

Later exposure to perches and nests reduces individual hens' occupancy of vertical space in an aviary and increases force of falls at night

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ABSTRACT Tiered aviaries are intended to improve laying hen welfare by providing resources that enable them to perform essential behaviors. However, hens must be able to navigate these complex systems efficiently and safely. This study investigated the influence of providing perches and nests starting at 17 or 25 wk of age (WOA) on hens' use of vertical space in an aviary at 36 and 54 WOA. Three treatments were applied to pullets raised in floor pens until 17 WOA (4 units/treatment; 100 hens/unit). Control (CON) pullets were placed into aviaries at 17 WOA. Floor (FLR) pullets were placed into aviaries at 25 WOA. Perches and nests were placed in enriched (ENR) pullets' floor pens at 17 WOA prior to moving ENR birds to aviaries at 25 WOA. Five focal hens/unit ($n = 20$ total hens/treatment) were fitted with accelerometers, and their diurnal movement (g) and frequency (n) and acceleration (g) of falls at night were recorded. Direct observation of focal hens was

conducted for 6 min/hen at morning, midday, and evening for 3 consecutive days at 36 and 54 WOA, and location and time spent on vertical tiers were recorded. At 36 WOA, FLR hens spent more time on litter than CON and ENR, which spent more time in the top tier (all $P \leq 0.05$). ENR hens exhibited higher vertical movement than CON and FLR hens (0.8, 0.6, and 0.3 g; $P = 0.003$). CON hens fell most often at night (16 vs. 9 FLR and 5 ENR), whereas FLR hens had higher acceleration and calculated collision force than CON and ENR hens during falls (0.8, 0.5, 0.3 g and 15, 10, 5 N, respectively; $P \leq 0.05$). At 54 WOA, hens' movement and vertical distribution were similar across treatments. Delaying birds' access to perches and nests until 25 WOA impacted their movement, vertical space use, and falls at night for at least 10 wk. However, providing perches and nests at 17 WOA, even in floor pens, considerably mitigated such impacts.

Key words: enrichment, adaptation, development, movement

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INTRODUCTION

The U.S. laying hen industry is transitioning the majority of laying hens to cage-free housing systems, such as aviaries, in response to announcements by the restaurant, grocery, and hotel chains and other food businesses that make up an estimated 70% of U.S. egg demand (Wong, 2017). The configurations of indoor, tiered aviaries are intended to alleviate the public's concern about animal welfare by providing laying hens with additional resources and space. The floor litter area, enclosed nests, and elevated tiers with perches are designed to fulfill the biologically driven needs of hens to lay eggs in nests (Cooper and Appleby, 1995), dust bathe (Vestergaard, 1982), and roost on elevated

structures (Brendler and Schrader, 2016). Hens have strong motivation to access these types of resources (Kruschwitz et al., 2008), and birds with fulfilled motivations are generally considered to have good welfare.

Hens in multi-tier aviaries frequently navigate among levels by jumping or flying. However, hens have been found to land poorly after 9 to 21% of all flights in a commercial aviary, with landings on perches failing more often than those on litter (Campbell et al., 2016a). Such failures could be the result of domestic hens' poor ability to control lift when flying due to high wing loading (i.e., increased weight per wing area compared to ancestral junglefowl, Moinard et al., 2004a) and exacerbated by the use of smooth perches (Scholz et al., 2014), obstructions (Moinard et al., 2005) the distance between takeoff and landing (Scott and Parker, 1994), or poor spatial navigation skills (Gunnarsson et al., 2000). Collisions with aviary structures or other hens as a result of either failed landings or falls from roosting locations at night are hypothesized to lead to injuries, such as keel bone damage, which is prevalent in up to

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80% of hens in non-cage systems (Wilkins et al., 2004; Moinard et al., 2004b; Harlander-Matauschek et al., 2015; Stratmann et al., 2015; Ali et al., 2016). Prevalence of fracture has been correlated with the height of these locations (Wilkins et al., 2011), with falls from relatively low perches (<77 cm above the ground), not increasing frequency of fracture (Sandilands et al., 2010). Therefore, it is likely that the greater kinetic energy at impact resulting from falling from greater heights increases the risk of keel fracture. Prior exposure to aviaries or to vertical arrays of perches may facilitate development of hens' spatial navigation abilities (Gunnarsson et al., 2000) and subsequent adaptation to the laying environment (Colson et al., 2008), which may reduce incidence of collisions and falls.

Typically, pullets are moved into laying hen housing before they have begun to lay, at about 17 wk of age (WOA) (Alm et al., 2015). This timing is primarily intended to ensure that the birds have access to nest areas from the start of lay to facilitate egg laying in nests. However, it is also important to consider when to provide pullets with access to perches and vertical space in order to prepare them for the challenges of navigating aviaries without injury. The early environment of animals, including that of our domestic livestock and poultry, influences how they develop and interact with their environment later in life (Denenberg, 1969; Appleby and Duncan, 1989; Hunniford and Widowski, 2016). There is evidence from commercial production systems (Gunnarsson et al., 1999; Häne et al., 2000) and experimental studies that hens should be exposed to features of laying systems when they are young in order to learn to use and benefit from them (Appleby and Duncan, 1989; Gunnarsson et al., 2000). Providing pullets with perches and nests or placing them in rearing systems identical to the later laying system improves hens' use of nests (Petersen, 1991), reduces floor egg laying (Appleby et al., 1988) and cloacal cannibalism (Gunnarsson et al., 1999), improves transition between vertical tiers (Michel and Huonnic, 2003), increases perch use (Faure and Jones, 1982), increases bone strength (Regmi et al., 2016), improves learning and memory (Tahamtani et al., 2015), and may even enhance production (Colson et al., 2005).

To maximize the adaptation of pullets to aviary housing, it has been recommended that rearing and laying environments should match as closely as possible (Colson et al., 2008; Janczak and Riber, 2015). However, for pullets destined for aviaries, floor pens are often a desirable alternative to rearing aviaries, as they are easier and cheaper to manage. If furnished with perches, which are important to both development of the spatial ability of hens (Colson et al., 2008) and reducing floor eggs (Appleby et al., 1988; Gunnarsson et al., 1999), it is possible that enriched floor pens could adequately prepare pullet for placement in aviaries.

The capacity of hens to learn to use vertical space appears to be slower after 16 WOA (see Janczak and Riber, 2015 for a review), yet as discussed above, pullets

are often moved from rearing environments to laying facilities after this age. At present, little experimental work has followed pullets introduced to arrays of perches or aviaries at later ages beyond their immediate response to evaluate the potential of hens to ultimately adapt. A more complete understanding of the full consequences of timing of resource provision on laying hen welfare throughout the lay cycle is needed. Such knowledge of the timing of resource provision would help in the understanding of when and what environmental features hens need to exposure to in order to help them adjust to complex non-cage laying environments. Placing pullets into laying facilities at ages later than 17 WOA has also been suggested as a practical solution that could be applied commercially to repopulate laying hen facilities following a disease outbreak. For instance, following outbreaks of highly pathogenic avian influenza, a lengthy cleaning and quarantine process occurs that disrupts the timing of pullet movement into hen houses. Delaying placement of pullets could be an alternative to depopulating the flock and starting again. The bigger project that included the current study investigated delayed placement from this perspective (Karcher et al., 2018).

Therefore, our objective was to investigate the influence of introducing pullets to perches and nests at 17 WOA on hens' subsequent use of vertical space and physical activity in an aviary system at 36 and 54 WOA. We predicted that hens given perches at 17 WOA in rearing floor pens and placed into aviaries at 25 WOA would behave comparably to birds placed directly into aviaries at 17 WOA. On the other hand, we expected that hens not given access to perches until 25 WOA would use floor litter areas and bottom tiers more frequently and exhibit less vertical movement among tiers during the day, and that these birds would roost on lower locations and exhibit more falls at night.

MATERIALS AND METHODS

Ethics

All research protocols were approved by the Michigan State University Institutional Animal Care and Use Committee prior to the start of data collection (AUF# 05/16-071-00).

Pullet Rearing and Treatments

A total of 3,000 Hy-Line W36 chicks were reared from hatch in an environmentally controlled, windowless building containing 5 pens on each side ($n = 10$ total pens) at the Michigan State University Poultry Teaching and Research Center (East Lansing, MI).

Each pen was 27.87 m² with 7 feed pans and 22 pin-metered drinkers. Six pens were used in the current study, each housing approx. 300 chicks. Chicks were brooded on platforms (1.2 m x 4.9 m x 0.46 m) with plastic flooring. From 3 WOA, chicks were given access

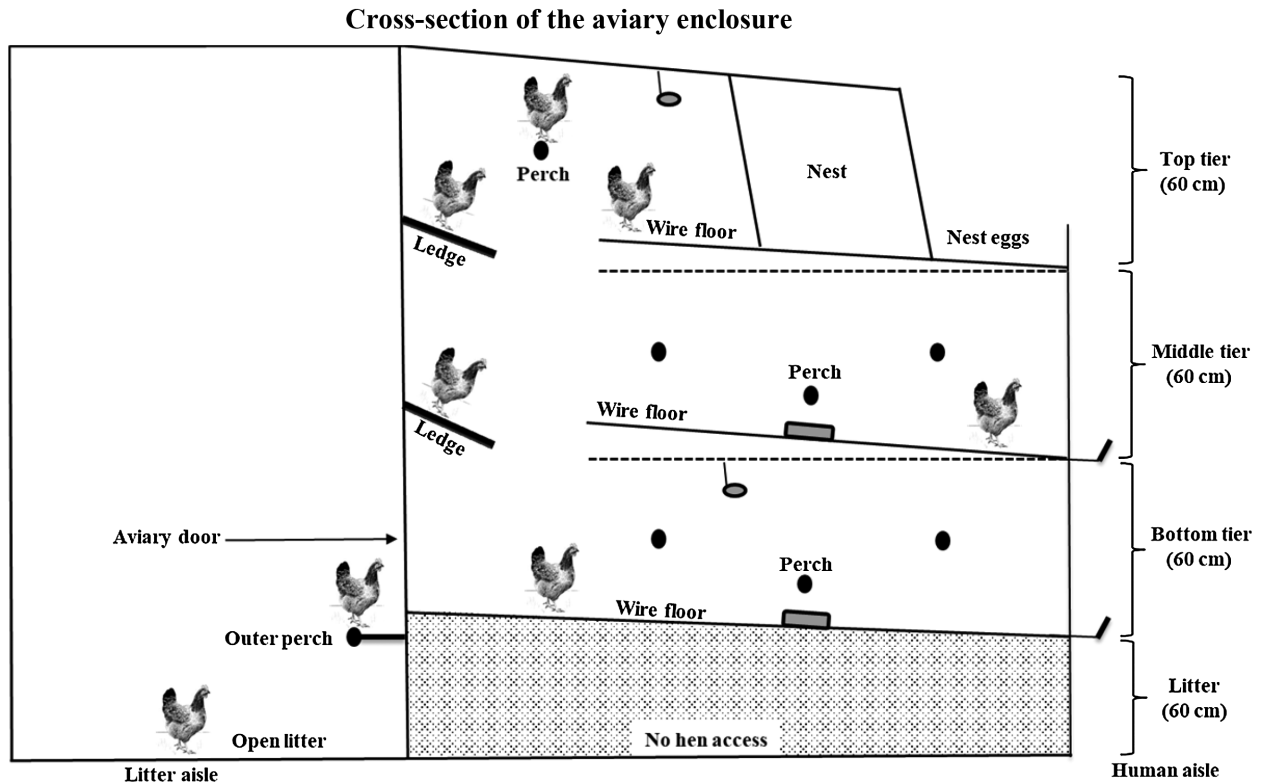


Figure 1. An end view of the aviary unit, showing human and litter aisles and locations of the litter area, solid metal ledges between the middle and upper tiers, wire floors, the colony nest, perches (black circles), drinkers (gray ovals), and external and internal feeders (gray boxes). The area under the aviary was not accessible by hens. The door allowing access to the litter remained open continuously. Adapted from Ali et al. (2016).

to a floor covered with wood shavings. Ramps made of the same plastic flooring as the platforms allowed chicks to move freely between the platform and the floor.

At 17 WOA, 3 different treatments were applied (929 cm²/bird; n = 2 rearing pens/treatment). Floor pullets (**FLR**) remained in unaltered floor rearing pens in the pullet-rearing building. Enriched (**ENR**) pullets also remained in their floor rearing pens but were provided with 56.8 cm² of nesting space per bird and 8.36 cm perch space per bird, with perches at variable heights. For further details on housing and system management, see Karcher et al. (2018). Control (**CON**) pullets were moved at 17 WOA to the laying hen facility and placed into 4 units of a commercial-style aviary system (NATURA60, Big Dutchman, Holland, MI).

At 25 WOA, the FLR and ENR birds were moved from their floor pens into units across the 4 aviary rooms, resulting in 1 unit per treatment per room and 4 total units for each treatment. To ensure that handling and movement of birds was consistent across treatments, when ENR and FLR hens were placed into their aviary units, CON hens were moved into new units spread across the 4 aviary rooms. Treatments were placed into units within the room so that across the 4 rooms, each treatment occupied a different location to account for any effects of being located near the door or at the end vs. the center of a row. Each unit was initially populated with 144 hens. Due to euthanasia

for tissue sample collection as a part of another project and any naturally occurring mortality, the total number of birds per unit at the start of the current study was 100 hens/unit.

Layer Housing and Management

The details of aviary design and system management are identical to those reported in Ali et al. (2016). Briefly, aviary units were composed of a 3-level, wire-mesh enclosure and an open litter area in front of each unit (Figure 1). As measured from the center of the enclosure, the floor of the bottom tier was 51 cm from the aviary floor, whereas the floors of the middle tier and the top tiers were 112 and 173 cm from the aviary floor, respectively. Each tier contained round metal perches (3.1 cm diameter) at all levels that extended the full length of the unit (244 cm; Figure 1). Two solid, metal ledges, intended to help hens' transition between tiers within the enclosure, ran the full length of each unit in front of the middle and top tiers.

Feeders provided 5 cm feeder space per bird, whereas water lines in the bottom and top tiers provided water access at a rate of 9 hens per pin-metered nipple drinker (Figure 1). A colony nest ran the length of the unit in the top tier, with a central divider creating 2 equally sized nesting compartments in each unit. The nest was 52 cm wide and each compartment was 122 cm long.



Figure 2. A fabric vest was used to secure the accelerometer to the hen. Vests were wrapped around hens' bodies behind the wings and secured using Velcro. Elastic bands around the base of the wings ensured the vest did not rotate or slip off the hen. The accelerometer was oriented so the X axis captured a hen's forward and backward movement and the Z axis captured vertical movement.

Nesting areas closed automatically 2 h before lights off, and this space was not available at night. Eggs, including those rolling out of nests onto an external egg belt, were collected by hand each morning.

Aviary doors to litter areas were opened 24 h after birds were placed in the unit and remained open thereafter, providing hens with continuous access to litter. Starting at 36 WOA, lights turned on at 05:00 and off at 20:00. Feed belts delivered fresh feed at 06:00, 14:00, and 19:30. To stimulate hens to eat food already present, the belts ran for approximately 10 s at 09:00 and 16:45.

Focal Hen Selection and Marking

Two days prior to observation, 5 hens in each unit (5% of birds per unit) were caught after lights off. Birds were selected from among different resources and levels throughout the aviary in an attempt to sample hens that were representative of the flock in that unit. In total, 20 focal hens were selected per treatment (5 hens/unit \times 4 units/treatment).

Each focal bird was fitted with a fabric vest (Figure 2) used to secure the accelerometer and of a different color for identification purposes (yellow, brown, black, green, and blue with each pen containing one hen of each color). An acceleration data logger (Onset HOBO PendantG acceleration data loggers, Onset Computer Corporation, Bourne, MA) was attached to the dorsal side of each vest. The loggers used in the current study were $58 \times 33 \times 23$ mm in size and 18 g in weight, with a ± 3 g; 29.4 m/s^2 measuring range, and ± 0.105 g; 1.03 m/s^2 accuracy level when operating between -20°C and 70°C . Loggers were oriented on the hens so the X-axis captured forward and backward movement (Cranio-caudal movement), the Y-axis captured sideways movement (Mediolateral movement), and Z-axis captured vertical movement (Dorsoventral movement) of the hens as shown in Figure 2. Loggers were firmly attached to vests to reduce noise in the data due to movement of the loggers themselves and to prevent changes in logger orientation. After fitting focal

birds with vests and accelerometers, hens were given 1 D to habituate to wearing the equipment. During this period, hens were monitored to ensure that vests were not impacting behavior, and locomotion abilities. After acclimation, loggers recorded hens' movement across 3 consecutive days (72 h) at each age, with scanning frequency of 20 Hz (-3 g to $+3$ g) in 3 axes.

Individual Hen Tracking

Direct observation of individual hens was conducted during the day (i.e., lights on) and night (i.e., lights completely off) over 3 consecutive days at 36 and 54 WOA, when hens were at the peak and middle of their lay cycles, respectively. Prior to starting data collection, 2 observers trained for 3 d to ensure high inter-observer reliability. During the day, direct observations were conducted starting 15 min after lights on (morning: 05:15 to 07:15), during the middle of the day (midday: 12:15 to 14:15) and 2 h before lights off (evening: 18:00 to 20:00). Nighttime direct observations were conducted 30 min after full darkness (PM: 21:30 to 23:30) and 2 h before lights on (AM: 03:00 to 05:00). Disturbance of hens during the night was minimized by using green headlamps, which allowed observers to see hens in the darkened room without rousing them to movement (Ali et al., 2016; Campbell et al., 2016c).

At each time of day, 2 rounds of observation were conducted with the second round occurring about 1 h after the first. In each round, direct observations were conducted for 3 min per focal hen. Location of the hen was recorded, along with the time spent at each location within the 3-min observation. The sequence of observation across hens, units, and rooms was randomized across the rounds of observation. There was a total of 360 min of individual hen direct observation for each treatment during each time of day (morning, midday, and evening) at each age. This was calculated as follows: $360 = 3 \text{ min direct observation per hen} \times 2 \text{ rounds of observation per time of day} \times 3 \text{ d of observation} \times 5 \text{ hens per unit} \times 4 \text{ units per treatment}$.

During nighttime observations, the roosting locations of focal hens were recorded.

Data Processing and Statistical Analysis

The raw accelerometer data, consisting of the date, time, and the related impulse in the X, Y, and Z dimensions, were downloaded from the devices (HOBOWare Graphing & Analysis Software, Bourne, MA) at the end of each 3-D observation period. Data on hens' vertical (a_z : dorsoventral movement across vertical levels), horizontal (a_x : craniocaudal movement within the same vertical level), and lateral movement (a_y : mediolateral movement within the same vertical level), during light hours were obtained directly from loggers. Hens' tri-axial movement (A_s) was calculated by summing and averaging raw movement data:

$$A_s = \sqrt{a_x^2 + a_y^2 + a_z^2}$$

Acceleration data were post-processed using MATLAB (MATLAB and Statistics Toolbox Release 2012, The MathWorks, Inc., Natick, MA). In order to accurately calculate falls and force of collision, data from the dark period were smoothed from noisy components by removing all minor acceleration fluctuations using a loop function. Data smoothing included passing of the raw acceleration values (A_j) through an asymmetrical 3 point-moving average low-pass filter (i = the middle point in the 3 point-moving average low pass filter) and through a step function to define thresholds used to remove minor fluctuations (t = threshold values of minor fluctuations, i.e., between 0.001 and 0.043 g). After processing data, falls were recognized by detecting massive shifts in acceleration from the static condition of hens during dark hours.

$$A_i = \frac{1}{3} \sum_{j=i-1}^{i+1} A_j \quad A'_i = \begin{cases} \mu, & \text{if } |A_i - \mu| < t \\ \mu, & \text{if } |A_i - \mu| \geq t' \end{cases}$$

Hens were considered to be in a stationary state when a constant acceleration (1 g) was acting on the hen. A fall during the night was detected when the recorded acceleration was lower than the constant/static one. Force of collision ($F = N$) due to falling was calculated using the hen's exact body weight ($M = \text{kg}$) and the summed acceleration recorded during falling ($A'_i = m/s^2$):

$$F = M \times A'_i$$

The distance of the fall ($D = \text{cm}$) was calculated using initial velocity ($V_i = m/s$), assumed to equal zero during the stationary condition before falling), time

($t = s$) elapsed during falling, and the summed acceleration during falling ($A'_i = m/s^2$):

$$D = \left\{ V_i \times t + \frac{1}{2} A'_i t^2 \right\} \times 100$$

During the night, hens' roosting locations were recorded during observations immediately after lights off (PM) and before lights on (AM). Using these data, falls and corresponding displacements indicated by the sensor were confirmed by comparing hens' initial nighttime roosting location during PM observations to their final roosting location during AM observations. Data obtained from direct observation of individual hens during the day were converted into percentages of time hens spent on the different vertical levels (litter, bottom tier, middle tier, and top tier) out of the total time of observation.

Statistical analyses were performed using R software (version 3.3.1) with the "stats" package (R Core Team, 2013). Descriptive statistics were calculated using the "psych" package, and data are presented as mean \pm standard error of the mean (SEM), and $P \leq 0.05$ was considered significant. All models included fixed effects of treatment, time of day, hen age, and their interactions. Aviary unit, day of observation, and individual hen were included as random effects for all models.

To describe the influence of different treatments on hens' ability to navigate through and use various resources in an aviary system during the day, the percentages of time hens used different resources were compared among different treatments, times of day, and hen ages. Generalized linear mixed models (GLMM) were developed with family set to "binomial" (to fit residuals to a normal distribution), using the "lme4" package (Bates, et al., 2014). Following the same approach, acceleration, distance, and force of collision of falls recorded during the night were compared among different treatments and across different ages. GLMM were developed with family set to "Poisson" (to fit residuals to a normal distribution), using the "lme4" package.

Statistically significant effects in all models were further analyzed with Tukey's honestly significant difference multiple comparison procedures using the "multcomp" package (Hothorn et al., 2008). Finally, since 2 observers collected the data, inter-observer reliability was calculated using Cohen's kappa Agreement coefficient (K), following Landis and Koch (1977) and using "cohen.kappa" function in the "psych" package. Inter-observer reliability was measured during the training period, before data collection took place, when trainees observed the same areas of the aviary simultaneously. Inter-observer agreement was considered good (Kappa = 0.96 ($P < 0.001$), CI (0.90, 0.99)). Graphs visualizing time hens spent per vertical level by time of day, and roosting sites and incidence of fall during night observations were generated using MATLAB (MATLAB

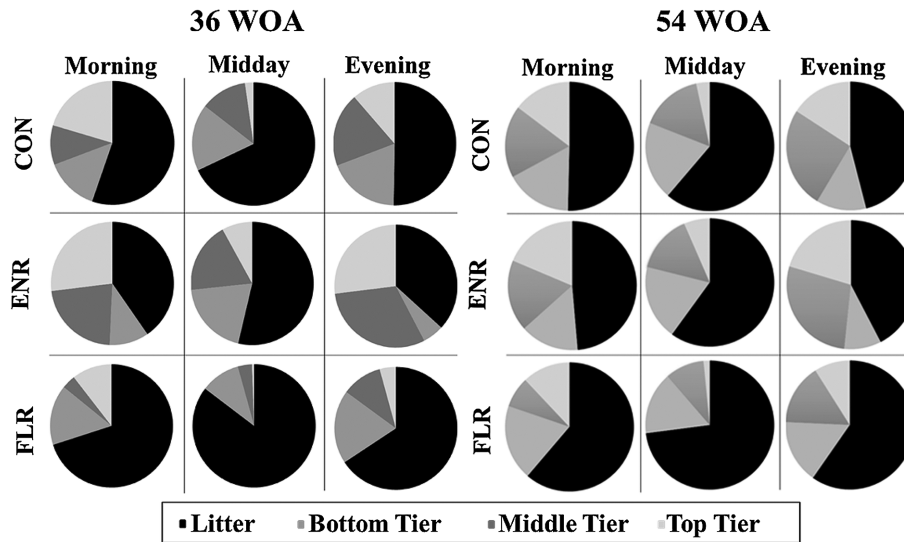


Figure 3. Percent of observation time spent by focal hens ($n = 20$ hens/treatment) across treatments (CON: control, ENR: enriched, and FLR: floor group) in different vertical levels of the aviary, during morning, midday, and evening at 36 and 54 WOA. Data are expressed as average % of the total time observed.

and Statistics Toolbox Release 2012, The MathWorks, Inc.).

RESULTS

Interaction of treatment, time of day, and hen age were significant at 36 WOA for the percentage of time spent by individual hens in different areas (Figure 3a, $Z = 7.25$; $P = 0.001$). At 36 WOA, FLR hens spent more time on litter across the day (morning: $P = 0.023$, midday: $P = 0.032$, and evening: $P = 0.035$) compared to CON and ENR hens (Figure 3a). On the other hand, ENR hens spent more time than CON and FLR hens on middle (Figure 3a; morning: $P = 0.035$, midday: $P = 0.036$, and evening: $P = 0.037$) and top tiers (morning: $P = 0.031$, midday: $P = 0.041$, and evening: $P = 0.021$) across the day (morning: $P = 0.035$, midday: $P = 0.036$, and evening: $P = 0.037$). ENR hens spent the least time on the bottom tier (5.58%) compared to CON (18.96%) and FLR (19.37%) hens during evening observations ($P = 0.023$; Figure 3a).

Fewer interactions among treatment, time of day, and hen age were found at 54 WOA for the percentage of time spent by individual hens in different areas (Figure 3b, $Z = 3.25$; $P = 0.041$). FLR hens spent more time on litter during midday than CON and ENR hens ($P = 0.039$; Figure 3b), and during evening observations, ENR hens spent more time on the top tier than CON and FLR hens ($P = 0.045$; Figure 3b).

CON hens followed similar patterns of time spent per area across the day between 36 and 54 WOA (Figure 3b, $Z = 2.56$; $P = 0.096$). However, as they aged, FLR hens spent less time on litter ($P = 0.032$) and more time on middle ($P = 0.036$) and top tiers ($P = 0.032$; Figure 3). Conversely, ENR hens spent more time on litter ($P = 0.042$) and less time on top tiers ($P = 0.039$) over time (Figure 3).

Treatment affected individual hen daily movement at 36 WOA (Figure 4, $Z = 7.25$; $P = 0.001$). Vertical movement was higher in ENR than CON and FLR hens ($P = 0.003$; Figure 4), and FLR hens had higher horizontal movement than CON and ENR hens ($P = 0.036$; Figure 4). However, total triaxial movement was not significantly different among treatments.

Table 1 shows the incidence, distance, acceleration, and force of collision of nighttime falls from locations higher than the bottom tier wire floors (because hens roosting here could not fall lower in the system). ENR hens fell less often during nighttime observations at 36 WOA compared to CON and FLR hens, and at 54 WOA, the incidence of falls was reduced across all treatments. At 36 WOA, more CON and ENR hens fell from higher roosting locations (i.e., longer distances) when compared to FLR hens ($P = 0.032$). However, though FLR hens fell shorter distances (i.e., from lower roosting locations), the acceleration and collision forces during these falls were higher than those of CON and ENR hens (36 WOA: $P = 0.029$; 54 WOA: $P = 0.036$). A comparison between accelerometer (Table 1) and observational data (Figure 5) confirms that each fall identified by accelerometer data matches with an observed downward change in roosting location of that same focal hen. CON focal hens fell from locations on the top tier 16 times out of 54 high-roosting observations (i.e., 30%). In contrast, ENR and FLR focal hens were recorded falling from locations on the top tier in 6 of 55 observations (i.e., 11%) of ENR hens, and 9 of 46 observations (i.e., 20%) of FLR hens (Figure 5).

Of the 60 total nighttime recordings per treatment, ENR and CON focal hens were recorded roosting on locations higher than bottom tier-wire floors more often than floor FLR hens (55 and 54 vs. 46 occurrences). At least 4 ENR hens were observed roosting either on or above the top tier ledge during all PM observations

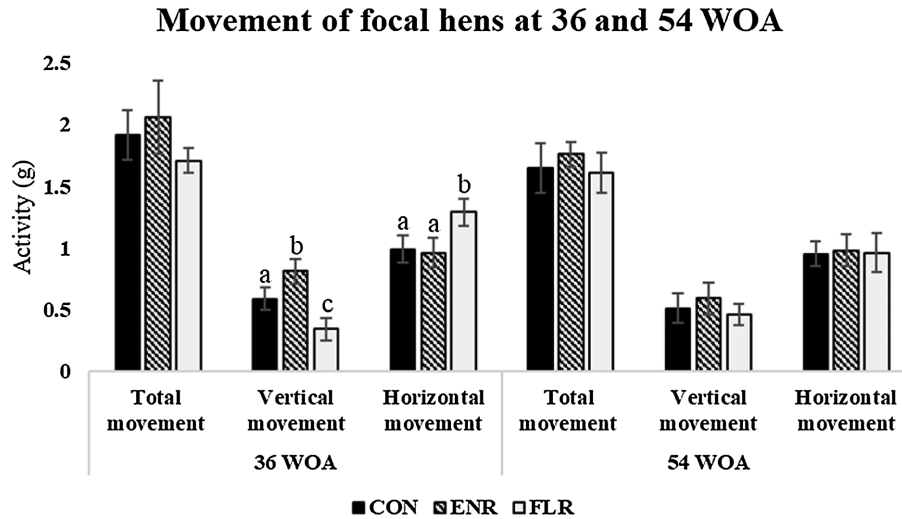


Figure 4. Daily individual hen movement (total = Triaxial, vertical = Dorsoventral, and horizontal = Craniocaudal movement) recorded across treatments (CON: control, ENR: enriched, and FLR: floor group) expressed as average movement (g) per individual hen. All parameters are expressed as mean movement \pm SEM. Different superscripts indicate statistically significant differences ($P < 0.05$) among different treatments.

Table 1. Incidence, distance, acceleration, and force of collision of nighttime falls of focal hens during 36 and 54 WOA across different treatments as recorded by accelerometers

	36 WOA			54 WOA		
	CON	ENR	FLR	CON	ENR	FLR
Fall incidence (n)	16	6	9	7	3	4
Fall distance (cm)	126.35 \pm 21.23 ^a	122.85 \pm 24.02 ^a	83.75 \pm 15.96 ^b	115.52 \pm 16.96 ^a	110.36 \pm 14.58 ^a	105.63 \pm 16.96 ^a
Acceleration (g)	0.51 \pm 0.21 ^a	0.26 \pm 0.11 ^b	0.78 \pm 0.31 ^c	0.31 \pm 0.12 ^a	0.27 \pm 0.11 ^a	0.43 \pm 0.16 ^b
Collision force (N)	10.31 \pm 2.21 ^a	5.27 \pm 1.48 ^b	14.94 \pm 2.67 ^c	5.23 \pm 1.25 ^a	4.25 \pm 1.96 ^a	8.85 \pm 1.58 ^b

Parameters are presented as means \pm SEM per treatment (n = 60 nighttime recordings per treatment). Different superscripts indicate statistically significant differences ($P < 0.05$) among different treatments within each age.

(Figure 5). In 50% of observations at 36 WOA, FLR focal hens were recorded roosting on locations lower than the top tier ledge; at 54 WOA, FLR hens were observed roosting on upper locations 58% of the time (Figure 5). CON hens roosted across more locations at both ages compared to both FLR and ENR hens (Figure 5). At 36 WOA, few hens showed site fidelity (i.e., roosting in the same location) across the 3 nights of observations. For example, 4 ENR hens and 4 CON hens retained their nightly roosting location, and only 1 FLR hen did so. However, at 54 WOA, more hens of all treatments were recorded roosting in the same location across the 3 nights of observation (Figure 5; ENR = 7, CON = 6, and FLR = 5).

DISCUSSION

The aim of this study was to examine the influence of access to perches and nests starting at 17 or 25 WOA on hens' subsequent use of vertical space and physical movement in a multi-tier aviary system at 36 and 54 WOA. Investigating the adaptability of hens at later ages and at the time when birds typically transition between rearing and laying extends our knowledge regarding what kind of exposure to spatial features hens

need to help them adjust to complex non-cage laying environments.

Previous research has indicated that pullets' ability to learn to use perches slows as they age, and learning before 16 to 18 WOA is important to avoid permanently impairing hens' subsequent spatial skills (Faure and Jones, 1982; Gunnarsson et al., 2000; Brantsæter et al., 2016). Furthermore, exposure to perches prior to 18 WOA increases later adult perching behavior (Faure and Jones, 1982; Appleby and Duncan, 1989). Thus, we know that birds are sensitive to environmental effects during early life (Bateson, 1979), and early exposure to enrichment has a long-lasting influence on development of certain abilities or reducing the risk of developing abnormal behaviors (Johnsen et al., 1998).

In the same context, Colson et al. (2008) conducted a study testing the influence of rearing environment (i.e., floor pens furnished with platforms and perches versus rearing aviaries) on birds' adaptation to laying aviaries in terms use of vertical tiers, jumps, flights, and egg location (i.e., nest vs. floor laid eggs). They reported that hens reared in furnished floor pens from 1 to 16 WOA stayed on litter and bottom tiers and laid more floor eggs, whereas aviary-reared hens showed greater use of elevated tiers, had higher accuracy of long flights

Focal hen nighttime roosting sites recorded by direct observations

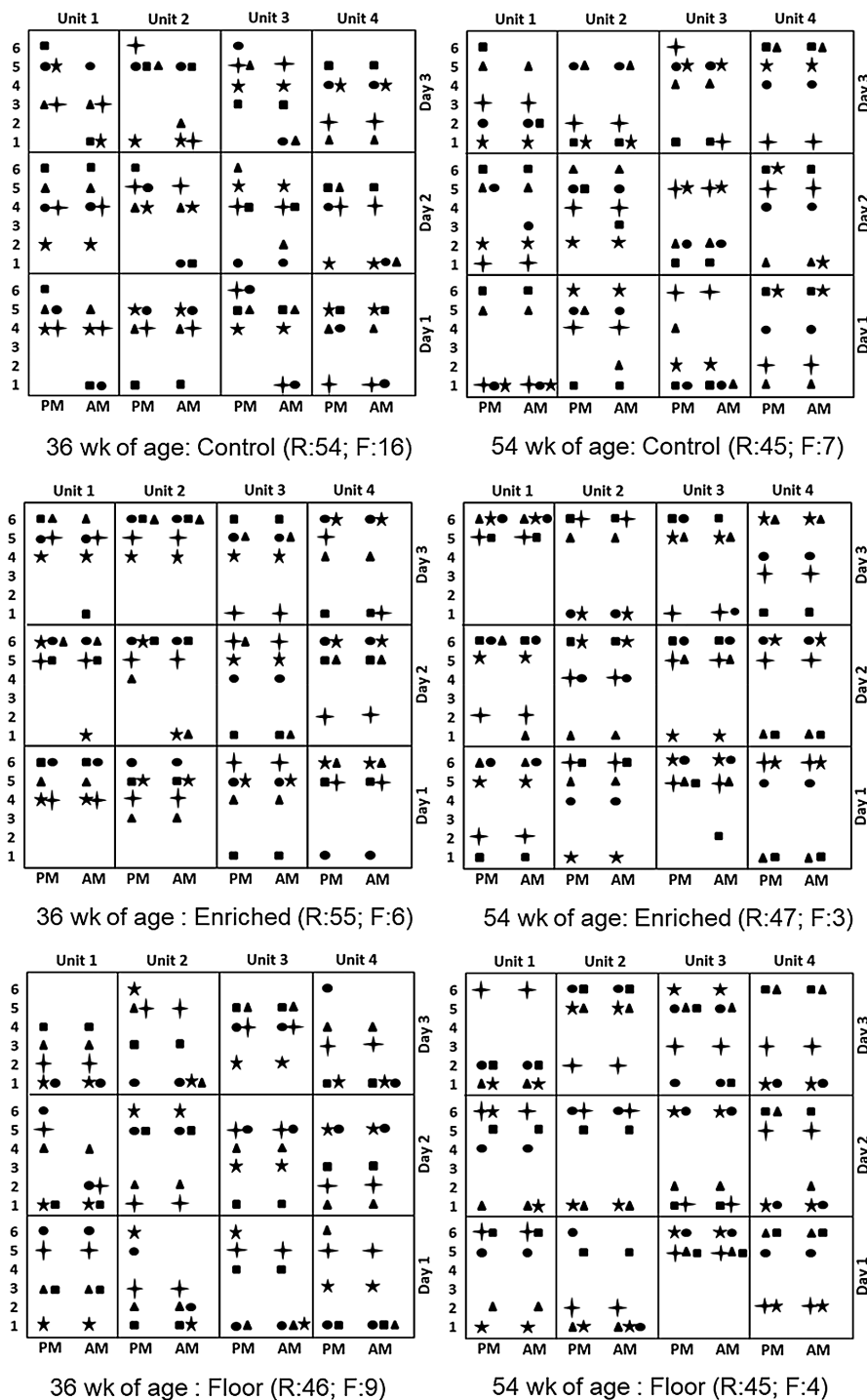


Figure 5. Nightly observation of focal hen roosting site, incidence of roosting on locations higher than bottom-tier floors (R), and incidence of falls (F) between PM (30 min after full darkness) and AM (2 h before lights on) observations, over the 3 nights of observation, across treatments (CON: control, ENR: enriched, and FLR: floor group) at 36 and 54 WOA. Each of the focal hens per unit is represented by a specific symbol, whereas roosting sites within aviaries are expressed with a number as follows: 1: bottom tier wire floor; 2: middle tier ledge; 3: middle tier wire floor; 4: top tier ledge; 5: top tier wire floor; 6: top tier perch. (Note: focal hens were not observed roosting on bottom or middle tier perches, litter, or the external perch.)

and jumps, and laid fewer floor eggs during adulthood. They attributed such differences to the fact that aviary-reared hens might have developed a better motor system and vertical navigation capabilities enabling them to better find nests. However, in the current study ENR

hens used top tiers more frequently and performed more vertical movement between tiers than CON hens, which partially contradicts findings from that previous study. These differences between studies might suggest that placing pullets into aviaries at 17 WOA (i.e., in the

current study) was not as effective as rearing them in these systems from 1 WOA as in Colson et al. (2008). Alternatively, enriching floor pens with perches at the later age of 17 WOA (i.e., as in the current study) was able to promote hens' development of spatial navigation skills more effectively than providing enrichments to floor pens at earlier ages (i.e., 1 WOA; Colson et al., 2008).

Daytime Vertical-Level Space Use and Movement

Hens in the FLR group, which were not given access to perches of any type until 25 WOA, tended to spend more time on litter floors and performed more horizontal than vertical movement. In contrast, ENR and CON hens were found in larger numbers at higher aviary levels and performed more vertical movement. These behavioral differences among treatment groups could be the result of preferences the birds developed earlier in life (Denenberg, 1969; Gvoryahu et al., 1989; Reed et al., 1993). Alternatively, FLR hens might have a poorer ability to perform the long jumps or flights needed to reach upper aviary levels, whereas ENR and CON hens had better-developed motor systems or cognitive spatial navigation abilities (Colson et al., 2008), enabling them to navigate through vertical spaces in the aviary more efficiently regardless of whether their earlier initial exposure to vertical space was in floor pens or the aviary itself.

The effects of rearing environments on birds' subsequent resources use and behavior in laying facilities have been reported to be temporary, gradually disappearing as hens adapt (Colson et al., 2008; Brantsæter et al., 2016). Similarly, in the current study, differences among treatments in use of vertical levels by hens were less pronounced at 54 WOA than at 36 WOA. By 54 WOA, hens from ENR and FLR treatments matched patterns of distribution across vertical tiers and floor areas, shown by CON hens that had been placed in aviaries at 17 WOA and by aviary-housed hens in previous studies (Carmichael et al., 1999; Odén et al., 2002; Michel and Huonnic, 2003; Colson et al., 2005).

Similarly, differences among treatments in horizontal and vertical physical movement levels were also reduced or absent by 54 WOA. This suggests that even FLR hens that were not given access to perches until 25 WOA were adapting to the complex configurations of the laying aviary. In parallel with research examining younger birds, our findings suggest that hens' ability to learn to use vertical space is slower after the rearing period (Gunnarsson et al., 2000); however, it does not appear hens are permanently impaired if learning does not occur during the rearing phase.

Nighttime Roosting Locations and Falls

Nighttime observations at 36 WOA revealed that both ENR and CON hens roosted at higher locations

within aviary tiers more frequently than FLR birds, which roosted more frequently in lower locations. Hens have been found to be highly motivated to roost on higher locations at night (Olsson and Keeling, 2000; Schrader and Müller, 2009; Ali et al., 2019). This is consistent with an anti-predator hypothesis, which postulates that like their wild ancestors, domestic fowl choose high roosts to ensure their safety from ground predators (Newberry et al., 2001). However, FLR hens were repeatedly observed roosting on lower locations within aviaries. Such a shift from natural hen preference might be attributed to FLR hens being unable to recognize perches as resting sites (Appleby et al., 1988), because, as discussed earlier, exposure to perches before 18 WOA has been found to encourage adult perching behavior (Faure and Jones, 1982; Appleby and Duncan, 1989). Another possible explanation might be that FLR hens developed preferences for sleeping on the floor while housed in the floor pens from 0 to 25 WOA, and they continued to express that preference by roosting on lower locations within the aviary.

Despite roosting at height in greater numbers during the night, ENR hens fell less compared with CON and FLR hens. Pullets' use of perches has been found to increase muscle deposition and to allow development of strong leg muscles (Enneking et al., 2012), and perch use in the laying phase improves mineral deposition in bones (Hester et al., 2013). CON and ENR hens were exposed to perches and vertical structures for a similar length of time; however, ENR hens might have developed better leg muscles, joints, and bones compared to CON and FLR hens. The open perch array in the ENR birds' floor pen may have permitted more long jumps between structures, whereas the closed-front design of the aviary used in our study prevented CON hens from making long jumps up or flights down.

As previously mentioned, FLR hens fell shorter distances at night, which is a natural consequence of their tendency to roost on lower locations within aviaries. However, despite falling from the shortest distances, FLR hens had the highest acceleration whereas falling and highest calculated force of collision. In contrast, ENR hens fell longer distances but had the lowest acceleration and calculated collision forces. In the current study, forces of collision during nighttime falls were calculated based on the recorded accelerations and birds' body weight. As average body weights during both ages were similar for hens of the different treatments, differences in acceleration or collision force cannot be attributed to differences in weight. The simplest scenario is that ENR hens had more time to extend and flap their wing to initiate drag to reduce acceleration and subsequent force of collision. It is also likely that ENR hens, due to their earlier exposure to perches compared to CON hens, had practice jumping and landing or perhaps even falling. Thus, they may also have developed better landing reflexes than FLR hens. During planned jumps, hens extend their

wings to provide resistance to straight falls, and during the landing after jumps or flights the hens flap their wings to reduce the impact force (Banerjee et al., 2014). Thus, differences in collision forces in the current study are largely related to differences in acceleration during falling. This is a result of ENR hens' ability to better extend and flap their wing to initiate drag to reduce acceleration and subsequent force of collision—whether due to experience or longer fall time or both.

Clear differences were detected across treatments in hens' nighttime roosting patterns. FLR hens roosted on lower locations, whereas ENR hens consistently occupied higher locations across observations. Such variability among treatment groups in occupancy of vertical levels might be derived from preferences that developed at earlier ages (Denenberg, 1969; Gvoryahu et al., 1989; Reed et al., 1993), from hens' attempts to avoid competition over resources (Campbell et al., 2016b), or, as mentioned previously, as a result of differences in spatial navigation abilities (Gunnarsson et al., 2000).

Across treatments, some individual hens occupied the same roosting location nightly. For example, some hens never roosted on the top level, whereas others always did. Such patterns shown by individual animals are usually diluted and masked by the overall behavior pattern of the flock, due to the technical difficulties of tracking individual hens within large flocks. Recently, Rufener et al. (2018) assessed movement and location patterns of individual hens in a commercial-style aviary and similarly concluded that individual hens demonstrated consistent patterns of visits to areas in the aviary across days of observations. Campbell et al. (2017) also reported consistent differences among individual hens in diurnal litter use, with some spending considerably more time on litter, whereas others never visited litter areas. The reasons behind individual consistency warrant further study to determine what motivations drive hens to show consistent patterns of behavior or site fidelity or, in some cases, to lack consistency. A possible explanation behind hens retaining the same roosting locations in the current study might be that individuals maintain a consistent pattern of behavior across days, as also shown in Rufener et al. (2018) or Campbell et al. (2017). Consistent roosting in more preferable higher areas each night might be more possible for dominant hens, whereas less dominant hens that are prevented from occupying preferable roosting locations might experience decreased welfare, lessening the benefits of aviary systems for subordinate birds (Shimmura et al., 2007). Understanding the relationship between consistent patterns of aviary space occupancy within individual hens and variability in patterns across individual hens should be examined further to determine how aviary systems fulfill behavioral needs at the level of the individual hen, which, ultimately, is where welfare is experienced.

CONCLUSIONS

Providing perches and nests to pullets starting at 17 WOA resulted in more use of higher tiers and greater vertical movement in a laying hen aviary at 36 WOA, regardless of whether these resources were provided to birds in floor rearing pens or in the aviaries themselves. Birds given perches and nests in floor pens from 17 to 25 WOA spent more time at higher aviary levels, including roosting at night, but fell less and with lower force and acceleration at 36 WOA than hens who had been living in those aviaries since 17 WOA. Delaying birds' access to perches and nests until 25 WOA reduced their total vertical movement and the time they spent in higher areas of the aviary, and at night, these birds fell faster and with more force for at least 20 wk after moving into the aviary. Together the findings suggest that even if perches and nests are provided near the start of lay and not in a configuration identical to that of the aviary, they can still benefit hens while lack of these resources up to the start of lay has negative consequences.

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