

Providing elevated structures in the pullet rearing environment affects behavior during initial acclimation to a layer aviary

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ABSTRACT Spatial abilities of hens are particularly sensitive to development during early life. Experiences in pullet housing may have lasting consequences on adult hens' movements in cage-free environments. We tested whether opportunities to access elevated spaces during rearing improved hens' use of a multitiered aviary. Female Dekalb White pullets were reared in either floor pens (**FL**), single-tiered aviaries (**ST**), or 2-tiered aviaries (**TT**; $n = 5$ pens/environment) through 16 wk of age. Rearing structures were replaced with identical multitiered aviaries at 17 wk. The distribution of the flock within the aviary and the vertical transitions of 10 focal hens/pen across the aviary were determined from videos recorded during their first (**D1**) and seventh (**D7**) day of aviary access, as well as at 19, 23, and 27 wk of age. Prevalence of floor eggs was recorded weekly from 17 to 28 wk of age. On D1, more ST and TT

hens utilized the aviary during the daytime ($P = 0.0077$), made more vertical transitions when searching for a roosting spot in the evening ($P = 0.0021$), and maintained a consistent distance traveled during transitions compared to FL hens ($P = 0.02$). These differences disappeared by D7, except that ST and TT hens continued to roost on the highest perches of the aviary more ($P < 0.0001$) than FL hens through 27 wk of age. FL hens laid more floor eggs than ST and TT hens for the first 2 wk of lay ($P < 0.0001$). The majority (97.9%) of vertical transitions was controlled. Uncontrolled transitions were highest at D1 and decreased by D7 ($P = 0.0009$) and were not affected by rearing ($P = 0.33$). The results suggest that hens reared with minimal height are hesitant to use the laying hen aviaries when they are first transferred. They acclimate within 1 to 2 wk, but continue to roost less in the highest accessible level.

Key words: aviary, pullet, rearing, layer, behavior

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INTRODUCTION

The multitiered laying hen aviary is a cage-free housing system that is particularly popular in the United States. The housing system features litter space on the floor and multiple levels of perches and platforms, which provide hens access to a variety of resources (e.g., feed, water, perches, nests, roosting space) and enables performance of important species-specific behaviors (Weeks and Nicol, 2006). To access these resources and

associated behavioral opportunities, aviary-housed hens must skillfully move through the complex structures. Failing to do so can result in welfare concerns, such as increased risk of falls and collisions that can lead to injury (Campbell et al., 2016a; Stratmann et al., 2015, 2019). Management and production issues, such as higher prevalence of eggs laid outside of the designated nest boxes (i.e., floor eggs), may also arise when hens fail to access higher aviary tiers (Colson et al., 2008).

Before they are placed in laying hen housing, pullets are raised in rearing systems until 16 to 18 wk of age. This period is particularly important for development of spatial abilities, defined as the birds' abilities to move in 3-dimensional space (Wichman et al., 2007; Campbell et al., 2019). Therefore, the design of, and behavioral opportunities provided within, pullet housing can have important consequences for how laying hens utilize aviary structures later

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in life. For example, providing perches within pullet housing has been shown to reduce the likelihood of floor eggs (Appleby et al., 1988; Gunnarsson et al., 1999). It is likely that experience with elevated spaces or more complex environments during rearing improves adult use of elevated spaces, where nest boxes are typically found, later in life. At 19 wk of age, aviary-reared hens were observed to use elevated tiers within their floor pens more than hens reared in barren cages (Brantsæter et al., 2016). Similarly, hens used the highest levels of a multitiered laying hen aviary more and laid fewer eggs outside of the nest boxes when reared in a multitiered pullet aviary, as compared to those reared on the floor with access to relatively lower perches and tiers (Colson et al., 2008). The pullet housing systems used in the latter 2 studies differed in many ways. It is, therefore, unclear which aspects of pullet housing design (e.g., floor and perch space allowances, group size, location of feed, or available height) contributed to the observed developmental effect.

We hypothesized that opportunities to interact with elevated structures during early life would improve spatial abilities of hens, leading to increased use of elevated resources. We predicted that hens reared with access to elevated spaces would use the aviary structure more, particularly its highest perches and tiers, and make more controlled movements as they moved up and down the aviary. In association with increased aviary use, we predicted that hens reared with elevated structures would lay fewer floor eggs. We expected these effects to be particularly pronounced as hens initially acclimated to their laying hen housing. To explore whether these outcomes were affected by the amount of elevated space provided to pullets, we compared the impacts of 3 rearing treatments: floor, single-tiered aviary, and 2-tiered aviary.

MATERIALS AND METHODS

All experimental procedures were reviewed and approved by the University of California, Davis

Institutional Animal Care and Use Committee (Protocol #20307).

Pullet Housing and Management

A total of 835 Dekalb White pullets were obtained at 1 d of age from a commercial hatchery and randomly distributed across 15 pens ($3.05 \times 3.05 \times 2.74$ m, L \times W \times H) located in one building at the Hopkins Avian Facility, University of California, Davis (Davis, CA). The resulting groups of 55 to 56 pullets were raised in 1 of 3 rearing environments ($n = 5$ pens/treatment): floor (FL), single-tiered aviary (ST), or 2-tiered aviary (TT; Figure 1). To address location effects, the 15 pens were subdivided into 5 blocks of 3 pens within the barn, with 1 replicate of each treatment randomly assigned to each block. Blocks were positioned from the north to south end of the barn.

All pens contained 4 round metal perches (3.8 cm \times 121.9 cm, D \times W). Within the FL pens, all 4 perches were 10.5 cm high. ST pens contained one 10.5 cm high perch and 3 perches within a single-tiered aviary structure. The ST structure consisted of 2 perches 35.4 cm high, 1 perch 64.7 cm high, and a 62.9 cm high plastic slatted tier (61.0 cm \times 121.9 cm, L \times W; Dura-Slat Poultry and Kennel Flooring, Southwest Agri-Plastics, Inc., Addison, TX), which was connected to the floor by a mesh ramp (96.5 cm \times 31.8 cm, 40° angle; McNichols Wire Mesh, McNichols Co., Inc., Livermore, CA). The TT pens contained a single 10.5 cm high perch, and 3 perches (29.4, 89.9, and 125.7 cm high) within a 2-tiered structure comprised of 2 plastic slatted tiers (30.5 cm \times 121.9 cm, L \times W) positioned 62.9 and 123.8 cm off the ground. A wire mesh ramp facilitated access to both tiers (190.5 cm \times 31.8 cm, 40° angle).

To keep the accessible space allowance constant across the rearing environments, chicken wire was used to prevent pullets from accessing the litter area directly



Figure 1. Female Dekalb White pullets were reared in 1 of 3 environments for the first 16 wk of life: floor rearing environment with 10 cm high floor perches (A), a single-tiered aviary with 3 elevated perches and 1 elevated tier (B), and 2-tiered aviary with 3 elevated perches and 2 elevated tiers (C). Pullets were reared in groups of 55 to 56 pullets/pen from 1 d to 8 wk of age, then 45 pullets/pen from 8 to 16 wk of age ($n = 5$ pens/rearing environment). Pullets were 16 wk of age at the time of the photographs.

underneath the tiers of the ST and TT structures. As part of data collection procedures for another study (Lu et al., 2021), stocking density was reduced to 45 pullets/pen during the eighth wk of age and further reduced to 28 to 30 pullets/pen during the 16th wk of age. By the end of rearing, pullets had access to at least 16.2 cm linear perch space/pullet and 0.31 m²/pullet floor space.

All pens were lined with pine wood shavings (Mallard Creek Inc., Rocklin, CA). Automatic water lines (Lubing USA, Cleveland, TN; 12 nipples/pen) and two 13.6 kg round feeders/pen (52 cm circumference/feeder) were accessible from the litter. A start and grow diet (Purina Start and Grow Medicated Crumbles, Purina Animal Nutrition LLC, Gray Summit, MO) was fed ad libitum. An artificial lighting schedule was maintained according to the Dekalb White Commercial Management Guide for Aviary-Barn Systems (Dekalb, n.d.) using lights directly above pens and in the adjacent hallway. Lights in the hallway were turned off 30 min prior to lights above the pen to create a dimming effect. Natural lighting entered the house through heavy plastic curtains permanently fitted to the sides of the building. Opaque plastic tarps reduced visibility into adjacent pens and the hallway passageway that was used by husbandry personnel and researchers. The tarps covered approximately half of the pen height, such that TT birds standing on the highest tier in their pen could see over the tarp for the second half of their rearing period.

Layer Housing and Management

At 17 wk, all rearing structures were removed from the pens and replaced with a multitiered layer aviary (Figure 2). Changing housing structures took place over a period of 2 consecutive days. Three pens/rearing treatment were changed on the first day and 2 pens/rearing treatment were changed on the second day. The layer aviary consisted of 5 round metal perches (3.8 cm × 121.9 cm, D × L) installed at 4 heights from the floor (50.0, 125.7, and 181.1 cm and 2 perches at 245.3 cm) and 3 plastic slatted tiers (69.2, 137.2, and 198.2 cm high; 121.9 cm × 61 cm, L × W). A colony nest (121.9 cm × 30.5 cm, L × W; Large Reversible Roll Out Chicken Nest Box, Best Nest Box, Hudson, OH) was installed on the bottom tier. The hens were able to access litter under the aviary and had access to approximately 11.2 m² accessible floor/tier space (0.37 m²/hen).

Pine wood shavings covered the pen floor (4 cm depth; Mallard Creek Inc., Rocklin, CA). Water and feed (16% Protein Layer Mini-Pellet, Bar Ale Inc., Williams, CA) were provided with the same equipment previously described. Lighting schedule was initially maintained with artificial and natural lighting according to the Dekalb White Commercial Management Guide for Aviary-Barn Systems (Dekalb, n.d.), but was subsequently modified to manage pecking behavior. Wounds on combs, wattles, and feet were first noted at 18 wk of age. Artificial lights directly above pens were turned off at 19 wk, 5 d prior to video recording for this age, to dim light



Zone D: Top two perches

Zone C: Top Tier, includes tier and one perch

Zone B: Middle Tier, includes tier and one perch

Zone A: First Tier, includes nest box and one perch

Litter Zone: Floor

Figure 2. At 17 wk, rearing environment structures were removed from pens and replaced with a layer multitiered aviary for female Dekalb White hens (28–30 hens/pen; $n = 5$ pens/rearing environment). The aviary was divided into 5 zones to record vertical transition behavior between zones during the d and the proportion of hens within each zone across the daytime and nighttime. Hens were photographed at 17 wk of age.

intensity. Natural light and artificial lights in the hallways continued to be maintained according to the schedule recommended by the Dekalb White Commercial Management Guide for Aviary-Barn Systems (Dekalb, n.d.). Pecking blocks (11.3 kg Flock Block Premium Poultry Supplement, Purina Animal Nutrition LLC, Gray Summit, MO) were added to each pen at 20 wk of age. At 25 wk of age, artificial lighting in the hallway was turned off and only natural lighting was used via the opaque plastic curtains permanently fitted to the sides of the building (14 h light, 10 h dark; NOAA, n.d.). Husbandry procedures were the same as for pullets with the addition of daily egg collection.

Data Collection

Hen behavior was recorded via 3 video cameras per pen (4K Ultra HD IP Security Camera, Lorex Corporation, Irvine, CA) connected to a network video recorder with pentaplex operation (4K Ultra HD Security NVR, Lorex Corporation, Irvine, CA). At 17 wk of age, we recorded 24 h of video on the first (**D1**) and seventh (**D7**) full day after all aviaries were installed. Video was recorded for 48 consecutive hours at 19, 23, and 27 wk of age.

The hens' movement within the aviary was evaluated for 10 focal pullets/pen ($N = 150$ focal pullets). Focal birds were randomly selected at 1 d of age, and individually marked across the back with nontoxic food coloring (Student Kit Soft Gel Paste Food Color, AmeriColor Corp., Placentia, CA). The markings were touched up as needed, with nontoxic food coloring for down feathers and nontoxic livestock marker (Markal All-Weather Paintstik Livestock Marker, LA-CO Industries, Inc., Elk Grove Village, IL) once primary feathers developed. A single focal pullet (**FL**) died during the rearing period; therefore, there were 149 total focal hens at the start of the layer period. Focal hen behavior was assessed using continuous sampling of 3 h of video/d (2 d/age), including a morning hour (immediately after artificial lights turned on), a mid-day hour (1–2 pm at 17 and 19 wk, 12–1 pm at 23 and 27 wk), and an evening hour (immediately before artificial lights turned off). Only natural light was available at 28 wk of age. Therefore, video was reviewed for the hour immediately after all cameras changed from black and white to color (>1 lux; morning), and the hour immediately before the cameras changed from color to black and white ($=/ < 1$ lux; evening). Following Rufener et al. (2019), we defined a vertical transition as a movement that results in the hen's location changing from 1 aviary zone to another while staying in the destination zone for 1 s or longer. We specified 5 distinct zones within the multitiered layer aviary (Figure 2). The Litter Zone delineated the floor area. Aviary zone A included the bottom tier, nest box, and the lowest elevated perch. Aviary zone B contained the middle tier and associated elevated perch. Aviary zone C contained the top tier and associated elevated perch. Finally, aviary zone D contained the 2 perches located at the top of the aviary. For each observed transition, we recorded hen ID, origin, and destination

zones, and whether the transition was controlled. A transition was considered controlled if a focal hen landed on both feet in an upright body posture without excessive wing flapping (modified from Stratmann et al., 2015). If her feet were not visible, then landing in an upright body position with her body centered over her feet was also considered controlled. An uncontrolled transition occurred when a focal hen landed on 1 foot, contacted the landing surface with a body part other than her feet (most commonly the chest, abdomen, or a wing), performed excessive wing flapping, made physical contact with a pen structure or another hen during the transition (for less than 1 s) or at the end of the transition, or various combinations of these characteristics. If the landing was out of view due to the camera angle, then the transition control was marked as unidentifiable, but origin and destination zones were still recorded, if visible.

A total of 18 observers were trained to identify focal hen vertical transitions and score their criteria on 2 h of video ($N = 100$ focal hen transitions). Interobserver agreement was high for identifying the number of focal hen transitions and their criteria ($\geq 90\%$). Uncontrolled transitions were rare, so observer reliability for this criterion was also assessed using a 55-question video test (24 controlled, 31 uncontrolled). Uncontrolled transition reliability was ≥ 0.82 (Cohen's kappa; irr package, version 0.84.1, Gamer et al., 2019). Trained observers were blind to rearing environment treatment, were randomly assigned video from different rearing environments and ages, and viewed videos for analysis using either IINA video player (version 1.1.2, <https://iina.io/>) or Behavioral Observation Research Interactive Software (**BORIS**, version 7.10.7, <http://www.boris.unito.it/>, Friard and Gamba, 2016). The lead observer (A. P.) randomly checked the work of each observer through the study to ensure that inter observer reliability remained high.

The distribution of hens within the aviary (in aviary zones A–D) was determined using instantaneous scan sampling of all hens (focal and unmarked). Daytime aviary use was determined using 30-min scan intervals conducted during all daylight hours (27 intervals at 17 wk and 29 intervals at 19, 23, and 27 wk). Nighttime use was evaluated based on 2 scans taken 4 h apart. Our preliminary observations indicated that, once birds settled into a zone for their nighttime roosting position, they did not move from that zone until the lights came on. A hen was counted as being in a zone when the majority (over half) of her body was in the zone at the time of the scan. Two observers were trained on 1 d of video (daytime and nighttime) for 3 pens (1 pen/rearing environment) and achieved 0.99 inter observer reliability (intraclass correlation coefficient; irr package). The first observer, who was blind to rearing treatment, analyzed all 19, 23, and 27 wk videos in a randomized order for rearing environment and age to prevent order bias. The second observer analyzed all 17 wk videos in a randomized order for rearing environment. This observer was not completely blind to rearing treatment as they were involved in the experimental design. However, they

could not readily identify the rearing treatments associated with the majority of pen numbers. The videos were viewed using VLC media player (version 3.0.16, VideoLAN, Paris, France).

Eggs were collected once daily in the afternoon by husbandry personnel from 17 to 28 wk of age. The first eggs were laid during wk 17, and hens across all pens were laying eggs at 18 wk age. Egg location data (number of eggs collected from the floor, next box, and aviary) were recorded on 4 d/wk from 18 to 27 wk, and on 3 d during wk 28. Several days were excluded from the dataset. This occurred when the nest box perch was inadvertently left up preventing hens from accessing the nest box ($N = 4$ d for 1 FL pen, 2 d for 1 ST and 1 TT pen, 1 d for 1 ST pen); and when the datasheet was not properly filled out ($N = 1$ d for 3 FL, 1 ST, and 2 TT pens, 2 d for 1 ST pen, and 2 d during 23 wk for all pens).

Statistical Analysis

All statistical analyses were conducted in R statistical software (version 4.2.0; R Core Team, 2021) using RStudio (version 2022.02.2+485) for macOS Catalina 10.15.7. All model fits were assessed for deviations from their expected distribution, overdispersion, outliers, and homogeneity of variance via plot and test functions in the DHARMA package (version 0.4.5; Hartig, 2022) for generalized linear mixed models or sjPlot package (version 2.8.10; Lüdecke, 2021) for linear mixed models. For all analyses, the final models were obtained by a stepwise backward reduction using ANOVA for model comparison with a P value of >0.05 as the criterion of exclusion. The significance of main effects was reported if their interaction terms were not significant. Model estimates and 95% confidence intervals were obtained with the effects package (version 4.2.1, Fox and Hong, 2009). After significant effects/interactions were determined, differences between specific treatments and ages are described based on patterns of nonoverlapping confidence intervals.

Number of Focal Hen Vertical Transitions.

The total number of vertical transitions/hour/day was summarized for each focal hen and analyzed with generalized linear mixed models (glmmTMB package, version 1.1.3, Brooks et al., 2017) with a negative binomial distribution. At 17 wk, rearing environment (factor with 3 levels: FL, ST, and TT), days in the aviary (factor with 2 levels: D1, D7), time of day (factor with 3 levels: morning, afternoon, evening), and their 2-way and 3-way interactions were included as fixed effects, with focal hen ID nested in pen as a random effect. A similar model structure was used for 19 to 27 wk, where rearing environment, time of day, age (factor with 3 levels: 19, 23, and 27 wk), and all interactions were included as fixed effects. Focal hen ID nested in pen was included as a random effect.

Uncontrolled Transitions. Data were summarized as the number of uncontrolled transitions/day/pen. A

generalized linear mixed model (glmmTMB package, version 1.1.3, Brooks et al., 2017) with a negative binomial distribution was used with separate models for 17 and 19 to 27 wk. Rearing environment (factor with 3 levels: FL, ST, and TT), age (17 wk model: days in the aviary as a factor with 2 levels, D1 and D7; 19 to 27 wk model: age as a factor with 3 levels, 19, 23, and 27 wk), and their interaction were assessed as fixed effects. An offset variable was also included as the log of the total number of transitions/day/pen with an identifiable landing to adjust the model for different numbers of transitions performed in each pen (Hutchinson and Holtman, 2005). Pen was included as a random effect.

Number of Aviary Zones Crossed During a Vertical Transition.

For each vertical transition ($N = 19,709$ total focal hen vertical transitions across all ages), the number of aviary zones crossed was calculated as the number of zones between the destination zone and the origin zone, resulting in 1, 2, 3, or 4 aviary zones crossed during a vertical transition. Vertical transitions were also classified as ascending ($N = 12,499$ focal hen vertical transitions) or descending ($N = 7,210$ focal hen vertical transitions). Ascending transitions were excluded from further analysis due to lack of variation in the data: 99.7% crossed 1 aviary zone and the remaining 0.3% crossed 2 aviary zones. There were no ascending transitions crossing 3 or 4 zones. Descending transitions had a greater distribution of transitions over the number of aviary zones crossed (1 zone: 57.0%, 2 zones: 21.5%, 3 zones: 20.5%, and 4 zones: 1.0%). These data were dichotomized into 1 aviary zone vs. crossing >1 aviary zone transitions, and analyzed using 2 generalized linear mixed models (lme4 package, version 1.1.29; Bates et al., 2015) with a binomial distribution. The 2 models represented 17 wk and 19 to 27 wk data, where the effect of rearing environment (factor with 3 levels: FL, ST, and TT), age (17 wk model: days in the aviary as a factor with 2 levels, D1 and D7; 19 to 27 wk model: age as a factor with 3 levels, 19, 23, and 27 wk), time of day (factor with 3 levels: morning, afternoon, evening), and their interactions were assessed. Focal hen ID nested in pen was included as a random effect.

Location of Hens on the Aviary

We calculated the average proportion of hens utilizing the aviary, regardless of zone. Data were analyzed with generalized linear mixed models (glmmTMB package, version 1.1.3, Brooks et al., 2017) with a β distribution. Separate models were utilized for the first week of aviary access (wk 17) and 19 to 27 wk of age. Within the 2 age models, separate models were also fitted for daytime vs. nighttime data. Each model assessed the effect of rearing environment (factor with 3 levels: FL, ST, and TT), age (17 wk model: days in the aviary as a factor with 2 levels, D1 and D7; 19 to 27 wk model: age as a factor with 3 levels, 19, 23, and 27 wk), and the 2-way interactions of the predictors. Pen was included as a random effect in all models, and date was added as a crossed random effect in the 19 to 27 wk models.

In a separate analysis, we calculated the average proportion of hens utilizing each zone of the aviary/day/pen during daytime and nighttime observations. Similar as above, data were analyzed with generalized linear mixed models (glmmTMB package, version 1.1.3, Brooks et al., 2017) with a β distribution and the same random effect structure described previously. Separate models were also used for the 2 age groupings and the daytime and nighttime data. Each model assessed the effect of rearing environment (factor with 3 levels: FL, ST, and TT), age (17 wk model: days in the aviary as a factor with 2 levels, D1 and D7; 19 to 27 wk model: age as a factor with 3 levels, 19, 23, and 27 wk), zone (factor with 4 levels: A, B, C, and D), and the 2-way and 3-way interactions of the predictors.

Distribution of Eggs. The prevalence of eggs laid on aviary tiers but outside of the nest box was low (0.15% of all eggs collected throughout the study). The proportion

of floor eggs/week/pen was analyzed using generalized linear mixed models (glmmTMB package, version 1.1.3, Brooks et al., 2017) with a β distribution that included rearing environment (factor with 3 levels: FL, ST, and TT), age (continuous, 11 timepoints from 18 to 28 wk), and their interaction, with pen included as a random effect. A separate model with the same structure was also run for the total proportion of eggs laid/week/pen.

RESULTS

Number of Focal Hen Vertical Transitions

During initial acclimation at 17 wk, there was an interaction among rearing environment, days in the aviary, and time of day ($\chi^2 = 16.81$, $df = 4$, $P = 0.0021$; Figure 3). In the evening, ST and TT hens made 3 times more vertical transitions than FL hens on D1, then

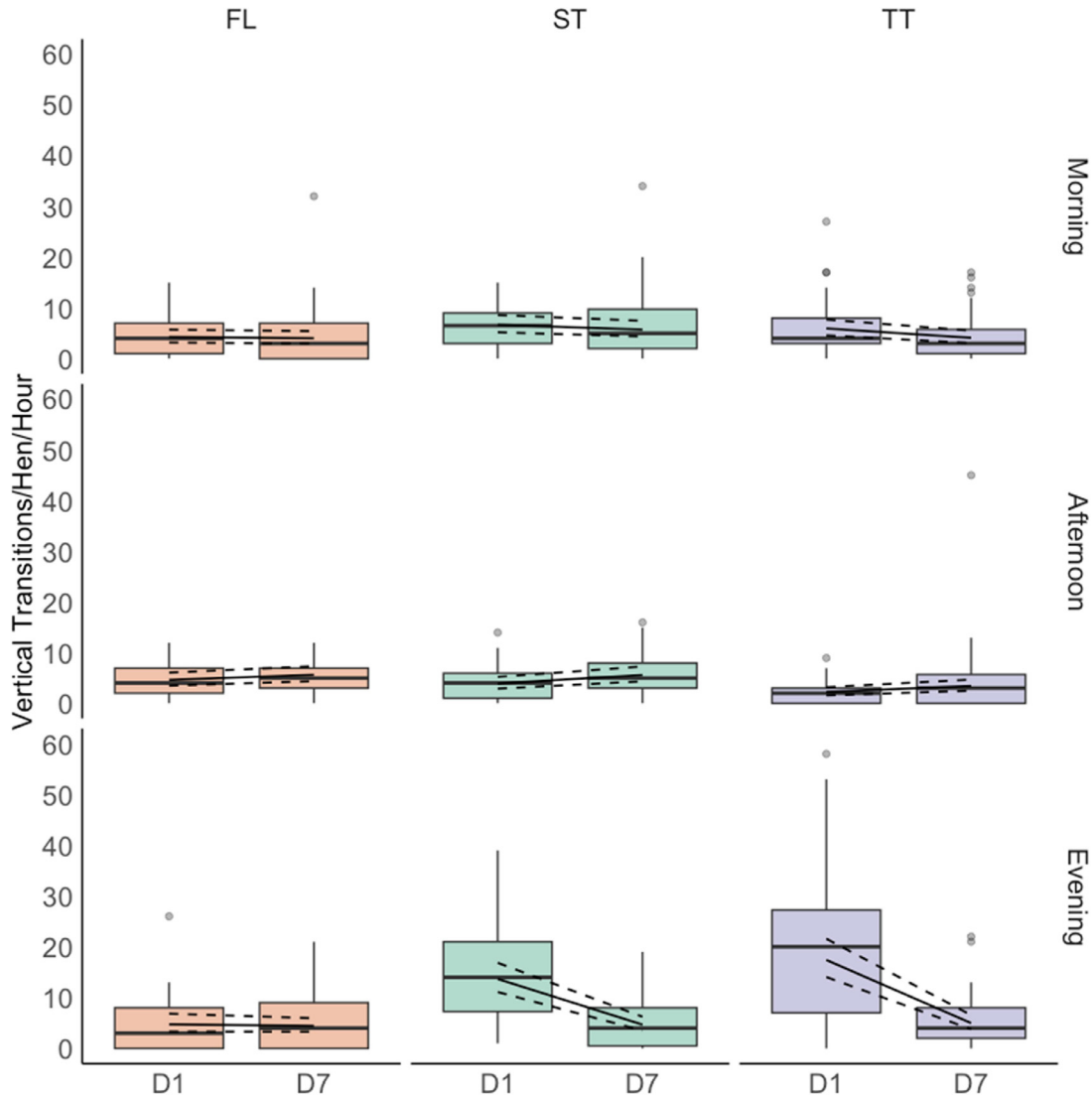


Figure 3. The number of vertical transitions made by Dekalb White focal hens at 3 h (morning, afternoon, and evening) during the first week of housing in a multitiered layer aviary. Hens were reared in 1 of 3 pullet rearing environments: floor (FL), single-tiered aviary (ST), or a 2-tiered aviary (TT; 10 focal hens/pen, $n = 5$ pens/rearing environment). The first full day that hens were housed in the layer aviary without atypical human disturbance is D1, followed by the seventh full day (D7). Raw data are displayed in box plots, where boxes represent the interquartile range, where the black line represents the median, the top of the box represents the 75th quartile, and the bottom of the box represents the 25th quartile. Whiskers represent the minimum and maximum values, while gray dots indicate outliers. The solid line overlaying the box plots is the estimated mean values from the generalized linear mixed effects model and the dashed lines are the 95% confidence intervals of the estimated means.

Table 1. Number of vertical transitions performed by Dekalb White hens at 19, 23, and 27 wk of age.

Time of day and age (wk)	FL estimate (95% CI)	ST estimate (95% CI)	TT estimate (95% CI)
Morning			
19	2.46 (1.84, 3.29)	3.44 (2.62, 4.51)	3.10 (2.36, 4.07)
23	1.78 (1.31, 2.42)	2.49 (1.87, 3.31)	1.95 (1.45, 2.64)
27	2.78 (2.06, 3.74)	4.01 (3.07, 5.23)	2.88 (2.17, 3.83)
Afternoon			
19	4.79 (3.72, 6.17)	6.05 (4.75, 7.72)	5.25 (4.09, 6.74)
23	4.74 (3.67, 6.11)	5.11 (3.98, 6.56)	4.55 (3.54, 5.86)
27	4.02 (3.07, 5.26)	5.24 (4.07, 6.73)	3.55 (2.72, 4.63)
Evening			
19	6.56 (5.14, 8.37)	8.17 (6.46, 10.33)	7.13 (5.62, 9.06)
23	5.94 (4.64, 7.61)	9.93 (7.90, 12.49)	9.66 (7.68, 12.14)
27	5.56 (4.31, 7.18)	6.59 (5.17, 8.39)	7.25 (5.72, 9.19)

As pullets, hens were reared in either a floor pen (FL), single-tiered aviary (ST), or 2-tiered aviary (TT) through 16 wk. At 17 wk, all hens were housed in a multitiered layer aviary where vertical transitions were observed. Presented model estimates and confidence intervals reflect the full model.

decreased by D7 to a similar number as FL hens. All hens were less active in the morning and afternoon compared to the evening, and they remained consistent from D1 to D7 during those times of day.

From 19 to 27 wk, there was also an interaction among rearing environment, age, and time of day ($\chi^2 = 15.73$, $df = 8$, $P = 0.046$; **Table 1**). ST and TT hens made more transitions than FL hens during the evening at 23 wk of age. Across ages, all hens were most active in the evening compared to the morning and afternoon.

Uncontrolled Transitions

Uncontrolled transitions were rare ($N = 404$ uncontrolled transitions out of 19,512 focal hen vertical transitions with an identifiable landing across all ages). At 17 wk, neither rearing environment ($\chi^2 = 2.23$, $df = 2$, $P = 0.33$) nor interaction with days in the aviary ($\chi^2 = 1.78$, $df = 2$, $P = 0.41$) affected the rate of uncontrolled transitions/day/pen. Uncontrolled transitions decreased between D1 and D7 ($\chi^2 = 10.95$, $df = 1$, $P = 0.00093$; estimated mean [95% CI]; D1: 8.03 [6.22,

10.36], D7: 3.99 [2.81, 5.67] uncontrolled transitions/day/pen). From 19 to 27 wk, TT hens displayed approximately one more uncontrolled transition/day/pen than ST hens ($\chi^2 = 6.42$, $df = 2$, $P = 0.04$; FL: 2.87 [2.11, 3.89], ST: 2.21 [1.63, 2.98], TT: 3.69 [2.89, 4.72] uncontrolled transitions/day/pen). All hens experienced 1 fewer uncontrolled transition/day/pen between 19 and 23 wk and maintained a similar level to 27 wk ($\chi^2 = 7.17$, $df = 2$, $P = 0.028$; 19: 3.77 [2.98, 4.78], 23: 2.26 [1.65, 3.10], 27: 2.74 [2.01, 3.72] uncontrolled transitions/d/pen). There was no interactive effect between rearing environment and age on uncontrolled transitions ($\chi^2 = 8.43$, $df = 4$, $P = 0.077$).

Number of Aviary Zones Crossed During a Descending Vertical Transition

There was a 3-way interactive effect of rearing environment, age, and time of day on the proportion of descending transitions crossing more than one aviary zone during the first week of layer aviary housing ($\chi^2 = 11.64$, $df = 4$, $P = 0.02$; **Table 2**). Between D1 and

Table 2. Proportion of descending transitions crossing more than 1 zone by Dekalb White hens.

Time of day and age (wk)	FL estimate (95% CI)	ST estimate (95% CI)	TT estimate (95% CI)
Morning			
17, D1	0.32 (0.23, 0.43)	0.41 (0.32, 0.52)	0.40 (0.30, 0.51)
17, D7	0.56 (0.44, 0.68)	0.45 (0.35, 0.55)	0.41 (0.30, 0.53)
19	0.46 (0.35, 0.57)	0.34 (0.26, 0.42)	0.35 (0.27, 0.44)
23	0.41 (0.31, 0.53)	0.29 (0.21, 0.39)	0.43 (0.33, 0.54)
27	0.095 (0.054, 0.16)	0.11 (0.072, 0.17)	0.12 (0.075, 0.19)
Afternoon			
17, D1	0.41 (0.30, 0.53)	0.44 (0.33, 0.56)	0.43 (0.26, 0.61)
17, D7	0.59 (0.47, 0.69)	0.49 (0.38, 0.60)	0.55 (0.43, 0.67)
19	0.56 (0.47, 0.65)	0.50 (0.42, 0.59)	0.60 (0.52, 0.68)
23	0.58 (0.49, 0.67)	0.43 (0.35, 0.53)	0.54 (0.44, 0.63)
27	0.48 (0.38, 0.59)	0.42 (0.34, 0.51)	0.59 (0.49, 0.69)
Evening			
17, D1	0.71 (0.56, 0.83)	0.53 (0.46, 0.61)	0.51 (0.43, 0.58)
17, D7	0.46 (0.35, 0.57)	0.51 (0.40, 0.62)	0.50 (0.38, 0.61)
19	0.54 (0.45, 0.62)	0.45 (0.37, 0.52)	0.47 (0.39, 0.55)
23	0.48 (0.39, 0.57)	0.32 (0.26, 0.39)	0.40 (0.33, 0.47)
27	0.52 (0.42, 0.62)	0.37 (0.29, 0.46)	0.42 (0.33, 0.51)

Hens were reared in either a floor (FL), single-tiered aviary (ST), or 2-tiered aviary (TT) pullet rearing environment through 16 wk, then housed in a multitiered layer aviary where descending transitions were recorded. Presented model estimates and confidence intervals reflect the full model.

D7, FL hens increased their proportion of morning transitions crossing more than one aviary zone and decreased their proportion of evening transitions crossing more than one aviary zone. FL hens were consistent across days in the afternoon, and ST and TT hens remained consistent across days for all times of day.

From 19 to 27 wk, rearing environment had a main effect on the proportion of descending transitions crossing more than 1 zone, such that FL hens displayed a higher proportion of these transitions than ST hens across ages ($\chi^2 = 7.05$, $df = 2$, $P = 0.029$; [Table 2](#)). Age and time of day had an interactive effect ($\chi^2 = 75.67$, $df = 4$, $P < 0.0001$), where the proportion of descending transitions crossing more than 1 zone remained consistent across ages for afternoon and evening but sharply decreased at 27 wk in the morning. Furthermore, hens made the lowest proportion of transitions crossing more than 1 zone in the morning compared to the afternoon and evening. There was no interactive effect between rearing environment and age ($\chi^2 = 4.27$, $df = 4$, $P = 0.37$), rearing environment and time of day ($\chi^2 = 6.12$, $df = 4$, $P = 0.16$), nor a 3-way effect among rearing environment, age, and time of day ($\chi^2 = 7.09$, $df = 8$, $P = 0.53$).

Location of Hens on the Aviary

In the first week of aviary access, there were interactive effects among the predictor variables during the daytime and nighttime. During the daytime, fewer FL hens were observed on the aviary overall on D1 than ST and TT hens, but FL hens increased their use by D7 (rearing \times day interaction; $\chi^2 = 9.72$, $df = 2$, $P = 0.0077$; [Table 3](#)). All hens increased use of aviary zone C by the end of the first week (aviary zone \times day interaction; $\chi^2 = 19.03$, $df = 3$, $P = 0.0003$), but more ST and TT hens were observed in aviary zone C than FL hens (rearing \times aviary zone interaction; $\chi^2 = 53.24$, $df = 6$, $P < 0.0001$; [Table 4](#)). At nighttime, FL and ST hens increasingly used the aviary for roosting by the end of the first week, while a majority of TT hens consistently roosted in the aviary from D1 to D7 ($\chi^2 = 9.67$, $df = 2$, $P = 0.0079$; [Table 3](#)). There was a 3-way

interaction between the rearing environment, day, and aviary zone ($\chi^2 = 14.40$, $df = 6$, $P = 0.03$). More FL hens roosted in aviary zone C from D1 to D7, while more TT hens roosted in aviary zone D and less in aviary zone A between D1 to D7 ([Table 4](#)). ST hens were consistent in roosting locations.

From 19 to 27 wk, hens decreased use of the aviary overall during the daytime ($\chi^2 = 23.93$, $df = 2$, $P < 0.0001$; [Table 3](#)). All hens increased use of aviary zone D by 27 wk and decreasingly used aviary zones B and C (aviary zone \times age interaction; $\chi^2 = 87.86$, $df = 6$, $P < 0.0001$; [Table 4](#)). More ST and TT hens were observed in aviary zone D than FL hens (rearing \times aviary zone interaction; $\chi^2 = 43.22$, $df = 6$, $P < 0.0001$). During the nighttime, hens increasingly used the aviary from 19 to 27 wk ($\chi^2 = 98.06$, $df = 2$, $P < 0.0001$; [Table 3](#)). All hens increased use of aviary zones C and D for roosting at 27 wk (aviary zone \times age interaction; $\chi^2 = 169.02$, $df = 6$, $P < 0.0001$; [Table 4](#)). TT hens roosted in aviary zone D the most, followed by ST hens and FL hens roosted in aviary zone D the least (rearing \times aviary zone; $\chi^2 = 78.25$, $df = 6$, $P < 0.0001$).

Distribution of Eggs

Total egg production was not influenced by rearing environment ($\chi^2 = 2.60$, $df = 2$, $P = 0.27$) or its interaction with age ($\chi^2 = 0.50$, $df = 2$, $P = 0.78$). The number of eggs laid increased in all pens as hens aged ($\chi^2 = 57.52$, $df = 1$, $P < 0.0001$). Rearing environment and age had an interactive influence on the proportion of floor eggs ($\chi^2 = 24.83$, $df = 2$, $P < 0.0001$), such that FL pens laid a higher proportion of floor eggs than ST and TT pens during the first 2 wk of lay ([Figure 4](#)). The proportion floor eggs decreased throughout the trial ($\chi^2 = 60.05$, $df = 2$, $P < 0.0001$; [Figure 4](#)).

DISCUSSION

The objective of this study was to determine whether providing access to elevated spaces during pullet rearing influenced hens' use of elevated resources after they

Table 3. Proportion of Dekalb White hens utilizing a multitiered aviary.

Time of day and age (wk)	FL estimate (95% CI)	ST estimate (95% CI)	TT estimate (95% CI)
Daytime			
17, D1	0.12 (0.10, 0.15)	0.30 (0.25, 0.34)	0.30 (0.26, 0.35)
17, D7	0.18 (0.15, 0.22)	0.30 (0.26, 0.35)	0.28 (0.24, 0.33)
19	0.20 (0.18, 0.23)	0.25 (0.22, 0.28)	0.23 (0.20, 0.26)
23	0.15 (0.13, 0.18)	0.16 (0.14, 0.19)	0.19 (0.16, 0.21)
27	0.11 (0.10, 0.13)	0.14 (0.12, 0.16)	0.14 (0.12, 0.16)
Nighttime			
17, D1	0.17 (0.070, 0.35)	0.45 (0.24, 0.68)	0.49 (0.27, 0.72)
17, D7	0.29 (0.13, 0.52)	0.62 (0.38, 0.81)	0.50 (0.28, 0.72)
19	0.33 (0.16, 0.56)	0.53 (0.31, 0.75)	0.53 (0.30, 0.74)
23	0.39 (0.20, 0.62)	0.61 (0.37, 0.80)	0.66 (0.43, 0.83)
27	0.72 (0.49, 0.87)	0.80 (0.60, 0.91)	0.89 (0.74, 0.95)

Hens were reared in either a floor (FL), single-tiered aviary (ST), or 2-tiered aviary (TT) pullet rearing environment through 16 wk, then housed in a multitiered layer aviary. These data represent the total proportion of hens observed on the aviary, regardless of zone. Presented model estimates and confidence intervals reflect the full model.

Table 4. Proportion of Dekalb White hens utilizing each zone of a multitiered aviary.

Time of day and age (wk)	FL estimate (95% CI)	ST estimate (95% CI)	TT estimate (95% CI)
Zone A			
Daytime			
17, D1	0.052 (0.035, 0.075)	0.042 (0.028, 0.063)	0.044 (0.030, 0.066)
17, D7	0.045 (0.030, 0.067)	0.037 (0.024, 0.057)	0.037 (0.024, 0.057)
19	0.038 (0.030, 0.048)	0.042 (0.034, 0.053)	0.031 (0.024, 0.040)
23	0.042 (0.033, 0.052)	0.040 (0.032, 0.051)	0.038 (0.030, 0.047)
27	0.036 (0.028, 0.045)	0.036 (0.029, 0.046)	0.034 (0.027, 0.043)
Nighttime			
17, D1	0.024 (0.009, 0.064)	0.066 (0.028, 0.15)	0.13 (0.061, 0.25)
17, D7	0.048 (0.020, 0.11)	0.075 (0.033, 0.16)	0.041 (0.016, 0.10)
19	0.029 (0.014, 0.058)	0.14 (0.079, 0.23)	0.063 (0.033, 0.12)
23	0.031 (0.015, 0.063)	0.077 (0.042, 0.14)	0.059 (0.030, 0.11)
27	0.012 (0.006, 0.025)	0.009 (0.004, 0.019)	0.014 (0.007, 0.030)
Zone B			
Daytime			
17, D1	0.052 (0.036, 0.075)	0.11 (0.083, 0.14)	0.089 (0.067, 0.12)
17, D7	0.060 (0.042, 0.084)	0.069 (0.049, 0.095)	0.051 (0.035, 0.074)
19	0.049 (0.040, 0.060)	0.057 (0.047, 0.069)	0.043 (0.035, 0.054)
23	0.027 (0.020, 0.035)	0.027 (0.020, 0.035)	0.028 (0.021, 0.036)
27	0.012 (0.009, 0.018)	0.021 (0.016, 0.028)	0.017 (0.012, 0.023)
Nighttime			
17, D1	0.010 (0.045, 0.20)	0.084 (0.037, 0.18)	0.051 (0.02, 0.13)
17, D7	0.082 (0.036, 0.18)	0.10 (0.046, 0.21)	0.11 (0.048, 0.22)
19	0.10 (0.058, 0.18)	0.051 (0.026, 0.097)	0.066 (0.035, 0.12)
23	0.062 (0.032, 0.12)	0.071 (0.038, 0.13)	0.085 (0.045, 0.15)
27	0.012 (0.006, 0.026)	0.009 (0.004, 0.020)	0.013 (0.006, 0.028)
Zone C			
Daytime			
17, D1	0.017 (0.009, 0.031)	0.15 (0.12, 0.18)	0.17 (0.14, 0.21)
17, D7	0.068 (0.049, 0.094)	0.18 (0.15, 0.22)	0.20 (0.17, 0.24)
19	0.11 (0.096, 0.13)	0.14 (0.12, 0.16)	0.15 (0.13, 0.17)
23	0.085 (0.072, 0.10)	0.095 (0.081, 0.11)	0.12 (0.10, 0.14)
27	0.062 (0.051, 0.075)	0.075 (0.063, 0.089)	0.08 (0.068, 0.095)
Nighttime			
17, D1	0.059 (0.025, 0.13)	0.204 (0.11, 0.35)	0.107 (0.048, 0.22)
17, D7	0.194 (0.10, 0.34)	0.244 (0.13, 0.40)	0.166 (0.084, 0.30)
19	0.24 (0.15, 0.36)	0.19 (0.11, 0.30)	0.24 (0.15, 0.36)
23	0.31 (0.21, 0.44)	0.22 (0.13, 0.33)	0.26 (0.17, 0.39)
27	0.53 (0.39, 0.65)	0.43 (0.31, 0.57)	0.56 (0.42, 0.69)
Zone D			
Daytime			
17, D1	0.002 (0.001, 0.005)	0.005 (0.002, 0.011)	0.003 (0.001, 0.007)
17, D7	0.003 (0.001, 0.007)	0.005 (0.002, 0.012)	0.006 (0.003, 0.013)
19	0.0015 (0.0008, 0.003)	0.007 (0.004, 0.011)	0.003 (0.002, 0.006)
23	0.0016 (0.0009, 0.003)	0.002 (0.001, 0.004)	0.005 (0.003, 0.008)
27	0.0023 (0.001, 0.004)	0.005 (0.003, 0.008)	0.013 (0.009, 0.019)
Nighttime			
17, D1	0.014 (0.005, 0.038)	0.045 (0.018, 0.11)	0.057 (0.023, 0.13)
17, D7	0.011 (0.004, 0.030)	0.059 (0.024, 0.14)	0.20 (0.10, 0.34)
19	0.013 (0.006, 0.028)	0.037 (0.018, 0.077)	0.13 (0.071, 0.21)
23	0.033 (0.016, 0.066)	0.076 (0.041, 0.14)	0.23 (0.14, 0.34)
27	0.042 (0.021, 0.083)	0.13 (0.074, 0.22)	0.31 (0.20, 0.44)

Hens were reared in either a floor (FL), single-tiered aviary (ST), or 2-tiered aviary (TT) pullet rearing environment through 16 wk, then housed in a multitiered layer aviary. The aviary was divided into zones, with Zone A representing the bottom tier and nest box, Zone B containing the middle tier, Zone C as the top tier, and Zone D having the highest perches on the aviary. Presented model estimates and confidence intervals reflect the full model.

transitioned to layer housing. Behavioral differences related to multitiered aviary use were most pronounced as hens acclimated to these housing structures. FL hens made fewer movements across the aviary tiers when they settled to roost, used higher aviary zones less, and laid more floor eggs as compared to aviary-reared hens (ST and TT). While most of the differences disappeared within 1 to 2 wk, ST and TT continued to use the highest levels of the aviary structure more than FL hens throughout the study.

Having been reared only with access to low perches, FL pullets may have been less able and/or more hesitant to access the multitiered aviary when first encountered.

Movement onto and across elevated spaces can be influenced by physical and cognitive ability, both of which can be impacted by rearing (Campbell et al., 2019). The FL hens increased their movement within the aviary and their use of higher aviary tiers for roosting within the first week of transitioning into multitiered aviary housing. The rapid acclimation to their laying aviary suggests that any potential physical or cognitive deficiencies from rearing had short-lasting effects on elevated space use by hens. Previous work has similarly found that, regardless of rearing environments, hens use elevated space to some degree. Gunnarsson et al. (2000) reared pullets with early or delayed access to perches,

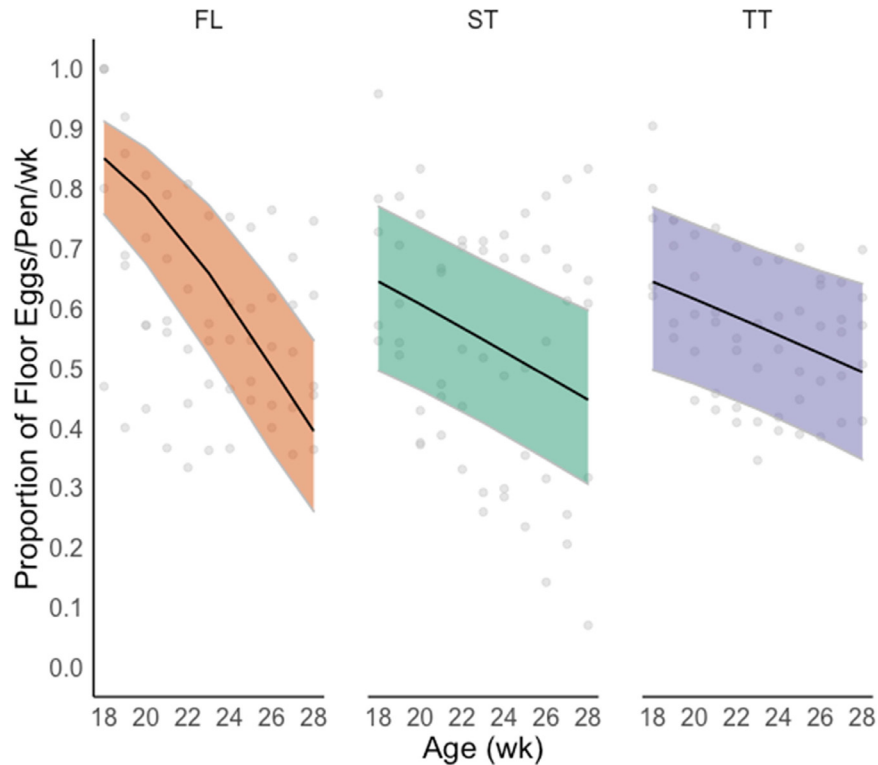


Figure 4. The proportion of floor eggs laid/pen by Dekalb White hens on a weekly basis from 18 to 28 wk of age. Hens were reared in 1 of 3 pullet rearing environments: floor (FL), single-tiered aviary (ST), or a 2-tiered aviary (TT). Raw data points for each rearing environment appear in gray. The solid black line overlaying the raw data points corresponds to the estimated mean values from the generalized linear mixed effects model and the colored ribbon represents the 95% confidence intervals of the estimated means.

and found that all pullets reached a 40 cm elevated platform within the same amount of time when tested at 16 wk of age. Delayed access to perches did, however, impact the success with which pullets retrieved a food reward from the height of 80 cm, which the authors suggested could reflect reduced spatial cognitive abilities more so than impaired physical abilities. [Rentsch et al. \(2023\)](#) also found that aviary-reared pullets performed better on a spatial task that involved accessing elevated platforms compared to pullets reared in conventional cages. [Colson et al. \(2008\)](#) reared pullets either in floor pens with 3 perches and 2 platforms or 1 of 2 types of aviaries. After the birds were transferred into layer aviaries at 17 wk of age, [Colson et al. \(2008\)](#) observed a similarly delayed use of elevated spaces as in our study, where the floor reared birds used the middle and high tiers of the layer aviary less than aviary-reared hens. Taken together, the results of our study compliment previous findings. Rearing pullets without adequate access to elevated spaces impacts later use of elevated spaces for some period of time. It remains unclear whether physical and/or cognitive changes are driving these differences.

It would be reasonable to speculate that the initial differences in the FL hens' use of aviaries could be due to differences in the birds' abilities to perceive depth. However, available research does not support this hypothesis. [Jones et al. \(2023\)](#) reared pullets according to the methods described in our study and tested their ability to perceive depth at 7 to 8, 15 to 16, and 29 to 30 wk of

age using a modified Y-maze with uneven arms and a visual cliff test. These approaches did not require birds to make vertical movements, which reduced potentially confounding influences of physical and cognitive abilities. Rearing did not affect the birds' ability to discriminate depth; regardless of rearing treatment. Most birds selected the shorter arm to escape the Y-maze and were more likely to cross the visual cliff when the fake floor was set at a depth of 15 cm vs. 30 or 90 cm. However, at 8 and 16 wk of age, FL pullets took longer and were less likely to cross the visual cliff and looked down at the gap more than ST and TT pullets. The results point to a reluctance or hesitation by FL pullets to cross depth, rather than an impairment in depth perception ([Jones et al., 2023](#)). Moreover, at 16 wk of age, these same pullets did not differ in neurological markers for spatial information processing, demonstrating that rearing did not impair neural functioning ([Pullin et al., 2022](#)). In other words, it is possible that FL birds are simply more cautious due to lack of experience with the system. [Norman et al. \(2021\)](#) also linked the lack of experience with aviary structures with the pullets' hesitancy to use these structures when transitioning to their layer housing environment. They reared pullets with perches but provided ramps to one of the treatments, which facilitated access to the elevated spaces. Pullets reared with ramps were confirmed to use the elevated spaces earlier during rear compared to pullets reared without ramps, and ramp-reared birds showed less crouching, pacing, and wing-flapping behavior when using ramps at 17 wk of

age (1 wk after the ramps were introduced to all flocks). It could be that the decreased use of elevated spaces noted during the acclimation period in our study, and in previous studies (e.g., Colson et al., 2008), does not reflect a lack of ability per se, but rather a hesitancy to move across a novel and complex (multitiered) spatial layout (Norman et al., 2021; Jones et al., 2023). This could be coupled with a preference by floor-reared birds for staying on the floor (Colson et al., 2008).

In addition to influencing spatial distribution, early experiences can have short- or long-lasting consequences on animal welfare and productivity (Rodenburg et al., 2008; Janczak & Riber, 2015). In our study, decreased use of elevated spaces by FL during the first week corresponded with an increased proportion of floor eggs laid during the first 2 wk after the birds were introduced into the multitiered aviaries. These findings support results from Colson et al. (2008), where floor-reared hens laid fewer eggs inside the nest boxes for the first 2 wk of lay and laid more floor eggs until the end of the study (at 27 wk). The impact of early experience with elevated structures, especially perches, on the occurrence of floor eggs have long been noted (Appleby et al., 1988; Gunnarsson et al., 1999). Appleby et al. (1988) reported that hens reared in a floor pen with perches were more likely to lay their first egg in a nest box than hens reared without perch access. Since the nest boxes were positioned only 15 cm off the floor, the authors concluded that the hens from both treatments were likely physically capable of entering the nest boxes. All hens in our study, including FL hens, utilized the zone containing the nest box (Zone A) starting the first day post-transfer, further demonstrating that hesitancy to use a novel resource, rather than physical ability, likely contributed to the increased prevalence of floor eggs.

Lowering the occurrence of floor eggs by promoting exploratory behavior of elevated spaces in the initial weeks of aviary housing could have practical benefits. Floor eggs are associated with increased food safety risks due to higher bacterial load (De Reu et al., 2008; Jones et al., 2015) and increased labor costs due to added time required to collect these eggs (Matthews and Sumner, 2015). The prevalence of floor eggs in the present study was high (weekly rates ranging from 39.4 to 85.1%), as compared to several previous reports (e.g., 1.1–28.7%, Gunnarsson et al., 1999; 3.5–6.6%, Colson et al., 2008; 0.3–4.2%, Oliveira et al., 2019). Such a high prevalence may be partially explained by our decision not to train the hens to use the nest boxes to avoid confound rearing treatment effects, as well as providing feed and water on the floor. Oliveira et al. (2019) reported that preventing hens from coming onto the pen floor during oviposition can reduce floor egg prevalence. It is commonplace on many farms to encourage hens to remain in aviaries overnight and at the time of oviposition with the goal of promoting nest box use.

Although behavioral changes related to space use were most pronounced for FL hens, all birds needed some time to learn the layer aviary system. Uncontrolled transitions were highest on the first day of aviary access

for all hens and decreased by the end of the first week. Falls and collisions in enriched colony cages and aviaries have also reportedly decreased over time as hens acclimated to their new housing, became less active with age, or both (Stratmann et al., 2015, 2019; Pullin et al., 2020). Similarly, across all rearing treatment groups in the present study, the number of floor eggs was highest when the hens were first introduced into the system and declined over time. Similar patterns of floor laying have been observed in commercial flocks (e.g., Oliveira et al., 2019).

We anticipated that access to elevated structures early in life would result in improved spatial skills, translating to fewer collisions and other types of uncontrolled movements in the aviary. However, rearing environment did not influence the rate of uncontrolled transitions, which were rare across all treatments in the current study (2.07% of all transitions). Downward movements, particularly those spanning longer distances, are thought to be more difficult for hens to land than upward movements (Scott et al., 1997; Moinard et al., 2004a,b). We observed an increase in the frequency of longer (i.e., crossing more than 1 zone) descending movements made by FL birds as they began using higher tiers for roosting. The increase in “risky” movements was not accompanied with an increase in uncontrolled landings. This could reflect that, in many cases, the birds moved from the aviary onto the pen floor from increasing roosting heights. In addition to distance traveled, the available landing surface affects the difficulty of vertical movements. For example, descending onto a perch is more difficult than landing on the litter floor due to the smaller landing surface on the perch (Scott et al., 1997; Moinard et al., 2004a,b). Indeed, Campbell et al. (2016a) reported lower rates of failed landings when hens moved down onto the litter floor than onto perches.

Our experimental housing design may have contributed to the rarity of uncontrolled transitions we observed compared to other studies. Uncontrolled movements and collisions were previously reported to account for up to 32% of descending transitions (Stratmann et al., 2015) and up to 21% of flights (Campbell et al., 2016a) in aviary settings. Factors such as flock size and space allowance likely contribute to the differences in uncontrolled transition frequencies among studies. Whereas we housed hens in groups of 28 to 30 hens/pen (0.37 m²/hen), the aforementioned research took place in either a small-scale (225 hens/pen and 0.14 m²/hen; Stratmann et al., 2015) or a large-scale commercial setting (>49,500 hens/house; 0.13 m²/hen; Campbell et al., 2016c). Increased space allowance means that our hens had more space for take-offs and landings. Stratmann et al. (2019) reported that increased fall rates within multitiered aviaries were observed during times of day when the hen activity was the highest (dusk and dawn), suggesting that less space per hen in larger flock sizes contribute to uncontrolled transitions.

In our and in other studies (Colson et al., 2008; Norman et al., 2021), hens acclimated quickly to their

environments, and most experience-based differences in the distribution of hens across the aviary disappeared within a few weeks. The only difference between FL and aviary-reared hens that persisted throughout the current study was zone preference for nighttime roosting. Hens typically prefer to roost on perches more than flat plastic grids or flat shelves, but roosting on all types of surfaces has been observed (Brendler and Schrader, 2016; Campbell et al., 2016b). Preferences for roosting surface in multitiered aviaries have previously been associated with the height of the surface, flock age, and available space on the surface (Brendler and Schrader, 2016; Campbell et al., 2016b). From 19 to 27 wk, all hens increasingly roosted in the aviary, but FL hens roosted least in aviary zone D (highest zone with perches only). It is not possible to disentangle whether the use of this zone was due to the FL hens' preference for lower heights, or slatted areas vs. perches for roosting. FL hens also maintained a different strategy for descending transitions between 19 and 27 wk of age than ST hens, such that FL hens displayed a higher proportion of longer descents than ST hens regardless of time of day or age. However, TT hens did not differ from either group, making this finding difficult to interpret. All hens displayed a lower proportion of long descents during the morning hour at 27 wk. This finding was likely an artifact of shifting to natural lighting at this age to manage aggressive behavior in the flock. Once all cameras in the barn turned to color from the natural light stimulus to begin recording behavior in the morning, the majority of hens had already descended from their nighttime roosting spot, thus missing the bulk of transitions that crossed more than 1 zone at this timepoint.

This research was carried out research facility and focused on a single laying hen breed, and the results should be interpreted with these study limitations in mind. The research setting allowed us to address the experimental question related to the role of early experience on the development of space use in a controlled manner, and facilitated longitudinal observations of focal bird movements. Many aspects of management within the research setting differ from commercial practices, including group size, total amount of space available to the hens, aviary design, drinker and feeder location, and the decision on to train hens to use nest boxes. The outcomes related to specific patterns of movement and resource use may be unique to the research conditions. Study outcomes may also be impacted by our choice to use Dekalb White hens. Previous studies have highlighted breed differences related to aviary use, particularly noting differences between white and brown breeds. White hens seem to use aviary ledges and perches to a greater extent, and prefer to roost on the highest offered tiers, whereas brown breeds tend to distribute themselves on the tier floors or in the litter area, and roost in lower parts of the aviary (Ali et al., 2016, 2019; Ciarelli et al., 2023). Whereas our study only considered a single white hen breed, the results might have been more pronounced in hens of a brown breed.

CONCLUSIONS

The experience-based differences in the use of the layer aviary observed in our study lend support to recommended practice of matching behavioral opportunities provided in rearing and layer environments (Gunnarsson et al., 1999; Janczak & Riber, 2015; Norman et al., 2021). As compared to aviary-reared hens, hens reared in FL pens temporarily reduced their utilization of elevated structures and had a higher prevalence of floor eggs. These differences seem to reflect an initial hesitancy for using novel resources rather than a deficiency in physical ability or depth perception. Few differences were noted between ST and TT hens, suggesting that it is most important to allow hens to gain experience with relevant behavioral opportunities (e.g., perching, transitioning among different heights) rather than providing more height or matching the environment perfectly (Gunnarsson et al., 1999).

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DISCLOSURES

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REFERENCES

- Ali, A. B. A., D. L. M. Campbell, D. M. Karcher, and J. M. Siegford. 2016. Influence of genetic strain and access to litter on spatial distribution of 4 strains of laying hens in an aviary system. *Poult. Sci.* 95:2489–2502.
- Ali, A. B. A., D. L. M. Campbell, D. M. Karcher, and J. M. Siegford. 2019. Daytime occupancy of resources and flooring types by 4 laying hen strains in a commercial-style aviary. *J. Vet. Behav.* 31:59–66.
- Appleby, M. C., I. J. H. Duncan, and H. E. Mcrae. 1988. Perching and floor laying by domestic hens: experimental results and their commercial application. *Br. Poult. Sci.* 29:351–357.

- Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67:1–48.
- Brantsæter, M., J. Nordgreen, T. B. Rodenburg, F. M. Tahamtani, A. Popova, and A. M. Janczak. 2016. Exposure to increased environmental complexity during rearing reduces fearfulness and increases use of three-dimensional space in laying hens (*Gallus gallus domesticus*). *Front. Vet. Sci.* 3:14.
- Brendler, C., and L. Schrader. 2016. Perch use by laying hens in aviary systems. *Appl. Anim. Behav. Sci.* 182:9–14.
- Brooks, M. E., K. Kristensen, K. J. van Benthem, A. Magnusson, C. W. Berg, A. Nielsen, H. J. Skaug, M. Maechler, and B. M. Bolker. 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J.* 9:378–400.
- Campbell, D. L. M., E. N. De Haas, and C. Lee. 2019. A review of environmental enrichment for laying hens during rearing in relation to their behavioral and physiological development. *Poult. Sci.* 98:9–28.
- Campbell, D. L. M., S. L. Goodwin, M. M. Makagon, J. C. Swanson, and J. M. Siegford. 2016a. Failed landings after laying hen flight in a commercial aviary over two flock cycles. *Poult. Sci.* 95:188–197.
- Campbell, D. L. M., M. M. Makagon, J. C. Swanson, and J. M. Siegford. 2016b. Perch use by laying hens in a commercial aviary. *Poult. Sci.* 95:1736–1742.
- Campbell, D. L. M., M. M. Makagon, J. C. Swanson, and J. M. Siegford. 2016c. Laying hen movement in a commercial aviary: enclosure to floor. *Poult. Sci.* 95:176–187.
- Ciarelli, C., G. Pillan, F. Bordignon, G. Xiccato, M. Birolo, and A. Trocino. 2023. Space use and navigation ability of hens at housing in the aviary for the laying phase: effect of enrichment with additional perches and genotype. *Poult. Sci.* 102:102962.
- Colson, S., C. Arnould, and V. Michel. 2008. Influence of rearing conditions of pullets on space use and performance of hens placed in aviaries at the beginning of the laying period. *Appl. Anim. Behav. Sci.* 111:286–300.
- Dekalb. (n.d.) Dekalb White commercial management guide for aviary-barn systems. Guide is now known as “Dekalb White commercial management guide – North American version” with modified lighting schedule as of May 2023. Accessed Nov. 2019. https://www.dekalb-poultry.com/documents/1827/Dekalb_White_CS_management_guide__North_American_Version_L2221-1.pdf.
- De Reu, K., W. Messens, M. Heyndrickx, T. B. Rodenburg, M. Uyttendaele, and L. Herman. 2008. Bacterial contamination of table eggs and the influence of housing systems. *World’s Poult. Sci. J.* 64:5–19.
- Fox, J., and J. Hong. 2009. Effect displays in R for multinomial and proportional-odds logit models: extensions to the effects package. *J. Stat. Softw.* 32:1–24.
- Friard, O., and M. Gamba. 2016. BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods Ecol. Evol.* 7:1325–1330.
- Gamer, M., J. Lemon, I. Fellows, and P. Singh. 2019. irr: Various Coefficients of Interrater Reliability and Agreement. *R Package v. 0.84.1*. Accessed April 2022. <https://cran.r-project.org/web/packages/irr/irr.pdf>.
- Gunnarsson, S., L. J. Keeling, and J. Svedberg. 1999. Effect of rearing factors on the prevalence of floor eggs, cloacal cannibalism and feather pecking in commercial flocks of loose housed laying hens. *Br. Poult. Sci.* 40:12–18.
- Gunnarsson, S., J. Yngvesson, L. J. Keeling, and B. Forkman. 2000. Rearing without early access to perches impairs the spatial skills of laying hens. *Appl. Anim. Behav. Sci.* 67:217–228.
- Hartig, F. 2022. DHARMa: Residual Diagnostics for Hierarchical (Multi-Level/Mixed) Regression Models. *R Package v. 0.4.5*. Accessed April 2022. <https://cran.r-project.org/web/packages/DHARMa/index.html>.
- Hutchinson, M. K., and M. C. Holtman. 2005. Analysis of count data using poisson regression. *Res. Nurs. Health* 28:408–418.
- Janczak, A. M., and A. B. Ribber. 2015. Review of rearing-related factors affecting the welfare of laying hens. *Poult. Sci.* 94:1454–1469.
- Jones, D. R., N. A. Cox, J. Guard, P. J. Fedorka-Cray, R. J. Buhr, R. K. Gast, Z. Abdo, L. L. Rigsby, J. R. Plumbee, D. M. Karcher, C. I. Robison, R. A. Blatchford, and M. M. Makagon. 2015. Microbiological impact of three commercial laying hen housing systems. *Poult. Sci.* 94:544–551.
- Jones, C. T., A. N. Pullin, R. Blatchford, M. M. Makagon, and K. Horback. 2023. Effects of rearing with vertical structures on the ontogeny of depth perception in laying hens. *Appl. Anim. Behav. Sci.* 259:105837.
- Lu, Q., C. B. Rufener, R. A. Blatchford, M. M. Makagon, M. J. Toscano, and J. Tarlton. 2021. The effect of rearing system design on bone mineral density and skeletal development in laying hens. *Poult. Sci.* 100(E-suppl. 1):177 [abstract].
- Lüdecke, D. 2021. sjPlot: Data Visualization for Statistics in Social Science. *R Package v. 2.8.10*. Accessed April 2022. <https://cran.r-project.org/package=sjPlot>.
- Matthews, W. A., and D. A. Sumner. 2015. Effects of housing system on the costs of commercial egg production. *Poult. Sci.* 94:552–557.
- Moinard, C., P. Statham, and P. R. Green. 2004a. Control of landing flight by laying hens: implications for the design of extensive housing systems. *Br. Poult. Sci.* 45:578–584.
- Moinard, C., P. Statham, M. J. Haskell, C. McCorquodale, R. B. Jones, and P. R. Green. 2004b. Accuracy of laying hens in jumping upwards and downwards between perches in different light environments. *Appl. Anim. Behav. Sci.* 85:77–92.
- National Oceanic and Atmospheric Administration (NOAA). n.d. NOAA Solar Calculator: Sunrise, Sunset, and Noon for any Place on Earth. National Oceanic and Atmospheric Administration, Washington, DC. Accessed May 2023. <https://gml.noaa.gov/grad/solcalc/>.
- Norman, K. I., C. A. Weeks, J. F. Tarlton, and C. J. Nicol. 2021. Rearing experience with ramps improves specific learning and behaviour and welfare on a commercial laying farm. *Sci. Rep.* 11:8860.
- Oliveira, J. L., H. Xin, L. Chai, and S. T. Millman. 2019. Management and production: effects of litter floor access and inclusion of experienced hens in aviary housing on floor eggs, litter condition, air quality, and hen welfare. *Poult. Sci.* 98:1664–1677.
- Pullin, A. N., V. S. Farrar, J. W. Loxterkamp, C. T. Jones, R. M. Calisi, K. Horback, P. J. Lein, and M. M. Makagon. 2022. Providing height to pullets does not influence hippocampal dendritic morphology or brain-derived neurotrophic factor at the end of the rearing period. *Poult. Sci.* 101:102161.
- Pullin, A. N., S. M. Temple, D. C. Bennett, C. B. Rufener, R. A. Blatchford, and M. M. Makagon. 2020. Pullet rearing affects collisions and perch use in enriched colony cage layer housing. *Animals* 10:1269.
- R Core Team. 2021. R: A language and environment for statistical computing. Accessed April 2022. <https://www.r-project.org/>.
- Rentsch, A. K., E. Ross, A. Harlander, L. Niel, J. M. Siegford, and T. M. Widowski. 2023. The development of laying hen locomotion in 3D space is affected by early environmental complexity and genetic strain. *Sci. Rep.* 13:10084.
- Rodenburg, T. B., H. Komen, E. D. Ellen, K. A. Uitdehaag, and J. A. van Arendonk. 2008. Selection method and early-life history affect behavioural development, feather pecking and cannibalism in laying hens: a review. *Appl. Anim. Behav. Sci.* 110:217–228.
- Rufener, C., Y. Abreu, L. Asher, J. A. Berezowski, F. Maximiano Sousa, A. Stratmann, and M. J. Toscano. 2019. Keel bone fractures are associated with individual mobility of laying hens in an aviary system. *Appl. Anim. Behav. Sci.* 217:48–56.
- Scott, G. B., N. R. Lambe, and D. Hitchcock. 1997. Ability of laying hens to negotiate horizontal perches at different heights, separated by different angles. *Br. Poult. Sci.* 38:48–54.
- Stratmann, A., E. K. F. Fröhlich, S. G. Gebhardt-Henrich, A. Harlander-Matauschek, H. Würbel, and M. J. Toscano. 2015. Modification of aviary design reduces incidence of falls, collisions and keel bone damage in laying hens. *Appl. Anim. Behav. Sci.* 165:112–123.
- Stratmann, A., S. Mühlemann, S. Vögeli, and N. Ringgenberg. 2019. Frequency of falls in commercial aviary-housed laying hen flocks and the effects of dusk phase length. *Appl. Anim. Behav. Sci.* 216:26–32.
- Weeks, C. A., and C. J. Nicol. 2006. Behavioural needs, priorities and preferences of laying hens. *World’s Poult. Sci. J.* 62:296–307.
- Wichman, A., M. Heikkilä, A. Valros, B. Forkman, and L. J. Keeling. 2007. Perching behaviour in chickens and its relation to spatial ability. *Appl. Anim. Behav. Sci.* 105:165–179.