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Journal of Veterinary Physiology and Pathology. 2025; 4(4): 64-68.

DOI: 10.58803/jvpp.v4i4.76

http://jvpp.rovedar.com/

Research Article

Modulatory Effects of Melatonin and Artificial Light on Testicular Morphometry in Guinea Fowl (*Numida Meleagris*)

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ARTICLE INFO

Article History:
Received: 02/11/2025
Revised: 29/11/2025
Accepted: 15/12/2025
Published: 29/12/2025

Keywords:
Guinea fowl
Melatonin
Morphometry
Photoperiod
Testis

ABSTRACT

Introduction: Guinea fowls have sophisticated seasonal mechanisms that make them an excellent model for studying reproductive activities. The present study aimed to investigate the impact of exogenous melatonin and artificial light on testicular morphometry in sexually mature guinea fowls.

Materials and methods: A total of 65 mature male guinea fowls, with an average weight of 1-2 kg, were randomly selected for the present study. Five guinea fowls were utilized for the primary evaluation study on day 0. Sixty guinea fowls were divided into six groups with 10 guinea fowls in each group. The first group (SD) was exposed to 8 hours of light and 16 hours of darkness (8L:16D). The second group (SD-Mel) was exposed to 8L:16D with an additional melatonin injection. The third group was the control group (CTL) with 12L:12D. The fourth group was maintained on CTL with an additional injection of melatonin (CTL-Mel). The fifth group (LD) was exposed to 16L:8D, and the sixth group was exposed to 16L:8D with an injection of melatonin (LD-Mel). Melatonin was administered intramuscularly at 1 mg/kg body weight 2 hours before the end of each light cycle. The entire study was conducted in 60 days. Body weight (BW), testicular weight, and gonado-somatic index (GSI) were recorded at days 0, 30, and 60 of the study.

Results: There was a consistent increase in the BW, testicular weight, and GSI from days 0 to 60 in all groups. The results of BW, testis weight, and GSI were statistically significant on days 0, 30, and 60 across all groups. The testicular weight was higher in SD and LD-Mel, while the lowest weight was in SD-Mel. The GSI was higher in SD and LD-Mel, while the lowest was in SD-Mel. In the LD-Mel and LD groups, there was a strong positive correlation between testes and GSI.

Conclusion: Melatonin exposure, particularly under long-day conditions, enabled guinea fowl to improve reproductive potential by day 30 of light treatment.

1. Introduction

Efficient reproductive activities in the poultry industry is crucial for sustainable production. In the guinea fowl, season has been recognized as one of the major limitations to large-scale production[1]. The guinea fowl restricts its reproductive activity to the rainy season, when green vegetation and insects are abundant[2]. Photoperiod is a key factor in the seasonal reproductive cycle of most birds[3,4]. The duration of scotophase or photophase has been reported to have developmental effects on the male reproductive system[5]. The most important indices in

Cite this paper as: Gosomji IJ, Akinsola OM, Baso A, Ahmed N, Omirinde JO, Azeez IA, Plang NJ, Koplamma NB, and Hena SA. Modulatory Effects of Melatonin and Artificial Light on Testicular Morphometry in Guinea Fowl (*Numida Meleagris*). Journal of Veterinary Physiology and Pathology. 2025; 4(4): 64-68. DOI: 10.58803/jvpp.v4i4.76

seasonal breeding birds is the testicular size[6]. The size of the testis influences the number of sperm and volume of ejaculate[7]. In seasonal breeding birds such as tree sparrows, short photoperiod decreases testicular size, while long photoperiod reverses the effect by increasing the testicular size[8,9]. An increase in testicular size is related to testosterone concentration, which is often associated with increased reproductive activity in birds[10,11].

Previous investigations revealed that artificial light plays a critical role in the reproductive performance of male guinea fowls[12-14]. In addition, exogenous melatonin, which acts as a potent antioxidant, positively influences male reproductive activities by protecting testicular cells from damage, enhancing sperm quality, and supporting testosterone production[14-17]. The ability of melatonin to cross the blood-testis barrier and its protective effects against oxidative damage have been reported to be both receptor-dependent and independent, making it a potential therapeutic agent for male fertility issues[18,19].

There is a lack of information on the modulatory effect of melatonin and light regimens on testicular morphometry in guinea fowl. Hence, the present study aimed to determine the modulatory and protective potentials of photoperiodic regimens and exogenous melatonin on the testicular morphometry of the guinea fowl during and after breeding seasons.

2. Materials and Methods

2.1. Ethical approval

The experimental procedures followed international guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Ethics Committee of Animal Use and Care of Ahmadu Bello University, Zaria, Nigeria (ABUCAUC/2021/056).

2.2. Study area

The study was conducted at the Animal House Facility, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. The facility is in the Northern Guinea Savannah zone (11.158337'N and 7.652635'E)[20].

2.3. Experimental design

Guinea fowl keets (*Numida meleagris)* were sourced from the National Veterinary Research Institute, Vom, Nigeria. The keets were brooded in deep litter from day old until six months of age, when they were mature for the experiment. A total of 65 mature male guinea fowls, with an average weight of 1-2 kg, were randomly selected based on the wattle inclining at a right angle to the axis of the upper jaw[21]. Five guinea fowls were utilized for primary evaluation on day 0, while 60 guinea fowls were divided into six groups based on the duration of artificial light (L) and darkness (D) as short day (SD), control (CTL), and long day (LD), with 10 chickens in each group. The first group (SD) was exposed to eight hours of light and 16 hours of darkness (8L:16D). The second group was exposed to 8L:16D with an injection of melatonin (SD-Mel). The third group was maintained on CTL with 12 hours of light and 12 hours of darkness (12L:12D). The fourth group was maintained on CTL with a melatonin injection (CTL-Mel). The fifth group (LD) was exposed to 16 hours of light and eight hours of darkness (16L:8D), and the last group was exposed to 16L:8D with melatonin injection (LD-Mel). Melatonin was injected intramuscularly at a dose of 1 mg/kg body weight (BW) 2 hours before the end of each light cycle 14. The entire experiment lasted 60 days at 27 ± 2°C. The BW, testicular weight, and gonado-somatic index (GSI) were recorded on days 0, 30, and 60 of the study.

The six groups were compartmentalized with black, light-tight spaces measuring 152.4 × 182.88 × 274.32 cm, with an LED lighting system (12 W) that provided an approximate light intensity of 387 lux. A standard Nigerian poultry commercial Vital Feed®, with an average composition of 18% crude protein, 6% fat, 5% crude fibre, 1.0% calcium, 0.35% phosphorus, and 3100 kcal/kg metabolizable energy, was supplied along with water, provided *ad libitum*.

2.4. Morphometry

Primary evaluation was performed on day 0, when five clinically healthy guinea fowls were euthanized. As there were no reports of death during the present study, all guinea fowls at days 30 and 60 of the study were sampled, and the feed was withdrawn before euthanasia. The guinea fowls were euthanized, using a combination of ketamine (Rotex Medica®, German, 10 mL ampoule, 50 mg/mL) and xylazine (Xylased®Bioveta, Czech Republic, 50 ml vials, 20 mg/mL) at dosages of 35 mg/kg and 5 mg/kg, respectively, via the jugular vein[22]. Thereafter, the thoraco-abdominal cavity of each guinea fowl was opened, and the testes and adjoining epididymis were carefully removed from the carcasses. Weight was measured with a Mettler electronic scale (Startorius SE2 Model 4108, Germany) with recordings precise to 0.1 mg[23]. The GSI, the testicular mass as a proportion of the total body mass, was calculated using the following formula[24].

GSI = Paired testicular weight / Total body weight × 100.

2.5. Data analysis

Data analysis was conducted using JMP® software, version 10 for Windows (2012). Results were presented as mean ± standard error of the mean (SEM). Differences between the artificial light and melatonin treatment groups were assessed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Statistical significance was defined as a p-value less than 5% ($p < 0.05$). In addition, the correlation coefficient was calculated to evaluate the relationship between testicular weight and both BW and the GSI in guinea fowls exposed to artificial light and exogenous melatonin.

3. Results

The morphometric changes in the BW, testes, and GSI exposed to different light regimes from day 30 to day 60 of exposure were recorded in Table 1. There was no significant difference ($p > 0.05$) in the BW and GSI parameters of guinea fowl in SD, CTL, and LD groups. However, there was a

significant difference in testis weight in the LD and SD groups ($p < 0.05$). The testis weight was significantly higher in the LD (1.78 g) and SD (1.74 g) groups compared to the control group (1.27 g; Table 1).

Table 1. Evaluation of body weight and testicular parameters in mature guinea fowls in 60 days of exposure to the photoperiodic regimen

Parameters \ Groups	SD (8L:16D)	CTL (12L:12D)	LD (16L:8D)	SEM	P value
Body weight (g)	1120	1169.17	1244.17	46.47	0.19^{ns}
Testicular weight (g)	1.74^{a}	1.27^{b}	1.78^{a}	0.16	0.05*
Gonado-somatic index (%)	0.15	0.11	0.14	0.01	0.06^{ns}

SD: Short day with 8 hours of light and 16 hours of darkness, LD: Long day with 16 hours of light and 8 hours of darkness, CTL: Control group with 12 hours of light and darkness, SEM: Standard error of mean, ns: Not significant. ^{a,b} Means with different superscript letters in each row are significantly different ($p < 0.05$).

The results of morphometric parameters in the guinea fowl exposed to exogenous melatonin in combination with different artificial light regimes are recorded in Table 2. There was no significant difference in the BW of guinea fowl exposed to exogenous melatonin and light regimes compared to the control group ($p > 0.05$). However, BW values were insignificantly higher in LD (1275.00 g) and CTL-Mel (1246.67 g) compared to other groups. The testicular weight was statistically significant in the guinea fowl exposed to exogenous melatonin and light regimes ($p < 0.05$; Table 2). The testicular weight was higher in SD (2.25 g) and LD-Mel (2.09 g) compared to other groups ($p < 0.05$), while the lowest testicular weight was observed in SD-Mel (1.24 g) and CTL (1.12 g). In comparison to the CTL (1.12 g), the testicular weight was higher in SD (2.25 g), LD-Mel (2.09 g), and SD-Mel (1.24 g). The GSI was statistically significant in the guinea fowl exposed to exogenous melatonin and light regimes ($p < 0.05$; Table 2). The GSI was higher in SD (0.19%) and LD-Mel (0.18%), compared to other groups ($p < 0.05$), while SD-Mel (0.12%) and LD (0.11%) had lower GSI, slightly above the control (0.10%). Regarding morphometric changes in BW, testicular weight, and GSI with days, the BW increased significantly in the guinea fowl at day 30 compared to that of days 0 and 60 ($p < 0.05$). Additionally, testicular weight and GSI showed a similar trend, with significant increases at day 60 compared to days 0 and 30 ($p < 0.05$; Table 3).

Table 2. Evaluation of body weight and testicular parameters in mature guinea fowls in 60 days of exposure to photoperiodic regimens and melatonin

Parameters	Groups						SEM	P value
	SD-Mel	SD	CTL-Mel	CTL	LD-Mel	LD		
Body weight (g)	1050	1190	1246.67	1091.67	1213.33	1275	65.72	0.09^{ns}
Testicular weight (g)	1.24^{c}	2.25^{a}	1.42^{b}	1.12^{c}	2.09^{a}	1.46^{b}	0.22	0.002
Gonado-somatic index (%)	0.12^{b}	0.19^{a}	0.12^{b}	0.10^{b}	0.18^{a}	0.11^{b}	0.1	0.01

SD: Short day with 8 hours of light and 16 hours of darkness, LD: Long day with 16 hours of light and 8 hours of darkness, CTL: Control group with 12 hours of light and darkness, Mel: Melatonin, SD-Mel: Short day with 8 hours of light and 16 hours of darkness and melatonin injection. CTL-Mel: Control group with 12 hours of light and darkness with a melatonin injection. LD-Mel: Long day with 16 hours of light and 8 hours of darkness with melatonin injection. SEM: Standard error of mean, ns: Not significant. $^{a, b, and c}$ Means with different superscript letters in each row are significantly different ($p < 0.05$).

Table 3. Evaluation of morphometric changes of body weight and testicular parameters in mature guinea fowls at days 0, 30, and 60 of exposure to photoperiodic regimens and melatonin

Parameters \ Days	0	30	60	SEM	P value
Body weight (g)	844.44^{c}	1222.22^{a}	1148.89^{b}	57.04	0.0002
Testicular weight (g)	0.75^{c}	1.53^{b}	1.70^{a}	0.18	0.003
Gonado-somatic index (%)	0.09^{c}	0.12^{b}	0.15^{a}	0.02	0.04

SEM: Standard error of mean. $^{a,b, and c}$ Means with different superscript letters in each row are significantly different ($p < 0.05$).

The correlative relationship among the BW, testicular weight, and GSI, light regimes, and melatonin is presented in Table 4. There was a positive correlation between the testis and GSI in the SD-Mel ($r = 0.9807$) and SD ($r = 0.9234$) groups. In the CTL and CTL-Mel groups, the correlation between testis and BW or GSI was positive. However, the correlation was moderate ($r = 0.3116$) between testis and CTL-Mel and BW, whereas it was high ($r = 0.8515$) between testis and CTL-Mel and GSI. There was a strong positive correlation between testes and GSI in the LD-Mel ($r = 0.8435$) and LD ($r = 0.9280$) groups. The correlation between testis and LD-Mel and BW ($r = 0.0194$) was low (Table 4).

Table 4. Correlation coefficients of testicular weight with body weight and gonado-somatic index of guinea fowls exposed to photoperiodic regimens and exogenous melatonin from day 30 and day 60

Correlated parameters	Testis correlation coefficient (r)
SD-Mel and BW	- 0.0182
SD-Mel and GSI	0.9807
SD and BW	- 0.4083
SD and GSI	0.9234
LD-Mel and BW	0.0194
LD-Mel and GSI	0.8435
LD and BW	- 0.0682
LD and GSI	0.9280
CTL-Mel and BW	0.3116
CTL-Mel and GSI	0.8515
CTL and BW	0.7517
CTL and GSI	0.8657

SD: Short day, LD: Long day, CTL: Control, Mel: Melatonin, BW: Body weight, GSI: Gonado-somatic index.

4. Discussion

In the guinea fowl, seasonal changes have been reported to have positive or negative impacts on GSI[25]. Malecki et al.[26]

reported a GSI of 0.15% in the reproductive phase of the guinea fowl during the breeding season. Even though the GSI was high enough to stimulate reproductive activities in the guinea fowl, the result was less than the present findings, where GSI was 0.18 and 0.19% in the LD-Mel and SD, respectively. However, the GSI results of the present study were lower than those obtained in the Nigerian local domestic fowl (1.1 %)[24,27]. These differences could reflect interspecies variation resulting from genetic, physiological, ecological, and evolutionary adaptations[28]. Increased testicular weight in the guinea fowls exposed to the long photoperiodic regime was consistent with the reports in Japanese quail and European starlings[29,30]. The long photoperiodic regime stimulates rapid gonadal growth through gonadotropin-releasing hormone (GnRH)-dependent activation of the testes and upregulation of androgen synthesis, resulting in increased testicular size[31].

The present study confirmed that exogenous melatonin could have reproductive inhibitory and stimulatory effects in the guinea fowl. The reproductive inhibitory effect of melatonin was expressed by the reduced testicular size obtained in the SD group. However, exogenous melatonin stimulates testicular increase in the LD group. The administration of exogenous melatonin demonstrated the reproductive pathways or pattern during the non-breeding seasons in guinea fowls. Melatonin downregulates GnRH, either directly or via increased expression of gonadotropin-inhibitory hormone (GnIH), which is consistent with the findings of Bao et al.[32] and Ubuka et al.[33]. In addition to its regulatory role in testicular morphophysiology, melatonin protects testicular integrity from oxidative damage caused by corticosterone due to stress and photoperiodic changes in tree sparrow[34].

The observed testicular changes from day 0 to day 60 in the present study underscored the importance of photoperiod in reproductive development. There was heightened sensitivity to photoperiod at the earliest exposure to light. This was observed in the first 30 days of the current study. The current findings were consistent with the findings in turkey[34,35] and Eurasian tree sparrow[11] where artificial light was observed to stimulate reproductive activities by increasing both testicular weight and GSI in the guinea fowl.

5. Conclusion

The long-day photoperiodic regime (16L:8D) promoted testicular development in sexually mature guinea fowl. Additionally, exogenous melatonin did not inhibit testicular development in sexually mature guinea fowl exposed to a long-day regime; instead, melatonin preserved testicular structural and functional integrity despite stress induced by artificial light. Further studies should be conducted to investigate the testicular genes expressed in guinea fowls exposed to artificial light and exogenous melatonin.

Declarations

Ethical considerations

The authors confirmed that this manuscript is an original submission, prepared exclusively for the Journal of Veterinary Physiology and Pathology, and not under consideration elsewhere. The manuscript was thoroughly checked for plagiarism, data fabrication, and duplication to ensure scientific integrity. The authors confirmed that they have not used AI in preparing and writing the manuscript.

Competing interests

There was no conflict of interest in the present study.

Authors' contributions

Innocent Jonah Gosomji conceptualized, supervised, validated, funded the study, wrote the original draft, reviewed, and edited the manuscript. Oludayo Michael Akinsola supervised, validated, and analyzed the generated data. Abdullah Baso was involved in supervision of the study, validation and reviewing the manuscript. Nura Ahmed was involved in funding and supervising the study, and also writing, reviewing, and editing the manuscript. Jamiu Oyewole Omirinde was involved in the supervision of the study and also writing, reviewing, and editing the manuscript. Idris Ayodeji Azeez, Naanman James Plang, and Nenchini Bala Koplamma supervised, validated, and analyzed the generated data. Sunday Akau Hena was involved in supervising the study and in writing, reviewing, and editing the manuscript. All authors read and approved the final edition of the manuscript for publication in the present journal.

Funding

The present study did not receive any specific grant from any funding agency in the private, government, or not-for-profit sectors.

Availability of data and materials

All datasets generated and analysed during the present study were included in this manuscript and will be available upon reasonable request from the corresponding author.

Acknowledgments

The authors of the present study wish to express most profound appreciation to Professor Tom A. Aire (St. George's University, True Blue, Grenada), Prof. Augustine Arukwe (Norwegian University of Science and Technology, Norway), and Prof. Umar M. Bello (Ahmadu Bello University, Zaria, Nigeria) for their unwavering support and mentorship.

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