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Source: Canadian Journal of Animal Science, 100(1) : 37-46

Published By: Canadian Science Publishing

URL: <https://doi.org/10.1139/cjas-2019-0009>

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# Diurnal rhythms of group-housed layer pullets with free choices between light and dim environments

Guoming Li, Baoming Li, Zhengxiang Shi, Yang Zhao, Qin Tong, and Yu Liu

**Abstract:** Chickens under appropriate light–dark programs can develop diurnal rhythms. However, actual needs of layer pullets for the light and dark environments are not fully understood. This study was to investigate the diurnal rhythms of pullets in the light (30 lx) and dim (<1 lx) environments. The preferences of pullets on the light and dim environments were examined. The testing system contained four identical compartments (0.96 m length × 1.20 m width × 2.00 m height for each). A light-emitting diode tube, a camera, and weighing sensors were installed in each compartment. Four groups of eight Chinese domestic layer pullets, Jingfen, were used at the weeks 15–18. Choices of environments and feeding behaviors were monitored by weighing sensors, and activity was measured by digital image processing. The results show that pullets spent on average  $35.5 \pm 2.2$  min under the light and  $24.5 \pm 2.1$  min under the dim in each hour. Human inspection can stimulate bird feeding and activity. Overall, pullets behaved more actively under the light than under the dim environment. Pullets stayed in the light and dim environments throughout each hour of a day, which may suggest that lighting environments with free choices in a pullet house might better serve for pullet preference.

**Key words:** layer pullet, diurnal rhythm, light and dim, preference test, behavior.

**Résumé :** Les poulets dans les programmes de lumière-noirceur appropriés peuvent développer des rythmes diurnes. Par contre, les besoins réels des poulettes pour la ponte pour les environnements lumineux et sombres ne sont pas bien compris. Le but de cette étude était d'examiner les rythmes diurnes des poulettes dans les environnements lumineux (30 lx) et sombres (<1 lx). Les préférences des poulettes des environnements lumineux et sombres ont été examinées. Le système de test contenait quatre compartiments identiques (0,96 m de longueur × 1,20 m de largeur × 2,00 m de hauteur pour chacun). Un tube à lumière DEL, un appareil photo, et des détecteurs de poids ont été installés dans chaque compartiment. Quatre groupes de huit poulettes chinoises domestiques (Jingfen) destinées à la ponte ont été utilisées des semaines 15 à 18. Les choix d'environnements et les comportements d'alimentation ont été surveillés par les détecteurs de poids, et l'activité a été mesurée par traitement numérique d'images. Les résultats montrent que les poulettes passaient, en moyenne,  $35,5 \pm 2,2$  min sous la lumière et  $24,5 \pm 2,1$  min dans les conditions sombres chaque heure. L'inspection humaine peut stimuler l'alimentation et l'activité des poulettes. De façon générale, les poulettes avaient un comportement plus actif sous la lumière que dans l'environnement sombre. Les poulettes demeuraient dans les environnements lumineux et sombres pendant chaque heure de la journée, ce qui pourrait suggérer que les

Received 17 January 2019. Accepted 16 July 2019.

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environnements avec choix de lumière dans les logements pourraient mieux servir le bien-être des poulettes.  
[Traduit par la Rédaction]

**Mots-clés :** poulette pour la ponte, rythme diurne, lumière intense et faible, test de préférence, comportement.

## Introduction

A light program in poultry production commonly consists of light and dark phases. Proper light phases stimulate bird activity (Newberry et al. 1985) and promote feed intake (Bhatti and Morris 1978). Appropriate dark phases improve bird rest and sleep (Newberry et al. 1985) and alleviate bird stress (Olanrewaju et al. 2006). Chickens under appropriate light–dark programs can develop diurnal rhythms (Bessei 2006). Diurnal rhythms are physical, mental, and behavioral changes within a daily cycle, and they respond primarily to light and dark phases in an organism–environment system. With appropriate diurnal rhythms, chickens can regulate their normal behaviors (Bessei 2006; Deep et al. 2012) and maintain biological and physiological performance (Refinetti 2010). Therefore, the appropriate diurnal rhythms are considered to be important indicators of welfare and health in poultry production (Bessei 2006). However, improper light–dark programs could lead to poor sleep quality (Malleau et al. 2007), increase of mortality (Lewis et al. 1992) and stress (Manser 1996), and abnormal behaviors (Schwean-Lardner et al. 2014).

Hy-Line (2016) has recommended for commercial layer production a step-down light–dark cycle (L:D) during the first 11 wk beginning with 20L:4D and ending with 12L:12D; then a constant cycle of 12L:12D at the weeks 12–16; and subsequently a step-up cycle at the weeks 17–30 ending with 16L:8D which is maintained in the following ages.

Besides continuous light programs, intermittent light programs are also applied to layer production due to economic and productive benefits. Coenen et al. (1988) investigated the effects of two programs [14L:10D vs. 14(0.25L:0.75D):10D, 15 min light and 45 min dark in each of 14 h] on general activity, behavioral patterns, and sleep for laying hens. They concluded that the hens can maintain their diurnal rhythms under both light programs and be more active during the light phases and under the former program. Riskowski et al. (1977) conducted the experiments under three light programs of 14L:10D, 2L:4D:8L:10D, and (1L:3.75D)4×:5D, 1 h light and 3.75 h dark with four repetitions. They found that the daily heat loss of White Leghorn laying hens was not affected by the three light programs while the daily feed consumption was significantly lower under the two intermittent light programs.

The light and dark phases in the above-mentioned light programs are designed mainly based on the consideration of farm operations and production profits. Such light programs may not be optimal from the birds' standpoints. Poultry needs for their living environments can be

fully understood based on choice/preference tests. Ma et al. (2016) investigated the choices of five light intensities (<1, 5, 15, 30, and 100 lx) for laying hens and found that the hens spent more time in the 5 lx environment. Li et al. (2018b) tested the light color preferences (blue, red, green, and white) of layer pullets and found that the pullets preferred the blue light the most. Although previous preference tests have provided crucial insights to the management of poultry-specific light, few studies focused on the preferences of the light and dark environments for 15- to 18-wk-old layer pullets.

Fifteen to eighteen (15–18) weeks of age are the key periods for pullet oviduct growth (Hy-Line International 2013). Appropriate light management during these periods are critical for later production performance and welfare (van der Klein et al. 2018). Assessment of light and dark rhythms based on pullet standpoints helps to understand the real light needs of pullets, thus having crucial economic and welfare implications for pullet production. The objective of this study was to investigate the diurnal rhythms of 15- to 18-wk-old layer pullets in the light (30 lx) and dim (<1 lx) environments provided by the lighting preference test system. Moreover, the preference of the light and dim environments by pullets was also examined in this study.

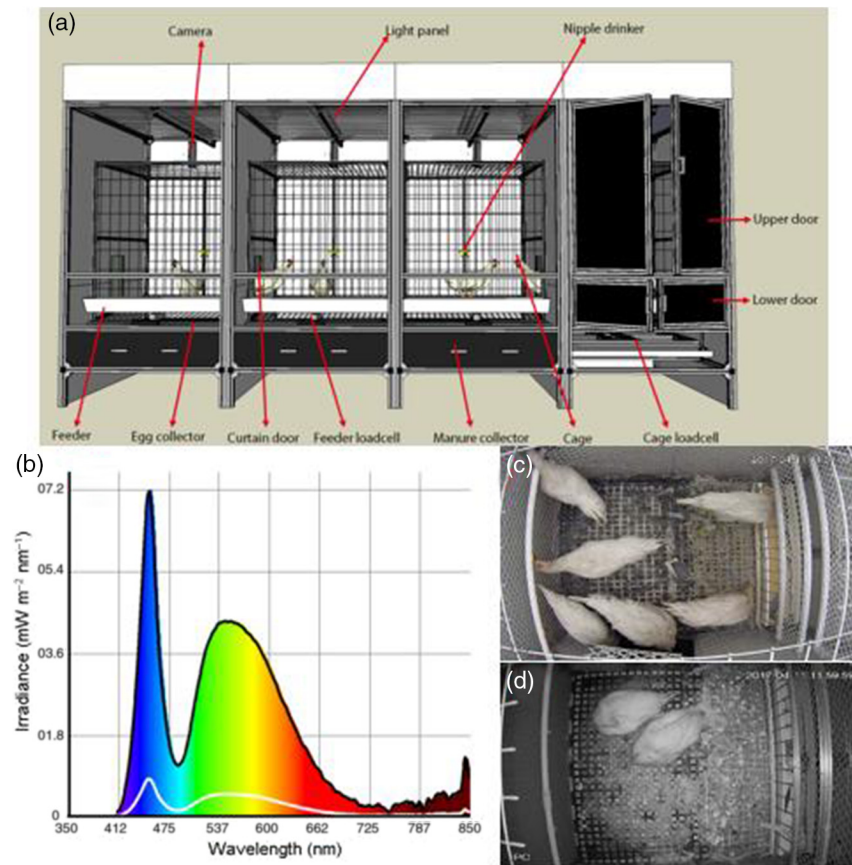
## Materials and Methods

All procedures in this experiment were approved by the Animal Physiology and Behavior Lab, Department of Agricultural Structure and Bioenvironmental Engineering, China Agricultural University (Beijing, People's Republic of China).

### Lighting preference test system

The experiment was conducted in the lighting preference test system [3.84 m × 1.20 m × 2.00 m, length (L) × width (W) × height (H)], which contained four identical compartments (0.96 m × 1.20 m × 2.00 m, L × W × H) (Fig. 1a). Compartments were blocked by 3 mm thick polyvinyl chloride boards. Each compartment consisted of an aluminum cage (0.85 m × 0.85 m × 1.20 m, L × W × H), a camera (V1.1.0, Zhejiang Dahua Technology Co., Ltd., Hangzhou, People's Republic of China), a cage loadcell (50 ± 0.0084 kg, MT1241, Mettler-Toledo International Inc., Changzhou, People's Republic of China), and a feeder loadcell (7 ± 0.002 kg, MT1022, Mettler-Toledo International Inc., Changzhou, People's Republic of China). The camera was installed on the top of each compartment to continuously monitor pullet behaviors. The cage loadcell and feeder loadcell were installed underneath the cage and feeder trough, respectively. They continuously recorded the weights of the cage and feeder.

**Fig. 1.** Illustrations and photos for the preference test: (a) a schematic drawing of the lighting preference test system (note: each compartment has the upper and lower doors and a manure collector); (b) the light spectrum distribution of the light-emitting diode light at 260 lx (black curve) and at 30 lx (white curve); (c) a photo of the experimental cage under the light environment; and (d) a photo of the experimental cage under the dim environment. [Colour online.]



Besides these, each compartment also included a manure collector, an egg collector, and two nipple drinkers. The upper and lower doors were installed for daily management (e.g., manure removal, egg collection, and system maintenance). Four compartments were separated into two groups, which meant each group contained two compartments/lighting environments. The air speed of the four compartments measured by an anemometer (TSI 9545, TSI Inc., Shoreview, MN, USA) was  $0.7\text{--}0.9\text{ m}\cdot\text{s}^{-1}$  at bird level. The system showed a great light tightness based on the previous validation (Li et al. 2018a), which can guarantee data quality under different lighting environments.

#### Lighting environment

Two white light-emitting diode (LED) tubes were used in the preference test. The light intensities of the LED tubes at maximal output were 200 lx. The corresponding light spectrum is shown in Fig. 1b. The tubes were partially wrapped with aluminum foil paper and the light intensities of the tubes were reduced to 30 lx at bird head level. According to the observations of Ma et al. (2016), wrapping the light tubes with aluminum foil paper did not change their light spectrum property. The

light intensity in each compartment was measured using a LED grow light spectrometer (SRI-PL-6000+, Optimum Optoelectronics Corp., Taiwan). For each compartment, the light intensities were tested three times. The 30 and  $<1$  lx light intensities were assigned to each group of compartments to create the desired light (Fig. 1c) and dim (Fig. 1d) environments for the test.

#### Animals and management

Two batches of sixteen 13-wk-old Chinese domestic layer pullets (Jingfen, Beijing Huadu Yukou Poultry Co. Ltd., Beijing, People's Republic of China) were used for the test. The 16 birds were equally assigned to two groups, i.e., four compartments, and they could only move freely between two adjacent compartments/lighting environments within the same group. The environmental management for the pullets at the weeks 1–13 followed commercial recommendations (Table 1). Upon arrival at the laboratory, each batch of 16 pullets was kept in the system to acclimate to the new lighting environments for 1 wk. The curtain doors were fully opened on the first acclimation day, and then the curtain strips were gradually dropped down the next 4 d (1/4 of curtain strips per day). After acclimation, these 16 birds were randomly



**Table 1.** Environmental management of Jingfen layer pullets at the weeks 1–13.

Age	Light program (L:D)	Light intensity (lx)	Temperature (°C)	Relative humidity (%)
<b>Days</b>				
1–3	24L:0D	60	36	60
4–7	22L:2D	30	34	58
<b>Weeks</b>				
2	20L:4D	30	32	58
3	18L:6D	30	30	58
4	16L:8D	30	28	58
5	14L:10D	30	26	58
6	12L:12D	30	24	58
7–13	12L:12D	30	22	58

**Note:** L:D, light–dark cycle.

distributed to four compartments, eight birds for each group. Feeding and egg collection were performed at 08:00 every day. Manure was removed every 3 d. Temperature and relative humidity were monitored separately and identical in each compartment. They were maintained at  $25.1 \pm 0.4$  °C and  $23\% \pm 1\%$ .

#### Pullet occupancy in compartments

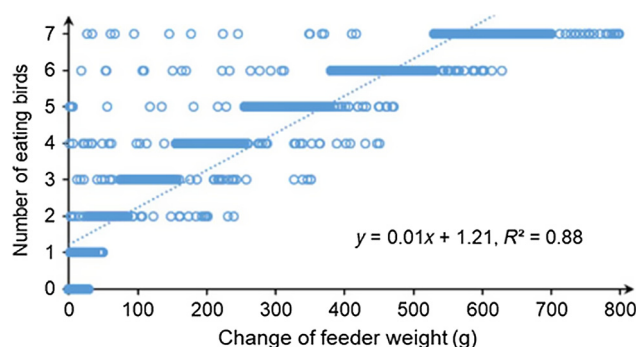
The weights of the cages and feeders were continuously monitored and stored at 1 s intervals in the LabVIEW-based data acquisition system (DAQ). Weight data were exported as .csv files from the DAQ and analyzed using visual basic application in Excel 2013. The number of pullets was determined using weight data in eqs. 1 and 2. In the  $i$ th second, overall body weight in two testing compartments ( $W_{i1}$  and  $W_{i2}$ ) of each group was divided by eight pullets to get the instantaneous average weight ( $\bar{w}_i$ ). The number of pullets ( $N_i$ ) in a compartment was determined by dividing total pullet weight in that compartment with the instantaneous average weight ( $\bar{w}_i$ ) and rounding to the nearest integer. The algorithm in terms of the pullet number detection was validated previously, and the accuracy was 99% or better (Li et al. 2018a).

$$(1) \quad \bar{w}_i = (W_{i1} + W_{i2})/8$$

$$(2) \quad N_i = \lfloor W_i / \bar{w}_i \rfloor$$

#### Feeding behaviors for pullets

Videos collected in each compartment were converted to images. The manually counted number of eating birds was compared with the corresponding change of the feeder weight within the same second. The scenarios of 0–8 eating birds were validated, each of which was validated by 1000 randomly selected images. The results showed an obvious linear relationship between the number of eating birds and the corresponding change of the feeder weight ( $R^2 = 0.88$ , Fig. 2). Puma et al. (2001) also reported that the range of the feeder weight change was 50–100 g when a bird was eating, and the change linearly increased as more birds used the feeder. Therefore, the linear relationship in this case was reasonable. Few scenarios

**Fig. 2.** The change of feeder weight and the corresponding number of eating birds. [Colour online.]

of eight birds being simultaneously at the feeder were observed. The number of eating pullets in a compartment was determined by the feeder weight change in every second according to the linear equation (Fig. 2).

Daily feed intake (DFI) was calculated by summing the differences of the feeder weights between 00:00 and 08:00, and between 08:00 and 24:00. The resolution of the feeder loadcell was 2 g. When the feeder weight change was less than 2 g for 5 s, which meant no eating bird, the feed intake was determined based on such periods.

#### Activity index

Activity index (AI) was used to quantify activity levels of the pullets in this study. The principle and calculation procedure of the AI were described by Aydin et al. (2010). Image processing was implemented to calculate the AI of the pullets using MATLAB (MATLAB R2014b; the MathWorks, Inc., Natick, MA, USA). The software automatically converted the video files to images second by second, and every RGB image was converted into a grayscale image. The difference between the current grayscale image and previous grayscale image was binarized based on the threshold in eq. 3.

$$(3) \quad I_b(x, y, t) = \begin{cases} 1 & \text{if } I(x, y, t) - I(x, y, t-1) > \tau \\ 0 & \text{otherwise} \end{cases}$$

**Table 2.** Behavioral responses during the preference test.

Behavioral responses	Unit	Definition
Hourly time spent at compartment (HTS)	min·pullet <sup>-1</sup> ·h <sup>-1</sup>	Time spent at compartment within each hour of a day
Hourly feeding time (HFT)	min·pullet <sup>-1</sup> ·h <sup>-1</sup>	Time spent at feeder within each hour of a day
Hourly cumulative activity indexes (HCAI)	pixels·pullet <sup>-1</sup> ·h <sup>-1</sup>	Activity indexes summed within each hour of a day
Daily time spent (DTS)	h·pullet <sup>-1</sup> ·d <sup>-1</sup>	Overall time spent in a compartment of the light/dim environment within a day
Daily percentage of time spent (DPTS)	%	DTS/24 h × 100%
Daily trip between compartments (DTBC)	times·pullet <sup>-1</sup> ·d <sup>-1</sup>	Number of trips to a compartment within a day
Duration per trip (DT)	min·trip <sup>-1</sup>	DTS/DTBC
Daily feed intake (DFI)	g·pullet <sup>-1</sup> ·d <sup>-1</sup>	Feed consumption within a day
Daily feeding time (DFT)	h·pullet <sup>-1</sup> ·d <sup>-1</sup>	Overall time spent at feeder within a day
Daily cumulative activity indexes (DCAI)	pixels·pullet <sup>-1</sup> ·d <sup>-1</sup>	Activity indexes summed within a day
Distribution of pullet occupancy (DPO)	%	Percentage of different numbers of birds in a compartment

where  $t$  is time;  $I_b(x,y,t)$  is the intensity at the coordinate  $(x,y)$  of the binary image;  $I(x,y,t)$  is the intensity at the coordinate  $(x,y)$  of the current grayscale image;  $I(x,y,t-1)$  is the intensity at the coordinate  $(x,y)$  of the previous grayscale image; and  $\tau$  is the threshold for binarization. The threshold considering electrical noises and small lighting variations was set to 40 pixels, which was estimated by observing the intensity of the difference in grayscale images without moving birds.

The total pixel value of each binary image was summed and then the resultant value was normalized in eq. 4:

$$(4) \quad V(t) = \frac{\sum_{(x,y) \in Z} I_b(x,y,t)}{\sum_{(x,y) \in Y} 1/N}$$

where  $V(t)$  is the summed pixel of a binary image at time  $t$ ;  $Z$  is the certain zone containing moving pixels in the binary image;  $Y$  is the area of a binary image in which pullets are clearly segmented from the background; and  $N$  is the number of pullets in  $Y$ . The normalization was used to eliminate errors due to different bird sizes and installation heights of cameras among compartments.

#### Definition of behavioral responses

Pullet compartment occupancy, feeding behaviors, and activity indexes were determined based on the above-mentioned methods. Detailed definition of the behavioral responses in this case is shown in Table 2.

#### Statistical analysis of results

The last 3 d of data in each week were used for data analysis. Therefore, total 12 observations of each behavioral response in each week were included in the statistical analyses. All data were analyzed using a two-way generalized linear model in the statistical analysis software (SAS version 9.3, SAS Institute Inc., Cary, NC, USA). The effects of lighting environment, bird age, and their interaction on the behavioral responses were analyzed through the model in eq. 5:

$$(5) \quad Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

where  $Y_{ijk}$  is the measured behavioral responses,  $\mu$  is the overall mean,  $\alpha_i$  is the fixed effect of lighting environment ( $i$  = the light or dim environment),  $\beta_j$  is the fixed effect of bird age ( $j$  = 15, 16, 17, and 18 wk),  $(\alpha\beta)_{ij}$  is the interaction effect of lighting environment and bird age, and  $\varepsilon_{ijk}$  is the random error for the model,  $\varepsilon_{ijk} \sim N(0, \sigma^2)$ . Effects of the factors on the behavioral responses of layer pullets were compared by adopting least significant difference statement. Effects were considered significant when  $P < 0.050$ .

## Results

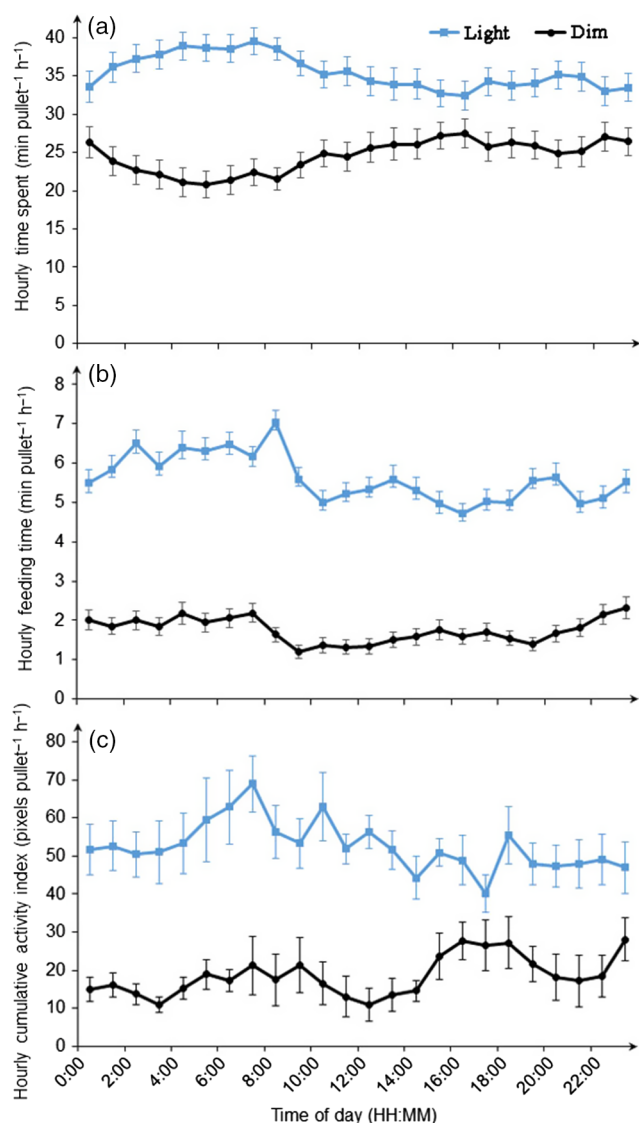
### Hourly behavioral responses under the light and dim environments

The HTS, HFT, and HCAI under the light and dim environments are shown in Fig. 3. The peaks of HTS, HFT, and HCAI were observed at 07:00–08:00. Time spent at compartment, feeding time, and activity for each pullet were distributed both under the light and dim environments throughout each hour of a day. The mean HTS under the light and dim environments were  $35.5 \pm 2.2$  and  $24.5 \pm 2.1$  min·pullet<sup>-1</sup>·h<sup>-1</sup>, respectively. The average HFT was  $5.6 \pm 0.6$  min·pullet<sup>-1</sup>·h<sup>-1</sup> under the light and  $1.8 \pm 0.3$  min·pullet<sup>-1</sup>·h<sup>-1</sup> under the dim. Pullets spent more time eating under the light than under the dim environment throughout a day. The average HCAI under the light and dim environments were  $52.6 \pm 6.3$  and  $18.6 \pm 5.2$  pixels·pullet<sup>-1</sup>·h<sup>-1</sup>, respectively. Pullets were more active under the light than under the dim environment throughout each hour of a day.

### Significance levels of lighting environment, age, and their interaction on behaviors

The significance levels of lighting environment, bird age, and their interaction on the behavioral responses of the pullets are shown in Table 3. Except for DTBC ( $P = 0.826$ ), other behavioral responses of pullets were significantly affected by lighting environment ( $P \leq 0.007$ ). Bird age had effects on DTBC, DT, DFI, and

**Fig. 3.** (a) Hourly time spent, (b) hourly feeding time, and (c) hourly cumulative activity index under the light and dim environments. Each point is the least-square mean of 48 observations, and the error bars represent standard errors. [Colour online.]



DFT ( $P \leq 0.024$ ). The interaction of the two factors significantly affected DTS, DPTS, DT, and DFT ( $P \leq 0.031$ ). The interaction effect indicated that the behaviors of pullets were affected by both examined factors interactively rather than independently.

#### Time spent under the light and dim environments

Table 3 shows least-square means of overall time spent under the light and dim environments. A pullet spent on average  $14.2 \pm 0.6$  h under the light and  $9.8 \pm 0.6$  h under the dim, corresponding to  $59.1\% \pm 2.4\%$  and  $40.9\% \pm 2.4\%$  of a day. Pullets spent significantly longer time under the light than under the dim environment ( $P < 0.001$ ). The L:D based on birds' choices were 14L:10D at the week 15, 15L:9D at the week 16, 17L:7D at the week 17, and

12L:12D at the week 18. The time spent under the light of the pullets increased at the weeks 15–17 while it decreased at the week 18.

#### Travel to different lighting environments

Table 3 shows the travel frequency of the pullets within a day. A pullet traveled on average  $75 \pm 5$  and  $74 \pm 5$  times to the light and dim environments, respectively. The DTBC under the light of each pullet was not significantly different from that under the dim ( $P = 0.826$ ). For each single trip, a pullet spent  $18.9 \pm 2.2$  min under the light and  $9.8 \pm 2.2$  min under the dim ( $P = 0.007$ ).

#### Feeding behaviors

Table 3 shows feeding behaviors of the pullets. Feed intake of a pullet was  $68.7 \pm 1.3$  g under the light and  $7.0 \pm 1.3$  g under the dim ( $P < 0.001$ ). The total feed intake of each pullet increased with ages ( $P = 0.024$ ), which was  $68.4$  g·pullet<sup>-1</sup>·d<sup>-1</sup> at the week 15 and  $83.3$  g·pullet<sup>-1</sup>·d<sup>-1</sup> at the week 18. Overall, a pullet spent 2.2 h eating under the light and 0.7 h under the dim within a day ( $P < 0.001$ ). The total feeding time of each pullet also increased with ages from  $2.3$  h·d<sup>-1</sup> at the week 15 to  $3.8$  h·d<sup>-1</sup> at the week 18 ( $P < 0.001$ ).

#### Activity index

Daily activity levels are shown in Table 3. The average DCAI was  $1.3 \pm 0.1 \times 10^3$  pixels·pullet<sup>-1</sup>·d<sup>-1</sup> under the light and  $0.4 \pm 0.1 \times 10^3$  pixels·pullet<sup>-1</sup>·d<sup>-1</sup> under the dim ( $P < 0.001$ ). The activity level of each pullet did not increase as the bird age increased ( $P = 0.197$ ).

#### Distribution of pullet occupancy

The DPO are shown in Fig. 4. The cases of 7–8 birds at the compartment were higher under the light than under the dim (29.6% under the light vs. 8.5% under the dim). The scenarios of 2–6 birds at the light compartment were similar to those at the dim compartment. These results may further help to explain why pullets stayed longer time under the light than under the dim.

## Discussion

#### Definition of the dim/dark environment

The dim environment with the  $<1$  lx light intensity did not mean “totally” dark in this study. It was hard to create an absolute dark compartment while providing a free access to the neighboring light compartment (light was leaking when a bird passed through the curtain door). Berk (1995) determined a room with the 0.5 lx light intensity as a dark environment. Ma et al. (2016) also defined a chamber with the  $<1$  lx light intensity as a dark environment. Therefore, treating the dim environment as a dark phase of a light program in this study is reasonable.

#### Hourly behavioral responses under the light and dim environments

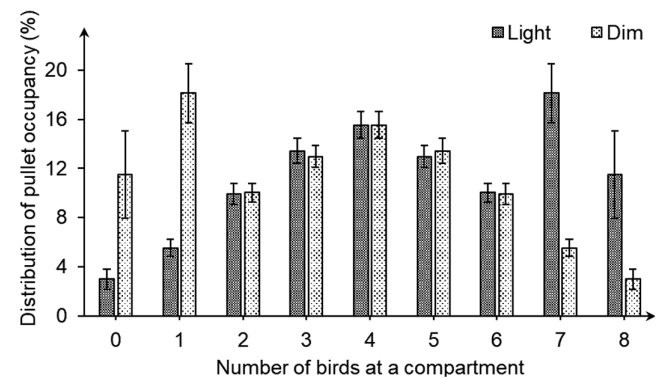
Typically, poultry had behavioral peaks right after light was turned on and before light was turned off

**Table 3.** Behavioral responses of the Jingfen layer pullets under two lighting environments and at the weeks 15–18.<sup>a</sup>

Lighting environment	Bird age (weeks)	DTS (h·pullet <sup>-1</sup> ·d <sup>-1</sup> )	DPTS (%)	DTBC (times·pullet <sup>-1</sup> ·d <sup>-1</sup> )	DT (min·trip <sup>-1</sup> )	DFI (g·pullet <sup>-1</sup> ·d <sup>-1</sup> )	DFT (h·pullet <sup>-1</sup> ·d <sup>-1</sup> )	DCAI (×10 <sup>3</sup> pixels·pullet <sup>-1</sup> ·d <sup>-1</sup> )
Light	15	13.7abc	57.2abc	69ab	13.0b	64.2a	1.8b	1.4a
	16	14.5ab	60.5ab	86ab	23.1ab	65.4a	1.9b	0.9ab
	17	17.3a	72.1a	51ab	35.2a	66.2a	2.6a	1.2a
	18	12.5bc	52.0bc	104a	7.9b	72.9a	2.5a	1.4a
Dim	15	10.3bcd	42.8bcd	69ab	9.5b	4.2b	0.5d	0.4b
	16	9.5cd	39.5cd	85ab	13.1b	5.7b	0.6d	0.3b
	17	6.7d	27.9d	46b	8.5b	7.5b	0.6d	0.3b
	18	11.5bc	48.0bc	103a	7.1b	10.4b	1.3b	0.7ab
Standard error		1.2	4.9	13	4.7	2.4	0.1	0.2
P value of lighting environment		<0.001	<0.001	0.826	0.007	<0.001	<0.001	<0.001
P value of bird age		1.000	1.000	0.002	0.022	0.024	<0.001	0.197
P value of interaction		<0.001	<0.001	0.996	0.031	0.897	0.002	0.516

**Note:** Means within a column not sharing a lowercase letter differ significantly at the  $P < 0.05$  level. DTS, daily time spent; DPTS, daily percentage of time spent; DTBC, daily trip between compartments; DT, duration per trip; DFI, daily feed intake; DFT, daily feeding time; DCAI, daily cumulative activity index.

<sup>a</sup>Data are means of 12 observations.

**Fig. 4.** Distribution of pullet occupancy at the light and dim compartments.

(Li et al. 2018c). However, we did not have light on and off operations in this study. That was probably why these peaks did not appear. The behaviors peaked at 07:00–08:00, which may be caused by the daily flock inspection. This phenomenon may not be associated with the hunger (feed was provided ad libitum for the pullets) nor related to the addition of new feed (feed were added only when necessary). Although the underlying cause of this phenomenon was unknown, human inspection can be used to stimulate bird activity or feeding.

In each hour, a pullet spent on average  $35.5 \pm 2.2$  min under the light and  $24.5 \pm 2.1$  min under the dim, which was consistent with the results of Ma et al. (2016), who also found that the mean HTS by the laying hens was 35 min under the light and 25 min under the dark during the preference test of light intensities. The hourly intermittent light program was in accordance with the biomimetic light program, in which each hour of a program was split into 15 min light and 45 min dark (0.25L:0.75D) for birds (Morris and Butler 1995). Morris and Butler (1995) compared production performance under two light programs. In one program, 8L:16D was maintained from 1 d of age to 18 wk of age, then photoperiod was increased by 1 h step at the weeks 18–21 and by 0.5 h step at the weeks 22–27 ending with 15L:9D, which was maintained until the week 72. In the other program, 8L:16D was held at the 1 d of age to 20 wk of age, and the program was abruptly transferred to 24(0.25L:0.75D) at 21 wk of age. They found that total egg outputs were the same under these two light programs and feed intake was less under the latter program. These results seemed to suggest that lighting environments with free choices in the house might better serve for pullet preference. Further studies to assess the impact of the lighting environments on the production performance are advisable.

The feeding time and cumulative AI were also distributed under the light and dim phases throughout each hour of a day. No existing literature directly described the diurnal feeding and activity rhythms in an



intermittent light–dim environment. [Squibb and Collier \(1979\)](#) demonstrated that the feeding time of the hens under the 24 h light and 24 h dark illumination programs were detected every hour of a day, whereas feeding activity under a light program of 12L:12D was only detected in each lighting hour. With a light program of 17L:7D, [Deep et al. \(2012\)](#) also observed that the birds in different light intensities ate in every lighting hour. [Newberry et al. \(1988\)](#) examined the behaviors and performance of broilers under the 24 h light, and the data showed that the broilers were active in each hour of a day. [Coenen et al. \(1988\)](#) investigated the effects of two different programs [14L:10D vs. 14(0.25L:0.75D):10D] on general activity, behavioral pattern, and sleep for laying hens. Their results showed that the hens were active only in the lighting hours. Typically, poultry eat and are active during the day, and rest at night. However, in this study, the pullets frequently traveled to the light and dim compartments and were exposed to the light for average 19 min and to the dim for average 10 min within each round trip. In the intermittent light, the absence of one long period of a dark phase, which was distinctly longer than any other dark phases, prevented birds from identifying a common “day” and “night” ([Lewis et al. 1992](#)). This may result in pullets eating and being active in every hour throughout the day in this study. Continuous eating throughout the day might decrease the feed efficiency ([Matsoukas et al. 1980](#)) and increase feed cost for farmers. Therefore, a distinctly long period of darkness should be considered when producers want to optimize the benefits of intermittent lights (e.g., reducing electrical utilization, saving feed, and lessening heat stress).

#### Time spent under the light and dim environments

A pullet spent more time under the light than under the dim environment. [Ma et al. \(2016\)](#) conducted the experiment to test the choices of light intensities (<1, 5, 15, 30, and 100 lx) and found that the hens spent significantly longer under the light (sum of 5, 15, 30, and 100 lx) than under the dim (<1 lx) ( $14.0 \pm 0.7$  h vs.  $10.0 \pm 0.7$  h). [Davis et al. \(1999\)](#) found that the birds spent the most time in the bright light (200 lx) at the week 2 but the least in the dim light (6 lx) at the week 6. The time spent at different lighting environments was mainly associated with the duration of expressed behaviors ([Davis et al. 1999](#)). In this case, feeding behaviors and activity were present more under the light than under the dim environment, which may result in a significantly higher proportion of time spent under the light environment.

The early lighting environments to which birds were exposed may have an effect on the later lighting choices ([Gunnarsson et al. 2008](#)). The pullets were reared under a program of 12L:12D before arrival, which may have effects on the light choices in this case. At the weeks 15–17, time spent under the light gradually increased,

which may be caused by the nature of poultry. Under natural conditions, most hens lay eggs in spring and summer, in which the daylight hours increase, and taper production in fall and winter, in which the photoperiod decreases. This nature of hens prevents their offspring from being exposed to suboptimal conditions ([Lewis 2006](#)) because weather and food in fall and winter are commonly unfavorable for young chicks. Based on this nature, a step-up light program in commercial farms was provided to pullets ([Hy-Line 2016](#)), and it was also consistent with the program based on pullets' choices. Through the manual observation, some birds were found to start laying eggs at the week 18 under the dim environment, which might be the reason why the average time spent under the light decreased to 12 h at the week 18. The Jingfen laying hen was selected to achieve high production performance, and its egg production rate could be up to 35% at the week 18 under optimal commercial conditions. Moreover, laying hens preferred to laying eggs in the dark nest box instead of the bright one ([Appleby et al. 1984](#)).

#### Travel to different lighting environments

Although the pullets spent more time under the light, they traveled to the light the same times as to the dim for each age, which indicated that the pullet preferred dynamic lighting environments rather than a constant one ([Rierson 2011](#)). The pullets spent significantly more time under the light than under the dim environment for a single trip. The assessment of DT can provide information on animal preference/aversion to a specific environment ([Kristensen et al. 2000](#)). Our results showed that while the pullets spent more time under the light, they also spent time in the dim environment. This may indicate that both light and dark phases are important for poultry. Some light programs, such as near-continuous lighting (23 h lighting in a 24 h basis) which is designed to maximize feed intake and body weight in broiler production ([Schwean-Lardner et al. 2012](#)) may not match poultry preference.

#### Feeding behaviors

The total feed intake of each pullet was slightly higher than that of [Hy-Line \(2016\)](#) recommendation due to 24 h available feed. In this case, the birds were observed to eat under the dim setting. Although poultry was thought to rest instead of feeding under the dim ([Kristensen et al. 2007](#)), some literature reported that poultry could eat under the dim. [Prescott and Wathes \(2002\)](#), [Ma et al. \(2016\)](#), and [Squibb and Collier \(1979\)](#) found that if the light intensity was gradually decreased, the birds could eat under the darkness. In this case, the pullets gradually acclimated the new lighting environments (the light and the dim) for 4 d. During this period, the pullets probably recognized where the feeder was and further consumed feed in the dim compartment. Overall, the feeding

behaviors under the light were significantly higher than under the dim. This was consistent with that of Prescott and Wathes (2002) and Davis et al. (1999), who reported that laying hens were motivated to eat under the bright environment rather than the dim one. According to the hypothesis of Prescott and Wathes (2002), pullets had more chances to get damage with a forceful peck under a relatively dim environment, in which case eating under bright environments may be a preferred option. In summary, pullets may be averse to eating in dim environments, thereby, a bright environment at feeders is recommended in a commercial farm to improve feeding preference of poultry.

#### Activity index

Although the method to calculate the AI in this study could not differentiate the individual active birds, it has been applied to evaluate the overall activity level of the whole flock (Aydin et al. 2010). Through observation, the AI of pullets were mainly associated with some active behaviors, such as walking, flapping, pecking, stretching, etc. Our results show that the pullets were more active under the light than under the dim, which was consistent with that of previous studies. Boshouwers and Nicaise (1987) found that the movement of laying hens increased as the light intensity increased. Newberry et al. (1988) observed that the standing, walking, and total activity of broilers were significantly higher under the bright light (180 lx) than the dim light (6 lx). These findings, including that of this study, demonstrated that farmers should provide sufficient light for poultry to let them express their natural behaviors in commercial houses.

#### Distribution of pullet occupancy

Pullets are social species with a strong tendency to form groups (Estevez et al. 2007), within which dominant birds have the priority to use the resources (e.g., feeders, nest boxes, etc.), and accordingly subordinate birds are unable to use the facilities fully (Shimmura et al. 2008). In this study, sufficient resource allowances (e.g., feed, water, feeder and drinker space, and compartment space) could avoid fierce resource/space competitions. Moreover, 7–8 birds stayed together under the light for most of the time, resulting in a space allocation of  $900\text{--}1032\text{ cm}^2\cdot\text{bird}^{-1}$ . That is greater than the minimal space requirement of  $748\text{ cm}^2\cdot\text{bird}^{-1}$  for cage-reared hens (California Department of Food and Agriculture 2013). Therefore, the resource allowance and the stocking density in this case had little effect on pullet lighting choices. Our data also showed that although most of the eight birds stayed in the light compartment, the rest preferred the dim one, which meant that different individual birds had different light preferences at the same moment. However, current light programs at a commercial farm allow all birds to see light and dark simultaneously, which may not meet the actual light

requirement of every bird. Again, a better solution might be to provide constant light in a hen house with dark compartments, so that the birds can choose light/dark environments at their will. Nevertheless, the effect of this light program on hen production remains unknown. More production experiments are recommended to verify this setting.

#### Conclusion

This study assessed the diurnal rhythms of the Jingfen layer pullets (15–18 wk of age) provided with light (30 lx) and dim (<1 lx) environments and their choices of the lighting environments using the lighting preference test system. Human inspection can stimulate bird feeding and activity. Pullets spent on average  $35.5 \pm 2.2$  min under the light and  $24.5 \pm 2.1$  min under the dim environment. The feeding time and activity of each pullet were distributed both under the light and dim environments throughout each hour of a day. Pullets spent more time, ate more, and were more active under the light than under the dim.

#### Acknowledgements

This work was supported by the National Key R&D Program of China (grant number 2017YFB0404000) and by the China Agricultural Research System (grant number CARS-40).

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