This is the way: The effect of artificial cues on early life ramp use behaviour of laying hen chicks

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ABSTRACT

Providing laying hen chicks with ramps during the rearing phase has been shown to increase inter-tiers transitions and use of elevated surfaces within aviaries. To maximize these benefits, we aimed to investigate if artificial cues that utilize the behavioural predispositions of domestic chicks would increase ramp use during the rearing phase and whether this increase would benefit the birds by improving their spatial cognition and skeletal strength. For this, we conducted two experiments: E1 compared two light cues (Dotter and Flicker) and E2 three moving cues (Beak, Hen, and LED) with respective control groups. Identical pens with two vertically stacked tiers connected by two ramps that housed 22 LSL chicks each were used for both experiments (N = 4/treatment). Cues were applied in intermittent bouts spread throughout the day until 60 days of age (DoA). We counted the number of inter-tier transitions using ramps, as well as the active use of ramps (e.g., walk/run, jump) that did not result in a transition by scan sampling recorded videos both when the cues were on and off. In E1, 47 birds (N = ~12/ treatment) underwent a spatial cognition test during 64 – 75 DoA and biomechanical properties of the tibiae and humeri were assessed for 20 birds per treatment by performing a three-point bending test at 87 DoA. Generalized linear mixed model analysis for E1, revealed that birds from both light cue groups performed more transitions when the cues were on compared to when the cues were off ($\chi^2 = 6.18$, p = 0.03). A three-way interaction between treatment, cue status, and DoA was found for active use of ramps ($\chi^2 = 5.19$, p = 0.02) with birds performing more active behaviours on ramps when cues were on compared to when cues were off until 38 and 24 DoA for Dotter and Flicker treatments, respectively. No differences between light cues and control groups were found in bone biomechanical properties and spatial cognition. In E2, birds from the Beak treatment performed 1.5 times fewer transitions than the Control birds ($\chi^2 = 10.16$, p = 0.02). Active use of ramps was affected by an interaction between treatment and DoA ($\chi^2 = 13.33$, p < 0.01) with birds from LED groups performing more active behaviours with increasing age while it decreased in the Hen and Control group groups. Overall, birds were more responsive to the cues that utilized predispositions based on the foraging aspects than the cues based on predispositions that aided in social interactions.

1. Introduction

Laying hens are being increasingly housed in cage-free systems such as avaries, wherein resources such as food, water and nestboxes are distributed in vertically stacked tiers. The vertical distribution of the resources requires birds to negotiate movements across elevated structures that are separated by varying angles and heights, which can be difficult for the birds (Scott et al., 1997; Scott and Parker, 1994). The height and complexity of aviaries are also associated with falls and collisions (Campbell et al., 2016; Stratmann et al., 2019), which are thought to influence the incidence of keel bone fractures (Toscano et al., 2020), one of the biggest welfare concerns in the laying hen industry (Harlander-Matauschek et al., 2015). To reduce falls and collisions, and facilitate transitions between tiers, several studies have investigated the use of ramps as an aid for movement between different tiers. Provision of ramps has been shown to increase the movement between tiers and reduce behaviours indicative of hesitancy before transitions, incidence of falls, collisions, keel fractures, and footpad disorders during the laying period (Heerkens et al., 2016; Pettersson et al., 2017; Stratmann et al., 2015).

Recently, studies have focused on the provision of ramps in the rearing phase, a critical period for the behavioural, cognitive and physical development of the birds. Providing ramps during the rearing phase has been shown to facilitate earlier use of elevated surfaces and more movement between aviary tiers (Norman et al., 2021; Stratmann et al., 2022). Furthermore, Norman et al. (2021) found that providing ramps in rearing aviaries had long-lasting effects into the laying period. Birds showed fewer behaviours indicative of hesitancy when transitioning between tiers in a laying aviary and had reduced incidence of keel bone fractures compared to birds reared without ramps. The authors hypothesized that the reduction in keel fractures might be due to developmental benefits in spatial cognitive as well as skeletal properties due to early access of vertical surfaces facilitated by ramps. In support of this notion, studies on rearing birds in complex environments indicate that early access to three dimensional areas benefits the spatial cognitive (Gunnarsson et al., 2000; Norman et al., 2019; Tahamtani et al., 2015) and skeletal (Casey-Trott et al., 2017; Regmi et al., 2015) properties of the birds.

Given the short and long-term welfare benefits of early life use of ramps, it is important to explore factors that can maximize these effects. The innate predispositions of laying hen chicks to certain stimuli in combination with learned preferences from early life exposure offers a powerful tool that can be used to guide and direct the locomotion of the chicks in the rearing phase. Domestic chicks exhibit predispositions to various stimuli that aid them in important biological functions such as imprinting on the mother hen, social learning and foraging (Bolhuis, 1991; Hogan, 1973; Miura and Matsushima, 2016). For example, visually-naive domestic chicks preferentially approach a stimulus that resembles a conspecific (stuffed fowl) rather than a less naturalistic one (a scrambled version of the stuffed fowl) (Johnson and Horn, 1988; Mayer et al., 2016). Chicks also show predispositions to several characteristics that are associated with conspecifics. The chicks show unlearned preference to signs of animacy such as speed changes (Rosa-Salva et al., 2016), self-propulsion (Mascalzonia et al., 2010) and biologically relevant semi-rigid motion (Vallortigara et al., 2005) even when presented as two-dimensional shapes in simple cartoon animations. The predispositions also extend to simple characteristics such as colours (blue and red, Ham and Osorio, 2007), repetitive tones (Fischer, 1972; Tolman, 1967), flickering lights (Simner, 1975, 1974) and pecking motions made by beak-shaped objects (Suboski and Bartashunas, 1984; Tolman, 1964). These predispositions of domestic chicks can be applied to commercial settings to aid their development.

The overall aim of this study was to investigate different artificial cues that utilize the innate predispositions aiding laying hen chicks in the early life responses and behavioural development to encourage the use of ramps in their early life. We therefore conducted two experiments in which a) two different light cues (E1) and b) three different moving cues (E2) were tested against control groups without cues. In both experiments, we made two broad types of comparisons:

a) across all treatment groups in which we predicted that birds provided with artificial cues would perform more inter-tier transitions using ramps, more active behaviours on ramps that do not result in transitions (e.g., walk/run, wing-assisted incline running (WAIR)), and use elevated surfaces earlier than the birds from the respective control groups.
b) within-cue treatment groups (i.e., groups with cues only) in which we compared the ramp use behaviour during periods of time when the cues were active (cue-on) or not (cue-off) in the same group of birds. We hypothesized that birds would perform more inter-transitions using ramps and more active behaviours on ramps not resulting in transitions when the cues were on compared to when they were off.

Given the potential positive associations between spatial cognitive and skeletal properties and ramp use, we also investigated the effect of ramp use on spatial cognition and bone biomechanics of birds in E1. We hypothesized that the increase in ramp use due to the light cues would lead to improved spatial cognitive properties and greater skeletal strength in birds from the light cue groups compared to the control group.

2. Methods

2.1. Ethical approval

The experiments were approved by the Veterinary Office of the Canton of Bern, Switzerland (BE 106/19) and met all federal and cantonal regulations for the ethical treatment of animals involved in research.

2.2. Animals and housing

For both experiments, identical pens (2 m × 2 m × 2.5 m (L × W × H)) were custom-built in a barn at the Aviforum research facility, Zollikofen, Switzerland. Each pen was visually isolated from each other with opaque metal sheets up to 1.5 m of the pen walls from the floor and thick opaque plastic sheets above that, although auditory and olfactory influences were possible. The pens were equipped with two vertically stacked plastic grid tiers, a litter area on the floor spread with wood shavings (2 m × 0.75 m (L × W)), and two round metal perches (2 m × 0.34 cm (L × diameter)) at the heights of 0.28 and 0.55 m above the second tier. The first tier (2 m × 1.15 m (L × W)) was at a height of 0.25 m and the second tier (2 m × 0.6 m (L × W)) at a height of 1.2 m from the floor. Two ramps made of metal grid (1.30 m × 0.24 m (L × W), at an angle of 35) were positioned in a criss-cross manner, connecting the first floor to two small platforms made of metal grid (0.65 m × 0.25 m (L × W)) on either side of the second tier (Fig. 1A). The ramp and the platforms were 2.5 mm in thickness and the grids were 1.6 cm × 1.6 cm (L × W) in size. The birds had *ad libitum* access to food and water with feed provided on a plate on the first tier until two weeks of age (WoA) which was replaced by a food dispenser placed in the litter area from three WoA. Additionally, the birds were provided with a feed plate on the second tier until five WoA. Starter feed (Egli Mühlen AG, Nebikon, Switzerland) was provided from one until nine WoA and pullet feed (Egli Mühlen AG, Nebikon, Switzerland) from nine to 17 WoA. Water was provided through nipple drinkers positioned on the first tier. An additional bell drinker was positioned on the first tier in the first WoA. Each pen was illuminated uniformly by a light bulb (Silox basic, 120 – 3000 K) attached to the ceiling of the pen. Daylight was prevented from entering the barn to prevent any bias in bird behaviour due to differential lighting across the pens. Artificial light was provided according to the standard rearing management for LSL pullets with 24 h of light for the first two days of life, which was gradually reduced to nine hours in the fifth WoA and stayed the same until 17 WoA. The light program included a dimming phase of five minutes in the dawn and 30 min in the dusk phase.

E1 was conducted from February – May 2020 and used 264 birds arbitrarily assigned to 12 pens (N = 22 birds/pen). One bird died at seven DoA. The birds were provided with ramp access from 08:00–16:00 on the second DoA. From three DoA, birds got uninterrupted access to ramps. Access to the litter area was given from five DoA until which they were confined to the first tier using a wire mesh.

E2 was performed from September – December 2020 and used 351 birds arbitrarily assigned to 16 pens. All pens housed 22 birds each, except for one pen with 21 chicks only. One bird died at 17 DoA. The birds were provided with uninterrupted access to ramps from four DoA and litter from five DoA. Eight birds per pen were colour marked for individual identification from one DoA and served as focal birds for another study. No difference in behaviour or body weight was found between focal and non-focal birds and no aggression towards focal birds was observed.
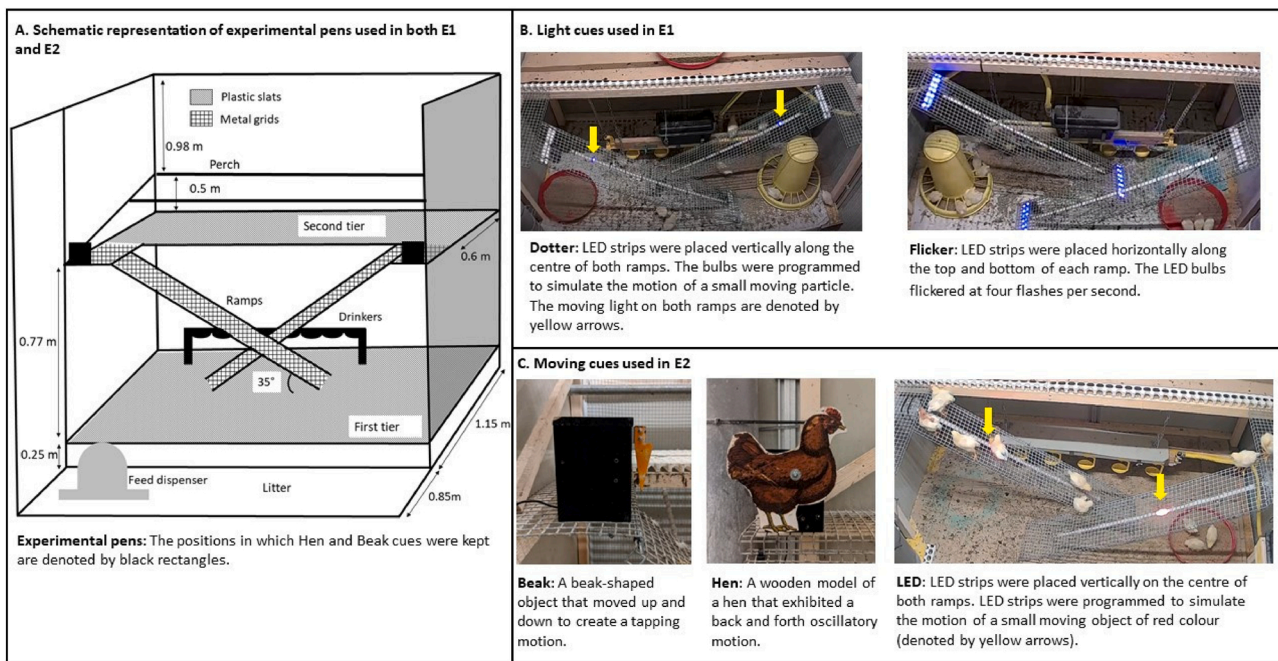
Fig. 1. A) Schematic representation of experimental pens used in both E1 (light cues) and E2 (moving cues). B) Light cues used in E1. C) Moving cues used in E2.

2.3. Experimental design

The following artificial cues were tested in the two experiments:

2.3.1. E1: Light cues

Two different light cues (both blue (RGB = [0, 0, 205])) were used and compared against a control group with no light cues resulting in a sample size of four pens randomly assigned per treatment (N = 4 pens/treatment). The light cues were provided using LED strips attached to the ramps that varied in placement and lighting pattern as follows (Fig. 1B):

1. Dotter: a 96 cm long LED strip with 28 bulbs was placed vertically along the centre of both ramps each. The bulbs were programmed to go on and off sequentially to simulate the motion of a small moving particle, i.e., only one LED on the strip was lit at a time. It took 24 s for the sequence to move from top to bottom and vice versa, at a speed of 4 cm/s.
2. Flicker: two LED strips of seven bulbs each across a length of 24 cm were placed horizontally along the top and bottom of each ramp. The LED bulbs flickered at four flashes per second at a flash duration of 0.8 ms.
3. Control: non-functional LED strips were placed both vertically along both ramps identical to the Dotter treatment and horizontally at the top and bottom of the ramps identical to the Flicker treatment.

In addition, non - functional LED strips were outfitted on ramps in both light cue treatments in positions identical to the LED strip locations of the other light treatment. For example, the ramps in the pens of the Dotter treatment were fitted with non – functional LED strips at the top and bottom of the ramps. The positioning of non-functional strips was done to ensure that ramps looked identical in all the pens except when the cues were applied.

The birds were exposed to the light cues on the second DoA from 08:00–16:00 continuously to facilitate the formation of a preference to the light stimuli as chicks are more responsive and learn best during this early sensitive period (Gray, 1960). From three DoA, the light cues were applied daily as 10 min bouts with an interval of 20 min between each bout. The number of light cue bouts was reduced as the chicks aged.

Light cues were applied for approximately 33.3% (16 bouts) of the total light period (16 h) in the first WoA, after which it was reduced sequentially to 20.4% (9 bouts) of the total light period (10 h) at five WoA after which it stayed the same.

2.3.2. E2: Moving cues

Three different moving cues were tested against a control group with no cues resulting in a sample size of four pens per treatment (N = 4 pens/treatment). The following three moving cues were used (Fig. 1C):

1. Beak: A beak-shaped orange coloured object that mechanically moved up and down to create a tapping motion. The beak was in the shape of an isosceles triangle with sides of 7.7 cm each and a base of 3 cm. The mechanical beak was attached to a black wooden box (12.5 cm × 7 cm × 16 cm (L × W × H)). The tapping speed of the beak changed after every minute and followed the sequence of 90, 120, 60, and 240 taps per minute.
2. Hen: A wooden model of a hen (18 cm × 0.6 cm × 18.5 cm (L × W × H)) with an image of a hen pasted over it that exhibited a back and forth oscillatory motion on a fixed axis. The model hen was attached to a black wooden box (10 cm × 15 cm × 14 cm (L × W × H)) and changed speed after every minute following the speed sequence of 10 oscillations per 12, 8, 14, and 10 s each.
3. LED: Two LED strips of 100 cm and 60 LED bulbs each were placed vertically on the centre of the ramps. The LED strip was programmed to stimulate the motion of a small moving object of red colour ((RGB = [225, 48, 0]). Three LED bulbs went on and off simultaneously in a sequential manner to simulate the continuous movement of a moving object. The speed of the flashing LED bulbs changed after every minute following a sequence of 76, 30, 23, and 46 cm/s respectively. The LED cue was comparable to the dotter cue from E1 but more animated because of the continuity in motion due to more closely spaced LED bulbs and speed change.
4. Control: The control pens did not have any cues.

The pens were assigned to treatments in a semi-random manner (Supplementary information, Fig. 1). Since the beak cues produced a tapping sound, we assigned the pens in a manner that controlled for the effect of tapping sound on other cues. The beak cues were assigned to

pens at the corners of the barn to minimize the contact with other pens. The three pens surrounding the beak pens belonged to three different treatments and were assigned randomly. We measured the sound levels in all pens using a sound meter (Voltcraft, SL-100 3 1) to quantify the sound produced by the beak cue. There was no significant difference between sound levels at the centre of the pens when the beak cues were operational or not (Supplementary information, Table 1). Non-operational cues as used in E1 were not used in E2 due to the multiple differences in appearance of the cues, hence we did not control for the appearance of the pens and ramps when the cues were not running. The moving cues were placed on the first tier close to the feeder plate and bell drinker for the first three DoA to induce a familiarity with the cues. During this time, the cues ran intermittently in four-minute bouts every hour. From four DoA onwards, the LED strips were fitted on both ramps and the Beak and Hen cues were placed on the second tier near the top end of both ramps. The cues were applied as four-minute bouts for a total of 6.25% (15 bouts) of the total light period which was gradually reduced to 5% (7 bouts) of the total light period at five WoA after which it stayed the same.

For both experiments, application of cues in bouts and the reduction in the number of bouts with increasing age served to lessen habituation to the cues. The cues were not applied for 90 min after lights went on and 80 min before lights went off as birds feed maximally during these periods (Savory, 1980). The cues were not applied during the routine care management of the chicks, which took place at predetermined slots of one hour in the morning and 40 min in the afternoon for E1 and two slots of 40 min each in the morning and one slot of 50 min in the afternoon for E2. In both experiments, the artificial cues were applied until nine WoA.

Table 1
Ethogram of the behaviours analysed from video observations for E1 (light cues) and E2 (moving cues).

Behaviours	Mode		Definition
Transition: The bird moves from first tier to second tier or vice-versa	Using ramp[a]	Walk/ run	The bird moves from one tier to another by walking /running on the ramp
		WAIR[b]	The bird moves from one tier to another by WAIR on the ramps
	Without using ramp	-	The bird jumps or flies from one tier to another without using ramps
Active use of ramps:[c] The bird performs a movement on the ramp which does not result in transition	-	Walk/ run	The bird walks/runs on ramp that does not result in a transition
	-	WAIR	The bird performs WAIR on ramp that does not result in a transition
	-	Jump/ fly	The bird jumps on or from a ramp
Distribution	Second tier	-	Number of birds on the second tier of the pen
	Perch	-	Number of birds on both perches of the pen

[a] When a bird employed multiple modes of locomotion to complete a transition using a ramp, the transition was assigned to the mode used to traverse more than 50% of the distance on the ramp. For example, if a bird moved from the first to the second tier using a ramp by employing WAIR to move 25% of the distance of the ramp and walked for the rest, the transition was recorded in walk/run mode.

[b] Wing-assisted incline running

[c] The active use of ramps was counted as two events if they were separated by at least 5 s

2.4. Data collection

2.4.1. Video observations and analysis

The behaviour of the birds was recorded at regular intervals until nine WoA using a single camera (Samsung SCO-2080R, IR, Samsung Techwin CO., Korea) fitted in each pen and customized recording software (Multieye Hybrid Recorder Version 2.3.1.8, Artec Technologies AG, Diepholz, Germany).

For E1, the recorded videos were analysed at 3, 4, 10, 11, 17, 24, 31, 38, and 59 DoA on pen level to quantify the behaviours as described in Table 1. Bouts analysed for each day were selected in a manner that accounted for the changing light period and thereby controlling for circadian rhythm-related activity pattern. Each day was divided into four quarters and the fifth and sixth minute of the first light cue bout that occurred in each quarter was analysed (i.e., cue-on). To compare behaviours in periods when the cues were on and off in the cue treatment groups, two minutes per video when the light cues were off, which followed the analysed light bout by twenty minutes, were analysed as well (i.e., cue-off). For example, if the light cue bout was applied from 09:00–09:10, the 9:05 and 9:06th minutes were analysed for the cue-on period, and 9:25 and 9:26th minutes were chosen for the cue-off period. The distribution of birds within the pen was recorded by pausing the video at the end of the two-minute observation for each bout and counting the number of birds in each zone as specified in Table 1.

For E2, the same behaviours were analysed at 4, 6, 10, 12, 20, 27, 41, and 55 DoA. For E2, the bouts for analysis were chosen in the same manner as for E1, but three minutes of video were analysed per bout compared to two minutes for E1.

For both experiments, the number of transitions using ramps was obtained by summing all transitions performed using WAIR as well as walk/run for each observation period (two minutes for E1 and three minutes for E2), resulting in eight observation bouts (four observation bouts each for cue-on and cue-off) per day per pen. Active use of ramps was obtained similarly by summing all WAIR, walk/run and jump/fly events that did not result in a transition (i.e., four observation bouts for both cue-on and cue-off per day per pen).

2.4.2. Spatial cognition test - E1

In the 10th and 11th WoA, three birds per pen (N = 12 birds per treatment) underwent a spatial cognition test in an experimental pen (3 m × 2 m × 2.5 m (L × W × H)) located in a separate room adjacent and connected to the barn. The test involved birds navigating two ramps made of a metal grid to obtain a food reward located on a platform at a height of 2 m from the floor. The first ramp (1.30 m × 0.24 m (L × W), at an angle of 50) connected the floor to a platform (P1, 40 cm × 30 cm (L × W)) at a height of 1 m. The second ramp (1.70 m × 0.24 m (L × W), at an angle of 36) connected P1 to another platform (P2, 40 cm × 30 cm (L × W)) located 90° to its right, at a height of 2 m from the ground and 1 m away horizontally from P1 (Supplementary information, Fig. 2). Additionally, a removable platform (P3), identical and situated opposite to P1 was used during training of the birds. The aim of training to P3 was to provide the birds with the experience of accessing the reward from a platform using a ramp without providing experience to the path they had to navigate for the tests. The habituation and training procedures are provided in Supplement 1. For the test, the birds were placed on the floor, and the reward was placed on P2 after tapping the cup on the platform three times. P3 was absent during the test. The birds were given five minutes to access the reward on P2 using the ramp. We recorded the time taken to reach the floor from P2 and behaviours indicative of hesitancy such as head orientations, crouched walk, pause on the ramp, and turning around while using the ramp, following the ethogram from Pettersson et al. (2017).

2.4.3. Bone Biomechanics - E1

At 13 WoA, 60 birds (5 birds per pen, 20 birds per treatment) were arbitrarily selected from different pen areas (i.e., two from the first tier,
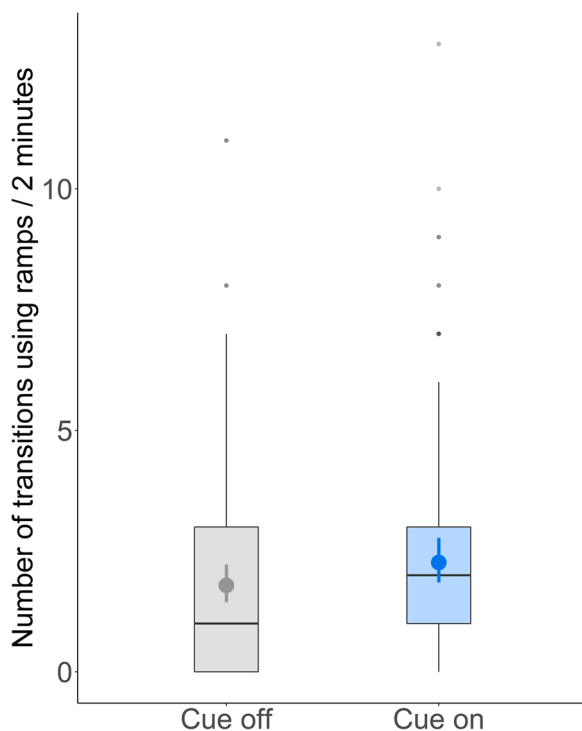
Fig. 2. Inter-tier transitions performed using ramps: Within-cue treatment comparison in E1 (light cues). (GLMM, main effect: Cue status, $\chi^2 = 6.18$, p = 0.03). Boxplots represent raw data and point range is back transformed model estimates with 95% confidence intervals.

and one each from litter, second tier and perches in each pen), killed by an overdose of barbiturate (Eskonarkon; active substance: Pentobarbitalum natricum 300 mg/1 mL) administered via an intravenous injection (120 mg/kg). Birds were subjected to cervical dislocation after testing reflex actions such as pupil response, to ensure death. The birds were then weighed and dissected to remove the right tibiae and humeri. The bones were stored at − 20 °C until they underwent three-point biomechanical testing following the ASABE Standards 2007 (ANSI/ASAE S459 MAR1992 (R2007)) using a Zwick and Roell Universal Testing Machine with a 2.5 kN load cell after thawing for at least 24 h at 15 °C (Toscano et al., 2015). The bones were laid in the test apparatus with the flattest side down and the force applied to the mid-shaft by a loading bar at a speed of 10 mm/min from which the force deformation curve was read (Toscano et al., 2015) and the peak force (Newtons) recorded. Bone stiffness (N/mm) was calculated as the slope of the load/displacement curve. Total work (J) done to fracture was obtained by calculating the total area under the entire load/displacement curve.

2.4.4. Statistical analysis

All analyses were performed using R (version 4.1.1, R Core Team, 2021) with R studio (RStudio Team, 2021) as the graphical interface. We used the package 'lme4′ (Bates et al., 2015) to fit linear mixed effects models (LMM) and the package 'glmmTMB' (Brooks et al., 2017) to fit generalized linear mixed effects models (GLMM) to account for the hierarchical structure of the data. The model assumptions for LMM were checked visually using q-q plots and model residual plots. The package 'DHARMa' (Hartig, 2020) was used to inspect the normality and homoscedasticity of the residuals for GLMM. Data were transformed when the model assumptions were not met. Likelihood ratio tests were used to test the effect of explanatory variables. Non-significant interactions (p > 0.05) were dropped from the model. *Post-hoc* comparisons of explanatory variables were performed by Tukeys HSD with a Bonferroni correction using the R package 'emmeans' (Lenth, 2021). The package 'ggplot2' (Wickham and Chang, 2016) was used to visualize the data

and the package 'tidyverse' (Wickham et al., 2019) was used to clean and organize data.

In both experiments, two different types of comparisons were conducted in order to compare the effect of the cues across all treatments including the control groups as well as within-cue groups for differences between the cue-on and cue-off periods.

Across treatments comparison: For the across treatment comparison, only observations during the cue-on period were used and compared across all treatments including the control groups. Both behaviours, i.e., transitions and active use of ramps were used as response variables and each fitted with a GLMM including treatment, DoA, and their interaction as explanatory variables. For E1, the variable treatment had three levels (i.e., Dotter, Flicker, and Control) and E2 had four levels (i.e., Beak, Hen, LED and Control). For both experiments, DoA was included as a continuous variable (E1: 3, 4, 10, 11, 17, 24, 31, 38, and 59; E2: 4, 6, 10, 12, 20, 27, 41, and 55). Each model included bouts nested in pen as a random factor. To account for the hierarchical dependency arising from observations on the same day we added date as a crossed random factor. All models were fitted with a generalized Poisson distribution. For transitions using ramps in E2, the model also included DoA as zero-inflation parameter to account for the zeros that arose due to seldom use of ramps at younger ages and the short length of the observation time points.

Within-cue treatments comparison: The within-cue treatments analysis (i.e., cue-on vs. cue-off) was performed for both transitions and active use of ramps, and only included treatment groups that had cues. For each response variable, we fitted a GLMM with treatment (i.e., Dotter and Flicker for E1 and Beak, Hen, LED for E2), cue status (i.e., cue-on vs. cue-off), and DoA (i.e., 3, 4, 10, 11, 17, 24, 31, 38, 59 for E1 and 4, 6, 10, 12, 20, 27, 41, 55 for E2 as continuous variables) as well as their two-way and three-way interactions as explanatory variables. Each model included bouts nested in cue status nested in pen crossed with date as random factors. A zero-inflation parameter that specified that the probability of observing structural zeroes depended on DoA was included for transitions using ramps in E2. Each model was fitted with a generalized Poisson distribution.

Mode of ramp use: The mode of transitions as well as active behaviours on ramps for both E1 and E2 were summarized and expressed as percentage occurrence per day.

Use of elevated surfaces: To assess the effect of light cues on the use of elevated surfaces, a GLMM was fitted with the sum of number of birds on the second tier and perches as a response variable. Explanatory variables included in the model were treatment (i.e., E1: Dotter, Flicker, and Control; E2: Beak, Hen, LED, and Control), DoA (i.e., 10, 11, 17, 24, 31, 38, and 59 DoA for E1 and 10, 12, 20, 27, 41, and 55 DoA for E2 as continuous variables) and their interaction. Bouts nested in pen crossed with date was added as a random factors in each model. For E2, an additional quadratic term of DoA was included as an explanatory term and DoA was included as a zero-inflation parameter. All models were fit with a generalized Poisson distribution.

Spatial cognition - E1: Time taken to reach the reward was square root-transformed and the number of hesitancy behaviours was log-transformed before LMM analysis, with treatment (i.e., Dotter, Flicker, and Control) as an explanatory variable and pen as a random factor.

Bone biomechanics – E1: To assess the effect of light cue application on bone biomechanical properties, peak force required to fracture the bones, bone stiffness, and work required to fracture were used as response variables and analysed using LMM with body weight included as a covariate. Bone stiffness and work required to fracture was log-transformed before analysis. Bone type (i.e., humerus and tibia), treatment (i.e., Dotter, Flicker, and Control) and their interaction were used as explanatory variables and bird ID nested in pen was used as a random factor.

The data and code for all analysis can be found at doi.org/10.17605/OSF.IO/3VWJ6.

3. Results

3.1. Behaviours on ramps

E1 light cues: Birds used ramps almost exclusively for transitions with 98.4%, 98.2% and 98.2% of all transitions occurring with ramps in the Control, Dotter, and Flicker groups, respectively. Birds performed three, six, and five transitions without ramps while 238, 289, and 221 transitions happened through ramps in Control, Dotter, and Flicker groups, respectively. No transitions were observed on the third DoA and only seven transitions occurred on the fourth DoA. These days were excluded from the analysis of transitions using ramps.

Across treatments comparison: No effect of treatment, DoA or their interaction was found for the number of transitions ($p > 0.05$, Estimated marginal means (EMM) $\pm$ CI of treatment groups: Dotter = 2.47 [2.02, 3.03], Flicker = 2.11 [1.70, 2.62], Control = 2.04 [1.64, 2.55]) as well as for active behaviours on ramps ($p > 0.05$, EMM $\pm$ CI of treatment groups: Dotter = 4.10 [3.01, 5.18], Flicker = 3.50 [2.55, 4.45], Control = 3.44 [2.51, 4.38]).

Within-cue treatments comparison: Birds from both light cue groups performed more transitions when the cues were on compared to when the cues were off ($\chi^2 = 6.18$, $p = 0.03$, EMM $\pm$ CI: cue-on = 2.26 [1.92, 2.67], cue-off = 1.79 [1.50, 2.13], Fig. 2). In addition, the number of transitions increased with DoA in both groups ($\chi^2 = 6.18$, $p = 0.01$, EMM $\pm$ CI for selected ages: DoA 11 = 1.66 [1.27, 2.16], DoA 38 = 2.29 [1.86, 2.83], DoA 59 = 2.96 [2.06, 4.24]).

A three-way interaction between treatment, cue status, and DoA was found for active behaviours on ramps ($\chi^2 = 5.19$, $p = 0.02$, Fig. 3). *Post-hoc* analysis revealed that birds from the Dotter treatment performed more active behaviours on ramps when cues were on compared to when cues were off until 38 DoA after which no difference was found (cue-off/cue-on ratio of EMM for selected ages in Table 2). In comparison, birds from the Flicker group performed more active behaviours on ramps when cues were on until 24 DoA. The trend of birds performing more active behaviours continued until 40 DoA after which it was reversed (Table 2).

E2 moving cues: Similar to E1, birds highly preferred ramps for transitions with 97.9%, 98.7%, 98.3% and 97.8% of all transitions occurring through ramps in Beak, Hen, LED, and Control treatments, respectively. Birds performed 16, 9, 13, and 18 transitions without the use of ramps while 745, 664, 750, and 804 transitions were using ramps in Beak, Hen, LED, and Control groups, respectively. Birds performed six transitions on the fourth DoA and 39 on the sixth DoA.

Across treatments comparison: Treatment affected the number of transitions ($\chi^2 = 8.27$, $p = 0.04$, EMM $\pm$ CI: Control = 3.67 [2.62, 5.15], Beak = 2.60 [1.88, 3.60], Hen = 3.11 [2.23, 4.32], LED = 3.32 [2.38, 4.63]) with birds from the Beak treatment performing 1.4 times fewer transitions than the Control birds ($p = 0.02$). No difference was found when comparing the Beak to the LED and Hen treatment ($p > 0.05$) and no effect of DoA was observed for the number of transitions.

Active use of ramps was affected by an interaction between treatment and DoA ($\chi^2 = 13.37$, $p < 0.01$, Fig. 4). The analysis for differences in slopes for predicted trend lines between treatment groups and DoA (Estimated slopes $\pm$ CI: Control = - 0.01 [- 0.02, 0.001], Beak = - 0.005 [- 0.02, 0.008], Hen = - 0.01 [- 0.03, −0.002], LED = 0.009 [- 0.02, 0.02]) revealed a difference between the LED and Control ($p = 0.02$) as well as Hen ($p < 0.01$) groups with birds from LED groups performing more active behaviours with increasing age while the number of active behaviours decreased with age in both Control and Hen groups (Table 2). No difference was found for the other treatment comparisons ($p > 0.05$).

Within-cue treatments comparison: The number of transitions was affected by an interaction of treatment and cue status ($\chi^2 = 8.73$, $p = 0.01$). *Post-hoc* analysis revealed that birds from the Beak group performed fewer transitions when the cues were on compared to when the cues were off ($p = 0.03$, EMM $\pm$ CI, cue-on = 2.94 [1.89, 4.58], cue-off = 4.54 [2.96, 6.96]). There was no difference in other treatment groups and DoA did not affect the number of transitions.

A three-way interaction between treatment, cue status, and DoA affected the active use of ramps ($\chi^2 = 12.80$, $p < 0.01$). *Post-hoc* analysis revealed a difference in slopes for predicted trend lines between cue status (cue-on vs. cue-off) and DoA in the Hen group ($p = 0.03$,
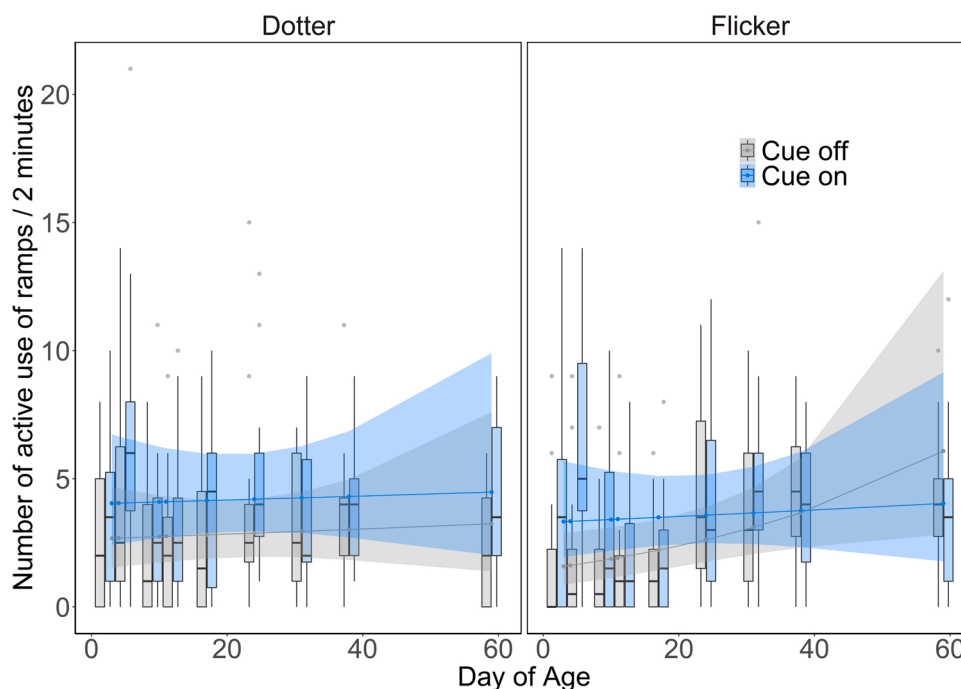

Fig. 3. Effect of treatment and cue status on active behaviours on ramps that did not result in transitions with increasing age (GLMM, effect of Treatment*Cue status*DoA, $\chi^2 = 5.19$, $p = 0.02$) from within-cue treatment comparison in E1 (light cues). Points connected by lines represent back-transformed estimated marginal means and the shaded ribbon represents 95% confidence interval.

Table 2

Estimated Marginal Means (EMM) from post-hoc analysis of GLMM models for active use of ramps for selected days from E1 (light cues) and E2 (moving cues).

| Analysis	Explanatory variable	Comparison	Ratio of EMM	SE	p-value
E1, Within-cue treatments	Treatment*Cue status*DoA	Dotter, cue-off vs cue-on, DoA 11	0.67	0.08	< 0.01
		Dotter, cue-off vs cue-on, DoA 24	0.69	0.07	< 0.01
		Dotter, cue-off vs cue-on, DoA 38	0.70	0.09	0.05
		Dotter, cue-off vs cue-on, DoA 59	0.73	0.17	1.00
		Flicker, cue-off vs cue-on, DoA 11	0.56	0.08	< 0.001
		Flicker, cue-off vs cue-on, DoA 24	0.73	0.08	0.03
		Flicker, cue-off vs cue-on, DoA 38	0.98	0.10	1.00
		Flicker, cue-off vs cue-on, DoA 59	1.50	0.32	0.40
E2, Across treatments	Treatment*DoA	Hen/LED, DoA 12	1.17	0.17	1.00
		Hen/LED, DoA 27	0.82	0.11	0.83
		Hen/LED, DoA 41	0.59	0.11	0.03
		Hen/LED, DoA 55	0.43	0.11	0.01
		Control /LED, DoA 12	1.34	0.19	1.00
		Control /LED, DoA 27	1.01	0.13	1.00
		Control /LED, DoA 41	0.77	0.13	0.84
		Control /LED, DoA 55	0.59	0.15	0.21
E2, Within-cue treatments	Treatment*Cue status*DoA	Hen, cue-on vs cue-off, DoA 12	0.80	0.12	1.00
		Hen, cue-on vs cue-off, DoA 27	1.08	0.13	1.00
		Hen, cue-on vs cue-off, DoA 41	1.44	0.26	0.36
		Hen, cue-on vs cue-off, DoA 55	1.93	0.45	0.08
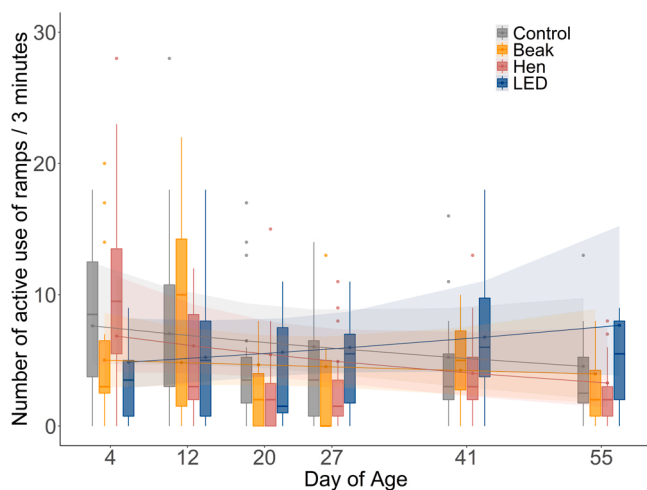
Fig. 4. Effect of treatment and DoA on active behaviours on ramps that did not result in transitions in E2 (moving cues) from across treatment analysis (GLMM, effect of Treatment*DoA, $\chi^2 = 13.37$, p < 0.01). Points connected by lines represent back-transformed estimated marginal means and shaded ribbons represent 95% confidence intervals. Two points above 30 (data point 43 from DoA 12/Hen group, and 42 from DoA 4/Beak group) were removed for better visualization.

Estimated slopes ± CI: cue-on = - 0.03 [- 0.04, - 0.01], cue-off = - 0.006 [- 0.02, 0.007]). However, the pairwise comparison of cue-on vs cue-off for individual days did not reveal any differences in the number of active behaviours performed on ramps (Table 2). No difference was found for the other treatment comparisons (p-value and Estimated slopes ± CI: LED, p = 1.00, cue-on = - 0.003 [- 0.02, - 0.009], cue-off = - 0.01 [- 0.03, - 0.0003], Beak, p = 0.6, cue-on = - 0.02 [- 0.03, - 0.003], cue-off = - 0.003 [- 0.02, 0.009]).

3.2. Mode of ramp use

Walking and running were the predominant modes of behaviour when using the ramps accounting for 87.2% and 89.3% of all transitions and 73.2% and 66.9% of all active behaviours that did not result in a transition for E1 and E2, respectively (Table 3). Transitions and active use of ramps using WAIR decreased with increasing age for both E1 and E2. Jump/fly behaviours increased with age peaking at three WoA for E1 and four WoA for E2, after which they showed a slight decrease.

3.3. Use of elevated surface

E1: Only two and four birds were observed on elevated surfaces at DoA three and four, respectively thus these days were excluded from the analysis. Use of elevated surface was affected by DoA ($\chi^2 = 3.91$, p = 0.04) with more birds using elevated surfaces with increasing DoA (EMMs ± CI for selected DoA: DoA 10 = 3.14 [2.28, 4.33], DoA 24 = 3.88 [3.08, 4.88], DoA 38 = 4.79 [3.68, 6.23]).

Table 3

Percentage occurrence of different modes of ramp use for transitions and active behaviours from E1 (light cues) and E2 (moving cues).

	Transitions with ramps					Active behaviours on ramps						
	E1			E2			E1				E2		
Week of age	Percentage of		Total no.	Percentage of		Total no.	Percentage of			Total no	Percentage of			Total no
	WAIR*	walk/run		WAIR	walk/run		WAIR	Jump /fly	Walk/run		WAIR	Jump/fly	Walk/run	
1	0	100	9	20	80	5	18.64	10.65	70.7	413	17.99	15.31	66.70	1117
2	29.84	70.16	191	17.22	82.78	482	12.13	10.46	77.4	239	10.12	26.14	63.73	830
3	17.48	82.52	206	9.52	90.48	504	3.64	26.72	69.63	247	4.41	28.54	67.05	431
4	7.33	92.67	232	6.33	93.67	537	0.00	31.37	68.62	408	6.08	26.13	67.79	444
6	1.79	98.21	223	1.89	98.11	530	1.02	24.1	74.87	390	2.15	26.90	70.96	606
8	3.21	96.79	218	0.75	99.25	534	0.00	22.94	77.05	340	2.02	18.20	79.78	544

The modes of behaviours were summarized and percentages calculated for 4, 11, 17, 24, 38, and 59 DoA for E1 (N = 4 pens/treatment, 12 pens in total) and 4, 12, 20, 27, 41, and 55 DoA for E2 (N = 4 pens/treatment, 16 pens in total). For E1 the time slot analysed per bout was two minutes while for E2 it was three minutes.
*Wing-assisted incline running

E2: No birds were observed on elevated surfaces at four DoA. Sixty-nine birds distributed over 19 observations over a total of 128 observations (8 observations each for 16 pens) were observed on elevated surfaces on the sixth DoA. These days were excluded from analysis because of the large number of zeros as observations. No effect of DOA was found for the use of elevated surfaces. However, the number of birds using elevated surfaces rose steeply from DoA six to ten after which it plateaued.

3.4. Spatial Cognition – E1

In the spatial cognition test, all birds except four (two birds each from Flicker and Dotter groups) reached P2, which were excluded from the analysis. Additionally, one bird from the Control group that failed to access the reward from P3 after seven learning trials was dropped from further training and subsequently from the test. No difference between treatments were found for the time taken to reach the reward ($\chi^2 = 1.58$, p = 0.45, EMM ± CI: Dotter (in seconds) = 41.8 [11.9, 90.9], Flicker = 55.8 [19.4, 111.0], Control = 32.1 [6.89, 75.7]) nor the total number of hesitancy behaviours performed ($\chi^2 = 2.40$, p = 0.30, EMM ± CI: Dotter = 4.36 [2.66, 7.13], Flicker = 5.87 [3.59, 9.61], Control = 4.26 [2.62, 6.92]) by the birds.

3.5. Bone biomechanics – E1

Peak force ($\chi^2 = 56.44$, p < 0.001), total work needed to fracture the bone ($\chi^2 = 32.98$, p < 0.001), and stiffness ($\chi^2 = 176.9$, p < 0.001) were affected by bone type with humeri having greater magnitude for all three biomechanical properties than tibiae. No differences were found for peak force (p > 0.05, EMM ± CI (in Newton), Humeri: Dotter = 132.0 [126.0, 165.0], Flicker = 131.0 [126,138], Control = 138.0 [132,144]; Tibiae: Dotter = 110.0 [105.0, 115.0], Flicker = 119.0 [113.0, 124.0], Control = 117 [112,123]), total work needed to fracture the bone force (p > 0.05, EMM ± CI (in Joules), Humeri: Dotter = 0.42 [0.39, 0.451], Flicker = 0.42 [0.39, 0.45], Control = 0.43 [0.40, 0.46]; Tibiae: Dotter = 0.37 [0.37, 0.40], Flicker = 0.36 [0.34, 0.39], Control = 0.35 [0.33, 0.38]) and bone stiffness (p > 0.05, EMM ± CI (in N/mm), Humeri: Dotter = 157.8 [149.6, 166.0], Flicker = 157.2 [149.0, 169], Control = 165.0 [157.7, 174]; Tibiae: Dotter = 96.4 [88.2, 105], Flicker = 98.3 [90.1, 106], Control = 99.7 [91.6, 108]) for the different treatments.

4. Discussion

The overarching aim of the study was to investigate if artificial cues would increase the early life use of ramps in laying hen chicks. We predicted that birds provided with artificial cues during the rearing phase (i.e., light and moving cues) would use the ramps more, perform more inter-tier transitions and use elevated surfaces earlier compared to birds reared without cues, which could only be partly confirmed in this study. Our results showed that birds increased their overall ramp use when the light cues (Dotter, Flicker, and LED) were on, while the ramp use was less in the Beak treatment compared to the Control, and the Hen treatment did not seem to alter the overall ramp use of chicks. No effect of cues was found on the use of elevated surfaces as well as outcomes of spatial cognition and skeletal properties.

Behavioural responses of chicks to objects in their environment and the development of these responses are influenced to a great extent by certain innate and learned preferences, social learning and facilitation, and age related tendencies in exploration and avoidance of novelty (Andrew and Brennan, 1983; Bolhuis, 1991; Nicol, 2004; Mascalzoni et al., 2010). A combination of these preferences and motivations might have driven the responses of the chicks to the cues in the current study.

Birds increased ramp use as a response to light cues (Dotter and Flicker from E1, LED from E2) with the cues based on predispositions related to foraging aspects of the birds (LED and Dotter) being

particularly effective throughout the observation period. Chicks were observed pecking at the LED bulbs and following the sequentially flashing lights with their head oriented downwards towards the light dots in both Dotter and LED groups, which might be indicative of following prey and increased ramp use. Meyer et al. (2019) used an approach similar to Dotter and LED and found that a moving red-light dot projected on to the floor improved the walking distance and active behaviours in broiler chicks. The authors hypothesized that the response of the birds might be explained by either the pecking preference of the birds to small particles (Hogan, 1973) or the predatory nature inherent to the domestic hen's red jungle fowl ancestors (Fernandez-Juricic et al., 2004). The response of following lights observed in the current study seemed to be governed by the properties of the light cues such as its speed and size as chicks were observed to be moving away from the LED cue during the first two weeks, especially at the highest speed, which may be an indicator for a fear response (Mills and Faure, 1990). Consequently, the LED cue was only able to evoke a following response from three WoA onwards, which was reflected in the ramp use behaviour as LED birds performed more active behaviours on ramps than Control birds after three WoA. Unlike the LED and Dotter, the Flicker cue was not able to sustain the response of the birds beyond four WoA. Although not studied for flickering lights, certain early life predispositions are shown to be governed by the developmental processes of maturation and decline with age. For example, early life predisposition to preferentially attend to changes in speed has been shown to disappear by three DoA in at least three chicken breeds (Padovana, Polverara, and Robusta maculate) (Versace et al., 2019). Early life predispositions are not rigid responses that are present throughout the life of chicks and this might be a reason that the effectiveness of the Flicker cue diminishes after 4 WoA.

Compared to the light cues, the Beak and Hen cues that were designed to utilize the early life predispositions aiding in imprinting and social interactions were not effective in promoting ramp use. Behavioural predispositions of birds that help in imprinting and conspecific recognition are known to be influenced by social context, which could have affected the effectiveness of the two cues (James, 1960). Chicks become familiar with and somewhat attracted to each other on their first day together (Porter et al., 2005) and in commercial hatcheries they might imprint on each other (Nicol, 2015). Hence rearing chicks together with their conspecifics might have rendered the Beak and Hen cues less effective. The Beak cue that was designed to stimulate the social facilitation of feeding behaviour (Suboski and Bartashunas, 1984) had an opposite effect as expected as the number of transitions was reduced when compared to the Control group. The birds did not appear to be fearful of the Beak, thus further investigations are required to elucidate the response of the birds to the Beak cue.

Overall, we did not find differences between treatment and control groups which might be due to several reasons. First, the noise associated with moving on the ramps, including the vocalizations, as a response to the cues in the treatment pens could have influenced the behaviour of birds in the adjacent control pens as the pens in both experiments were only visually isolated but birds could still hear one another. Thus the increase in ramp use when the cues were on in the treatment pens might have led to birds using the ramps in the control pens as well. Another reason for the lack of differences between control and treatment groups might be a ceiling effect. The frequency of ramp use might have been close to the upper limit due to the small group size and small experimental pens in which the probability of birds encountering and using ramps might be very high per se, which might have reduced the magnitude of change in ramp use the cues could generate. If this was indeed the case, even if the cues were effective, the amount of ramp use could not be increased, an explanation supported by the high numbers of transitions with ramps observed in all groups.

Our study also found an age-dependent change in ramp use. Birds showed an increase in the number of transitions with age in both replicates. Active use of ramps stayed the same throughout the observation

period in E1, while it showed a slight decrease with age in all treatment groups except for the LED groups in E2. Use of elevated surfaces was also related to age with sparse use of the second tier in the first WoA, which then increased between the second WoA and fourth WoA where it stabilized in both E1 and E2, which is consistent with other studies (Kozak et al., 2016; Norman et al., 2019). A combination of factors such as behavioural, physical and morphological development of the birds as well as resource distribution within the home pen might explain the influence of age on the use of ramps and elevated surfaces. In nature, chicks begin to explore their environment and move away from their mother intermittently from the second WoA (Wood-Gush and Duncan, 1976). Similarly, chicks are motivated to seek opportunities to explore novel stimuli in commercial settings (Newberry, 1999), which was also observed in our study. Birds in all groups, including the Control, started using ramps immediately after they were provided even though only a few transitions were observed in their first week of life. The active exploration of ramps before using them for transitions suggests that the birds show a gradual progression in the vertical space use including the use of ramps, which might be driven by a trade-off between the natural explorative tendencies and social reinstatement motivation (Vallortigara et al., 1990) of the chicks. Ramp use in chicks began with initial exploration of the lower parts of the ramps by few chicks, which gradually expanded to higher parts of the ramps over the first week. We also observed that seeing the pen mates use the ramp encouraged chicks to use ramps. Even though we could not identify individuals, it is possible that the initial use of ramps and second tier is driven by highly explorative individuals. However, the exploration of ramps is also regulated by a tendency of the chicks to stay within the flock. Hence, the explorative, social reinstatement and social facilitative tendencies of the birds might work in tandem to gradually regulate the progression of ramp use.

The increase in transitions on ramps and the number of chicks on elevated surfaces with age might be explained by the differential distribution of the resources within the tiers of the pen and age-dependent change in resource use as well. Essential resources such as food, water, and litter were provided on the first tier and ground. Even though a food tray was present on the second tier the only different resource available on the second tier were perches. In nature, daytime perching behaviour in red junglefowl was reported to start from four WoA (McBride et al., 1969), which was observed in the current study as well. Similar resource-dependent regulation of vertical space use has been reported by other studies as well (Dotta, 2022; Kozak et al., 2016). The changes in morphological and physical characteristics of the birds with growth might also explain the age-related changes in ramp and elevated surface use. For instance, decrease in use of WAIR and increasing jump/fly on ramps are possibly related to increased feather coverage that improves balance (Leblanc et al., 2016) and to the development of long curved claws that enable the hens to grasp the grid wires of ramps more effectively (Birn-Jeffery et al., 2012).

The spatial cognition and bone biomechanical tests in E1 revealed no differences between the light cue treatments and control groups, which is probably due to the fact that differences in vertical movement (i.e., ramps use) and space use (i.e., number of birds on elevated surface) between the treatments were minimal. In addition, birds from all groups had access to ramps from the first WoA and used these comparably often. Previous studies that have specifically looked at the impact of facilitated access to elevated surfaces by ramps on bone properties within a multitier aviary (Stratmann et al., 2022) and on spatial cognition within an experimental setup (Norman et al., 2019) showed only minor effects. In order to investigate potential effects on spatial cognition and skeletal properties further, future studies would benefit from including information on individual birds in order to link behaviour with other responses such as spatial cognition.

5. Conclusion

Dotter, Flicker and LED cues seemed to improve the early life ramp use in laying hen chicks. While the Dotter and LED cues seemed to be effective throughout the observation period, the effectuality of the Flicker cue was limited until four WoA. Overall, no differences in ramp use between cue and control groups were found, potentially due to the cross-treatment influences and ceiling effect. Future work is needed to understand the mechanism of functioning of the LED and Dotter cues. Also, the applicability of the results would need to be confirmed in commercial settings to assess its practicality in commonly used rearing aviaries. Although not within the aims, the study also adds to growing evidence that laying hen chicks can be given access to elevated surfaces facilitated by ramps without negative welfare consequences such as early life mortality. We also demonstrated that the birds showed a gradual progression in use of ramp and elevated surfaces in their early life.

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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2023.105873.

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