

CHOICE BETWEEN FLUORESCENT AND POULTRY-SPECIFIC LED LIGHTS BY PULLETS AND LAYING HENS

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ABSTRACT. Light plays an important role in poultry development, production performance, health, and well-being. Light technology continues to advance, and accordingly new light products are finding applications in poultry operations. However, research concerning responses of young and adult laying hens to light sources is relatively lacking. This study assessed the choice between a Dim-to-Red poultry-specific light-emitting diode (LED) light (PS-LED, correlated color temperature or CCT = 2000K) and a warm-white fluorescent light (FL, CCT = 2700K) by pullets and laying hens (W-36 breed) via preference test. Birds with different prior lighting experiences were evaluated for their light choice, including (1) pullets (14 to 16 weeks of age or WOA) reared under incandescent light (designated as P_{INC}), (2) layers (44 to 50 WOA) under PS-LED (L_{LED}) throughout the pullet and laying phases, and (3) layers under FL (L_{FL}) throughout the pullet and laying phases. Each bird category consisted of 12 replicates, three birds per replicate. Each replicate involved a 6-day preference test, during which the birds could move freely between two interconnected compartments that contained PS-LED and FL, respectively. Time spent and feed intake by the birds under each light were measured and then analyzed with generalized linear mixed models. Results showed that regardless of prior lighting experience, birds in all cases showed stronger choice for FL ($p = 0.001$ to 0.030), as evidenced by higher proportions of time spent under it. Specifically, the proportion of time spent (mean \pm SEM) under FL versus PS-LED was $58.0\% \pm 2.9\%$ vs. $42.0\% \pm 2.9\%$ for P_{INC} , $53.7\% \pm 1.6\%$ vs. $46.3\% \pm 1.6\%$ for L_{LED} , and $54.2\% \pm 1.2\%$ vs. $45.8\% \pm 1.2\%$ for L_{FL} . However, the proportions of daily feed intake occurring under FL and PS-LED were comparable in all cases ($p = 0.419$ to 0.749). The study thus reveals that prior lighting experience of the pullets or layers did not affect their choice of FL versus PS-LED. While the birds exhibited a somewhat stronger choice for FL, this tendency did not translate into differences in the proportion of feed use under each light type.

Keywords. Behavior and welfare, Computer vision, Poultry Lighting, Preference assessment.

Light plays an important role in the behavior, development, production performance, health, and well-being of poultry (Manser, 1996; Lewis and Morris, 2000; Olanrewaju et al., 2006; Rajchard, 2009; Lewis, 2010). As such, extensive research on poultry lighting has been conducted over the past eight decades, leading to the establishment of general guidelines on photoperiod and light intensity for improved animal performance and energy efficiency (ASABE, 2014). As light technology continues to advance, new light products (specific to the animal or production stage) constantly emerge, and some are increasingly finding applications in animal operations. However, controlled comparative research is relatively limited regarding the behavioral and performance responses of animals, es-

pecially pullets (young hens before lay) and laying hens, to emerging light technologies.

Poultry have a different light spectral sensitivity compared to humans (Prescott and Wathes, 1999; Prescott et al., 2003; Saunders et al., 2008). In particular, poultry have five types of retinal cone photoreceptors that are sensitive to ultraviolet (UV) and short-, medium-, and long-wave radiation (Osorio and Vorobyev, 2008), and they can perceive light not only through their retinal cone photoreceptors but also through extra-retinal photoreceptors in the brain (e.g., pineal gland and hypothalamic gland) (Mobarkey et al., 2010). It has been demonstrated that the retinal cone photoreceptors produce the perception of colors by receiving light at peak sensitivities of approximately 415, 450, 550, and 700 nm and that they are more related to poultry activities (e.g., feeding, drinking, and locomotion) and growth. In contrast, the extra-retinal photoreceptors can only be activated by long-wave radiation (e.g., yellow-red and red) that can penetrate the skull and deep tissues of poultry and that impacts sexual development and maturity (Lewis and Morris, 2000). Because different lighting sources (e.g., incandescent, high-pressure sodium or HPS, fluorescent, and light-emitting diode or LED lights) have different spectral characteristics, the retinal and extra-retinal photoreceptors of birds may be stimulated differently when exposed to different lighting sources, thus causing different impacts on the animals. For example, research found that red light was associated with sexual devel-

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opment and maturity of pullets (Harrison et al., 1969; Gongtananun, 2011; Min et al., 2012; Baxter et al., 2014; Li et al., 2014), while blue light was associated with improving broiler growth, calming the birds (albeit with no delineation of the underlying mechanism), and enhancing the immune response (Prayitno et al., 1997; Rozenboim et al., 2004; Cao et al., 2008; Xie et al., 2008; Sultana et al., 2013).

A lighting study investigating broilers reported that a Dim-to-Blue poultry-specific LED light (correlated color temperature or CCT = 5000K) and a NextGen poultry-specific LED light (CCT = 3500K) resulted in better well-being (better plumage, hock, and/or footpad conditions) and improved production (better feed conversion) when compared to a daylight compact fluorescent light (CFL, CCT = 5000K) (Huth and Archer, 2015). No explanation was provided regarding the underlying mechanism for the improvement. In contrast, another study reported no differences in growth, feed intake, feed conversion, mortality, ocular development, or immune response of broilers reared under the same two types of LED lights, an incandescent light (CCT = 2010K), and a warm-white CFL (CCT = 2700K) (Olanrewaju et al., 2016). Another recent study revealed that a Dim-to-Blue poultry-specific LED light and a warm-white CFL led to comparable W-36 pullet performance in body weight, body weight uniformity, and mortality (Liu et al., 2017). Similarly, when applying a Nodark poultry-specific LED light (CCT = 4100K) and warm-white fluorescent lights in commercial aviary hen houses, no differences between the two types of light were detected in egg weight, egg production, feed use, mortality rate, or egg quality parameters for DeKalb white hens (Long et al., 2016a, 2016b). In addition, a study found that the effects of LED lights on broiler growth were age-related (Yang et al., 2016). These inconsistent results, along with the increasing number of novel lights intended for poultry production, and the increasing focus on animal well-being, make it necessary to further investigate the responses of poultry to lighting conditions. Performance-based studies, such as those reported in the literature, although important and necessary, can be subject to the influence of other factors, such as thermal conditions, nutrition, feeding practices, space allowance, and indoor air quality. On the other hand, behavior-based assessment of animal responses to lighting conditions in an otherwise uniform environment may provide insights into lighting preferences of the animals.

Preference tests investigate the instantaneous behavioral responses of animals to various environmental stimuli, rather than the long-term physiological impacts; thus, they can offer an efficient assessment of animal preferences (Ma et al., 2016). As a result, preference tests have been used extensively in poultry studies to assess different environmental conditions, including floor type (Hughes, 1976), nest box (Appleby et al., 1984; Millam, 1987), perch height and shape (Struelens et al., 2008; Lambe and Scott, 1998), ammonia level (Green, 2008; Kashiha et al., 2014), and various light regimens, as cited below. Broilers (Cobb breed) at 1 to 6 weeks of age (WOA) were shown to have no preference for white or yellow LED lights at a light intensity of 5 lux (Mendes et al., 2013). Turkeys (BIG6 breed) at 6 to 13 WOA preferred fluorescent light with supplementary UV radiation

at a light intensity of 15 lux (Moinard and Sherwin, 1999). Turkeys (BUT8 breed) at 6 to 19 WOA were found to spend significantly longer time under a light intensity of 25 lux when given free choice among <1, 5, 10, and 25 lux (Sherwin, 1998). Laying hens (Shaver 288 breed) at 24 WOA preferred CFL lighting over incandescent lamps at a light intensity of 12 lux because they spent on average 73.2% of the time under CFL and only 26.8% under incandescent light (Widowski et al., 1992), but they did not have a preference for high ($\geq 20,000$ Hz) or low (120 Hz) flicker frequency of CFL at 19 WOA (Widowski and Duncan, 1996). Laying hens (Leghorn breed) at 20 to 23 WOA also had no preference for HPS or incandescent light (Vandenbert and Widowski, 2000). In addition, preference studies on pullets (LSL breed) reared under incandescent light or natural daylight revealed that the early lighting experience of pullets affects their later preference for lights. Birds reared under incandescent light showed a preference for incandescent light as compared to birds reared under natural daylight at 14 WOA (Gunnarsson et al., 2008, 2009). More energy-efficient, readily dimmable, and long-lasting LED lights are increasingly finding applications in poultry operations. There is anecdotal evidence that some commercial poultry-specific LED lights are advantageous for the performance and behavior of poultry over traditional fluorescent lights; however, concrete research data are lacking. Thus, it is of socio-economic as well as scientific value to evaluate the behavioral responses of poultry to various lighting sources through preference testing.

The objectives of this study were to (1) assess the preference of pullets and layers between a Dim-to-Red poultry-specific LED light (PS-LED) and a warm-white fluorescent light (FL) and (2) evaluate the potential influence of prior lighting experience on subsequent light preference. The results are expected to contribute to improvement of current lighting guidelines on light sources for pullet rearing and laying hen production.

MATERIALS AND METHODS

The study was conducted in a controlled-environment animal research laboratory at Iowa State University (Ames, Iowa). The experimental protocol was approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC No. 3-15-7982-G).

EXPERIMENT BIRDS, BIRD HUSBANDRY, AND TESTING APPARATUS

Hy-Line W-36 commercial layers were used in this study. A total of 36 pullets and 72 layers were tested for their light preferences. All birds were non-beak-trimmed and individually identified with wing bands. The same lighting program, based on the Hy-Line Commercial Layer Management Guide (Hy-Line, 2016), was followed while the birds were reared or kept in respective light environments prior to commencement of the preference test. Specifically, the pullets were reared in litter-floor rooms that used only incandescent light and were randomly selected for the preference test at 14 to 16 WOA. The layers, transferred from litter-floor

rooms as pullets at 16 WOA, were kept in conventional cages that used a Dim-to-Red PS-LED (AgriShift JLL, 8 W, Once, Inc., Plymouth, Minn.) or a warm-white FL (MicroBrite MB-801D, cold cathode fluorescent light or CCFL, 8 W, Litetronics, Alsip, Ill.). The layers were randomly selected for the preference test at 44 to 50 WOA. Half of the layers (36) had been reared under a Dim-to-Blue PS-LED (Agrishift MLB, 12 W, Once, Inc.) in the pullet phase, and the other half had been reared under a warm-white FL (EcoSmart CFL, 9 W, EcoSmart Lighting Australia, Sydney, Australia). The characteristics of the light sources used in the study and their spectral distributions are shown in table 1 and figure 1, respectively. Therefore, the birds were divided into three categories based on age or production stage and prior lighting experience: pullets reared under incandescent light (P_{INC}), layers under PS-LED throughout the pullet and laying phases (L_{LED}), and layers under FL throughout the pullet and laying phases (L_{FL}). Each category consisted of 12 groups or replicates (experimental units), with three birds per group.

A light preference test tunnel and an acclimation chamber were used for the study (fig. 2). The preference test tunnel was modified from an existing system. It consisted of five identical compartments, each measuring 61 × 91 × 198 cm (W×D×H) and containing a 60 × 60 × 90 cm cage and an 18 cm plenum space (35 cm above the cage top). The test tunnel was equipped with mechanical (push-pull) ventilation

so that all compartments were maintained at an essentially identical constant temperature of 21°C throughout the experiment. All inner walls and ceilings of the compartments were covered with white plastic sheets. Each compartment had a rectangular feeder (50 × 15 × 10 cm) outside the front wall and two nipple drinkers (35 cm high) on the back wall of the cage. An access door on the front side of each compartment allowed caretakers to refill the feeder and collect eggs with minimum disturbance to the birds. The false ceiling of the plenum was made of perforated plastic panel (1.27 cm dia. holes and 48% open area). A light bulb under study was situated on the false ceiling panel of the plenum, pointing upward. The coefficient of variation (CV) for the light distribution uniformity within the cage was <8% for all cases based on 16-spot floor-level measurements. The acclimation chamber, measuring 216 × 91 × 150 cm, was used to house two interconnected cages, each measuring 74 × 64 × 46 cm. The purpose of the acclimation chamber was to train the birds to use the passageway and expose them to the lights under study. Detailed specifications of the test tunnel and the acclimation chamber, including their construction, ventilation system (air duct, inlet and exhaust fans), and egg and manure collection systems, were given in a previous article (Ma et al., 2016).

For the modified test tunnel, two pairs of light preference test compartments (LPTC) were formed by grouping the two

Table 1. Characteristics of incandescent light, warm-white fluorescent light, Dim-to-Blue PS-LED, and Dim-to-Red PS-LED used in this study.

Light Type	Power at Full Intensity (W)	Light Output Equivalence to Incandescent (W)	CCT ^[a] (K)	Flicker Frequency (Hz)	Spectral Distribution
Incandescent ^[b]	40	40	2550	None	Continuous spectrum, with increasing contributions at longer wavelengths.
Warm-white fluorescent ^[c]	8 or 9	40	2700	120	Discrete spectrum, main spectral spikes at 545 and 610 nm.
Dim-to-Blue PS-LED ^[d]	12	100	4550	120	Continuous spectrum, spectral spikes at 450 and 630 nm with a predominant spectral output at 430-460 nm.
Dim-to-Red PS-LED ^[d]	8	40	2000	120	Continuous spectrum, spectral spikes at 450 and 630 nm with a predominant spectral output at 610-640 nm.

[a] CCT = correlated color temperature.

[b] Measures to ban incandescent lamps have been implemented in the European Union, the U.S., and many other countries.

[c] Fluorescent light refers to both compact fluorescent light (CFL, 9 W) and cold-cathode fluorescent light (CCFL, 8 W); CFL and CCFL have essentially identical spectral characteristics.

[d] PS-LED = poultry-specific LED light.

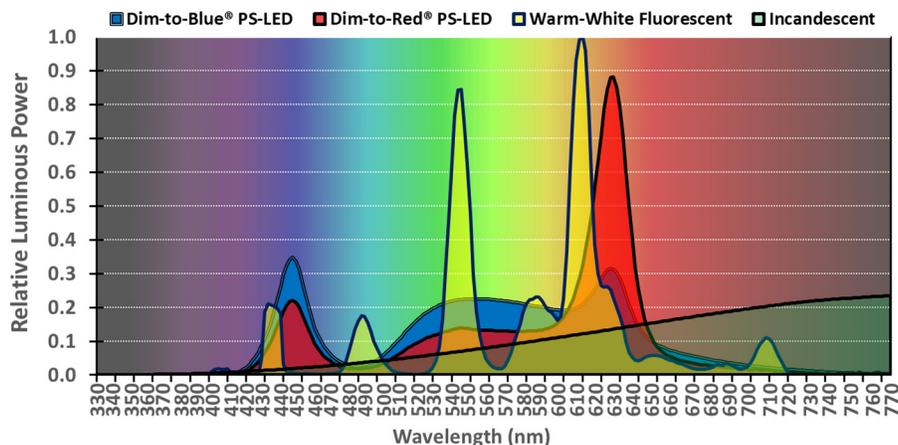


Figure 1. Spectral characteristics of the incandescent light, warm-white fluorescent light, Dim-to-Blue PS-LED, and Dim-to-Red PS-LED used in this study (PS-LED = poultry-specific LED light). Fluorescent light refers to both compact fluorescent light (CFL) and cold-cathode fluorescent light (CCFL); CFL and CCFL have essentially identical spectral characteristics.

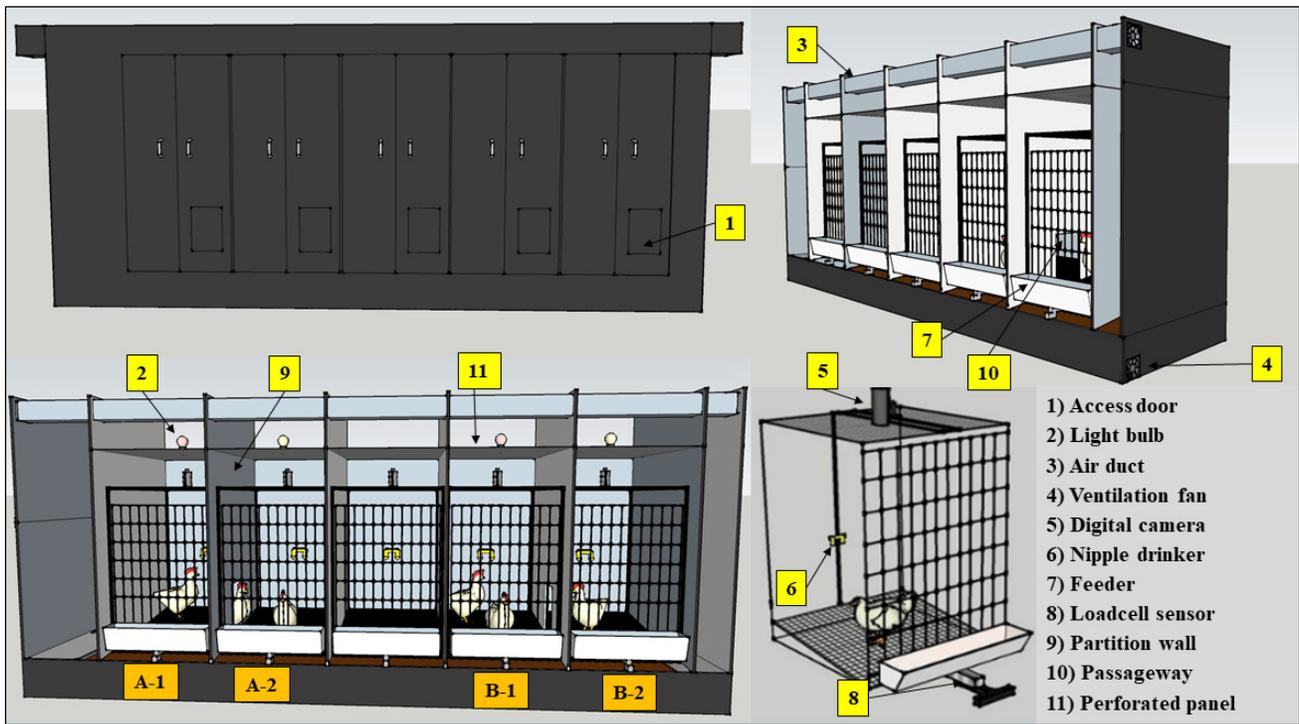


Figure 2. Schematic representation of the light preference test system.

adjacent compartments from both ends of the tunnel, with the middle compartment used as a separation space between the two pairs. A rectangular passageway, measuring 20×25 cm (W×H), was located in the lower portion of the partition wall (floor to 20 cm high) for each pair of LPTC, allowing birds to move freely between the two interconnected cages (one bird at a time). As such, two groups of birds could be tested simultaneously in the test tunnel. Feed and water were available *ad libitum* in all cages. The same amount of feed was added to each feeder before assigning the birds, and the feeders were refilled daily during the dark period. Eggs were also collected daily during the dark period. At the end of each trial, euthanasia procedures were performed on the test birds according to the IACUC protocol, and manure inside the compartments was removed. The test and acclimation systems were disinfected before the next trial.

LIGHTING REGIMENS

The preference or choice of light was tested between the Dim-to-Red PS-LED and the warm-white FL (fig. 1). Light intensity was determined using a spectrometer (GL SPECTIS 1.0 Touch, JUST-Normlicht, Inc., Langhorne, Pa.) coupled with software (SpectraShift 2.0, Once, Inc.) for measuring poultry-perceived light intensity in p-lux (Saunders et al., 2008; Liu et al., 2017). Arrangement of the lights was made according to the experimental design, as described below. In the acclimation chamber, light intensity varied from 18 to 30 p-lux, depending on the distance from the floor to the lights. In the LPTC, light intensities were adjusted to similar levels (i.e., 25 p-lux on the floor and 20 p-lux at the feeder) and maintained constant throughout the testing period. Constant photoperiods for pullets and layers were used, i.e., 10 h light and 14 h dark (10L:14D) for pullets at 14 to 16 WOA and 16L:8D for layers at 44 to 50 WOA.

EXPERIMENTAL PROCEDURES

A total of 36 groups of birds (12 groups for each bird category) were tested in 18 trials to evaluate light preference or choice by the birds. For each trial, six birds in two groups of the same category were tested simultaneously. The six test birds first underwent a 7-day acclimation period in the acclimation chamber ($1578 \text{ cm}^2 \text{ bird}^{-1}$ space allowance), during which they became used to passing through the passageway between the interconnected cages. The acclimation chamber was alternately lit by the PS-LED and the FL from one day to the next, thus allowing the birds to experience both test lights before being assigned to LPTC. After the acclimation period, these two groups of birds were randomly assigned to the two pairs of LPTC ($2400 \text{ cm}^2 \text{ bird}^{-1}$) for a 6-day test period. During the test period, the PS-LED and the FL were randomly assigned to the compartments and alternated daily (during the dark period) to avoid potential compartment effect (e.g., location preference). The first two days in LPTC were used as an acclimation period for the birds, and these data were excluded from the analysis. Thus, the results were analyzed based on data collected during the last four days.

DATA COLLECTION

A real-time sensor-based monitoring system was built by incorporating four load-cell scales (RL1040-N5, Rice Lake Weighing Systems, Rice Lake, Wisc.), four thermocouples (type-T, Omega Engineering, Inc., Stamford, Conn.), and a relative humidity (RH) sensor (HMT100, Vaisala, Inc., Woburn, Mass.) with a LabView-based data acquisition system (ver. 7.1, National Instruments, Austin, Tex.). The system consisted of a compact FieldPoint controller (NI cFP-2020, National Instruments) and multiple thermocouple input modules (NI cFP-TC-120, National Instruments). The data

were collected at 1 s intervals. Air temperature in each compartment, RH in the air duct near the exhaust fan (10 cm in front), and the weight of each feeder were monitored continuously. Air temperature was used for adjusting the ventilation rate to maintain a consistent temperature in the compartments. Feeder weight was used for determining daily feed use in each compartment by calculating the feeder weight difference between the beginning and end of the day.

A real-time vision system was built and used by incorporating four infrared video cameras (GS831SM/B, Gadspot, Inc., Tainan City, Taiwan) and a PC-based video capture card (GV-600B-16-X, GeoVision, Inc., Taipei, Taiwan) with surveillance system software (ver. 8.5, GeoVision, Inc.). One camera was installed on top of each cage and recorded top-view images. This vision system could record images from all four cameras simultaneously at 1 frame per second (FPS). Distribution of the birds in the LPTCs was analyzed using an automated image processing program in MATLAB (R2014b, MathWorks, Inc., Torrance, Cal.) and VBA programs in Excel (Microsoft Office 2016, Redmond, Wash.).

DETERMINATION OF TIME-SERIES DISTRIBUTION OF THE BIRDS

Images were recorded at 1 FPS. Thus, each image represented a momentary state of the birds in the LPTCs. The algorithm for determining the distribution of birds in the LPTCs consisted of four procedures: (1) extracting the pixels representing birds in each image (figs. 3a through 3e), (2) counting the number of bird blobs detected in each image (fig. 3e), (3) determining the area of each blob (fig. 3f), and (4) determining the number of birds in each cage (table 2 and fig. 4). The two simultaneous images from each pair of LPTCs were analyzed separately for each cage. As such, if a bird was passing through or staying in the passageway, one bird would be detected as two blobs, one per image, as shown in scenarios 8, 9, and 10 in figure 4. A blob could also be a single bird, as shown in scenarios 5 and 6, or multiple contacting birds, as shown in scenarios 1, 2, and 4. In this study, contacting birds were not individually segmented during the image processing. Instead of implementing a computationally intensive segmentation procedure, a simple enumeration method was applied. Specifically, with only three

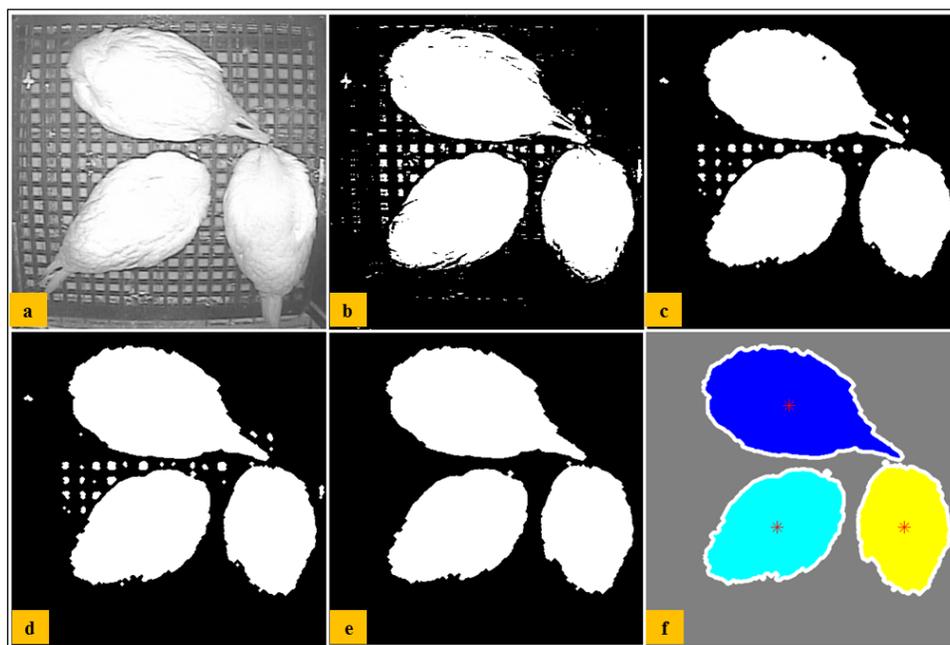


Figure 3. Image processing procedure: (a) RGB image of birds, (b) binary image of birds without enhancement, (c) binary image of birds with morphological opening operation, (d) binary image of birds with morphological closing operation, (e) binary image of birds with small objects removed, and (f) detected blobs in the binary image.

Table 2. Criteria for scenario classification of bird distributions in the light preference test compartments.

Scenario	Criteria for Scenario Classification ^[a]
1	All three birds were in one cage and in contact with each other.
2	All three birds were in one cage, with one bird apart from the other two that were in contact with each other.
3	One bird was in one cage, and the other two contacting birds were in the other cage.
4	One bird was passing through or staying at the passageway, with at least one contact among the birds.
5	All three birds were in one cage and apart from one another.
6	One bird was in one cage, and the other two birds were in the other cage without body contact.
7	One bird was passing through or staying in the passageway and in contact with one bird while the third bird was by herself.
8	One bird was passing through or staying in the passageway while the other two were away and in contact with each other.
9	One bird was passing through or staying in the passageway while the other two were away in one cage without body contact.
10	One bird was passing through or staying in the passageway while the other two were in separate cages with no contact among the birds.

^[a] Distribution of the birds in the light preference test compartments was classified by scenario based on the total number of detected blobs, the number of blobs detected in each cage, and the number of birds with body contacts to each other.

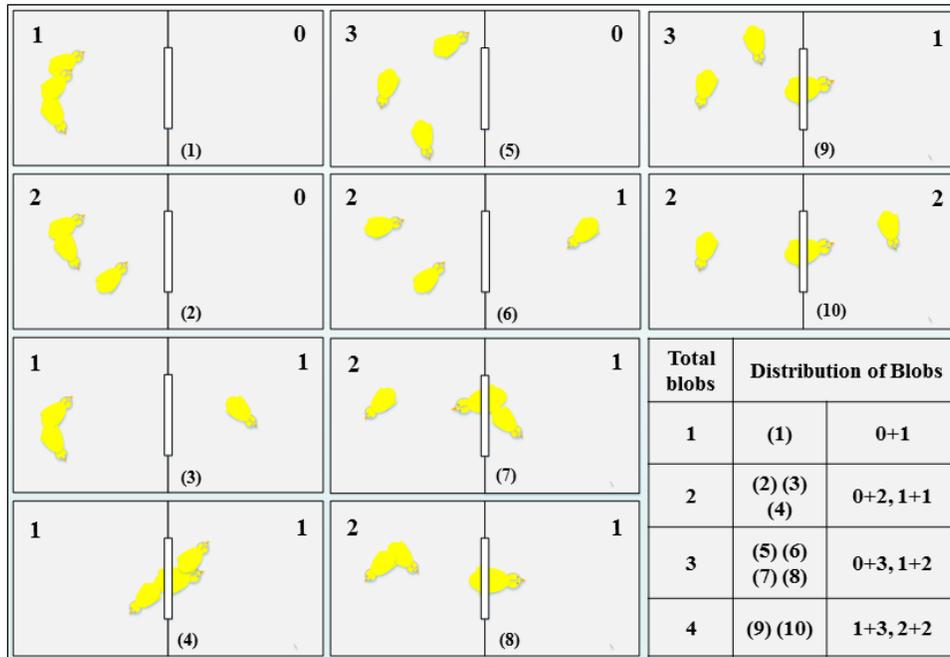


Figure 4. Representative distributions of birds in the light preference test compartments. Numbers in parentheses are scenarios. For each scenario, three birds were present in two adjoining compartments. The small rectangle represents the passageway between the compartments. The number in the corner of each compartment is the number of blobs detected in that compartment.

birds in the LPTC, there was a maximum of four total detected blobs and ten possible scenarios for distribution of the birds (fig. 4). The possibilities are one blob for scenario 1, two blobs for scenarios 2 through 4, three blobs for scenarios 5 through 8, and four blobs for scenarios 9 and 10. The criteria for scenario classification for the distribution of birds are described in table 2.

With the knowledge of the number of blobs in each cage and the area of each blob, the number of birds in each cage was determined using an automated VBA program in Excel. Specifically, the VBA program first checked the number of detected blobs in each cage. When there was an empty cage (no detected blob), all three birds had to be in the other cage (i.e., scenarios 1, 2, or 5). A threshold for blob area (6000 pixels for a pullet and 8000 pixels for a layer) was then applied to the blobs because a blob consisting of a single bird had approximately 12,000 pixels for a pullet and approximately 16,000 pixels for a layer. If both cages had only one blob and each blob area was larger than the threshold, the cage with the larger blob was considered to have two birds (i.e., scenario 3 or in certain cases scenario 4). If one cage had two blobs and the other cage had only one blob, and all the blobs were larger than the threshold, the cage with two blobs was considered to have two birds (i.e., scenario 6 or in certain cases scenario 7). If four total blobs were detected in two cages or if any blob was smaller than the threshold (6000 or 8000 pixels), a bird was passing through or staying in the passageway (i.e., scenarios 8, 9, and 10, or in certain cases scenarios 4 and 7). For scenarios that had a bird passing through or staying in the passageway, the blob smaller than the threshold could be excluded. Thus, these scenarios were analyzed similarly to others (i.e., scenario 4 similar to 1 or 3, scenario 7 similar to 3 or 6, scenario 8 similar to 2 or 3, scenario 9 similar to 5 or 6, and scenario 10 similar to 6). Con-

sequently, for every recorded frame, the number of birds in the corresponding cage could be determined. The algorithm applied in the analysis was validated by human observation of the time-series images, with an accuracy of 98% or better. The false determinations of bird number were mainly attributed to the infrequent wing-flapping of the birds or sudden frame loss from the cameras.

CALCULATION OF BEHAVIOR VARIABLES

With the knowledge of the time-series distributions of the birds in the LPTC, time budgets and moving frequency of the birds were calculated and summarized using a separate VBA program in Excel. The proportion of daily feed intake of birds under the PS-LED or the FL (PDFI, %) was also calculated. All the behavior variables analyzed in this study are described in table 3. The amount of time spent under the PS-LED or the FL was calculated by dividing the time the birds spent under the PS-LED or the FL by the length of the photoperiod on a per-bird basis (min bird^{-1}). The amount of time with no bird, one bird, two birds, or three birds under the PS-LED or the FL was calculated by dividing the respective durations by the length of the photoperiod. In this study, birds were not individually identified with the vision and

Table 3. Behavior variables of birds measured during preference test.

Variable	Description
LMF	Light-period moving frequency of birds between lights ($\text{times bird}^{-1} \text{ h}^{-1}$)
PLTS	Proportion of light period spent under PS-LED or FL (%)
L3F0	Proportion of light period with all three birds under PS-LED (%)
L2F1	Proportion of light period with two birds under PS-LED and one bird under FL (%)
L1F2	Proportion of light period with one bird under PS-LED and two birds under FL (%)
L0F3	Proportion of light period with all three birds under FL (%)
PDFI	Proportion of daily feed intake under PS-LED or FL (%)

sensor systems; thus, all behavior variables are presented as group averages.

STATISTICAL ANALYSIS

Statistical analyses were performed using SAS Studio 3.5 (SAS Institute, Inc., Cary, N.C.). The behavior variables shown in table 3 were analyzed to determine light preference and to compare differences among the three categories of birds (P_{INC} , L_{LED} , and L_{FL}). The behavior variables (i.e., LMF, PDFI, PLTS, L3F0, L2F1, L1F2, and L0F3) were analyzed with generalized linear mixed models by implementing PROC GLIMMIX procedures. A Gaussian distribution was specified for the analysis of LMF, whereas a beta distribution was specified for analysis of PDFI, PLTS, L3F0, L2F1, L1F2, and L0F3. All statistical models were of the following form:

$$Y_{ijkd} = \mu + B_i + P_j + (BP)_{ij} + G(BP)_{ijk} + D(BPG)_{ijkd} + e_{ijkd} \quad (1)$$

where Y_{ijkd} denotes the independent observation on day d for group k in LPTC $_j$ of bird category i , μ is the overall mean, B_i is the bird category effect (fixed), P_j is the LPTC effect (fixed), $(BP)_{ij}$ is the interaction effect (fixed) of bird category and LPTC, $G(BP)_{ijk}$ is the group effect (random) tested within each LPTC for each bird category, $D(BPG)_{ijkd}$ is the day effect (random) for each group, adjusted with first-order autoregressive or AR (1) covariance structure, and e_{ijkd} is the random error with a normal distribution with mean μ and variance $\sigma^2 [N \sim (\mu, \sigma^2)]$.

Evaluation of the light preference was accomplished by testing the null hypothesis that the proportion of the light period (PLTS) or the proportion of daily feed intake (PDFI) under each light equals 0.5. As the beta distribution used a logit link function, the evaluation was actually testing if the intercept equals zero [$\text{logit}(0.5) = 0$]. In addition, Tukey-Kramer tests were used for pairwise comparisons among bird categories for all the behavior variables. Differences were considered significant at $p < 0.05$. Normality and homogeneity of variance of the data were examined by residual diagnostics. Unless otherwise specified, data are presented as least squares means along with the standard error of the mean (SEM).

RESULTS AND DISCUSSIONS

TIME SPENT UNDER DIFFERENT LIGHTS

As shown in figure 5, all three categories of birds showed a stronger choice for the FL than for the PS-LED in terms of light-period time spent ($p = 0.011$, 0.030 , and 0.001 for P_{INC} , L_{LED} , and L_{FL} , respectively), and the tendency of this choice was not affected by prior lighting experience ($p = 0.422$). Specifically, PLTS under the FL was $58.0\% \pm 2.9\%$, $53.7\% \pm 1.6\%$, and $54.2\% \pm 1.2\%$ for P_{INC} , L_{LED} , and L_{FL} , respectively. Correspondingly, PLTS under the PS-LED was $42.0\% \pm 2.9\%$, $46.3\% \pm 1.6\%$, and $45.8\% \pm 1.2\%$ for P_{INC} , L_{LED} , and L_{FL} , respectively. The results of the current study were similar to the findings of an earlier study that reported laying hens' preference for CFL over incandescent light at a

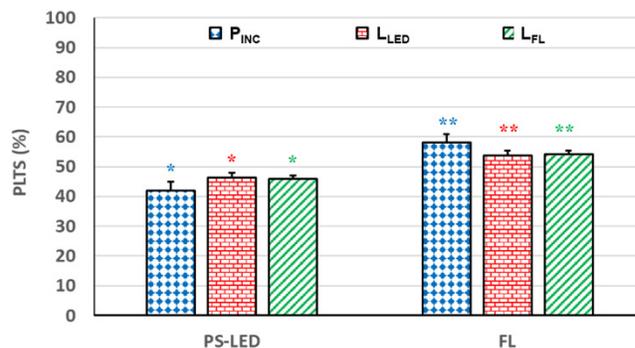


Figure 5. Proportion of light-period time spent (PLTS) under poultry-specific LED (PS-LED) and fluorescent light (FL): P_{INC} = pullets reared under incandescent light, L_{LED} = layers under PS-LED throughout the pullet and laying phases, and L_{FL} = layers under FL throughout the pullet and laying phases. Bars with a single asterisk (*) are significantly lower than 50% at $p < 0.05$; bars with double asterisks (**) are significantly higher than 50% at $p < 0.05$. For PS-LED or FL, no distinct differences were detected among the three bird categories at $p < 0.05$.

light intensity of 12 lux by spending on average 73.2% of the time under CFL and only 26.8% of the time under incandescent light (Widowski et al., 1992); however, there was no explanation as to why the birds preferred CFL over the other light. Laying hens were reported to show no preference for HPS or incandescent light (Vandenbert and Widowski, 2000). Broilers were reported to show no behavioral sign of preference between white and yellow LED lights at a light intensity of 5 lux (Mendes et al., 2013). However, turkeys were found to prefer fluorescent light with supplementary UV radiation compared to fluorescent light without UV radiation at a light intensity of 15 lux (Moinard and Sherwin, 1999). Research has demonstrated that poultry have a fourth retinal cone photoreceptor that allows them to see in the UVA range (315-400 nm) (Prescott and Wathes, 1999; Cuthill et al., 2000). As a result, they may use UVA perception to modify various behavioral functions such as feeding, peer recognition, mate selection, and social encounters (Lewis and Gous, 2009). With UVA radiation forming 3% to 4% of fluorescent light, but almost none in incandescent light and most of the newly emerging LED lights (Lewis and Gous, 2009), attraction of the birds to the FL, as observed in the current study, may be a reflection of the UVA light effect. Further investigation of bird preference for UVA light seems warranted.

LIGHT-PERIOD DISTRIBUTIONS OF BIRDS

Light-period distributions of the birds between the two light types provide a more detailed illustration of their choices (fig. 6). In general, birds in all three categories spent significantly more time splitting into the two cages than staying together in one cage, with a tendency for choosing the FL when more birds stayed together. Specifically, L1F2 ($40.7\% \pm 2.4\%$) and L2F1 ($33.6\% \pm 2.5\%$) for P_{INC} were significantly higher than L0F3 ($18.9\% \pm 2.6\%$, $p = 0.001$ and 0.021 , respectively) or L3F0 ($6.8\% \pm 0.8\%$, $p < 0.001$ and $P < 0.001$, respectively). L1F2 ($31.6\% \pm 1.4\%$) for L_{LED} was significantly higher than L0F3 ($22.6\% \pm 1.7\%$, $p = 0.031$) or L3F0 ($15.3\% \pm 1.5\%$, $p < 0.001$), and L2F1 ($30.5\% \pm 1.6\%$) for L_{LED} was also significantly higher than L3F0 ($p < 0.001$).

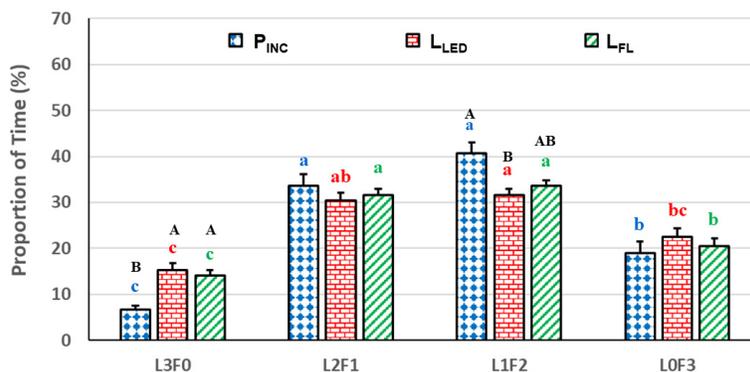


Figure 6. Light-period bird distributions under poultry-specific LED light (PS-LED) and fluorescent light (FL): P_{INC} = pullets reared under incandescent light, L_{LED} = layers under PS-LED throughout the pullet and laying phases, L_{FL} = layers under FL throughout the pullet and laying phases, and L_xF_y = proportion of the light period with *x* birds under PS-LED and *y* birds under FL. Within a distribution pattern (L_xF_y), bars with different uppercase letters differ significantly at $p < 0.05$. For each of the three bird categories (P_{INC}, L_{LED}, or L_{FL}), bars with different lowercase letters differ significantly at $p < 0.05$.

Likewise, L1F2 (33.6% ± 1.2%) and L2F1 (31.6% ± 1.4%) for L_{FL} were significantly higher than L0F3 (20.6% ± 1.7%, $p = 0.005$ and $p < 0.001$, respectively) or L3F0 (14.2% ± 1.2%, $p < 0.001$ and $p < 0.001$, respectively). These distribution patterns differed from those found in a previous study in which laying hens spent about 60% of the time during the light period with three or four hens in the same cage when four hens were housed in five interconnected cages (Ma et al., 2016).

As mentioned earlier, laying hens were reported to spend on average 73.2% of the time under CFL and only 26.8% of the time under incandescent light (Widowski et al., 1992). By comparison, the degree of preference was not as strong in the current study, as reflected by the time spent by the birds (55% vs. 45%). The lower degree of preference in the current study might have arisen from a dominant-subordinate relationship among the birds, which tends to exist in small groups. The establishment of dominance hierarchies in pullets and laying hens housed in small groups usually starts as early as the first encounter and remains relatively consistent during subsequent production stages. Where dominance hierarchies exist, the subordinate birds usually benefit from avoiding encounters with the dominant birds (Pagel and Dawkins, 1997; D'Eath and Keeling, 2003). In the current study, the floor space, feeder space, and nipple drinkers provided in each cage were considered sufficient for all birds, which might have weakened the significance of hierarchy. However, aggressive pecking was observed among the test pullets and layers during the early rearing period, and the behavior seemed to continue after assignment to the test environments.

LIGHT-PERIOD MOVING FREQUENCY

Birds were observed to move frequently between the interconnected cages for feeding, drinking, resting, foraging, and nest-seeking during the light period. LMF of P_{INC}, L_{LED}, and L_{FL} averaged 19.8 ± 1.0, 31.9 ± 2.4, and 29.9 ± 1.9 times bird⁻¹ h⁻¹, respectively (fig. 7). L_{LED} and L_{FL} had significantly higher LMF than P_{INC} ($p < 0.001$), while LMF was highly comparable for L_{LED} and L_{FL} ($p = 0.804$). The higher LMF for layers than for pullets probably stemmed from the intensive nest-seeking behavior of the hens because nest boxes

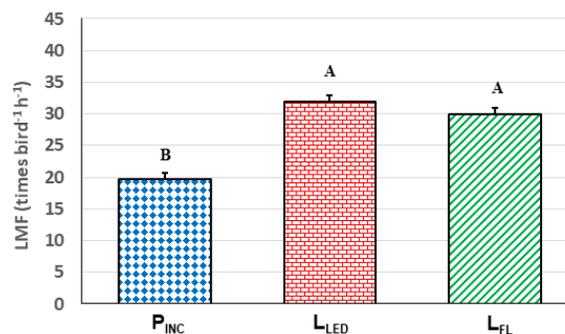


Figure 7. Light-period moving frequency (LMF) between poultry-specific LED (PS-LED) and fluorescent light (FL): P_{INC} = pullets reared under incandescent light, L_{LED} = layers under PS-LED throughout the pullet and laying phases, and L_{FL} = layers under FL throughout the pullet and laying phases. Bars with different letters differ significantly at $p < 0.05$.

were not provided in the current study. Hens were highly motivated to gain access to nest boxes prior to oviposition and displayed frustration when nests were not available (Cooper and Appleby, 1996). They tended to aggressively compete to lay eggs in a curtained nest area when housed in small cages (Hunniford et al., 2014). This was not a behavioral characteristic for the 14 to 16 WOA pullets. In an earlier study, a significant negative correlation was found between the birds' degree of preference for a particular light and their movement between lights (Widowski et al., 1992); namely, birds having a stronger preference for a particular light moved less frequently between lights. However, this relationship was not apparent in the current study, as the birds in all three categories showed similar degrees of preference for the FL light during the light period.

DAILY FEED INTAKE

Birds in all three categories showed no light preference for feeding, as reflected by PDFI ($p = 0.419$, 0.566, and 0.749 for P_{INC}, L_{LED}, and L_{FL}, respectively, fig. 8). Specifically, 51.8% ± 2.3%, 51.2% ± 2.0%, and 49.6% ± 1.4% of daily feed intake occurred under the PS-LED for P_{INC}, L_{LED}, and L_{FL}, respectively. Correspondingly, 48.2% ± 2.3%, 48.8% ± 2.0%, and 50.4% ± 1.4% of daily feed intake

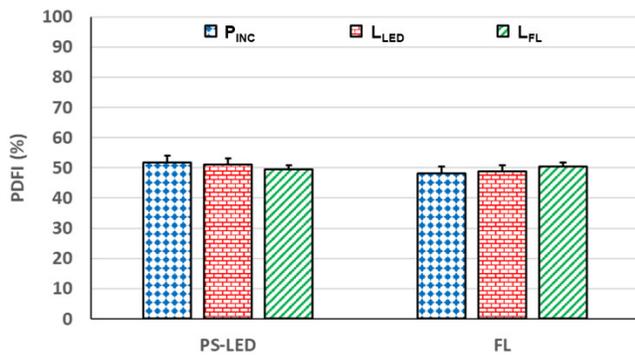


Figure 8. Proportions of daily feed intake (PDFI) under poultry-specific LED light (PS-LED) and fluorescent light (FL): P_{INC} = pullets reared under incandescent light, L_{LED} = layers under PS-LED throughout the pullet and laying phases, and L_{FL} = layers under FL throughout the pullet and laying phases. For all bird categories, PDFI was not significantly different from 50%. Within PS-LED or FL, no distinct differences were detected among the three bird categories.

occurred under the FL for P_{INC}, L_{LED}, and L_{FL}, respectively. This result of no light preference for feeding did not parallel the findings of some earlier studies. Shaver hens were found to perform more ingestion behaviors (feeding, drinking, and ground pecking) under fluorescent light than under incandescent light (Widowski et al., 1992). Broilers were found to eat substantially more feed in chambers equipped with white LED light than with yellow LED light (Mendes et al., 2013). However, the preference for light type was confounded by light intensity in these earlier studies, as the bird-perceived light intensities were not equal when the light levels applied to the cages or chambers were adjusted using light meters based on human vision (Prescott and Wathes, 1999; Prescott et al., 2003; Saunders et al., 2008). Indeed, feed intake by birds seemed to be more associated with light intensity than with light type or spectrum. Broilers reared in high light intensity (2.5 to 35 lux) were found to have significantly higher feed consumption than broilers reared in low light intensity (2.5 lux) (Purswell and Olanrewaju, 2017). ISA Brown hens were observed to eat for the longest time under the brightest light intensity (200 lux) and for the shortest time under the dimmest light intensity (<1 lux) when given free choice of light intensities of <1, 6, 20, or 200 lux (Prescott and Wathes, 2002). In contrast, Hy-Line W-36 commercial layers were found to have the highest feed intake at 5 lux (32.5%) and the lowest feed intake at 100 lux (6.7%) when given free choice of light intensities of <1, 5, 15, 30, or 100 lux (Ma et al., 2016).

CONCLUSIONS

In this study, light preference of Hy-Line W-36 pullets and laying hens between a Dim-to-Red poultry-specific LED light (PS-LED) and a warm-white fluorescent light (FL) was assessed in free-choice light preference test compartments. Three categories of birds, each with different prior lighting experience, were tested: pullets reared under incandescent light (P_{INC}), layers under PS-LED throughout the pullet and laying phases (L_{LED}), and layers under FL throughout the pullet and laying phases (L_{FL}). Each category consisted of

12 groups (replicates), with three birds per group. The following observations and conclusions were made:

- The pullets and layers showed a moderate degree of preference for the FL versus the PS-LED during the light period (53% to 58% vs. 47% to 42%), although the proportions of time spent under the respective light types were statistically different.
- The pullets and layers had comparable proportions of daily feed intake for the FL and PS-LED conditions.
- Prior lighting experience of the pullets and layers did not influence their choice for the LF or the PS-LED nor the proportions of daily feed intake under each light during subsequent exposure to the lights.

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NOMENCLATURE

- CCFL = cold-cathode fluorescent light
- CCT = correlated color temperature
- CFL = compact fluorescent light
- CV = coefficient of variation
- FL = fluorescent light
- FPS = frames per second
- HPS = high-pressure sodium
- L0F3 = proportion of the light period with all three birds under the FL (%)
- L1F2 = proportion of the light period with one bird under the PS-LED and two birds under the FL (%)
- L2F1 = proportion of the light period with two birds under the PS-LED and one bird under the FL (%)
- L3F0 = proportion of the light period with all three birds under the PS-LED (%)
- LED = light-emitting diode
- L_{FL} = layers under FL throughout the pullet and laying phases
- L_{LED} = layers under PS-LED throughout the pullet and laying phases
- LMF = light-period moving frequency of birds between lights (times bird⁻¹ h⁻¹)
- LPTC = light preference test compartments
- PDFI = proportion of daily feed intake under the PS-LED or the FL (%)
- P_{INC} = pullets reared under incandescent light
- PLTS = proportion of light-period time spent under the PS-LED or the FL (%)
- p-lux = poultry-perceived light intensity (lux)
- PS-LED = poultry-specific LED light

RH = relative humidity (%)
SEM = standard error of the mean
UV = ultraviolet
WOA = weeks of age

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