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Journal

Poultry Science, 103(12)

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Publication Date

2024-08-22

DOI

10.1016/j.psj.2024.104245

Peer reviewed

Poultry Science

Effects of pullet housing on bone development in aviary-housed Dekalb White hens

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ABSTRACT The skeletal health of laying hens improves when birds are given opportunities to perform load-bearing movements with elevated structures, such as perches. We investigated how early access to elevated structures varying in complexity and height would affect bone quality and subsequent keel bone fractures in a layer multitiered aviary. Female Dekalb White pullets were reared in floor pens furnished with floor perches (**FL**), single-tiered aviaries (**ST**), or 2-tiered aviaries (TT; n = 5 pens/treatment) through 16 wk of age. At 17 wks, all structures were replaced with identical multitiered layer aviaries. The keel, both tibiae, and both humeri were collected from 60 euthanized birds from each rearing treatment at 8, 16 and 30 wk of age, and analyzed with dual X-ray absorptiometry (**DEXA**) for bone mineral density and length. At 18, 26, 28, and 30 wk of age, 10 focal hens/pen were radiographed repeatedly and the presence, severity of keel bone fractures were assessed with a tagged visual analogue scale. The number of fractures was also recorded. At 16 wk of age, FL pullets had lower BMD of the tibia ($P = 0.003$), keel ($P = 0.013$), and humerus ($P = 0.004$) compared to ST and TT pullets. Most of the observed treatment differences disappeared after pullets were transferred to the aviary. BMD continued to increase for all hens through 30 wk of age. Pullet rearing did not affect the presence or severity of keel bone fractures, or number of new fractures incurred between ages ($P > 0.05$). The prevalence and severity of keel bone fractures increased between 26 to 28 wk and remained high to 30 wk of age ($P < 0.0001$). Hens experienced more new fractures between 26 to 30 wk than between 18 to 26 wk of age ($P = 0.0046$). The effects of pullet housing on bone quality were short-term when hens had access to adult housing with multiple opportunities for load-bearing movements. Keel fractures with minor severity were high in prevalence reflecting the use of radiography to assess this injury.

Key words: aviary, bone, hen, keel, pullet

2024 Poultry Science 103:104245
https://doi.org/10.1016/j.psj.2024.104245

INTRODUCTION

Access to perches and other elevated structures can benefit the musculoskeletal health of laying hens (reviewed by Hester, 2014; Bist et al., 2023). The addition of perches to conventional cages has been associated with improved bone strength and increased bone mineralization in hens (Hester, 2014). Hens housed in furnished cages also have improved bone quality over hens housed in conventional cages, which has been attributed to opportunities to perch and/or move through a larger space (Leyendecker et al., 2005; Jendral et al., 2008; Casey-Trott et al., 2017c). Likely for the same reasons, housing hens in aviaries or free-range systems has been linked with improved bone parameters as compared with hens in conventional cages (Fleming et al., 2006; Regmi et al., 2015) and furnished cages (Rodenburg et al., 2008). Although the specific benefits to bone strength and quality differ by study, positive effects are broadly associated with opportunities for hens to perform load-bearing movements.

On the other hand, access to perches and other elevated structures has been linked to the development of keel bone fractures (Fleming et al., 2006; Wilkins et al., 2011; Hester et al., 2013; Rufener and Makagon, 2020).

Received April 24, 2024.
Accepted August 18, 2024.

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Keel bone fractures are an animal welfare concern due to their high prevalence and links with reduced mobility, pain, neurological correlates for negative affective states, and lower egg production (Riber et al., 2018; Rufener et al., 2019a,b; Armstrong et al., 2020). Although these injuries occur in all types of housing systems, the keel fracture prevalence is lowest among hens housed in conventional cages (reviewed by Rufener and Makagon, 2020). Some studies have reported lower keel bone fracture prevalence in housing systems that limit the amount of elevated space provided, such as single-tiered aviaries (Riber and Hinrichsen, 2016) or furnished cage systems (Rodenburg et al., 2008), compared with housing systems that provide hens with access to multiple heights (but see Rufener and Makagon, 2020).

Early-life experiences in pullet barns (during the first 16−18 wk of life) are known to affect a multitude of animal welfare outcomes in laying hens, including bone integrity (Janczak and Riber, 2015). Cage-housed pullets raised with access to perches had higher bone mineral content of the tibia, keel, and humerus by 12 wk of age compared with pullets raised in conventional cages (Enneking et al., 2012). Similarly, pullets raised in aviaries had higher bone mineral density and bone mineral content values as compared with cage-reared pullets when assessed at 16 wk of age (Casey-Trott et al., 2017b). Experiences with elevated structures in the layer system can make up for differences in bone quality from pullet rearing (Hester et al. 2013; Anderson et al., 2024).

The relationship between hens' early-life experiences and keel bone fracture risk has received less attention. A recent review of publications referencing keel fracture prevalence from the past 30 years noted that 46.9% of articles provided no information about pullet housing (Rufener and Makagon, 2020). This suggests that early experiences were not considered or discussed as a possible contributing factor to keel fracture formation in nearly half of the published studies. Studies that have explored the role of early-life experiences on later keel bone fracture prevalence yielded varied results. Casey-Trott et al. (2017c) reported fewer keel bone fractures among aviary-reared hens that were subsequently housed in conventional or furnished cage systems. Others reported that early improvements to bone quality from the rearing environment were not sufficient to prevent keel bone fractures later in life (Hester et al., 2013; Regmi et al., 2015).

The current study aimed to determine whether opportunities to access elevated areas during rearing would improve bone quality (bone mineral density, **BMD**) and mitigate the development of keel bone fractures after hens were transferred to multitiered aviaries. We hypothesized that opportunities to interact with elevated structures in pullet housing would result in higher BMD in pullets and reduced prevalence of keel bone fractures as the hens matured. We expected that birds reared with opportunities to access higher aviary levels would have more opportunities to make vertical transitions and thus, increased participate in more bone loading activities (jumping up and down perches and aviary tiers, using wings during balancing movements and wing assisted includes). Consequently, we predicted those birds would have higher BMD of the humerus, keel, and tibia and fewer or less severe keel bone fractures than those reared in floor pens. We further expected bone parameters and keel fracture prevalence to increase with age.

MATERIALS AND METHODS

This study took place at the Hopkins Avian Facility, University of California, Davis (Davis, CA). All procedures were reviewed and approved by the University of California, Davis Institutional Animal Care and Use Committee (Protocol #20307).

Animals, Housing and Management

Pullet Housing A total of 835 day-old Dekalb White pullets with intact beaks were obtained from a commercial hatchery and placed in groups of 55 to 56 birds/pen across 15 pens (3.05 × 3.05 × 2.74 m, L x W x H) located within a research barn. Five pens were assigned to each of 3 rearing treatments: floor (**FL**), single-tiered aviary (**ST**), or 2-tiered aviary (**TT**). A block design was used to address location effects. Each treatment was represented once, in random order, within a block of 3 pens. Plastic tarps were hung across the bottom half of the fencing that separated the pens to reduce visual access into adjacent pens.

Pine wood shavings (Mallard Creek Inc., Rocklin, CA) were used as litter in all pens. Drinking water was provided *ad libitum* through automatic water lines (Lubing, Cleveland, TN; 12 nipples/pen). A start and grow diet (Purina Start and Grow Medicated Crumbles, Purina Animal Nutrition LLC, Gray Summit, MO) was provided *ad libitum* in two 13.6 kg round feeders/pen (52 cm circumference/feeder). Ceiling lights mounted within the pens and in the adjoining hallways supplied artificial lighting in accordance with the schedule described in the Dekalb White Commercial Management Guide for Aviary-Barn Systems (Dekalb, n.d.). Natural light entered the house through curtains that covered barn windows.

The 3 rearing treatments differed in the type of elevated structures provided within the pens. FL pens were furnished with 4 round metal perches, with a 3.8 cm and 121.9 cm length, installed at 10.5 cm high. ST pens contained a single 10.5 cm high perch with 3 additional perches mounted onto a single-tired aviary structure (2 at 35.4 cm high; one 64.7 cm high). The aviary structure included a single 61.0 × 121.9 cm tier (Dura-Slat Poultry and Kennel Flooring, Southwest Agri-Plastics, Inc., Addison, TX) located 62.9 cm off of the pen floor, which was connected to the floor by a mesh ramp (96.5×31.8 cm, 40-degree angle; McNichols Wire Mesh, McNichols Co., Inc., Livermore, CA). TT pens also contained a single 10.5 cm high perch and an aviary structure with 3 additional perches (29.4, 89.9, and 125.7 cm high). The TT structure featured 2 slatted tiers

(30.5 × 121.9 cm, L x W) located 62.9 and 123.8 cm off the floor. A ramp made of wire mesh connected both tiers to the floor (190.5 × 31.8 cm, 40-degree angle). The amount of usable space available to pullets was standardized across all treatment pens. Chicken wire prevented pullets from accessing the litter area directly underneath the tiers in ST and TT pens, and all pens contained 4 perches of identical dimensions. Group sizes were reduced to 45 pullets/pen and 28 to 30 pullets/pen during the 8th and 16th wk of age.

Layer Housing At 17 wk of age, pullet housing structures were replaced with a multitiered aviary across 2 d due to logistics (3 pens/rearing treatment on 1 d, 2 pens/rearing treatment on the following day). The aviary featured 3 plastic slatted tiers (121.9 × 61 cm, L x W) located 69.2, 137.2, and 198.2 cm off the ground and 5 round metal perches identical to those used in pullet rearing (1 perch at 50.0, 125.7, and 181.1 cm high, and 2 perches at 245.3 cm). The bottom tier housed a colony nest (121.9 × 30.5 cm, L x W; Large Reversible Roll Out Chicken Nest Box, Best Nest Box, Hudson, OH). The hens were able to access litter under the aviary to have 11.2 m^2 total accessible floor/tier space. Water and feed (16% Protein Layer Mini-Pellet, Bar Ale Inc., Williams, CA) were provided as described above. Pine wood shavings (Mallard Creek Inc., Rocklin, CA) were used as litter. Natural light and artificial lights in the hallways supplied light based on the schedule recommended by the Dekalb White Product Guide (Dekalb, n.d.). The lights located within the pens were turned off at 19 wk of age to prevent aggressive pecking. 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. All lights in the barn were turned off at 25 wk of age. Natural lighting from the covered windows supplied light through to the end of the study (14 h light, 10 hr dark; https://gml.noaa.gov/grad/solcalc/). Additional management details, including photos of the housing systems and description of data collection methods conduct for other studies on the same flock, have been described by Jones et al. (2023) and Pullin et al. (2024).

Data Collection

Bone Mineral Density and Bone Length At 8, 16, and 30 wk hens were weighed, and the keel, both tibiae, and both humeri were collected from 180 birds (60 per time point and treatment), which were previously examined to identify birds with intact keel bones. Dissected bones were visually inspected ahead of further analysis, and those containing visible cracks or breaks were removed from the sample. As a result, only 42 keels were included from the 30 wk data collection time point (10, 17 and 15 from FL, ST and TT respectively). The bones were stored at −80°C, then thawed at room temperature before they were analyzed using dual X-ray absorptiometry (DEXA; InAnalyzer, Medikors, Inc., South Korea). The BMD of the full tibia, center of the tibial diaphysis, full humerus, center of the humerus, and full keel bone, as well as bone lengths were measured by a single observer. Following Tarlton et al. (2013), the center of the bone was defined as a 0.08cm region adjacent to the midsection of the bone. Intraobserver reliability was established to be >0.9 (intraclass correlation coefficient; irr package; Gamer et al., 2017).

Keel Bone Integrity Ten focal hens/pen (N = 150 focal pullets) were randomly selected at 1 d of age and individually marked with nontoxic food coloring (Student Kit Soft Gel Paste Food Color, AmeriColor Corp., Placentia, CA) until their primary feathers developed, after which they were marked with nontoxic livestock marker (Markal All-Weather Paintstik Livestock Marker, LA-CO Industries, Inc., Elk Grove Village, IL). Markings were reapplied as needed. Focal hens were radiographed within the barn in which the hens were housed at 18, 26, 28, and 30 wk of age. We used a portable X-ray unit (Citation Digital Radiography, MyVet Imaging, Inc., Closter, NJ; Battery Powered Portable X-ray System, Poskom, Goyang, South Korea; Xmaru 1012 WCC/WGC Flat Panel Detector, Rayence, Closter, NJ) with film-focus distance of 60 cm and voltage of 50 kV/2.5 mAs. Following Širovnik and Toscano (2017), images were taken with the hen inverted. The procedure took approximately 10 sec/hen. During the first 3 time points all hens were radiographed within a single day. At 30 wk of age, focal hens were radiographed over the course of 9 d to accommodate data collection for other portions of the larger project involving the same flock of hens (Jones et al., 2023; Pullin et al. 2022). Due to focal hen mortality, the number of focal hens slightly varied at each age (18 wk, N = 149; 26 wk, N = 144; 28 and 30 wk, N = 142 focal hens).

Radiographs (N = 577) were converted to JPEG files, then scored by a trained observer for the presence (binary variable) and severity of fractures (continuous variable using the tagged visual analogue scale descried by Rufener et al. (2018). Briefly, the scale included 6 visual analog reference tags, allowing the observer to assign each bone a cumulative keel bone fracture severity score ranging from 0.0 (no fracture) to 10.0 (severe fractures present). We tabulated the number of fractures present at each time point. We additionally calculated the number of new fractures present at wk 26 relative to wk 18, and 30 relative to wk 26. The observer was blind to rearing environment and scored radiographs in an order randomized by rearing environment and age to prevent order bias. A sub-sample of 80 radiographs was scored by a second observer and inter-observer reliability was determined to be 0.96 (intraclass correlation coefficient; irr package).

Statistical Analysis

Bone Mineral Density and Bone Length Analyses were conducted using R software and RStudio (R Core Team, 2021) using linear mixed models, following graphical inspection for normal distribution of residuals and homoscedasticity. Final models were obtained by a stepwise backwards reduction using ANOVA for model comparison with a P-value of > 0.05 as the criterion of exclusion. Model estimates and 95% confidence intervals were obtained with the effects package (version 4.2.1, Fox and Hong, 2009). Separate linear mixed models (lme4 package) were run for each bone measurement. Additional analyses were completed with bone length measurements were corrected for BW to account for possible treatment effects on overall growth. Fixed effects included rearing treatment (factor with 3 levels FL, ST, and TT), bird age (factor with 3 levels: 8, 16, and 30 wk), and their interaction. Hen nested in pen (tibia and humerus measures) or pen (keel bone measures) and were included as crossed random effects. The collection date was included as a crossed random effect, except for outcome variables corrected for BW.

Keel Bone Integrity Statistical analyses were conducted in R statistical software using Rstudio. All model fits for keel bone damage data were assessed for deviations from their expected distribution, overdispersion, outliers, and homogeneity of variance via plot and test functions in the DHARMa package (version 0.4.6; Hartig, 2022) for generalized linear mixed models or sjPlot package (version 2.8.11; Lüdecke, 2021) for linear mixed models. Data were transformed if necessary to improve model fit. The final models were obtained by a stepwise backwards reduction using ANOVA for model comparison with a P-value of > 0.05 as the criterion of exclusion. Model estimates and 95% confidence intervals were obtained with the effects package (version 4.2.1, Fox and Hong, 2009).

Keel bone fractures were not detected on any of the focal hens at 18 wk of age, therefore this age was excluded from prevalence and severity analyses to improve the model fit (N = 149 radiographs). Keel bone fracture prevalence was analyzed with a generalized linear mixed model (lme4 package, version 1.1.32) with a binomial distribution. For keel bone fracture severity, a linear mixed model (lme4 package, version 1.1.32) was used and radiographs that did not have a fracture (score of 0, N = 76, 39, and 30 radiographs at 26, 28, and 30 wk, respectively) were also excluded to improve model fit. Both models included rearing environment (factor with 3 levels: FL, ST, and TT), age (factor with 3 levels: 26, 28, and 30 wk), and their interaction, with focal hen nested within pen as a random effect. The change in the number of new fractures were analyzed with a generalized linear mixed model with a poisson distribution (glmmTMB package, version 1.1.5) where rearing environment (factor with 3 levels: FL, ST, and TT), age (factor with 2 levels: 18−26 and 26−30 wk), and their interaction were included as fixed effects. Focal hen nested within pen was included as a random effect.

RESULTS

Bone Mineral Density and Bone Length A treatment*age interaction effect was shown for most BMD measurements, and keel bone length (Table 1). BMD for the tibia diaphysis was higher in FL and ST pullets than TT pullets at 8 wk of age, and lower in FL pullets as compared to ST and TT pullets at 16 wk of age ($P = 0.012$). At 16 wk of age BMD of the whole tibia ($P = 0.003$) and keel ($P = 0.013$) were lower in FL pullets than ST and TT pullets, and the BMD of the whole humerus ($P = 0.004$) were lower in FL pullets than TT pullets with bones from ST pullets showing intermediate values. At 30 wk of age, ST hens had shorter keel bones as compared with FL or TT hens ($P = 0.008$). However, after adjusting for BW, only age ($P < 0.0001$) affected keel length. As expected, all BMD and bone lengths values increased with age (Table 1, Figures 1−3).

Keel Bone Fractures The prevalence of keel bone fractures increased between 26 to 28 wk of age, and remained high to wk 30 ($\chi^2 = 70.30$, df = 2, $P < 0.0001$; Table 2). Keel fracture prevalence was not affected by

Table 1. Effects of rearing treatment by age on bone mineral content (BMD) and bone length.

Bone parameter	Age (wk)	FL Estimate (95% CI)	ST Estimate (95% CI)	TT Estimate (95% CI)	Age x Rearing (P-value)
Tibia whole bone BMD (mg/cm^2)	8	137.4 (131.0, 143.7)	141.7 (135.4, 148.0)	134.4 (128.0, 140.7)	$P = 0.003$
	16	201.7 (195.3, 208.2)^a	211.0 (204.5, 217.40)^b	215.4 (209.0, 221.8)^b	
	30	261.2 (254.9, 267.6)	266.3 (260.0, 272.7)	262.7 (256.3, 269.0)	
Tibia diaphysis BMD (mg/cm^2)	8	177.4 (170.2, 184.7)^b	176.7 (169.6, 183.9)^b	168.9 (161.7, 176.1)^a	$P = 0.0.012$
	16	180.6 (173.4, 187.8)^a	187.3 (180.1, 194.5)^{ab}	190.6 (183.5, 197.7)^b	
	30	265.1 (258.0, 272.3)	261.1 (253.9, 268.2)	259.3 (252.2, 266.4)	
Humerus whole bone BMD (mg/cm^2)	8	113.4 (109.7, 117.1)	115.1 (111.4, 118.8)	113.1 (109.3, 116.8)	$P = 0.004$
	16	192.4 (188.6, 196.2)^a	195.0 (191.1, 198.8)^{ab}	200.0 (196.3, 203.8)^b	
	30	212.4 (208.6, 216.2)	212.7 (208.9, 216.5)	211.1 (207.3, 214.9)	
Keel whole bone BMD (mg/cm^2)	8	57.1 (54.0, 60.2)	59.7 (56.5, 62.8)	58.0 (54.9, 61.0)	$P = 0.013$
	16	95.0 (91.7, 98.2)^a	100.4 (97.2, 103.7)^b	101.8 (98.5, 105.0)^b	
	30	110.0 (104.3, 115.7)	107.9 (103.3, 112.4)	111.2 (106.5, 116.0)	
Keel length (mm)	8	114.8 (112.7, 116.9)	114.5 (112.4, 116.6)	114.4 (112.3, 116.5)	$P = 0.008$
	16	152.5 (150.3, 154.7)	153.3 (151.1, 155.5)	151.6 (149.4, 453.8)	
	30	160.6 (156.8, 164.3)^b	155.3 (152.3, 158.3)^a	160.6 (157.4, 163.8)^b	

Hens were reared on the floor with access to perches (FL), in single-tier (ST) or 2-tier (TT) aviaries through 16 wk of age, then moved to multitier aviary systems. Bone parameters were determined by dual-energy X-ray absorptiometry (DEXA). Superscripts denote treatment differences by age determined based on overlap in estimated means and 95% intervals.

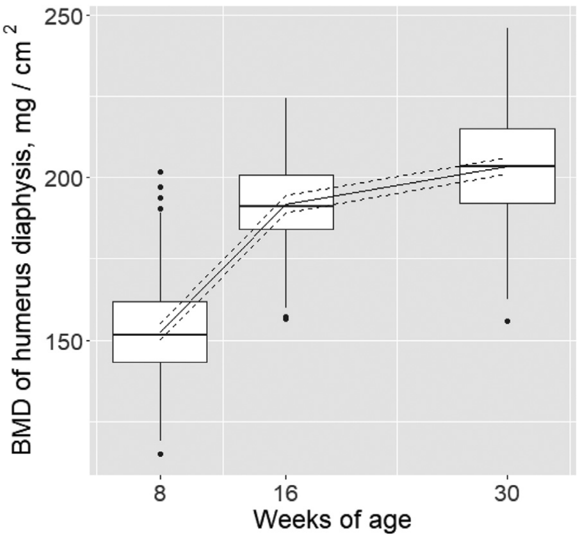

Figure 1. Bone mineral density (BMD) of humerus diaphysis from 8, 16 wk old pullets and 30 wk old hens**.** BMD was affected by age ($P < 0.001$), but not treatment or the interaction of treatment and age. Boxplots show medians, interquartile, and absolute ranges of raw data. The solid line represents the estimated mean, the dashed lines show the estimated 95 % confidence interval.

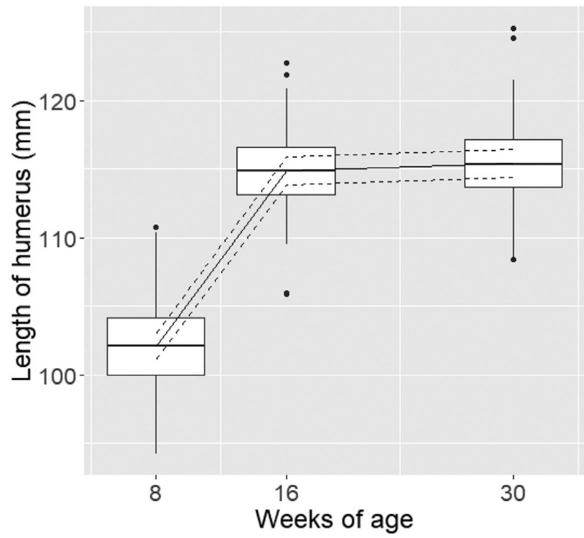

Figure 3. Length of humerus bones from 8, 16 wk old pullets and 30 wk old hens. The length of the whole humerus was estimated from dual-energy X-ray absorptiometry (DEXA), and was affected by age ($P < 0.001$), but not treatment or the interaction of treatment and age. Boxplots show medians, interquartile, and absolute ranges of raw data. The solid line represents the estimated mean, the dashed lines show the estimated 95 % confidence interval.

rearing environment ($\chi^2 = 0.44$, df = 2, $P = 0.80$), or its interaction with age ($\chi^2 = 2.26$, df = 4, $P = 0.69$). The severity of keel bone fractures increased with age ($\chi^2 = 9.05$, df = 2, $P < 0.0001$; Figure 4). Neither rearing environment ($\chi^2 = 2.70$, df = 2, $P = 0.26$) nor its interaction with age ($\chi^2 = 3.54$, df = 4, $P = 0.47$) influenced keel bone fracture severity. Compared to the number of new fractures incurred between 18 to 26 wk of age, hens experienced more new fractures between 26 and 30 wk ($\chi^2 = 8.02$, df = 1, $P = 0.0046$; 18−26 wk: 0.54 [0.43, 0.68], 26−30 wk: 0.82 [0.68, 0.98] new fractures/hen, estimated mean [95% CI]). Rearing environment ($\chi^2 = 1.38$, df = 2, $P = 0.50$) and its interaction with age ($\chi^2 = 0.81$, df = 2, $P = 0.67$) did not affect the number of new fractures.

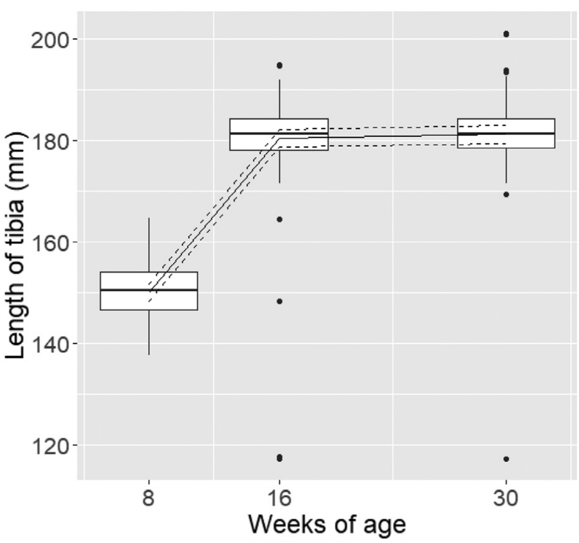

Figure 2. Length of tibia bones from 8, 16 wk old pullets and 30 wk old hens. The length of the whole tibia was estimated from dual-energy X-ray absorptiometry (DEXA), and was affected by age ($P < 0.001$), but not treatment or the interaction of treatment and age. Boxplots show medians, interquartile, and absolute ranges of raw data. The solid line represents the estimated mean, the dashed lines show the estimated 95 % confidence interval.

DISCUSSION

We hypothesized that opportunities to interact with elevated structures during early life would result in higher BMD in pullets and reduced prevalence of keel bone fractures in adult laying hens. The most pronounced differences in BMD were observed at 16 wk of age. Compared to FL, aviary-reared (ST and/or TT) pullets had higher BMD of the tibial diaphysis, whole tibia, humerus, and keel at this age. BMD values and keel length measures continued to increase in all treatments after hens were transferred to multitiered aviaries. Our initial analysis suggested that the keels of ST hens were shorter that hose of FL and TT hens at this age. This difference likely reflects variation in body size within treatment groups as neither treatment nor the interaction of treatment and age affected keel length when valuated per unit of bodyweight. Early differences

Table 2. Prevalence of keel bone fractures by rearing treatment.

Age (wk)	FL Estimate (95% CI)	ST Estimate (95% CI)	TT Estimate (95% CI)
18	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)
26	0.42 (0.11, 0.80)	0.40 (0.11, 0.77)	0.28 (0.07, 0.67)
28	0.95 (0.72, 0.99)	0.81 (0.44, 0.96)	0.88 (0.56, 0.98)
30	0.96 (0.77, 1.00)	0.95 (0.74, 0.99)	0.94 (0.71, 0.99)

Hens were reared in a floor pen with low perches (FL), a single-tiered (ST), or 2-tiered aviary (TT) through 16 wk of age. Radiographs were taken on focal hens at 18, 26, 28, and 30 wk of age. Values represent the proportion of hens with keel fractures.

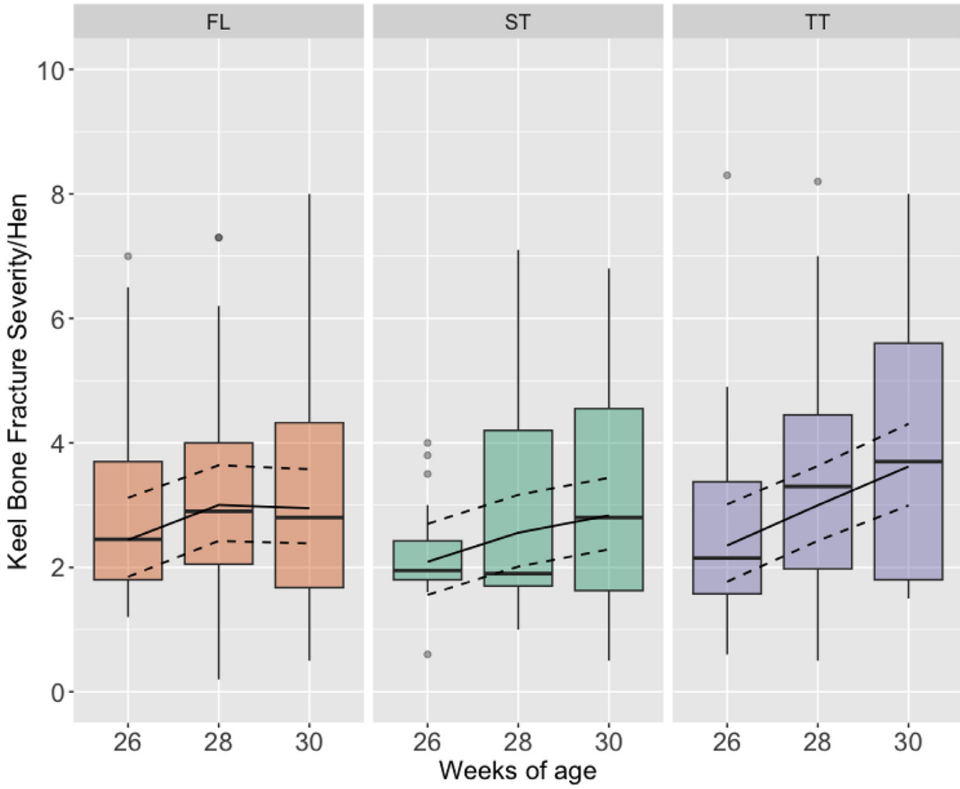

Figure 4. Keel bone fracture severity by rearing treatment and age in floor pens with perches (FL), a single-tiered (ST), or a 2-tiered aviary (TT). Boxplots show medians, interquartile, and absolute ranges of raw data. The solid line represents the estimated mean, the dashed lines show the estimated 95 % confidence interval.

in BMD, including the keel bone, did not translate to treatment differences in keel fracture at any age.

The results of our BMD analyses echo findings previously reported by others. Pullet rearing environment, and specifically the opportunity to use elevated spaces, has short-term effects on bone mineralization. Differences in bone mineralization among mature hens seem to be more heavily influenced by perching opportunities during the laying period (Hester et al., 2013; Casey-Trott et al., 2017c). Enneking et al. (2012) reported that rearing pullets in cages fitted with perches vs. conventional cages resulted in increased BMC of the keel, tibia, and humerus, but not BMD, at 12 wk of age. However, bone mineralization measures did not differ by rearing treatment at 71 wk of age. Alternatively, hens provided with perch access in their layer environment (from 16 wk of age) had higher BMD of the keel, humerus, and the wing bones and higher BMC of the keel at 71 wk compared to hens housed without perches during lay (Hester et al., 2013). Similarly, Casey-Trott et al. (2017b) found that pullets reared in multitiered aviaries had higher BMD and BMC of the humerus, tibia, and radius at 16 wk of age as compared with pullets reared in conventional cages. After they were transferred to their adult layer environment, BMC of several of the bones remained higher among the aviary-reared hens through 73 wk of age but BMD was higher among cage-reared hens (Casey-Trott et al., 2017c). Housing during the laying period (i.e., conventional cage or 2 sizes of furnished cages) influenced bone mineralization at 73 wk of age (Casey-Trott et al., 2017c). Finally, Anderson et al. (2024) reported that hens that had been provided with perch access in their rearing and layer environments had higher BMD at 40 wk of age, but pullet housing had a less pronounced effect on bone quality than layer housing.

Casey-Trott et al. (2017a) reared pullets in either conventional cages or multitiered aviaries before moving them to either conventional or furnished cages at 16 wk of age. The aviary-reared hens had fewer keel bone fractures at 30 to 70 wk of age as compared to hens reared in conventional cages. The authors speculated that this could be due to increased keel bone growth promoted by the load-bearing exercise opportunities in the aviary rearing environment (Casey-Trott et al., 2017b). Other studies reported that rearing-related differences in bone properties were not sufficient to protect against keel bone fracture formation (Hester et al., 2013; Gebhardt-Henrich et al., 2017). However, rearing environments that offer opportunities for pullets to access height could mitigate keel fracture development in other ways. Repeated collisions with housing structures have been associated with the development of keel bone fractures in aviaries (Stratmann et al., 2015) and furnished cages (Baker et al., 2020; Baker et al., 2024). Therefore, familiarizing pullets with elevated structures during rearing could facilitate their use of elevated spaces in the layer environment (Colson et al., 2008; Pullin et al., 2024) and consequently reduce the occurrence of collisions and keel bone fractures (Stratmann et al., 2015). This relationship was not observed in the current study though. The pullet rearing environment did not affect keel bone fracture prevalence at any age in the current study, nor did it influence the use of multitiered aviary structures long-term (reported in Pullin et al., 2024). Other than utilizing the highest aviary perches, which remained lower among FL hens through 27 wk, rearing-related differences in the use of layer aviary structures were limited to the first 1 to 2 wk post-transfer (at 17 wk of age; Pullin et al., 2024). Furthermore, collisions and unsuccessful vertical transitions were rare (<2% of all vertical transitions; Pullin et al., 2024) and not affected by rearing treatment, suggesting that behavior-related trauma to the keel was uncommon in the current study. Although seemingly contradictory to the results presented by Casey-Trott et al. (2017a) linking pullet rearing to keel fracture prevalence during lay, our findings could also point to the importance of providing pullets with exposure to utilizing and gripping perches, even if the height is minimal. All pullets in the current study had access to perches, although the FL pullets were only 10.5 cm from the ground. Where Casey-Trott et al. (2017a) found rearing-related effects on keel fractures, the conventional cage-reared pullets did not have access to any perches during early life. Similar to the current study, DePaoli et al. (2024) reported that pullet rearing environment did not affect keel fracture prevalence at the end of lay, and they reared pullets in either cages or in furnished floor pens, both of which contained perches only 7 cm high.

As predicted, keel fracture prevalence increased over time: from 0% at 18 wk of age to >90% at 30 wk of age. The latter prevalence is higher than the average prevalence reported for white laying hen strains at this age (23.1%) or for aviary housed hens at over 49 wk of age (37 to 39%; reviewed by Rufener and Makagon, 2020). Keel bone assessment method could have contributed to the discrepancy. Palpation, or manually feeling for the presence of callouses and fractures, is the most common technique of keel bone fracture assessment (Casey-Trott et al., 2015; Rufener and Makagon, 2020). However, the method is associated with low inter-observer reliability and low sensitivity for identifying fractures as compared with radiography, which was used in this study (Casey-Trott et al., 2015; Tracy et al., 2019). A recent study using radiography on hens from 22 to 61 wk of age found that 97% of hens experienced at least 1 keel bone fracture, and most fractures occur at the caudal tip of the keel (Baur et al., 2020). The majority of fractures in the present study were minor in severity, falling within the first or second visual analogue tag of fractured keels (Rufener et al., 2018), and might have been overlooked if assessed by palpation.

The presented results support and expand on the growing knowledge related to the role of early experiences on bone development and keel integrity in laying hens. We conclude that (1) opportunities to access more complex environments during rearing affect bone mineralization development in pullets, however the differences disappear if hens are provided opportunities to access elevated spaces and/or engage in other load-bearing activities in their layer housing, and (2) rearing pullets in systems that provide more opportunities for accessing elevated space, beyond the perches present in FL, does not protect hens from sustaining keel fractures through 30 wk of age. The results represent findings from hens housed in multitiered aviary systems during the laying period. This system was selected based on the popularity of these systems in the United States, where the study was conducted. The 3 rearing treatments allowed us to examine the implications of raising pullets in increasingly complex environments that differed primarily in system height. The study examined a single laying hen breed, Dekalb White. Previous research has established that breed effects the behavior of pullets and hens in aviaries (Ali et al., 2016, 2019; Pufall et al., 2021; Rentsch et al., 2023a, b), as well as keel fracture development (Regmi et al., 2016; Eusemann et al., 2018; DePaoli et al., 2024; and as reviewed by Rufener and Makagon, 2020). Future research should, therefore, incorporate multiple breeds.

ACKNOWLEDGMENTS

Research reported in this publication was supported by the Foundation for Food & Agriculture Research under award number − Grant ID: 550830. The content of this publication is solely the responsibility of the authors and does not necessarily represent the official views of the Foundation. The research was further supported by a donation of aviary housing components from Lubing. We thank Dr. Suzanne Millman, Iowa State University, for providing valuable input and feedback on study design, and helpful comments on previous manuscript drafts. We acknowledge Cloude Lu, University of California, Davis, for assistance with processing bone images, and the avian facility managers, staff and undergraduate interns for helping care for the birds. We thank Andrew Blandino and the Stat Lab, University of California, Davis, for statistical consultation.

DISCLOSURES

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Maja Makagon reports financial support was provided by Foundation for Food and Agriculture Research. Maja Makagon reports equipment, drugs, or supplies was provided by Lubing. Richard Blatchford reports was provided by Foundation for Food and Agriculture Research. Richard Blatchford reports equipment, drugs, or supplies was provided by Lubing. John Tarlton reports financial support was provided by Foundation for Food and Agriculture Research. Michael Toscano is a Section Editor for Poultry Science; Maja Makagon is an Associate Editor for Poultry Science If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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