

ANIMAL WELL-BEING AND BEHAVIOR

Preference testing for UV light spectrum and intensity in laying hens

Md Sohel Rana,^{*,†,‡} Andrew M. Cohen-Barnhouse,[†] Caroline Lee,[†] and Dana L. M. Campbell^{†,1}

^{*}*School of Environmental and Rural Science, University of New England, Armidale, NSW 2351, Australia;*

[†]*Agriculture and Food, Commonwealth Scientific and Industrial Research Organisation (CSIRO), Armidale, New South Wales 2350, Australia; and* [‡]*Department of Livestock Services, Ministry of Fisheries and Livestock, Dhaka 1215, Bangladesh*

ABSTRACT Sunlight intensity and UV radiation may affect free-range hens' use of the outside range, particularly when sunlight is intense with a high UV index. However, it is uncertain what aspect of sunlight (brightness or UV) may be most aversive to hens to discourage them from leaving standard indoor lighting conditions to venture outdoors. A controlled indoor-based choice study was conducted to determine whether hens showed preferences for different light wavelengths and intensities that may affect outdoor range usage. Cage-reared ISA Brown laying hens ($n = 84$) at 44 wk of age in 3 groups (28 hens/group) were tested for preferences of indoor standard light emitting diode (**LED**) white light (control) vs. one of three different treatment lights: 1) visible spectrum plus infrared wavelengths (VIS); 2) visible spectrum plus UVA wavelengths (UVA); and (iii) visible spectrum plus UVA and UVB wavelengths (UVA/B) presented successively at low, medium, or high levels of

intensity. Hens within each group were individually tested for 2 h in an apparatus with 2 compartments (control vs. treatment) connected by a tunnel on both sides. Videos of hens' time spent in each compartment and behaviors were decoded and analyzed using GLMM. Hens spent more time under the low intensity of the UVA/B light treatment (62%), the low intensity of VIS light (61%), medium intensities of both UVA/B light (60%), and UVA light (59%), and the high intensity of the VIS light (58%) when compared with control light (all $P \leq 0.05$). Hens spent less time feeding under all intensities of UVA light (all $P \leq 0.03$) and showed more foraging, ground pecking, and preening at lower levels of UVA/B light ($P < 0.05$). The study suggests that UVA/B light (sunlight) may have positive effects for hen range use, but during peak sun intensities, hens may need additional measures (e.g., shelter) to protect themselves. Confirmation of these findings in a free-range setting is needed.

Key words: behavior, free-range, laying hen, light preference, sunlight, UV light

2021 Poultry Science 100:101063
<https://doi.org/10.1016/j.psj.2021.101063>

INTRODUCTION

Light is a critical factor in the laying hen industry as it has significant impact on a hen's physiology, behavior, production, and welfare (Manser, 1996; Mohammed et al., 2010; Jacome et al., 2014). The physical properties of light include photoperiod, intensity (brightness) and wavelength (color), and all have potential effects in layer production systems (Lewis and Morris, 2006). There has been much previous research demonstrating that the photoperiod of light is essential for sexual maturity

and egg production (Lewis et al., 1997; Min et al., 2012), intensity can affect the development of feather pecking behaviors (Kjaer and Vestergaard, 1999; Janczak and Riber, 2015; Shi et al., 2019), and wavelength is processed by different photoreceptors in the eye that can then stimulate performance and physical activities (Huber-Eicher et al., 2013; Baxter et al., 2014). Typically, assessment of sources for artificial illumination in intensive commercial layer farms have been based on perception via human vision (Maddocks et al., 2001; Prescott et al., 2003), but a chicken's visual perception is different (Goldsmith, 1990; Bowmaker et al., 1997). Owing to the presence of a fourth (extra) retinal cone (Govardovskii and Zueva, 1977; Osorio et al., 1999), avian species are able to see part of the UV light spectrum (315–400 nm), namely the UVA wavelengths (Prescott and Wathes, 1999; Lewis and Morris, 2000; Rajchard, 2009). The UVB portion of

© 2021 The Authors. Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Received August 25, 2020.

Accepted February 6, 2021.

¹Corresponding author: dana.campbell@csiro.au

the light spectrum (280–315 nm) is not visually perceived by hens but can penetrate the skin of a chicken's feet, comb, and wattles. These UVB wavelengths play a key beneficial role in the production of vitamin D₃ that promotes intestinal absorption of calcium and phosphorus and improves bone mineralization and bone growth (Edwards Jr, 2003; de Matos, 2008; Schutkowski et al., 2013).

Preference testing of indoor-housed chickens indicates both laying hen chicks and broilers prefer the UVA spectrum in comparison with lights with no UVA component (Kristensen et al., 2007; Liu et al., 2018). UVA and/or UVB light supplementation can increase behavioral activity, reduce fear responses, improve bone composition, and improve egg production (Sobotik et al., 2020; Wei et al., 2020). However, these previous studies have typically used low levels of UV supplementation. In contrast, sunlight is a direct source of high levels of UV radiation. The wavelengths of sunlight contain infrared radiation (700 nm–1 mm) (49.4%), human (and chicken) visible radiation (400–700 nm) (42.3%), and 8.3% UV radiation (100–400 nm) (Gibson, 2000). This UV radiation comprises 3 different types with the majority of the UV radiation that reaches the earth surface being UVA (95%), with a small remainder UVB (5%), and all the UVC (100–280 nm) is screened out by the ozone layer (Holick, 2016). For chickens that are exposed to direct sunlight in a free-range system that provides outdoor access, the high levels of UV radiation may be visually aversive or cause skin damage and sunburn (Lewis and Gous, 2009). Consequently, this may be a contributing factor to low levels of range access that are often seen in free-range chickens (Dawkins et al., 2003; Hegelund et al., 2005). Previous studies have shown that hens typically range less during sunny and hot days compared with when the weather remains calm and dull (Richards et al., 2011; Gilani et al., 2014) and broilers range less with increasing solar radiation (Stadig et al., 2017). Further studies in broiler chickens (Jones et al., 2007; Fanatico et al., 2016) and laying hens (Chielo et al., 2016) found that time of day impacted the birds' ranging as fewer birds went outside during midday/early afternoon as compared with morning and late afternoon. This corresponds with changing patterns of sunlight intensity throughout the day where the sun reaches its peak around midday. However, when different types of shelters are used in the ranging area, whether artificial (Nagle and Glatz, 2012) or natural (Dal Bosco et al., 2014; Larsen et al., 2017), birds use more of the outdoor range, and this could be related to their protection from direct sunlight (Rault et al., 2013; Stadig et al., 2017 [meat chickens]). The effects of sunlight on range usage is particularly pertinent to Australia as free-range systems are increasing in prevalence (Australian Eggs: Annual report, 2019) but the sunlight is also strong and potentially damaging (McKenzie et al., 2003; Lewis and Gous, 2009). Across the summer period, the UV index is extremely high and is typically greater than 8 where the recommended exposure level for humans is UV index ≤ 3 (Lemus-

Deschamps and Makin, 2012). Lower range use on commercial Australian farms has been observed on days with bright sunshine (de Koning et al., 2019).

In addition to the effects of UV radiation, the intensity (brightness) of sunlight in the visual spectrum (human and chicken, 400–700 nm) may also impact hens' ranging behavior. Indoor poultry lighting is typically kept at a low lux level, approximately 10–20 lux which is in stark contrast to the sunlight intensity 10,000–40,000 lux/130,000 lux experienced on a cloudy/sunny day (sun at zenith), respectively, when outside (Norton and Siegwart, 2013), although some commercial producers in Australia report keeping free-range hens at a higher lux inside (~ 100 lux, DLMC. Pers comm. 2019). Some previous indoor-based preference testing compared choices and behaviors of chickens under different lux illuminations. Light intensity preference testing by Davis et al. (1999) in laying hens with different light intensities (6, 20, 60, and 200 lux) revealed that birds at 2 wk of age preferred to spend more time under bright illumination (200 lux); however, at 6 wk of age they had changed their preference toward the dimmest lighting (6 lux). Mohammed et al. (2010) studied the effects of varying light sources of low (5 lux) or high (50 lux) intensity on laying hen behavior, showing that hens exhibited more pecking and aggression under 50 lux, but other observed behaviors (e.g., walking, sitting, standing, feeding, resting) were not significantly affected. Another study by Vandenberg and Widowski (2000) found that laying hens preferred to spend more time sitting and feeding under dimmer (27 lux) incandescent light but showed more pecking, preening, and nesting behavior under high-pressure sodium light (426 lux). Laying hens with choices between different intensities of fluorescent light spent the most time at 5 lux and the least time at 100 lux relative to <1 lux, 15 lux, or 30 lux (Ma et al., 2016). In contrast, Prescott and Wathes (2002) found that the feeding preference of laying hens was greater in high-intensity light (200 lux) rather than low intensities (<1 , 6, or 20 lux) of incandescent light. Laying hens have also shown increased levels of physical activity and energy expenditure corresponding with rising light intensity between 0.5 and 120 lux (Boshouwers and Nicaise, 1987). However, to the authors' knowledge, there has been no study comparing standard light intensities with those closer to what are found in sunlight in a controlled indoor setting.

Therefore, the objectives of this study were to use controlled indoor testing to determine hen preferences for different light wavelengths and intensities that may affect range usage. We predicted that hens would avoid light of high intensities, particularly the UVB wavelengths.

MATERIALS AND METHODS

The study was conducted in the Rob Cumming Poultry Innovation Centre at the University of New England, Armidale, NSW, Australia. The research protocol

was approved by the Animal Ethics Committee of the University of New England (AEC18-137).

Animals and Husbandry

A total of 108 ISA Brown laying hens of 44 wk of age procured from 1 local commercial cage farm were used for this experimental trial. All the hens were from the same flock with no outdoor exposure to natural daylight except during transport from the farm to the research facility (approximately 2-hour duration). On arrival at the facility, hens were equally distributed among 9 home pens within 1 room, separated by wire paneling with shade cloth. Each of the pens measured 3.2 m L $\times$ 1.75 m W and contained 1 nest box (34 cm L $\times$ 29 cm W $\times$ 24 cm H), 1 round feeder (34 cm L $\times$ 29 cm W $\times$ 24 cm H), 1 round feeder (34 cm L $\times$ 29 cm W $\times$ 24 cm H), 2 nipple drinkers,

and a single two-tiered perch (1.07 m L $\times$ 80 cm W $\times$ 64 cm H), with wood shavings as floor litter. Standard commercial layer mash and water were provided *ad libitum*. Hens were stocked at an approximate density of 3 hens/m². The home pens were illuminated with poultry specific white LED bulbs (IP65 Dimmable LED Bulb, B-E27: 10W, 5K, no flicker, Eco Industrial Supplies, China) with an average light intensity level of 20.3 ± 2.1 lux at bird's eye height level across the pens (Lutron Light Meter, LX-112850; Lutron Electronic Enterprise CO., Ltd., Taipei, Taiwan). The hens were maintained on a 16L: 8D lighting schedule (lights on at 5 am and off at 9 pm) during the study period. The home pens (and testing room – see the following section) were environmentally controlled with mechanical ventilation set to maintain an average temperature of $21^\circ\text{C} \pm 1$ when possible, based on

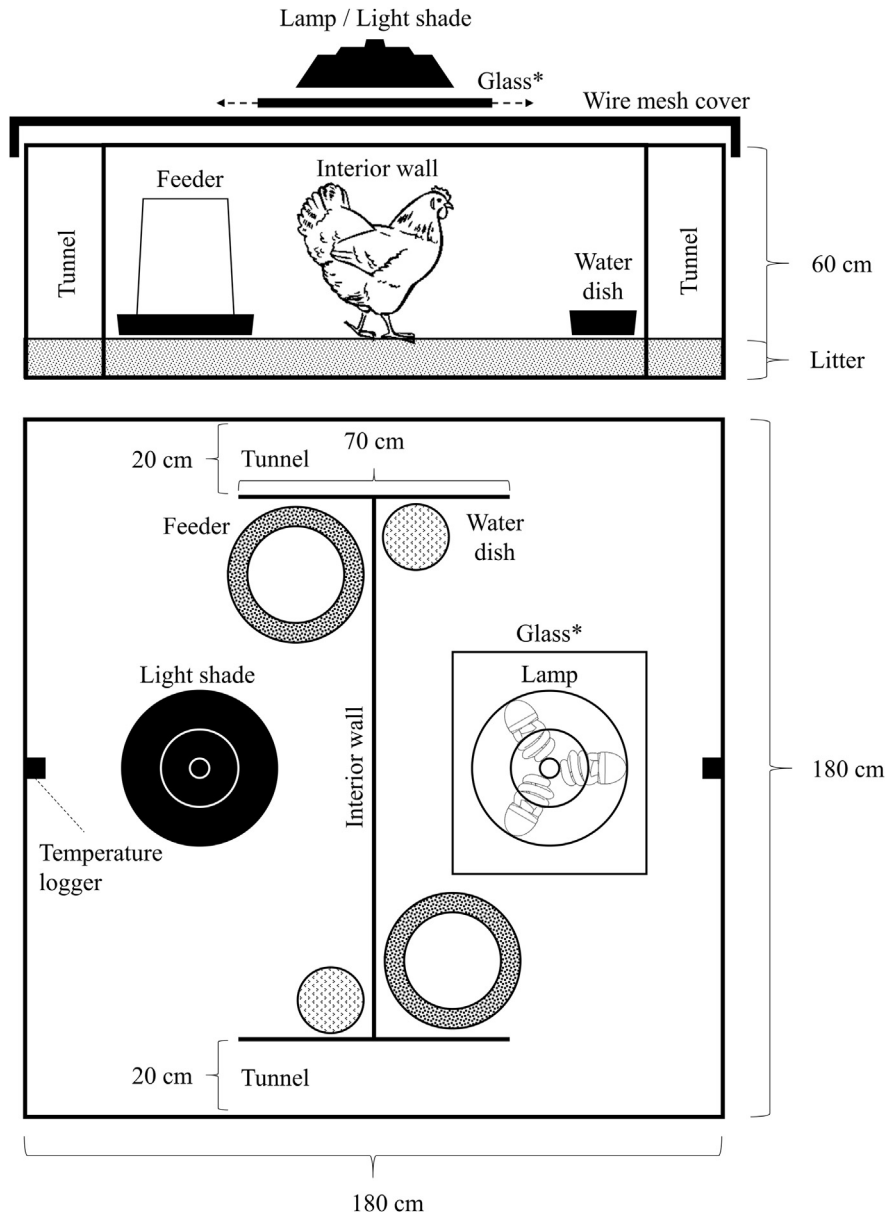


Figure 1. A schematic of the Light Preference Testing Apparatus (LPTA) showing the dimensions of the LPTA and placement of the food, water, lights and temperature loggers. *For the UVA treatments, 3-mm glass was placed between the lamp and the wire mesh cover to filter the UVB wavelengths.

outdoor temperatures. Hens were allowed 12 days to adjust to the new facility before starting acclimation within the testing apparatus.

Light Preference Testing Apparatus

Six Light Preference Testing Apparatus (LPTA) boxes were set up in the adjacent room (testing room) at the facility. The 6 boxes were evenly distributed within the 6.2 m L x 9.6 m W room (which was divided into thirds by wire and shade cloth) so that 2 boxes of the same light treatment were across from each other; test hens could hear but not see each other. Each of the black Formply, square-shaped LPTA was divided in half and comprised 2 identical adjacent compartments (180 cm L x 90 cm W x 60 cm H, [Figure 1](#)) joined by access tunnels (70 cm L x 20 cm W x 60 cm H) on each end. The tops of the compartments were covered with wire mesh to prevent the hens from escaping. Each compartment contained a round feeder (34 cm H x 21 cm D x 120 cm C) with standard commercial mash and a water dish (8 cm H x 12 cm D x 42 cm C) with wood shavings as floor litter (10 cm H). A temperature logger (Tinytag Plus 2, TGP-4500; Gemini Data Loggers Ltd, West Sussex, UK) was set up in each side of the apparatus for 3 LPTA ([Figure 1](#)) all on the same side of the room to record temperature in 10-min intervals (there were only sufficient loggers available to record in 1 box from each treatment).

The testing room was illuminated by standard lighting consistent with the home pens, resulting in an average light intensity level of 20.3 ± 2.1 lux at a bird's eye height across the compartments. One side of each LPTA served as the control condition with illumination provided only by the standard room lighting. The other side was the treatment condition where in addition to standard room lighting, different lights (see following section) under a round metal light shade were suspended just above the covering mesh (60 cm from the floor in the center of the compartment, [Figure 1](#)). For balance, an empty metal light shade was placed on top of the center of the mesh on the control side. The position of the standard lights was such that no shadows were cast within the LPTA. Video cameras (Hikvision network turret cameras DS-2CD2355FWD-1) for each LPTA were installed at 150 cm above the floor and connected to a network video recorder in a separate room (Hikvision DS-7608NI-I2-8P CCTV network video recorder).

Test Lights

The selected lights for preference testing were commercially available Exo Terra (Rolf C. Hagen, Montreal, QC, Canada) pet reptile bulbs. The lights were selected to determine broadly what part of the sunlight spectrum was aversive to the hens in high intensities but within the scope of what artificial light spectrums were commercially available. Three different types were selected as being representative of 1) the

human/chicken visible spectrum (VIS) but including infrared wavelengths (Halogen Basking Spot Lamp PT2181 50W, PT2182 75W, and PT2183 100W), 2) the human/chicken visible spectrum including UVA (Reptile UVB200, 25W, PT2341), and 3) the human/chicken visible spectrum including UVA and UVB (UVA/B) (Reptile UVB200, 25W, PT2341, [Figure 2](#)). The peaks in the visible spectrum across the 3 bulb types were not equivalent. The same bulbs were used for the UVA and UVA/B treatments with 3-mm glass placed under the bulbs in the UVA treatment to filter out the UVB wavelengths. Three light intensity levels (low, medium, and high) of each 3 different light treatments were also tested by using different bulb wattages (treatment 1) or increasing numbers of bulbs of the same wattage (treatments 2 and 3 up to 3 bulbs were used). The treatment lights were suspended directly above the wire mesh covering ([Figure 1](#)) to enable the high intensities to be simulated. There was approximately 10 cm of wood shavings placed on the concrete floor within each LPTA, and thus, standing chicken eye height was approximately 30 cm above the wood shavings or 20 cm from the light source. If the hens stretched their necks, they were only a few cm from the bulb (depending on potential movement of the wood shavings by the hens and how high the hen reached). All wavelengths and radiation intensities were measured over an average of 10 readings using an Ocean Insight Flame-S-XR1 Spectroradiometer (200–1,025 nm, Quark Photonics, Melbourne, VIC, Australia) set with an integration time of 180,000 μ s and integration range from 280 to 1,000 nm ([Figures 3A–3D](#)). The device was placed directly under the light source at a distance of 20 cm from the source (approximate chicken eye height at standing). Readings of the control light were taken at 15 cm owing to the comparatively low intensity of this light. All readings were taken from individual bulbs within an enclosed container to eliminate interference from other ambient light sources. The individual spectrums of the different bulbs (wavelengths) did not change with the different intensity treatments (Watts), only their irradiance. The measurements are indicative of the irradiance under the light source but not within the whole compartment owing to irradiance decay across distance. Lux of the lights was also measured using a digital lux meter (Lutron Light Meter, LX-112850; Lutron Electronic Enterprise CO., Ltd., Taipei, Taiwan), and the UV index was measured for the UVA/B treatment using a reptile UV index meter placed at 20 cm from the source (Solar-meter Model 6.5 R UVI Reptile, Solarmeter Australia, Noosaville Dc, QLD) ([Table 1](#)).

Bird Acclimation and Selection for Preference Testing

For acclimation to the LPTA, hens were placed in the apparatus on 4 separate occasions across different weeks, first as a group and then as individuals. Before commencing habituation, all hens were leg-banded

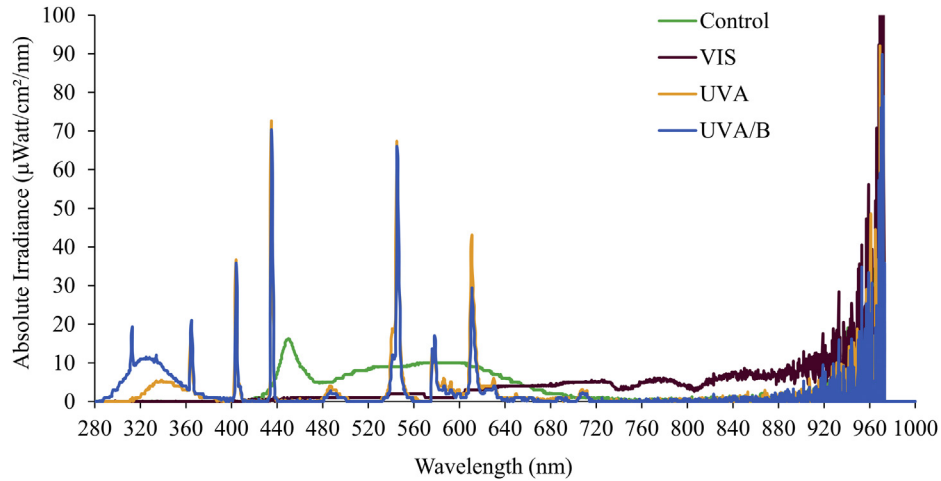


Figure 2. Spectral irradiance of different light treatments (high intensity) as measured by an Ocean Insight Flame-S-XR1 Spectroradiometer at 20 cm from the source (15 cm from source for control for visual appearance). Control: Poultry-specific LED white bulb (IP65 Dimmable LED Bulb, B-E27: 10W, 5K; VIS: Halogen Basking Spot Lamp (372–800 nm), PT2183 100W, Exo Terra; UVA: Reptile UVB200 light (320–712 nm), 25W, PT2341, Exo Terra with 3-mm glass placed under the bulbs; UVA/B: Reptile UVB200 light (288–714 nm), 25W, PT2341, Exo Terra. Abbreviation: VIS, visible spectrum plus infrared wavelengths.

with unique-colored/numbered rings, and they were returned to their home pens after every habituation session. During the habituation period, only the standard ambient light source was used to illuminate both compartments of the LPTA. At 46 wk of age, 4 hens (from the same home pen) were placed in each LPTA with 1 pair initially placed into each compartment. The hens were allowed to move around freely via the tunnels for

approximately 3 h during the daytime and this was repeated in groups of 4 for all hens (between approximately 9 am–5 pm). At 47 wk of age, hens were placed in their groups of 12 (the whole pen) into the LPTA for overnight habituation (5 pm–9 am). At 48 wk of age, hens were placed in the LPTA individually for acclimation for approximately 2 h during the daytime (between approximately 9 am–5 pm). Finally, at 50 wk of

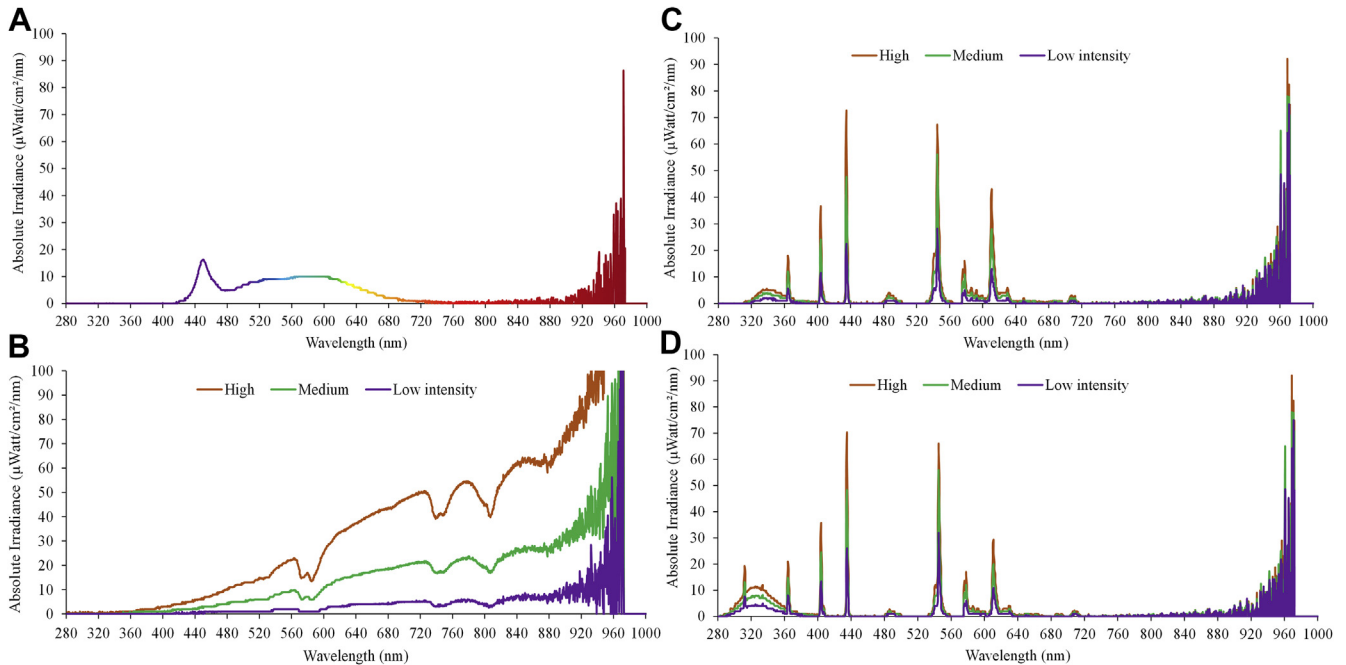


Figure 3. (A–D): Spectral composition of the lights of different intensities as measured by an Ocean Insight Flame-S-XR1 Spectroradiometer at 20 cm from the source (15 cm from source for Control for visual appearance). (A) Control: Poultry-specific white LED bulb (IP65 Dimmable LED Bulb, B-E27:10W-5 K); (B) VIS: Halogen Basking Spot Lamp, Exo Terra. Intensity of light was categorized as PT2181 50W (Low), PT2182 75W (Medium), or PT2183 100W (High); (C) UVA: Reptile UVB 200, 25W, PT2341, Exo Terra was used with 3-mm glass placed under the bulbs to block UVB wavelengths. Intensity was categorized as Low (1 × UVB 200 bulb), Medium (2 × UVB 200 bulbs), or High (3 × UVB 200 bulbs); (D) UVA/B: Reptile UVB 200, 25W, PT2341, Exo Terra. Intensity was categorized as Low (1 × UVB 200 bulb), Medium (2 × UVB 200 bulbs), or High (3 × UVB 200 bulbs). Abbreviation: VIS, visible spectrum plus infrared wavelengths

Table 1. Parameters of treatment lights at different intensity levels.

Treatment light	Wavelength (nm)	Level of intensity	Light intensity (lux) ¹	UV index ²
LED white	420–724	Control/ambient	20.3 ± 2.1	-
VIS	372–800	Low	1,930/81,000	-
		Medium	25,900/95,600	-
		High	47,000/98,000	-
UVA	320–712	Low	690/5,480	-
		Medium	1,590/5,800	-
		High	2,140/7,160	-
UVA/B	288–714	Low	712/5,640	6.5/33.3
		Medium	1,920/6,500	10.2/34.1
		High	2,640/8,630	14.1/42.4

¹All the light measurements were taken at hen's eye height: 30 cm above from the floor and/or 20 cm from the sources as well as 5 cm from the source to account for a hen that may stretch up. The curved shape of the VIS bulb resulted in variable lux readings, the highest center point reading was included in the table.

²The UV index was only available for the UVA/B as this is calculated based on a range of factors including the relative contributions of both of these types of UV light.

age, hens were placed in the box individually again for 2 h during the daytime (9 am–5 pm) but with the standard lights in the room increased to an intensity of approximately 200 lux and exposure to the low intensity of the treatment lights. This was to give the hens both some experience with the new spectrums and some exposure to higher lux in an effort to minimize the chance of adverse reactions (e.g., panic flight and injury) to their first exposure to much higher intensities than they were accustomed to. In total, each hen spent 23 h within the LPTA during the habituation phase. Hens were monitored via video cameras throughout the daytime habituation periods to observe their movement. In the final habituation session, only hens that had exhibited movement between the 2 compartments via the tunnels (minimum 2 times between compartments was observed) were selected to proceed with testing ($n = 84$ hens).

Preference Testing

After habituation, a total of 84 hens (28 hens/treatment) were individually tested for different light preferences. An individual hen was only tested within 1 of the 3 treatment groups but was exposed to all 3 intensities within that treatment. The 3 intensities (low, medium, high) were tested sequentially to minimize confounds of previous exposure on preferences and to simulate what a hen may experience when the pop holes first open within a free-range facility. The hens would gradually be exposed to increasing intensities of light as they slowly venture outside for the first time after indoor rearing (typical practice within Australia and other countries). Individual hens were tested with each level of light intensity from 51 to 53 wk of age. It took approximately 5 d to test all 28 hens of a specific treatment for 1 intensity. During testing, 2 LPTA were simultaneously used for each treatment allowing 6 hens to be tested on each occasion. Hens were captured from the home pens and transported to the neighboring testing room in carry bins. For consistency, birds were always placed in the

treatment compartment first and allowed 2 h to exhibit a choice before being returned to their home pen. The side placement of the treatment lights were balanced between the 2 LPTA per treatment (i.e., treatment lights were on either the left or right side of the box) and bird placement within boxes was rotated between intensities (i.e., if a bird was placed in box-A for the low-intensity test, she was placed in box-B for the medium-intensity test, and box-A for the high-intensity test). On each test day, 3 successive sessions (9 am–11 am, 12 pm–2 pm, and 3 pm–5 pm) were completed. Therefore, 18 hens (6 hens/treatment) were tested per day. All the testing sessions were recorded by video cameras for later analysis. Outside of the testing period, hens remained in their home pens with standard lighting and full access to all necessary resources.

Video Observations and Data Acquisition

Six overhead video cameras recorded the position and behavior of the hens throughout the testing period. Data were generated by watching the full-length (2 h) video records of all tested hens ($n = 252$ test sessions) individually by a single observer using the “Behavioral Observation Research Interactive Software” (Friard and Gamba, 2016).

Time Spent Measurements The entry and exit times of the hen to each LPTA were used to determine the absolute durations of testing. The durations of time that hens spent in the treatment light compartment, in the standard light (control) compartment, or in the tunnels were expressed in total number of s for each of the 2-hour test periods and used to calculate the percentages of time that hens spent in each compartment. The frequencies of movement between the compartments were also used to count the latency (s) until the hen first exited the treatment compartment, the number of transitions (visits to each compartment), and the duration of each compartment visit. Furthermore, during observation, each of the compartments were split into thirds (on screen) to document the time spent directly under the

light sources (the middle) and in either side (right/left). The food and water were located in the right and left thirds.

Exhibited Behaviors Behaviors of the hens during the testing periods were also recorded in each compartment by the same observer based on the ethogram displayed in Table 2. Behaviors of eating, foraging, drinking, and dust bathing were measured as state events in seconds, whereas other behaviors of body shaking, ground pecking, preening, leg stretching, wing flapping, and escape attempts were measured as point events (frequency).

Data and Statistical Analyses

All data were analyzed in JMP 14.0 (SAS Institute, Cary, NC) with α level set at 0.05. Data were compiled per individual hen separately for each light treatment and level of intensity. These data included the proportion of the 2-hour test period spent in each compartment (minus the time spent in the tunnels), the total time spent in the middle of each compartment (min), the latency to first exit the treatment compartment (hens were always placed on the treatment side), the number

of visits to each compartment, and the mean duration (min) of each compartment visit. The temperature data were averaged per test session to provide a single value for the test period of an individual hen. It was assumed that the values would be similar for the opposite box of the same light treatment (without logging devices), and thus, the temperature values were repeated for the hen that was tested at the same time in the opposite replicate treatment box. Data were transformed to approach normality with square root transformation applied to the count data and $\log_{10}$ transformation applied to the duration data. The proportional data (time spent) were logit-transformed after subtracting a constant of 0.001 from all data to allow transformation of “100” values ($n = 48$ of 252). The corresponding values of ‘0’ had a constant of 0.001 added so they could also be included in the analyses. General linear mixed models (GLMMs) were applied to each parameter separately for different intensity levels for each light treatment with compartment and temperature nested within compartment included as fixed effects and hen ID as a random effect. GLMMs were also applied to compare data from the treatment side only between the different light treatments separately at each intensity level

Table 2. Definition of time spent variables and behavioral ethogram used in the video observations.

Parameters	Event type	Unit	Definition
Time spent			
Time spent	State	min/hen	Total time spent in a compartment during the testing period.
Percentage of time spent	State	%	$\text{Time spent (min)} \div 120 \times 100\%$.
Intercompartment transitions (visits)	Point	count	The number of visits a hen makes to a compartment within the testing period.
Mean visit duration	State	min/hen	Mean time spent in a compartment during a single visit.
Time spent at middle	State	min/hen	Total time spent in the middle of the compartment (under the light sources) during the testing period.
Behavioral ethogram			
Feeding time	State	min/hen	Time spent at the feeder starting from when the hen commenced feeding until she turned away from the feeder. This time included brief pauses that hens may have made during feeding.
Foraging time	State	min/hen	Scratching at the litter substrate with feet followed by pecking at the litter.
Drinking time	State	min/hen	Time spent at the water dish starting from when the hen lowered her head and consumed water until the hen turned away from the dish. This time included brief pauses when the hen was not consuming water but was still facing the water dish.
Dust bathing	State	min/hen	Rolling or moving around in substrate, wings fluffed up, kicking substrate onto the feathers.
Preening	Point	count	Grooming of feathers with beak.
Ground pecking	Point	count	Pecking at substrate or fallen feathers on the ground (not preceded by feet scratching).
Body shaking	Point	count	Rapid whole-body movement associated with ruffling of the feathers that occurred randomly throughout the test period or at the end of the dust bathing sequence.
Wing flapping	Point	count	Opening of the wings while still standing.
Escaping (jump)	Point	count	Hens trying to escape from the apparatus by jumping upwards.
Leg stretching	Point	count	One leg stretched out on either side of the body.

including the proportion of time spent, time spent in the middle, mean visit duration, mean number of visits, and latency to first exit. Light treatment and temperature nested within compartment were included as fixed effects and hen ID as a random effect. The model residuals were plotted for visual inspection of homoscedasticity. Where significant differences were present, Student's *t* tests were applied to the least squares means, but the raw values are presented in the tables and graphs.

For behavioral response data analysis, feeding time, drinking time, foraging time, and dust bathing were recorded as state events and measured in seconds that were converted to min in the final analysis and log₁₀ transformed. Dust bathing occurred infrequently and thus was not statistically analyzed, but data are presented in the tables. The behavioral responses including body shaking, ground pecking, and preening were recorded as point events, and the count values were square-root-transformed with the raw values presented in the tables. There were insufficient observations of wing flapping, leg stretching, and escape attempts for statistical analysis. GLMMs were fitted to analyze each behavioral response separately for different intensity levels of each light treatment with compartment, and temperature nested within compartment included as fixed effects and hen ID as a random effect. Where significant differences were present, Student's *t* tests were applied to the least squares means. All the results are

presented by the level of intensity separately for each light treatment.

RESULTS

Time Spent

Visible Light The proportion of total time spent in the compartments was significantly affected by treatment for the low and high intensity of VIS light ($F_{(1, 26.86)} = 5.97$, $P = 0.02$; $F_{(1, 26.67)} = 5.58$, $P = 0.03$, respectively) with hens spending more time in the treatment side over the control side (Figure 4). In contrast, there was only a trend to spend more time on the treatment side at the medium intensity ($F_{(1, 25.64)} = 3.87$, $P = 0.06$, Figure 4). There were no significant differences in the time spent in the middle of the compartment (all $P \geq 0.15$), the frequency of visits between the compartments (all $P \geq 0.08$), and mean visit duration (all $P \geq 0.24$) for any intensity level of the VIS treatment but temperature did have a significant effect on the frequency of visits ($P = 0.01$) and mean visit duration ($P = 0.04$, Table 3) at the medium intensity level.

UVA Light The proportion of total time spent in the compartments was significantly affected by treatment for the medium intensity level ($F_{(1, 25.85)} = 4.75$, $P = 0.04$) with hens spending more time in the treatment

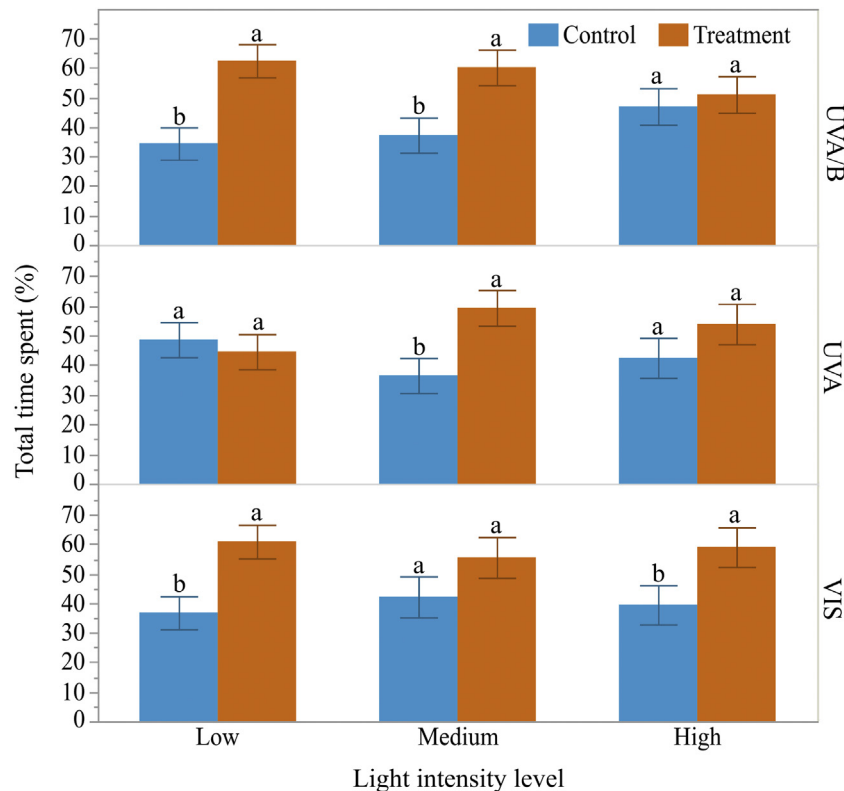


Figure 4. The least squares means $\pm$ SEM for percentage of time spent on the control or treatment side by hens at different levels of VIS, UVA, and UVA/B light intensity. Raw values are presented, but analyses were conducted on transformed data. ^{a,b}Dissimilar superscript letters indicate significant differences between control and treatment lights by a post hoc Student's *t* test ($P \leq 0.05$) separately for each intensity level of each treatment. Abbreviation: VIS, visible spectrum plus infrared wavelengths

Table 3. Hens' time spent at different intensity levels of the VIS light treatment.

Variable	Level of light intensity	Category	Time spent ¹ (min)	Test statistics (df, F-Ratio, <i>P</i> -value)	Temperature (⁰ C) LSM $\pm$ SEM (C/T) ² (df, F-Ratio, <i>P</i> -value)
Time spent at middle	Low	Control	15.95 $\pm$ 5.49	$F_{(1, 31.34)} = 2.15, P = 0.15$	24.44 $\pm$ 0.33/26.10 $\pm$ 0.33
		Treatment	36.01 $\pm$ 5.42		$F_{(2, 30.73)} = 0.37, P = 0.69$
	Medium	Control	21.55 $\pm$ 4.78	$F_{(1, 30.58)} = 0.60, P = 0.44$	24.51 $\pm$ 0.27/26.05 $\pm$ 0.27
		Treatment	38.95 $\pm$ 4.74		$F_{(2, 34.19)} = 3.10, P = 0.06$
	High	Control	24.34 $\pm$ 5.63	$F_{(1, 26.25)} = 0.19, P = 0.67$	23.60 $\pm$ 0.44/25.46 $\pm$ 0.44
		Treatment	30.07 $\pm$ 5.63		$F_{(2, 28)} = 0.16, P = 0.85$
Intercompartment transitions (visits)	Low	Control	10.02 $\pm$ 3.12	$F_{(1, 48.45)} = 3.29, P = 0.08$	$F_{(2, 38.54)} = 1.06, P = 0.36$
		Treatment	11.55 $\pm$ 3.11		
	Medium	Control	12.62 $\pm$ 4.44	$F_{(1, 48.52)} = 0.33, P = 0.57$	$F_{(2, 38.20)} = 5.35, \mathbf{P = 0.01}$
		Treatment	9.72 $\pm$ 4.43		
	High	Control	11.58 $\pm$ 2.94	$F_{(1, 37.12)} = 0.65, P = 0.43$	$F_{(2, 36.41)} = 2.96, P = 0.06$
		Treatment	9.68 $\pm$ 2.94		
Mean visit duration	Low	Control	16.52 $\pm$ 7.74	$F_{(1, 37.09)} = 1.13, P = 0.29$	$F_{(2, 26.56)} = 0.18, P = 0.84$
		Treatment	26.80 $\pm$ 7.65		
	Medium	Control	32.06 $\pm$ 8.52	$F_{(1, 39.63)} = 0.58, P = 0.45$	$F_{(2, 32.69)} = 3.57, \mathbf{P = 0.04}$
		Treatment	42.71 $\pm$ 8.46		
	High	Control	16.40 $\pm$ 8.34	$F_{(1, 23.99)} = 1.47, P = 0.24$	$F_{(2, 21.18)} = 1.93, P = 0.17$
		Treatment	44.94 $\pm$ 8.35		

Raw values are presented with the analyses conducted on transformed data. Significant *P*-values are indicated in bold.

¹The least squares means $\pm$ SEM are presented for each variable.

²(C/T): Control/Treatment.

side, but there was no effect of treatment for the low intensity level ($F_{(1, 26.06)} = 0.0002, P = 0.99$) and only a trend to spend more time on the treatment side at the high intensity level ($F_{(1, 25.56)} = 3.72, P = 0.06$, Figure 4). Hens also spent more time specifically in the middle of the treatment compartment at the medium intensity level ($P = 0.05$) but not at the low ($P = 0.99$) or high ($P = 0.28$) intensities (Table 4). Similarly, hens showed significantly more visits to the treatment side at the medium intensity level ($P = 0.001$) but not at the low ($P = 0.24$) or high ($P = 0.18$) intensities (Table 4). Temperature had a significant effect on the frequency of visits ($P = 0.04$) at the high intensity level (Table 4). There was no significant effect of light treatment on the mean visit duration at any intensity (all $P \geq 0.24$, Table 4).

UVA/B Light The proportion of total time spent in the compartments was significantly affected by treatment for the low ($F_{(1, 25.31)} = 5.09, P = 0.03$) and medium ($F_{(1, 25.51)} = 6.29, P = 0.02$) intensity levels with hens preferring to spend more time on the treatment side (Figure 4). In contrast, there was no preference exhibited at the high intensity level ($F_{(1, 26.77)} = 1.96, P = 0.17$, Figure 4). Hens also spent significantly more time in the middle of the treatment compartment at the low ($P = 0.01$) intensity level but not at the medium ($P = 0.21$) or high intensities ($P = 0.96$, Table 5). There was no effect of treatment on the frequency of visits at any intensity level (all $P \geq 0.29$, Table 5), but at the medium intensity level, temperature showed a significant effect on the frequency of hens' visits between the compartments ($P = 0.04$, Table 5). There were

Table 4. Hens' time spent at different intensity levels of the UVA light treatment.

Variable	Level of light intensity	Category	Time spent ¹ (min)	Test statistics (df, F-Ratio, <i>P</i> -value)	Temperature (⁰ C) LSM $\pm$ SEM (C/T) (df, F-Ratio, <i>P</i> -value)
Time spent at middle	Low	Control	24.12 $\pm$ 5.16	$F_{(1, 24.55)} = 0.0003, P = 0.99$	25.33 $\pm$ 0.32/25.37 $\pm$ 0.32
		Treatment	26.73 $\pm$ 5.16		$F_{(2, 31.35)} = 1.38, P = 0.27$
	Medium	Control	19.63 $\pm$ 5.49 ^b	$F_{(1, 24.70)} = 4.40, \mathbf{P = 0.05}$	25.22 $\pm$ 0.19/25.36 $\pm$ 0.19
		Treatment	37.05 $\pm$ 5.49 ^a		$F_{(2, 30.49)} = 0.54, P = 0.59$
	High	Control	16.55 $\pm$ 5.50	$F_{(1, 21.17)} = 1.24, P = 0.28$	24.29 $\pm$ 0.29/24.54 $\pm$ 0.29
		Treatment	28.14 $\pm$ 5.50		$F_{(2, 25.72)} = 0.45, P = 0.64$
Intercompartment transitions (visits)	Low	Control	19.52 $\pm$ 5.49	$F_{(1, 26.46)} = 1.42, P = 0.24$	$F_{(2, 35.08)} = 0.35, P = 0.71$
		Treatment	19.65 $\pm$ 5.49		
	Medium	Control	10.49 $\pm$ 2.76 ^b	$F_{(1, 34.10)} = 12.75, \mathbf{P = 0.001}$	$F_{(2, 35.43)} = 0.03, P = 0.97$
		Treatment	11.63 $\pm$ 2.76 ^a		
	High	Control	17.62 $\pm$ 7.72	$F_{(1, 46.61)} = 1.83, P = 0.18$	$F_{(2, 36.11)} = 3.50, \mathbf{P = 0.04}$
		Treatment	17.43 $\pm$ 7.72		
Mean visit duration	Low	Control	17.50 $\pm$ 5.90	$F_{(1, 20)} = 0.98, P = 0.33$	$F_{(2, 28.31)} = 0.89, P = 0.43$
		Treatment	13.08 $\pm$ 5.90		
	Medium	Control	13.72 $\pm$ 6.96	$F_{(1, 17.95)} = 1.48, P = 0.24$	$F_{(2, 25.31)} = 1.92, P = 0.17$
		Treatment	28.86 $\pm$ 6.96		
	High	Control	16.34 $\pm$ 8.10	$F_{(1, 16.47)} = 0.01, P = 0.91$	$F_{(2, 24.20)} = 1.95, P = 0.16$
		Treatment	39.46 $\pm$ 8.10		

^{a,b}Dissimilar superscript letters indicate significant differences between control and treatment lights ($P \leq 0.05$).

Raw values are presented with the analyses conducted on transformed data. Significant *P*-values are indicated in bold.

¹The least squares means $\pm$ SEM are presented for each variable.

²(C/T): Control/Treatment.

Table 5. Hens' time spent at different intensity levels of the UVA/B light treatment.

Variable	Level of light intensity	Category	Time spent ¹ (min)	Test statistics (df, F-Ratio, <i>P</i> -value)	Temperature (⁰ C) LSM $\pm$ SEM (C/T) (df, F-Ratio, <i>P</i> -value)
Time spent at middle	Low	Control	16.96 $\pm$ 3.93 ^b	$F_{(1, 26.32)} = 6.83, \mathbf{P} = 0.01$	25.09 $\pm$ 0.33/25.59 $\pm$ 0.33
		Treatment	33.50 $\pm$ 3.99 ^a		$F_{(2, 33.84)} = 0.30, P = 0.75$
	Medium	Control	16.54 $\pm$ 4.31	$F_{(1, 24.91)} = 1.66, P = 0.21$	24.88 $\pm$ 0.29/25.66 $\pm$ 0.29
		Treatment	36.44 $\pm$ 4.31		$F_{(2, 32.91)} = 1.11, P = 0.34$
	High	Control	21.72 $\pm$ 3.97	$F_{(1, 27.41)} = 0.002, P = 0.96$	23.96 $\pm$ 0.38/25.01 $\pm$ 0.38
		Treatment	26.74 $\pm$ 3.96		$F_{(2, 31.75)} = 0.89, P = 0.44$
Intercompartment transitions (visits)	Low	Control	16.23 $\pm$ 4.50	$F_{(1, 47.82)} = 0.05, P = 0.82$	$F_{(2, 37.27)} = 1.61, P = 0.21$
		Treatment	15.30 $\pm$ 4.50		
	Medium	Control	14.17 $\pm$ 3.14	$F_{(1, 39.75)} = 0.26, P = 0.61$	$F_{(2, 35.83)} = 3.57, \mathbf{P} = 0.04$
		Treatment	13.12 $\pm$ 3.14		
	High	Control	14.76 $\pm$ 5.19	$F_{(1, 36.65)} = 1.15, P = 0.29$	$F_{(2, 36.35)} = 2.00, P = 0.15$
		Treatment	15.24 $\pm$ 5.19		
Mean visit duration	Low	Control	12.22 $\pm$ 6.43 ^b	$F_{(1, 23.18)} = 4.35, \mathbf{P} = 0.05$	$F_{(2, 31.31)} = 2.53, P = 0.10$
		Treatment	29.44 $\pm$ 6.43 ^a		
	Medium	Control	10.01 $\pm$ 6.31	$F_{(1, 26.40)} = 2.13, P = 0.16$	$F_{(2, 28.34)} = 1.58, P = 0.22$
		Treatment	33.28 $\pm$ 6.32		
	High	Control	21.86 $\pm$ 4.25	$F_{(1, 32.07)} = 0.12, P = 0.73$	$F_{(2, 29.29)} = 2.51, P = 0.10$
		Treatment	32.35 $\pm$ 7.23		

^{a,b}Dissimilar superscript letters indicate significant differences between control and treatment lights ($P \leq 0.05$).

Raw values are presented with the analyses conducted on transformed data. Significant *P*-values are indicated in bold.

¹The least squares means $\pm$ SEM are presented for each variable.

²(C/T): Control/Treatment.

significantly longer visits to the treatment side at the low intensity level ($P = 0.05$) but not the medium or high intensities (both $P \geq 0.16$, Table 5).

Relative Preferences Among Light Treatments

Hens' latency to first exit the treatment compartment did not differ among the treatment groups at any level of intensity (all $P \geq 0.15$, Table 6), but there was an effect of temperature on the latency to exit at the medium intensity level ($P = 0.03$, Table 6). There were no significant effects of light treatment on hens' preferences for overall time spent and time spent at the middle of the compartment for any intensity level (all $P \geq 0.13$, and all $P \geq 0.29$, respectively, Table 6) except for a significant effect of temperature on hens time spent at the medium intensity ($P = 0.002$, Table 6). Although there were no significant differences in the number of visits to the treatment compartment across all intensities (all $P \geq 0.24$), hens did show a preference to spend a greater amount of time under the UVA/B light and the least amount of time under the UVA light during a single visit at the low intensity ($P = 0.04$, Table 6). There were no effects of treatment on the mean visit duration at the medium ($P = 0.64$) and high ($P = 0.83$) intensities (Table 6), but temperature did have a significant effect on the mean visit duration at the medium intensity ($P = 0.01$) and the frequency of visits at the high intensity ($P = 0.01$, Table 6).

Behavioral Responses

There were no significant effects of the VIS light treatment on the time spent feeding, drinking, and foraging or the frequency of ground pecking, and preening at any intensity level (all $P \geq 0.12$, Table 7), but more body shaking was observed under the low intensity of the VIS treatment light ($P = 0.04$, Table 7). In the UVA light treatment, hens spent more time feeding under the standard light at all 3 intensity levels (all $P \leq 0.03$, Table 7),

but there were no significant effects on any of the other measured behaviors (Table 7). In contrast, in the UVA/B light treatment, hens showed more foraging under the medium intensity of the treatment light ($P = 0.002$, Table 7), more ground pecking under both the low ($P = 0.004$) and medium ($P = 0.01$) intensities of the treatment light and more preening under the low intensity of the UVA/B light ($P = 0.01$, Table 7).

DISCUSSION

The aims of this experiment were to test in a controlled indoor setting, whether laying hens showed preferences toward light spectrums and intensities that free-range hens may experience during outdoor ranging, including the human and chicken visible spectrum of light and the UVA and UVB wavelengths. The spectrums and intensities tested approximated sunlight as close as logistically possible based on commercially available pet reptile light bulbs. The results showed that hens without substantial prior experience of daylight had significant preferences to spend more time under the different types of treatment lights over standard indoor lighting. The hens preferred the high intensity of the visual spectrum light and a trend toward the high intensity of the UVA, but did not prefer the high UVA/B wavelengths. However, the hens did not actively avoid this high intensity and instead showed equal preference with the standard control lighting. Preferences for some lights were also affected by corresponding differences in temperature but not consistently. There were some effects of the treatment lights on behaviors; however, behavioral expression may have been limited by the use of cage-reared hens. Further testing of free-range hens with daylight experience may confirm what aspects of sunlight could limit range access.

Hens showed a preference for light spectrums within their visual perception capabilities that were more

Table 6. Comparisons of hens' time spent at different intensity levels of treatment lights.¹

Light intensity level	Light treatments	Time spent (%)	Time spent middle (min)	Mean visit duration (min)	Visits to treatment compartment (n)	Latency to first exit (min)
Low	VIS	60.37 ± 5.97	36.56 ± 5.33	27.71 ± 7.34 ^a	11.74 ± 4.55	31.04 ± 7.54
	UVA	43.76 ± 5.91	27.21 ± 5.28	14.23 ± 7.27 ^b	19.35 ± 4.5	20.08 ± 7.47
	UVA/B	62.72 ± 5.82	33.65 ± 5.20	28.53 ± 7.15 ^a	16.45 ± 4.44	26.31 ± 7.35
	Test statistics	$F_{(2,78)} = 2.08, P = 0.13$	$F_{(2,76)} = 1.27, P = 0.29$	$F_{(2,78)} = 3.31, P = 0.04$	$F_{(2,78)} = 1.45, P = 0.24$	$F_{(2,78)} = 1.92, P = 0.15$
Medium	Temperature x light treatments	$F_{(3,78)} = 0.16, P = 0.92$	$F_{(3,76)} = 0.17, P = 0.91$	$F_{(3,78)} = 0.41, P = 0.74$	$F_{(2,76)} = 1.27, P = 0.29$	$F_{(3,78)} = 1.70, P = 0.17$
	VIS	59.88 ± 6.22	34.64 ± 5.33	36.10 ± 8.00	10.31 ± 3.63	43.69 ± 8.34
	UVA	61.59 ± 6.19	39.03 ± 5.31	32.02 ± 7.97	11.42 ± 3.61	39.94 ± 8.30
	UVA/B	60.21 ± 6.03	35.22 ± 5.17	29.01 ± 7.76	14.09 ± 0.052	38.97 ± 8.08
High	Test statistics	$F_{(2,78)} = 0.02, P = 0.98$	$F_{(2,77)} = 0.66, P = 0.52$	$F_{(2,78)} = 0.45, P = 0.64$	$F_{(2,78)} = 1.41, P = 0.25$	$F_{(2,78)} = 0.08, P = 0.92$
	Temperature x light treatments	$F_{(3,78)} = 5.49, P = 0.002$	$F_{(3,77)} = 2.0, P = 0.12$	$F_{(3,78)} = 4.41, P = 0.01$	$F_{(2,76)} = 1.27, P = 0.29$	$F_{(3,78)} = 3.06, P = 0.03$
	VIS	59.71 ± 6.72	29.25 ± 5.49	41.46 ± 9.31	10.40 ± 5.62	49.15 ± 9.52
	UVA	55.99 ± 6.74	27.72 ± 5.51	39.69 ± 9.33	21.37 ± 5.64	41.76 ± 9.55
	UVA/B	51.10 ± 6.59	26.53 ± 5.38	31.10 ± 9.12	15.30 ± 5.51	36.19 ± 9.33
	Test statistics	$F_{(2,78)} = 0.57, P = 0.57$	$F_{(2,77)} = 0.50, P = 0.61$	$F_{(2,78)} = 0.18, P = 0.83$	$F_{(2,78)} = 5.53, P = 0.59$	$F_{(2,78)} = 0.28, P = 0.76$
	Temperature x light treatments	$F_{(3,78)} = 0.94, P = 0.43$	$F_{(3,77)} = 0.18, P = 0.91$	$F_{(3,78)} = 1.11, P = 0.35$	$F_{(2,78)} = 3.86, P = 0.01$	$F_{(3,78)} = 75, P = 0.52$

^{a-c}Dissimilar superscript letters indicate significant differences among treatment lights ($P \leq 0.05$).Raw values are presented with the analyses conducted on transformed data. Significant P -values are indicated in bold.¹The least squares means ± SEM are presented for each variable.

representative of natural light indicating attraction to these spectrums despite minimum prior exposure to daylight. This is in contrast with some previous research showing pullets reared in incandescent light also preferred this light over natural glass-filtered daylight (UVB wavelengths unlikely to penetrate glass) in comparison with the natural light preferences displayed by those birds reared under that light type (Gunnarsson et al., 2008). But layer chicks do show inherent preferences for LED lights with UVA supplementation in comparison with no UVA content (Liu et al., 2018). In addition, pullets and layers with different exposures to varying types of artificial lighting all preferred fluorescent lights in a choice scenario which may have been a result of the small component (<5%) of UVA in this light type (Liu et al., 2017). Although hens in this study showed preferences for the natural light spectrums, they still spent a considerable portion (36–49%) of their time under the control lighting. This is similar to preferences measured in captive wild avian species that were from different ecological backgrounds. Those birds that were typically forest dwellers and exposed to patchy light, showed lower preferences for UVA supplementation than those species from high sunlight evolutionary histories (Ross et al., 2013). The typical habitat of the domestic chicken's jungle fowl ancestors may preclude preferences to spend the majority of time under brighter light of natural spectrums. Hens in this study also showed preferences for higher intensities of the VIS light and UVA light (although the preference was only a trend at the highest UVA intensity) indicating the brightness of these lights was not aversive to the hens. Chicks similarly preferred a higher UVA content when given a choice between varying percentages of UVA wavelengths (Liu et al., 2018). All hens were always placed on the treatment side for consistency, but this may have increased the time spent under the treatment lights compared with always placing hens on the control side first. Alternatively, the hens in the present study may have also shown these preferences for greater intensities as they became more accustomed to the treatment lights across multiple test sessions. Chickens transferred from indoor rearing may thus be attracted to the daylight in the range area when the pop holes first open although it may take time to adapt to the brightness and initial range use could be inhibited by other factors such as the exposure in an open area.

Under the UVA/B light treatment, hens showed clear preferences for the treatment light under the low and medium intensities but not at the high intensity. When focused specifically on the middle of the treatment compartment where the UV radiation would not yet have greatly dispersed, the hens only preferred to spend more time in this area at the low intensity level. This suggests that while some degree of UVB was preferred, the higher intensities supplied too much UVB radiation which may have led to skin damage. This result is consistent with studies of free-range chickens where birds range less on sunny days (Gilani et al., 2014; Bestman et al., 2019), during the midday period (Chielo et al.,

2016; Fanatico et al., 2016) or with increasing solar radiation (Stadig et al., 2017). The UVB wavelengths were not provided in isolation in this present study as it was unknown if the hens would minimize their contact with the high radiation if only the UVB was present, but

the relative preferences between the hens exposed to the UVA or the UVA and B suggest the UVB wavelengths specifically were less preferred. However, hens did not actively avoid the treatment side and thus showed limited aversion. Although the lights we used

Table 7. Hens' behavioral responses under lights of different levels of intensity.

Variable	Level of light intensity	Category	VIS light		UVA light		UVA/B light	
			LSM $\pm$ SEM	<i>P</i> -value*	LSM $\pm$ SEM	<i>P</i> -value*	LSM $\pm$ SEM	<i>P</i> -value*
Feeding (min)	Low	Control	5.30 $\pm$ 1.50	0.24	8.10 $\pm$ 1.58 ^a	0.03	4.83 $\pm$ 1.65	0.78
		Treatment	4.21 $\pm$ 1.48		3.92 $\pm$ 1.52 ^b		7.58 $\pm$ 1.65	
	Medium	Control	4.44 $\pm$ 1.76	0.56	4.61 $\pm$ 1.34 ^a	0.03	6.56 $\pm$ 2.10	0.60
		Treatment	3.47 $\pm$ 1.75		2.81 $\pm$ 1.34 ^b		5.02 $\pm$ 2.10	
	High	Control	2.31 $\pm$ 0.89	0.83	7.65 $\pm$ 2.06 ^a	0.002	5.63 $\pm$ 1.75	0.68
		Treatment	3.08 $\pm$ 0.89		2.93 $\pm$ 2.06 ^b		4.34 $\pm$ 1.75	
Drinking (min)	Low	Control	3.32 $\pm$ 1.24	0.22	0.72 $\pm$ 0.33	0.46	1.25 $\pm$ 0.51	0.58
		Treatment	2.01 $\pm$ 1.23		0.85 $\pm$ 0.33		1.49 $\pm$ 0.51	
	Medium	Control	2.07 $\pm$ 0.55	0.12	0.70 $\pm$ 0.36	0.28	0.59 $\pm$ 0.45	0.83
		Treatment	0.68 $\pm$ 0.56		0.98 $\pm$ 0.36		2.11 $\pm$ 0.45	
	High	Control	1.16 $\pm$ 0.84	0.48	1.41 $\pm$ 0.48	0.40	1.09 $\pm$ 0.57	0.23
		Treatment	2.09 $\pm$ 0.84		0.87 $\pm$ 0.48		2.50 $\pm$ 0.57	
Foraging (min)	Low	Control	0.87 $\pm$ 0.37	0.32	1.10 $\pm$ 0.87	0.61	0.56 $\pm$ 0.88	0.34
		Treatment	0.54 $\pm$ 0.36		1.77 $\pm$ 0.87		1.88 $\pm$ 0.88	
	Medium	Control	4.13 $\pm$ 1.43	0.58	0.42 $\pm$ 0.54	0.84	0.97 $\pm$ 0.74 ^b	0.002
		Treatment	0.45 $\pm$ 1.42		1.68 $\pm$ 0.54		1.85 $\pm$ 0.74 ^a	
	High	Control	3.83 $\pm$ 1.67	0.40	1.41 $\pm$ 0.51	0.18	1.86 $\pm$ 1.01	0.68
		Treatment	0.92 $\pm$ 1.67		1.40 $\pm$ 0.51		1.23 $\pm$ 1.01	
Dust bathing (min)	Low	Control	1(4.37)		0		0	
		Treatment	1(30.89)		0		1(10.60)	
	Medium	Control	3(9.83 $\pm$ 2.93)	*	0	*	0	*
		Treatment	1(22.22)		0		2(9.74 $\pm$ 4.69)	
	High	Control	2(15.06 $\pm$ 12.92)		0		1(43.07)	
		Treatment	3(17.05 $\pm$ 9.79)		0		2(11.61 $\pm$ 4.79)	
Body shaking	Low	Control	1.23 $\pm$ 0.42 ^b	0.04	2.15 $\pm$ 0.47	0.60	1.63 $\pm$ 0.42	0.08
		Treatment	2.41 $\pm$ 0.41 ^a		1.71 $\pm$ 0.47		2.44 $\pm$ 0.42	
	Medium	Control	1.81 $\pm$ 0.39	0.23	1.18 $\pm$ 0.31	0.25	1.28 $\pm$ 0.31	0.34
		Treatment	1.52 $\pm$ 0.39		1.66 $\pm$ 0.31		1.59 $\pm$ 0.31	
	High	Control	1.44 $\pm$ 0.42	0.36	1.52 $\pm$ 0.30	0.39	1.18 $\pm$ 0.24	0.97
		Treatment	1.70 $\pm$ 0.42		0.75 $\pm$ 0.30		1.11 $\pm$ 0.24	
Ground pecking	Low	Control	2.53 $\pm$ 0.95	0.18	2.86 $\pm$ 0.72	0.62	1.93 $\pm$ 0.73 ^b	0.004
		Treatment	3.29 $\pm$ 0.94		2.94 $\pm$ 0.72		4.20 $\pm$ 0.73 ^a	
	Medium	Control	3.78 $\pm$ 0.94	0.49	2.46 $\pm$ 0.88	0.06	1.96 $\pm$ 0.58 ^b	0.01
		Treatment	2.43 $\pm$ 0.93		3.13 $\pm$ 0.88		3.81 $\pm$ 0.58 ^a	
	High	Control	3.22 $\pm$ 0.84	0.52	2.46 $\pm$ 0.59	0.79	2.30 $\pm$ 0.50	0.31
		Treatment	2.35 $\pm$ 0.84		1.61 $\pm$ 0.59		2.74 $\pm$ 0.50	
Preening	Low	Control	0.69 $\pm$ 0.35	0.16	1.45 $\pm$ 0.48	0.20	0.31 $\pm$ 0.23 ^b	0.01
		Treatment	1.42 $\pm$ 0.35		1.38 $\pm$ 0.48		1.23 $\pm$ 0.23 ^a	
	Medium	Control	0.37 $\pm$ 0.18	0.52	0.32 $\pm$ 0.20	0.56	0.31 $\pm$ 0.16	0.95
		Treatment	0.56 $\pm$ 0.18		0.55 $\pm$ 0.20		0.22 $\pm$ 0.16	
	High	Control	0.42 $\pm$ 0.27	0.65	0.71 $\pm$ 0.23	0.54	0.32 $\pm$ 0.15	0.88
		Treatment	0.56 $\pm$ 0.27		0.39 $\pm$ 0.23		0.32 $\pm$ 0.15	
Leg stretching	Low	Control	0		0		0	
		Treatment	0		0		0	
	Medium	Control	0	*	0	*	0	*
		Treatment	0		0		0	
	High	Control	0		0		0	
		Treatment	1(2)		1(1)		0	
Wing flapping	Low	Control	2(1 $\pm$ 0.0)		3(3.33 $\pm$ 1.86)		2(1 $\pm$ 0.0)	
		Treatment	2(3 $\pm$ 0.0)		1(2 $\pm$ 0.0)		5(3.16 $\pm$ 2.23)	
	Medium	Control	3(2 $\pm$ 0.58)	*	2(1.5 $\pm$ 0.5)	*	2(4 $\pm$ 1.0)	*
		Treatment	2(1.5 $\pm$ 0.5)		3(2.33 $\pm$ 1.33)		4(3.5 $\pm$ 1.03)	
	High	Control	4(2.75 $\pm$ 1.75)		4(2 $\pm$ 0.58)		2(6 $\pm$ 5.0)	
		Treatment	5(1.6 $\pm$ 0.4)		4(1.75 $\pm$ 0.48)		3(5 $\pm$ 3.51)	
Escaping	Low	Control	3(6.33 $\pm$ 2.73)		6(10 $\pm$ 4.91)		2(4 $\pm$ 2.0)	
		Treatment	7(2.71 $\pm$ 1.29)		8(9.25 $\pm$ 4.09)		9(2.33 $\pm$ 0.83)	
	Medium	Control	2(11 $\pm$ 7.0)	*	5(12.8 $\pm$ 7.63)	*	1(23)	*
		Treatment	9(9.89 $\pm$ 7.57)		6(12 $\pm$ 3.29)		3(11.67 $\pm$ 9.21)	
	High	Control	2(7 $\pm$ 6.0)		2(21.5 $\pm$ 10.5)		2(20 $\pm$ 16.0)	
		Treatment	5(2.4 $\pm$ 0.75)		5(7.2 $\pm$ 2.63)		3(7.67 $\pm$ 3.28)	

Notes: ^{a,b}Dissimilar superscript letters indicate significant differences between control and treatment lights ($P \leq 0.05$). Raw values are presented with the analyses conducted on transformed data. Significant *P*-values are indicated in bold. *Chi-square tests were not performed due to insufficient data, the raw values are presented as the number of hens that exhibited the behavior and the mean ($\pm$ SEM) durations or number of events in parentheses.

showed a high UV index equivalent to a peak summer day in Australia, they may not have sufficiently mimicked sunlight to result in avoidance by the hens. It is unclear if this was a result of their limited experience with sunlight and whether hens accustomed to ranging would show stronger avoidance of light with UVB components; a future study that warrants further investigation.

Measures of behavioral expression showed some differences in hen behavior under the different light types. Consistently, hens showed less time feeding under the UVA light, similar to a previous study that reported suppressed feed intake in young laying hens under UVA light (Lewis et al., 2000). Hens also showed more foraging, ground pecking, and preening at the low intensity of the UVA/B light suggesting hens were more comfortable and motivated to express active hen-typical behaviors (cf. sitting or standing) under this type of light. Many behaviors were not affected by treatment, and other studies have found no differences in hen behavior or activity under different light types (Widowski et al., 1992; Mohammed et al., 2010; Huber-Eicher et al., 2013). Overall, expression of key behaviors (e.g., dust bathing) was low, and this may have been a result of the testing environment or that these hens were previously cage-housed and accustomed to less space and wire flooring thus reducing performance of some activities (Black and Hughes, 1974).

CONCLUSION

This study demonstrated that hens with minimal sunlight experience preferred lights that approximated daylight including high intensities of these lights. When a combination of UVA and B wavelengths were presented, preferences were reduced at the higher intensity suggesting hens avoided the damaging radiation. Lower levels of UVA/B resulted in more behavioral expression of foraging and comfort behaviors. This suggests that hens in a free-range setting may positively respond to sunlight access but when the sunlight is intense, hens may need additional measures (e.g., shelter) to protect themselves from certain levels of UV radiation and intensity. It was impossible to completely mimic sunlight intensity and wavelengths in an indoor experimental setting for this study. In addition, older cage-reared hens were used which may have hindered behavioral expression. Therefore, further study is required to validate these findings in a free-range setting.

ACKNOWLEDGMENTS

Funding support for the project was provided by Australian Eggs. The authors would like to thank the Animal House staff at the University of New England (UNE) for set-up assistance and B. Burke for supply of the laying hens. Thank you to Jim M. Lea (CSIRO) and Tim R. Dyal (CSIRO) for technical and husbandry

assistance. Md Sohel Rana was supported by UNE and CSIRO postgraduate scholarships. Gratitude is expressed toward L. Hewitt, S. Harris, and J. Javorniczky for valuable discussions regarding experimental design and UV light.

DISCLOSURES

The authors declare no conflicts of interest.

REFERENCES

- Baxter, M., N. Joseph, V. R. Osborne, and G. Y. Bedecarrats. 2014. Red light is necessary to activate the reproductive axis in chickens independently of the retina of the eye. *Poult. Sci.* 93:1289–1297.
- Bestman, M., C. Verwer, T. van Niekerk, F. Leenstra, B. Reuvekamp, Z. Amsler-Kepalaite, and V. Maurer. 2019. Factors related to free-range use in commercial laying hens. *Appl. Anim. Behav. Sci.* 214:57–63.
- Black, A. J., and B. O. Hughes. 1974. Patterns of comfort behaviour and activity in domestic fowls: a comparison between cages and pens. *Br. Vet. J.* 130:23–33.
- Boshouwers, F. M. G., and E. Nicaise. 1987. Physical activity and energy expenditure of laying hens as affected by light intensity. *Br. Poult. Sci.* 28:155–163.
- Bowmaker, J. K., L. A. Heath, S. E. Wilkie, and D. M. Hunt. 1997. Visual pigments and oil droplets from six classes of photoreceptor in the retinas of birds. *Vis. Res.* 37:2183–2194.
- Chielo, L., T. Pike, and J. Cooper. 2016. Ranging behaviour of commercial free-range laying hens. *Animals* 6:28.
- Dal Bosco, A., C. Mugnai, A. Rosati, A. Paoletti, S. Caporali, and C. Castellini. 2014. Effect of range enrichment on performance, behavior, and forage intake of free-range chickens. *J. Appl. Poult. Res.* 23:137–145.
- Davis, N. J., N. B. Prescott, C. J. Savory, and C. M. Wathes. 1999. Preferences of growing fowls for different light intensities in relation to age, strain and behaviour. *Anim. Welfare.* 8:193–203.
- Dawkins, M. S., P. A. Cook, M. J. Whittingham, K. A. Mansell, and A. E. Harper. 2003. What makes free-range broiler chickens range? In situ measurement of habitat preference. *Anim. Behav.* 66:151–160.
- de Koning, C., S. M. Kiteessa, R. Barekatain, and K. Drake. 2019. Determination of range enrichment for improved hen welfare on commercial fixed-range free-range layer farms. *Anim. Prod. Sci.* 59:1336–1348.
- de Matos, R. 2008. Calcium metabolism in birds. *Vet. Clin. North Am. Exot. Anim. Pract.* 11:59–82.
- Edwards, Jr, H.M. 2003. Effects of u.v. irradiation of very young chickens on growth and bone development. *Br. J. Nutr.* 90:151–160.
- Fanatico, A. C., J. A. Mench, G. S. Archer, Y. Liang, V. B. Gunsaulis, C. M. Owens, and A. M. Donoghue. 2016. Effect of outdoor structural enrichments on the performance, use of range area, and behavior of organic meat chickens. *Poult. Sci.* 95:1980–1988.
- Friard, O., and M. Gamba. 2016. BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods Ecol. Evol.* 7:1325–1330.
- Gibson, J. H. 2000. UVB radiation: Definition and Characteristics. in *UV-B Monitoring and Research Program*. Accessed Aug. 2020. http://uvb.nrel.colostate.edu/UVB/publications/uvb_primer.pdf.
- Gilani, A. M., T. G. Knowles, and C. J. Nicol. 2014. Factors affecting ranging behaviour in young and adult laying hens. *Br. Poult. Sci.* 55:127–135.
- Goldsmith, T. H. 1990. Optimization, constraint, and history in the evolution of eyes. *Q. Rev. Biol.* 65:281–322.
- Govardovskii, V. I., and L. V. Zueva. 1977. Visual pigments of chicken and pigeon. *Vis. Res.* 17:537–543.
- Gunnarsson, S., M. Heikkilä, J. Hultgren, and A. Valros. 2008. A note on light preference in layer pullets reared in incandescent or natural light. *Appl. Anim. Behav. Sci.* 112:395–399.

- Hegelund, L., J. T. Sørensen, J. B. Kjaer, and I. S. Kristensen. 2005. Use of the range area in organic egg production systems: effect of climatic factors, flock size, age and artificial cover. *Br. Poult. Sci.* 46:1–8.
- Holick, M. F. 2016. Biological effects of sunlight, ultraviolet radiation, visible light, infrared radiation and vitamin D for health. *Anti-cancer Res.* 36:1345–1356.
- Huber-Eicher, B., A. Suter, and P. Spring-Stahli. 2013. Effects of colored light-emitting diode illumination on behavior and performance of laying hens. *Poult. Sci.* 92:869–873.
- Jacome, I. M. T. D., L. A. Rossi, and R. Borille. 2014. Influence of artificial lighting on the performance and egg quality of commercial layers: a review. *Rev. Bras. Cienc. Avic.* 16:337–344.
- Janczak, A. M., and A. B. Riber. 2015. Review of rearing-related factors affecting the welfare of laying hens. *Poult. Sci.* 94:1454–1469.
- Jones, T., R. Feber, G. Hemery, P. Cook, K. James, C. Lamberth, and M. Dawkins. 2007. Welfare and environmental benefits of integrating commercially viable free-range broiler chickens into newly planted woodland: a UK case study. *Agric. Syst.* 94:177–188.
- Kjaer, J. B., and K. S. Vestergaard. 1999. Development of feather pecking in relation to light intensity. *Appl. Anim. Behav. Sci.* 62:243–254.
- Kristensen, H. H., N. B. Prescott, G. C. Perry, J. Ladewig, A. K. Ersbøll, K. C. Overvad, and C. M. Wathes. 2007. The behaviour of broiler chickens in different light sources and illuminances. *Appl. Anim. Behav. Sci.* 103:75–89.
- Larsen, H., G. Cronin, C. L. Smith, P. Hemsworth, and J.-L. Rault. 2017. Behaviour of free-range laying hens in distinct outdoor environments. *Anim. Welfare.* 26:255–264.
- Lemus-Deschamps, L., and J. K. Makin. 2012. Fifty years of changes in UV Index and implications for skin cancer in Australia. *Int. J. Biometeorol.* 56:727–735.
- Lewis, P. D., and T. R. Morris. 2000. Poultry and coloured light. *World Poult. Sci. J.* 56:189–207.
- Lewis, P., and T. Morris. 2006. *Poultry Lighting: The Theory and Practice*. Northcot, Hampshire, UK.
- Lewis, P. D., and R. M. Gous. 2009. Responses of poultry to ultraviolet radiation. *World Poult. Sci. J.* 65:499–510.
- Lewis, P. D., G. C. Perry, and T. R. Morris. 1997. Effect of size and timing of photoperiod increase on age at first egg and subsequent performance of two breeds of laying hen. *Br. Poult. Sci.* 38:142–150.
- Lewis, P. D., G. C. Perry, and T. R. Morris. 2000. Ultraviolet radiation and laying pullets. *Br. Poult. Sci.* 41:131–135.
- Liu, K., K. Wang, T. Fei, L. Chai, and H. Xin. 2018. Computer vision-based animal preference assessment – do laying hen chicks prefer light with UVA radiation? *Agricultural and Biosystems Engineering Conference Proceedings and Presentations.* 567. https://lib.dr.iastate.edu/abe_eng_conf/567/.
- Liu, K., H. Xin, and L. Chai. 2017. Choice between fluorescent and poultry-specific LED lights by pullets and laying hens. *Transactions of the ASABE.* 60:2185–2195.
- Ma, H., H. Xin, Y. Zhao, B. Li, T. A. Shepherd, and I. Alvarez. 2016. Assessment of lighting needs by W-36 laying hens via preference test. *Animal* 10:671–680.
- Maddocks, S. A., I. C. Cuthill, A. R. Goldsmith, and C. M. Sherwin. 2001. Behavioural and physiological effects of absence of ultraviolet wavelengths for domestic chicks. *Anim. Behav.* 62:1013–1019.
- Manser, C. E. 1996. Effects of lighting on the welfare of domestic poultry: a review. *Anim. Welfare.* 5:341–360.
- McKenzie, R. L., L. O. Björn, A. Bais, and M. Ilyasd. 2003. Changes in biologically active ultraviolet radiation reaching the Earth's surface. *Photochem. Photobiol. Sci.* 2:5–15.
- Min, J. K., M. S. Hossan, A. Nazma, C. N. Jae, T. B. Han, K. K. Hwan, W. K. Dong, S. C. Hyun, C. C. Hee, and S. S. Ok. 2012. Effect of monochromatic light on sexual maturity, production performance and egg quality of laying hens. *Avian Biol. Res.* 5:69–74.
- Mohammed, H. H., M. A. Grashorn, and W. Bessei. 2010. The effects of lighting conditions on the behaviour of laying hens. *Arch. Geflügelkd.* 74:197–202.
- Nagle, T. A., and P. C. Glatz. 2012. Free range hens use the range more when the outdoor environment is enriched. *Asian-Aust. J. Anim. Sci.* 25:584–591.
- Norton, T. T., and J. T. Siegwart, Jr. 2013. Light levels, refractive development, and myopia—a speculative review. *Exp. Eye Res.* 114:48–57.
- Osorio, D., M. Vorobyev, and C. D. Jones. 1999. Colour vision of domestic chicks. *J. Exp. Biol.* 202:2951–2959.
- Prescott, N. B., C. M. Wathes, and J. R. Jarvis. 2003. Light, vision and the welfare of poultry. *Anim. Welfare.* 12:269–288.
- Prescott, N. B., and C. M. Wathes. 1999. Spectral sensitivity of the domestic fowl (*Gallus g. domesticus*). *Br. Poult. Sci.* 40:332–339.
- Prescott, N. B., and C. M. Wathes. 2002. Preference and motivation of laying hens to eat under different illuminances and the effect of illuminance on eating behaviour. *Br. Poult. Sci.* 43:190–195.
- Rault, J.-L., A. van de Wouw, and P. Hemsworth. 2013. Fly the coop! Vertical structures influence the distribution and behaviour of laying hens in an outdoor range. *Aust. Vet. J.* 91:423–426.
- Rajchard, J. 2009. Ultraviolet (UV) light perception by birds: a review. *J. Vet. Med.* 54:351–359.
- Richards, G. J., L. J. Wilkins, T. G. Knowles, F. Booth, M. J. Toscano, C. J. Nicol, and S. N. Brown. 2011. Continuous monitoring of pop hole usage by commercially housed free-range hens throughout the production cycle. *Vet. Rec.* 169:338.
- Ross, M. R., K. L. Gillespie, L. M. Hopper, M. A. Bloomsmith, and T. L. Maple. 2013. Differential preference for ultraviolet light among captive birds from three ecological habitats. *Appl. Anim. Behav. Sci.* 147:278–285.
- Schutkowski, A., J. Krämer, H. Kluge, F. Hirche, A. Krombholz, T. Theumer, and G. I. Stangl. 2013. UVB exposure of farm animals: study on a food-based strategy to bridge the gap between current vitamin D intakes and dietary targets. *PLoS One* 8:e69418.
- Shi, H., B. Li, Q. Tong, W. Zheng, D. Zeng, and G. Feng. 2019. Effects of LED light color and intensity on feather pecking and fear responses of layer breeders in natural mating colony cages. *Animals* 9:814.
- Sobotik, E. B., J. R. Nelson, and G. S. Archer. 2020. How does ultraviolet light affect layer production, fear and stress. *Appl. Anim. Behav. Sci.* 223:104926.
- Stadig, L. M., T. B. Rodenburg, B. Ampe, B. Reubens, and F. A. M. Tuytens. 2017. Effect of free-range access, shelter type and weather conditions on free-range use and welfare of slow-growing broiler chickens. *Appl. Anim. Behav. Sci.* 192:15–23.
- Vandenbert, C., and T. M. Widowski. 2000. Hens' preferences for high-intensity high-pressure sodium or low-intensity incandescent lighting. *J. Appl. Poult. Res.* 9:172–178.
- Wei, Y., W. Zheng, B. Li, Q. Tong, H. Shi, and X. Li. 2020. Effects of B-wave ultraviolet supplementation using light-emitting diodes on caged laying hens during the later phase of the laying cycle. *Animals* 10:15.
- Widowski, T. M., L. J. Keeling, and I. J. Duncan. 1992. The preferences of hens for compact fluorescent over incandescent lighting. *Can. J. Anim. Sci.* 72:203–211.