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HORMONAL PROFILE AND FOLLICULAR DYNAMICS OF TWO BREEDS OF OLD LAYING HENS ON MELATONIN ADMINISTRATION IN NIGERIA

PERFIL HORMONAL Y DINÁMICA FOLICULAR DE DOS RAZAS DE GALLINAS PONEDORAS ENVEJECIDAS QUE SE LE SUMINISTRÓ MELATONINA EN NIGERIA

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In a 20-week study, 117 laying hens, aged 52 weeks, of the Isa Brown and Nera Black breeds were used to evaluate the effects of melatonin on reproductive hormones and egg production. The study utilized a 2×3 factorial, a completely randomized block design with three treatments (0 mg, 5 mg, and 10 mg of melatonin) and three replicates, administered daily through drinking water. A total of 39 samples of blood were collected and analyzed. Melatonin at 5 mg (T_2) significantly improved levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in Isa Brown hens, with concentrations of 629.14 pg/mL and 55.48 mIU/mL, respectively. However, higher melatonin levels at 10 mg (T_3) led to reduced hormone levels (279.40 pg/mL and 39.64 mIU/mL). A similar trend was observed in Nera Black hens. Hen day egg production (HDEP) also improved with melatonin treatment, with the 5 mg dosage (T_2) yielding the highest production rates for both breeds. Specifically, Isa Brown hens showed HDEP percentages of 39.11 % (T_1), 86.33 % (T_2), and 61.66 % (T_3), while Nera Black hens had HDEP percentages of 31.10 % (T_1), 70.86 % (T_2) and 58.60 % (T_3). In conclusion, melatonin at 5 mg improved the reproductive hormonal profile, promoted follicle development and growth the latter will increase egg production in the two breeds, preferentially in the Nera black breed.

Keywords: *follicle stimulating hormone, luteinizing hormone, melatonin*

En un estudio de 20 semanas, se utilizaron 117 gallinas ponedoras, de 52 semanas de edad, de las razas Isa Brown y Nera Black para evaluar los efectos de la melatonina en las hormonas reproductivas y la producción de huevos. El estudio utilizó un diseño factorial 2×3, en bloques completamente aleatorizados con tres tratamientos (0 mg, 5 mg y 10 mg de melatonina) y tres réplicas, administradas diariamente a través del agua de beber. Se recolectaron y analizaron 39 muestras de sangre. La melatonina a 5 mg (T_2) mejoró significativamente los niveles de hormona foliculoestimulante (FSH) y hormona luteinizante (LH) en gallinas Isa Brown, con concentraciones de 629.14 pg/mL y 55.48 mUI/mL, respectivamente. Sin embargo, mayores niveles de melatonina a 10 mg (T_3) condujeron a niveles reducidos de hormonas (279.40 pg/mL y 39.64 mUI/mL). Una tendencia similar se observó en las gallinas Nera Black. La producción de huevos de gallina por día (HDEP) también mejoró con el tratamiento con melatonina, con la dosis de 5 mg (T_2), con la mayor tasa de rendimiento productivo para ambas razas. Específicamente, las gallinas Isa Brown mostraron porcentajes de HDEP de 39.11 % (T_1), 86.33 % (T_2) y 61.66 % (T_3), mientras que las gallinas Nera Black tuvieron porcentajes de HDEP de 31.10 % (T_1), 70.86 % (T_2) y 58.60 % (T_3). En conclusión, la melatonina a 5 mg mejoró el perfil hormonal reproductivo, promovió el desarrollo y crecimiento de los folículos. Esto último aumentará la producción de huevos en las dos razas, preferentemente en la raza Nera Black.

Palabras clave: *hormona estimulante del folículo, hormona luteinizante, melatonina*

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Introduction

Laying hens can continue to produce eggs for several years; however, their productivity often declines significantly after the first year, with this decline varying widely among breeds (Albatshan *et al.* 1994). To enhance the performance of aging hens, it is crucial to delay ovarian aging and manage the depletion of the primordial follicle reserve after the peak egg production period (Barzegar *et al.* 2020).

Previously, many researchers have reported the antioxidant and anti-inflammatory effects of melatonin (Bantounou *et al*. 2022). Melatonin (MT) has been shown to influence both the quantity and maturity of ovarian follicles, though research on its specific mechanisms in birds remains limited (Hao *et al*. 2020). Recent studies have highlighted melatonin's beneficial effects on reproduction in various animals. For instance, melatonin has been found to promote oocyte and early embryo development in mammals, including mice (Ganji *et al*. 2015). In laying hens, common issues during the later stages of production include reduced laying performance, decreased follicle counts in the ovaries (Zakaria *et al.* 1983 and Ferlazzo *et al*. 2020), and higher egg breakage rates (Albatshan *et al*. 1994).

Follicular development, maturation, and atresia play significant roles in various stages of follicular function, providing insight into the molecular mechanisms underlying egg production. FSH and LH work in tandem to ensure normal follicular growth (Raju *et al*. 2013). FSH, produced by the anterior pituitary in response to gonadotropin-releasing hormone (GnRH) from the hypothalamus, plays a crucial role in reproductive physiology and fertility in both male and female animals (Ferlazzo *et al*. 2020). FSH enhances follicular development by promoting angiogenesis in the theca externa follicles, especially in hens with low egg production (Bi *et al*. 2021). While FSH stimulates ovum development and maturation, LH is responsible for ovulation. Optimal levels of both hormones accelerate follicle growth, leading to increased egg production through the development and ovulation of more follicles (Prastiya *et al.* 2022). It also plays a role in regulating reproductive hormones by influencing FSH and LH synthesis and secretion, affecting the circadian rhythm, embryonic growth, and ovary development (Duo *et al.* 2014). Melatonin has been linked to improved immune responses and reduced oxidative stress in poultry. For example, a study by Liu *et al.* (2022) found that melatonin supplementation enhanced the immune system and reduced markers of oxidative stress in broilers, contributing to better overall health and productivity. Despite these findings, there are limited research on the effects of melatonin on laying hens beyond their egg-laying peak, particularly in Nigeria.

Therefore, this experiment was designed to evaluate the effect of melatonin on hormonal profile, follicular development and production performance of spent layers of two prominent breeds of laying hen in Nigeria.

Materials and Methods

Experimental site: This experiment was approved by the University Ethical and Research Community with reference number EBSU/2022/2087. After the approval, it was then carried out at the Poultry Unit of the Teaching and Research Farm of Ebonyi State University, Abakaliki, Nigeria. The experiment was carried out during the dry season between October 2022 and March 2023.The mean temperature was 37.8 ^{o}C - 39 ^{o}C during the hot dry season.

Experimental animals and management: The study involved Isa Brown and Nera Black hens, both 54 weeks old at the start of the 20-week experiment. The experiment was conducted in two phases, referred to as Experiment I and Experiment II, each focusing on a different breed of laying hens. A total of 117 laying hens from each breed were used, with the hens divided into three treatment groups for each experiment. Each treatment group consisted of 39 birds, which were further subdivided into three replicates of 13 hens each, housed on rice husk bedding.

The average live weight for Isa Brown birds was 1.65 kg, while Nera Black birds averaged 1.96 kg. These layers were sourced from Agrited, a breeding company based in Ibadan, Nigeria. Throughout the experiment, the birds were fed a layer's mash containing 16 % crude protein, 3.5 % ether extract, 4.7 % crude fiber, and 11.2968 MJ/kg metabolizable energy. Below is the diet composition (table 1)

Table 1. Feed Composition of the Experiment

Ingredients	**% composition**
Maize	48.50
Wheat offal	10.30
Palm kernel meal	12.70
Groundnut cake	10.70
Fish meal	6.30
Oyster shell	6.0
Bone meal	4.0
Salt	0.5
Premix	0.5
Lysine	0.25
Methionine	0.25
Total	100
Calculated	**Values**
Crude protein %	16
Crude fiber %	4.70
Ether extract %	3.20
Metabolizable (Mj/kg)	11.30

*Premix to provide the following per kg of feed; Vit A-500 iu, Vit D3-1200 mg, Vit.E-11 mg, Vit.K-2 mg, Riboflavin- 20 mg, Nicotinic acid-10 mg, Pantothenic acid- 7 mg, Cobalamin- 0.08 mg, Choline chloride-900 mg, Folic acid- 1.5 mg, Biotin-1.5 mg, Iron- 25 mg, Manganese-80 mg, Copper-2 mg, Zinc-50 mg, Cobalt-1.25 mg and Selenium-0.1 mg

Experimental materials: The experimental material was melatonin supplement. The melatonin was dissolved in 10 % ethanol at the rate of 5 mg/1 mL or 10 mg/1 mL and administered orally through drinking at the rate of 2 mL per liter of water daily to the layers.

Hormonal analysis: At the end of the experiment (72 weeks of age), blood samples (5 mL) (two samples in each replicate) were obtained from the brachial vein of each hen's wing and stored in a vacutainer plain tube in a cool box (4 °C) filled with ice gel and ice cubes before moving them to the laboratory. An enzyme-linked immunosorbent assay (ELISA) was used to determine the Progesterone, FSH, and LH levels in the blood samples. In the ELISA, blood samples were centrifuged. The plasma luteinizing hormone (L.H.), follicle-stimulating hormone (FSH), and progesterone were quantified utilizing a homologous RIA (Krishnan *et al.* 1993) or determined by carrying out a quantitative test based on a solid phase enzyme-linked immuno-absorbent assay (ELISA) kit.

Reproductive morphological evaluations: At the end of the experiment (72 weeks of age), 6 birds were randomly selected from each group (2 birds per replicate making it 18) and weighed and decapitated. The follicles were collected and promptly counted under the microscope for small white follicles, small yellow follicles and slightly larger follicles within 5mm to 2mm (SWF, SYF, F5, and F2).

Statistical analysis: A 2×3 factorial arrangement in a completely randomized block design was used for the study. The differences between the treatment groups and the control group were analyzed with a Mini-Tab Version 12. Duncan's new multiple-range tests after fisher test were used to identify which treatment conditions were significantly different from each other at a significance level of $p<0.05$ (Duncan 1955)

Results

Effect of Melatonin on the Hormonal Profile of Isa Brown: Results from the table 2 showed that melatonin significantly ($p<0.05$) influenced the hormonal profile of the Isa Brown breed of laying birds. The control group T_1 (2.96 pg/mL) had the lowest level followed by T_2 (6.15 pg/mL) of progesterone while T_3 (8.24 pg/mL) had the highest level. Follicle-stimulating hormone and Luteinizing hormone levels were equally significantly ($p<0.05$) affected by melatonin treatment. The highest average FSH levels were found in the hens under 5 mg of melatonin. Higher levels of FSH were seen in T_2 which had 629.140 pg/mL followed by T_3 (279.45 pg/mL) while control group T_1 (198.78 pg/mL) had the least level. A similar trend was followed in the LH level where T_2 (55.48 mIU/mL) had higher levels followed by T_3 (39.64 mIU/mL) and T_1 (30.68 mIU/mL), respectively

Results from table 3 indicate that melatonin significantly ($p<0.05$) improved the hen day egg production of layers after 50 weeks of age. Groups on 5 mg had 86.33 % followed by those on 10mg which had 61.66 % while the control group (0 mg) had the lowest HDEP. A similar trend was equally observed on the oviduct weight. The size of the follicles was equally influenced by melatonin administration. The LYF, SYF and SWF were all increased by melatonin administration. From F5-F1 there were numerical increases with increasing levels of melatonin, but it was not statistically significant.

Effect of Melatonin on the Hormonal Profile of Nera Black: Progesterone, Follicle-stimulating hormone, and Luteinizing hormone levels were significantly ($p<0.05$) affected by melatonin treatment (table 4). The highest average FSH levels were found in the hens under 5 mg of melatonin which was ($T_2$429.68 pg/mL) followed by groups on 10 mg ($T_3$198.46 pg/mL) and 0 mg ($T_1$52.74 pg/mL), respectively. A similar trend was equally recorded in LH where higher levels were recorded in T_2 followed by T_3 and T_1, respectively. Progesterone levels were equally increased with increased levels of melatonin across the groups.

Results from table 5 show that melatonin significantly ($p<0.05$) influenced some of the parameters. The HDEP was improved with melatonin administration. Treatment T_2 (70.86 %) on 5 mg of melatonin had higher HDEP followed by T_3 (58.60 %) while the control group had the least (31.10 %). The oviduct weight increased with increased level of melatonin where T_3 (84.18 g) was followed by T_2 (78.59 g) while T_1 (54.40 g) recorded the lowest weight.

Table 2. Mean Effect of Melatonin on the Hormonal Profile of Isa Brown

Parameter	T_1 (0 mg)	T_2 (5 mg)	T_3 (10 mg)	SEM	P - value
Progesterone, ng/mL	2.96^b	6.15^a	8.24^a	0.41± 0.01	0.000**
FSH, pg/mL	198.78^b	629.140^a	279.45^b	6.41± 0.07	0.053**
LH, mIU/mL	30.68^b	55.48^a	39.64^b	0.32 ± 0.0	0.000**

$^{a-b}$ Means in the same row with different superscripts differ significantly ($p<0.05$) according to Duncan's Multiple Range Test. FSH: Follicle Stimulating Hormone, LSH: Luteinizing Hormone

Table 3. Mean Effect of Melatonin on Follicular Dynamics of Isa Brown

Parameter	T_1 (0 mg)	T_2 (5 mg)	T_3 (10 mg)	SEM	p-value
HDEP (%)	39.11^b	86.33^a	61.66^a	3.63 ± 0.07	0.005**
Oviwt (g)	34.40^b	64.59^a	68.18^a	2.92 ± 0.02	0.000**
F_5 (mm)	3.00^b	6.00^a	5.00^a	0.86 ± 0.00	0.001**
F_4 (mm)	4.00	6.00	4.00	0.61 ± 0.00	0.012
F_3 (mm)	4.00	5.00	5.00	0.50 ± 0.00	0.057
F_2 (mm)	4.00	4.00	5.00	0.99 ± 0.10	0.001
F_1 (mm)	4.00	4.00	5.00	0.48 ± 0.00	0.058
LYF (mm)	30.55^b	46.33^a	38.11^a	4.60 ± 0.10	0.000**
SYF (mm)	18.55^c	40.66^a	30.12^a	0.59 ± 0.0	0.007**
SWF (mm)	20.35^a	44.44^a	35.33^b	2.03 ± 0.03	0.000**

$^{a-b}$ Means in the same row with different superscripts differ significantly (p<0.05) according to Duncan's Multiple Range Test. HDEP: Hen-Day-Egg Production, Oviwt: Oviduct weight.

F_5: Fifth yellow follicle, F_4: Fourth Yellow Follicle, F_3: Third Yellow Follicle, F_2: Second Yellow Follicle and F_1: First Yellow Follicle, LYF: Large Yellow Follicle, SYF: Small Yellow Follicle, SWF: Small White Follicle

Table 4. Mean Effect of Melatonin on the Hormonal Profile of Nera Black

Parameter	T_1 (0 mg)	T_2 (5 mg)	T_3 (10 mg)	SEM	p- value
Progesterone ng/mL	3.16^c	8.15^b	9.94^a	0.41± 0.01	0.012**
FSH pg/mL	52.748^c	429.68^a	198.45^b	6.41 ± 0.05	0.009**
LH mIU/mL	23.55^b	51.78^a	31.14^b	0.32 ± 0.00	0.000**

$^{a-b}$ Means in the same row with different superscripts differ significantly (p<0.05) according to Duncan's Multiple Range Test. FSH: Follicle Stimulating Hormone, LSH: Luteinizing Hormone

Table 5. Mean Effect of Melatonin on Follicular Dynamics of Nera Black

Parameters	0 mg	5 mg	10 mg	SEM	p-value
HDEP (%)	31.10^c	70.86^a	58.60^b	1.13 ± 0.00	0.007**
Oviwt (g)	54.40^b	78.59^a	84.18^a	2.12 ± 0.02	0.000**
F_5 (mm)	2.00^b	4.00^a	3.00^a	0.61 ± 0.00	0.000**
F_4 (mm)	2.00	4.00	4.00	0.31 ± 0. 00	0.031
F_3 (mm)	2.00	3.00	2.00	0.30 ± 0.00	0.001
F_2 (mm)	3.00	4.00	4.00	0.81 ± 0.01	0.000
F_1 (mm)	2.00	4.00	4.00	0.28 ± 0.1	0.120
LYF (mm)	18.55^b	28.30^a	27.11^a	4.60 ± 0.04	0.000**
SYF (mm)	10.00	15.60	15.10	0.20 ± 0.00	0.201
SWF (mm)	10.35	14.44	13.33	1.43 ± 0.02	0.057

$^{a-b}$ Means in the same row with different superscripts differ significantly (p<0.05) according to Duncan's Multiple Range Test. HDEP = Hen-Day-Egg Production, Oviwt = Oviduct weight. F_5: Fifth yellow follicle F_4: Fourth Yellow Follicle. F_3: Third Yellow Follicle. F_2: Second Yellow Follicle and F_1: First Yellow Follicle, LYF Large Yellow Follicle, SYF = Small Yellow Follicle, SWF Small White Follicle

Some of the follicles were equally influenced by the treatment while some had a numerical increase but were not statistically ($p>0.05$) significant.

Discussions

Aging and environmental stress significantly impact an animal's reproductive potential, suppressing its ability to reproduce effectively (Pandi-Perumal 2013 and Barzegar *et al*. 2020). The increased FSH and LH levels in both breeds, could be attributed to melatonin antioxidative and nutraceutical properties in disease management (Kamfar *et al.* 2024) The lowest FSH levels were observed in the 10 mg melatonin and control groups. Statistical analysis of the results confirmed that the 5 mg melatonin group exhibited significantly ($p<0.05$) higher FSH levels. This finding aligns with Ragil *et al.* (2022), who reported that elevated FSH and LH levels in laying hens promote follicle development and ovulation.

The increased follicle development, maturation, and growth in both Isa Brown and Nera Black hens in this study led to a notable increase in hen-day egg production. This outcome is consistent with Jonak *et al*. (2017), who observed that FSH enhances granulosa cell differentiation in pre-hierarchical follicles and supports steroid hormone synthesis in granular cells. Similarly, Ragil *et al.* (2022) noted that higher FSH levels were associated with increased egg-laying frequency in Isa Brown hens. FSH plays a key role in follicular development by promoting angiogenesis in the theca externa follicles of hens with low egg production rates and aged hens (Tamura *et al.* 2017 and Bi *et al*. 2022).

The hormonal status of a hen significantly influences egg production and quality (Prastiya *et al.* 2022). The high FSH levels in Isa Brown hens likely contributed to their higher follicle counts compared to Nera Black hens, resulting in greater hen-day egg production in the Isa Brown group. Additionally, progesterone levels were elevated in the melatonin treatment groups compared to the control, indicating that higher melatonin doses increased progesterone levels, unlike FSH and LH. However, high doses of melatonin (e.g., 10 mg) significantly reduced FSH and LH levels in both breeds, as observed in treatment 3. This result supports Kang *et al.* (2023), who reported that higher doses of melatonin (e.g., 10 mg/kg) reduced FSH and LH levels, potentially due to feedback mechanisms or altered endocrine responses.

Luteinizing hormone (LH) levels fluctuated across treatment groups, with the highest concentrations observed in those administered 5 mg of melatonin. This suggests that melatonin enhances follicle development, particularly of the largest follicles (F1). This finding is consistent with the study by Marques *et al*. (2022), which reported increased LH levels leading to a higher number of mature follicles in laying birds. The increase in progesterone synthesis observed in this study was linked to a reduction in estrogen levels, which occurs as larger follicles in the follicular hierarchy, especially F1, grow. This growth contributed to improved laying performance in the birds, despite the natural decline in production due to aging.

Hen-day egg production (HDEP) was significantly enhanced by melatonin administration. At 50 weeks (350 days), birds typically pass their egg production peak, and a decline in egg production often leads farmers to sell the layers as spent layers before recovering their production costs (Tamura *et al*. 2017). However, melatonin administration improved HDEP in both Isa Brown and Nera Black hens. Isa Brown achieved the highest HDEP at 86.33 %, followed by Nera Black at 70.86 %. These results align with Yaxiong *et al*. (2016), who found that melatonin improved egg production in layers after 400 days. The results of this experiment equally corroborate the work of Bocheva *et al.* (2024) who noted that melatonin improves egg production in laying birds. The improved production could be attributed to protective effect of melatonin against stress through its direct free radical scavenging activity and indirect antioxidant activity via production of antioxidant enzymes (Yuanyuan *et al*. 2021)

In this experiment, the highest HDEP levels were recorded in treatment 2 (5 mg melatonin), followed by treatment 3, with treatment 3 showing the lowest performance. This led to the decreased expression of inflammatory mediators, such as cytokines, adhesion molecules and enzymes (Ferlazzo *et al*. 2020) leading to improved follicular growth. This indicates that a lower melatonin dosage of 5 mg was more effective in both breeds compared to a higher dosage.

The improved performance could be attributed to the increase in follicle development, from F5 to F1, as well as the small white follicles (SWF) and small yellow follicles (SYF) in both breeds. This is in line with Yaxiong *et al.* (2016), who reported that a higher melatonin dosage (20 mg implant) had a slightly inhibitory effect on the egg-laying rate. Additionally, increased melatonin administration led to an increase in oviduct weight, which was also reflected in higher egg weights and average body weight in the birds.

Before the experiment, egg production occurred once every three to four days, but within five days of melatonin administration, production increased to once every two days, and eventually to daily laying, especially in Isa Brown hens. This suggests that melatonin may serve as a growth promoter in animals.

Conclusions

Enhancing hen-day egg production in aged birds beyond 364 days is a crucial goal that can significantly increase farmers' income in the poultry industry. In this experiment, we observed that melatonin positively influenced the hormonal profile of the birds, leading to a resurgence in their peak egg production due to the further maturation of ovarian follicles. Isa Brown birds exhibited superior performance with a higher number of follicles, resulting in greater hen-day egg production. However, Nera Black birds had a higher oviduct weight compared to Isa Brown, which contributed to increased egg weight and overall body weight.

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