# Effects of light regime on circadian rhythmic behavior and reproductive parameters in native laying hens

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The paper aimed to study the effects of ABSTRACT light regime on circadian rhythmic behavior and reproductive parameters in a native laying hen, Beijing You Chicken (**BYC**) during 22 to 30 wk. A total of 630 19wk-old BYC female chicken were allocated to 6 light regime groups with 3 replicates per group and 35 birds per replicate, reared in individually lit floor pens with separate outdoor areas. A  $2 \times 3$  factorial experiment (2) lighting patterns: continuous and intermittent lighting; 3 photoperiods: 16 h, 14 h, 12 h) was arranged, including 16L:8D for group 1; 12L:2D:4L:6D for group 2; 14L:10D for group 3; 10L:2D:4L:8D for group 4; 12L:12D for group 5, and 8L:4D:4L:8D for group 6, respectively. The circadian rhythmic behavior of the hens, including feeding, egg-laying and sleeping behaviors were observed by scan sampling and target sampling method for consecutive 3 d every other week during 22 to 30 wk. Infrared cameras were set outside each pen to record the rhythmic behaviors of the birds every (6:00-7:00;8:00-9:00;10:00-11:00;12:00other hour -13:00;14:00-15:00;16:00-17:00;18:00-19:00;20:00-21:00;22:00-23:00;0:00-1:00;2:00-3:00;4:00-5:00). Theovarian weight, oviduct weight, oviduct length, the number of large yellow follicles (LYF), and small yellow follicles (SYF) were measured at the end of 30 wk. The egg-laying rate during 22 to 30 wk was measured. The results showed that the highest feeding frequency was given by the 16L:8D, and the lowest given

by the 12L:12D (P < 0.05). The average feeding duration was the longest in the 8L:4D:4L:8D at 6:00 to 8:00 (18.67 min/hen) (P < 0.05), and the shortest in the 8L:4D:4L:8D at 18:00 to 20:00 (3.75 min/hen) (P <0.05). The highest egg-laying frequency was given by the 8L:4D:4L:8D (0.28), the lowest given by the 10L:2D:4L:8D (0.21) (P < 0.05). The shortest egg-laying duration occurred at 6:00 to 8:00, was given by the 16L:8D (8 min/hen), and the longest egg-laving duration occurred at 18:00 to 20:00, was given by 16L:8D and 8L:4D:4L:8D (>20.5 min/hen) (P < 0.05). The highest sleeping frequency was given by the 12L:2D:4L:6D, the lowest was given by the 12L:12D (P < 0.05). At 6:00 to 8:00, the shortest sleeping duration was given by 12L:2D:4L:6D (1.5 s/hen), at 18:00 to 20:00, the longest given by 8L:4D:4L:8D (14.3 s/hen) (P < 0.05). Lighting pattern and photoperiod alone or in interaction had no effect on egg-laying rate during 22 to 30 wk (P >(0.05), but had significant effects on ovarian weight, oviduct weight, oviduct length, the number of LYF and SYF (P < 0.05), and the continuous groups were all significantly higher than the intermittent groups (P < 0.05). The present study indicated that light regimes affected the frequency and duration of circadian behavior in BYC laying hens, and the continuous light was more beneficial to the reproductive development than the intermittent light in the early laving period.

Key words: lighting pattern, photoperiod, circadian behavior, reproductive parameters

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#### INTRODUCTION

Light plays an important role in the adjustment of many activities of poultry (Kristensen et al., 2007), such

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as feeding, egg-laying, sleeping, etc. When reared with moderate day length (12–14 h), Chicken consume most of their feed during the photophase, with little feeding during the scotophase (Buyse et al., 1996). The activity of chickens decreased during the dark phase (Ohtani and Leeson, 2000). Broiler chicken spent more time at feeding device under 23L:1D than under 14L:10D and 17L:7D (Appleby et al., 2004). The broiler chickens exposed to 200 lx light intensity were more active and fed more during the photophase but were less

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active and fed less during the scotophase than the birds exposed to 1 lx light intensity (Blatchford et al., 2012).

Light may be the most critical of all environmental factors affecting reproduction in birds (Olanrewaju et al., 2006). The egg-laying of poultry mainly depends on the growth and follicle development level in ovary and affected by breed, environment, etc. (Ci, 2009). The ovarian weight and number of large yellow follicles (**LYF**) responded positively to increasing light intensity (Renema et al., 2001), but the responses to lighting pattern and photoperiod are not clear.

Beijing You Chicken (**BYC**), a dual-purpose native chicken used for meat and egg production in Beijing district, was included in "National Animal and Poultry Breed Resource Protection List" by the Ministry of Agriculture of the People's Republic of China in 2000, and obtained the national geographical indications of agricultural products registration certificate in 2020. Due to its potential market value, BYC raising has boomed over the past 20 yr. Shen et al. (2011) found that the 8L:4D:4L:8D had beneficial effects on performance of BYC laying hens during the pre-laying and peak laying period. The feeding duration of growing BYC was longer in 16L: 8D than in 12L: 12D and 8L: 16D (Zhao et al., 2012). In a previous study we found that the egg-laying rate of BYC was significantly higher in intermittent 16 h group than in continuous 16 h group during 20 to 61 wk (Geng et al., 2014), and the egg production was not significantly affected by lighting pattern, but the photoperiod significantly affected AFI during 22 to 57 wk (Geng et al., 2018). The present study aimed to study the effects of light regime on rhythmic behavior and reproductive parameters of BYC at peak egg-laying period, in order to provide some references for the appropriate lighting of the native chicken.

### MATERIALS AND METHODS

## **Experimental Design and Birds**

The experiment was conducted at BYC Breeding Farm, Daxing district, Beijing, China. A total of 630 19wk-old BYC female chicken were moved from the rearing room (keeping 11–12 h of light) and randomly allocated to 6 light regime groups with 3 replicates per group and 35 birds per replicate under free-range condition. The birds were reared in individually lit floor pens with separate outdoor areas, the pen condition and the related management was the same as our previous study (Geng et al., 2018). The indoor density was about 6.8 birds /m<sup>2</sup>, and outdoor density was about 1.9 birds /m<sup>2</sup>. Nest boxes, perches, and rice husks litter were equipped in each pen, the nests were open all day, and the litter is 5 to 10 cm in thickness.

A 2 × 3 factorial experiment was arranged (2 lighting patterns: continuous and intermittent lighting; 3 photoperiods: 16 h, 14 h, 12 h), including 16L:8D (6:00–22:00) for group 1; 12L:2D:4L:6D (6:00–18:00. 20:00–24:00) for group 2; 14L:10D (6:00 $\sim$ 20:00) for group 3; 10L:2D:4L:8D (6:00–16:00,18:00–22:00) for group 4;

Table 1. Experimental design and lighting treatments.

Group	Lighting pattern	$\rm Photoperiod/(h)$	Lighting regimen
1	Continuous	16	16L:8D (6:00~22:00)
2	Intermittent	16	12L:2D:4L:6D (6:00
3	Continuous	14	-18:00, 20:00-24:00) 14L:10D (6:00-20:00)
4	Intermittent	14 14	14L:10D (0.00-20.00) 10L:2D:4L:8D (6:00
			-16:00, 18:00-22:00)
5	Continuous	12	12L:12D (6:00-18:00)
6	Intermittent	12	8L:4D:4L:8D (6:00 -14:00, 18:00-22:00)

12L:12D (6:00-18:00) for group 5; and 8L:4D:4L:8D (6:00-14:00,18:00-22:00) for group 6, respectively (see Table 1). Special light-proof cloth and the light controller were used in each pen. Light was provided by energy-saving lamps 2 m off the ground, and light intensity was 10 lux in the middle height of pen. Infrared cameras were installed on the ceiling of the walkway outside each pen to observe the pen and the birds.

In order to keep the same ranging time for the birds, the following arrangement was adopted: lights at 6:00 in the morning every day, birds fed 6:00 to 8:00, range freely 8:00 to 14:00, and return to the pens at 14:00when the second feeding time begins. The birds were fed commercial corn-sovbean-based diets formulated according to breed requirement in mash form, the composition and nutrient levels of the basal diet were seen in Table 2. The hens were fed twice a day: 6:00 and 14:00. Temperature, the relative humidity and other management were manipulated according to the "Technical regulation of Beijing You Chicken Feed and Management" (DB11/T 1378-2016).

The study was performed in accordance with local ethical guidelines and met the requirement of the Institutional Animal Care and Use Committee, the approved certificate is SYXK (Jing) 2017-0039.

 Table 2. Composition and nutrient levels of the basal diet.

Ingredients, $\%$	19 <b>-</b> 21 wk	22-30 wk
Corn	65.5	64.0
Soybean meal	21.5	23.2
Wheat bran	5.0	3.8
Limestone	4	5
Premix <sup>1</sup>	4	4
Total	100	100
Nutrient levels <sup>2</sup>		
ME/(MJ/kg)	11.20	11.08
Crude protein/%	15.07	15.51
Calcium/%	2.03	2.75
Total phosphorus/%	0.51	0.51
Available phosphorus/%	0.29	0.29

<sup>1</sup>Premix provided per kilogram of diet: Vitamin A, 100–250 KIU; Vitamin D3, 60–80 KIU; Vitamin E, 0.5 KIU; Vitamin K3, 80 mg; Vitamin B1, 45 mg; Vitamin B2, 180 mg; Vitamin B6, 100 mg; Vitamin B12, 0.5 mg; D-Calcium pantothenate, 220 mg; Nicotinamide, 720 mg; Folic acid, 20 mg; Biotin,2 mg; Copper, 0.2–0.8 g, Ferrous iron, 1.5–5 g; Zinc, 0.8 –2.4g; Manganese,1.5–3 g; Iodine, 10–30 mg; Selenium, 2–6 mg

<sup>2</sup>The nutrient levels were calculated from the data provided by Feed Database in China (2013). They were average of 3 measured values except that ME was calculated value.

#### Measurement and Methods

The circadian rhythmic behaviors of the hens, including feeding, egg-laying, and sleeping were mainly observed by scan sampling and target sampling method for consecutive 3 d every other week during 22 to 30 wk. Infrared cameras were set outside each pen to record the rhythmic behaviors of the birds every other hour (6:00-7:00;8:00-9:00;10:00-11:00:12:00-13:00:14:00-15:00:16:00-17:00:18:00-19:00:20:00-21:00;22:00-23:00;0:00-1:00;2:00-3:00;4:00-5:00).Simultaneously 2 people stayed in the walkway without entering the camera's field of view and disturbing the hens' normal activities were responsible for observation of the birds at 6:00 to 8:00, 12:00 to 14:00, and 18:00 to 20:00, 30 min for each group. The feeding, egg-laving, and sleeping behavior was mainly observed. The observers were trained in advance to ensure consistent observation methods. Scanning method was firstly used to confirm if there are target behaviors are occurring, for example, feeding, sleeping, and egg-laying, and the bird closest to the staff while the target behavior was occurring was selected for 2 min of observation, and then the next bird was selected and observed, 6 birds each replicate. The interobserver reliability was established for the two observers based on the behavior of one hen during the first day. The individual behavior of each target bird was instant recorded at regular interval (e.g., 6:00-8:00), including the feeding frequency, feeding duration, egg-laying frequency, egg-laying duration, sleeping frequency, and sleeping duration. The related behavior parameters were referred to Steinmeyer et al. (2010), Howie et al. (2011), and Jacobs et al. (2019), and defined in Table 3.

The number of hens and eggs of each replicate were recorded every day, the egg-laying rate during 22 to 30 wks was calculated, the calculation was total egg number/(hen number  $\times$  63 d). At the end of 30 wk, 4 birds from each replicate were randomly chosen and euthanized by cervical dislocation, and the ovarian weight, the oviduct weight, and length were measured. The number of large yellow follicles (LYF, 7–9 mm in diameter) and small yellow follicles (SYF, 5–6 mm in diameter) were measured.

## Statistical Analyses

The camera videos were mainly used for behavioral analysis, and the observers' recordings were used for further validation. When there was disagreement between the observer and the video, the observers will check the video, discuss the detail, and get the reconciliation. During the video observing, one replicate was observed in each group, after the statistics, the next replicate was conducted. The data were analyzed statistically using SPSS 25.0 Software for Windows (SPSS Inc. Chicago, IL). The feeding frequency, egg-laying frequency, and sleeping frequency were used to analyze the circadian changes. General linear model was used to analyze the effects of lighting pattern and photoperiod alone and in interaction on egglaving rate during 22 to 30 wk, behavioral duration, ovarian weight, oviduct weight and length, number of large yellow follicles, and small yellow follicles. Duncan's Test was used for multiple comparisons. The percentage was arcsine transformed before the normality test. P < 0.05was regarded as statistically significant.

## RESULTS

### **Feeding Behavior**

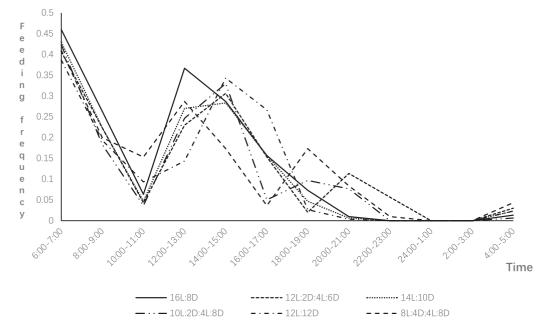
Figure 1 shows the circadian changes of the feeding frequency of BYC caused by the light regime. Within a day, there were about 3 feeding peaks, the first feeding peak focused at 6:00 to 7:00 and 12:00 to 13:00 when the feeding occurred, the highest feeding frequency was given by the 16L:8D, and the lowest given by the 12L:12D (P < 0.05). The second feeding peak focused at 14:00 to 15:00, the highest feeding frequency was given by the 12L:12D (P < 0.05), and the lowest feeding frequency given by the 8L:4D:4L:8D (P < 0.05). The third feeding peak was focused at 20:00 to 21:00, the highest feeding frequency for the 12L:2D:4L:6D when the lighting occurred at that time (P < 0.05), which indicated that the higher feeding frequency of the chickens was related with the feeding time and lighting time.

Figure 2 shows the average feeding duration of BYC affected by the light regime at different periods of time. The average feeding duration was the longest in the

Table 3. The rhythmic behavioral parameters and related definition.

Rhythmic behaviors	Behavioral parameters	Definition			
Feeding	The start of feeding	A hen peck at the feed in the trough			
0	The end of feeding	A hen did not peck at the feed for 5 s or more			
	Feeding duration	The time from the start of feeding to the end of feeding			
	Feeding frequency	The proportion of feeding hens in a replicate group during the observation period (10 min)			
Egg-laying	The start of egg-laying	A hen enter and sit in the nest			
	The end of egg-laying	A hen laid the egg and come out of the nest			
	Egg-laying duration	The time from the start of egg-laying to the end of the egg-laying			
	Egg-laying frequency	The proportion of laying hen in a replicate group during the observation period (10 min)			
Sleeping <sup>1</sup>	The start of sleeping	A hen closed her one or two eyes while standing or sitting, or beak pointing backwards and tucked under the scapular			
	The end of sleeping	A hen opened her two eyes while standing or sitting			
	Sleeping duration	The time from the start of sleeping to the end of the sleeping			
	Sleeping frequency	The proportion of sleeping hen in a replicate group during the observation period (10 min)			

 $^{1}$ About 30% of hens had their backs to the camera or the observers during the observation, therefore, the judge of sleeping depends on closed eyes combined with the posture of the hen.



**Figure 1.** Effects of light regime on circadian change of feeding frequency of BYC. Note: 16L:8D, 6:00–22:00; 12L:2D:4L:6D, 6:00–18:00. 20:00 – 24:00; 14L:10D, 6:00–20:00; 10L:2D:4L:8D, 6:00–16:00, 18:00–22:00; 12L:12D, 6:00–18:00; 8L:4D:4L:8D, 6:00–14:00, 18:00–22:00. Abbreviation: BYC, Beijing You Chicken.

8L:4D:4L:8D at 6:00 to 8:00 (18.67 min/hen) (P < 0.05), and the shortest in the 8L:4D:4L:8D at 18:00 to 20:00 (3.75 min/hen) (P < 0.05).

#### Egg-Laying Behavior

Figure 3 shows the circadian change of egg-laying frequency of BYC caused by light regime. The egg-laying peak was focused at 10:00 to 11:00, the highest egg-laying frequency was given by the 8L:4D:4L:8D (0.28), the lowest given by the 10L:2D:4L:8D (0.21), and the 16L:8D was in the middle (0.23). The egg-laying frequency increases at 8:00, decreased after 16:00, no eggs, or only sporadic eggs. It seemed that the circadian pattern of egg-laying was not affected by the feeding time and lighting time.

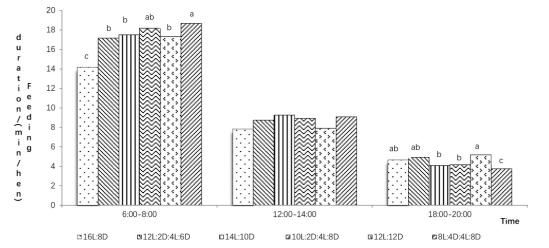
Figure 4 shows the average egg-laying duration of BYC affected by the light regime at different periods of time. The shortest egg-laying duration occurred at 6:00

to 8:00, was given by the 16L:8D (8 min/hen), and the longest egg-laying duration occurred at 18:00 to 20:00, was given by 16L:8D and 8L:4D:4L:8D (>20.5 min/hen) (P < 0.05).

#### Sleeping Behavior

Figure 5 shows the circadian change of sleeping frequency of BYC caused by light regime. There were about 2 sleeping peaks: the first sleeping peak was after 20:00, the highest sleeping frequency was given by the 12L:2D:4L:6D, the lowest was given by the 12L:12D (P < 0.05); the second sleeping peak focused on 12:00-13:00, when there was almost no differences for the light regime groups (P > 0.05).

Figure 6 shows the average sleeping duration of BYC affected by the light regime at different periods of time. At 6:00 to 8:00, the shortest sleeping duration was given by 12L:2D:4L:6D (1.5 s/hen), the longest was given by



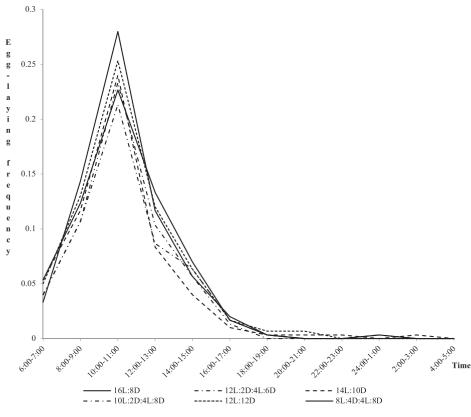


Figure 3. Effects of light regime on circadian change of egg-laying frequency of BYC. Abbreviation: BYC, Beijing You Chicken.

14L:10D and 10L:2D:4L:8D (2.3 s/hen); at 12:00 to 14:00, the shortest sleeping duration was given by 12L:2D:4L:6D (6.8 s/hen), and the longest was given by 14L:10D (8.3 s/hen); at 18:00 to 20:00, the shortest sleeping duration was given by 12L:2D:4L:6D (10.7 s/hen), and the longest given by 8L:4D:4L:8D (14.3 s/hen) (P < 0.05).

Table 4 shows that lighting pattern and photoperiod alone or in interaction had no effects on the feeding duration at 6:00 to 8:00, 12:00 to 14:00, 18:00 to 20:00, also had no effects on egg-laying duration at 6:00 to 8:00, 12:00 to 14:00, but the lighting pattern and photoperiod in interaction had significant effects on egg-laying duration at 18:00 to 20:00 (P < 0.05), though the egg-laying seldom occurs during this period. The continuous 16 h group, continuous 12h group and intermittent 12 h group had the longer egg-laying duration than the intermittent 16 h group at 18:00 to 20:00, but had no differences with 14 h groups (P > 0.05). The lighting pattern and photoperiod alone or in interaction had no effects on

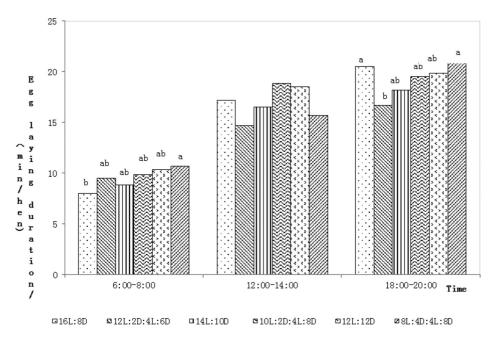


Figure 4. Effects of light regime on average egg-laying duration of BYC. Abbreviation: BYC, Beijing You Chicken.

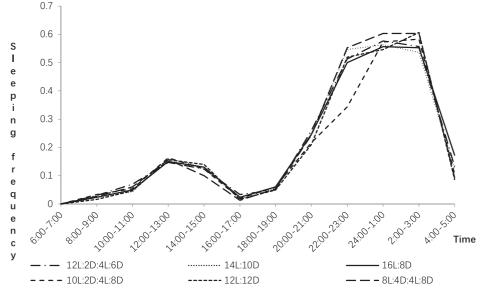


Figure 5. Effects of light regime on circadian change of sleeping frequency of BYC. Abbreviation: BYC, Beijing You Chicken.

sleeping duration at 6:00 to 8:00, 12:00 to 14:00, but the photoperiod alone significantly affected the sleeping duration at 18:00 to 20:00 (P < 0.05), the 12 h group had the longer sleeping duration than the 16 h groups (P < 0.05), but the 14h groups had no differences with the other groups.

#### Reproductive Parameters

Table 5 shows that lighting pattern and photoperiod alone or in interaction had no significant effects on body weight of laying hens at 30 wk of age, and the egg-laying rate during 22 to 30 wk (P > 0.05), but had significant effects on ovarian weight, oviduct weight, oviduct length, the number of LYF and SYF (P < 0.05). The ovarian weight, oviduct weight, oviduct length, the number of LYF and SYF in the continuous groups were higher than in the intermittent groups (P < 0.05), the ovarian weight, oviduct weight, oviduct length, the number of LYF and SYF in the 16 h groups and 12 h groups were higher than in 14 h groups (P < 0.05). The ovarian weight, oviduct weight, oviduct length, the number of LYF and SYF in continuous 16h group, intermittent 16 h group, continuous 12 h group were higher than those in 14h groups and intermittent 12 h group (P

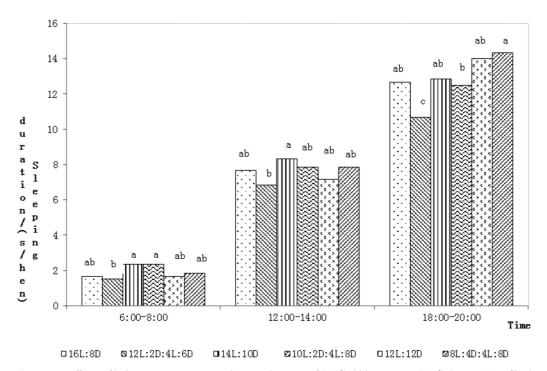


Figure 6. Effects of light regime on average sleeping duration of BYC. Abbreviation: BYC, Beijing You Chicken.

Table 4. Effects of light regime on rhythmic behavior duration.

Lighting		m Feeding  duration/(min/hen)			Egg-laying duration/(min/hen)			Sleeping duration /(min/hen)		
pattern	Photoperiod /(h)	6:00 - 8:00	12:00 - 14:00	18:00 - 20:00	6:00 - 8:00	12:00 - 14:00	18:00 - 20:00	6:00 - 8:00	12:00 - 14:00	18:00-20:00
Continuous	16	14.17	7.83	4.67	8.00	17.17	$20.50^{a}$	1.67	7.67	12.67
Intermittent	16	17.17	8.75	4.92	9.50	14.67	$16.67^{\rm b}$	1.50	6.83	10.67
Continuous	14	17.50	9.25	4.08	8.83	16.50	$18.17^{ab}$	2.33	8.33	12.83
Intermittent	14	18.17	8.92	4.17	9.83	18.83	$19.50^{\mathrm{ab}}$	2.33	7.83	12.50
Continuous	12	17.33	7.92	5.17	10.33	18.50	$19.83^{a}$	1.67	7.17	14.00
Intermittent	12	18.67	9.08	3.75	10.67	15.67	$20.83^{a}$	1.83	7.83	14.33
SEM		0.55	0.36	0.20	0.35	0.76	0.45	0.29	0.25	0.36
Main effects										
Lighting pattern	Continuous	16.33	8.33	4.64	9.06	17.39	19.50	1.89	7.72	13.16
	Intermittent	18.00	8.92	4.28	10.00	16.39	19.00	1.89	7.50	12.50
Photoperiod	16	15.67	8.29	4.79	8.75	15.92	18.58	1.58	7.25	$11.67^{b}$
-	14	17.83	9.08	4.13	9.33	17.67	18.83	2.33	8.08	$12.67^{ab}$
	12	18.00	8.50	4.46	10.50	17.08	20.33	1.75	7.50	$14.17^{a}$
P value	Lighting pattern	0.132	0.445	0.372	0.183	0.524	0.550	1.000	0.670	0.318
	Photoperiod	0.160	0.678	0.404	0.128	0.648	0.192	0.595	0.412	0.014
	Lighting pattern ×z Photoperiod	0.664	0.688	0.187	0.790	0.327	0.027	0.977	0.470	0.340

<sup>ab</sup>Values with different letter superscripts in the same column mean significant difference (P < 0.05).

Table 5. Effects of light regime on reproductive parameters of BYC at 30 wk of age.

Lighting pattern	Photoperiod /(h)	$\mathrm{BW}/\mathrm{(g)}$	${ m Ovarian}\ { m weight}/{ m (g)}$	$\begin{array}{c} \text{Oviduct wei} \\ \text{ght}/(\text{g}) \end{array}$	${ m Oviduct} \ { m length}/({ m cm})$	LYF/(n)	SYF/(n)	Egg laying rate $/(\%)$
Continuous	16	1,628.32	46.38 <sup>a</sup>	$63.25^{a}$	$90.45^{a}$	$5.65^{\mathrm{a}}$	$6.73^{\mathrm{a}}$	52.74
Intermittent	16	1,603.26	$47.68^{a}$	$64.55^{a}$	92.31 <sup>a</sup>	$5.68^{\mathrm{a}}$	$6.74^{\mathrm{a}}$	50.56
Continuous	14	1,534.89	$37.65^{b}$	$46.82^{b}$	$78.45^{b}$	$4.65^{b}$	$5.23^{\mathrm{b}}$	50.64
Intermittent	14	1,573.42	$38.16^{b}$	$49.71^{b}$	$76.42^{b}$	$4.32^{b}$	$5.38^{ m b}$	48.27
Continuous	12	1,617.53	$50.26^{a}$	$75.46^{a}$	$90.06^{\mathrm{a}}$	$6.03^{\mathrm{a}}$	$7.34^{\mathrm{a}}$	50.51
Intermittent	12	1,587.38	$38.74^{b}$	$55.32^{b}$	$87.32^{b}$	$5.34^{\rm b}$	$6.08^{\mathrm{b}}$	49.76
SEM		49.64	2.25	2.92	2.98	1.69	2.27	5.23
Main effects								
Lighting pattern	Continuous	1,593.58	$44.76^{a}$	$61.84^{a}$	$86.32^{a}$	$5.44^{a}$	$6.43^{\mathrm{a}}$	51.29
	Intermittent	1,588.02	$41.53^{b}$	$56.53^{b}$	$85.35^{b}$	$5.11^{b}$	$6.07^{\mathrm{b}}$	49.53
Photoperiod	16	1,615.79	$47.03^{a}$	$63.90^{a}$	91.38 <sup>a</sup>	$5.67^{\mathrm{a}}$	$6.74^{a}$	51.66
-	14	1,554.16	$37.91^{b}$	$48.27^{b}$	$77.44^{b}$	$4.49^{b}$	$5.31^{b}$	49.46
	12	1,602.46	$44.50^{a}$	$65.39^{a}$	$88.69^{\mathrm{a}}$	$5.69^{\mathrm{a}}$	$6.71^{a}$	50.14
P value	Lighting pattern	0.377	0.046	0.037	0.056	0.039	0.047	0.076
	Photoperiod	0.368	0.044	0.031	0.038	0.043	0.045	0.191
	$\begin{array}{c} \text{Lighting pattern} \\ \times \text{Photoperiod} \end{array}$	0.432	0.043	0.041	0.055	0.046	0.048	0.085

<sup>ab</sup>Values with different letter superscripts in the same column mean significant difference (P < 0.05). Abbreviations: BW, body weight; LYF, large yellow follicle; SYF, small yellow follicle.

< 0.05), and the continuous 12 h group had the largest value.

## DISCUSSION

Many animals have circadian rhythmic activities, such as resting and sleeping mainly occur in the dark period, while foraging mainly occurs in the daytime (Appleby et al., 2004). The chickens responded to hunger by foraging when light was first given, and foraging again only when the dark period was predicted before the lights were turned off (May and Lott, 1992).

The feeding behavior of poultry was affected by the lighting regime. Chickens reared in continuous light were less able to anticipate feeding cycles than those given dark periods (May and Lott, 1992); The broilers raised in continuous 8 h dark period can change their

feeding patterns substantially compared with those raised in intermittent light with a separate 8h dark period (Duve et al., 2011), while the broilers under 24 h continuous light did not have a stable feeding rhythm (Ferrante et al., 2006). These previous studies focused on the occurrence of the behaviors, there had little information about the detailed behavioral parameter, such as frequency, and duration, etc. affected by light regime. This present study investigated the frequency and duration during a fixed period, indicated that the highest feeding frequency was given by the 16L:8D, and the lowest given by the 12L:12D, the feeding frequency of the hens was influenced by the lighting time and feeding time, and the hens had the longest feeding duration in the morning, indicating that the birds can take more feed to compensate the loss during the previous night, which was partly in agreement with Schwean-Lardner et al. (2012) suggested that the chickens were able to adjust their rhythmic behaviors according to the lighting and their own requirements.

Egg-laying of poultry depends not only on the number of follicles, but also on the position of follicles in the ovary, the interaction between follicles and the oviduct development (Liu, 2006). Egg-laying is closely related to the follicular growth and development, and affected by the breed, feeding, and environment. Broiler breeders had the best laying performance when the light duration was 11 to 12 h (Ciacciariello et al., 2005). When the light duration was greater than 11 h, the number of LYF was not affected by the light duration, and the number of LYF in the 11L:13D, 13L:11D, 15L:9D and 17L:7D groups were 6.00, 7.75, 7.50, and 7.2, respectively (Chen et al., 2008). In this present study, the lighting regime had significant effects on egg-laying duration, and the number of LYF and SYF. The number of LYF and SYF in the continuous groups were all higher than in the intermittent groups, indicating the follicle development of continuous groups was quicker than the intermittent groups, but the egg-laying rate during 22 to 30 wk was not affected, though there was a trend that the egg-laying rate in continuous lighting groups was higher than the intermittent lighting groups. Our previous study indicated that the lighting regime had no effects on the egg-laying rate during 20 to 26 wk, 27 to 33 wk, 34 to 40 wk, but had significant effects on the egg-laving rate during 41 to 47 wk, which was related with the increased prolactin and luteinizing hormone levels (Geng et al., 2014). In this present study the native laying hens were in early increasing period (22-30 wk), the related hormone level may be still low and the egg-laying rate has not increased as fast as the quick development of reproductive organs.

Sleeping is an important widespread behavior enabling animals to recover from daily stress, sleep deprivation leads to reduced alertness and performance (Boerema et al., 2003). Sleeping is also associated with energy conservation (Malleau et al., 2007). The chicken has unihemispheric sleep and bihemispheric sleep, which means the chicken can sleep with one or both eyes closed (Mascetti, 2016) Raap et al. (2015) firstly demonstrated experimentally that artificial light disrupted sleep behavior in great tits, the light at night caused birds to wake up earlier and leave the nest-box earlier in the morning, and thus sleep less. Touitou et al. (2017) reported that the light at night suppressed melatonin secretion, and affected the sleep and circadian disruption. Our present study indicated that the average sleeping duration of the native laying hen was the shortest in the morning (1.5 s/hen) given by 12L:2D:4L:6D, and the longest in the evening (14.3 s/hen) given by 8L:4D:4L:8D, indicating the chicken can adjust their sleep behavior in response to the changing light regime, which partly agree with Raap et al. (2015) suggested the light pollution had a significant effect on sleep in free-living animals, in particular in the morning.

During the observation, we also found differences in the sleeping posture during the day and at night. During the day, most hens sleep standing up with one or both eye closed, while at night, most hens sleep on their squats with their heads tucked under the wings. The reason could be that during at daytime, the hens are usually alert and sleep for shorter periods, and have more alert posture, but at night, the enclosure is safe for them, and the birds tend to sleep in a more comfortable posture.

The ovarian weight, oviduct weight, and follicle numbers are closely related to the reproductive performance of laying hens Chen et al. (2007) indicated that 11L:13D limited ovarian follicle formation, whereas the 17L:7D restricted ovarian and oviduct development. The increase in the oviduct weight was positively correlated with the increase in light intensity (Renema et al., 2001). Under the light intensity of 11x and 500 lx, the oviduct weight of chickens showed extremely significant difference. An increase in relative and absolute oviduct weight was positively correlated with an increase in light exposure time (Chen et al., 2008). Photoperiod significantly affected the oviduct weight of Hy-Line Gray laying hens at 20 wk of age, the oviduct weight of 16L:8D and 12L:12D groups was significantly higher than that of 8L:16D group, but photoperiod had no significant difference on ovarian diameter and ovarian follicle quantities (Zhang et al., 2014).

Light increasing ways and photoperiod affected the egg quality of Hy-Line Brown layers, and this effect may be due to the change of the oviduct shape (Pan et al., 2007). Photoperiod had no significant effects on number of total follicles, large yellow follicle, and small yellow follicle at the onset of egg-laying, but the influence on total follicle number and small yellow follicle number was significant (Pan, 2008). In this present study, ovarian weight, oviduct weight, oviduct length, LYF, and SYF numbers at 30 wk were significantly affected by lighting pattern and photoperiod, the continuous groups were all significantly higher than the intermittent groups.

### CONCLUSIONS

The present study indicated that light regimes affected the frequency and duration of circadian behaviors in native laying hens, and the continuous light was more beneficial to the reproductive development than the intermittent light in the early laying period.

#### DISCLOSURES

The authors declared that we have no conflicts of interest to this work.

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