Providing ramps in rearing aviaries affects laying pullet distribution, behavior and bone properties

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Primary Audience: Flock Managers, Housing Manufacturers, Researchers, Veterinarians, Geneticist, Quality Assurance Personnel, Researchers, Plant Managers

SUMMARY

To ensure that laying hens can make full use of the various resources within an aviary barn and develop optimum bone health while minimizing keel bone fractures, appropriate cognitive and bone development during rearing is critical. Given previous work documenting the benefit of ramps that could be used by hens to transition between tiers and reduced incidence of keel bone fractures, the project examined the provision of ramps during the rearing period, which birds could use voluntarily from 10 d of age. We hypothesized that the provision of ramps would influence how pullets distribute within the aviary and how birds vertically move between the aviary tiers leading to greater bone strength in birds with access to ramps. The study used 2 flocks of laying hen chicks (Lohmann Selected Leghorn; 4,800 chicks/flock) that were reared in one of 2 commercial rearing facilities with each divided into 4 pens (600 chicks/pen) to allow for treatment assignments. In 2 pens of each facility, ramps were installed from each of the 3 tiers providing a walking path that birds could access from 10 d of age. Video recordings were made at 4 times per day at 3, 4, 5, 8, 11, and 14 wk of age to determine the relative distribution of birds and the number of transitions between each tier. At 16 wk of age, 10 birds per pen per flock were killed and the tibia and humerus collected for biomechanical assessment; the keel was also collected for bone mineral density via computed tomography. Chicks/pullets within pens provided ramps demonstrated a rapid use of the upper tiers of the aviary paralleling greater usage of ramps between all aviary levels. Despite the ramp and tier usage following the predicted pattern, differences in bone strength were opposite than expected for tibiae and may reflect the different behaviors pens with ramps and without ramps would allow. Results support the position that provision of ramps within a commercial system will lead to voluntary usage of the ramps with long term effects on the distribution of birds in the system throughout the rearing period.

Key words: keel, transition, rear, development, bone health

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DESCRIPTION OF PROBLEM

The housing of laying hens is of critical interest for consumers and retailers in light of increasingly negative perception of traditional battery cages in the United States, Europe, and across the world. As a consequence of this developing perspective, the need for viable alternative layer housing systems during rear and lay has become critical for hen welfare, production, and management. Aviaries, multitier structures within a barn characterized by several vertically stacked levels with different resources such as feed, water, perches, nest boxes distributed among these levels, are one cage-free option that is increasingly popular.

Aviaries provide for many benefits that are believed to lead to improved welfare, most importantly the ability to perform a greater repertoire of highly motivated behaviors such as dust-bathing, use of nest boxes, and roosting at elevated positions (Aerni et al., 2005). While aviaries offer these various resources to improve welfare, they also require that hens develop necessary spatial-cognitive abilities and musculoskeletal properties (Kozak et al., 2016a; LeBlanc et al., 2018a,b) to access resources, which are located throughout the system at different tiers. For instance, dust-bathing is typically performed in the litter at the lowest level, whereas roosting is performed preferentially at higher levels, while nest boxes are typically in a mid-level position. Hens develop the necessary cognitive and locomotive skills for successful adaptation to aviaries in the laying period during the rearing period. Early experiences are known to influence the development of individuals and how they interact with their future environment. The rearing period of laying hens is thus considered a crucial phase for pullets to develop adequate spatial-cognitive abilities and skeletal integrity.

One specific problem that likely results during the laying period from a combination of improper bone development and poor navigation skills is keel bone fractures. Despite the described benefits of more space and environmental complexity, keel fractures are common in cage-free systems with reports of 40% or more of laying hens within North American (Petrik et al., 2015) and European (Rodenburg et al., 2008; Käppeli et al., 2011; Wilkins et al., 2011) flocks manifesting some level of fracture. The high frequency of keel damage in hens is now considered one of the greatest welfare problems facing the commercial laying hen industry (Harlander-Matauschek et al., 2015) with concomitant likely detriments to productivity (Nasr et al., 2013; Rufener et al., 2019b) and mobility (Rufener et al., 2019a). Although the precise cause of keel injury is unknown (Toscano et al., 2020), previous investigations have suggested that locomotion within the aviary (Stratmann et al., 2015a) or specific arrangement and positions of internal structures that likely affect movement (Richards et al., 2011; Wilkins et al., 2011; Heerkens et al., 2015; Stratmann et al., 2015b) contribute to keel damage. Increased activity during rearing, especially those targeted at types of movements required during lay, could be beneficial to general bone health (Regmi et al., 2015). In addition to keel fractures, broken bones (including the keel as well as others) have been reported at the end-of-lay in response to depopulation (Gregory and Wilkins, 1989; Kristensen et al., 2001; Sandilands et al., 2007; Gerpe et al., 2021) suggesting that appropriate bone health in general is a theme that should be addressed.

To improve housing systems for laying hens in ways that facilitate access to resources, strengthen bones prior to lay, and reduce falls as well as keel bone fractures, efforts have been directed at aiding hens' transition between the vertical locations within an aviary system. Stratmann et al. (2015a) investigated ramps as a means to facilitate inter-tier movement by hens and found ramp introduction was associated with reduced incidence of falls and collisions as well as keel bone fractures, a finding supported by others (Heerkens et al., 2016; Norman et al., 2021). Given the relatively poor flight abilities of laying hens (compared to smaller, more agile bird species; Tobalske, 2015), the facilitation of walking to access various levels by providing ramps for routine movement is likely a safer mode of locomotion (vs. flying behavior) within the confined conditions of aviaries.

The aims of the study were to investigate the effects of providing ramps during the pullet rearing phase under semicommercial conditions and to compare behavior and bone health of pullets without access to ramps. We hypothesized that the provision of ramps would influence how pullets distribute within the aviary and how birds vertically move between the aviary tiers. As ramps would provide earlier access to upper aviary tiers and thus associated resources, we expected that pullets with ramps would use the upper levels earlier and distribute more evenly across the aviary tiers. In addition, as ramps provide a different way of moving between aviary tiers, we predicted that pullets with access to ramps would have improved mechanical bone properties such as shear strength and bone stiffness. We specifically hypothesize that pullets with access to ramps during the rearing phase would

a) Move to the upper tiers earlier
b) Distribute more evenly within the aviary
c) Perform more transitions between aviary tiers
d) Have stronger bones (stiffer bones and more force needed to break them)

compared to pullets without access to ramps during rearing.

MATERIALS AND METHODS

Ethical Note

Approval to conduct the study was obtained from the Veterinary Office of the Canton Bern in Switzerland. The experiment complied with all Swiss regulations regarding the treatment of experimental animals.

Animals and Housing

For the experiment, 2 flocks of Lohmann Selected Leghorn (**LSL**) laying hen chicks were raised from day of hatch until 18 wk of age (**WOA**) in a rearing facility with one flock from May to October 2017 and the other flock from January to May 2018. Each flock consisted of 4,800 chicks (N = 4,800 chicks per flock, N = 9,600 chicks in total) that were obtained from a commercial breeder and randomly assigned to 8 pens (600 birds / pen) of our on-site commercial rearing facility (Aviforum, Zollikofen, CH). Per barn side, 4 pens were used with each pen containing a tiered aviary structure placed in the middle of the pen. The four pens on one barn side contained an aviary structure with vertical tiers stacked directly on one another (Figure 1; direct; n = 4 pens / flock: Inauen Natura, Inauen AG, Appenzell, Switzerland; space allocation per bird: 42.74 m^2 (pens 1−3), 41.61m^2 (pen 4); total space per pen: 4.80 m × 3.50 m; wintergarden: 4.90 m × 2.55 m). The remaining 4 pens on the other barn side contained an aviary with tiers stacked in an offset configuration (Figure 1; offset; n = 4 pens / flock: Landmeco Harmony, Globogal AG, Lenzburg, Switzerland; space allocation per bird: 40.99 m^2; total space per pen: 4.90 m × 4.55; wintergarden: 4.95 m × 3.45 m).

The direct aviary consisted of two tiers where feeding chains and nipple drinkers were provided on both tiers with perches raised 50 cm above both tiers. Platforms were provided on each side of the direct rearing aviary to facilitate up- and downward movements. The total height of the direct aviary from floor to top perches was 195 cm. The offset aviary consisted of 3 tiers with feeding chains provided on the first and second tier and drinkers on each tier. Perches were placed on the first and third tier with a total height of 241 cm in the offset rearing aviary. To provide additional grid area, one platform per pen was placed along the wall in the offset aviary pens (width × length: 81 × 310 cm).

Aside from differences in the aviary structure, the 8 pens were identical in terms of animal numbers and bird density. Birds had access to a covered outside area (one porch per pen, with porch size varying between 15 and 21 m^2) that was equipped with 5 wooden perches. Daily access to the porch was allowed starting at 5 WOA until the end of the rearing phase from 1000 until 1600. Due to different seasons and associated outdoor temperatures, the second flock had later access to the porch (starting with 8 WOA) than the first flock. Artificial light was provided depending on WOA according to the LSL standard rearing procedure with light hours decreasing from 16 h per day to a minimum of eight hours in WOA 12 to WOA 16 and then increasing again to 10 h per day until WOA 18. Light was programmed with a 1-min

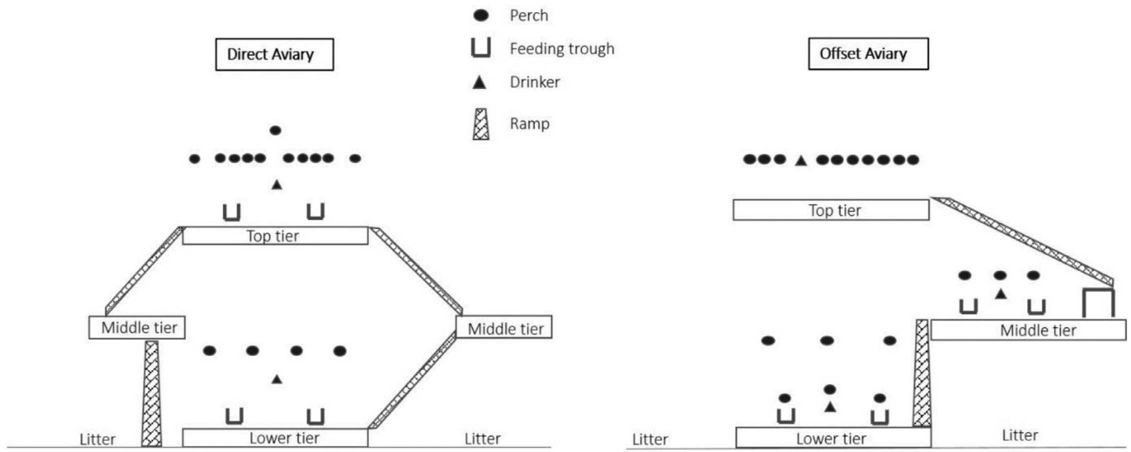

Figure 1. Schematic side view of the two rearing aviary designs including placement of ramps used to connect the different tiers in the ramp treatment condition (i.e., two ramps per placement). Length and angles of Offset Aviary ramps (lower tier to middle tier: 200 cm/34°, 200 cm, 32°; middle tier to upper tier: both ramps 83 cm/40°). Length and angles of Direct Aviary ramps (litter to middle tier: both ramps 130 cm/39°; middle tier to top tier: 101 cm/37°, 101 cm/38°; lower tier to middle tier: both ramps 100 cm/34°; middle tier to upper tier: 98 cm/37°; 98 cm, 38°).

dimming phase in the morning and 10 min dimming in the evening. Additionally, daylight was provided through windows that were automatically opened and closed. A standard feed was provided with a starter diet from WOA 1 to 8 followed by a pullet feed from WOA 9 to 17. The floors of all pens were covered with wood shavings. Chicks were initially contained on the lowest tier in each aviary until 7 d after hatch. Afterward the first tier was opened and birds had access to the whole pen including the litter. In the offset aviary structure, access to the litter in each pen was facilitated with ramps (122 × 59 cm) on both sides connecting the litter and the first tier (32.5 cm distance) whereas in the direct aviary structure, wooden bars were provided on both sides connecting the litter and the first tier (36 cm distance). These structures were provided in all pens until 4 WOA, after which the structures were removed as the pullets were able to jump these distances without the structures.

Experimental Design

For both flocks, 2 pens within each aviary structure contained ramps between tiers whereas the other 2 pens were left as controls without ramps. The configuration resulted in a 2 × 2 factorial design: aviary structure (direct & offset) × ramp treatment (ramp and control) with n = 2 pens / aviary structure / flock. Within the 4 ramp treatment pens, ramps were installed in a manner that allowed all vertical tiers of the pens to be accessed by walking rather than flying or jumping. Ramps were 24 cm wide with a mesh size of 2 × 2 cm and varied in length depending on their location within the aviary (Figure 1).

Data Collection

Behavior. To evaluate the benefit of ramps during rearing on the behavior of the pullets, videos were recorded of both rearing flocks using 2 infrared cameras per pen (Samsung SNO-6083R, Samsung Techwin CO., South Korea) and customized recording software (Multieye Green Watch, Recorder Version 2.4.2, Artec Technologies AG, Diepholz, Germany). Cameras were positioned on both sides of the aviary and placed in way to cover the entire height and width of the aviary.

Recordings were used to quantify the following behaviors: a) distribution of the pullets (e.g., number of birds on top tier) and b) number of transitions between tiers. The number of birds on the top tier was counted on both pen sides at 3, 4, 5, 8, 11, and 14 WOA at 4 times per day during 15-min periods within each recording day. Time of day (**TOD**) included 1) after lights on, 2) midday, 3) the dusk phase and 4) after lights off. For each 15-min period, birds were counted at the first, eighth, and 16th min per TOD. In order to standardize bird counting, a specific area within the top tier on both pen sides that was comparable between pens and treatments was labeled on the videos in which the birds were counted only. The observed area of the top tier per pen was 3.6 m^2, distributed between pen sides.

The number of transitions between the lower and middle tier and the number of transitions between the middle and top tier were counted at 3, 4, 8, and 14 WOA at the same 3 TOD as for pullet distribution. For each TOD, videos were analyzed continuously for 2 min and the number of transitions pullets made between tiers was counted. To standardize and compare the number of transitions between treatments, the same area per aviary level was labeled for each pen and all transitions occurring in that particular area were counted. In order to standardize the areas per pen, reference points within the aviary (e.g., beams etc.) were used. Transitions outside of designated areas were not included. For the pens including ramps, it was distinguished whether a transition was done with or without a ramp. The protocol for video recordings as well as analyses was identical for both flocks.

Biomechanical Testing of Humeri and Tibiae. At 16 WOA, 80 pullets per flock (10 birds per pen per flock) were selected in a stratified manner, sedated by an intraperitoneal injection of barbiturate (690 mg/kg), and killed by cervical dislocation. Each killed bird was weighed and the keel bone, tibia, and humerus removed, cleaned and stored at −20°C until assessment. Humeri and tibiae underwent 3-point biomechanical testing after thawing for 24 h at 15°C following the ASABE Standards

2007 (ANSI/ASAE S459MAR1992 (R2007)) using a Zwick and Roell Universal Testing Machine with a 2.5 kN load cell. The fulcrum was adjusted at 40 mm to get the requested length to bone diameter ratio greater than 10. The bones were laid in the test apparatus with the flattest side down and the force applied to the midshaft with a crosshead speed of the loading bar of 10 mm/min. The force deformation curve was read from the texture analyzer. From this curve, the Ultimate force (in N) required to break a bone was recorded. To take bone size into account, the value of the ultimate force was divided by the cross section of the bone (A), which was calculated as the product of π and the square of the radius of the thinnest part of the bone (using the small diameter, see ANSI/ASAE 8.2). The ultimate force value (i.e., F / 2*A) was taken as an approximation of the ultimate shear strength. Bones from pullets at this age were too thin to measure the outer and inner diameter required to get a more exact measurement of ultimate shear strength. Bone stiffness, defined as the slope of the linear region of the load-displacement curve, was derived by the regression between 0.3 and 0.5 mm. The system software also calculated the total area under the entire stress deformation curve to provide the total work required to reach structural failure.

Computed Tomography of the Keel. Computed tomography analyses of 5-cm distal sagittal sections (i.e., image taken from the side of tip farthest from the animal's center) of the keel bone were performed using a high-resolution tomograph ProMax 3D (Planmeca, Helsinki, Finland). The X-ray source was set at 90 kV and 800 mA, with a pixel size of 150 μm. For each specimen, 470 projection images were acquired over an angular range of 180°. The images were converted into DICOM format and imported into Bruker CTAn Software (Kontich, Belgium) for morphometric analysis in 2D and 3D. The cortical region of the bone (region of interest [**ROI**]) was interactively delimited in each of the images for micro-architectural measurements using a color mask denoting a particular threshold Hounsfield unit (HU). The threshold HU was chosen based on a profile line drawn across the bone cross-section. The image slices were reconstructed using the reconstruction software NRecon (Skyscan, Bruker). After ROI determination, the images were converted for three-dimensional calculation. Apparent bone mineral density (**BMD** (mg/cm^3)), HU, Grayscale index, and moment of inertia were calculated.

Production Data. In both rearing flocks, mortality (%) and weekly feed consumption per flock (kg) divided by the number of live birds at pen level was summarized every 4 wk (WOA 4, 8, 12, and 16). Values from the first week were not included in the analyses since birds were neither exposed to the different ramp treatments nor aviary structures since in all cases they were confined within the first tier of the aviaries for the first 7 d after population of the barn.

Statistical Analyses

Data were analyzed using linear mixed-effects models (**LMER**) and generalized linear mixed-effects models (**GLMER**) in R (R Core Team, 2017) and RStudio as the user interface (RStudio Team, 2018) applying the package lme4 (Bates et al., 2015). Model assumptions were checked visually using q-q plots for LMER and the package DHARMa (Hartig, 2018) for GLMER to check for a normal error distribution and homoscedasticity of the residuals. A stepwise backwards reduction of the full model was used to obtain the final model with a P-value of < 0.05 as the exclusion criteria. Model estimates were calculated and displayed using the package “effects”(Fox, 2003). Once model effects were identified, planned comparisons to separate means by Tukey's post hoc test with a Bonferroni correction were performed with package "emmeans"(Lenth, 2021).

Behavior. To assess the effect of ramps on the distribution of birds on the top tier across age, a GLMER model with a negative binomial function was fitted including number of birds on the top tier as a response variable. Explanatory variables included in the model were age (WOA 3, 4, 5, 8, 11, and 15), treatment (ramp vs. control), and TOD (after lights on, mid-day, dusk period and after lights off) as well as the interactions between treatment and age and treatment and TOD. A random factor of TOD nested in pen nested in barn side nested in flock was included in the model.

Data on number of transitions between tiers was analyzed using LMER, and the same explanatory variables and random terms as for the distribution data were used. In order to test how pullets would transition between tiers when they had the choice, the percentage of transitions made with ramps was calculated within the ramp group (N = 4 pens, i.e., where birds could choose whether they perform a transition with a ramp or jump/fly instead). Therefore, the percentage of transitions using ramps was calculated from the total number of transitions observed and analyzed using LMER with explanatory variables being age (WOA 3, 4, 8, and 14), TOD (lights on, mid-day, and dusk phase) and pathway within aviary (from lower to middle tier and from middle to top tier) as well as the interaction of age and pathway within aviary. Pen nested in flock was included as a random factor in the model.

Bone Properties. To assess the effect of ramps during rearing on pullet bone properties, the ultimate shear strength, bone stiffness, and total work to fracture were used as response variables and analyzed using LMER. Since ultimate shear strength was adjusted for bone size and therefore bird size using the bone diameter, body mass was not included in the analyses. For bone stiffness and total work to fracture, however, body mass was used to correct for bird size. Fixed factors included in all analyses were treatment (ramp vs. control), aviary structure (offset vs. direct), and bone type (humerus vs. tibia) as well as all two-way interactions. The random term included in each model was bone nested in bird nested in pen nested in barn side nested in flock.

Computed tomography outputs of keel bones (mean HU unit as an indication of bone mineral density and moment of inertia) were analyzed using LMER. Fixed factors included in both models were treatment (ramp vs. control) and aviary structure (offset vs. direct). The random term included in the model was pen nested in barn side nested in flock.

Production Data. To assess the effect of ramps during the rearing phase on pullet production parameters, cumulative values of 4 wk for mortality and feed consumption on a per bird basis were used as response variables. Data were analyzed using LMER with fixed factors included in each model being age (WOA 4, 8, 12, and 16) and treatment (ramp vs. control) as well their interaction. Random factors included in each model were pen nested in barn side nested in flock.

RESULTS AND DISCUSSION

The current study sought to investigate whether provision of ramps between tiers of a rearing aviary would lead to chicks/pullets performing more and earlier intertier transitions, a more balanced distribution of birds across tiers, and altered musculoskeletal characteristics. The effort was grounded in the belief that predicted changes via better design of the rearing environment could improve hen welfare by increasing access to resources in the rear and lay environments. Findings largely supported our expectations with strong differences between treatments that were age- and time of day-dependent.

Behavior

Number of Pullets on Top Tier. The number of pullets on the top tier was affected by the interaction of age and treatment (χ^2 = 291.5, P < 0.001) as well as the interaction of TOD and treatment (χ^2 = 21.5, P < 0.001). The number of pullets on the top tier was higher in the ramp treatment compared to the control group at 3, 4, 5 (P > 0.001), and 8 WOA (P = 0.03) with the difference between the treatments decreasing with increasing age. A tendency for greater numbers of pullets on the top tier in the ramp group was found at 11 WOA (P = 0.08). Numbers of birds present on the top tier were similar for both treatments at 15 WOA (P = 0.4; Figure 2).

In addition, more pullets were observed on the top tier at all TOD (P < 0.001), with the difference most pronounced during the dusk phase and after lights off compared to the morning and midday (Figure 3).

During earliest observations at 3 WOA, pullets housed with ramps were already accessing the top tier in greater numbers compared to

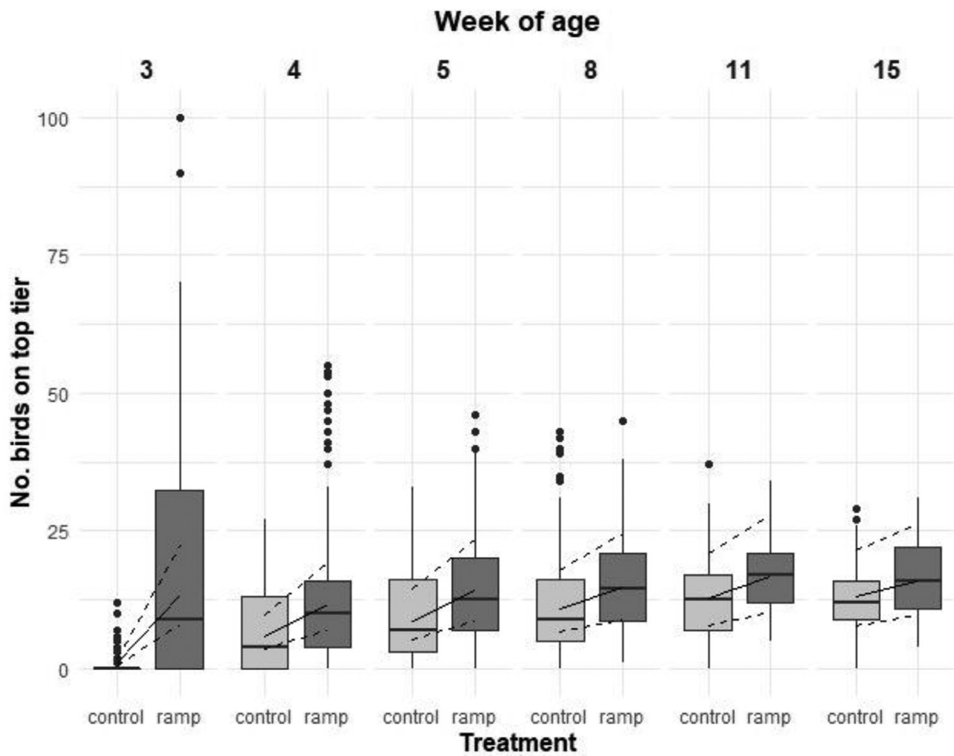

Figure 2. Effect of week of age and ramp treatment on the number of laying hen pullets on the top tier of the rearing aviary where an interaction of age and treatment was identified ($P < 0.001$). Boxplots represent raw data. The solid line represents the estimated means and the dashed lines the 95% confidence intervals.

pullets in pens without ramps. Within pens containing ramps transitions were most active on the ramps leading to the top tier. Aviary systems, including rearing aviaries, are characterized by the presence of multiple vertical tiers; therefore, enhancing birds' access to the tiers and associated resources is key to optimizing welfare. Ramps minimize the difficulty in transitioning (i.e., otherwise via jumping) between tiers by providing a direct walking path. The lack of ramps in the control pens likely hindered the capacity of pullets, particularly at the youngest ages, to access the middle and upper tiers as indicated by the comparatively few control pullets in the top tier in the first weeks of observation. The benefit of ramps to encourage vertical movement is also supported by Norman et al. (2021) who found pullets at upper perches as early as one WOA when provided ramps. Although the pattern for greater number of birds at the top tier continued in the current study, treatment differences were eliminated after eleven WOA, likely due to the control birds' increasingly enhanced ability to transition without ramps. Data from the current effort support the position that laying hen chicks are motivated to access elevated positions from early age and will do so when provided appropriate accommodations (i.e., ramps). The observed earlier access could be important for animal welfare if the changes lead to animals that are better prepared for the laying barn as well as ensuring improved access to all resources during rearing.

Transitions Between Tiers. The number of transitions pullets made between aviary tiers was affected by the interaction of age and treatment ($\chi^2 = 46.7$, $P < 0.001$) as well as by the interaction of TOD and treatment ($\chi^2 = 55.7$, $P < 0.001$). More transitions between tiers were observed in the ramp treatment compared to the control group ($P < 0.001$) where the magnitude of the difference decreased with increasing age (Figure 4).

Transitions were observed more often among pullets in the ramp treatment compared

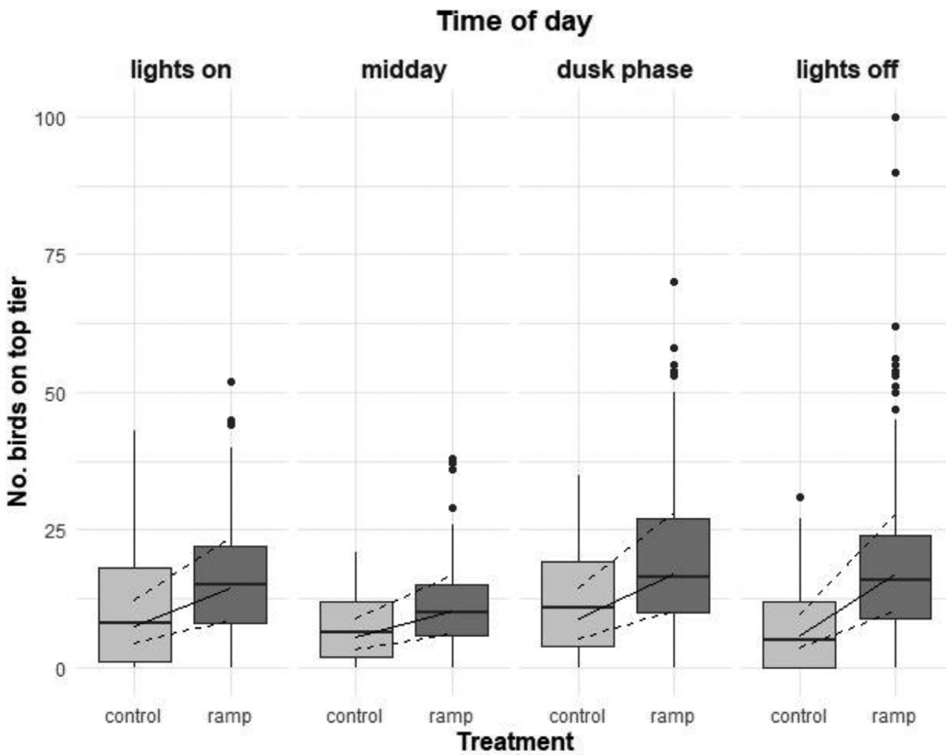

Figure 3. Effect of time of day and ramp treatment on the number of laying hen pullets on the top tier of the rearing aviary with more pullets observed on the top tier at all times of day ($P < 0.001$). Boxplots represent raw data. The solid line represents the estimated means and the dashed lines the 95% confidence intervals.

to the control group during all TOD ($P > 0.001$) with the difference being more pronounced after lights on in the morning compared to midday and the dusk phase (Figure 5).

The percentage of transitions pullets made between tiers using ramps was related to age as well as between specific tiers. Overall, the percentage of transitions with ramps compared to transitions without ramps was high but decreased with increasing pullet age ($\chi^2 = 43.2$, $P < 0.001$). However, even at 14 WOA about 80% of all transitions observed in the ramp group were made by pullets using ramps. Pullets made transitions between the middle tier to the top tier more often using ramps compared to transitions between the lower tier to the middle tier ($\chi^2 = 18.5$, $P < 0.001$).

In addition to chicks accessing the top tiers earlier and in greater numbers when ramps were present, ramps also generally provided for a greater number of transitions across all tiers. Increased transitions were observed in the ramp treatment from the first time point and continuing forward in time with the smallest magnitude of difference at 14 WOA suggesting differences were equalizing between 8 and 14 WOA. Similar findings at 14 WOA have been reported elsewhere in both commercial (Norman et al., 2021) and non-commercial settings (Norman et al., 2018). Even at 14 WOA, where the difference between the treatment and the control values was the smallest of all time points, the number of transitions by pullets in the control pens did not appear to reach the values of pullets in the ramp treatment suggesting birds of the latter never achieved their full potential for transitions. Future work should include additional time points to determine whether increased resolution of observations would provide for alternative interpretations.

Beyond gross treatment by age differences in total transitions, transitions between specific tiers manifested treatment and age patterns that were not evaluated statistically but, nonetheless, are interesting to examine. Transitions between low/mid and mid/high tiers for pullets

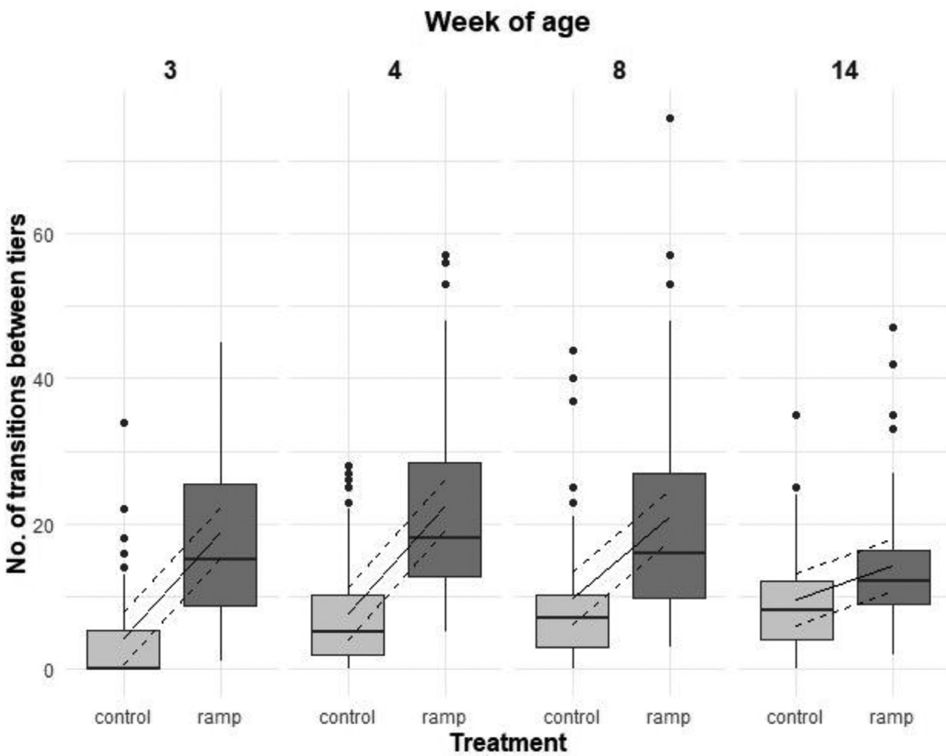

Figure 4. Effect of age and ramp treatment on the number of transitions by pullets between tiers in the rearing aviary where interactions of age and treatment ($P < 0.001$) and time of day and treatment ($P < 0.001$) were identified. Box-plots represent raw data. The solid line represents the estimated means and the dashed lines the 95% confidence intervals.

in ramp pens was relatively the same at all time-points (range: 42−60%) whereas the majority of transitions within control pens occurred between the low-to-mid tiers at the first time point (Direct: 86%; Offset: 100%) and remained above 63% until 8 WOA. Although comparable numbers of pullets in both treatments were accessing the upper tiers by 4 WOA, the greater number of overall transitions and the more uniform distribution among tiers in the ramp treatment suggests less impediments to the birds' natural inclination for movement throughout the rearing phase.

Whereas differences in pullet distribution until 3 WOA were likely due to their physical inability to perform certain types of transitions, differences in the types of transitions after 4 WOA likely reflect preferences and confidence in how birds choose to navigate. Within ramp pens, the vast majority of transitions occurred by walking on ramps with no less than 55% of all transitions involving ramps at all observed ages (though the percentage varied with location and type of aviary structure). For instance, transitions using ramps within the offset pens between the middle and top tiers never fell below 93% (at 14 WOA). However, ramp use to transition between low and middle tiers declined to 55% by 14 WOA (see Supplementary Table 1), a value that likely reflects the ability of pullets at this age to use perches as a midway point when transitioning. The three perches at the level of the middle tier in the offset structure are positioned in a way that older (larger) pullets would not need to use the ramps to transition between the lowest and middle tiers, which may explain the fewer number of transitions observed there. Nonetheless, the continued high rate of transitions via walking on ramps when birds could voluntarily transition by other means (e.g., jumping, flying, etc.) confirms the benefit of ramps to accommodate the pullets' natural behavior. The

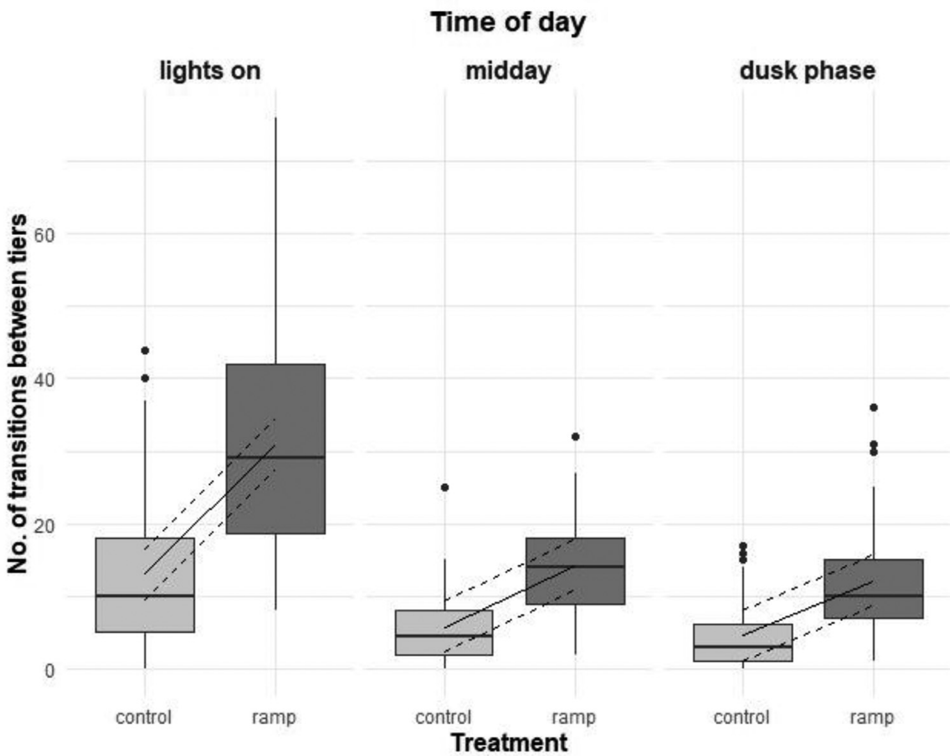

Figure 5. Effect of time of day and ramp treatment on the number of transitions by pullets between tiers with more transitions observed in the ramp treatment during all times of day ($P > 0.001$). Boxplots represent raw data. The solid line represents the estimated means and the dashed lines the 95% confidence intervals.

finding is particularly relevant for Switzerland from a regulatory perspective where housing is legally required to not infringe on the normal biological functioning of an animal (Art. 3, TSchV, Switzerland, 2008). Beyond allowing for greater exploration of their environment, ramps may have also indirectly allowed pullets to express other relevant species-specific behaviors such as roosting at elevated positions in the evenings. As observed in the supplemental table (Supplementary Table 2), a greater number of pullets within the ramp treatment were present on the upper tier with increasing age, which likely reflects a desire for elevated roosting positions, as this behavior is known to develop starting with around 9 WOA (Newberry et al., 2001). The greater use of ramps also likely confers safety benefits as usage of ramps is associated with reduced collisions (Stratmann et al., 2015a) and fractures (Stratmann et al., 2015a; Heerkens et al., 2016; Norman et al., 2021) as a consequence of easier movement.

While not able to make statistical comparisons between types of aviary structures, the inclusion of 2 rearing systems did allow for a side-by-side comparison that could benefit commercial implementation. Beyond the already noted variation across barn systems for type of transitions, there were also variations observed in the number of pullets on the top tier, especially in the early rearing phase (i.e., 3&4 WOA) with 4 to 10 times more birds in the direct/ramp compared to offset/ramp pens. Interestingly, the combination of differing numbers of pullets on the top tier in spite of similar transitions between types of aviary structures suggests birds were reaching the top tier but not necessarily remaining there in the offset aviary structure. Although only conjecture, it is likely that the lack of a feeder (and accessible drinker) on the top tier of the offset structure reduced the pullets' incentive to remain on the top tier for reasons beyond roosting. The lack of a feeder on the top tier of the offset structure also represented a management concern as it was feared that young chicks would gain access to the upper tier and not be able to find their way back to lower levels containing feed and water. Hence, we delayed chicks' access to the upper tier for 10 d after population of the barn until confidant chicks would be able to return to tiers containing feed and water. The difference in pullet distribution should be evaluated further to determine if adding feed and water to the top tier in the offset structure would minimize differences in occupancy to ensure optimized space use. Norman et al. (2021) did not report chicks becoming isolated on the elevated sections of the system used in their study despite providing access at one week of age. However, in the current study the increased structure height, grid over the entire aviary tier, and multiple ramps between tier sections likely introduced an added complexity that warrants additional caution.

Bone Properties

Both ultimate shear strength and bone stiffness of pullet long bones were linked to bone type with humeri having a higher ultimate shear strength (χ^2 = 7.8, P = 0.005) and stiffness (χ^2 = 588.3, P < 0.001) compared to tibiae. Total work to fracture was affected by an interaction of treatment and bone type (χ^2 = 7.2, P = 0.007) with tibiae from pullets reared without ramps needing more total work to fracture compared to tibiae from pullets reared with ramps. Work to fracture values for the humerus did not differ between treatment groups (Figure 6). Keel bone mineral density (χ^2 = 0.9831, P = 0.612) and moment of inertia (χ2 = 3.084, P = 0.214) did not differ between treatments nor aviary structure.

Despite treatment differences in the distribution and behavior of pullets across the observed ages, there were no differences between treatments for keel and humeri parameters while the reversal of expected effects on tibiae bone health was surprising. Several potential explanations are offered. First, although animals within ramp pens clearly made more vertical transitions between tiers, there was no measure of activity within tiers or on the litter, that is, motion in the

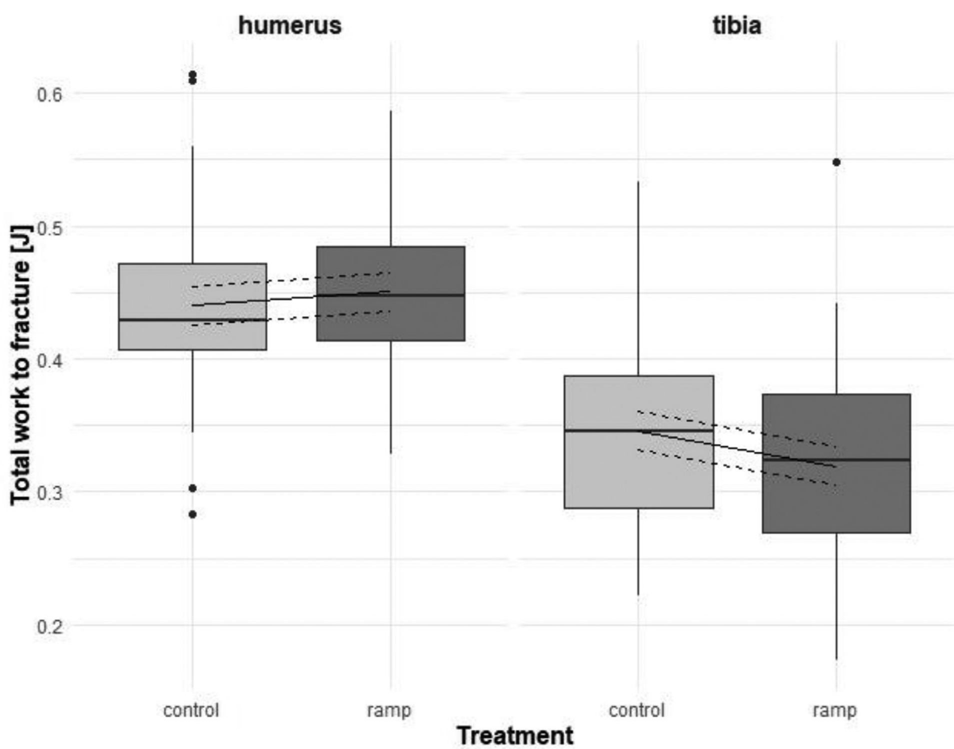

Figure 6. Effect of pullet bone type and ramp treatment on the total work to fracture were an interaction of treatment by bone type was observed (P = 0.007). Boxplots represent raw data. The solid line represents the estimated means and the dashed lines the 95% confidence intervals.

horizontal plane. Pullets in control pens may have compensated for fewer transitions between levels by increasing horizontal activity within the tiers leading to comparable or greater amount of bone loading. Alternatively, because animals within control pens did gradually increase transitions among tiers from four WOA onwards, this delayed response may have been adequate to compensate for the large treatment differences observed in transitions at earlier ages. The assumption was that earlier interventions would lead to greater bone strength benefits, a difference believed to also benefit cognitive aspects of navigation (Gunnarsson et al., 2000; Norman et al., 2019). While the usage of ramps in this study paralleled previous observations of chicks' voluntary activity in upper areas (Kozak et al., 2016a), future research should consider evaluating relevant ages for key musculoskeletal development processes to optimize when bone loading activities are most beneficial. Lastly, musculoskeletal differences among treatments could also be attributed to the methods used to transition by pullets within the different treatments. Animals within control pens, unable to walk between tiers, would be required to do some combination of jumping and flying that would involve a greater magnitude of leg bone loading than walking (Kozak et al., 2016b) . In this scenario, it could be expected jumping activity of control pullets lead to the increased work to fracture tibiae observed in control birds.

Production Parameter. Pullet mortality was linked to age and increased from 4 to 8 WOA with no further increase until WOA 16 (χ^2 = 29.8, $P < 0.001$). Overall mortality was 1.9% in flock 1 and 0.8 % in flock 2.

Feed consumption per bird differed depending on age, with increasing feed consumption per pullet with increasing age (χ^2 = 285.3, $P < 0.001$). On average, feed consumption per bird increased from 0.57 kg per bird in WOA 4 to affected mortality nor feed consumption per pullet.

CONCLUSIONS AND APPLICATIONS

1. Our study confirmed that ramps were actively used by the animals throughout the rearing period.
2. Ramps appeared to be chicks' preferred means of moving between aviary levels and allowed birds to distribute themselves throughout the aviary and likely benefit from the distributed resources between tiers.
3. Treatment differences were observed in bone health but in the opposite direction for tibiae to what was expected, suggesting birds without ramps were likely compensating by adopting alternative behaviors involving their legs, such as greater intratier activity or jumps between levels.

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DISCLOSURES

All authors declare no competing interests.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.japr.2022.100283.

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