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Effect and molecular regulatory mechanism of monochromatic light colors on the egg-laying performance of Yangzhou geese

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ABSTRACT

Photoperiodic control is essential for manipulating the reproductive performance of avian species. This study was conducted to assess the neuroendocrine mechanisms that regulate reproductive functions of Yangzhou geese when there are different monochromatic light colors from light emitter diode (LED) sources. A flock of geese was divided into four groups with white, red, blue, and green light treatments being imposed. The results indicated that peak laying rates and reproductive performance were greater in geese treated with white or red as compared with blue or green light treatments. The fertilization rate of eggs and hatchability of fertilized eggs were greater with the white or red as compared with blue or green light treatments. There was a greater abundance of *OPN5, Dio2*, *c-Fos*, and *GnRH-I* mRNA in the hypothalamus earlier in the treatment period and abundances of these hypothalamic factors were greater with the white or red light treatments. Abundances of pituitary *LH beta* and *FSH beta* mRNA increased at a lesser rate with the blue or green light treatments and were in greater abundances with the white or red light treatments. The lighting regimen also resulted in photo-refractoriness with there being greater abundances of *GnIH*, *VIP*, and *PRL* mRNA with the use of white or red light treatments. The results indicate that the use of white or red monochromatic lights while imposing a long photoperiod of 11 h daily could result in sustaining functions of the reproductive system of Yangzhou geese for considerably longer times, thus, resulting in greater egg-laying performance.

1. Introduction

Domestic geese are an economically important waterfowl that have long supplied eggs and feathers for human use in many parts of the world (Shi et al., 2008). Yangzhou geese have a long-day breeding cycle (Zhu et al., 2017a, b), egg-laying is normally concentrated between February and June (Shi et al., 2008). In modern goose production practices, photoperiod and light intensity are the main factors that control the seasonality of reproduction and are an essential part of the physical environment that controls physiological processes in birds (Shi et al., 2007, 2008; Chang et al., 2016; Zhu et al., 2017b, 2019).

In avian species, lighting stimuli are perceived in the deep brain photoreceptors (DBP), such as opsins or neuropsin, which are

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present in the paraventricular organ (PVO) within the medial basal hypothalamus (MBH) (Nakane et al., 2010; Yamashita et al., 2010). As a result of the lighting stiumulus, neural signals are transmitted to the pars tuberalis (PT) of the pituitary gland, which induces the synthesis of thyroid stimulating hormone (TSH). The TSH subsequently functions at ependymal cells to induce the thyroid hormone activating enzyme, type 2 deiodinase (Dio2). The Dio2 converts thyroxine (T4) to 3,5,3-triiodothyronine (T3) in the MBH which results in initiation of the nervous impulses that lead to the synthesis and release of gonadotropin-releasing hormone (GnRH) (Yoshimura, 2013; Bédécarrats et al., 2015). The GnRH is transported by the portal blood circulation to the anterior pituitary gland, where it stimulates the synthesis and release of the gonadotropins: luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Sharp and Blache, 2003; Bédécarrats et al., 2015). The LH and FSH functions are to stimulate gonad growth and development, as well as production of sex steroid hormones (Leska and Dusza, 2007). A gonadotropin-inhibitory hormone (GnIH), dodecapeptide, is another factor that affects GnRH secretion (Tsutsui et al., 2000). Increased GnIH release at the end of the breeding season (Ciccone et al., 2004) induces the end of the reproductive cycle. Greater GnIH secretion in the pituitary gland has inhibitory effects on LH synthesis and, therefore, secretion (Ciccone et al., 2004). The secretion of vasoactive intestinal peptide (VIP) and prolactin (PRL), as well as the abundance of the mRNAs for these factors, is highly responsive to increased photoperiods (Mauro et al., 1992; Deviche et al., 2000). Furthermore, VIP and PRL contribute to the development of photo-refractoriness and the inhibition of GnRH and LH secretions.

Artificial illumination is widely applied in modern poultry production. Different light colors can affect the development of the reproductive organs (Hassan et al., 2013), age of puberty, and time of sexual maturity (Baxter et al., 2014), as well as muscular-structural development (Rozenboim et al., 2004) and bird behavior (Parvin et al., 2014). The effect of wavelength has, however, not been elucidated, especially for the egg production of geese. Light emitter diode (LED) bulbs are a recent technology and, compared to incandescent and fluorescent lamps, have greater luminous efficiency and operate for longer periods of time before needing to be replaced. Furthermore, these lamps manufactured to transmit different wavelengths (Parvin et al., 2014). The present study was, therefore conducted to evaluate the effect of various monochromatic light colors on the timing and efficacy of reproductive activities, as well as on the underlying endocrine and molecular regulatory mechanisms in the hypothalamo-pituitary-gonadal (HPG) axis.

2. Materials and methods

2.1. Experimental design and animals

The experiment was approved by the Research Committee of the Jiangsu Academy of Agricultural Sciences and was conducted with adherence to the Regulations for the Administration of Affairs Concerning Experimental Animals (Decree No. 2 of the State Science and Technology Commission on November 14, 1988). The animal trial was conducted at Tianzhijiao Farm (118° 27′ E, 32° 01′ N) in Quanjiao, Chuzhou, Anhui Province, China. The experiment commenced on April 5, 2017 and used a flock of 119-day-old Yangzhou geese (n = 1200, female: male = 4:1) of the same genetic origin. The flock was divided into four equal groups and for each group there were three replicates. The geese in each of the four groups (n = 300, female: male = 4:1) initially had imposed on them a short photoperiod of 8 h (8 h light: 16 h dark) for 56 days and received monochromatic blue light. Subsequently, the photoperiod was increased to 11 h (11 h light: 13 h dark) for 174 days (Fig. 1A, B, C, and D), and Groups W, R, B, and G had imposed on them a monochromatic white (Fig. 1A), red (Fig. 1B), blue (Fig. 1C), and green (Fig. 1D) light regimen, respectively. Throughout the experimental phase, geese were maintained in a fully enclosed shed and had imposed on them white (400–700 nm, 100–120 lx), red (630–700 nm, 100–120 lx), green (500–565 nm, 100–120 lx), or a blue (440–484 nm, 100–120 lx) light regimen provided by an LED system (Shenzhen Hongda Technology Co., Ltd., Shenzhen, China). The light intensity was measured (in lx) using a digital light meter positioned at head height of the geese during night shifts, without interference from external light. During the 8 h short photoperiod, lights in the barns were turned on from the 0630 until the 1430 h when lights were turned off. The lights in the barns were turned on from the 0630 until 1730 h for the 11 h photoperiod. The daily egg-laying rate was calculated as the total number of eggs laid each day (merged the three replicates of each group) divided by the number of geese present in each treatment group on that same day.

The birds were fed *ad libitum* a mixed feed diet containing 14.28% crude protein (CP) during the 56 days of short photoperiod. Thereafter, the birds were fed *ad libitum* with a diet containing 16.28% CP (Table S1) when there was imposing of the long photoperiodic light regimen using previously published experimental methods (Zhu et al., 2019). Throughout the experiment (230 days), the geese diet was supplemented with green grass (about 100 g per goose/day) whenever possible. Feed was provided during the daytime, and geese had free access to water in drinking troughs.

Starting from the 14th week, to determine fertility and hatchability, all eggs were collected in weekly batches (total of 18 times) from each group. The transparency of the fertilized eggs was evaluated visually on the seventh day of incubation. Hatchability was defined as the percentage of goslings produced divided by the total number of eggs incubated. The incubation experiment was conducted in a Goose 6528 incubator (Ren's Machinery Technology Co., Ltd., Foshan, China). The incubation procedure was: first phase (from 1 to 5 days) with a temperature of 38 °C and humidity of 65%; second phase (from 6 to 15 days) with a temperature of 37.7 °C and humidity of 60%; third phase (from 18 to 22 days) with a temperature of 37.5 °C and humidity of 62%; fourth phase (from 23 to 27 days) with a temperature of 37.2 °C and humidity of 65%; and fifth phase (from 27 to 31 days) with a temperature of 37.0 °C and humidity of 70%. From day 56 of the experiment, blood samples from 12 geese were collected from wing veins using heparinized syringes every 2 weeks, for a total of 12 times. Plasma was separated by centrifugation of the blood at 2000 × g within 3 h of sample collection and was stored at -20 °C until measurements of hormone concentrations were conducted.

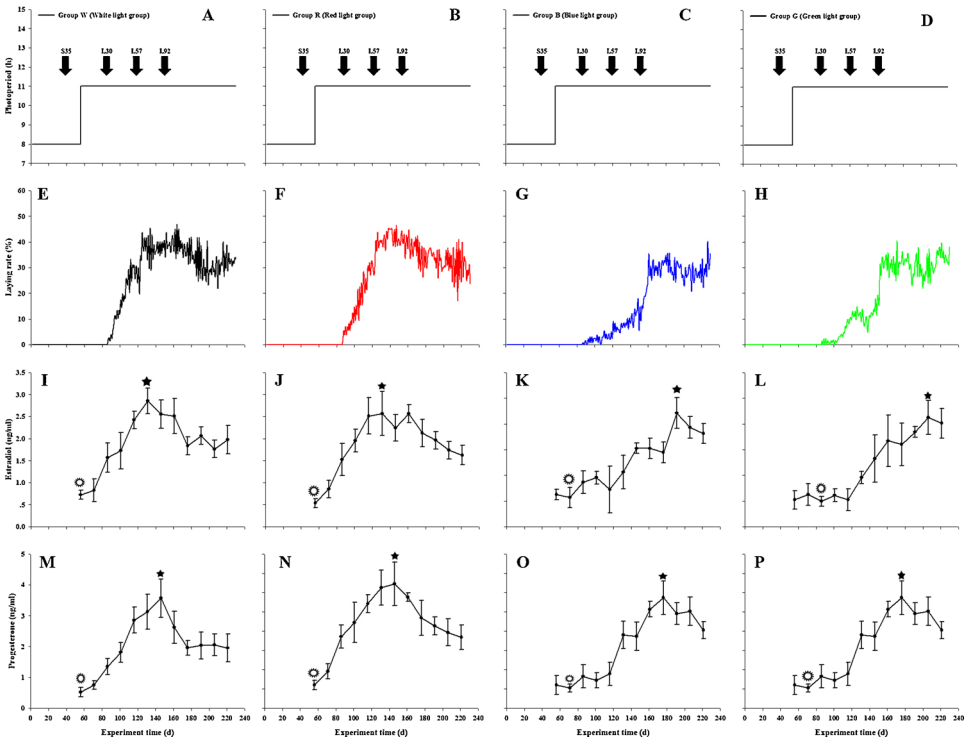

Fig. 1. Laying percentage (E–H), plasma estradiol (I–L), and progesterone concentrations (M–P) in geese subjected to different light colors with artificial photoperiodic regimens; For the artificial photoperiod group (A), there was an initial imposing of a short 8 h photoperiod (8 light:16 dark) for 56 days; Subsequently, the photoperiod was increased to 11 h (11 light:13 dark) for 174 days. Throughout the experimental phase, geese were kept in a fully enclosed shed with there being imposed a white (A), red (B), blue (C), and green LED light regimen (D); Data are presented as mean ± standard error of the mean (vertical bars); Star represents relatively greater hormone concentrations; lace box represents relatively lesser hormone concentrations (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

2.2. Tissue collection, microscopy, and histological evaluation

On day 35 of the 8 h short photoperiod (referred to hereafter as S35) and on days 30, 57, and 92 of the 11 h long photoperiod (referred to hereafter as L30, L57, and L92, respectively), eight geese were randomly selected from each group. After being weighed, the 32 geese were slaughtered, and tissue samples were collected between the 0900 and 1100 h. Immediately after collection, the hypothalamus and pituitary gland tissues were snap frozen in liquid nitrogen and stored at -80 °C until samples were assessed for relative abundance of mRNA. To investigate ovarian follicle development, the number of large white follicles (LWFs; white follicles greater than 6 mm in diameter), small yellow follicles (SYFs; yellow follicles with diameters between 8 and 10 mm), and large yellow follicles (LYFs; yellow follicles greater than 10 mm in diameter) were collected and counted in eight female geese.

Pieces of oviduct magnum (approximately 0.125 cm^3) were dissected from eight geese of each group and immediately fixed in 10% buffered neutral formalin solution for 24 h. The processing was the same as previously reported (Zhu et al., 2019). Stained sections were individually examined using a bright field Olympus B × 63 light microscope (OLYMPUS B × 63; Olympus Corporation, Tokyo) at 10 × and 40 × magnification to examine the changes in the pseudostratified columnar epithelium, ciliated cells, and folds of the oviduct magnum tissues from geese. Three folds and measurements in epithelia of each goose were measured. There were these assessments in eight geese of each experimental group.

2.3. Measurements of hormone concentrations

Plasma estradiol (E2) and progesterone (P4) concentrations were determined using an enzyme-linked immunosorbent assay (ELISA) from the Quantitative Diagnostic Kit for estradiol (North Institute of Biological Technology, Beijing, China). Assay sensitivity was 0.025 ng/mL and 0.2 ng/mL, and the intra- and inter-assay variation coefficients were both less than 15%. Serial dilutions of goose plasma samples resulted in an inhibition curve parallel to the standard curve. The r-values of the assay standard curves were greater than 0.99. The methods used for the hormone assays have been previously described (Zhu et al., 2017b).

2.4. RNA isolation, cDNA synthesis, and quantitative real-time polymerase chain reaction

Real-time quantitative polymerase chain reaction (qRT-PCR) was performed to quantify the relative abundance of mRNA for

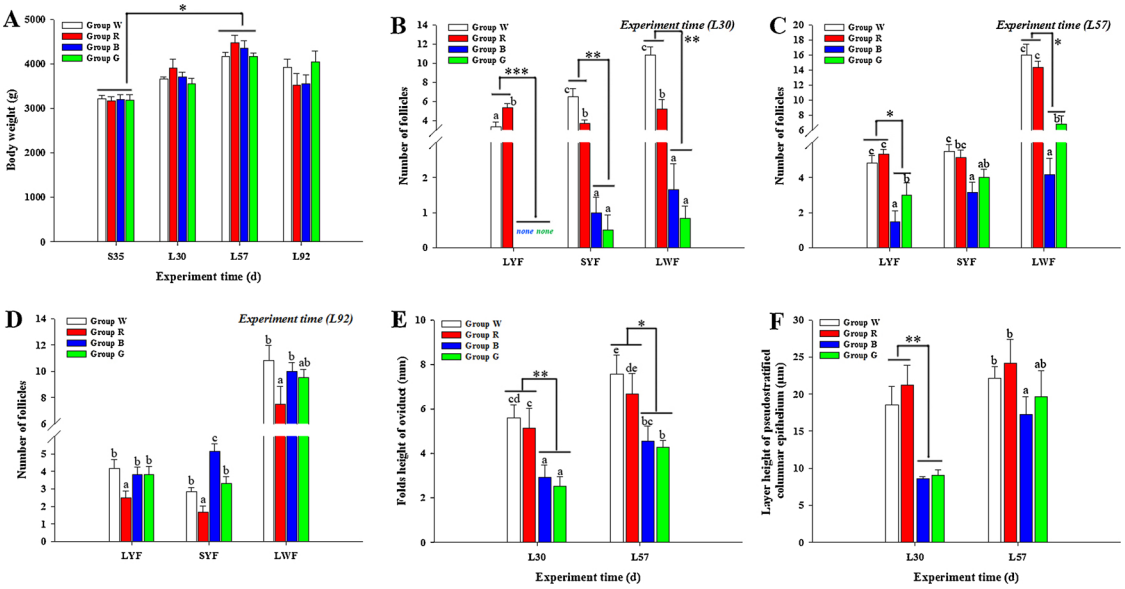

Fig. 2. Body weight, ovarian follicular development, and oviduct magnum index for the geese where there was imposing of the different light color regimens - either white, red, blue, or green LED lights; Phase changes in live weight (A), follicular development (B, C, and D), oviduct magnum index (E and F) are depicted; Each bar represents the mean value from eight determinations, including the standard error; *, **, and *** indicate significant differences $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively; Different letters above the bars indicate significant differences $P < 0.05$.

various genes (Figs. 4 and 5) in the hypothalamus and pituitary gland. Gene-specific primers were designed using the Primer 3.0 software (www.ncbi.nlm.nih.gov/tools/primer-blast/) using data from BLAST, Ensemble, and GenBank databases (Table S2). The methods used have been previously described (Zhu et al., 2019). Relative mRNA abundances were determined using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) and were normalized to an internal housekeeping gene, *β-actin*.

2.5. Statistical analysis

The results depicted in the figures represent the mean ± standard error of the mean of 12 (Fig. 1) and 8 (Fig. 2, 3, 4 and 5) geese for each treatment group. Differences in P4 plasma concentrations after imposing the different light regimens were analyzed using one-way analysis of variance (ANOVA) tests. Relative mRNA abundances were analyzed using one-way ANOVA tests for the main treatments of the four monochromatic light colors in the serial sampling experiment. The mean differences for each treatment were compared using the mean ± standard error of mean, and values were considered significant at $P < 0.05$. Duncan's multiple range test was used to rank the means. Statistical analyses were performed using IBM SPSS software (ver. 11.0; IBM SPSS, Armonk, NY, USA).

3. Results

3.1. Effect of monochromatic lights on egg laying

During the course of the experiment, the egg-laying rate rapidly increased and was greatest at 40% on Days L69 and L68 in Groups W and R (Fig. 1E and F), respectively. In Group B and G, the egg-laying rate increased, albeit at a lesser rate than in Groups W and R (Fig. 1G and H). Consequently, there was a peak of 30% laying rate on Days L107 and L96, respectively. During the entire experimental period, the peak egg production rate in Groups B and G was always less than 40%. In Groups W and R, the relatively greater egg laying rates (greater than 30%) were maintained for approximately 3 months, from Day L58 to L135 and L59 to L137, respectively. Results from statistical analyses indicated that the total number of eggs laid per goose in Groups W and R was 49.6 ± 4.6 and 47.2 ± 3.2, respectively. In Groups B and G, the number of eggs laid per goose was 25.6 ± 3.1 and 29.7 ± 3.6, respectively. Overall, more eggs were laid by geese in Groups W and R ($P < 0.001$) than in Groups B and G (Table 1). Furthermore, the fertilization rate of the eggs and the hatchability of fertilized eggs were greater in Groups W and R ($P < 0.05$) than B and G (Table 1).

3.2. Plasma estradiol and progesterone concentrations

The plasma E2 (Fig. 1I to L) and P4 (Fig. 1M to P) concentrations in geese from the four groups were less (less than 1.00 ng/mL and approximately 0.5 ng/mL) during the first 8 h of the short photoperiod phase (blue light). In Groups W and R, the E2 (Fig. 1I and

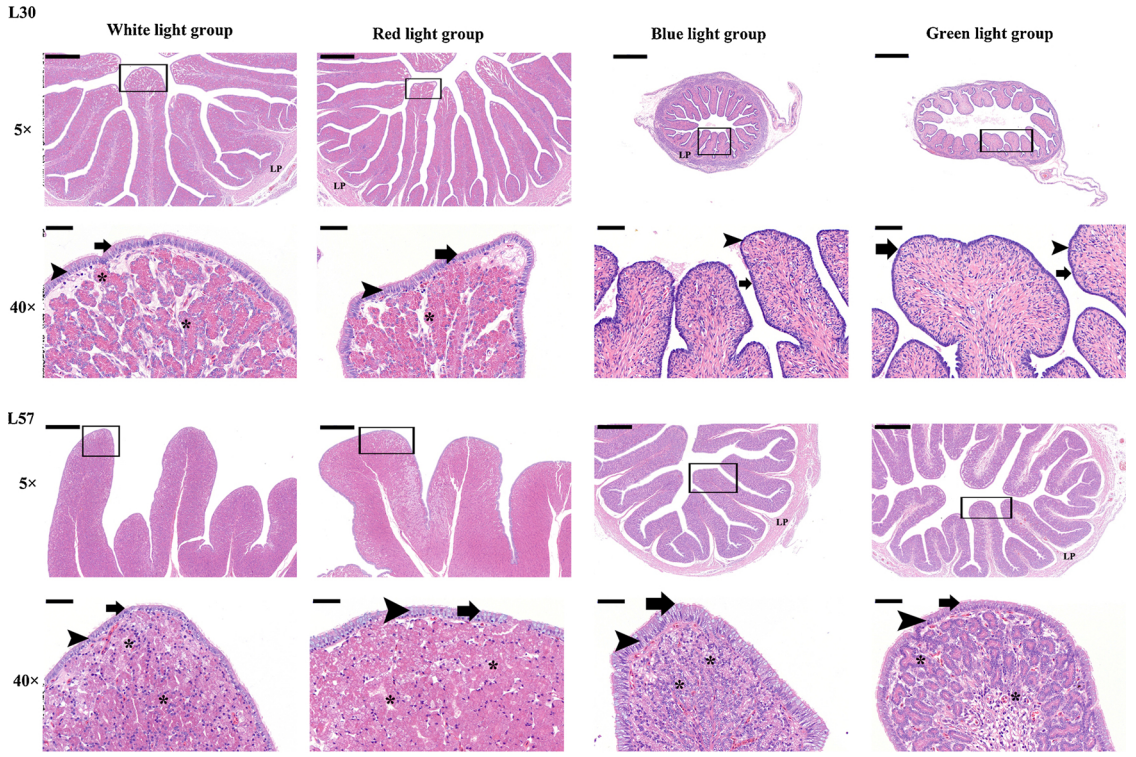

Fig. 3. Histological analysis of sections from Yangzhou geese folds of oviduct magnum in response to imposing regimens of different light colors; Sections from Yangzhou geese were collected on Days L30 and L57 and were stained with hematoxylin and eosin; Symbols are as follows: black arrowhead, pseudostratified columnar epithelium; black arrow, ciliated cells; *, eosinophilic cytoplasmatic granules; Scale bar represents 500 μm at 5× and 50 μm at 40× magnification.

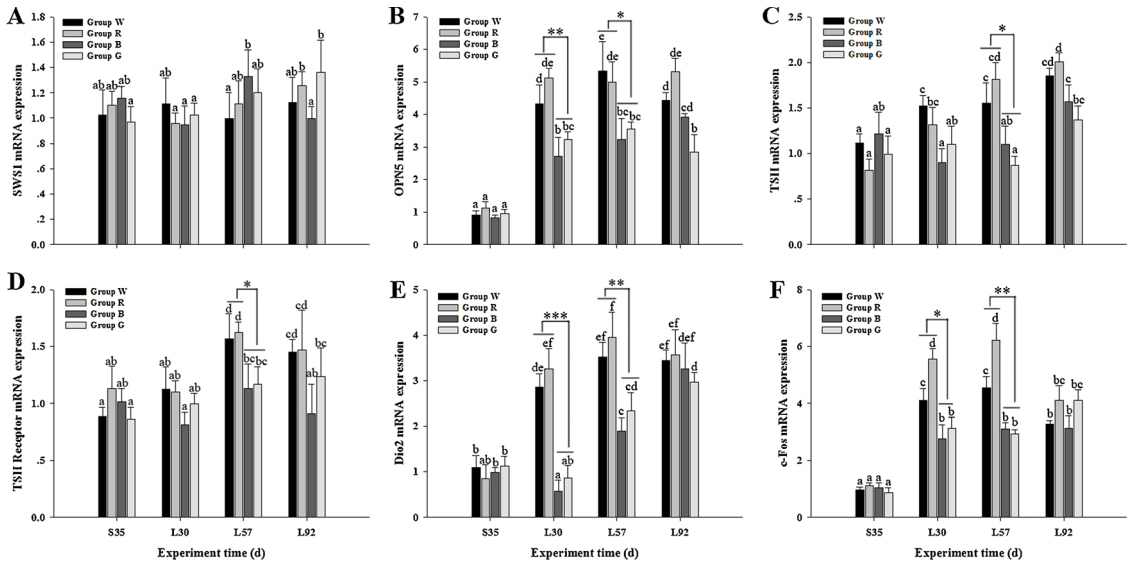

Fig. 4. Average relative abundance of mRNA transcripts in the hypothalamus of Yangzhou geese in response to imposing different light color regimens; Panels A to F represent the relative abundances of mRNA for hypothalamic *SWS1, OPN5, TSH, TSH receptor, Dio2,* and *c-Fos*, respectively; Each value represents the average data from eight geese; Data are shown as mean values ± standard error of the mean; *, **, and *** indicate differences $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively; Different letters above the bars denote differences $P < 0.05$.

J) concentration increased ($P < 0.01$) to approximately 2.86 ± 0.29 and 2.51 ± 0.41 ng/mL on Days 131 (day L75) and 116 (day L60) of the long photoperiod phase of the experiment. In Groups W and R, the plasma E2 trended to lesser concentrations starting on Days L90 and L105, respectively (Fig. 1I and *J*). In Groups B and G (Fig. 1K and L), however, the concentration of E2 increased to

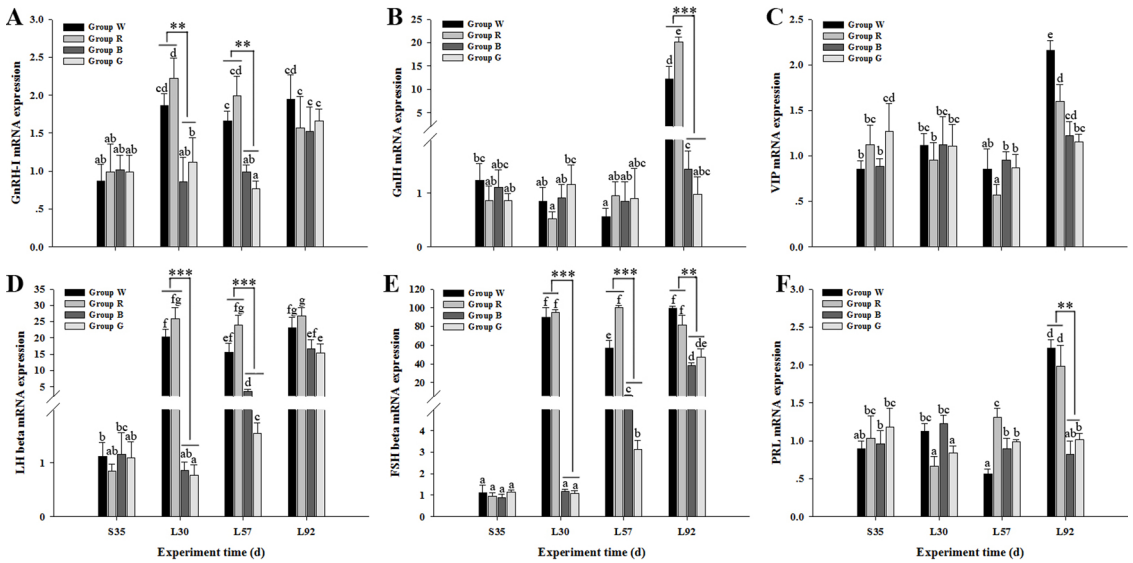

Fig. 5. Relative abundance of hypothalamic and pituitary mRNA in Yangzhou geese in response to imposing different light color regimens; Panels A–C represent relative abundances of mRNA for *GnRH-I, GnIH,* and *VIP*, respectively, and D-F represent relative mRNA transcript abundances for *LHβ, FSHβ,* and *PRL* (whichever is applicable), respectively; Each value represents the average data from eight geese; Data are shown as mean values ± standard error of the mean; *, **, and *** indicate differences $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively; Different letters above the bars denote differences $P < 0.05$.

Table 1
Egg production, fertility, and hatchability of eggs of breeding geese with imposing of different photoperiodic light regimens[1,2].

Item	Monochromatic lights			
	White	Red	Blue	Green
Egg production, (per/birds)	49.56 ± 4.63 ^{b}	47.18 ± 3.18 ^{b}	25.62 ± 3.14 ^{a}	29.72 ± 3.56 ^{a}
Fertility, %	91.32 ± 1.89 ^{bc}	94.49 ± 2.23 ^{c}	83.24 ± 3.11 ^{a}	86.03 ± 1.99 ^{ab}
Hatchability of fertilized eggs (%)	87.68 ± 2.11 ^{bc}	91.33 ± 1.78 ^{c}	85.36 ± 4.22 ^{ab}	82.95 ± 2.72 ^{a}

[1] Results were the means of all eggs for the same group of geese in the whole experimental period.
[2] Means in the same row with different letters were significantly different ($P < 0.05$).

approximately 2.21 ± 0.30 and 2.12 ± 0.33 ng/mL at Days 191 (day L135) and 206 (day L150), respectively. Towards the end of the experiment (day L165), the plasma E2 concentration remained at in relatively greater concentrations in birds of both groups (Fig. 1K and L).

Plasma P4 concentrations were less than 1.0 ng/mL, in the first half of the 56 day short photoperiod phase (Fig. 1M to P). The P4 then increased to peak concentrations around 146 days (day L90) in Groups W and R (Fig. 1M and N). Subsequently, P4 started to decrease with there being lesser concentrations (approximately 1.80 ng/mL) at 221 days (day L165) in Groups W and R. In Groups B and G (Fig. 1O and P), the P4 increased to greater concentrations of approximately 2.87 ± 0.43 and 2.66 ± 0.26 ng/mL on Days 176 (day L120) and 191 (Day L135) of the experiment, respectively. Plasma P4 remained at a relatively greater concentration of 2.10 ng/mL in Groups B and G until the end of the 11 h photoperiod phase.

3.3. Body weight, ovarian follicular development, and histology

During the 8 h short photoperiod, the body weights of the geese in the four groups were maintained at greater than 3100 g (Fig. 2A). When the photoperiod was switched to longer hours, the body weights of the geese in the four groups rapidly increased to approximately 4100 g ($P < 0.05$) on Day L57. On Day L92, the body weights decreased slightly and remained at approximately 3900 g in Groups W and G, and 3500 g in Groups R and B (Fig. 2A).

On day L30, there were LYFs and SYFs in Groups W and R (Fig. 2B), and the numbers were greater than the average number of LYFs for Groups B and G ($P < 0.001$) on the same day. On Day L57 (Fig. 2C), the number of LYFs and SYFs were not different ($P > 0.05$) between Groups W and R. The numbers of LYFs and SYFs decreased, however, to 2.50 ± 0.37 and 1.67 ± 0.36 in Group R from Days L30 to L57, and these numbers on Day L30 were less ($P < 0.05$) than those on Day L57. Until Day L92, there were greater numbers of LYFs in Groups B and G. Furthermore, the number of SYFs and LWFs on Days L57 and L92 were greater ($P < 0.05$) than those on Day L30 for Groups B and G.

On Day L30, the folds of the oviduct magnum had a reddish color in Groups W and R (Figs. 2E and F; Fig. 3). The histological microscopic graph assessments indicate the folds were lined with a layer of pseudostratified columnar epithelium with thick lamina propria and dense eosinophilic cytoplasmatic granules were located underneath (Fig. 3) the lamina propria in both of these groups. In contrast, the eosinophilic cytoplasmatic granules were less developed in Groups B and G than in Groups W and R (Fig. 3). The villus height of folds (Fig. 2E) was greater in Groups W and R ($P < 0.05$) than B and G on Days L30 and L57. Furthermore, the villus layer height of the pseudostratified columnar epithelium (Fig. 2F) was greater in Groups W and R ($P < 0.001$) than B and G on Day L30. Until Day L57 of the experiment, the pseudostratified columnar epithelium and lamina propria were thicker, and the eosinophilic cytoplasmatic granules were greater in number in Groups B and G than W and R (Fig. 3). The results indicate that the oviduct magnum began to develop on Day L57 in Groups B and G.

3.4. Relative abundances of mRNAs

3.4.1. Relative abundances of mRNAs in the hypothalamus

Relative abundances of *SWS1* and *TSH Receptor* mRNA in the hypothalamus (Fig. 4A and D) remained basal throughout the experimental phase, and there were no obvious trends associated with the imposing of monochromatic light color regimens. The relative abundances of mRNA for *OPN5*, *Dio2* and *c-Fos* (Fig. 4B, E and F) were initially less during the 8 h photoperiod phase in all four groups. With photostimulation, the relative abundances of mRNAs for these three genes increased ($P < 0.05$) from Day L30 in Groups W and R. In Groups B and G, relative abundances of *OPN5*, *Dio2* and *c-Fos* mRNAs also increased steadily from Day L30. Relative abundances of *OPN5*, *Dio2* and *c-Fos* mRNA were greater in Groups W and R ($P < 0.05$) than in Groups B and G on Days L30 and L57. The relative abundance of *TSH* mRNA increased steadily from the beginning of the experiment and was in peak abundance on Day L92 in Group W (Fig. 4C). On day L30, relative abundance of *TSH* mRNA was greater in Group W ($P < 0.05$) than Groups B and G. In Groups B and G, relative abundance of *TSH* mRNA was initially less before Day L57, but relative abundance increased in both groups by the end of the experiment (Fig. 4C). On Day L57, relative abundance of *TSH* mRNA was less in Groups B and G ($P < 0.05$) than W and R.

3.4.2. Relative abundances of mRNA for neurohormonal genes in the hypothalamus

Relative abundance of hypothalamic *GnRH-I* mRNA was initially less on Day S35 during the 8 h photoperiod phase in all four groups. Relative abundance of *GnRH-I* mRNA increased ($P < 0.01$) and there were greater relative abundances for *GnRH-I* in Groups W and R (Fig. 5 A) following photostimulation (from Day L30 to L92). In groups B and G, relative abundance of *GnRH-I* mRNA remained less on Day L57 and did not begin to increase until Day L92. On Days L30 and L57, relative abundance of *GnRH-I* mRNA was greater ($P < 0.01$) in Groups W and R than B and G. From Day S35 to L57, relative abundance of *GnIH* mRNA (Fig. 5B) was less in the four groups. In Groups W and R, relative abundance of *GnIH* markedly increased 12-fold and 20-fold by Day L92. Furthermore, the observed relative abundances were greater in Groups W and R ($P < 0.001$) than in B and G at this time. The relative abundance of *VIP* (Fig. 5C) mRNA transcripts in the hypothalamus was of a pattern of change similar to that of *GnIH* throughout the experiment. In Groups W and R, the relative abundance of *VIP* mRNA transcripts increased (two-fold increase, $P < 0.05$) on Day L92. The relative abundance of *VIP* mRNA transcripts was greater ($P < 0.05$) in Group W than in the other three groups on Day L92.

3.4.3. Relative abundances of mRNAs in the pituitary gland

The relative abundances of *LH β* and *FSH β* mRNA (Fig. 5D and E) in the pituitary gland were initially basal on Day S35 in the four groups. With photostimulation, the relative abundances markedly increased ($P < 0.001$) on Days L30 and L57 in Groups W and R, and the greater relative abundances were sustained on Day L92. From Day L57, relative abundances of mRNAs for the two genes gradually increased in Group B, but the relative abundances in Group G continued to be less. By the end of the experiment (Day L92), relative abundance of *LH β* and *FSH β* mRNA transcripts increased ($P < 0.001$) in Groups B and G. The values were, however, less ($P < 0.001$) than those observed in Groups W and R on Days L30 and L57 (Fig. 5D and E). The relative abundance of *PRL* (Fig. 5F) mRNA transcript was basal on Day S35 and these relative abundances were continued until Day S35 to L92 in the four groups. In Groups W and R, relative abundance of *PRL* mRNA transcript was greater ($P < 0.05$) on Day L92 than those of Groups B and G.

4. Discussion

In modern domestic geese husbandry, artificial lighting has been used in breeding practices to attain sexual maturity and the synchronization of timing of reproductive functions (Shi et al., 2007; Chang et al., 2016; Zhu et al., 2017b, 2019). Results of the present study indicate that the egg-laying rate of Yangzhou geese can be increased with use of white and red LED lighting treatments. With the blue and green light treatments (Groups B and G), the reproductive performance and laying rate of the geese were not as great. Compared to green or blue wavelengths, the penetration of red wavelength radiation to the hypothalamus results in a greater amount of reproductive stimulation because red monochromatic light has a longer wavelength, which produces short wavelengths closer to green and blue (Lewis and Morris, 2000). White light is a homogenous mixture of all colors, including red, which may explain why the egg production of birds when there was imposing of the white LED light regimen was similar to that of birds in the red LED light group (Baxter et al., 2014).

To assess the effects of different LED colors on reproductive functions, there was a long photoperiod of 11 h imposed following by imposing a shorter 8 h photoperiod, based on the methods used in previous studies with geese (Zhu et al., 2019). In Groups W and R the peak laying rates and reproductive performances were greater than those in Groups B and G. Furthermore, results from the

present study indicate changes in plasma E2 and P4 concentrations were consistent with changes in egg-laying rates in the four groups, and this reflects the close relationship between ovarian follicle development and egg-laying. By imposing the experimental conditions of the present study, the imposing of the blue and green light regimen failed to induce increases in circulating estradiol concentrations, whereas imposing of the white and red light was more effective, which is consistent with what has been reported to occur in chickens (Baxter et al., 2014). As reproductive tissues or organs develop in response to sex steroid hormones, the oviduct mucosa tunica functions increase and as a consequence of these tissues synthesizing egg albumin the development of these tissues are essential for egg-laying. The functions of these tissues were quite different after imposing the different lighting regimens in the present study.

In the avian species, the DBP receptors that perceive light signals are localized in the MBH (Nishiwaki-Ohkawa and Yoshimura, 2016). The OPN5 was localized in the neurons of the PVO of the hypothalamus in quail (Nakane et al., 2010) and there was a marked increase in neural depolarization with light stimulation in eye-patched and pinealectomized quail (Nakane et al., 2014). These findings indicate that the OPN5 receptor in the hypothalamus responded to and was involved in transmission of external photic signals. In addition, c-Fos was observed in the ME and IN, and responded to a photoperiodic stimulus in studies with quail (Meddle and Follett, 1995, 1997). In the present study, the abundance of mRNA of *OPN5* and *c-Fos* increased substantially on Day L30 in the four groups, but the relative abundances were greater in the groups with white and red light than blue and green light treatments. These results indicated that DBP (*OPN5* and *c-Fos*) responded to changes in the photoperiodic signal and that there was transmission of the signals to regulate *TSH* gene expression. These photoperiodic regulatory pathways in geese are similar to those reported in quail and chickens (Baxter et al., 2014). This explains why quail, chickens, and ducks (drakes) sustain reproductive functions when there is the imposing of a low-energy red light treatment regimen (Baxter et al., 2014). In the present study, the results also indicate that switching the photoperiod from 8 to 11 h resulted in a greater relative abundance of *TSH* and *Dio2* mRNA transcripts in Groups W and R, and that this response occurred earlier and was more pronounced with white and red light than blue and green light treatments.

In birds, FSH mainly promotes E2 secretion as a result of development of small ovarian follicles (Leska and Dusza, 2007). The LH promotes the development, maturation, and secretion of P4 from large yellow follicles. The production and secretion of LH is regulated by the hypothalamic-releasing hormone, GnRH, which in turn could be affected by PRL that is released upon hypothalamic secretion of VIP (Dawson and Sharp, 1998) and GnIH (Dawson and Sharp, 2007). Results of previous studies in drakes and other birds indicate blue light or green light alone did not induce a sustained HPG function when there was either acute or chronic imposing of these lighting regimens but that the red light regimen could sustain HPG function (Baxter et al., 2014; Haas et al., 2017). Drakes housed in facilities where there was a red light regimen had greater LH concentrations in blood plasma similar to those where there was imposing of a long-day white light treatment regimen(Haas et al., 2017). In the present study, switching the photoperiod from 8 to 11 h in Groups W and R resulted in a greater abundance of hypothalamic *GnRH* mRNA and there was suppression of *GnIH* mRNA to a basal abundance. Notably, geese in an activated reproductive state in Groups W and R had greater relative abundances of both *FSHβ* and *LHβ* mRNA earlier in the treatment period. This could explain the earlier follicular development and the increase in plasma P4 concentrations observed in the geese where there was imposing of the white and red light treatment regimens. With prolonged 11 h photoperiod treatments in Groups W and R of the present study, the phenomenon of photorefractoriness appeared by Day L92 and was associated with the timing of an increase in the relative abundance of *GnIH* mRNA transcripts, which is similar to what has been previously reported to occur in geese (Zhu et al., 2017a, b). In birds, peak concentrations of PRL coincide with the onset of gonadal regression (Sharp and Blache, 2003). In the present study, relative abundance of *PRL* mRNA was minimal when there was imposing of short photoperiodic conditions in the four groups of geese, but there was a three-fold increase following 92 days of the 11 h photoperiodic treatment regimen in Groups W and R. Relative abundance of *PRL* mRNA transcript remained much less in groups where there was a blue and green light treatment regimen. These results indicate that Yangzhou geese that were maintained while there was an imposing of a white and red light treatment regimen had a longer and greater peak egg production and larger cumulative egg number than those of geese where there was imposing of a blue and green light regimen.

In conclusion, white and red LED light treatments with a long photoperiod of 11 h induced the activation of the reproductive system of Yangzhou geese, resulting in maintenance of greater egg production. By imposing the white and red light treatment regimens, relative abundance of *OPN5* and *c-Fos* as well as *Dio2* and *GnRH-I* mRNA transcripts in the hypothalamus were greater. When the blue and green light regimen was imposed, there was a delay in initiation of reproductive functions and there was a longer period before the peak egg-laying rate occurred. The imposing of the white and red light treatment regimen led to a post-egg production peak regression as a result of photorefractoriness that occurred because of a greater *GnIH*, *VIP*, and *PRL* gene expression in the hypothalamus and pituitary gland tissues. The results of the present study indicate that the use of white and red monochromatic lights, together with an increase from a short 8 h to a modestly long photoperiod of 11 h daily resulted in a sustained functionality of the neuroendocrine system to support the reproductive functions of Yangzhou geese for a considerably longer time period, thus resulting in a greater egg-laying performance of geese when there was an imposing of these lighting and photoperiodic regimens.

Author contributions

HXZ and ZDS designed the study. HXZ, BBG, RC, MDH, and XLQ conducted the animal experiments, and collected and analyzed the samples. HXZ and ZDS prepared the manuscript with corrective input from ZC and MML. All authors read and approved the final manuscript.

Declaration of interests

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.anireprosci.2019.03.015.

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