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Green Light and Intermittent Lighting Modulate Testicular Gonadotropin Inhibitory Hormone without Central or Morphological Effects in Broiler Chickens

Research Article

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ABSTRACT

Environmental factors, especially light duration and wavelength (colour), affect reproductive physiology in broilers. In birds, light is perceived especially by extraretinal photoreceptors in the brain, including the hypothalamus, which regulates reproductive function. Gonadotropin-inhibitory hormone (GnIH), expressed in both the hypothalamus and gonads, suppresses gonadotropin release and modulates reproductive activity. Its expression is influenced by photoperiod and light colour. Rooster fertility is economically important, as one male can inseminate many females. This study investigated how green light and intermittent lighting affect GnIH levels and testicular development in prepubertal broiler males. 288 one-day-old male commercial broilers (Ross-308) were divided into four groups (n=12) and exposed to: Group I, 18 hours light - 6 hours dark (18L:6D) with white light; Group II, 18L:6D with green light; Group III, 17L:3D:1L:3D with white light; and Group IV, 17L:3D:1L:3D with green light. The study was conducted in four identical experimental rooms, each consisting of six pens (replicates). Two male broilers were randomly selected from each pen. A total of 48 chickens, 2 males from each pen (replicate group), were randomly selected for analysis. After 42 days under standard conditions, GnIH levels were measured in the hypothalamus and testes via ELISA. Testicular development was assessed histologically by evaluating seminiferous tubule diameter and epithelial height. Results showed that intermittent lighting and green light significantly increased testicular GnIH levels but had no effect on hypothalamic GnIH. The most pronounced increase in testicular GnIH was observed in Group IV, which received both intermittent lighting and green light. No significant differences were observed in testicular morphology. These findings suggest that intermittent lighting and green light may selectively influence gonadal GnIH levels without affecting central GnIH or morphology, offering insight into how lighting strategies may be optimized in poultry production.

KEY WORDS endocrinology, histology, hormones, lighting, physiology, reproduction.

INTRODUCTION

Lighting is a critical environmental factor that directly influences physiological processes such as metabolism, growth, and reproduction, as well as productivity traits in poultry (Pandey, 2019; Zhao *et al.* 2019). In recent years, various artificial lighting programs have been widely implemented to improve broiler (*Gallus gallus domesticus*) production performance. These programs commonly modify parameters such as light intensity, photoperiod, and colour (wavelength) (Sayin *et al.* 2022), all of which significantly influence reproductive functions in poultry. While changes in photoperiod affect reproductive physiology (Siopes and Pyrzak, 1990; Geng *et al.* 2022; Guo *et al.*

2022), variations in light colour have been shown to impact the development of sexual organs and the timing of sexual maturity (Hassan *et al.* 2013; Baxter *et al.* 2014).

In birds, light perception is mediated by both retinal and extraretinal photoreceptors (ERPR), which are involved in light detection and located in various brain regions, including the pineal gland, pituitary gland, and hypothalamus (Rozenboim *et al.* 2022). Consequently, lighting conditions can modulate reproductive behaviour and egg production in birds by altering the hypothalamic release of key reproductive hormones such as gonadotropin-releasing hormone (GnRH) and gonadotropin-inhibitory hormone (GnIH) (Mobarkey *et al.* 2010). Dixit *et al.* (2022) reported that house sparrows exposed to short photoperiods followed by only one day of long photoperiod exhibited increased GnRH and decreased GnIH expression in the hypothalamus.

GnIH was first identified in the hypothalamus of quail by Tsutsui *et al.* (2000) and was described as a key inhibitor of gonadotropin release and a suppressor of reproduction parameters in poultry. GnIH shows an inhibitory effect on GnRH activity (Bédécarrats, 2015) and reduces the secretion of LH and FSH (Tsutsui *et al.* 2000). In mammals, exogenous GnIH administration has been shown to suppress the hypothalamic-pituitary-gonadal axis by down-regulating key reproductive genes and proteins involved in steroidogenesis and spermatogenesis (Dai *et al.* 2024). GnIH and its receptors are predominantly expressed in the hypothalamus (Kriegsfeld *et al.* 2006), but their presence has also been confirmed in the peripheral tissues such as bone (You *et al.* 2025), pituitary gland, and gonads (Tsutsui, 2016), suggesting that GnIH may act not only centrally but also peripherally (Figure 1(A)). Studies have reported that short photoperiod exposure upregulates gonadal GnIH levels in quail (Zhou *et al.* 2022) (Figure 1(B)). In addition to photoperiod, different light colours have been shown to alter GnIH expression in birds (Tsutsui and Ubuka, 2021), mice and humans (You *et al.* 2025). For instance, Zhang *et al.* (2017) demonstrated that green light treatment significantly increased hypothalamic GnIH mRNA and protein levels in male Beijing Huadu chickens compared to white and other light treatments (Figure 1(B)). Moreover, in a study on gonadal development, Ubuka *et al.* (2006) found that GnIH administration reduced the size of seminiferous tubules and induced testicular apoptosis, thereby suppressing testicular growth and development (Figure 1(C)).

In addition to photoperiod and light colour, reproductive stage is also believed to influence GnIH levels. Manoochehri *et al.* (2021) reported that GnIH expression was more intense in prepubertal turkeys compared to other stages.

This finding was supported by Zubair *et al.* (2022), who observed similar results in male Rhesus monkeys. Furthermore, it has been suggested that light manipulations during the prepubertal period may reduce fertilization rates by prematurely triggering sexual development (Briere *et al.* 2011).

Efficient egg production is a primary objective in layer chicken breeding, and the fertility of roosters is considered even more critical than that of hens, as one rooster can fertilize eggs from multiple hens (Mohammadi *et al.* 2021). While previous studies have mainly focused on the effects of light conditions on reproduction in laying hens or sexually mature poultry, little is known about how photoperiod and light colour affect GnIH levels in prepubertal male broilers.

Although studies addressing GnIH expression in broiler chickens are limited, some research has demonstrated its physiological relevance in this species. For example, Ciccone *et al.* (2004) showed that GnIH suppresses gonadotropin subunit expression in the pituitary of domestic chickens, while Hadinia *et al.* (2020) investigated GnIH and GnIH receptor gene expression in Ross 308 broiler breeder pullets in response to energy intake and photostimulation. To our knowledge, this is one of the first studies to investigate the combined effects of intermittent lighting and green light on both hypothalamic and gonadal GnIH expression in broilers prior to sexual maturity. Based on this, the present study aimed to investigate the effects of intermittent lighting and green light treatments on hypothalamic and gonadal GnIH levels during the prepubertal period in male broiler chickens. Additionally, the histological impact of these treatments on testicular development was examined.

MATERIALS AND METHODS

Experimental animals

This study was approved by the Aydın Adnan Menderes University Animal Experiments Local Ethics Committee (Ethics Committee Approval No: 64583101/2023/41).

A total of 288 one-day-old male commercial broiler chicks (Ross 308) were randomly allocated into four groups based on their body weight. The study was conducted in four identical trial rooms, each containing six compartments (replicates) with a floor area of 1.65 m² (1.1×1.5 m) per compartment. After subtracting the area occupied by the feeder and drinker (0.65 m²), the remaining usable movement area was 1 m². Each compartment housed 12 animals, corresponding to a maximum stocking density of 33 kg/m² (European Union, 2007) (Table 1). Wood shavings, evenly spread to a height of 5–7 cm, were used as bedding material.

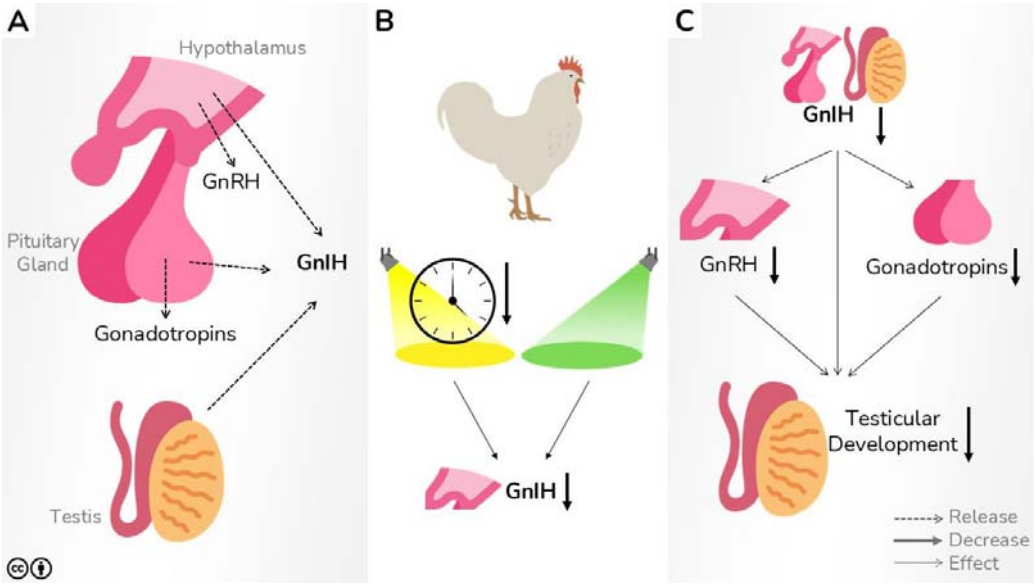

Figure 1 The role of GnIH and the effects of light treatments on testicular development. (A) Gonadotropin-inhibitory hormone (GnIH) is secreted by the hypothalamus, pituitary gland, and testes (Tsutsui, 2016), gonadotropin-releasing hormone (GnRH) is secreted by the hypothalamus (Mobarkey *et al.* 2010), and gonadotropins secreted by the pituitary gland (Tsutsui *et al.* 2000). (B) Exposure to green light (Zhang *et al.* 2017) and shorter photoperiods (Zhou *et al.* 2022) reduce GnIH secretion. (C) Reduced GnIH levels lead to a decrease in GnRH (Bédécarrats, 2015) and gonadotropins (Tsutsui *et al.* 2000), ultimately suppressing testicular development (Ubuka *et al.* 2006)

Table 1 Overview of experimental groups and treatment conditions

Groups	Photoperiod	Light colour	Replicate group number	Replicate group size	Total sample size	Sample size (n)
Group I	Continuous[1] (18L:6D)	White	6	12	72	12
Group II	Continuous (18L:6D)	Green	6	12	72	12
Group III	Intermittent (17L:3D:1L:3D)	White	6	12	72	12
Group IV	Intermittent (17L:3D:1L:3D)	Green	6	12	72	12
Total[2]					288	48

[1] Photoperiods indicate the number of hours of light (L) and dark (D) within a 24-h cycle; for example, 18L:6D means 18 hours of light and 6 hours of dark, and 17L:3D:1L:3D means 17 hours of light, 3 hours of dark, 1 hour of light, and 3 hours of dark.
[2] For this study, 48 broiler chickens (2 from each replicate group) were selected from a total of 288. The remaining chickens were used in other research projects.

Temperatures of the trial rooms were initially maintained at 32 ± 1 °C at chick-back level using electric thermostatic radiant heaters for the first three days and then gradually reduced by 3 °C per week until day 21. Afterward, it was kept constant until the end of the study. Relative humidity was controlled between 50% and 60% throughout the trial. The chickens were fed *ad libitum* using hanging-type feeders (2.5 cm feeder length per animal). Starter feed providing 3000 kcal/kg metabolizable energy (ME) and 230 g/kg crude protein (CP) was provided between days 0–10, grower feed (3100 kcal/kg ME, 215 g/kg CP) between days 11–24, and finisher feed (3200 kcal/kg ME, 195 g/kg CP) from days 25–42. Water was supplied *ad libitum* via hanging-type waterers.

Light treatments

Chicks were assigned into four groups based on photoperiod and light colour treatments (Table 1). To facilitate the adaptation and promote water and feed intake between days 0-6, a 23-hour light: 1-hour darkness (23L:1D) was applied. From day 7 to 42, Group I and II received an 18L:6D lighting program (European Union, 2007), while Group III and IV were exposed to 17L:3D:1L:3D intermittent lighting schedule. The 17L:3D:1L:3D program was selected over other intermittent regimens, such as 16L:2D:1L:2D:1L:2D, based on prior studies indicating its more pronounced antioxidant and immune-enhancing effects (Zheng *et al.* 2013; Zhao *et al.* 2019). Lighting was regulated by an automatic timer in each room.

White LED bulbs were used for Groups I and III, and green LED bulbs for Groups II and IV. To isolate the effect of light wavelength, the light intensity was kept constant at 20 lx at the eye level of the broilers.

Tissue sampling

On day 42 of the study, two chickens from each of the six replicate groups (12 chickens per group, 48 in total) were euthanized by decapitation. Testicular tissues and the brain region containing the hypothalamus were collected from the euthanized animals. Hypothalamic tissues and a portion of the testicular tissues were stored at -80 °C for subsequent ELISA analyses. The remaining testicular tissues were fixed in Bouin's solution for histological examination.

Determination of GnIH levels by ELISA analysis

To determine GnIH levels, 0.4 grams of hypothalamic tissue and testicular follicles were isolated, diluted threefold with cold phosphate-buffered solution (pH 7.4), and homogenized at 15000 rpm using a homogenizer (Isolab® 621.11.001, Light Load). The homogenates were then centrifuged at +4 °C for 10 minutes (Hettich® Mikro 220R). The resulting supernatants were analysed for GnIH levels using ELISA (CK-bio-22542, Shanghai Coon Koon Biotech Co. LTD) following the manufacturer's instructions.

Hypothalamic and gonadal GnIH concentrations (pg/mL) were measured spectrophotometrically at 450 nm. The manufacturer reports a detection sensitivity of <10 pg/mL, with intra- and inter-assay coefficients of variation below 10% and 15%, respectively.

Determination of testicular development by histological examination

Following tissue fixation in Bouin's solution, the samples were passed through a graded alcohol and xylene series and embedded in paraplast. The tissue blocks were stored at +4 °C until analysis. Serial sections of 5–6 µm thickness were cut at 300 µm intervals using a microtome. Wrinkled sections were floated in a water bath at 45–50 °C and subsequently mounted on APES-coated slides. The slides were left to dry in an oven set at 37 °C overnight. The following day, the sections were deparaffinized with xylene and rehydrated through a graded alcohol series. They were then stained using a triple staining technique, where haematoxylin, acid fuchsin, and aniline blue were used. After staining, the sections were mounted with Entellan (Merck®). The seminiferous tubules were visualized using the CellSens Entry (Olympus®) program with a microscope (Olympus®, BX43F) equipped with a digital microscope camera (Olympus®, SC50). The diameters and epithelial heights of the tubules were measured at 20x magnification, scaled according to Güleş *et al.* (2019), and photographed at 100x magnification. All measurements were performed in a blind manner by two independent observers.

Statistical analysis

Power analysis was conducted using G*Power® 3.1 software to determine the appropriate number of animals per study group. With an alpha level of 0.05 and an effect size of 0.50, the required sample size (n) was calculated as 12. Data obtained during the study were statistically analysed using SPSS® 20.0 (SPSS, 2011). Appropriate correction methods were applied when the data did not meet the assumptions of normality. No data transformation was required. A generalized linear model (GLM) was employed to evaluate the effects of the experimental factors. The model included lighting pattern and light colour as fixed effects, and individual birds as the experimental unit. All treatment combinations included 12 animals, ensuring a balanced design. Results are presented as mean ± SEM. Group differences were assessed using Duncan's multiple range test. While this method identified significant differences, future studies may consider using polynomial contrasts to better capture underlying trends. A P-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

This study, conducted on prepubertal male broiler chickens (Ross 308), aimed to investigate the effects of intermittent lighting and green light treatments—both individually and in combination—on hypothalamic and gonadal GnIH levels, as well as testicular development. Notably, it is one of the first to detect and quantify GnIH hormone levels at the testicular level in this population.

As shown in Table 2, intermittent lighting led to a significant increase (297±41.6 pg/mL) compared to continuous lighting (184±12.6 pg/mL) (P=0.003). Green light also significantly elevated testicular GnIH levels (313±39.6 pg/mL) compared to white light (168±12.0 pg/mL) (P=0.000). The combination of intermittent lighting and green light produced a synergistic effect, as evidenced by Group IV showing the highest testicular GnIH levels (414±66.1 pg/mL), significantly higher than Group I (157±14.2), Group II (212±18.3), and Group III (179±19.6) (P=0.013). No significant differences were found among Groups I, II, and III. Unlike the pronounced changes in testicular GnIH levels, neither the lighting pattern and light colour, nor their interaction significantly affected hypothalamic GnIH levels (P>0.05).

Histological analysis of the testes also revealed no significant differences among groups in seminiferous tubule diameter and epithelial height (P>0.05) (Figure 2).

Table 2 Gonadotropin-inhibitory hormone (GnIH) levels in the hypothalamus and testes, seminiferous tubule diameters, and epithelial heights (mean±SE)

Factors	Variables			
	Hypothalamic GnIH (pg/mL)	**Testicular GnIH (pg/mL)**	**Seminiferous tubule diameters (µm)**	**Seminiferous tubule epithelial height (µm)**
Lighting				
Continuous	177±9.35	184±12.6	84.4±5.28	29.8±1.29
Intermittent	191±19.8	297±41.6	89.3±4.98	30.9±0.99
Light colour				
White	201±18.6	168±12.0	85.7±5.17	29.7±1.08
Green	167±10.5	313±39.6	87.9±5.14	31.0±1.21
Lighting x light colour				
Continuous-white	196±14.2	157±14.2^{b}	75.7±3.90	27.4±1.20
Continuous-green	158±9.96	212±18.2^{b}	93.1±9.37	32.2±2.13
Intermittent-white	206±35.3	179±19.6^{b}	95.8±8.85	31.9±1.60
Intermittent-green	175±18.7	414±66.1^{a}	82.8±4.24	29.8±1.18
Significance (P-value)				
Lighting	0.535	0.003*	0.491	0.495
Light colour	0.123	0.000*	0.756	0.401
Lighting x light colour	0.419	0.013*	0.173	0.138

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05).

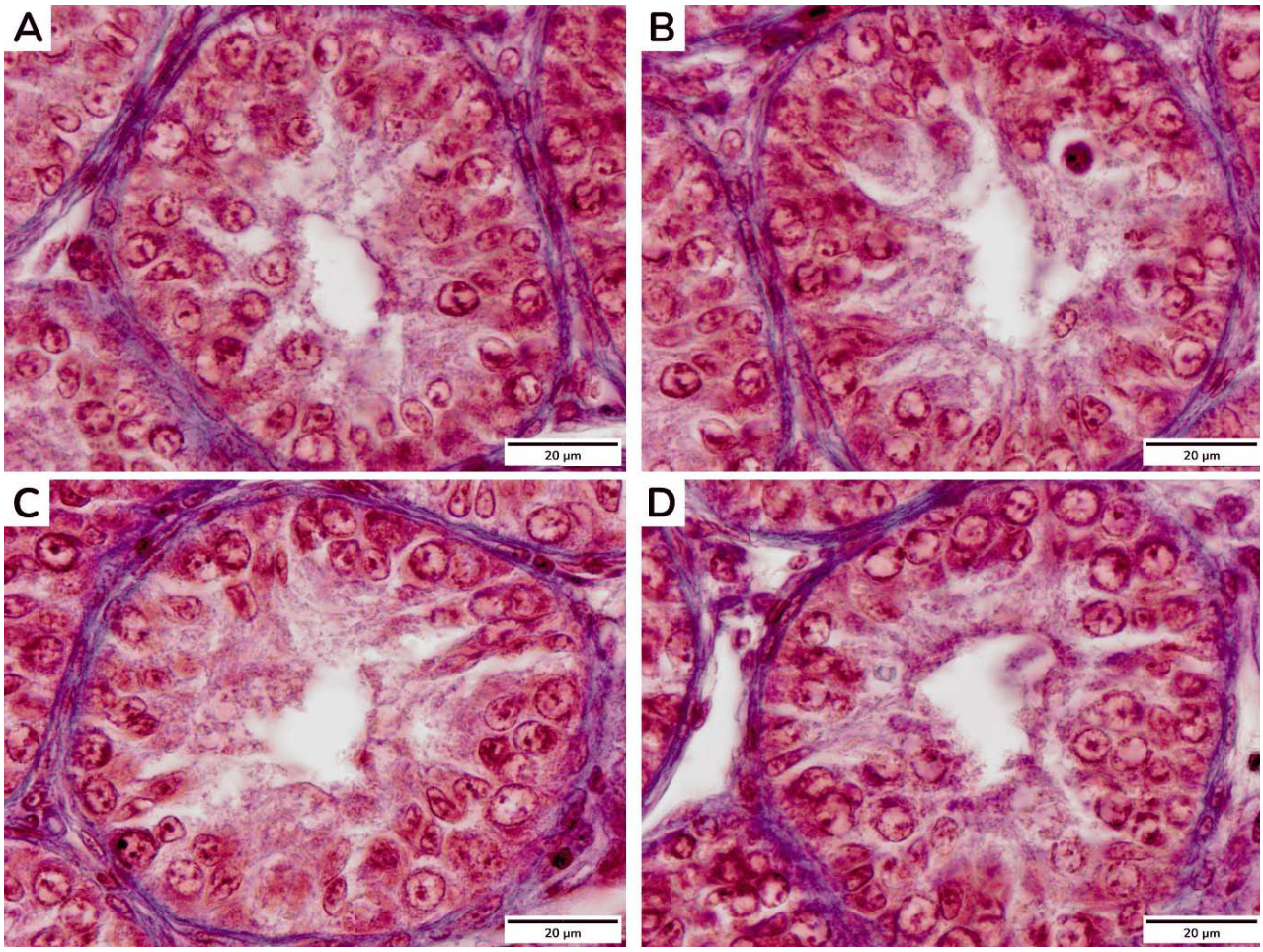

Figure 2 Representative triple-stained testicular tissues at 100× magnification. No significant morphological differences were observed. (A) Continuous-white light (group I), (B) continuous-green light (group II), (C) intermittent-white light (group III), and (D) intermittent-green light (group IV)

The present study aimed to investigate how photoperiod and light colour affect GnIH levels and testicular development in prepubertal male broiler chickens – an area that remains relatively underexplored. Previous studies have shown that GnIH expression is influenced by photoperiod (Dixit *et al.* 2020) and light colour (Mobarkey *et al.* 2013). You *et al.* (2025) found that green light exposure increased serum GnIH levels and mitigated bone loss in both mice and humans, supporting the light-induced peripheral activity of GnIH beyond the reproductive axis. For instance, Zhou *et al.* (2022) reported that short photoperiods upregulate gonadal GnIH levels in 8-week-old laying quails, leading to reduced egg production and follicle numbers. Similarly, a study on Beijing You Chickens showed that continuous light exposure during the early laying period promoted sexual development more effectively than intermittent lighting (Geng *et al.* 2022). These findings are further supported by additional studies (Ubuka *et al.* 2005; Bédécarrats *et al.* 2009; Dixit *et al.* 2017; Ouyang *et al.* 2021).

The presence of GnIH and its receptors at the gonadal level is well established in previous researches (Oishi *et al.* 2012; Tsutsui, 2016). In our study, intermittent lighting and green light both increased testicular GnIH levels and the combination of the two (Group IV) produced the highest levels. This finding, along with the lack of significant differences among groups I-III, suggests a synergistic interaction between photoperiod and light colour, indicating that dual manipulation of lighting conditions may be more effective in modulating reproductive neuroendocrine activity than either factor alone. Green and yellow light perceived through the retina have been shown to stimulate GnIH and subsequently inhibit reproduction in sexually mature broiler breeder hens (Mobarkey *et al.* 2013). In addition, photoperiodic changes were found to alter melatonin receptor expression in GnIH neurons, indicating that the photoperiodic regulation of GnIH is mediated by melatonin (Soni *et al.* 2021). It has also been demonstrated that melatonin can bind to its receptors at the gonadal level, influencing the GnIH system (McGuire *et al.* 2011), and that it suppresses testicular development by inhibiting testosterone secretion (Xu *et al.* 2023). In a study by Zhou *et al.* (2022), reproductive activity was suppressed in quails exposed to short photoperiods, accompanied by increased GnIH and GnIH receptor expression levels in the ovaries and follicles. Taken together, these findings indicate that GnIH expression is regulated within the gonads, and that both photoperiod and green light exposure may modulate this regulation via melatonin signalling in poultry.

In contrast to the significant differences observed in gonadal GnIH levels, no significant group differences were detected in hypothalamic GnIH levels in our study. This finding contradicts some previous reports (Dixit *et al.* 2022; Zhou *et al.* 2024; You *et al.* 2025). The hypothalamic-pituitary-gonadal (HPG) axis in poultry functions through a feedback mechanism (Rose *et al.* 2022). For instance, injections of oestradiol or an oestradiol/progesterone combination in chickens have been shown to reduce GnIH receptor mRNA levels in the pituitary gland (Maddineni *et al.* 2008), suggesting that gonadal steroidal feedback regulates pituitary function. It is also known that elevated GnRH levels in the gonads suppress hypothalamic GnRH expression (Maeda *et al.* 2010), implying that GnIH may be similarly regulated via gonadal feedback. Additionally, the presence of GnIH mRNA in the testes, along with GnIH receptors in the gonads, indicates that GnIH is synthesized not only in the hypothalamus but also in the testes. Exogenous administration of GnIH has been shown to significantly inhibit testicular function by suppressing testosterone biosynthesis in the testes (Bentley *et al.* 2008; McGuire and Bentley, 2010). Considering the feedback mechanism of the HPG axis (Rose *et al.* 2022), the significant increase in testicular GnIH levels observed in this study – despite no change in hypothalamic levels – may reflect a compensatory feedback response from gonads. To confirm this hypothesis, further studies investigating other reproductive hormones are needed. The observed divergence between increased testicular GnIH and unchanged hypothalamic levels may be attributed to the developmental stage of the animals, as the responsiveness of different components of the HPG axis can vary with age. For instance, a study by Xin *et al.* (2024) on laying hens showed that the expression levels of GnRH and GnIH in the hypothalamus, as well as the receptor levels of these hormones in the pituitary, are significantly affected by age. GnRH expression increased from 9 to 40 weeks and then declined, while GnIH expression peaked at 70 weeks. These findings suggest that HPG axis activation may vary by age in broiler chickens. Moreover, since melatonin can influence GnIH expression at the gonadal level (McGuire *et al.* 2011), the significant increase in testicular GnIH levels – despite the absence of changes in hypothalamic expression – in animals exposed to intermittent lighting and green light in our study may be explained by this mechanism.

Another possible age-related finding in our study is the lack of GnIH effect on gonadal development. Most studies examining the effect of photoperiod have reported a negative correlation between GnIH levels and gonadal development (Ubuka *et al.* 2005; Bédécarrats *et al.* 2009; Dixit *et al.* 2017; Ouyang *et al.* 2021). In our study, no significant differences were observed on seminiferous tubule diameter or epithelial height. This finding contrasts with the study by Ubuka *et al.* (2006), which demonstrated that exogenous GnIH administration significantly reduced seminiferous

tubule diameter in the testes. Similarly, Jiang *et al.* (2023) observed significant reductions in testicular weight, volume, and semen quality following exogenous GnIH administration in roosters. The main difference between these two studies and ours is that the former used mature quails (12 weeks old) and roosters (27 weeks old), whereas our study focused on broiler chickens that had not yet reached sexual maturity. This raises the question of whether the significant difference observed in testicular GnIH levels in our study is age-related, despite the absence of morphological differences. A study on testicular development in Ross 308 broilers reported that testicular diameter increases linearly with age. While no significant difference was found in seminiferous tubule diameters on days 14, 21, and 28, significant increases were observed on days 35 and 42 compared to earlier ages (Kara and Tekiner, 2024). These findings suggest that seminiferous tubule development primarily occurs after 5 weeks of age. In our study, the absence of histological differences may be attributed to the animals being euthanized at 6 weeks, potentially before statistically significant changes had time to develop. Further studies considering the age factor are needed to confirm this hypothesis and to better delineate the timeline of light-induced morphological responses in broiler testes.

A limitation of this study is that it was conducted on broilers that had not yet reached sexual maturity and are primarily raised for meat production. Therefore, hormonal and morphological outcomes may differ from those observed in sexually mature or layer-type poultry, and the findings should be interpreted within this biological context. Since most research on reproductive functions in poultry has focused on breeding animals, there are relatively few studies available for direct comparison with our findings – particularly in broilers. One of the strengths of this study is that, to our knowledge, it is among the few that examine both hypothalamic and gonadal GnIH expression in prepubertal male broilers exposed to different light conditions. The use of a balanced experimental design with equal group sizes adds to the reliability of the results. Furthermore, the inclusion of both molecular (ELISA) and histological analyses enables a more comprehensive evaluation of reproductive responses.

CONCLUSION

In conclusion, this study demonstrates that intermittent lighting and green light exposure during the prepubertal period significantly increase gonadal GnIH levels in male broilers, while having no effect on hypothalamic GnIH expression or testicular morphology. These findings show that testicular GnIH levels are sensitive to light treatments, suggesting that GnIH may function as an early regulatory factor in the reproductive axis, acting before any morphological changes become evident. The absence of testicular structural changes despite elevated GnIH levels suggests that hormonal responses to photoperiod and light colour may occur earlier than measurable tissue changes. Importantly, the results suggest that even in broilers—primarily raised for meat production—light regimes can influence reproductive endocrine pathways. This opens new possibilities for understanding and optimizing lighting strategies in poultry management.

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