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# Effect of Melatonin and Lighting Regime on Physiological Responses and Haematological Profile of Isa Brown laying Birds

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## ABSTRACT

**Background:** Melatonin is the main neurohormone synthesized and released by the pineal gland. It stimulates several antioxidative enzymes which increase its efficiency as an antioxidant and enhance the maturation of oocytes and the development of follicles in animals. Artificial lighting on the other hand improves bird's performance especially reproductive performance and behavior. By providing artificial light, growers can manipulate this natural cycle to their advantage and increase the egg laying. Therefore, this current study was aimed to evaluate the effect of melatonin and increased lighting on the welfare of laying birds.

**Methods:** In November 2018 to December 2019, a total 162 sixteen weeks Isa Brown hens were used for the experiment which was grouped into 9 treatments. Melatonin and lighting at three levels (0mg, 5mg, 10mg and 12hrs, 15hrs and 18hrs) were administered orally to the birds four times weekly within a day interval for 30 weeks from 16 weeks to 46 weeks. Data on rectal temperature, respiratory rate and heart rate were collected and analysed. Blood samples were also collected and analysed for haematological parameters

**Result:** Results showed that rectal temperature (RT), respiratory rate (RR) and heart rate (HT) were significantly ($p<0.05$) influenced by both melatonin and lighting. Melatonin at 5mg significantly ($p<0.05$) reduced the rectal temperature (40.55°C), respiratory rate (139.44bpm) and heart rate (320bpm) while lighting at 18 hours significantly ($p<0.05$) increased the rectal temperature, respiratory rate and heart rate due to heat stress among the parameters. The haematological profiles were influenced ($p<0.05$) by the administration of melatonin. Treatments levels on melatonin administration (5mg and 10mg) had increased circulating packed cell volume of 28.00% and 28.11% respectively compared to the control group which had 25.66%. The results indicates that melatonin at 5mg and 10mg improved the welfare of the birds but increased lighting beyond 12 hours compromised immunity of the birds.


**Key words:** Haematology, Lighting, Melatonin, Isa brown, Vital signs.

## INTRODUCTION

Prolonged heat stress results in dramatic physiological changes in chicken especially in broiler birds which is associated with elevated body temperature, increased respiratory, panting and cannibalism (Abbas *et al.,* 2000). These changes are used as indicators of heat stress (Melesse *et al.*, 2011). In addition to high ambient temperature, relative humidity also plays an important role in bringing about stress in the chickens leading to production and economic losses (Ajakaiye *et al.*, 2011). These losses are heavily incurred in animals like cows, sows, broilers, layers, and turkeys and contribute to heavy much economic loss in livestock production. In the USA, St Pierre *et al.* (2003) noted that economic losses in the layer sector are very high due to heat stress and when the heat stress was reduced, it was observed that the losses also reduced. When environmental temperature exceeds the limits of the thermoneutral zone, body temperature of the animal increases leading to distress.

Melatonin (N-acetyl-5-methoxytryptamine) with a chemical formula $C_{13}H_{16}N_2O_2$ is the main neurohormone synthesized and released by the pineal gland. This hormone appears to be a potent free radical scavenger opposing the most toxic hydroxyl radicals (Webb *et al.*, 1995). Besides its ability to directly neutralize a number of free radicals and reactive oxygen and nitrogen species, it stimulates several antioxidative enzymes which increase its efficiency as an antioxidant (Reiter *et al.*, 2000). It is one of the important hormones that prevent metabolic and physiologic disorders in poultry but does not attract attention by poultry scientists (Suleyman *et al.*, 2018). Melatonin helps regulate feed consumption, energy metabolism and body heat. It provides elimination of free radicals in the body and also stimulates growth hormone secretion and thus, affects growth performance of poultry positively (Suleyman *et al.*, 2018). Melatonin sets the internal biological clock that regulates different daily and seasonal rhythms in various physiological systems including the cardiopulmonary, reproductive, excretory, thermoregulatory, behavioural, immune and

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neuroendocrine systems in birds (Melamud *et al*., 2012). Therefore the objective of this research is to evaluate the effect of melatonin and lighting on the physiologic responses and health status of laying birds during hot dry season.

## MATERIALS AND METHODS

The experiment was carried out between November 2018 to December 2019 at the Poultry Unit of the Teaching and Research Farm, Ebonyi State University, Abakaliki in Nigeria. A total of one hundred and sixty-two (162) 16 weeks Isa Brown hens were used for the experiment which was grouped into 9 treatments. They were further subdivided into three replicates of six birds each in a 2×3 factorial in a completely randomized design at 20 weeks when the birds had started laying. Anymetre weathermeter which measures both temperature and humidity was used to record the temperature and relative humidity within and outside the pen. The weekly mean temperature and relative humidity index recorded outside the pen ranged from 39.06°C-40.06°C and 60% while inside the poultry house had the value range of 43.06°C-46.02°C and 56% but highest within the treatment area that had highest duration of lighting with the value range of 47.02°C-48.04°C and 55%. Feed and water were served to the birds *ad libitum*.

The experimental factors in the experiment were melatonin supplement and lighting regime. Melatonin was purchased from Roban Pharmacy in Abakaliki. The melatonin was dissolved in warm water at the rate of 5mg/2mls or 10mg/2mls and administered orally after cooling by drenching four times weekly (Monday, Wednesday, Friday and Sunday) while 100 watt bulbs were used to provide lighting whose light intensity were measured using photometer with light intensity of 1700Lux from 06:00pm daily in each experiment according to the treatment specifications in each group. The three levels of melatonin administered were 0mg, 5mg and 10mg while lighting groups were 12 hours, 15 hours and 18 hours daily. A standard digital clinical thermometer was used to measure the rectal temperature (RT) at 07:00h and 15:00h weekly after the light (6:00pm - 12:00am) introduction from 16$^{th}$ week to 46$^{th}$ week according to treatment groups. Three birds were randomly selected out of each treatment weekly and with a digital stethoscope and thermometer the heart rates, respiratory rate and rectal temperature were determined according to Sturkie, (1976). Three birds per treatment was randomly selected and blood samples were collected and analysed according to Coles, (1986) and Cheesbrough, (2000).

Data collected from the experiment were subjected to statistical analysis using Mini-tab version 18 software analysis of variance (ANOVA). Treatment means were separated using Fisher pairwise comparisons

## RESULTS AND DISCUSSION

### Effect of melatonin on physiological responses

Results of the physiological response is presented in Table 1.1. The rectal temperature and respiratory rate of layers administered different dosages of melatonin differed significantly ($p<0.05$) among the treatment groups. The rectal temperature was significantly ($p<0.05$) higher on control group $ISM_0$mg (43.56°C) compared to those on levels of melatonin which recorded lower rectal temperatures of 40.54°C and 41.52°C for $ISM_5$ and $ISM_{10}$ respectively.

The reduced rectal temperature among the treatments on levels of melatonin indicated that melatonin alleviated the negative impact of heat stress. The high rectal temperature in the $ISM_0$ mg group showed that the layers were already in a stressful condition as the temperature already increased from 41°C to 43°C. This finding is in agreement with the report of Nalini *et al*. (2008) who stated during heat stress physiological adjustment can occur when the temperature is above 41°C. Exposure of poultry to ambient temperature outside the thermo-neutral zone during the course of production may affect production and feed conversion efficiency due to decreased feed intake, immune response and mortality (Howlinder and Rose, 1989). Results from the study showed that inclusion of melatonin decreased the rectal temperature of the Isa Brown laying birds. This report is in agreement with the report of Zeman *et al*. (2001), who noted that the supplementation of melatonin at a dose of 150mg/kg of feed resulted in a highly significant decline in heat production of 2-week and 3-week-old chickens (8.38% and 13.05%, respectively). Report from the experiment is consistent with report of Apeldoorn *et al.* (1999) who reported that melatonin supplementation in a broiler diet reduces heat production and more significantly, the physical activity related to heat production. Siegel (1995) also stated that melatonin can be used in controlling temperature stress, since epiphyseal gland and its metabolites play an important role in the circadian thermoregulation in many animal species (Reiter, 1995). Saarela and Reiter (1994) also observed that melatonin aids in circadian thermoregulatory adjustments of body temperature. The decline in heat production could be attributed to the hypothermic effect of melatonin (George, 1999). A dose-dependent reduction in basal temperature after a single melatonin injection was recorded in chickens by Rozenboim *et al.,* (1998). This is also in line with the report of Minka and Ayo, (2012) who observed that the mean colonic temperature value of 40.8 ± 0.2°C was recorded in melatonin-treated quails during transportation and the value was significantly ($p<0.05$) lower than that of 42.4 ± 0.7°C recorded in the control group. The respiratory rates of the laying birds were significantly influenced ($p<0.05$) by the

**Table 1.1:** Main effect of Melatonin on the physiological responses of Isa Brown.

| Parameters | $ISM_0$ | $ISM_5$ | $ISM_{10}$ | SEM |
|---|---|---|---|---|
| Rectal Temperature (°C) | 43.56$^a$ | 40.55$^c$ | 41.52$^b$ | 0.39 |
| Respiratory Rate (bpm) | 195.22$^a$ | 139.44$^c$ | 143.00$^b$ | 1.23 |
| Heart Rate (bpm) | 337.77 | 320.22 | 334.33 | 8.71 |

$^{a-c}$ Means in the same row with different superscripts are significantly different ($p<0.05$).

treatment. There were drastic reductions in the respiratory rate of the layers on the 5mg and 10mg of melatonin compared to those on 0mg. This indicates that melatonin maintained the thermo-neutral or comfort zone of the thermally stressed layers. This finding is in agreement with the report of Sinkalu *et al*. (2010) who noted that broilers presented a significant increase of respiratory rate under high temperatures with values up to 165 breaths per minute. Result from this finding is also in agreement with the report of Rozenboim *et al*. (1998) who recorded a dose dependent reduction in basal temperature after a single melatonin injection was recorded in chickens. The decreased basal temperature subsequently resulted in a lower dissipation of heat to environment. The finding of the present study has, thus, demonstrated that the increases in internal heat energy generation from feed metabolism, associated with rearing layers under continuous photoperiods may be ameliorated by melatonin.

**Effect of lighting on physiological responses**

The result of the effect of lighting on physiological responses are presented in Table 1.2. Significant difference was observed in the rectal temperature, respiratory rate and heart rate of the birds. There were significant differences ($p<0.05$) on the mean of respiratory rate recorded. Significant difference ($p<0.05$) was also obtained on the mean of heart rate among the treatment groups. Isa Brown on $ISL_{18}$ (350 bpm) had the highest mean values compared to $ISL_{15}$ (328.11 bpm) and $ISL_{12}$ (320.00 bpm) groups. Results from this study showed that longer duration of lighting increased the environmental temperature of the poultry house which in turn elevated the rectal temperature with increased respiratory rate of the layers, the birds were also deprived of sleep due to continues lighting which led to increased activity. These report are in agreement with the findings of Aengwanich, (2008) who stated that sleep deprivation increased body temperature and activity due to lack of rest. The increased respiratory rate is as a result of high heat production. This finding is in agreement with the report of Zhou and Yamamoto (1997) who stated that higher environmental temperature is responsible for high heat production thereby increasing respiratory rate (209.25 bpm) after 3 hours of heat exposure than normal condition of (48.50/minute). Bottje *et al.* (1990) also reported similar findings as respiratory rate of (140-170 breaths/minute) increased with increasing body temperature when chicken was exposed to 40.5°C.

**Effect of melatonin on haematological parameters**

The result of the effect of melatonin on the haematological parameters are presented in Table 2.1. Layers on 5 mg $ISM_5$ (28.00%) of melatonin had the highest mean value of PCV followed by group $ISM_{10}$ (28.11%) while the control $ISM_0$ (25.66%). RBC significantly increased with increased melatonin, layers in melatonin administration had the highest mean values compared to the control. The mean values of MCV, MCH and MCHC did not differ significantly ($p>0.05$) among the treatment groups. MCH and MCHC mean values decreased with increased melatonin administration compared to the control. There was significant difference ($p<0.03$) among the treatment means of WBC. Heterophils also differed among the treatment means. The layers in the control had increased number of heterophils compared to those on melatonin treatment. Lymphocyte differed significantly with increased mean values on those with melatonin treatment compared to the control. Melatonin increased the circulating monocytes but decreased eosinophils concentration while basophils mean values were not significantly ($p>.005$) influenced by melatonin administration. The monocytes mean values were numerically high on melatonin treated group compared to the control. The beneficial effects of melatonin are not confined to direct modulation of the immune system it diminished the parasite load in blood and tissue (Erbaş *et al.,* 2012). Indeed, melatonin increases total antioxidant capacity and/or reduces the production of reactive oxygen species (Espino *et al.,* 2012). The antioxidant activity of melatonin and its metabolites may also account for its anti-apoptotic effects on immune cells (Maestroni, 2001).

**Table 1.2:** Effect of lighting on physiological response of Isa Brown.

| Parameters | $ISL_{12}$ | $ISL_{15}$ | $ISL_{18}$ | SEM |
|---|---|---|---|---|
| Rectal temperature (°C) | $42.51^{b}$ | $42.65^{b}$ | $44.46^{a}$ | 1.44 |
| Respiratory rate (bpm) | $185.33^{b}$ | $196.85^{a}$ | $198.00^{a}$ | 4.39 |
| Heart rate (bpm) | $320.00^{b}$ | $328.11^{b}$ | $350.22^{a}$ | 9.57 |

$^{a-b}$ Means in the same row with different superscripts are significantly different ($p<0.05$).

**Table 2.1:** Main effect of melatonin on haematological parameters of Isa Brown.

| Parameter | $ISM_0$ | $ISM_5$ | $ISM_{10}$ | SEM |
|---|---|---|---|---|
| PCV (%) | $25.66^{b}$ | $28.00^{a}$ | $28.11^{a}$ | 1.50 |
| Hb (g/dl) | $6.22^{b}$ | $8.80^{a}$ | $8.75^{a}$ | 1.32 |
| RBC ($\times10^{12}$/L) | $1.65^{b}$ | $1.72^{a}$ | $1.75^{a}$ | 0.50 |
| MCV (fl) | 155.30 | 162.79 | 155.62 | 1.18 |
| MCH (pg) | 49.81 | 45.81 | 47.28 | 1.05 |
| MCHC (g/dl) | 32.03 | 28.14 | 27.57 | 2.98 |
| WBC ($\times10^{9}$/L) | $6.90^{b}$ | $8.13^{a}$ | $8.63^{a}$ | 2.25 |
| HETERO (%) | $57.88^{a}$ | $49.44^{b}$ | $45.22^{b}$ | 3.58 |
| LYM (%) | $58.66^{b}$ | $69.00^{a}$ | $64.44^{a}$ | 4.24 |
| MON (%) | $4.22^{b}$ | $6.00^{a}$ | $6.20^{a}$ | 1.45 |
| EOS (%) | $2.55^{a}$ | $1.20^{b}$ | $1.00^{b}$ | 0.32 |
| BASO (%) | 1.87. | 1.60 | 1.52 | 0.02 |

$^{a-b}$ Means in the same row with different superscripts are significantly different ($p<0.05$).

PCV = Packed cell volume, Hb = Hemoglobin, RBC = Red blood cell, MVC = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration, WBC = White blood Cell, HETERO = Heterophils, LYM = Lymphocytes, MON= Monocytes, EOS = Eosinophils, BASO = Basophils.

**Table 2.2:** Main effect of lighting regime on Haematological parameters of Isa Brown.

| Parameter | $ISL_{12}$ | $ISL_{15}$ | $ISL_{18}$ | SEM |
|---|---|---|---|---|
| PCV (%) | 28.55[a] | 25.44[b] | 22.77[b] | 1.25 |
| Hb (g/dl) | 8.44 | 7.68 | 7.73 | 0.29 |
| RBC ($\times 10^{12}$/L) | 1.63 | 1.72 | 1.77 | 0.04 |
| MCV (fl) | 156.74 | 165.34 | 153.24 | 2.92 |
| MCH (pg) | 51.77 | 44.65 | 49.67 | 1.95 |
| MCHC (g/dl) | 33.03 | 27.00 | 28.85 | 2.08 |
| WBC ($\times 10^{9}$/L) | 8.64 | 7.21 | 7.81 | 1.74 |
| HETERO (%) | 51.00[b] | 51.80[b] | 55.66[a] | 4.01 |
| LYM (%) | 50.44 | 50.66 | 50.00 | 0.56 |
| MON (%) | 3.22[b] | 3.50[b] | 5.24[a] | 0.45 |
| EOS (%) | 2.28 | 2.27 | 2.00 | 0.32 |
| BASO (%) | 1.80. | 1.90 | 2.0 | 0. |

[a-b]Means in the same row with different superscripts are significantly different ($p<0.05$).

PCV = Packed cell volume, Hb = Hemoglobin, RBC = Red blood cell, MVC = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration, WBC = White blood cell, HETERO = Heterophils, LYM = Lymphocytes, MON= Monocytes, EOS = Eosinophils, BASO = Basophils.

The results from this study indicate that melatonin improved the health status of Isa Brown laying birds. Melatonin level with immune processes and the immune-hematopoietic system has been shown to improve immune response (Pacini and Borziani, 2016). Results of this study affirms the findings of Ben *et al.* (1995) who stated that administration of daily melatonin starting three days before stress increased PCV, significantly reduced heterophils circulation, mortality, and postponed the onset of disease and death. This also affirms the report of Rodriguez *et al.* (2001) observation that melatonin is also able to enhance the phagocytic capacity of heterophils. Melatonin administration upon reperfusion decreased the migration of circulatory neutrophils and macrophages/monocytes into stressed rats and inhibited microglial activation following transient focal cerebral ischemia in rats (Ben *et al.*, 1995).

**Results of effect of lighting regime on haematological parameters as presented in Table 2.2**

There were significant differences on the PCV, heterophils and monocytes mean values. The PCV mean values were $ISL_{12}$ (28.55%), $ISL_{15}$ (25.44%) and $ISL_{18}$ (22.77%). This shows that the control group on 12 hours lighting $ISL_{12}$ had the highest PCV mean values compared to those on $ISL_{15}$ AND $ISL_{18}$ respectively. This suggests that lighting decreased the PCV mean values across the treatment means. Heterophil values were significantly ($p<0.05$) higher in the treatment groups with the longest duration of lighting compared to the others. The mean values were $ISL_{12}$ (51.00%), $ISL_{15}$ (51.80%) and $ISL_{18}$ (55.66%). Lighting also increased the monocytes mean values compared to the control group. Those on group $ISL_{15}$ and $ISL_{18}$ had higher number of monocytes compared to ISL12. The increased heterophils and monocytes values indicate possible presence of pathogenic organisms which results to increased body temperature which ultimately made the body unfavourable for further pathogenic growth.

## CONCLUSION

The results from this study have shown that 5mg of melatonin reduced the rectal temperature, respiratory rate and heart rate of Isa Brown laying birds during thermal stress. Increased lighting had a deleterious effect on the physiological responses of the birds especially with 18hrs of lighting which increased the heart rate and rectal temperature leading to distress n the birds. The results of the haematological profiles of the birds indicate that melatonin improved the overall health status of the Isa Brown laying birds especially those in 5mg of melatonin and 15hrs of lighting. Lighting alone did not affect the haematological indices.

It is therefore recommended that 5 mg of melatonin be used to reduce thermal stress in Isa Brown laying birds during thermal stress to improve the wellbeing of the birds which in turn enhance productivity of the birds.

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