPages/c&m.htm 3/26/2011 5:12:28 AM.

Jones, R. B. 1996: Fear and adaptability in poultry: insights. *World's Poult. Sci. J.,* 52, 131 – 170.

Kedzierska, M., Olas, B., Wachowicz, B., Jeziorski, A. and Piekarski, J. 2012: Relationship between thiol, tyrosine nitration and carbonyl formation as biomarkers of oxidative stress and changes of haemostatic function of plasma from breast cancer patients before surgery. *Clin. Biochem.,* 45, 231 – 236.

Keim, S. M., Guisto, J. A. and Sullivan, Jr., J. B. 2002: Environmental thermal stress. *Ann. Agric. Environ. Med.,* 9, 1 – 15.

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

Korkmaz, A., Ma, S., Topal, T., Rosales-Corral, S., Tan, D. X. and Reiter, R. J. 2012: Glucose: A vital toxin and potential utility of melatonin in protecting against the diabetic state. *Mol. Cell. Endocrinol.,* 349, 128 – 137.

Korkmaz, A., Tan, D. X. and Reiter, R. J. (2011). Melatonin; an established radioprotective agent against Japan's nuclear disaster. *TAF Prev. Med. Bull.,* 10, 2 – 4.

Korkmaz, A., Topal, T., Oter, S., Tan, D. X. and Reiter, R. J. 2008: Hyperglycemia-related pathophysiologic mechanisms and potential beneficial actions of melatonin. *Med. Chem.,* 8, 1144 – 1153.

Lin, H., Decuypere, E. and Buyse, J. 2006: Acute heat stress induces oxidative stress in broiler chickens. *Comp. Biochem. Physiol., Part A,* 144, 11 – 17.

Marin, R. H., Freytes, P., Guzman, D. and Jone, R. B. 2001: Effects of an acute stressor on fear and on the social reinstatement response of domestic chicks to cagemates and strangers. *Appl. Anim. Behav. Sci.,* 71, 57 – 66.

Murakami, N., Kawano, T., Nakahara, K., Nasu, T. and Shiota, K. 2001: Effects of melatonin on circadian rhythm, locomotor activity and body temperature in the intact house sparrow, Japanese quail and owl. *Brain Res.,* 889, 220 – 224.

Nardone, A., Ronchi, B., Lacetera, N., Ranieri, M. S. and Bernabucci, U. 2010: Effects of climate changes on animal production and sustainability of livestock systems. *Liv. Sci.,* 130, 57 – 69.

Nisbet, D. J., Edrington, T. S., McReynolds, J. L., Callaway, T. R. and Byrd, J. A. 2008: Influence of exogenous melatonin administration on *Salmonella enteritidis* colonization in molted layers. *Poult. Sci*., 87, 1083 – 1088.

Nordquist, R. E., Heerkens, J. L. T., Rodenburg, T. B., Boks, S., Ellen, E. D. and van der Staay, F. J. 2011: Laying hens selected for low mortality: Behaviour in tests of fearfulness, anxiety and cognition. *Appl. Anim. Behav. Sci.,* 131, 110 – 122.

Oden, K., Gunnarsson, S., Berg, C. and Algers, B. 2005: Effects of sex composition on fear measured as tonic immobility and vigilance behaviuor in large flocks of laying hens. *Appl. Anim. Behav. Sci.,* 95, 89 – 102.

Oladele, S. B., Ayo, J. O., Ogundipe, S. O. and Esievo, K. A. N. 2003: Seasonal and species variations in erythrocytes osmotic fragility of indigenous poultry species in Zaria, Northern Guinea Savannah zone of Nigeria. *Bull. Anim. Health Prod. Afr.*, 51, 204 – 214.

Oluyemi, J. A. and Adetowun, A. 1979: Measures applied to combat thermal stress in poultry under practical tropical environment. *Poult. Sci*., 58, 767 – 773.

Piestun, Y., Halery, O., Shinder, D., Ruzal, M., Druyan, S. and Yahav, S. 2011: Thermal manipulations during broiler embryogenesis improves post-hatch performance under hot conditions. *J. Therm. Biol.,* 36, 469 – 474.

Poeggeler, B. 2004: Neuroprotection by indole and nitrone compounds acting as mitochondrial metabolism modifiers with potent antioxidant activity. *9th Intern. Conf. Alzheimer's Dis. Rel. Disorders,* Philadelphia, Pp. 398.

Saint-Dizier, H., Leterrier, C., Levy, F. and Richard, S. 2008: Selection for tonic immobility duration does not affect the response to novelty in quails. *Appl. Anim. Behav. Sci.,* 112, 297 – 306.

Salmeto, A. L., Hymel, K. A., Carpenter, E. C., Brilot, B. O., Bateson, M. and Sufka, K. J. 2011: Cognitive bias in the chick anxiety-depression model. *Brain Res.,* 1373, 124 – 130.

Schwean-Lardner, K., Fancher, B. I. and Classen, H. L. 2012: Impact of daylength on behavioural output in commercial broilers. *Appl. Anim. Behav. Sci.,* 137, 43 – 52.

Sen, C. K. 2001: Antioxidants in exercise. *Nutr. Sports Med*., 31, 891 – 908.

Sinkalu, V. O. and Ayo, J. O. 2009: Modulatory role of vitamin A on individual and diurnal fluctuations in rectal temperature of Black Harco pullets. *Intern. J. Appl. Res. Vet. Med*., 7, 102 – 111.

Sinkalu, V. O. and Ayo, J. O. 2011: Behavioural and tonic immobility responses of broiler chickens administered with melatonin during the early rainy season of Northern Nigeria. *In*: Iguisi, E. O., Ati, O. F., Yusuf, R. O. and Ubogu, A. E. (Editors). *Proc. Intern. Conf. Nig. Meteorol. Soc.,* Ahmadu Bello University, Zaria, Kaduna State, Nigeria, Pp. 463 – 472.

Sinkalu, V. O., Ayo, J. O., Adelaiye, A. B. and Hambolu, J. O. 2007: Modulatory role of vitamins C and E on diurnal fluctuation in rectal temperature of Black Harco pullets during the hot-dry season. *Trop. J. Anim. Sci.*, 10, 249 – 255.

Sinkalu, V. O., Ayo, J. O., Adelaiye, A. B. and Hambolu, J. O. 2008: Effects of vitamin E on diurnal variations in rectal temperature of Black Harco pullets during the hot-dry season. *J. Therm. Biol.*, 33, 32 – 36.

Spinka, M. 2012: Social dimension of emotions and its implication for animal welfare. *Appl. Anim. Behav. Sci.,* 138, 170 – 181.

Tan, D. X., Hardeland, R., Manchester, L. C., Poeggeler, B., Lopez-Burillo, S., Mayo, J. C., Sainz, R. M. and Reiter, R. J. 2003: Mechanistic and comparative studies of melatonin and classic antioxidants in terms of their interactions with the ABTs cation radical. *J. Pineal Res.*, 34, 249 – 259.

Tan, D. X., Manchester, L. C., Reiter, R. J., Plummer, S. F., Limson, J., Weintraub, S. T. and Qi, W. 2000a: Melatonin directly scavenges hydrogen peroxide: A potentially new metabolic pathway of melatonin biotransformation. *Free Radical Biol. Med*., 29, 1177 – 1185.

Tan, D. X., Manchester, L. C., Reiter, R. J., Qi, W., Karbownik, M. and Calvo, J. R. 2000b: Significance of melatonin in antioxidative defense system: reactions and products. *Biol. Signals Receptors*, 9, 137 – 159.

Tauler, P., Aguilo, A., Gimeno, I., Fuentespina, E., Iur, J. A. and Pons, A. 2003: Influence of vitamin C diet supplementation on endogenous antioxidant defense during exhaustive exercise. *Eur. J. Physiol.*, 446, 658 – 664.

Togo, F., Watanabe, E., Park, H., Shephard, R. J. and Aoyagi, Y. 2005: Meteorology and the physical activity of the elderly: the Nakanojo study. *Intern. J. Biometeorol.,* 50, 83 – 89.

Verbrugghe, E., Boyen, F., Gaastra, W., Bekhuis, L., Leyman, B., Van Parys, A., Haesebrouck, F. and Pasmans, F. 2012: The complex interplay between stress and bacterial infections in animals. *Vet. Microbiol.,* 155, 115 – 127.

Vielma, J. R., Bonilla, E., Bonilla, L. C., Mora, M., Leendertz, S. M. and Bravo, Y. 2014: Effects of melatonin on oxidative stress, and resistance to bacterial, parasitic, and viral infections: A review. *Act. Trop.,* 137, 31 – 38.

Wang, S., Ni, Y., Guo, F., Sun, Z., Ahmed, A. and Zhao, R. 2014: Differential expression of hypothalamus fear- and stress-related genes in broiler chickens showing short or long tonic immobility. *Dom. Anim. Endocrinol.*, 47, 65 – 72.

Zagron, G. and Weinstock, M. 2006: Maternal adrenal hormone secretion mediates behavioural alterations induced by prenatal stress in male and female rats. *Behav. Brain Res.,* 175, 323 – 328.

Zisapel, N. and Cardinali, D. P. 2008: Physiological effects of melatonin receptors and signal transduction pathways. *Prog. Neurobiol.,* 85, 335 – 353.