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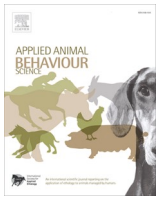

Effects of ultraviolet light supplementation on hen behaviour and welfare during early lay

Md Sohel Rana [a,b,c], Caroline Lee [b], Stephen W. Walkden-Brown [a], Dana L.M. Campbell [a,b,*]

[a] Department of Animal Science, School of Environmental and Rural Science, University of New England, Armidale, NSW 2351, Australia
[b] Agriculture and Food, Commonwealth Scientific and Industrial Research Organisation (CSIRO), Armidale, NSW 2350, Australia
[c] Livestock Research Institute, Department of Livestock Services, Ministry of Fisheries and Livestock, Dhaka 1212, Bangladesh

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ABSTRACT

Poultry can see in the ultraviolet (UV) spectrum and supplementation of UVA has been shown to reduce laying hens' fear and stress. The shorter wavelengths (UVB) also facilitate vitamin D_3 production. However, UV spectrums are typically not included in indoor commercial poultry lighting which may result in less than optimum welfare of laying hens. This study predicted that UVA and UVA/B supplementation would increase desirable behaviours such as foraging and exploratory pecking and reduce measures of fear and stress. The effects of UV light supplementation were assessed on 252 ISA Brown hens during the period from 16 to 27 weeks of age. Birds were housed in eighteen pens (14 hens/pen) under three different light treatment groups each with six replications: (i) UV0: standard control lighting with LED white light, (ii) UVA: control lighting plus a supplemental 'daylight' avian bulb providing UVA, and (iii) UVA/B: control lighting plus a supplemental full spectrum reptile bulb containing both UVA and UVB wavelengths. Behavioural time budgets were assessed in the home pens at 18 and 24 weeks. At 20, 23, and 26 weeks, assessments included: individual behavioural tests of tonic immobility and open field, pen-level novel object tests and blood heterophil/lymphocyte (H/L) ratios. At 27 weeks the UV supplemental lights were turned off for 3 days as a 'stressor' with bird locations in the pen measured before and during the stressor, along with H/L ratio and attention bias test assessments. Results showed limited effects of the supplemental lights. At 26 weeks both UV light treatments reduced tonic immobility duration ($P = 0.006$) and vocalisations across time in the open field test ($P = 0.0003$), while the UVA/B hens had lower H/L ratios than the UV0 hens ($P = 0.01$). However, removing the treatment lights did not result in greater stress and anxiety and there were few differences observed in the home pen time budgets. Overall, these results align with complementary findings in the same birds showing minimal impact of the UV treatments on measures of egg production, egg quality, bone health, and circulating plasma P and Ca but with an increase in serum vitamin D ($25(OH)D_3$) for the UVA/B hens. The supplemental UV lights in this study were only on one side of the pens which could have minimised their impacts. Similarly, effects may vary at different points in the production cycle than included in this study.

1. Introduction

Light intensity, duration, timing of the photoperiod, and spectrum are all critically important for poultry behaviour, health, growth, and production throughout the flock cycle (Lewis and Morris, 1998; El-Sabrout et al., 2022; Oso et al., 2022). Distinct from humans, chicken vision is highly sensitive to different light wavelengths (colour), including being able to perceive ultraviolet A (UVA) radiation (320–400 nm) (Prescott and Wathes, 1999a; Lewis and Morris, 2000; Rajchard, 2009). Ultraviolet B (UVB) radiation (280–315 nm) also has beneficial physiological effects by stimulating vitamin D_3 synthesis (Rana and Campbell, 2021). As commercially available indoor poultry lights are designed to human specifications, they are typically deficient in UV spectrum which may not fulfil hens' lighting needs, negatively impacting their welfare (Prescott et al., 2003; Lewis and Gous, 2009; Benson et al., 2013; Rana and Campbell, 2021).

In light preference testing, when hens were given a free choice of lighting environment, day-old layer chicks across an eight-day period were shown to spend more time under the illuminated area containing 15% UVA compared to light devoid of the UV spectrum, with their

* Corresponding author at: Agriculture and Food, Commonwealth Scientific and Industrial Research Organisation (CSIRO), Armidale, NSW 2350, Australia.
E-mail address: dana.campbell@csiro.au (D.L.M. Campbell).

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strength of preference decreasing as the percentage of UVA in the supplemented light reduced to 10% or 5% (Liu et al., 2018). Laying hens aged 44 weeks also showed preferences towards medium intensity UVA light as well as low and medium intensity lights containing both UVA and UVB wavelengths (UVA/B) relative to the standard indoor LED white lighting (Rana et al., 2021). UV radiation has reflective properties and can be reflected from materials commonly used in poultry houses, which could influence hens' natural behaviours including environmental pecking and foraging (Prescott and Wathes, 1999b). Birds performed more exploratory behaviours such as ground scratching (foraging), ground pecking, and environmental pecking in the presence of UVA and/or UVA/B spectral lights (Maddocks et al., 2001; Rana et al., 2021; Wichman et al., 2021), although effects in some studies were only subtle (Maddocks et al., 2001; Wichman et al., 2021).

UVA supplementation has previously lowered physiological stress indicators of plasma corticosterone concentrations in layer chicks (Maddocks et al., 2001) as well as the heterophil/lymphocyte (H/L) ratio in adult cage-housed hens at 44 and 72 weeks (Sobotik et al., 2020). In the same group of hens, the behavioural testing of inversion and tonic immobility also documented lower fear responses in the UVA supplemented birds (Sobotik et al., 2020) demonstrating positive welfare impacts of UVA wavelengths. However, contrasting with positive impacts, UVA light supplementation tested in a commercial facility significantly increased plumage damage of laying hens in the production phase due to increased feather pecking behaviours resulting in early trial termination (Spindler et al., 2020). In a contrasting result, plumage coverage of 32-week-old adult hens exposed to full-spectrum daylight lamps (UVA/B) and/or UVB for 6 weeks in experimental floor pens was not affected by the UV treatments (Kühn et al., 2019). UV radiation, particularly UVB wavelengths, can have damaging effects on the skin, thus there may be a difference between optimum versus detrimental radiation exposure (McKenzie et al., 2003; Lewis and Gous, 2009). The ancestors of today's domestic chickens lived in forested environments and so some degree of full spectrum light may be preferable to laying hens in indoor housing (Wichman et al., 2021). As natural light contains UVA and UVB, artificial lighting that includes these wavelengths is likely to lead to more positive consumers' views on hen welfare (Schröder and McEachern, 2004; Pettersson et al., 2016). The presence of supplemental UVA/B wavelengths may mitigate hens' fear and stress responses and improve hens' natural behaviours and welfare but this needs to be tested further. Indoor UV supplementation may also be a tool for free-range producers to facilitate adaptation to an outdoor environment when indoor-reared pullets arrive at the laying hen facility.

Therefore, the objective of this study was to investigate the effects of both supplemented UVA and UVA/B light over standard lighting on the behaviour, fear, and stress responses of laying hens during early lay. Based on the literature (Rana and Campbell, 2021), it was predicted that the UV supplementation would increase desirable behaviours such as foraging and exploratory pecking and reduce measures of fear and stress, but it may increase undesirable feather pecking. The data presented here complement a separate set of results from the same study on external hen health, bone health and blood parameters associated with bone physiology (Rana et al., 2023).

2. Materials and methods

2.2. Animal housing and husbandry

The animals and experimental set-up have previously been described in Rana et al., 2023. The study was conducted in the Rob Cumming Poultry Innovation Centre of the University of New England, Armidale, NSW, Australia. A total of 252 ISA-Brown layer pullets (*Gallus gallus domesticus*) at 16 weeks of age were procured from a commercial supplier. The birds had been infrared beak-trimmed at the hatchery which is common practice within the Australian laying hen industry. Birds had been reared in a commercial facility with deep litter and curtained sides before transferring into the indoor experimental setting. The curtains were opened to facilitate ventilation into the house when birds were fully feathered, so pullets did have some exposure to natural daylight during rearing. The commercial supplier farm was selected based on pullet availability and minimal transport distance to the research facility with rearing conditions outside of control of the experimenters. Once pullets arrived at the experimental facility, they were given an oral (via gavage) preventive medication (Exzolt®) for mite infestation. This was repeated 7 days later along with an oral dewormer (CCD® Levamisole, CCD® Animal Health, Tamworth, NSW, Australia). In no specific order, birds were allocated into eighteen floor pens (14 pullets/pen), within three rooms (6 pens/room). The pens were separated by wire panelling with shade cloth for visual isolation between birds and confinement of specific light treatments. Shade cloth did not extend to the ceiling to allow air flow throughout the room. Hens could potentially see over the shade cloth if they stretched their necks while on the upper tier of the perch. Each of the pens (3.2 m L × 1.75 m W) contained one three-rung perch (1.07 m L × 64 cm W × 80 cm H), a rollaway nest box (34 cm L × 29 cm W × 24 cm H), a round feeder (40 cm H × 43.5 cm D × 1.36 m C), two nipple drinkers, and wood shavings as a litter substrate (5 cm depth) (Figures S1, S2 and see Fig. 1 in Rana et al., 2023). Birds had *ad libitum* access to feed and water. Birds were fed on a standard commercial grower mash the first week after arrival, replaced with commercial layer mash until the end of the study. Pens were fitted with poultry-specific LED white light bulbs (IP65 Dimmable LED Bulb, B-E27–10 W-5 K, Eco-Industrial Supplies, Zhenjiang, China) with an average light intensity of 30 lux at birds' eye height across the pens (Testboy GmBH TV 335 Digital LED Luxmeter, Vechta, Germany; settings were calibrated to the light source as per the manufacturer guidelines). The hours of lighting followed ISA Brown management recommendations where at 16 weeks, birds were provided with 14 L:10D lighting, increased by 15 min light every week until a fixed 16 L:8D schedule at 24 weeks onwards (lights on at 05:00 h and off at 21:00 h). The hen stocking density was approximately 2.5 hens/m^2, with indoor temperature and humidity controlled mechanically through an automatic ventilation system. Different coloured, numbered leg bands uniquely identified each hen to facilitate the experimental procedures.

2.3. Experimental lighting set-up

Pens were assigned one of three different lighting treatment groups the day after arrival. There were 6 pen replicates per treatment balanced for location within the rooms such that each treatment was in each different pen location across the three rooms: (i) UV0 (control): standard lighting by LED-white light (IP65 Dimmable LED Bulb, B-E27–10 W-5 K, Eco-Industrial Supplies, Zhenjiang, China) with no UV supplementation; (ii) UVA: standard lighting by LED-white light plus full-spectrum avian daylight (containing UVA and UVB) supplementation (PureSun Compact Bird Lamp, E27–20 W, Flicker free, Arcadia, Germany) with 3-mm glass used directly under the bulb to block the UVB spectrum; and (iii) UVA/B: standard lighting by LED-white light plus full-spectrum Exo-Terra® pet reptile bulbs (containing UVA and UVB) supplementation (Reptile UVB200, 25 W, PT2341, Rolf C. Hagen, Montreal, QC, Canada). There was more UVB in the reptile bulb, hence the use of two different bulb types for the supplemental treatments (see Figure S3). An LED Filament Warm-white light (Edison Screw LED GLS Filament Globe, E27–4 W, Mirabella, Victoria, Australia) was also added into the control pens to balance for the increased light intensity in the UV treatment pens. Supplementary Figure 3 displays the light spectrums and radiation irradiance of each bulb as measured over an average of 10 readings using an Ocean Insight Flame-S-XR1 Spectroradiometer (200–1025 nm, Quark Photonics, Melbourne, Australia) set with an integration time of 180,000 ms and integration range from 280 to 1000 nm. All supplemental bulbs were enclosed under an Arcadia ceramic reflector clamp lamp E27 (200 mm, RARM160X) hung from the ceiling 76 cm above the floor on one side of the pen. Thus, birds had a choice to be under the

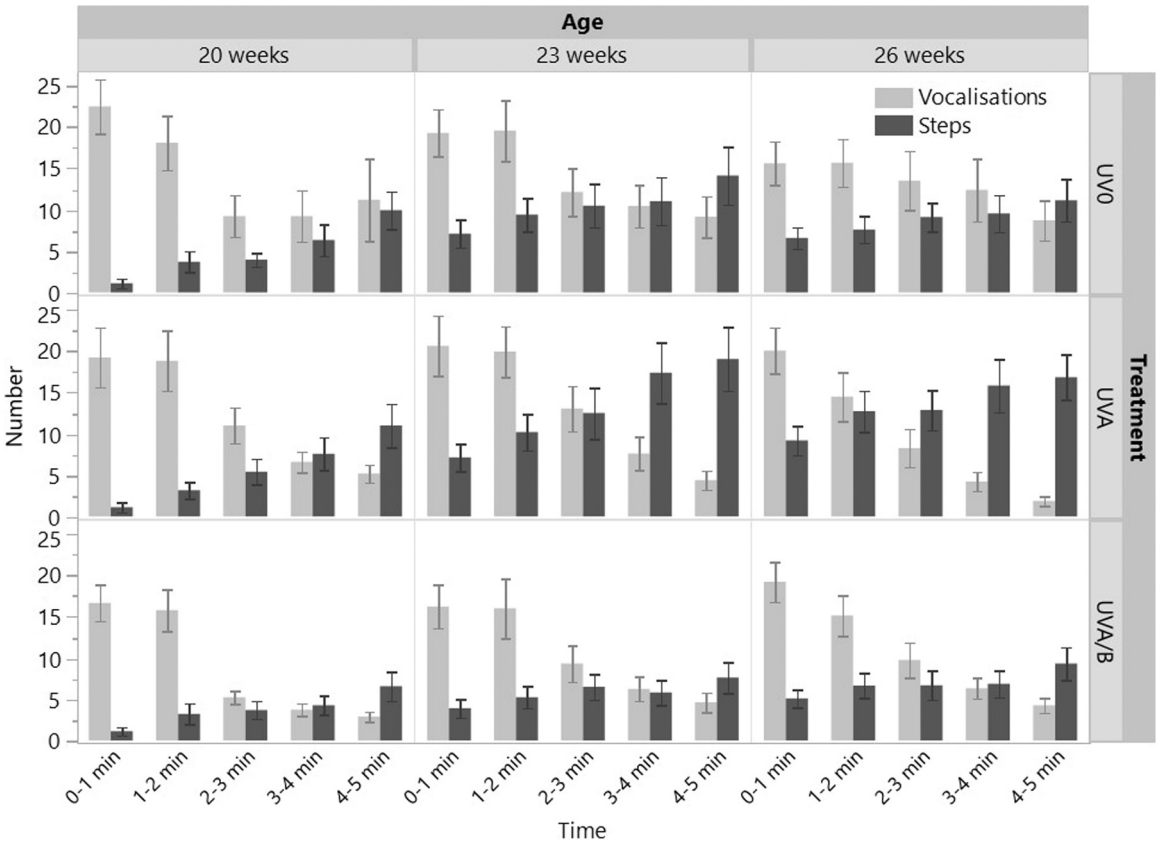

Fig. 1. The number of vocalisations and steps in a 5-min open field test conducted on individual hens from three different light treatments (UV0: Control light with no ultraviolet (UV) spectral light, UVA: Control light plus UVA spectral light, UVA/B: Control light plus both UVA and UVB spectral light; n = 30 hens/treatment) across 20, 23, and 26 weeks of age.

supplemental light or standard lighting area. The light intensity and UV irradiance was maximal directly underneath the treatment light source and gradually decreased with the distance from the source due to scattering effects. At floor level and chicken eye height (approximately 40 cm accounting for 5 cm of litter substrate) the respective light intensities were: UV0: 77.6 ± 3.33 lux and 207.8 ± 9.80 lux; UVA: 69.5 ±1.77 lux and 174.8 ± 6.04 lux; and UVA/B: 75.3 ± 2.94 lux and 181.4 ± 10.34 lux. The UV index under the UVA/B light was 0.4 and 0.9 at floor and chicken eye height respectively. The light intensities were measured using a lux meter (Testboy GmbH TV 335 Digital LED Luxmeter, Vechta, Germany; settings were calibrated to the light source as per the manufacturer guidelines), and the UV index was measured for the UVA/B treatment using a reptile UV index meter (Solarmeter Model 6.5 R UVI Reptile, Solarmeter Australia, Noosaville Dc, QLD, Australia). The perch was intentionally located on the treatment light side only to facilitate UV irradiance exposure to legs and feet (Schutkowski et al., 2013) and maximise physiological impacts that were assessed in the complementary study by Rana et al. (2023). Based on the treatment light positioning, half of each pen was under the treatment light source and the remaining half of the pen was under the standard lighting condition (Figures S1, S2). An analogue timer was set in each pen to operate the treatment lights in accordance with the standard lighting schedule. Every 2 weeks, the dust on the UV-filtering glass in the UVA treatment was removed with an alcohol wipe.

2.4. Behavioural observations

To facilitate behavioural observations, pens were recorded for 7 days across 18 and 24 weeks of age using overhead cameras in each pen (Hikvision network turret cameras DS-2CD2365G1-I 6MP) connected to a network video recorder in a separate room (Hikvision DS-7732NI-I4–16 P CCTV network video recorder, Figure S1). Later, a single observer observed the hens' behavioural repertoire on two consecutive days each at 18 and 24 weeks, with two sampling periods per day (afternoon: 12:00 −14:00 h; evening: 17:00 −19:00 h). The observer conducted scan samples every 15 min to determine the specific behaviour hens were performing based on the ethogram displayed in Table 1. The birds were watched for up to 30 s to confirm the behaviour they were exhibiting with the first observed behaviour per hen recorded. The number of hens performing each behaviour were counted, with a total of 14 observations per scan matching the 14 birds/pen. The raw observation data resulted in 8 scans per 2-h period totalling 112 counts of behaviours (14 birds x 8 scans). These values were summed per behaviour so there was a single value across the 2-h observation period (e.g., if all hens in a pen were only observed walking, the total count of walking for the 2-h period would be 112).

2.5. Behavioural tests

2.5.1. Tonic immobility (TI)

A subset (A) of 30 hens per treatment group (5 hen/pen, n = 90) were assessed via a tonic immobility (TI) test across 2 days each at 20, 23, and 26 weeks. Testing occurred from approximately 09:00 until 16:00 h. The majority of eggs had been laid by the time testing commenced. Individual hens were caught and picked up in hand by the experimenter and carried from their home pens to a separate testing room with a small cloth over their head to reduce stress and daylight exposure. The testing room was empty of birds but was within the same facility as the home pens (one of 4 rooms at the facility which had external-only connecting doors). The order of testing each day was balanced across treatment groups within each room where all pens were completed before going to the next room. Following transport to the testing room each hen was

Table 1
Behavioural ethogram used during home pen observations.

Behaviour	Definition
Side preference	Pens were divided into equal halves on the screen. Hens' side preferences were recorded as hen presence in either the supplemental light or standard light side at the beginning of each scan. If in the middle, the side with the majority of the hen's body located on it was counted as the location.
Perching	The hen is on the perch.
Feeding	The hen is feeding at the feed trough.
Drinking	The hen is drinking at the drinkers.
Standing	The hen is upright on both legs and inactive.
Nesting	The hen is inside the nest box.
Preening	The hen is grooming its feathers with its beak by moving their head.
Ground pecking	The hen has its head lowered below the midline while in a standing position and is pecking at substrate on the ground.
Ground scratching	The hen is scratching the ground substrate with its feet. This typically occurs in conjunction with ground pecking.
Environmental pecking	The hen has its head lowered below the midline while in a standing position and is pecking atresources other than the substrate on the ground.
Resting	The hen is sitting with hocks on the ground in a resting position, eyes remain open or closed, the head could be upright and moving around.
Gentle pecking	The hen is gently pecking at another hen that does not result in displacement of the conspecific.
Severe feather + aggressive pecking	The hen is directing rapid aggressive pecking at the head and/or body area of another hen, typically resulting in retreat or submissive crouching by the recipient. Feathers may be removed.
Dust bathing	The hen is rubbing its head on the ground, rolling or moving around in the substrate, wings opened and shaking, feathers ruffled, kicking substrate onto the body.
Leg stretching	The hen is laying down and head is touching on the ground. One leg is stretched out from either side of the body (not observed to be part of a dust bathing sequence).
Lying	The hen is lying down on either side, with its head stretched out or folding over the neck and both feet extended on the same side (not observed to be part of a dust bathing sequence).
Body shaking	The hen suddenly jerks its whole body including head and tail (not observed to be part of a dust bathing sequence).
Wing flapping	The hen is opening and shaking its wings while in a standing position (not observed to be part of a dust bathing sequence).
Wing-leg stretching	The hen stretches out one leg behind and opens its wing while in a standing position.
Walking	The hen is actively moving around the pen while not showing other listed behaviours.

placed in a supine position in a cradle with its head hanging down over the end. The right hand of the experimenter was placed on the breast of the bird, while the left hand gently held the bird's head down. Hens were restrained in this position for 10 s. Once the hen was released, TI duration was recorded until the hen returned to an upright position, up to a maximum of 5 min. If the hen righted within 10 s after release, TI was re-induced up to a maximum of five attempts. The number of induction attempts and duration of TI was recorded. Any hen that reached the maximum duration, was assigned a TI duration of 300 s; whereas any hen that reached 5 induction attempts was assigned the maximum duration they remained in TI. The hens that reached 5 induction attempts were not excluded from the dataset as across all testing ages, there were 44 tests (out of 270 individual tests across 90 hens) that reached this maximum. Hens were returned to their home pen immediately after testing concluded with additional leg bands to identify which birds had been tested.

2.5.2. Open field test

The same subset (A) of hens used for TI testing were also assessed in an open field test (OFT) at 20, 23, and 26 weeks. The individual tests were conducted from approximately 09:00 until 16:00 h across 2–3 days once the TI testing was finished for all birds. As per TI testing, individual hens were caught in hand from their home pens by the same experimenter and carried to the testing room with a small cloth over their head. Any impact of the catching procedure on test responses was presumed to be equal across treatments. The order of testing each day was balanced across treatment groups where all pens within a room were completed before moving onto the next room. Hens were placed via a hen-sized opening flap into a wooden square open field box (1.8 m L x 1.8 m W x 1.8 m H with a painted green floor and green sides (#00AD3D) up to 60 cm height). Shade cloth was placed over the roof to darken the test box and prevent bird escape. The hen was placed in the dark (approximately 5 lux: Testboy GmbH, TV 335 Digital LED Luxmeter, Vechta, Germany), then a light was switched on inside the arena (illuminated the arena to approximately 90 lux at bird's eye height) and the 5 min test commenced. Two video cameras (Sony Handycams HDR-PJ410, Sony Corporation, Tokyo, Japan) directly above the test box (underneath the shade cloth), recorded bird behaviour or connected to a screen for real-time observation by an observer within the room but out of sight. The trained observer recorded the latency to move more than just the head (seconds), latency to first step (seconds), latency to first vocalise (seconds), and the number of vocalisations (individual vocalisations, not bouts with no differentiation between type of vocalisation) per min across the 5 min test duration. The overhead light was then switched off and hen was removed via one side of the box that opened up to allow a person to enter and return the hen to their home pen. Additional leg bands allowed for identification of those hens that had already been tested. Later, two trained observers blinded to the light treatment groups counted the hens' number of steps in the test arena per min across the 5 min test duration and live measurements were double-checked against the recorded video clips.

2.5.3. Novel object test

Following completion of all the OFTs, two novel object tests (NOTs) was carried out at the pen level one morning each at 20, 23, and 26 weeks from approximately 09:00 – 11:00 h (six NOTs in total). All pens within a single room were tested simultaneously before moving to the next room. An experimenter placed a novel object within all the pens (standard light side) of each room, set a timer for 5 min (based on the last object placed) and left the room. Following test completion, the novel objects were removed and placed into the pens of the next room and so forth. Once three rooms had been exposed to the first novel object, the second novel object was placed in the pens of the first room and the procedure repeated so that two separate tests were conducted per pen at each age point. Six objects were used in total: at 20 weeks, a green plastic disk holding 3 different coloured plastic balls followed by a yellow plastic container holding an orange funnel on top containing a green spiky ball; at 23 weeks, a blue plastic scrubbing brush followed by an aluminium foil pipe; and at 26 weeks, a red plastic basket followed by a brown cardboard box holding a green duster. The behaviour of the hens toward the novel object was video recorded by the overhead cameras installed in each pen. The observation period of the test commenced 10 s after the experimenter placed the novel object on the pen floor and ended after 3 min. A trained observer who was blinded to the light treatments decoded the recorded videos and measured the latencies (seconds) for the first 3 hens to approach the object, the number of hens approaching the object across the test, the latency (seconds) of the first hen to peck the object, and the number of pecks to the object across the 3 min test duration. To obtain these measurements, a circle with a 25 cm radius was drawn on the computer screen from the centre of the object. An approach was considered as a hen in close proximity (within <25 cm radius) to the novel object where the hens' head and neck were within the circle. If none of the hens approached the object

within 3 min then the latency to approach was recorded as 3 ×180 s. For the number of hens that approached, the same hen could be counted for multiple approaches, but they must have turned away or displayed no visible interest in the object before returning to it for a second approach to be tallied if they remained in the vicinity of the object. Individual hens could not be reliably identified on the screen; thus, it was not possible to determine if some hens never approached. The peck was counted if the hen approached the novel object and touched/pecked the object with its beak. If no hens pecked within the 3 min test duration, then the latency was counted as 180 s. The number of pecks at the novel object were counted regardless of bird identification for the test duration; a single hen could do multiple pecks. Due to the first novel object (green plastic disk holding 3 different coloured plastic balls) being highly attractive to the hens and able to be dismantled (hens spread the balls out across the pen), accurate observations could not be completed and thus data from this novel object test were excluded from analyses.

2.6. Stress measurements

2.6.1. Blood sampling and heterophil/lymphocyte (H/L) ratio

Blood samples were taken from another subset (B) of hens (5 hens/pen; 30/treatment) in the morning between 09:00 and 12:00 h at 20, 23, and 26 weeks. The majority of hens had finished laying prior to the sampling time period. The same randomly selected subset of hens was sampled at each age point (identified by their leg bands). Hens were captured from their home pen, and approximately 1.5–2 ml of blood per hen was collected from the brachial vein by three experienced operators using a 20-gauge needle into a lithium-heparin plasma separation gel vacutainer (BD Vacutainer® PST Lithium Heparin Tubes, BD 368056, Franklin Lakes, NJ, United States). Once clotting was confirmed on the sampled vein, the hen was immediately returned to the home pen. The vacutainers were immediately stored in an ice bath and shifted to the laboratory within 3 h after collection. On the same day, one/two drops of blood from the vacutainer was used for a blood smear slide. A blood smear was prepared using the 'Modified Wright-Giemsa stain' following the manufacturer's instructions and procedures (Sigma-Aldrich® Solutions, Cat# WG32–1 L, Saint Louis, MO, United States). In brief, one drop of blood was taken out from the tube using a glass pipette and placed at the centre of a glass microscope slide with the frosted end used for labelling (Livingstone International Pty Ltd, O'Riordan St., Mascot, NSW, Australia). The drop was spread on the glass slide using the wedge smear technique to minimise artificial cell rupture and then air-dried. The dried smear slide was dipped in a Coplin jar (feather-edge down) containing methanol (Sigma-Aldrich® Solutions, Cat# M1775–1GA, Saint Louis, MO, United States) for one min to fix the film, and then air-dried. Subsequently, the dried film was dipped in a Coplin jar with Modified Wright-Giemsa stain for 30 seconds. After removing the slide from the stain, it was again dipped in another Coplin jar containing phosphate-buffered saline (Sigma-Aldrich® Solutions, Cat# P3288–1vl, Saint Louis, MO, United States) for 2–3 min. Finally, the slide was rinsed slowly in running deionized water and then kept at room temperature overnight for air drying. On the following day, the slide was covered with a cover slip using the mounting medium DPX (Sigma-Aldrich® Solutions, Cat# 06522, Saint Louis, MO, United States) and stored in the refrigerator at 4–8 ℃ for further analysis. Later, the slides were scanned using a NanoZoomer 2.0RS (Hamamatsu Photonics, Shizuoka, Japan) slide scanner and observed using NDP.view2 image viewing software (www.hamamatsu.com/all/en/U12388-01.html) with a magnification of 80X to determine the H/L ratio. An experienced observer counted the number of leukocyte cells including heterophils, eosinophils, basophils, lymphocytes, and monocytes until the total observed number of cells reached one hundred. A keystroke counter was used to keep track of the number of observed cells. The H/L ratio was then calculated by dividing the number of heterophils by the number of lymphocytes (Campo et al., 2008).

2.6.2. Induced stressor

At 27 weeks, the supplemental treatment lights were turned off in the home pens (including in the control pen) for 3 days to assess if their removal induced a stress response in the hens, indicating their value to the birds. Behavioural and physiological measures were taken before and after light removal to assess within-bird differences. The day before turning off the treatment lights, a blood sample from the same subset (A) of 90 behavioural test hens (see Section *2.5 Behavioural tests*) was taken in the morning (after laying was finished) to measure baseline H/L ratio (see Section *2.6.1 Blood sampling and heterophil/lymphocyte (H/L) ratio*), which was repeated after 3 days. An attention bias test (ABT) of the sampled hens was also performed twice. Once in the morning (commencing approximately 09:00 h) of the day following blood collection before turning off the treatment lights as well as in the morning (commencing approximately 09:00 h) after 3 days of light removal and following the day of the repeated blood collection. Additionally, 2 consecutive days before turning off the lights and the entire duration of the induced stressor was video recorded via the overhead pen cameras to record hen locations in the pen. Following these measurements, the treatment lights were turned back on.

2.6.2.1. Attention bias test. The same open field box (Section *2.5.2 Open field test*) was used for the attention bias test (ABT) with the same subset (A) of behavioural test hens. In this test, a small pile of mixed grain was present in the back centre of the arena, but no playback of alarm calls was included. The ABT was based on the protocol of Campbell et al. (2019) which demonstrated that latency to eat was the most informative measure to determine treatment effects. While the hens were not familiar with the mixed grain, previous experiments carried out by the authors with different brown layer flocks indicated this was an attractive feed to the hens. The individual hen was captured by the experimenter from the home pen and carried with a small cloth over their head to the test room. The hen was placed in the arena in the dark via the hen-sized entry flap. The arena light was switched on and the test began, concluding when the hen ate the grain. The maximum duration of the test was 5 min where any hen that did not consume the grain was assigned a latency of 300 s. Two overhead cameras (Sony Handycams HDR-PJ410, Sony Corporation, Tokyo, Japan) enabled the behaviour of the hens in the arena to be video recorded and observed live via an experimenter located out of sight within the room. The observer recorded the hen's latency to first step (seconds), latency to eat (seconds) and latency to first vocalise (seconds). All latencies were later double-checked via the video recordings by a second observer blind to treatment.

2.6.2.2. Home pen location preference observations. Two trained observers watched the recorded videos of the home pens across two days before and two days during (excluding the day of the ABT) the removal of the supplemental lights to measure the differences in the distribution of hens within the pen due to the implemented stressor. For this purpose, the observer divided the pen into equal halves on the screen and took a snapshot every 15 min across three 2 h periods (morning: 06:00 – 08:00 h; afternoon: 12:00 – 14:00 h; evening: 17:00 – 19:00 h). The number of hens on each pen side (supplemental light/standard light) were counted to determine if location preferences changed when the supplemental lights were turned off.

2.7. Data and statistical analyses

Analyses were conducted in JMP 17.2.0 (SAS Institute, Cary, NC, USA). Raw values are presented in the tables and figures. Statistical significance was set at $\alpha < 0.05$. Where significant differences were present in the general linear mixed models (GLMM), post-hoc Student's t-tests were applied to the least squares means. The studentised residuals were visually inspected to ensure homoscedascity.

The number of hens in the two home pen locations (standard or supplemental light side) were converted to proportions of total hens in the pen and averaged across each observation period (n = 288: 1 mean value x 2 sides x 2 observation periods x 2 days x 2 age points x 6 pen replicates x 3 treatments). The proportional values were logit-transformed and analysed using separate GLMMs per age point that included the fixed effects of 'side', the interactions between 'treatment and side', 'side and time period', and 'treatment, side, and time period' and the random effects of 'room' and 'pen' (fixed effects of 'treatment' and 'time period' alone were not included in the model given the proportional values between sides added to one). The final dataset on behaviours observed in the home pen consisted of one summed value per pen per observation time per day per age point (n = 144 per behaviour: 1 summed total x 2 observation periods x 2 days x 2 age points x 6 pen replicates x 3 treatments). These values were converted to proportions of observations (e.g., 12 birds counted as walking across 8 scans x 14 birds (112): 12/112 = 0.11). A constant of 0.0001 was added to each value in the datasheet to enable logit transformation of zero values. Some behaviours were observed infrequently and were combined resulting in a total of 11 behaviours including: perching, feeding, drinking, preening, resting, standing, walking, 'pecking and foraging' (included environmental pecking, ground pecking, and ground scratching), 'comfort behaviour' (included: dust bathing, leg stretching, lying, body shaking, wing flapping, wing-leg stretching), severe feather/aggressive pecking and gentle pecking. Nest box occupancy was observed only 103 times out of a total of 16126 possible counts and therefore was not included in any analyses. The behaviours were each analysed using separate GLMMs per age point that included the fixed effects of 'treatment', 'time', and their interaction and random effects of 'room' and 'pen'. Severe feather/aggressive pecking and gentle pecking could not be transformed to approach normality and were thus analysed using separate Kruskal-Wallis tests to compare 'treatment' and 'time' per age point.

The individual bird data on number of attempts to induce tonic immobility and tonic immobility duration (seconds) from Subset A hens (n = 90) at 20, 23, and 26 weeks were compiled. The count data on attempts were square-root transformed and the duration data were $\log_{10}$ transformed before analysing with GLMMs separately by 'age' including the fixed effect of 'treatment', and random effects of 'room' and 'pen'.

The individual bird data on the number of vocalisations and number of steps per min of the 5-min OFT from Subset A hens (n = 90 hens x 5 mins = 450) at 20, 23, and 26 weeks were compiled. The counts of steps for 9 birds were missing at 23 weeks due to failure to record the videos of these birds (UVA = 4 birds from one pen; UV0 = 5 birds from one pen). The number of vocalisations for these birds had been recorded live. The count data were square-root transformed and analysed with GLMMs separately by 'age' including the fixed effects of 'treatment', 'time', and their interaction, and random effects of 'room', 'pen', and 'bird ID'.

The latencies for the first 3 birds to approach the novel object were compiled per pen across 5 NOT occurring at 20 (n = 1), 23 (n = 2), and 26 (n = 2) weeks of age (n = 270; 6 pens x 3 treatments x 5 tests x first 3 birds). The data on the latencies for the first peck at the novel object, the total number of hens that approached across the test, and the total number of pecks at the novel object across the test were compiled (n = 90 per parameter, 6 pens x 3 treatments x 5 tests). The data were unable to be transformed to normality and thus were analysed using nonparametric Kruskal-Wallis tests applied separately per age point.

The individual bird data for H/L ratios from Subset B hens (n = 90) were compiled for 20, 23, and 26 weeks of age. Some blood samples were unable to be analysed with 4 samples missing at 20 weeks across all treatment groups, and one sample missing from the UVA group at 26 weeks (n = 265 across 3 age points). The ratio data were $\log_{10}$ transformed and analysed per age point using GLMMs with the fixed effect of 'treatment' and random effects of 'room' and 'pen'.

For the stressor period, the individual bird data for H/L ratios from Subset A hens (n = 90) were compiled for samples taken before and after the stressor. Samples were unable to be processed from 3 birds in a UVA pen and 3 birds in a UVA/B pen in the 'before' period resulting in n = 84, with all samples processed in the 'after' period (n = 90). The ratio data were $\log_{10}$ transformed and analysed using a GLMM with the fixed effects of 'treatment', 'stressor', and their interaction and random effects of 'room', 'pen', and 'bird ID'. The individual bird latency data (step, eat, and vocalise) from the ABT were compiled for before and after the stressor. The data were $\log_{10}$ transformed and analysed using a GLMM with the fixed effects of 'treatment', 'stressor', and their interaction and random effects of 'room', 'pen', and 'bird ID'. The count data on locations of birds in the home pens were converted to the proportion of total birds in each pen located on the supplemental light or standard light side averaged within the morning, afternoon, and evening time periods. The total dataset comprised an average proportional value with n = 432 (1 mean value x 2 pen sides x 2 observation days x 3 time of day periods x before and after the stressor period x 6 pen replicates x 3 treatments). The proportional values were logit-transformed and analysed using separate GLMMs per time period (morning, afternoon, evening) and included the fixed effects of 'side', 'treatment and side', 'side and stressor', 'treatment, stressor, and side', and the random effects of 'room' and 'pen' (main effects of treatment and stressor alone were not included as proportional values summed to the same total between pens).

3. Results

3.1. Home pen behaviours (18 and 24 weeks)

At 18 weeks of age, across all treatments, there were no significant differences in the proportion of hens that were observed on the standard or supplemental light side ($F_{(1,\ 120)} = 2.50$, $P = 0.12$), no significant interaction between side and treatment ($P = 0.62$), no significant interaction between side and time period ($P = 0.35$) nor treatment, side, and time period ($F_{(2,\ 120)} = 0.23$, $P = 0.79$, Table 2). At 24 weeks of age, there was a significant effect of side ($F_{(1,\ 120)} = 175.5$, $P < 0.0001$) with birds spending more time on the supplemental light side but no significant interaction between treatment and side ($P = 0.53$, Table 2). There was a significant interaction between side and time period ($P = 0.0005$) with hens spending the most time on the supplemental light side in the afternoon (12:00 – 14:00 h), followed by the supplemental light side in the evening (17:00 – 19:00 h), standard light side in the evening, and the least time on the standard light side in the afternoon (Table 2). There was no significant interaction between treatment, side, and time period ($F_{(2,\ 120)} = 2.37$, $P = 0.10$).

There were few significant effects of treatment on behaviours observed in the home pens at 18 or 24 weeks of age. At 18 weeks of age, the birds in the UVA treatment showed more resting than birds in the UVA/B treatment ($P = 0.03$), the UVA/B birds showed more preening than the UV0 birds at 18 weeks of age ($P = 0.07$, post-hoc tests showed group differences), and the UV0 birds showed more walking than the UVA birds ($P = 0.04$, Table 2). There were no other behavioural differences observed between treatments at 18 or 24 weeks of age (all $P \geq 0.10$, Table 2). There were, however, differences between the afternoon and evening time periods in most behaviours at both age points with some behaviours being observed more in the afternoon and vice versa (Table 2). There were no significant interactions between treatment and time (all $P \geq 0.08$, Table 2). Numerically, the most frequently observed behaviours were pecking and foraging, perching, preening, and feeding (Table 2).

3.2. Behavioural tests (20, 23, 26 weeks)

There were no significant effects of treatment on the number of attempts to induce tonic immobility or the duration of tonic immobility at 20 and 23 weeks of age (both $P \geq 0.11$), but there were significant differences in both variables at 26 weeks of age (both $P \leq 0.04$) where

Table 2
Home pen behavioural observations under different light treatments for hens at 18 and 24 weeks of age[1].

Behaviour[2]	Age (weeks)	Treatment (%)[3]			F stat, *P-value*	Time period (%)		F stat, *P-value*	Treatment x Time period (F stat, *P-value)*
		UV0	UVA	UVA/B		Afternoon	Evening		
Location					Treatment x side			Time period x side	
Standard side	18	47.9 ± 3.2	47.0 ± 2.6	49.5 ± 2.2	$F_{(2, 120)} = 0.48$, $P = 0.62$	49.3 ± 2.6	47.0 ± 1.6	$F_{(1, 120)} = 0.88$, $P = 0.35$	
Light side		52.1 ± 3.2	53.1 ± 2.6	50.3 ± 2.2		50.7 ± 2.6	53.0 ± 1.6		
Standard side	24	33.5 ± 2.4	36.3 ± 3.0	34.3 ± 2.0	$F_{(2, 120)} = 0.64$, $P = 0.53$	31.3 ± 2.5[d]	38.1 ± 1.2[c]	$F_{(1, 120)} = 12.82$, **$P = 0.0005$**	
Light side		66.5 ± 2.4	63.6 ± 3.0	65.6 ± 2.0		68.5 ± 2.5[a]	62.0 ± 1.2[b]		
					Treatment			Time period	
Perching	18	18.7 ± 1.7	16.4 ± 1.7	15.5 ± 1.1	$F_{(2, 13)} = 0.39$, $P = 0.68$	13.7 ± 1.5[b]	20.0 ± 1.1[a]	$F_{(1, 51)} = 25.52$, **$P < 0.0001$**	$F_{(2, 51)} = 0.90$, $P = 0.41$
	24	13.8 ± 1.8	14.7 ± 1.8	12.0 ± 1.1	$F_{(2, 13)} = 0.19$, $P = 0.83$	17.2 ± 1.5[a]	9.8 ± 0.7[b]	$F_{(1, 51)} = 17.94$, **$P < 0.0001$**	$F_{(2, 51)} = 2.26$, $P = 0.11$
Feeding	18	14.6 ± 0.8	13.9 ± 0.8	13.0 ± 0.9	$F_{(2, 13)} = 0.99$, $P = 0.40$	15.0 ± 0.6[a]	12.6 ± 0.8[b]	$F_{(1, 51)} = 8.12$, **$P = 0.006$**	$F_{(2, 51)} = 0.97$, $P = 0.39$
	24	18.9 ± 1.3	19.8 ± 1.1	18.9 ± 1.0	$F_{(2, 13)} = 0.18$, $P = 0.84$	16.9 ± 0.8[b]	21.6 ± 0.9[a]	$F_{(1, 51)} = 17.99$, **$P < 0.0001$**	$F_{(2, 51)} = 1.21$, $P = 0.31$
Drinking	18	3.1 ± 0.4	2.0 ± 0.3	2.2 ± 0.4	$F_{(2, 13)} = 1.83$, $P = 0.20$	2.3 ± 0.3	2.5 ± 0.3	$F_{(1, 51)} = 0.35$, $P = 0.56$	$F_{(2, 51)} = 1.22$, $P = 0.30$
	24	3.6 ± 0.5	3.8 ± 0.5	3.5 ± 0.4	$F_{(2, 13)} = 0.73$, $P = 0.50$	3.0 ± 0.3[b]	4.3 ± 0.3[a]	$F_{(1, 51)} = 5.47$, **$P = 0.02$**	$F_{(2, 51)} = 1.19$, $P = 0.31$
Preening	18	14.2 ± 1.6[b]	19.3 ± 1.8[a,b]	19.9 ± 1.9[a]	$F_{(2, 13)} = 3.32$, **$P = 0.07$**	23.6 ± 1.4[a]	12.0 ± 0.8[b]	$F_{(1, 51)} = 63.73$, **$P < 0.0001$**	$F_{(2, 51)} = 0.88$, $P = 0.42$
	24	9.0 ± 1.2	10.3 ± 1.7	10.9 ± 1.5	$F_{(2, 13)} = 0.15$, $P = 0.86$	15.6 ± 1.0[a]	4.6 ± 0.4[b]	$F_{(1, 51)} = 122.40$, **$P < 0.0001$**	$F_{(1, 51)} = 1.42$, $P = 0.25$
Resting	18	6.1 ± 1.0	6.7 ± 1.1	6.7 ± 1.2	$F_{(2, 13)} = 0.40$, $P = 0.68$	10.9 ± 0.7[a]	2.2 ± 0.5[b]	$F_{(1, 51)} = 45.70$, **$P < 0.0001$**	$F_{(2, 51)} = 1.07$, $P = 0.35$
	24	2.3 ± 0.5[a,b]	2.6 ± 0.6[a]	1.9 ± 0.6[b]	$F_{(2, 13)} = 4.56$, **$P = 0.03$**	4.2 ± 0.5[a]	0.3 ± 0.1[b]	$F_{(1, 51)} = 125.02$, **$P < 0.0001$**	$F_{(2, 51)} = 1.66$, $P = 0.20$
Standing	18	1.8 ± 0.4	1.7 ± 0.4	1.9 ± 0.5	$F_{(2, 13)} = 0.20$, $P = 0.82$	1.2 ± 0.2	2.4 ± 0.4	$F_{(1, 51)} = 2.31$, $P = 0.14$	$F_{(2, 51)} = 0.24$, $P = 0.79$
	24	1.2 ± 0.4	1.3 ± 0.3	1.2 ± 0.4	$F_{(2, 13)} = 0.07$, $P = 0.93$	2.0 ± 0.3[a]	0.4 ± 0.1[b]	$F_{(1, 51)} = 19.97$, **$P < 0.0001$**	$F_{(2, 51)} = 1.01$, $P = 0.37$
Walking	18	7.8 ± 1.1[a]	4.7 ± 0.9[b]	5.3 ± 0.8[a,b]	$F_{(2, 13)} = 4.12$, **$P = 0.04$**	3.2 ± 0.6[b]	8.7 ± 0.7[a]	$F_{(1, 51)} = 20.49$, **$P < 0.0001$**	$F_{(2, 51)} = 1.15$, $P = 0.32$
	24	3.4 ± 0.5	2.7 ± 0.5	2.7 ± 0.5	$F_{(2, 13)} = 0.67$, $P = 0.53$	3.5 ± 0.5	2.3 ± 0.3	$F_{(1, 51)} = 0.92$, *$P = 0.34$*	$F_{(2, 51)} = 0.60$, $P = 0.55$
Pecking and foraging	18	25.2 ± 1.8	24.0 ± 1.3	26.5 ± 2.0	$F_{(2, 13)} = 0.34$, $P = 0.72$	19.8 ± 0.9[b]	30.7 ± 1.2[a]	$F_{(1, 51)} = 64.73$, **$P < 0.0001$**	$F_{(2, 51)} = 2.33$, $P = 0.11$
	24	40.3 ± 2.7	35.6 ± 2.5	39.5 ± 3.2	$F_{(2, 13)} = 0.70$, $P = 0.10$	26.5 ± 1.0[b]	50.5 ± 1.2[a]	$F_{(1, 51)} = 207.61$, **$P < 0.0001$**	$F_{(2, 51)} = 1.58$, $P = 0.22$
Comfort behaviour	18	4.6 ± 1.1	6.0 ± 1.0	5.5 ± 1.1	$F_{(2, 13)} = 0.56$, $P = 0.59$	7.7 ± 1.0[a]	3.0 ± 0.4[b]	$F_{(1, 51)} = 9.58$, **$P = 0.003$**	$F_{(2, 51)} = 0.10$, $P = 0.91$
	24	4.1 ± 0.9	3.6 ± 0.6	5.1 ± 1.0	$F_{(2, 13)} = 1.28$, $P = 0.31$	7.1 ± 0.6[a]	1.5 ± 0.3[b]	$F_{(1, 51)} = 33.24$, **$P < 0.0001$**	$F_{(2, 51)} = 2.66$, $P = 0.08$
Severe feather/ aggressive pecking	18	1.4 ± 0.3	2.0 ± 0.5	1.3 ± 0.3	$\chi2 = 0.24$, df = 2, $P = 0.89$	1.2 ± 0.2	1.9 ± 0.4	$\chi2 = 1.11$, df = 1, $P = 0.29$	
	24	2.0 ± 0.3	3.4 ± 0.6	2.3 ± 0.5	$\chi2 = 2.24$, df = 2, $P = 0.33$	2.0 ± 0.4[b]	3.1 ± 0.4[a]	$\chi2 = 4.43$, df = 1, **$P = 0.04$**	
Gentle pecking	18	1.3 ± 0.3	1.6 ± 0.3	1.4 ± 0.3	$\chi2 = 0.74$, df = 2, $P = 0.69$	1.3 ± 0.2	1.5 ± 0.2	$\chi2 = 0.12$, df = 1, $P = 0.73$	
	24	1.2 ± 0.3	2.1 ± 0.6	1.6 ± 0.4	$\chi2 = 0.64$, df = 2, $P = 0.73$	1.5 ± 0.4	1.5 ± 0.4	$\chi2 = 0.48$, df = 1, $P = 0.49$	

[1]The raw means ± SEM are presented for each variable with the general linear mixed models conducted on transformed data. Significant P-values (< 0.05) are indicated in bold. [a-d]Dissimilar superscript letters indicate significant post-hoc differences between the groups ($P < 0.05$).
[2]The behaviours are described in the ethogram in Table 1. 'Pecking and foraging' included environmental pecking, ground pecking, and ground scratching, 'comfort behaviour' included: dust bathing, leg stretching, lying, body shaking, wing flapping, and wing-leg stretching.
[3]Treatments: UV0: Control light with no ultraviolet (UV) spectral light, UVA: Control light plus UVA spectral light, UVA/B: Control light plus both UVA and UVB spectral light. There were 6 pen replicates per treatment with 14 hens per pen.

the UV0 treatment hens showed fewer attempts and a longer duration (Table 3). There were no significant effects of treatment at 20, 23, or 26 weeks of age on the latencies to first move, step, and vocalise in the open field test (all $P \geq 0.13$, Table 3). There was a significant interaction between treatment and time in the number of vocalisations at 26 weeks of age only, where visually, the UVA and UVA/B hens reduced the number of vocalisations across time more than the UV0 hens ($F_{(8, 348)} = 3.80$, $P = 0.0003$; 20 weeks: $F_{(8, 348)} = 0.80$, $P = 0.61$; 23 weeks: $F_{(8, 348)} = 0.97$, $P = 0.46$, Fig. 1). There was no significant main effect of treatment on the number of vocalisations in the open field test at 20 weeks ($F_{(2, 13)} = 1.64$, $P = 0.23$), 23 weeks ($F_{(2, 13)} = 0.55$, $P = 0.59$), or 26 weeks ($F_{(2, 13)} = 1.09$, $P = 0.36$). There were significant effects of time at each age point ($F_{(4, 348)} = 47.43 – 74.59$, all $P < 0.0001$) with the number of vocalisations generally decreasing across the 5-min test (Fig. 1).

Similarly, there was no significant effect of treatment on the number

Table 3
Effects of light treatments on attempts and duration in 5-min tonic immobility (TI) tests and latencies to move, step, and vocalise across 5-min open field tests at 20, 23 and 26 weeks in hens[1].

Age (weeks)	Treatments[2]	Attempts	TI duration (s)	Latency to move (s)	Latency to step (s)	Latency to vocalise (s)
20	UV0	2.30 ± 0.25	106.03 ± 15.55	8.58 ± 2.62	156.67 ± 19.77	6.30 ± 1.34
	UVA	1.93 ± 0.20	88.07 ± 13.91	12.46 ± 3.29	125.7 ± 17.24	7.17 ± 1.02
	UVA/B	2.63 ± 0.29	72.23 ± 13.03	5.83 ± 1.15	139.67 ± 17.85	12.47 ± 6.63
	Test statistics	$F_{(2, 13)} = 2.35$,	$F_{(2, 13)} = 1.49$,	$F_{(2, 13)} = 2.43$,	$F_{(2, 12.92)} = 0.35$,	$F_{(2, 13)} = 0.69$,
	(df, F-ratio, P-value)	$P = 0.13$	$P = 0.26$	$P = 0.13$	$P = 0.71$	$P = 0.52$
23	UV0	3.00 ± 0.23	106.17 ± 19.47	5.97 ± 0.87	53.53 ± 15.13	18.77 ± 9.74
	UVA	2.67 ± 0.28	91.27 ± 18.31	7.47 ± 3.40	78.60 ± 18.16	29.80 ± 13.58
	UVA/B	3.63 ± 0.29	81.73 ± 17.30	8.23 ± 2.60	109.60 ± 20.90	28.93 ± 13.54
	Test statistics	$F_{(2, 13)} = 2.65$,	$F_{(2, 13)} = 0.65$,	$F_{(2, 13)} = 0.92$,	$F_{(2, 13)} = 3.00$,	$F_{(2, 13)} = 0.005$,
	(df, F-ratio, P-value)	$P = 0.11$	$P = 0.54$	$P = 0.42$	$P = 0.09$	$P = 0.99$
26	UV0	2.37 ± 0.23[b]	135.33 ± 20.16[a]	6.17 ± 1.04	73.83 ± 19.14	36.60 ± 16.32
	UVA	3.00 ± 0.26[a]	79.00 ± 16.62[b]	6.83 ± 2.20	53.47 ± 12.69	17.97 ± 9.80
	UVA/B	3.13 ± 0.27[a]	104.10 ± 20.51[b]	7.57 ± 3.83	61.77 ± 14.03	23.43 ± 11.98
	Test statistics	$F_{(2, 13)} = 4.22$,	$F_{(2, 13)} = 7.71$,	$F_{(2, 13)} = 2.06$,	$F_{(2, 13)} = 0.15$,	$F_{(2, 13)} = 1.07$,
	(df, F-ratio, P-value)	**$P = 0.04$**	**$P = 0.006$**	$P = 0.17$	$P = 0.86$	$P = 0.37$

[1]The raw means ± SEM are presented for each variable with analyses conducted on transformed data. Significant P-values (< 0.05) are indicated in bold. [a,b]Dissimilar superscript letters indicate significant post-hoc differences between the groups ($P < 0.05$).
[2]Treatments: UV0: Control light with no ultraviolet (UV) spectral light, UVA: Control light plus UVA spectral light, UVA/B: Control light plus both UVA and UVB spectral light. N = 30 hens/treatment were tested.

of steps in the open field test at 20 weeks ($F_{(2, 13)} = 0.36$, $P = 0.70$), 23 weeks ($F_{(2, 11.8)} = 1.81$, $P = 0.21$), or 26 weeks ($F_{(2, 13)} = 2.01$, $P = 0.17$) nor a significant interaction between treatment and time at any age point (($F_{(8, 312–348)} = 0.46 – 1.90$, $P \geq 0.0.06$, Fig. 1). There were significant effects of time at each age point ($F_{(4, 312–348)} = 4.74 – 33.59$, all $P \leq 0.0010$, Fig. 1) with steps generally increasing across the 5-min test (Fig. 1).

There was no significant effect of treatment on any of the variables measured in the novel object test at 20, 23, or 26 weeks including the latency to approach the novel object, latency for the first bird to peck at the novel object, number of birds that approached, and number of pecks directed at the object (all $P \geq 0.37$, Table 4). Numerically, there was more interaction with the novel objects at 20 and 26 weeks than at 23 weeks (Table 4).

3.3. H/L ratio (20, 23, 26 weeks)

There was a significant effect of treatment ($P = 0.01$) on the H/L ratio at 26 weeks of age where the hens in the UVA/B treatment showed a lower ratio than the hens in the UV0 treatment group (Table 4). There was no significant effect of treatment at 20 or 23 weeks of age (both $P \geq 0.16$, Table 4).

3.4. Induced stressor (H/L ratio, ABT, home pen locations: 27 weeks)

There was no significant effect of treatment, stressor, nor their interaction on the H/L ratio (all $P \geq 0.13$, Table 5). There was no significant effect of treatment or the interaction between treatment and stressor in the latency to step (both $P \geq 0.29$), but there was a significant effect of stressor ($P = 0.004$) with birds having a shorter latency to step after the stressor (Table 5). There was only a trend for a significant effect of treatment on the latency to eat ($P = 0.07$) where post-hoc tests showed the UVA birds were quicker to eat than the UV0 birds but neither differed from the UVA/B birds (Table 5). There was no significant interaction between treatment and stressor in the latency to eat ($P = 0.42$), but there was a significant effect of stressor ($P < 0.0001$) with birds eating quicker after the stressor (Table 5). There were no significant effects of treatment, stressor, or their interaction for the latency to vocalise (all $P \geq 0.32$, Table 5).

In the morning observation period, there was a significant effect of

Table 4
Effect of light treatments on hens' latency to approach, latency to peck and the total number of birds that approached and total number of pecks at a novel object across 3-min novel object tests at 20 (n = 1), 23 (n = 2) and 26 weeks (n = 2) as well as the heterophil/lymphocyte (H/L) ratio at 20, 23, and 26 weeks[1].

Age (weeks)	Treatments[2]	Latency to approach (s)	Latency to peck (s)	Number of hens approached	Number of pecks	H/L ratio
20	UV0	98.39 ± 16.76	148.33 ± 25.96	4.00 ± 1.79	2.83 ± 2.14	0.29 ± 0.02
	UVA	89.67 ± 17.77	107.50 ± 33.28	8.33 ± 4.37	6.00 ± 3.64	0.34 ± 0.04
	UVA/B	83.11 ± 18.12	130.17 ± 32.25	7.00 ± 3.33	11.33 ± 11.13	0.34 ± 0.03
	Test statistics	$\chi2 = 0.54$, df = 2, $P = 0.76$	$\chi2 = 0.66$, df = 2, $P = 0.72$	$\chi2 = 0.94$, df = 2, $P = 0.62$	$\chi2 = 0.48$, df = 2, $P = 0.79$	$F_{(2, 13.40)} = 0.19$, $P = 0.83$
23	UV0	129.47 ± 10.17	180.00 ± 0	2.50 ± 0.84	0.00 ± 0	0.58 ± 0.07
	UVA	111.78 ± 11.86	180.00 ± 0	3.67 ± 1.11	0.00 ± 0	0.45 ± 0.05
	UVA/B	105.97 ± 13.13	175.50 ± 4.5	4.92 ± 1.52	0.08 ± 0.08	0.42 ± 0.04
	Test statistics	$\chi2 = 1.91$, df = 2, $P = 0.38$	$\chi2 = 2.0$, df = 2, $P = 0.37$	$\chi2 = 1.31$, df = 2, $P = 0.52$	$\chi2 = 2.0$, df = 2, $P = 0.37$	$F_{(2, 13.85)} = 2.06$, $P = 0.16$
26	UV0	72.47 ± 12.74	96.33 ± 23.61	11.33 ± 3.05	19.92 ± 7.24	0.55 ± 0.05[a]
	UVA	48.75 ± 11.58	64.67 ± 17.83	14.33 ± 2.86	27.08 ± 6.64	0.46 ± 0.05[a,b]
	UVA/B	51.83 ± 12.23	72.00 ± 23.41	13.00 ± 2.97	51.83 ± 23.32	0.39 ± 0.05[b]
	Test statistics	$\chi2 = 1.86$, df = 2, $P = 0.39$	$\chi2 = 0.88$, df = 2, $P = 0.64$	$\chi2 = 0.82$, df = 2, $P = 0.66$	$\chi2 = 1.02$, df = 2, $P = 0.60$	$F_{(2, 13.09)} = 5.98$, **$P = 0.01$**

[1]The raw means ± SEM are presented for each variable with H/L ratio analyses conducted on transformed data. Different novel objects were presented for each of the 5 tests conducted in the home pens. Only data from one test are presented at 20 weeks due to one novel object being dismantled by the birds. Significant P-values (< 0.05) are indicated in bold. [a,b]Dissimilar superscript letters indicate significant post-hoc differences between the groups ($P < 0.05$).
[2]Treatments: UV0: Control light with no ultraviolet (UV) spectral light, UVA: Control light plus UVA spectral light, UVA/B: Control light plus both UVA and UVB spectral light. N = 6 pens/treatment for the novel object tests, with n = 30 hens/treatment sampled for the H/L ratio.

Table 5
Effect of a stressor where supplemental treatment lights[1] were turned off, on the heterophil/lymphocyte (H/L) ratio and latencies to step, eat, and vocalise during an attention bias test (ABT) prior to and after the stressor was applied[2].

Stressor	Treatments[1]	H/L ratio	ABT: Latency to step (s)	ABT: Latency to eat (s)	ABT: Latency to vocalise (s)
Before	UV0	0.75 ± 0.07	60.43 ± 17.0	218.20 ± 19.22	16.83 ± 9.89
	UVA	0.59 ± 0.06	42.33 ± 13.75	165.70 ± 21.70	6.50 ± 1.37
	UVA/B	0.60 ± 0.06	56.27 ± 17.25	226.73 ± 18.73	6.83 ± 1.10
After	UV0	0.61 ± 0.07	44.17 ± 12.96	173.70 ± 23.37	9.20 ± 1.99
	UVA	0.56 ± 0.04	22.83 ± 10.46	111.90 ± 22.38	5.03 ± 1.01
	UVA/B	0.56 ± 0.08	46.1 ± 16.10	157.67 ± 22.80	8.43 ± 1.97
Test statistics (df, F-ratio, P-value)	Treatment	$F_{(2, 12.09)} = 1.68$, $P = 0.22$	$F_{(2, 13)} = 1.26$, $P = 0.32$	$F_{(2, 13)} = 3.33$, $P = 0.07$	$F_{(2, 13)} = 1.26$, $P = 0.32$
	Stressor	$F_{(1, 85.72)} = 2.30$, $P = 0.13$	$F_{(1, 87)} = 8.90$, **$P = 0.004$**	$F_{(1, 87)} = 56.25$, **$P < 0.0001$**	$F_{(1, 87)} = 0.0$, $P = 0.99$
	Interaction	$F_{(2, 85.7)} = 0.66$, $P = 0.52$	$F_{(2, 87)} = 1.24$, $P = 0.29$	$F_{(2, 87)} = 0.88$, $P = 0.42$	$F_{(2, 87)} = 0.97$, $P = 0.38$

[1]Treatments: UV0: Control light with no ultraviolet (UV) spectral light, UVA: Control light plus UVA spectral light, UVA/B: Control light plus both UVA and UVB spectral light. N = 30 hens/treatment were sampled for the ABT and H/L ratio.
[2]The raw means ± SEM are presented for each variable with analyses conducted on transformed data. Significant P-values (< 0.05) are indicated in bold.

side ($F_{(1, 120)} = 27.95$, $P < 0.0001$), with more hens located on the standard side. There was also a significant interaction between side and stressor in the morning period where more hens were located on the standard side before the supplemental lights were switched off, but during the stressor period (supplemental lights off), there were equal numbers of birds on each side of the pen (Fig. 2). There was no significant interaction between treatment and side ($F_{(2, 120)} = 2.54$, $P = 0.08$), nor treatment, stressor, and side ($F_{(2, 120)} = 10.6$, $P = 0.35$). In the afternoon observation period, there was a significant effect of side ($F_{(1, 120)} = 109.47$, $P < 0.0001$) with more hens located on the supplemental side and a significant effect of treatment by side with the most hens located on the supplemental light side in the UVA, UV0 and then UVA/B group than their respective standard sides (Fig. 2). There was no significant interaction effect of side and stressor ($F_{(1, 120)} = 0.39$, $P = 0.53$), or treatment, stressor, and side ($F_{(2, 120)} = 0.05$, $P = 0.95$). In the evening observation period, there was again a significant effect of side with more hens located on the supplemental light side ($F_{(1, 120)} = 93.22$, $P < 0.0001$). There was also a significant interaction between treatment and side ($F_{(2, 120)} = 7.71$, $P = 0.0007$) where the most hens were located on the supplemental light side in the UV0, followed by UVA and UVA/B treatments than their respective standard sides (Fig. 2). There was no significant interaction effect of side and stressor ($F_{(1, 120)} = 0.06$, $P = 0.81$), nor treatment, side, and stressor ($F_{(2, 120)} = 1.68$, $P = 0.19$).

4. Discussion

The aims of this research were to determine whether supplemental UVA and UVA/B light available on one side of the pen would have positive impacts on hen behaviour, fear, and stress responses during the early laying period. Across multiple behavioural and blood sampling assessments, there were some impacts of the supplemental light treatments relative to hens under standard lighting conditions, but for the majority of measures there were no significant differences between treatment groups. There were limited effects on home pen behaviours at both 18 and 24 weeks of age. More hens were observed on specific sides of the pen, but this varied between the standard or the treatment light side depending on the bird age and time of day with limited difference in pen side preference depending on the treatment group. When the hens were older, both UV light treatments did reduce tonic immobility durations, vocalisations across time in the open field test, and the UVA/B hens had lower H/L ratios than the UV0 hens indicative of reduced fear and stress. However, removing the treatment lights did not result in greater stress and anxiety. Overall, these results align with the complementary findings presented in Rana et al. (2023) showing minimal impact of the UV treatments. The measures from that study taken on the same hens as the current study showed limited physiological effects of the UV supplementation on measures of egg production, egg quality, bone health, and circulating plasma P and Ca but with an increase in serum vitamin D ($25(OH)D_3$) for the UVA/B hens (Rana et al., 2023). The hens in these studies were able to choose to be in proximity of the supplemental lights as they were only on one side of the pens. This could have reduced the impacts of the light treatments. The effects of light supplementation may also vary across hen age when the birds are in the mid lay or end lay stages of the flock cycle as only the early lay stage was covered in the current study.

The reduced measures of fear and stress in the hens supplemented with UV light aligns with previous research that has shown similar positive impacts of providing supplemental UV light manifested as reductions in tonic immobility duration, flapping during inversion testing, H/L ratio and plasma corticosterone concentrations in hens assessed at mid- and end-of-lay (Sobotik et al., 2020). Given the ability of chickens to perceive UV wavelengths, it is hypothesized that this mechanism of effect comes from the hens being exposed to biologically relevant lighting spectrums that facilitate the hens' natural interaction with their environments (Rana and Campbell, 2021). These differences were only seen in the final assessment point at 26 weeks of age suggesting there may have been a required exposure period for effects to occur, or that effects only became discernible between treatment groups once all hens had established their peak laying patterns. However, positive effects were not clearly observed when looking at time budgets of the birds within their home pens. It was predicted that hens would exhibit more foraging and environmental pecking (desirable) under UV supplementation, but no differences were detected between lighting treatments. Behaviours may have been impacted differently if the birds had not been beak trimmed. Different supplemental spectrums may have also resulted in different impacts. The treatment lights were selected to provide UVA and the most UVB possible in a commercially available pet bulb (hence a reptile bulb was selected), but the bulbs also differed in their peak wavelengths and overall spectral composition. Thus, UV bulbs with different spectral peaks aligned with chicken visual sensitivities (Wilby et al., 2015) may have resulted in different impacts. There was more preening in the UVA/B treatment than the UV0 treatment which aligns with previous observations of hens under UVA/B lighting relative to standard lighting in choice chambers (Rana et al., 2021), but only at 18 weeks of age. Wichman et al. (2021) also did not observe lighting effects on behaviour in the home pens when birds were exposed to 'daylight (contained UV)', 'forest understory (contained UV)' or standard (no UV) lighting, yet observations in preference tests did show behavioural differences under the different light environments with more active behaviours (foraging, locomotion, standing) under the UV spectrum lights (Wichman et al., 2021). It may be that the home pens had sufficient space and resources for all hens to exhibit normal time budgets with little scope for changes as a result of the supplemental lights. The UV lights also did not increase any abnormal/undesirable behaviours. It was predicted that aggressive and severe feather pecking may have increased

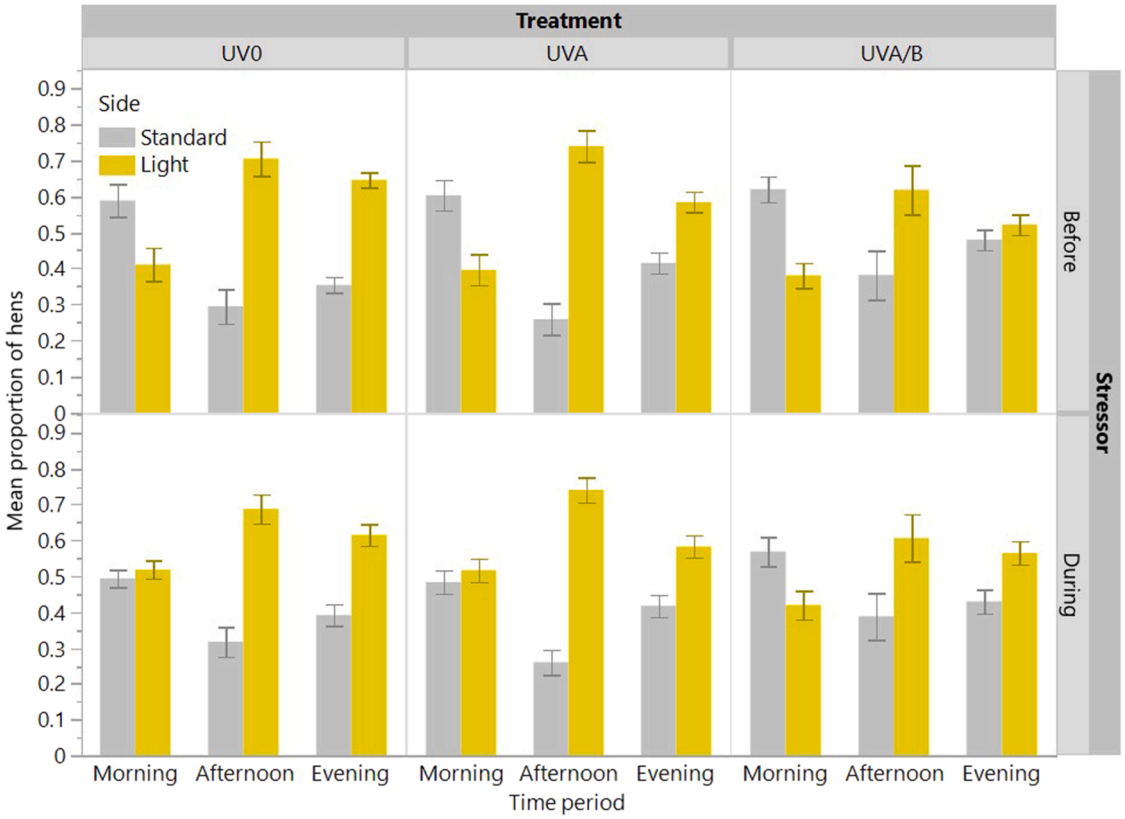

Fig. 2. The proportion of hens from three light treatment groups (UV0: Control light with no ultraviolet (UV) spectral light, UVA: Control light plus UVA spectral light, UVA/B: Control light plus both UVA and UVB spectral light; n = 6 pens/treatment) on the standard or supplemental light side of the pen in the morning (06:00 – 08:00 h), afternoon (12:00 – 14:00 h) and evening (17:00 – 19:00 h) before and during a stressor period where the supplemental lights were turned off. The standard room light schedule was 05:00 h to 21:00 h.

based on other research with UVA supplementation in a commercial setting (Spindler et al., 2020), although those commercial birds were not beak trimmed as per the hens in the current study. There were more comb wounds observed in the UV treatments in the complementary assessments conducted on the same set of hens as the current study (Rana et al., 2023) suggesting aggressive pecking may have increased under UV supplementation as the hens aged. Overall, low percentages of aggressive and severe feather pecking were observed across the early lay phase, but this behaviour may have increased across the flock cycle and/or if the birds were not beak trimmed, allowing further conclusions on the potential negative impacts of UV supplementation.

The locations of hens in the home pens and whether hens occupied the supplemental light side more than the standard side were inconsistent across age as well as time of day. When the hens were older, there was a preference for the supplemental light side, particularly in the afternoon and evening. However, this preference was not always differentiated among the treatments nor in favour of the UV supplementation. This contrasts with other studies showing preferences for UV supplemented light over standard lighting (Liu et al., 2018; Rana et al., 2021; Wichman et al., 2021) and suggests the additional lux, regardless of spectrum could be preferable. It is unclear the impact that exposure to some natural light during rearing had on the birds' preferences and this could be explored in future studies. Alternatively, the lack of clear treatment preference may be due to confounding of the resource distribution in the pen with the supplemental lights hung over the only perch in the pen. This placement of light was deliberate to ensure the hens were able to expose their legs to the supplemental light to facilitate maximum physiological benefit of the UVB wavelengths through skin absorption (Rana et al., 2023; Schutkowski et al., 2013). A single perch was used to encourage exposure to the treatment lights. However, this did mean any perching behaviour could only be on the supplemental light side. The stressor period had limited impact on the birds in terms of their pen locations as well as H/L ratios and attention bias testing. This suggests the resource removal was something the birds were able to adjust to, at least in the shorter term, aligning with the minimal effects observed on other home pen behaviours. The quicker latencies in the attention bias test following the stressor period were likely a reflection of the hens' adaptation to the arena (Campbell et al., 2019) and overcoming possible neophobia of the novel mixed grain rather than an indication that the supplemental light removal reduced anxiety.

In conclusion, the supplemental UV lights had some positive impacts for reducing fear and stress in the hens during the early lay period which aligns with other research. There were no negative impacts observed but testing with older birds in commercial settings when feather pecking may be more likely to develop could determine if UV supplementation is a risk factor for this negative behaviour.

Ethical statement

The research protocol was approved by the Animal Ethics Committee of the University of New England (AEC21–034) and housing met the requirements of the Model Code of Practice for the Welfare of Animals: Domestic Poultry (Primary Industries Standing Committee, 2002).

CRediT authorship contribution statement

Md Sohel Rana: Writing – review & editing, Visualization, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Caroline Lee:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Stephen W Walkden-Brown: Writing – review & editing, Supervision, Resources, Methodology. **Dana L.M. Campbell:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2024.106235.

References

Benson, E.R., Hougentogler, D.P., McGurk, J., Herrman, E., Alphin, R., 2013. Durability of incandescent, compact fluorescent, and light emitting diode lamps in poultry conditions. Appl. Eng. Agric. 29, 103–111. https://doi.org/10.13031/2013.42531.

Campbell, D.L.M., Dickson, E.J., Lee, C., 2019. Application of open field, tonic immobility, and attention bias tests to hens with different ranging patterns. PeerJ 7, e8122. https://doi.org/10.7717/peerj.8122.

Campo, J.L., Prieto, M.T., Dávila, S.G., 2008. Association between vent pecking and fluctuating asymmetry, heterophil to lymphocyte ratio, and tonic immobility duration in chickens. Appl. Anim. Behav. Sci. 113, 87–97. https://doi.org/10.1016/j.applanim.2007.10.008.

El-Sabrout, K., El-Deek, A., Ahmad, S., Usman, M., Dantas, M.R.T., Souza-Junior, J.B.F., 2022. Lighting, density, and dietary strategies to improve poultry behavior, health, and production. J. Anim. Behav. Biometeorol. 10, 2212–22112 doi/10.31893/jabb.22012.

Kühn, J., Wassermann, C., Ebschke, S., Schutkowski, A., Thamm, K., Wensch-Dorendorf, M., von Borell, E., Stangl, G.I., 2019. Feasibility of artificial light regimes to increase the vitamin D content in indoor-laid eggs. Poult. Sci. 98, 5177–5187. https://doi.org/10.3382/ps/pez234.

Lewis, P.D., Gous, R.M., 2009. Responses of poultry to ultraviolet radiation. World Poult. Sci. J. 65, 499–510. https://doi.org/10.1017/S0043933909000361.

Lewis, P.D., Morris, T.R., 1998. Responses of domestic poultry to various light sources. World Poult. Sci. J. 54, 7–25. https://doi.org/10.1079/WPS19980002.

Lewis, P.D., Morris, T.R., 2000. Poultry and coloured light. World Poult. Sci. J. 56, 189–207. https://doi.org/10.1079/WPS20000015.

Liu, K., Wang, K., Fei, T., Chai, L., Xin, H., 2018. Computer vision-based animal preference assessment – Do laying hen chicks prefer light with UVA radiation?, ASABE Annual International Meeting, Detroit, MI, USA. DOI: 10.13031/aim.201800193, p. 1.

Maddocks, S.A., Cuthill, I.C., Goldsmith, A.R., Sherwin, C.M., 2001. Behavioural and physiological effects of absence of ultraviolet wavelengths for domestic chicks. Anim. Behav. 62, 1013–1019. https://doi.org/10.1006/anbe.2001.1842.

McKenzie, R.L., Björn, L.O., Bais, A., Ilyasd, M., 2003. Changes in biologically active ultraviolet radiation reaching the Earth's surface. Photochem. Photobiol. Sci. 2, 5–15. https://doi.org/10.1016/S1011-1344(98)00182-1.

Oso, O.M., Metowogo, K., Oke, O.E., Tona, K., 2022. Evaluation of light emitting diode characteristics on growth performance of different poultry species: a review. Worlds Poult. Sci. J. 78, 337–351. https://doi.org/10.1080/00439339.2022.2007509.

Pettersson, I.C., Weeks, C.A., Wilson, L.R.M., Nicol, C.J., 2016. Consumer perceptions of free-range laying hen welfare. Bri. Food J. 118, 1999–2013. https://doi.org/10.1108/BFJ-02-2016-0065.

Prescott, N.B., Wathes, C.M., 1999a. Spectral sensitivity of the domestic fowl (*Gallus g. domesticus*). Br. Poult. Sci. 40, 332–339. https://doi.org/10.1080/00071669987412.

Prescott, N.B., Wathes, C.M., 1999b. Reflective properties of domestic fowl (*Gallus g. domesticus*), the fabric of their housing and the characteristics of the light environment in environmentally controlled poultry houses. Br. Poult. Sci. 40, 185–193. https://doi.org/10.1080/00071669987584.

Prescott, N.B., Wathes, C.M., Jarvis, J.R., 2003. Light, vision and the welfare of poultry. Anim. Welf. 12, 269–288. https://doi.org/10.1017/S0962728600025689.

Primary Industries Standing Committee. 2002. Model Code of Practice for the Welfare of Animals—Domestic Poultry. 4th ed. Collingwood: CSIRO Publishing.

Rajchard, J., 2009. Ultraviolet (UV) light perception by birds: a review. Vet. Med. 54, 351–359. https://doi.org/10.17221/110/2009-VETMED.

Rana, M.S., Campbell, D.L.M., 2021. Application of ultraviolet light for poultry production: a review of impacts on behavior, physiology, and production. Front. Anim. Sci. 2, 699262 https://doi.org/10.3389/fanim.2021.699262.

Rana, M.S., Cohen-Barnhouse, A.M., Lee, C., Campbell, D.L.M., 2021. Preference testing for ultraviolet light spectrum and intensity in laying hens. Poult. Sci. 100, 101063 https://doi.org/10.1016/j.psj.2021.101063.

Rana, M.S., Clay, J., Regmi, P., Campbell, D.L.M., 2023. Minimal effects of ultraviolet light supplementation on egg production, egg and bone quality, and health during early lay of laying hens. PeerJ 11, 314997. https://doi.org/10.7717/peerj.14997.

Schröder, M.J., McEachern, M.G., 2004. Consumer value conflicts surrounding ethical food purchase decisions: a focus on animal welfare. Int. J. Consum. Stud. 28, 168–177. https://doi.org/10.1111/j.1470-6431.2003.00357.x.

Schutkowski, A., Krämer, J., Kluge, H., Hirche, F., Krombholz, A., Theumer, T., Stangl, G. I., 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, 10.1371/journal.pone.0069418.

Sobotik, E.B., Nelson, J.R., Archer, G.S., 2020. How does ultraviolet light affect layer production, fear and stress. Appl. Anim. Behav. Sci. 223, 104926 https://doi.org/10.1016/j.applanim.2019.104926.

Spindler, B., Weseloh, T., Eßer, C., Freytag, S.K., Klambeck, L., Kemper, N., Andersson, R., 2020. The effects of UV-A light provided in addition to standard lighting on plumage condition in laying hens. Animals 10 (6), 1106. https://doi.org/10.3390/ani10061106.

Wichman, A., Groot, R.D., Håstad, O., Wall, H., Ruben, D., 2021. Influence of different light spectrums on behaviour and welfare in laying hens. Animals 11, 924. https://doi.org/10.3390/ani11040924.

Wilby, D., Toomey, M.B., Olsson, P., Frederiksen, R., Cornwall, M.C., Oulton, R., Kelber, A., Corbo, J.C., Roberts, N.W., 2015. Optics of cone photoreceptors in the chicken (*Gallus gallus domesticus*). J. R. Soc. Interface 12, 20150591. https://doi.org/10.1098/rsif.2015.0591.