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Histomorphological Changes in the Thyroid Gland of Guinea Fowl (*Numida meleagris*) Exposed to Different Photoperiod Regimens and Exogenous Melatonin

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ABSTRACT

Investigations established that birds used photoperiod to predict and adjust seasonal changes in their environment through predictive changes. Understanding the interaction of photoperiods and exogenous melatonin will provide information on physiological activities and guide in better poultry management. In this study, thirty matured male helmeted guinea fowls were utilized. They were randomly assigned to three groups (n = 10) of different photoperiodic regimes: short-day (SD; 8L:16D), control (CTR; 12L:12D) and long-day (LD; 16L:8D). Each of these groups was further divided into two groups: melatonin (Mel; 1 mg/kg) or without melatonin. The experiment lasted for a period of eight weeks. The results obtained from the SD + Mel group showed that the thyroidal follicles were lined by simple squamous follicular cells and serrated edges, while the SD without Mel had smooth follicular edges with similar lining. The LD + Mel and LD without Mel appeared normal, with similar histological features observed in the CTR; the lining was dominated with simple cuboidal epithelium with very few squamous cells. The follicular diameter across the groups was statistically significant ($p < 0.0001$). The LD + Mel group had the largest follicular diameter, while the lowest was in SD without melatonin. The thyroid glands were both positive for Periodic Acid Schiff and Alcian Blue with varied percentage of positive areas. Overall, the activity of the thyroid gland in the guinea fowl is influenced by long photoperiod and exogenous melatonin. Its influence on the thyroid glands mimics the reproductively active phase of the guinea fowl.

Keywords: Guinea fowl; Histomorphology; Melatonin; Photoperiod; Thyroid gland

INTRODUCTION

Melatonin is a potent antioxidant and antiapoptotic agent that act by reducing the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Fischer *et al*., 2013; Salehi *et al*., 2019). It inhibits cell proliferation of thyroid gland, thyroid hormone synthesis and potentially decreasing its size (Gordon *et al*., 1980; Baltaci *et al.,* 2003; Baltaci *et al*., 2004). Conversely, melatonin antioxidant properties protect the thyroid gland from oxidative damage thereby supporting immune function. Additionally, melatonin may lower thyroid hormone levels by reducing synthesis and increase clearance, impacting energy, stress and immune responses (Karbownik *et al*., 2005; Rao and Chhunchha, 2010). Its concentration in the systemic circulation is utterly dependent on the degree of exposure to the light and dark cycle (Bhattacharya *et al*., 2019).

Light and dark cycles are used by animals to predict and adjust seasonal changes through predictive changes such as body weight, sleep, body fat, reproduction, immune function as well as behavior (Walton *et al*, 2011). This provides the animals with optimal ability to adapt and survive their changing seasonal environment (Renthlei *et al*., 2022). As photoperiod transducer, the melatonin hormone is mainly produced and secreted by the pineal gland during the night (Migaud *et al*., 2006; Xia *et al*., 2019). Through this activity, the pineal gland converts light information into a melatonin signal and thus relays on information such as the time of the day and year for cells (Kulczykowska *et al*., 2010).

Guinea fowls are good models for seasonal breeding since they restrict their reproductive activities to the rainy season (Avornyo *et al*., 2016; Okyere *et al*., 2020). Several reports

have been documented on biological manipulation of avian reproduction through the combined effect of photoperiodic regimes (artificial light) and exogenous melatonin (Baso *et al*., 2022; Baso *et al*., 2023; Gosomji *et al*., 2024). Understanding the interaction between artificial light cycles and exogenous melatonin will provide better insights into hormonal adaptation mechanisms and also guide in better poultry management.

MATERIALS AND METHODS

Animal Husbandry and Management

The study was conducted in a Commercial Poultry Farm (N 9.2182º, E 9.5179º), Bukuru Express Road, Plateau State Nigeria. The guinea fowls utilized for this study were managed under an intensive system with different photoperiodic regimes in a demarcated pen. The pen was covered with a black light-tight chamber to avoid external interruption of light. Each demarcation was partitioned 200 x 110 x 100 cm; illuminated by two cool-white LED lights (12 W) that provided a light intensity of 350 lux, for the control (CTR), short photoperiod (SD) and long photoperiod (LD) exposed groups. Water and commercial feed was provided *ad libitum.*

Experimental Design

Thirty (30) mature apparently healthy male helmeted guinea fowls (*Numida meleagris*) were used for this study. Wattle and vent sexing were used for the selections as described by Umosen *et al*. (2008) and Abdul-Rahman *et al*. (2015). Simple random sampling was used to group the guinea fowls (n = 10) into three (3) according to the hours of exposure of light (L) and darkness (D) as 8L:16D (short photoperiod; light exposure was eight hours and sixteen hours of darkness), 16L:8D (long photoperiod; light exposure was sixteen hours and eight hours of darkness) and 12D:12L (control; light exposure was twelve hours and twelve hours of darkness) a modified method by Thiele, (2009). Each group was further divided into two (2) making a total of six (6) groups i.e. one exposed to photoperiod regimes only (Non-Mel), and the other subjected to both artificial light regimes and melatonin (Mel) (Baso *et al*., 2022; Gosomji *et al*., 2024). The experiment lasted for eight (8) weeks. The use of animals for this experiment was performed in accordance with the guidelines for the Committee on Animal Use and Care, University of Jos, Plateau state, Nigeria where UJ / FPS/ F17-00379, was assigned for the study as the ethical clearance code.

Samples Collection

The birds were euthanized using a combination of ketamine and xylazine, at a dose of 35 mg/kg and 5 mg/kg, respectively via the jugular vein (Azeez *et al*., 2023). Thereafter, feathers were removed from the neck, sternum and chest, and the skin was incised using surgical scalpel. The neck, thoracic inlet, celomic cavity, the ribs were then cut opened. From both sides of the incision areas and the ribs cage were lifted to expose the heart, major blood vessels and the thyroid glands. As soon as the thyroid was exposed, its major adhesions, blood vessels and nerves were incised so that it can be removed for further study. The tissues were immediately fixed in 10% neutral buffered formalin (Azeez *et al*., 2022).

Histomorphometry

The fixed thyroid gland tissues were dehydrated through a series of graded alcohol (70%, 80%, 90%, 95%, and 100%). They were cleared in xylene and infiltrated in molten paraffin wax (Kiernan, 2015). Transverse sections of 5 µm thick were cut from the embedded tissue using disposable microtome knives. These sections were mounted on clean grease-free slides and stained at room temperature using Haematoxylin and Eosin (H and E) to observe the general features of the organ (Bancroft and Gamble, 2013). Periodic Acid Schiff (PAS) and Alcian Blue (AB) were used to determine the amount neutral and acid mucopolysaccharide as described by Hena *et al*. (2018) and Bancroft and Gamble (2013), respectively.

The histomorphometric changes in the thyroid were determine using Motic Image Plus (MI plus) version 2.0, using the modified approach of Azeez *et al*. (2022). In this approach, photomicrograph of twenty (20) rounded or nearly rounded thyroid follicle were selected to determine the follicular diameter (FD) and these were determined using four orthogonal lines, while four straight lines at different points of the capsule were used to determine the capsular thickness.

Statistical analysis

The data obtained were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Neuman Keuls post hoc statistical tests. Statistical significance was set at $p < 0.05$. GraphPad Prism software (GraphPad Prism version 5 for Windows, GraphPad Software, San Diego, California, USA) was used for the analyses.

RESULTS

Histology

The histological changes of the thyroid gland of guinea fowls at different photoperiods and exogenous melatonin groups are explained below in Figure 1.

The result in SD + Mel presents a serrated / rough edge of the thyroidal follicles lined by squamous and cuboidal cells. Lining the luminal part of some of the follicles are evenly distributed vacuolation giving it a patchy appearance (Figure 1A). Within the interfollicular spaces are the parafollicular cells, red blood cells and network of connective tissues (Figure 1A - B). In the SD, thyroid follicles were of varied sizes and shapes, lined by squamous epithelium. The vacuolations were fewer (1C). The parafollicular cells were numerous (Figure 1D). The colloids in both SD and SD + Mel appeared lighter (Figure 1A - D).

The follicular cells in CTL + Mel and CTL were of varied shapes and sizes. In-between the thyroid follicles were the interfollicular spaces occupied by parafollicular cells and red blood cells enmeshed in the connective tissue and lining the follicles were simple cuboidal epithelium with very few squamous epithelium (Figure 1E - H).

In the LD + Mel and LD, the follicular cells were of different sizes and shapes lined by simple cuboidal epithelium. Although there were few squamous epithelia

lining the thyroid gland exposed to LD, the colloids appeared darker in both groups (Figure 1I - L).

Histochemistry

Periodic Acid Schiff (PAS) Quantification

The thyroid glands subjected to PAS stain in this experiment were observed to be positive (Figure 2A – F; i – iii). The positivity of the thyroid gland to the stain was determined by percentage of the positively stained area. In the SD groups, the percentage staining area of the thyroid gland was not statistically significant ($p > 0.43$) in the Non-Mel and Mel of the groups. Although more of the positive area were observed in the Non-Mel group than in the Mel group (Figure 2A – B; i).

In the CTR groups, the percentage staining area of PAS was statistically insignificant ($p > 0.49$) when comparing the Non-Mel and Mel groups (Figure 2C – D; ii). The positive response was higher in the Mel group than the Non-Mel group. Similar findings were observed LD groups where the PAS staining area was statistically insignificant ($p > 0.81$). However, the positive responses of both Non-Mel and Mel groups were similar (Figure 2E – F; iii).

Alcian Blue (AB) Quantification

The AB stained thyroid gland was positive in this study (Figure 3A – F; i – iii). The positivity of the thyroid gland to the stain was determined by percentage of the positively stained area. The percentage staining area of the thyroid gland in the SD groups was not statistically significant ($p > 0.17$). The Non-Mel had higher positive response than Mel group in the short photoperiodic group. Although more of the positive area were observed in the Non-Mel group than in the Mel group (Figure 3A – B; i).

In the CTR group, the percentage staining area of AB was statistically insignificant ($p > 0.79$) when comparing the Non-Mel and Mel groups (Figure 3C – D; ii). The positive response was higher in the Mel group than the Non-Mel group. Similar findings were observed in the CTR groups where the AB staining area was statistically insignificant ($p > 0.83$). However, the positive responses of both Non-Mel and Mel groups were similar (Figure 3E – F; iii).

Histomorphometric Assessment

The follicular diameter across the photoperiodic groups were statistically significant ($p < 0.0001$) with the higher follicular diameter at LD than SD (Table 1). For the photoperiodic groups exposed with exogenous melatonin, there was a significant difference in the follicular diameter with highest values in long photoperiod LD + Mel while the lowest diameter was short photoperiod SD + Mel. Overall, the photoperiodic with melatonin groups had higher follicular diameter than photoperiodic group only (Table 1).

The thyroid gland of the helmeted guinea fowl was enveloped by a tightly adhered capsule, ` a connective tissue that contains blood vessels and lymph nodes, in some cases the parathyroid glands were obvious. Following the exposure to photoperiod and exogenous melatonin, the capsule thickened base on the amount of exposure. The capsular thickness exposed to photoperiodic regime is statistically significant ($p < 0.003$) where the capsule of the thyroid gland exposed to LD was more than the short photoperiod but both lesser than the control (Figure 4).

There was no significant difference in the capsular thickness of the thyroid glands exposed to melatonin and non-melatonin groups (Figure 4). However, the interaction of photoperiod and exogenous melatonin indicates a significant difference ($p < 0.0001$) in the capsular thickness of the thyroid gland of the helmeted guinea fowl. The lowest thyroidal capsular thickness was LD + Mel while the LD was highest. Compared to the CTR, the LD was thicker while LD + Mel was thinner. There were significant differences when comparing SD and CTR + Mel ($p < 0.004$); SD + Mel and CTR + Mel ($p < 0.0003$); CTR and CTR + Mel ($p < 0.002$); CTR + Mel and LD ($p < 0.04$); CTR + Mel and LD + Mel ($p < 0.0001$).

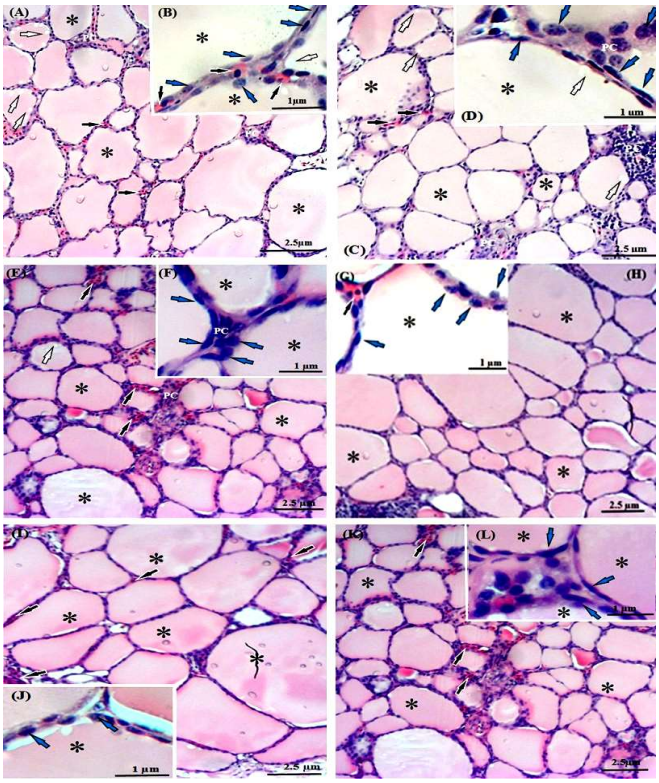

Figure 1: Photomicrograph of the guinea fowl (*Numida meleagris*) showing vacuolation (white arrows) and serrated edges of the thyroidal follicles (black asterisks), parafollicular cells (PC), red blood cells (black arrows) between follicles, cuboidal and squamous follicular cells (blue arrows) lining the follicles (black asterisks). (A - B) SD + Mel; (C - D) SD; (E - F) CTL + Mel; (G - H) CTL; (I - J) LD + Mel; (K –L) LD. H and E stain; Scale bar: (A, C, E, G, I and K) 2.5 µm, (B, D, F, H, J and L) 1 µm.

DISCUSSION

Light period is an exogenous factor affecting poultry performance, which is regulated by hormones including thyroid hormones (Yoshimura *et al*., 2003). Our results demonstrated that the exposure to varying photoperiod regimens and treatment with exogenous melatonin potentiated the activation of thyroid gland in the guinea fowl and this corroborated the findings of Dzerzhynsky *et al*. (2006). Several reports on the influence of light – dark cycle and artificial light on the thyroid gland of birds has been documented (Follet and Nicholls, 1984; Wilson and Reinert, 1993; Dawson *et al*., 2001; Yoshimura, 2013). The reports confirmed the stimulatory effect of melatonin and its impact on metabolic activity (Sakamoto *et al*., 2000), as such a slight change in metabolism could affects anatomic

presentation of vital organs especially the thyroid gland. This therefore confirms the activity of melatonin which accelerating the process of thyroid gland activation as such increases the immune system of the animal (Dzerzhynsky *et al.*, 2006).

The thyroid gland histoarchitecture of the guinea fowl in this study had similar presentations with previous reports of other birds (Moghanlo and Mohammadpour, 2018). However, there was a transition of normal appearance of cuboidal epithelium lining the colloids to squamous epithelium and also the serrated edges of the thyroid follicles in the short photoperiod either with melatonin or without melatonin probably due to increase in the endogenous melatonin in the circulation which further indicated that the follicle was inactive in the groups. This corroborate the findings by Moghanlo and Mohammadpour (2018) in guinea fowl which was further confirmed by the lighter appearance of colloids in the SD groups as indication of inactive thyroid glands while the darker appearance in the long duration of artificial light either with melatonin or without melatonin were confirmed in active thyroid glands.

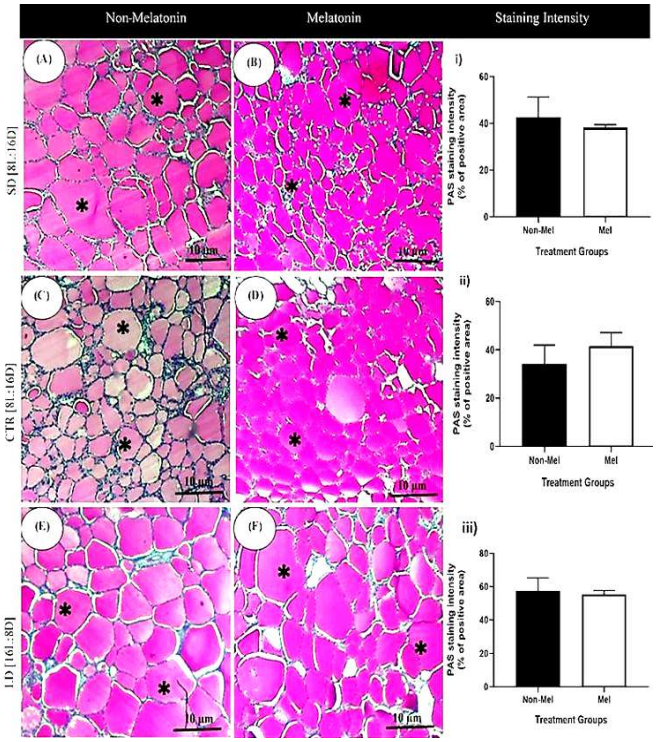

Figure 2: Photomicrographs showing the periodic Acid-Schiff (PAS) expression: PAS +ve areas in the thyroid follicles and interfollicular spaces of the thyroid gland of guinea fowl (*Numida meleagris*) exposed to different photoperiodic regimes: (Fig. 7A-F), in melatonin (Mel) and non-Mel groups. (i-iii) are the corresponding bar charts, representing area intensities (%) in the PAS-stained tissues of each photoperiodic regimen. Scale bar: 10 µm

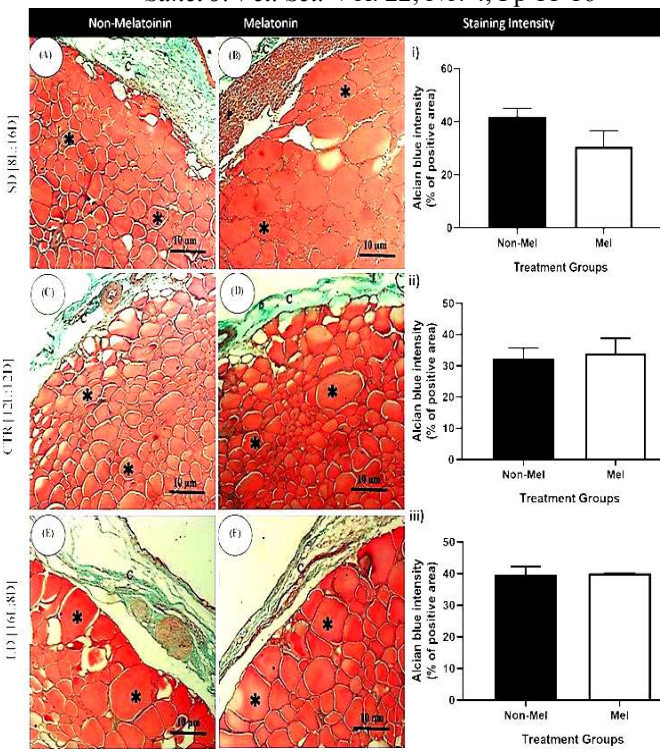

Figure 3: Photomicrographs showing the Alcian Blue (AB) expression: AB +ve areas in the interfollicular spaces and loose connective tissue area of the thyroid gland capsule of guinea fowl (*Numida meleagris*) exposed to different photoperiodic regimes: (Fig. 8A-F), in melatonin (Mel) and non-Mel groups. (i-iii) are the corresponding bar charts, representing area intensities (%) in the AB-stained tissues of each photoperiodic regimen. Scale bar: 10 µm.

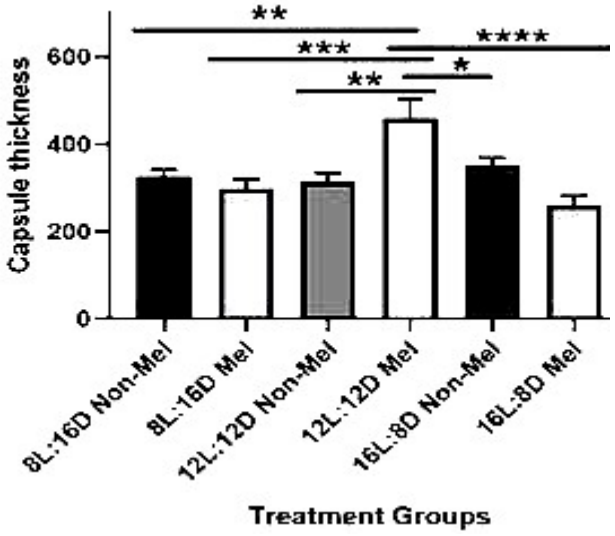

Figure 4: Chart illustrating thyroid gland capsular thickness of the guinea fowl exposed to different photoperiod and exogenous melatonin; n = 3 per group; significant difference in SD and CTR + Mel ($P < 0.004$); SD + Mel and CTR + Mel ($P < 0.0003$); CTR and CTR + Mel ($P < 0.002$); CTR + Mel and LD ($P < 0.04$); CTR + Mel and LD + Mel ($P < 0.0001$).

Table 1: Thyroid follicular diameter of guinea fowl (*Numida meleagris*) subjected to artificial lights and melatonin

Factors	8L:16D (µm)	12L:12D (µm)	16L:8D (µm)
Photoperiods (No melatonin)	340.6 ± 5.34^{b}	329.2 ± 4.70^{c}	362.3 ± 5.17^{b}
Photoperiods + Melatonin	357.1 ± 10.02^{c}	374.8 ± 19.85^{b}	404.0 ± 6.38^{a}

a, b, c values with different letter superscripts in each row are significantly different ($p < 0.0001$)

In this study, increase follicular diameter is associated with exogenous melatonin. This agrees with the findings of Paul *et al.* (2011) on matured male chicken. The increase in the diameter could be as a result of enlarged

thyroid gland and increases total thyroid hormone in the blood stream due to either endogenous or exogenous melatonin (Gordon *et al*., 1980; Baltaci *et al.,* 2003; Baltaci *et al*., 2004). Thus, the reduced size/height of the follicular cells in the present study along with the slight reduction of follicular size may have contributed to the reduction of serum thyroid hormone levels. Meanwhile, the decrease in relative colloid area suggests slightly reduced hormone storage (Mattsson *et al*., 2019).

In conclusion, the thyroid gland of helmeted guinea fowl is influenced by the long photoperiod with exogenous melatonin. Its influence on the thyroid glands mimics the reproductively active phase of the guinea fowl.

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Conflict of Interest

The authors have no conflict of interest to declare

Author Contribution

GIJ conceived and designed the experiment, supervised, reviewed the original and final draft of the manuscript; KNB analyzed generated data and reviewed the final draft of the manuscript; PSA analyzed the generated data and reviewed the final draft of the manuscript; IAJ collected samples and reviewed the final draft of the manuscript; WN reviewed the original and final draft of the manuscript; BA reviewed the original and final draft of the manuscript; MI collected samples and analyzed the generated data; AIA supervised and reviewed the final draft of the manuscript; GS collected samples and wrote the original manuscript; OJO supervised and reviewed the final draft of the manuscript; HSA supervised and reviewed the final draft of the manuscript. All authors approved the final manuscript for publication.

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