



Interaction Effect of Melatonin and Lighting Regime on Behavioral Responses, Hematological Indices and Production Performance of Isa Brown Laying Birds

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ABSTRACT

Background: Poor poultry production performance of laying birds because of reduced immunity leading to economic loss is a major problem facing egg production in the poultry industry especially in Nigeria. Melatonin is a hormone that regulates the brain's biological clock and acts on physiological functions and the immunity system. It also provides the elimination of free radicals in the body, but lighting affects melatonin secretion and light is also necessary to enhance feeding and photoperiod which enhance egg laying.

Methods: A total of 164 Isa Brown laying Birds were used to evaluate the interaction effect of melatonin and lighting regime on behavioural responses, haematological indices and laying performance of Isa Brown laying birds. The experiment was grouped into 9 treatments which were further subdivided into three replicates of six birds in a 2x3 factorial in a completely randomized design. Melatonin and lighting at three levels were administered to the birds daily for 30 weeks. The three levels of melatonin were 0 mg, 5 mg and 10 mg while lighting was 12 hours, 15 hours and 18 hours daily. Data were collected on behavioural responses, haematological indices and production performance of the birds.

Result: Results from the experiment indicate that the interaction of melatonin and lighting significantly ($p < 0.05$) reduced the dust-bathing, feather pecking and panting rate of the birds. The interaction level at $M_{10}L_{15}$ reduced dustbathing to 80.30% as against the control M_0L_{12} which was at 95.43%. The interaction significantly ($p < 0.05$) improved feed conversion ratio, body weight gain and hen-day egg production for $M_{10}L_{12}$, M_5L_{15} , $M_{10}L_{15}$ respectively. The haematological parameters were significantly improved by the effect of the interaction. It is therefore concluded that the interaction of melatonin and lighting in different levels of administration improved the behavioural responses and production performances and maintained the health status of the Isa brown laying birds.

Key words: Behavioral, Dust-bathing feather pecking, Lighting, Melatonin, Panting.

INTRODUCTION

Stress affects the growth, behavioural, physiology and reproductive performance of animals negatively. This leads to a decline in the production and welfare of animals (Etim *et al.*, 2014). Melatonin effectively protects against heat stress, by a variety of mechanisms. As animals in tropical countries are exposed to heat stress, melatonin with its potential beneficial effects may be useful as an anti-heat stress agent to prevent the loss of production (Iqbal *et al.*, 2016). Some animals especially photoperiodic animals require additional lighting to attain maximum productive potentials although additional lighting beyond certain periods elucidated some negative behavioural and physiological responses that lower production potentials. Behavior can be seen as what animals do to interact with, respond to and control their environment. It is generally the animal's first line of defense in response to environmental change (Al-Obaidi *et al.*, 2002). Animals should be housed to maximize species-specific behavior and minimize stress-induced behaviors (Etim *et al.*, 2014). The performance of certain behaviours can lead to improvements or damage in physical health. For example, dairy cows who walk daily have fewer leg problems, including noninfectious leg and hoof disorders, as well as lower incidence of mastitis, bloat and calving-related disorders, than cows kept in tie stalls (NRC, 1999). Feather pecking in laying is both a welfare

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and economic problem. Pulling out feathers causes pain (Grieves *et al.*, 2012), increases risk of injuries and can trigger an outbreak of cannibalism (Keeling 1995). Behaviour is very important in indicating illness, pain, fear, stress, or tension. If possible, animals will move away from a fear-producing stimulus by fleeing or seeking cover (or will sometimes attempt to escape detection or injury by becoming immobile). Sick animals show many behavioural changes, including anorexia, sleepiness, depression and

a reduction in grooming activity, which help to conserve energy and thus facilitate healing. Animals use behavioural mechanisms to deal with short-term stressors like heat and social interactions.

In laying houses, increasing lighting duration above the normal 12 hours daytime improves egg laying. Increasing the duration of lighting day and night, can improve the health and immune status of the birds. It equally improves mobility and alertness. Increased lighting equally increases rate of feather pecking, panting and high feed conversion ratio. Light decreases melatonin production and signals the body to prepare for being awake. Light and dark alterations constitute the principal timing signal of melatonin secretion from the pineal gland. Light influences melatonin synthesis.

Melatonin helps the immune responses in poultry (Moore and Siopes, 2003) by increasing lymphocyte proliferation (Kliger *et al.*, 2000) and antibody formation (Zheng *et al.*, 2013). Clark and Classen (1995) noted that melatonin improves feed utilization by stimulating growth hormone in poultry (Zeman *et al.*, 1999), energy metabolism (Apeldoorn *et al.*, 1999) and regulating thermoregulatory mechanisms (Rozenboim *et al.*, 1998; Sahin *et al.*, 2004). Its application compensated for eggshell and skeletal defects in chickens (Taylor *et al.*, 2013). Antioxidants both natural (plant) or synthetic improves the laying performance of birds (Yildirim *et al.*, 2017). Melatonin given with drinking water changed the behaviour of quails and increased the amount of certain peripheral hormones, especially growth hormone. The stimulation level of growth hormone was 140% when melatonin was administered alone, whereas the level of growth hormone stimulation was 285% when melatonin was administered with quipazinin (Zeman *et al.*, 1999).

In tropical regions, high ambient temperature is a major problem facing the egg production industry, since it adversely affects feed intake, egg production, egg quality, behavioural and physiological responses and the welfare of birds. This leads to a seasonal decline in the production of poultry products like chicken and egg. This necessitated the need to combine the two factors to mitigate the effect of a hostile environment and improve production within this region.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm of Ebonyi State University Abakalilik, Ebonyi State Nigeria between October 2022 to March 2023. The annual rainfall is 1200/mm to 1400/mm. The mean temperature is 30°C during raining season and 38°C during hot dry season (Ofomata, 1997).

Experimental animals and management

One hundred and sixty-two (162) Isa brown laying birds were used for the experiment. The birds were grouped into nine

treatments consisting of eighteen (18) birds per treatment. Each treatment was further replicated three times with each replicate having six (6) birds each.

Experimental materials

The experimental materials used for the study were a melatonin supplement and a lighting regime. The Melatonin supplement from Health Vit West-Coast company was used for experiment and each container has 60 tablets. The melatonin was dissolved in distilled water at the rate of 5 mg/2 mls or 10 mg/2 mls and administered orally to 20-week laying birds according to the treatment specifications in each group. Artificial light in the form of a 100w electric bulb was used to provide light according to the specifications of each treatment.

Experimental design

A two-by-three (2×3) factorial arrangement in a randomized complete block design was used to design the experiment.

Experimental procedures

The experiment lasted for a total of one hundred and eighty-three (183) days (November-April). This is the period of high environmental temperature in the region. The experimental birds were weighed at the beginning and end of the experiments. A formulated layer's diet with 17% crude protein and 2800 kcal ME/kg of energy was fed to the birds. A wall and desk thermometer and hydrometer (Brannan, UK) were used to measure the temperature and relative humidity of the experimental site. Melatonin was dissolved in 2 mls of distilled water and given orally according to the treatment specifications in each group between the hours of 05:00 pm-06:00 pm. The birds were exposed to lighting according to the specification in the treatment group during the experimental period, while treatment 1 which is the control was raised under normal lighting without melatonin.

Behavioural response (BR)

Cameras and videotapes were set at strategic locations in the pen to monitor the activities of the birds. The videotapes were analyzed four times at 2 hrs- intervals between the hours of 9:00 pm-11:00 pm and 2 pm to 4 pm daily. The birds were also observed for levels of panting, dust bathing and feather pecking. Panting: this was graded into three categories. Namely; Normal < 1 hour, Alert 1-2 hours and Danger > 2 hours.

Dust bathing

this was also scored in three stages, which include; Normal < 30 minutes, Alert 30 minutes-1 hour and Danger 1-2 hours.

Performance evaluation

Hen day egg production (%) (HDEP)

This was determined by dividing the number of eggs picked in each treatment by the number of hens in that treatment.

$$\text{HDEP} = \frac{\text{Number of eggs picked}}{\text{Number of birds}} \times 100$$

Body weight gain (BWG)

The birds were weighed on an individual basis at the beginning of the experiment and weekly thereafter. At the end of the experiment, the total body weight gain was calculated by subtracting the initial body weight from the final body weight.

Feed intake

A weighed quantity of feed was served to the birds daily. Leftover was collected per treatment group, weighed and recorded. From the data collected, the daily feed intake of each treatment group was determined by the difference between the quantity served and the leftovers.

Feed conversion ratio

This was determined by dividing the average daily feed intake by the average egg weight.

$$\text{FCR} = \frac{\text{Amount of feed consumed in grams}}{\text{Egg weight in grams}}$$

Egg weight

Three eggs were picked in each treatment and weighed using a sensitive electronic balance.

Hematological parameters

At the end of the experiments, blood samples were collected from three birds per treatment in each of the experiments to determine the hematological parameters. The blood samples were collected from birds slaughtered for follicular dynamics. The samples were collected into specimen bottles containing

Ethylene diamine tetra-acetic acid (EDTA) as an anticoagulant. The parameters were determined using microhematocrit method (Dacic and Lewis, 1991).

RESULTS AND DISCUSSION

Interaction effect of melatonin and light on behavioural response of isa brown laying birds

Results of the interaction effect of melatonin and lighting on the behavioural response of Isa brown laying birds are presented in Table 1.

The interaction effect of melatonin and lighting at 5 mg and 10 mg and 15 hrs and 18 hrs respectively reduced the dust-bathing, panting and feather pecking rate of the birds. Groups on longer lighting duration (18 hrs) without melatonin had higher dust-bathing, panting and pecking rate compared to other groups but the interaction effect of melatonin brought reduction in the parameters. The result of this finding is in agreement with the findings of Kjaer and Vestergaard, (1999) who noted that high light intensity enhances development of feather pecking and cannibalism in laying hen. Taylor *et al.* (2013) reported that farmers routinely use reduced light intensities and duration to prevent feather pecking which, on the other hand, may restrict the movement of hens around the house and thus decrease their welfare status. The interaction of melatonin and lighting improved the behavioral status of the birds.

Interaction effect of melatonin and lighting regime on the production performance of isa brown laying birds

Results of the interaction effect of melatonin and lighting on the production performance of Isa brown laying birds are presented in Table 2.

Table 1: Interactive effect of melatonin and lighting on behavioral responses of isa brown.

PAT (%)	M ₀ L ₁₂	M ₀ L ₁₅	M ₀ L ₁₈	M ₅ L ₁₂	M ₅ L ₁₅	M ₅ L ₁₈	M ₁₀ L ₁₂	M ₁₀ L ₁₅	M ₁₀ L ₁₈	SEM
DB	95.43 ^{ab}	98.86 ^a	100 ^a	95.86 ^{ab}	90.43 ^b	90.86 ^{ab}	94.43 ^{ab}	80.30 ^c	88.16 ^{bc}	4.67
PNT	100.00 ^a	100.00 ^a	100.00 ^a	94.43 ^a	88.86 ^{ab}	77.73 ^{bc}	77.73 ^{bc}	72.60 ^{bc}	72.01 ^c	6.97
FPCK	80.43 ^a	83.30 ^{ab}	94.30 ^a	88.86 ^{ab}	72.16 ^b	83.30 ^{ab}	72.15 ^b	77.73 ^{ab}	72.16 ^b	4.57

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

PAT= Parameter, DB= Dust-bathing, PNT= Panting, FPCT= Feather pecking.

Table 2: Mean Interaction effect of melatonin and lighting regime on the productive performance of isa brown laying birds.

Parameter	M ₀ L ₁₂	M ₀ L ₁₅	M ₀ L ₁₈	M ₅ L ₁₂	M ₅ L ₁₅	M ₅ L ₁₈	M ₁₀ L ₁₂	M ₁₀ L ₁₅	M ₁₀ L ₁₈	SEM
IBWT (kg)	0.95	0.97	0.93	0.94	0.93	0.92	0.96	0.94	0.96	0.05
BWTG (kg)	0.33 ^b	0.34 ^b	0.38 ^{ab}	0.34 ^b	0.45 ^a	0.42 ^{ab}	0.34 ^b	0.39 ^{ab}	0.42 ^a	0.02
FBWT (kg)	1.28 ^c	1.31 ^{ab}	1.31 ^{bc}	1.29 ^{cd}	1.39 ^a	1.35 ^a	1.30 ^{ab}	1.30 ^{ab}	1.29 ^{cd}	0.02
DFI (g)	102.66 ^d	106.33 ^c	109.33 ^{ab}	103.33 ^d	106.33 ^c	107.66 ^{bc}	103.66 ^d	107.66 ^{bc}	1110.00 ^{ab}	1.99
TFI (g)	8624 ^c	8932 ^b	9184 ^a	8680 ^c	8932 ^b	9156 ^a	8708 ^c	9044 ^a	9212 ^a	149.5
HDEP (%)	78.00 ^d	80.00 ^{cd}	79.33 ^{cd}	83.33 ^{bc}	90.00 ^a	85.33 ^{bc}	91.00 ^a	94.00 ^a	90.00 ^a	1.27
FCR	1.73 ^d	1.80 ^{ab}	1.82 ^a	1.73 ^{bc}	1.68 ^d	1.87 ^a	1.68 ^d	1.70 ^{cd}	1.80 ^{ab}	0.29
MORT	1.66 ^{ab}	2.00 ^{ab}	2.33 ^a	1.00 ^{bc}	0.66 ^{cd}	0.00 ^d	0.33 ^d	0.00 ^d	0.66 ^{cd}	0.46

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

IBWT= Initial body weight, BWTG = Body weight gain, FBWT= Final body weight gain.

DFI= Daily feed intake, TFI, = Total feed intake, FCR = Feed conversion ratio, HDEP = Hen day egg production.

The body weight gain increased significantly ($p < 0.05$) compared to the control groups. Layers on treatment $M_{10}L_{18}$ had higher body weight gain compared to those in other treatments. This implies that the interaction lead to improved feed utilization and production efficiency. The final body weight gain, daily feed intake and total feed intake were all influenced by the interaction. The hen day egg production, feed conversion ratio and mortality rate were also influenced by the treatments with layers in treatment $M_{10}L_{15}$ performing better than those in other treatments. The results of the study demonstrated that layers rose under 15 hrs and 18 hrs lighting photoperiods with 5 mg of melatonin consumed less feed and produced more eggs compared to layers raised on other lighting photoperiods without melatonin. This report agrees with the findings of Kumar *et al.* (2024) who noted that melatonin supplementation showed positive effects on the preantral follicular development in combination with different growth factors in sheep. Those rose on 15 hrs and 18 hrs lighting photoperiods with 10 mg of melatonin consumed more feed but no significant difference on egg production performance compared to those on 5 mg their counterparts on other treatments. Egg production was observed better when birds were provided 5 luxes for 16 hrs photoperiod during growing and 15 luxes for 20 hrs photoperiod during laying period (Khaskheli *et al.*, 2020). Results from this study has shown that melatonin can ameliorate the negative effect of continues lighting or photoperiod required in laying birds' production (Abbas *et al.*, 2008). The results obtained in this research suggest that melatonin administration to layering birds enhanced their growth and productivity. The marked improvement in the overall growth, egg production characteristics and performance observed may be attributed to the ability of melatonin to effectively counter the adverse effect of oxidative stress, induced by heat stress, increased lighting duration and feed metabolism. This is because increased lighting photoperiod induces

sleep deprivation and causes severe physiological stress responses (Campo and Davila, 2002). Interestingly, the group reared on 5 mg of melatonin and 15 hours of lighting had consistently higher body weight compared to those on 10 mg of melatonin for 18 hrs, this finding collaborate with the findings of Sinkalu *et al.* (2010) but contradicts the result of Rahimi *et al.* (2005), who obtained no significant difference in body weights at 42 d between lighting schedule treatment groups. Melatonin supplements could improve the feeding efficiency of chickens and promote their growth (Poon *et al.*, 1993). This finding agreed with the results of Apeldoorn *et al.* (1999), who subjected broiler chickens to varied lighting schedules of continuous 23L:1D and intermittent 1L:3D photoperiods and repeatedly found that the amount of feed consumed by melatonin-treated groups was consistently the lowest. The interaction resulted in a significant increase in the day production of layers under $M_{5}L_{15}$, $M_{5}L_{18}$ and $M_{10}L_{15}$, $M_{10}L_{18}$ compared to other treatment groups. Results from this studies on the effect of lighting on the performance of the laying birds indicates that continuous lighting up to 18 hours increased feed intake and this brought about increased body weight and enhanced HDEP. This report collaborated with the report of Jia *et al.* (2016) that melatonin implantation at a dose of 10 mg significantly improved the egg-laying rate of laying birds. This is because melatonin may have enhanced the egg-laying productivity of hens physiologically.

Interaction effect of melatonin and lighting on haematological indices of isa brown laying birds

Results of the interaction effect of melatonin and lighting on the haematological Indices of Isa brown laying birds are presented in Table 3.

Results from the present study indicate that melatonin improved the packed cell volume (PCV), the haemoglobin concentration (Hb), red blood cells (RBC) and RBC indices.

Table 3: Mean effect of melatonin and light on the haematological indices of isa brown laying birds.

PMT	M_0L_{12}	M_0L_{15}	M_0L_{18}	M_5L_{12}	M_5L_{15}	M_5L_{18}	$M_{10}L_{18}$	$M_{10}L_{15}$	$M_{10}L_{18}$	SEM
PCV (%)	25.00 ^c	26.00 ^{bc}	26.00 ^{bc}	27.33 ^{abc}	29.33 ^{abc}	27.33 ^{abc}	27.33 ^{abc}	30.00 ^a	29.33 ^{ab}	0.85
Hb (g/dl)	8.26 ^{ab}	8.60 ^a	7.8 ^{ab}	8.86 ^a	6.53 ^b	8.26 ^{ab}	8.20 ^{ab}	7.93 ^{ab}	7.13 ^{ab}	0.32
RBC ($\times 10^{12}/L$)	1.58	1.66	1.69	1.66	1.67	1.85	1.60	1.82	1.78	0.045
MCV (fl)	158.22 ^b	156.62 ^{bc}	153.84 ^c	162.65 ^b	175.62 ^a	147.72 ^c	170.81 ^a	164.83 ^{ab}	164.77 ^{ab}	2.98
MCH (pg)	52.72 ^a	51.80 ^a	46.15 ^{ab}	53.37 ^a	39.10 ^b	44.62 ^b	51.25 ^a	43.57 ^b	40.05 ^b	1.05
MCHC (g/dl)	33.04 ^a	33.07 ^a	30.00 ^a	32.81 ^a	22.26 ^b	30.22 ^a	30.000 ^a	26.43 ^{ab}	24.30 ^b	1.11
WBC ($\times 10^9/L$)	5.31 ^c	7.23 ^{bc}	8.15 ^b	10.43 ^a	6.75 ^c	7.21 ^b	10.18 ^a	7.66 ^{bc}	8.05 ^b	0.64
HETRO (%)	31.66 ^b	31.67 ^b	50.33 ^{ab}	44.33 ^{ab}	49.00 ^{ab}	55.00 ^a	46.66 ^{ab}	51.33 ^a	52.66 ^a	4.85
LYM (%)	70.60 ^a	64.66 ^{ab}	46.66 ^c	55.66 ^{abc}	66.00 ^{ab}	55.33 ^{abc}	52.00 ^{bc}	54.33 ^a	48.00 ^b	5.56
MON (%)	0.66 ^{ab}	2.33 ^{ab}	2.66 ^{ab}	1.00 ^{ab}	0.33 ^b	0.66 ^{ab}	1.00 ^{ab}	1.00 ^{ab}	0.33 ^b	0.45
EOS (%)	1.33 ^a	0.66 ^{ab}	0.66 ^{ab}	0.33 ^b	0.66 ^{ab}	0.60 ^{ab}	0.33 ^{ab}	0.00 ^b	0.00 ^b	0.32
BASO (%)	1.33	0.66	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.42

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

PMT= Parameters, PCV= Packed cell volume, Hb= Hemoglobin, RBC= Red blood cell.

WBC= White blood cell, HETRO Hetrophils, LYM= Lymphocytes, MON= Monocytes, EOS= Eosinophils, BASO= Basophils.

The increase in RBCs count, PCV and Hb concentration obtained in the present study may be attributed either to its direct stimulatory effect on bone marrow or through stimulation of cytokines. The results from these findings corroborate the report of Igwe *et al.* (2020) and Sinkalu (2012) who recorded higher values of erythrocytes, PCV, Hb, thrombocytes and total WBC obtained in broiler chicks groups administered with melatonin compared with the control groups.

These findings are also consistent with the report of Hodallah *et al.* (2011) who noted that RBC, PCV and Hb concentration of broiler birds treated with melatonin increased compared to the control groups. In other animals, Anwar *et al.* (1998) found that melatonin treatment in rats numerically increased RBCs, Hb and PCV. Results from the white blood cell differentials indicate that there were significant differences ($p < 0.05$) in the values of the heterophil and lymphocytes. Birds in the melatonin group had lower values of heterophil but higher values of lymphocytes. This present investigation showed that melatonin treatment significantly decreased the heterophil percentage in both experiments. This indicates that melatonin significantly increased all the immune parameters of the two strains of laying birds used in this study. The report of this study also suggested that melatonin is immune-enhancing for tropical birds and plays an important role in the maintenance of immunity. These results are consistent with the previous reports of Hodallah *et al.* (2011) who indicated that administration of melatonin decreased heterophil percent and lymphocyte percentage in broiler chicks.

CONCLUSION AND RECOMMENDATION

Results from this study have shown that some of the adverse effects of increased lighting duration in laying were reduced by the interaction of melatonin and lighting. Increased lighting is very important in stimulating egg laying but it leads to behavioral abnormality like aggressive feather pecking which can lead to cannibalism. Lighting also suppresses melatonin production but the interaction of the two treatments led to improved performance of the birds. The behavioural responses of the birds were also improved by the administration of melatonin at all levels. Panting rate, feather pecking and dust-bathing were drastically reduced and this improved the general well-being of the birds. The interaction improved hen day egg production and health status and reduced the mortality rate of the birds.

Conflict of Interest

There is no conflict of interest among the authors.

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