The effect of new monochromatic light regimes on egg production and expression of the circadian gene BMAL1 in pigeons

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ABSTRACT We examined the effect of monochromatic light supplementation on pigeon reproductive performance and on the expression of the brain and muscle aryl hydrocarbon receptor nuclear translocator-like (BMAL1) protein in the hypothalamic–pituitary–gonadal (HPG) axis. White King pigeons were selected randomly from 4 lofts (510 pairs/loft) with 3 subgroups/loft. The lofts were exposed to one of 4 light treatments for 3 months administered in the morning and evening as follows: blue light (480 nm), green light (540 nm), red light (660 nm), and control white light. The laying rate, fertility rate, and birth rate were recorded. After 3 months, 48 birds were selected randomly from the 4 lofts (6 females and 6 males from each loft), sacrificed, and the HPG axis was isolated. Following exposure to red light, laying rate was greater than the control group (P = 0.013), but there were no significant differences in the fertility rate (P = 0.41) or birth rate (P = 0.66). Expression of BMAL1 in the hypothalamus was unaffected by the light regime but was greater in the pituitary of females exposed to red light (P = 0.046) and in the pituitary of males exposed to the control white light (P = 0.059). The change in BMAL1 expression in the pituitary of females was negatively correlated with birth rate in monochromatic light (P = 0.021). We suggest that reproductive performance of pigeons is improved by light supplementation in the morning and evening. According to these data, 100 pigeons exposed to red light could lay 26.68 more eggs per month than the control group. Additionally, BMAL1 expression in the HPG axis of pigeons exposed to monochromatic light correlated with birth rate.

Key words: monochromatic light, egg production, BMAL1, pigeon

INTRODUCTION

Light is a major environmental factor affecting livestock and poultry reproductive activity, and light supplements are widely used in the modern poultry industry to promote animal reproductive performance. Light of different wavelengths affects broilers (Johnson et al., 1982), quails (Foster and Follett, 1985), and turkeys (Levenick and Leighton, 1988). In chicks, skeletal muscle development was stimulated by in ovo green-light illumination (Haley, 2006; Liu et al., 2010), and egg production and quality were reduced in response to light of 880 nm (Rozenboim et al., 1998).

Light schedules affect photoperiodism in poultry, which is controlled by the mediobasal hypothalamus (Ohta et al., 1984). The core circadian clock, which regulates the circadian rhythm, is located in the suprachiasmatic nucleus (SCN) of the hypothalamus. Ikegami et al. (2009) identified the localization of the circadian clock protein known as the brain and muscle aryl hydrocarbon receptor nuclear translocator-like (BMAL1) protein in the chicken brain and concluded that the circadian clock in the pars tuberalis of the pituitary and ependymal cells modulates the photoperiodic signal transduction cascade. BMAL1 is a core component of the circadian rhythm, a transcription/translation-based oscillatory loop that serves as an internal biological clock. The oscillatory loop involves BMAL1, Clock, and Period (PER; Reppert and Weaver, 2002). Expression of BMAL1 in chicken is modulated by the duration of light, and the expression patterns of the clock genes are affected by photoperiod in the SCN and pineal gland (Yasu et al., 2003). Luteinizing hormone acts additively with Clock/BMAL1 to enhance StAR gene expression and stimulates Per2 gene expression in the presence of Clock/BMAL1. This latter observation is consistent with control of the timing of ovulation by the circadian rhythm (Nakao et al., 2007).

This background information led us to suspect that monochromatic light supplements would affect egg production, and we investigated this in pigeons. Since the change of illumination time could alter BMAL1
expression, we wondered if BMAL1 expression profiles would be differentially affected by monochromatic light of different wavelengths. Specifically, we analyzed the correlation of reproductive performance and the expression of BMAL1 gene in the hypothalamic-pituitary-gonadal (HPG) axis under different light regimes.

MATERIALS AND METHODS

Animals and Light Regime

White King pigeons (Columba) were selected at random from 4 lofts with similar populations (510 pairs/loft) in Taizhou Pigeon Breeding Co., Ltd. (Taizhou, China). The pigeons were raised according to the farm’s practice. Each loft was exposed to blue light (480 nm), green light (540 nm), red light (660 nm), or white light (400 to 760 nm; Shenzhen Hongda Technology Co., Ltd., Shenzhen, China) for 3 months and the white light group was the control group. The pigeons of each loft were divided into 3 subpopulations. Incandescent bulbs (7 W) were hung from the roof of the house. Birds were exposed to monochromatic lights in the morning (from 05:00 to 06:30) and evening (from 17:30 to 20:00) for a total of 15 h of light every 24 h. The birds were housed as pairs in laying batteries (length × width × height = 50 × 33 × 40 cm³). Water and food were available ad libitum.

Quantitative Analysis of BMAL1 Expression

Birds (n = 48) were selected randomly from the 4 lofts (6 females and 6 males from each loft), sacrificed, and the HPG axes isolated. Tissues were collected at the same time of day (between 09:00 and 10:30 a.m.) from each of the light groups. The pigeons were anesthetized with sodium pentobarbital with the dosage of 2.5 mg/100 g body weight and all efforts were made to minimize suffering. Collected tissues were immediately frozen in liquid nitrogen and stored at −80°C until analyzed. Total RNA was isolated by TRNzol and reverse transcribed using the Fast Quant RT Kit (catalog numbers DP405 and KR106, respectively; Tiangen Biotech Co., Ltd, Beijing, China).

BMAL1 expression was detected by real-time PCR and the following primers were designed against the coding region of the BMAL1 gene (GenBank accession no. KF906247.1): forward 5'-AAGAAGAACGACCCGAAGGAC-3' and reverse 5'-TGCCGTCCTTATGCAACA-3'. Primers were also designed for the glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH; GenBank accession no. AF036934.1): forward 5'-TGAAAGTCCGGATCTCAACCG-3' and reverse 5'-ACGGCTCCTGGAAGATATGTA - 3'. SuperReal PreMix (SYBR Green; catalog number FP204; Tiangen Biotech Co., Ltd., Beijing, China) was used to detect the expression of BMAL1. PCR reactions, 20 μL volume, consisted of 10 μL 2 × SuperReal Premix, 0.4 μL 50× ROX Reference Dye, 0.6 μL of each primer, 1 μL cDNA, and 7.4 μL double-distilled H₂O. PCR was performed under the following conditions: cDNA was denatured at 95°C for 15 min, followed by 40 cycles of 95°C for 10 s and 60°C for 32 s. Assays were repeated independently 3 times and the studies were carried out in the Yangzhou University Animal Science Department.

Statistical Analysis

Laying rate, fertility rate, and birth rate of the different groups were recorded daily during the experimental period. The 2−ΔΔCT method was used to analyze relative BMAL1 expression across the different light groups (Livak et al., 2001). The average Ct value of BMAL1 in HPG axis tissue of the control white light group was defined as 1.0, so that the relative expression levels of this gene in other tissues could be quantified.

Data were analyzed by one-way ANOVA and the Pearson’s correlation coefficients were analyzed by bivariate correlations using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). The significance of differences among the different groups was evaluated by a least significant difference post hoc multiple comparisons test. The significance level was set at P < 0.05.

RESULTS

Reproductive Performance of Pigeons

Egg production of birds illuminated with monochromatic light of different wavelength is shown in Fig. 1. Laying rate in the group treated with red light was significantly greater than the control group (P = 0.013). There were no significant differences among the other groups, although the laying rate of birds exposed to green light was slightly greater (P = 0.41). The fertility rate of birds exposed to red light was highest among the 4 groups, while that of birds exposed to blue light was lowest (Fig. 1C). The opposite was observed when birth rate was assessed; birds exposed to blue light had the highest rate and birds exposed to red light the lowest rate. However, these trends were not statistically significant (P = 0.31 and 0.19, respectively).

We suggest that monochromatic light supplementation enhances the reproductive performance of pigeons. Optimal light spectra supplementation dramatically increased the laying rate, a finding useful for production on the pigeon farm.

BMAL1 Expression Analysis

The photoperiodism of the circadian clock genes in the avian brain is well-known. We wanted to know if monochromatic light supplementation affects expression of BMAL1 in the HPG axis. Examination of the
pituitary and testis of males revealed that *BMAL1* expression in the birds exposed to red and blue light was down-regulated compared to the control. In both groups, the expression in the pituitary was approximately 7-fold less than in the control and expression in testicular tissue was approximately 3-fold less than the control group (Fig. 2). In the hypothalamus, however, there was no difference in *BMAL1* expression among the 4 groups (Figs. 2 and 3). The expression of *BMAL1* in the ovary of females was down-regulated compared to the control, and the pattern was similar to that of the male. However, the expression of *BMAL1* in the pituitary was different in females and males (Figs. 2 and 3).
**Correlation Analysis**

Table 1 shows the correlations between BMAL1 and production in pigeons that had been treated with monochromatic light. Expression in the pituitary in both males and females was negatively correlated with birth rate ($P = 0.021$). In the pituitary of female pigeons, BMAL1 expression was positively correlated with both laying and fertility rates; however, these variables were negatively correlated with BMAL1 expression in the pituitary of males.

Expression of BMAL1 in the hypothalamus of males had little correlation with birth rate but a relatively strong negative correlation with laying and fertility rates. The correlations between BMAL1 expression in male testis and reproductive performance were similar to those observed for the female ovary. The change in BMAL1 expressions in the HPG axis may be related to the reproductive performance of pigeons.

**DISCUSSION**

Our results show that monochromatic light supplementation affects bird reproductive performance and that laying rate can be significantly increased with supplemental red light. They also predict that egg production will be enhanced through improvement in laying and fertility rates, variables that are of utmost concern to the pigeon breeder.

The effects of light on reproductive performance may be related to the wavelength of light. Foss and White (1983) suggested that red light increases egg production in brown-egg laying hens. Also, supplementation with short wavelength light generally has a minimal effect on laying rate in turkeys (Jones et al., 1982). These results are in agreement with our data. The birth rates of pigeons exposed to green and blue lights were slightly higher than those of pigeons exposed to red and white lights. Cao et al. (2007) concluded that testes development was affected by blue and green light. The testis weight, testis volume, seminiferous tubule area, and the percentage of germ cells were higher in animals exposed to blue and green light than in those exposed to red light. Our findings also suggest that red light affected spermatogenesis of male pigeons.

Although we found that fertility rate and birth rate were not affected by light color, other research has indicated that sexual maturation is affected by monochromatic light. The greatest stimulation of sexual maturation in males was achieved by exposure to radiation in the red part of the spectrum in quail (Woodard et al., 1969) and in turkeys (Gill et al., 1988). Gongruttanun (2011) observed higher blood estradiol concentrations and growth rate of ovarian follicles in native Thai-pullet hens (Gallus domesticus) that had been exposed to red light. Estradiol plays a key role in reproductive functions; specifically, a surge of estradiol indicates ovulation. Baxter (2014) suggested that red light was most effective in elevating circulating estradiol, while green light was completely ineffective. Furthermore, stimulation of the retina with green light resulted in the synthesis of serotonin, which was subsequently converted to melatonin during the dark phase, inhibiting the reproductive axis via gonadotropin releasing hormones.

Bunger et al. (2000) demonstrated that BMAL1 is an essential component in the circadian oscillator in

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**Table 1.** Correlation between production and BMAL1 expression in male (M) and female (F) pigeons exposed to monochromatic light.

<table>
<thead>
<tr>
<th></th>
<th>Hypothalamus (F)</th>
<th>Pituitary (F)</th>
<th>Ovary (F)</th>
<th>Hypothalamus (M)</th>
<th>Pituitary (M)</th>
<th>Testis (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laying rate</td>
<td>-0.268</td>
<td>0.728</td>
<td>-0.420</td>
<td>-0.826</td>
<td>-0.405</td>
<td>-0.438</td>
</tr>
<tr>
<td>Fertility rate</td>
<td>-0.259</td>
<td>0.760</td>
<td>-0.397</td>
<td>-0.808</td>
<td>-0.388</td>
<td>-0.382</td>
</tr>
<tr>
<td>Birth rate</td>
<td>-0.539</td>
<td>-0.979</td>
<td>-0.467</td>
<td>0.035</td>
<td>-0.458</td>
<td>-0.447</td>
</tr>
</tbody>
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1Pearson’s correlation coefficient; *$P < 0.05$. 

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**Figure 3.** Relative mRNA expression ($2^{-\Delta\Delta CT}$) of expression levels of BMAL1 gene in the HPG axis of female pigeons reared under the supplement of red light (R), green light (G), blue light (B), or white light (W) were obtained after normalization to GAPDH gene. Values marked with different letters on the bars are significantly different ($P < 0.05$).
mammals and it has been well established that the circadian clock regulates mammalian reproductive physiology. In our study, supplementation with monochromatic light affected BMAL1 expression. The different effect of red light on BMAL1 expression in the pituitary of male and female pigeons in our study may have an important effect on hormone secretion, which affects the reproduction of pigeons. BMAL1 expression in the female pituitary had a significant negative correlation with birth rate, which suggests that BMAL1 influences hormone secretion from the pituitary. Protein hormones (growth hormone, luteinizing hormones, and follicular stimulating hormone) secreted by the pituitary gland have an important influence on reproduction. Nakao et al. (2007) concluded that the circadian clock controlling the timing of ovulation is in the ovary and that Clock/BMAL1 acts additively with luteinizing hormone, accelerating the development of a preovulatory surge. Alvarez et al. (2008) found that both male and female BMAL1 knockout mice were infertile. Wunderer et al. (2013) also suggested that clock genes and their protein products may be directly involved in the photoperiod-dependent regulation and adaptation of hormone synthesis and release.

In conclusion, our study shows that reproductive performance of pigeons is improved by light supplementation in the morning and evening. Compared with the control group, our results suggest that each hundred pigeons could lay 26.68 more eggs each month if they are supplemented with red light. We also observed that the expression pattern of the positive circadian gene BMAL1 in the pigeon HPG axis was affected by monochromatic lights supplementation and the changes were correlated with birth rate.

REFERENCES