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Influence of keel impacts and laying hen behavior on keel bone damage

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ABSTRACT Keel bone damage, which presents as fractures and/or deviations of the keel, has been detected in laying hens housed in all types of systems. Factors leading to keel bone damage in hens housed with limited vertical space, such as those housed in furnished systems, are not well understood, and are the topic of this study. Ten focal hens from each of 12 furnished cages (4 rooms of 3 cages) were fitted with keel mounted tri-axial accelerometers. Their behavior was video recorded continuously over two 3-wk trials: the first when the hens were between 52 and 60 wk of age, and the second approximately 20 wk later. The integrity of each hen's keel was evaluated at the start and end of each 3-wk trial using digital computed tomography. We identified predominant behaviors associated with acceleration events sustained at the keel (collisions, aggressive interactions and grooming) by pairing accelerometer outputs with video data. For each recorded acceleration event we calculated the acceleration magnitudes as the maximum summed acceleration recorded during the event, and by calculating the area under the acceleration curve. A principle components analysis, which was used as a data reduction technique, resulted in the identification of 4 components that were used in a subsequent regression analysis. A key finding is that the number of collisions a hen has with structures in her environment, and the number of aggressive interactions that a hen is involved, each affect the likelihood that she will develop 1 or more fractures within a 3-wk time span. This relationship between hen behavior and keel fracture formation was independent of the magnitude of acceleration involved in the event. Observed behavior did not have an impact on the formation of keel bone deviations, further supporting reports that the mechanisms underlying the 2 types of keel bone damage are different.

Key words: laying hen, keel, behavior, enriched cage, accelerometer

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INTRODUCTION

The presence of keel bone damage (fractures and/or curvature of the bone) in laying hens has long been documented (e.g., Darwin, 1868; Gregory et al., 1990; Abrahamsson and Tauson, 1993). In recent years, the topic has gained widespread attention as a hen welfare problem (Lay Jr. et al., 2011; Harlander-Matauschek et al., 2015; Rufener and Makagon, 2020). Of the 2 types of keel damage, the formation of keel bone fractures is of key concern due to its association with pain (Nasr et al., 2012a). Keel bone damage, and particularly keel factures, is more prevalent in housing systems that provide hens with more behavioral opportunities than traditional battery cage systems (Wilkins et al., 2011; Rufener and Makagon, 2020). In aviary systems, navigating onto, between and from perches and platform tiers has been cited as a key source of fractures (Moinard et al., 2004; Stratmann et al., 2015a; Campbell et al., 2016, Rufener et al., 2019a). Potential causes of keel bone damage for hens housed in furnished cages, which limit the amount of vertical space available to the birds, are less well understood. Yet, keel fracture prevalence in these systems can be high. Based on 24 reports published over a 10 yr period, a mean keel bone fracture prevalence of 50.3% has been estimated for hens housed in furnished cage systems (Rufener and Makagon, 2020), with some studies reporting up to 98% prevalence (Thøfner et al., 2021).

Multiple studies have implicated the presence of perches as a factor contributing to keel bone damage in furnished cage systems. Higher percentages of hens with

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keel fractures have been reported when perches are present within a cage system (Vits et al., 2005; Scholz et al., 2008; Wilkins et al., 2011). However, these flock level associations are based on the assumption that the individuals experiencing keel damage are the same individuals that are interacting with the perches (see Siegford et al., 2016; Rufener and Makagon, 2020). Furthermore, they do not specify the types of perch interactions that may be leading to keel damage. This information is critical for an effective array of mitigation strategies to be considered. Our recent study (Baker et al., 2020) evaluated potential causes of keel bone damage in hens housed in furnished cage systems by identifying behaviors and cage structures associated with acceleration events experienced by individual hens at their keels. Collisions with perches, specifically during ascents, were associated with the highest proportion of acceleration events at the keel, lending evidence to the role of perches in keel bone damage. However, keel bone damage was not evaluated, leaving the link between perch collisions and keel damage unclear. The current study aimed to fill current knowledge gaps by evaluating the contributions of behaviors associated with accelerations experienced at the keel bone and the magnitude of those accelerations (representative of impact strength) to the development of keel bone fractures and deviations.

MATERIALS AND METHODS

This study was approved by the Michigan State University Animal Care and Use Committee prior to the start of data collection.

Housing and Birds

Hy-Line W-36 pullets were reared in conventional cages without perches. At 17 wk of age, the birds were moved into furnished cage systems distributed across 4 rooms at the Laying Hen Research Facility at Michigan State University, USA. Each room held 9 cages in a 3 × 3 formation. All cages were stocked with hens, but only the top 3 cages per room were used in this study (3 cages × 4 rooms). Two rooms contained AVECH furnished cages (Big Dutchman, Holland, MI) that featured round, metal perches and 62 hens/cage, and the other 2 rooms contained VERSA systems (ChoreTime, Milford, IN), with square, plastic perches and 65 hens/cage). All hens were stocked at 116 sq in/hen (748.4 cm^2/hen). All cages had a curtained nest box, scratch pads, external feeders, hanging nipple drinkers, and perches. All rooms were exposed to a 16 h photoperiod for the duration of the study.

Ten focal hens were randomly selected from each of the 12 cages. Data on the same group of focal hens were recorded over two 3 wk trials (Timepoint A and Timepoint B). Because of the limited number of accelerometers available for this project, 1 pen per room was observed at a time within each trial. Once a 3 wk trial was completed for the 10 focal birds in the first cage, the ten focal birds in the next cage were studied for 3 wk, after which the accelerometers were moved to the focal hens in the third cage. It took 9 wk to complete data collection within Timepoint A and B. Timepoint A and B were conducted when hens were 52 to 60 and 74 to 83 wk of age, respectively. Cage order was randomized per room. Three types of data were collected for each focal hen: keel bone integrity, accelerations experienced at the keel ("acceleration events"), and hen behavior.

Keel Integrity

Computed tomography (**CT**) scans were taken at the beginning and end of each 3 wk trial. Focal hens were removed from cages at night, while barn lights were off, and placed in temporary transportation cages to be scanned at the Michigan State University College of Veterinary Medicine (16 slice, GE Brightspeed, General Electric Healthcare, Princeton, NJ). Hens were fitted with colored leg bands so that they could be individually identified during the scanning process, and with vests with darkened facial coverings to keep birds calm and still during the scan. Details of the CT scanning protocol, including vest design, are detailed by Chargo et al. (2019a), whose study was conducted concurrently with ours.

Mimics software (MaterialiseNV, Leuven, Belgium) was used to convert CT scans into 3-dimensional

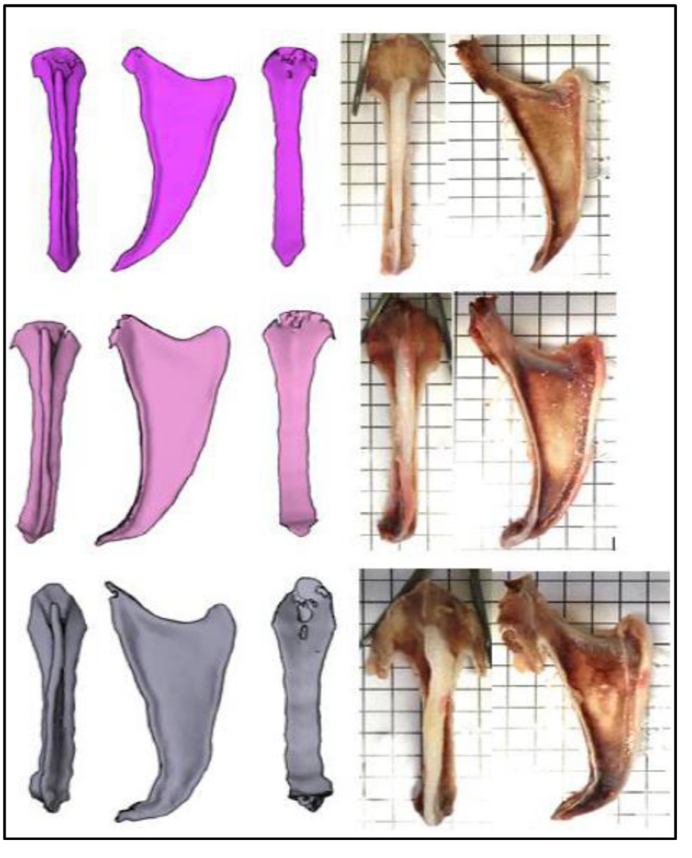

Figure 1. Visual comparison of 3D keel bone modes (ventral, sagittal, and dorsal plane) with the dissected bone from the same hen (ventral and sagittal plane). Top row: keel bone with minimal damage; middle row: keel bone with a slight deviation and tip damage; bottom row: keel bone with a severe deviation (score 2) and tip fractures.

computer models of keel bones for integrity scoring (Figure 1). Keel models were scored for type, location, and severity of damage. All scans were scored for the presence and absence of fractures and deviations at any location on the bone. For fractures, damage severity was defined as the number of fractures on the bone (F0 = no fractures, F1 = 1 fracture, F2 = multiple fractures). Deviation severity was subjectively scored by a single scorer based on a visual evaluation of how much the bone deviated from a straight line overlaid over top of the image of the bone (S0 = no deviation, S1 = slight deviation, S2 = severe deviation). The presence of damage located on the tip of the keel, defined as the caudal ¼ of the crest length, was additionally noted. Figure 1 shows examples of 3 of many possible bone score combinations. The change in the severity of keel fracture (*ChangeF)* and keel deviations (*ChangeD*) was determined for each hen by comparing the 3 wk scan relative to the initial scan within each trial using the same 0 to 2 scales. For example, a bone that sustained no additional fractures over the course of a trial received a score of +0, one that sustained a single additional fracture received a score of +1, and one that sustained multiple additional fractures received a score of +2.

Acceleration Event Data

On the first day of each 3 wk trial focal hens were fitted with custom jackets containing tri-axial accelerometers. Most hens appeared to return to their normal behaviors (e.g., feeding, drinking, walking around, perching) within 5 to 30 min of being fitted. A unique symbol located on the dorsal side of each jacket made it possible to individually identify each focal hen during each trial; leg bands were used to confirm that the same hens were observed during Timepoint A and B. The double-layer jacket was designed so that a small sensor could be threaded through the material layers and fitted into a pocket located over the hen's keel. The accelerometer loggers were programed to save 1 s of data surrounding a keel acceleration event, which was defined as an event with a summed acceleration in 3 dimensions of over 12 G-units. The 12 G-unit cut off was established based on preliminary observations, which revealed that events that fell below this acceleration value were associated primarily with preening, stretching and other comfort behaviors vs. the types of behaviors likely to cause keel fractures. Accelerometers were removed from focal birds approximately half-way through each 3 wk trial (on d 10) so that batteries could be recharged. All removal and replacement of loggers took place after the lights turned off for the night to minimize bird disturbance. Accelerometer data were downloaded during the charging event and at the end of each trial and sorted into the following categories according to combined acceleration peak values: <20 (12−19.9), 20 to 39.9, 40 to 59.9, 60 to 79.9, ≥80 G-units. Three of the accelerometers failed during the course of the study, therefore acceleration event data were available for a total of 119 and 118 hens from Timepoint A and B respectively.

Behavior Observations

Three overhead cameras (VF540 Bullet Camera, Clinton Electronics, Loves Park, IL) per focal cage were set to record video continuously for the duration of each 3 wk trial. We reviewed (GeoVision Digital Surveillance System; ViewLogEx v.8.5.6.0) video clips associated with the date and time stamps from the keel loggers to determine each hen's behavior at the moment a keel acceleration event was detected. Nighttime behaviors proved difficult to identify due to hen clustering during roosting. Therefore, only videos recorded during the 16 h per d when the barn lights were on were included in the study. Observed behavioral categories included collisions, aggressive interactions, preening activity, wing flapping, and mass scattering (defined in Table 1). Due to the low prevalence of acceleration events caused by wing flapping (4.2%) and mass scattering (2.2%; see Baker et al., 2020), these behavioral categories were excluded from the analysis.

Statistical Analysis

Principal Component Analysis Individual hen behavior and acceleration event data were analyzed by principal component analysis (**PCA**) and subsequent regression analysis. Data from Timepoints A and B were included in the same analysis given that the PCA was used purely for data reduction, and because previous analyses of the data showed that the number and type of acceleration events at the keel experienced by a hen during Timepoint A was unrelated to those experienced

Table 1. Behavioral definitions.

Behavior	Description
Collision events	Hitting an obstacle
Aggressive interactions	Sharp, deliberate pecks at face or neck of another bird, fighting, chasing after, or body slamming into another bird (not a clear collision event)
Grooming	Self-maintenance, preening, ruffling, or gentle pecking
Wing flapping	Both wings fully extended, moving up and down (not due to collision or grooming)
Mass scattering	Multiple bird movement (example: major cage disturbance), with no clear collision of focal hen
Cannot ID	Impact occurred during lights off
	Hen behavior was obstructed from viewer by cage structure or another bird
	No clear behavior could be determined at the moment of impact
	Video error impairing observations

during Timepoint B (Baker et al., 2020). A total of 7,384 acceleration events, representing 59.37% of all logged acceleration events, were paired with 1 of the 3 behavioral categories of interest (collision, grooming, aggression). Within each behavioral category the data were binned by acceleration output (<20, 20−39.9, 40−79.9, and >80 G). These behavior-by-acceleration output categories were included in the PCA as 12 distinct variables. Because all of the acceleration events a hen experienced could have potentially resulted in keel bone damage, the total number of acceleration events per hen per trial was also included in the PCA. In addition, the area under the curve (**AUC**) of all logged acceleration events experienced per hen per 3 wk time period was summed for a Sum AUC variable. This variable provided an estimate of the combined acceleration experienced at the keel within a 3 wk period by taking into account the duration and magnitude of the acceleration event. AUC was calculated using R statistical software (R v.3.2.3; RStudio v.1.0.136; "pracma::trapz" [v.1.9.9]; "gtools::mixedsort" [v.3.5.0]), and only included G values reaching over a threshold of 20 G. Therefore, accelerations with peaks <20 G or with single, short peaks had a calculated AUC of 0 G × ms.

The final PCA contained 14 total variables: 12 behavioral categories, total number of acceleration events, and Sum AUC. The PCA was run in R statistical software (R v.3.2.3; RStudio v.1.0.136). All categories were transformed with a log(x + 1) transformation. A correlation matrix of the 14 variables was created, and checked for accuracy of values using Bartlett's test for Identity Matrix ($X^2 = 1{,}520.87$; $P < 0.001$; df = 91) and Kaiser, Meyer, Olkin Measure of Sampling Adequacy (Overall MSA = 0.79) ("psych::cortest.bartlett"; "psych::KMO"; "psych" [v.1.7.5]). A Cattell's scree plot identified 4 components (RC) with eigenvalues greater than 1 ("pysch::VSS.scree" [v.1.7.5]). A PCA extracting 4 components explained the most variability among factors, while also having the best overall fit (Figure 2). The final analysis was performed with a varimax (orthogonal) rotated PCA for the 4 principal components of the correlation matrix containing the 14 variables ($n = 237$) (mean item complexity = 1.5; RSMR = 0.07; $X^2 = 230.23$; $P < 0.001$; fit of diagonal values = 0.95) ("psych::principal" [v.1.7.5]). Eigenvalues, proportion of variance, and cumulative variance of each extracted component were as follows: RC1: 4.1, 0.29, 0.29; RC2: 2.24, 0.16, 0.45; RC3: 1.5, 0.11, 0.56; RC4: 1.18, 0.08, 0.64.

Table 2. Loading values associated with each of the 4 main components (RC 1−4) and the 14 variables included the PCA: number of collisions (C), aggressive interactions (A), grooming (G) events associated with each of 4 acceleration output levels (<20, 20−39.9, 40−79.9, and >80), total number of acceleration events, and Sum AUC.

Variables	RC1	RC2	RC3	RC4
C <20	**0.819**		0.261	
C 20−39	**0.879**			
C 40−79	**0.885**			
C >80	**0.815**	0.121		0.190
G <20		0.224	**0.864**	
G 20−39	0.119	−0.108	**0.594**	**0.461**
G 40−79		0.192		**0.827**
G >80	0.253	−0.137	0.104	0.282
A <20	0.102	**0.744**	0.164	
A 20−39	0.235	**0.720**		−0.189
A 40−79		**0.668**	0.108	0.141
A >80	0.222	**0.641**	−0.106	0.237
Acceleration events	**0.772**	**0.312**	**0.488**	
Sum AUC	**0.648**	0.235	−0.130	0.208

Bolded numbers highlight loading values with absolute values >0.3, indicating moderate (>0.3) to excellent (>0.70) correlations.

Table 2 summarizes the loadings for all 14 variables for each of the 4 RCs.

Following Comrey and Lee (1992), only variables with loading values greater than 0.3 were considered for each component, with loading values of 0.55 and above considered good to very good, and values of 0.71 and above considered excellent. Collision behaviors from across all acceleration peak categories loaded strongly onto component RC1 (0.85 average). Likewise, all aggression event categories grouped strongly in RC2 (0.69 average). Grooming behaviors with acceleration values of <20 G and 40 to 79.9 G loaded strongly onto RC3 (0.86) and RC4 (0.83), respectively. The remaining grooming events (20 to 39.9 G) loaded onto RC3 (0.59) and RC4 (0.46). Total acceleration events loaded strongly onto RC1 (0.77), and to a lesser extent onto RC2 (0.31), and

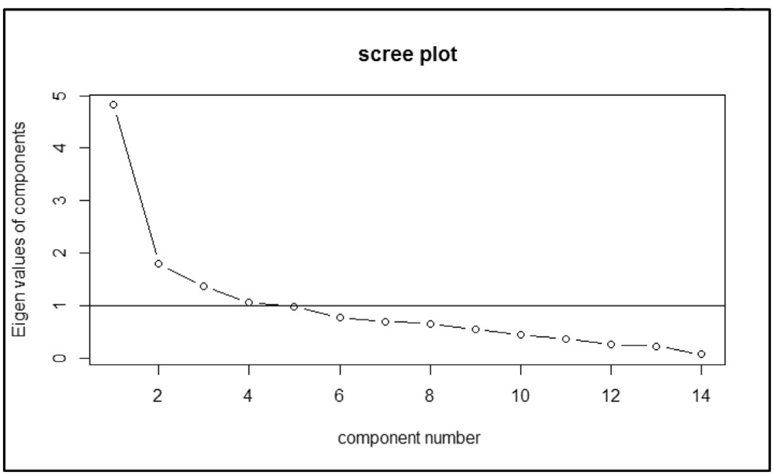

Figure 2. Plot of successive eigenvalues for each corresponding PCA component.

Table 3. Simplified component groupings of variables.

Component	Comprising variables	Description	Simplified Comp. ID
RC1	C <20, C 20−39, C 40−79, C >80, Acceleration Events, Sum AUC	Hens with high number of collisions across all acceleration ranges; high number of total events; high summed area of events	Collision component
RC2	A <20, A 20−39, A 40−79, A >80, Acceleration Events	Hens with a high number of aggressive interactions across all acceleration ranges; moderate number of total events	Aggression component
RC3	G <20, G 20−39, Acceleration Events	Hens with a high number of low grooming events and moderate level of total events	Lower energy grooming component
RC4	G 20−39, G 40−79	Hens with a high number of low and mid-level grooming events	Higher energy grooming component

Fourteen variables were included in the PCA: the number of collisions (C), aggressive interactions (A), grooming (G) events associated with each of 4 acceleration output levels (<20, 20−39.9, 40−79.9, and >80), total number of acceleration events, and the Sum AUC experienced within a 3 wk period.

RC3 (0.49). Sum AUC loaded onto RC1 (0.65). This information was used to describe and name the 4 components to simplify groups of variables during regression analysis (Table 3), and hens received a factor score for each component per 3 wk trial depending on the distribution of their specific acceleration events across each component.

Regression Analysis RC factor scores taken from the PCA were regressed with bone damage scores. The keel bone fracture (*BaseF*) and deviation (*BaseD*) severity scores associated with the initial bone scan taken from each hen were included as predictor variables alongside RC factor scores; change in fracture (*ChangeF*) and deviation (*ChangeD*) scores were response variables. Baseline and 3-wk keel scans were available for 37 hens from the VERSA housing system, and 43 hens from AVECH system for Timepoint A, and for 46 hens and 52 hens from each of the respective housing systems for Timepoint B. Some hens only had a complete set of data for either Timepoint A or Timepoint B data but not both.

All regression analyses were completed in R statistical software (R v.3.2.3; RStudio v.1.0.136). An ordinal logistic regression model ("MASS::polr" [v.7.3-45]) was used because of its ability to predict categorical variables on an ordered scale without congruous distance. Effect on *ChangeF* and effect on *ChangeD* were run as 2 separate models, each containing the same 6 predictors (RC1, RC2, RC3, RC4, *BaseF*, and *BaseD*). Both initial models contained interactions between all RC values, and interactions between *BaseF* and *BaseD*, but predamage scores were found nonsignificant in *ChangeF* model and removed for better fit. Assumption of proportional odds was tested and met for both final ordinal logistic regression models ("oridinal::clm"; "ordinal::nominal_test" [v.2015.6-28]).

RESULTS

Summary Data

Tables 4 and 5 summarize changes in keel bone integrity occurring within Timepoints A and B. Of the 80 hens with a full set of CT scans completed during Timepoint A, 52.5% had single keel bone fracture (score F1) and another 32.5% had 2 or more fractures (score F2) at start of the data collection period (*BaseF*). Over the next 3 wk, 53.75% of the hens sustained additional keel bone fractures (+1 or +2; *ChangeF*). During Timepoint B, 91.8% of the 98 sampled hens had either one (57.1% hens) or more (34.7% hens) keel fractures (*BaseF*). In total, 40.8% of hens sustained additional fractures during this Timepoint (+1 or +2; *ChangeF*). Tip fractures were observed in 79.4 and 91.1% of hens that had fractures at the start of Timepoint A and B, respectively.

Keel bone deviations were observed in 63.8% of the hens sampled at the start of Timepoint A (*BaseD*). New or more pronounced deviations (*ChangeD*) were noted for 32.5% of hens by the end of this Timepoint. Keel

Table 4. Summary of keel fracture data.

		Additional damage sustained during Timepoint (*ChangeF*)		
Timepoint	Baseline fracture score (*BaseF*)	+0	+1	+2
A	F0	4	8	0
	F1	17	24	1
	F2	16	9	1
B	F0	3	5	0
	F1	31	21	4
	F2	24	6	4

The number of hens with baseline keel fracture scores (*BaseF*) of F0 (no fractures), F1 (single fracture) or F2 (multiple fractures) that sustained zero (+0), one (+1) or more (+2) additional fractures (*ChangeF*) in the course of a 3 wk data collection period during Trial A and B. Images from a total of 80 and 98 hens were included in Timepoint A and B, respectively.

Table 5. Summary of keel deviation data.

		Additional damage sustained during Timepoint (*ChangeD*)		
Timepoint	Baseline deviation score (*BaseD*)	+0	+1	+2
A	S0	15	14	0
	S1	30	11	1
	S2	9	0	0
B	S0	22	9	0
	S1	44	9	0
	S2	12	1	1

The number of hens with baseline keel deviation scores (*BaseD*) of S0 (no deviation), S1 (minor deviation), or S2 (severe deviation) that experienced changes in the number of magnitude of bone deviations (*ChangeD*) in the course of a 3 wk data collection period during Trial A and B. *ChangeD* ranged from +0 = no change, to +2 = deviations in new areas of the bone or visibly larger than recorded based on the baseline scan. Images from a total of 80 and 98 hens were included in Timepoint A and B, respectively.

deviations were observed on 68.4% of the hens at the start of Timepoint B (*BaseD*), with 20.4% of the hens showing new or more pronounced deviations of the keel by the end of Timepoint B (*ChangeD*). Fractures and deviations commonly co-occurred: 55 and 61.2% of the hens had both types of damage at the start of Timepoints A and B, respectively. Overall, 93.8 and 99% of hens sampled at the start of Timepoint A and B had some form of keel damage (fracture, deviation or both).

Collisions were the most frequent source of acceleration events of the 3 behaviors included in the analysis (62.9%). This was followed by grooming (27.9%), and aggressive interactions (9.2%). Results from the analysis of the full behavioral data set and accelerometer outputs collected as part of this project can be found elsewhere (Baker et al., 2020).

Regression Analysis

Baseline damage (*BaseF* and *BaseD*) did not influence the change in fracture score and was removed from the final regression model. The ordinal logistic regression using RC values to predict change in fractures (n = 178, AIC = 315.95, SE 0|1 = 0.17, SE 1|2 = 0.36) showed an effect of RC1 (P = 0.029, SE = 0.18), RC2 (P = 0.042, SE = 0.18), and the interaction of all RC values (P = 0.005, SE = 0.35) on whether a hen received a +1 or +2 change in fracture damage ($P < 0.001$, SE = 0.36) during a 3 wk time period. There was no effect on the difference between a +0 and a +1 *ChangeF* (P = 0.22, SE = 0.36). There was no effect of RC3 (P = 0.85, SE = 0.19) or RC4 (P = 0.3, SE = 0.24) on receiving a +1 or +2 *ChangeF*.

The ordinal logistic regression using RC values and baseline damage to predict change in deviations (n = 178, AIC = 247.37, SE 0|1 = 0.85, SE 1|2 = 1.08) showed no main or interaction effects of any of the RC values on *ChangeD*. However, Change D was affected (P = 0.002, SE = 1.01) by the presence of deviations (BaseD score of 2; $P < 0.001$, SE = 1.89), or the presence of deviations in combination with fractures in the baseline scan (BaseF score 1 with BaseD score 2; $P < 0.001$, SE = 1.85; BaseF score 2 and BaseD score of 2; P = 0.001, SE = 3.54).

DISCUSSION

A high prevalence of keel bone damage was detected in our flock of focal birds, with 93.8% of the focal hens showing some form of damage by 52 to 57 wk of age (start of Timepoint A), and all but 1 hen showing damage by 74 to 80 wk of age (start of Timepoint B). A large proportion of keel damage occurred at the bone's tip. The tip of the keel is the last section of the bone to develop and has previously been noted to be especially susceptible to damage (Zheng et al., 2012; Casey-Trott and Widowski, 2016; Heerkens et al., 2016; Chargo et al., 2019a,b; Baur et al., 2020; Thøfner et al., 2021). The overall observed prevalence is numerically higher than what has been reported for hens housed in furnished cage systems. A mean keel fracture prevalence of 50.3% has been estimated for hens housed in these types of system (Rufener and Makagon, 2020), although higher occurrence of damage has been reported on some farms (e.g., up to 98% in Thøfner et al., 2021). A number of factors may have contributed to the high prevalence values in our study. CT scans could have increased the likelihood of detecting damage relative to methods such as palpation by increasing detection of fractures on the dorsal side of the bone and the tip (Casey-Trott et al., 2015; Rufener and Makagon, 2020). The study was conducted at a research facility, where several projects were simultaneously taking place, including in the 2 tiers of cages directly under our focal birds. Therefore, over the 68 wk the hens spent in the housing (from 16 to 83 wk of age) the focal hens in our study may have experienced more frequent disruptions than typical, due to presence of researchers in the barn, removal of birds from cages for weigh-ins and other assessments, and noise associated with research and facility maintenance. Indeed, the average occurrence of keel bone fractures experienced by hens housed in furnished cages seems to be lower when assessed on farm vs. in experimental settings (Rufener and Makagon, 2020). It is important to note that this study focused on the development of keel bone damage sustained by individual hens over two 3 wk trials during which management and research related disruptions were minimized.

Despite the high prevalence of keel bone damage at the beginning of the study, a large proportion of hens sustained new fractures or deviations during each 3 wk trial. Previous research on keel bone damage development has largely focused on prevalence across age groups, where overall prevalence seems to stabilize after 49 wk of age (reviewed by Rufener and Makagon, 2020). Only a few studies have quantified the integrity of the keel bone in the same focal birds over time. Rufener et al. (2019a,b) reported that the severity of keel bone damage, specifically fractures, also seemed to stabilize at older ages. Eusemann et al. (2018) noted an increase in the occurrence of keel bone fractures, and the occurrence and severity of keel bone deviations, as birds aged. The different keel damage scoring systems, housing systems, and laying hen strains used across studies complicate results comparisons.

The principal component analysis identified 4 main components: 1) RC1 = collision component, 2) RC2 = aggression component, 3) RC3 = lower energy grooming component, and 4) RC4 = higher energy grooming component (Table 3). The collision component showed heavy grouping of collisions of all summed acceleration peaks; <20, 20 to 39.9, 40 to 79.9, and >80 G-unit all strongly loaded together (>0.8 loading value). This implies that there was a subsample of hens prone to repeated acceleration events in the form of collisions (hens with a high collision component) across acceleration categories. The same result holds true for the aggression component, in which all summed acceleration categories loaded between 0.64 and 0.74. This likely reflects the relatively similar distribution of specific behaviors across summed acceleration shown in our previous work (Baker et al., 2020).

The highest peak category for grooming (>80 G), did not load onto any of the 4 components, most likely because there were so few grooming events with summed acceleration peaks in that range. We had previously reported (Baker et al., 2020) that acceleration events in the 12 to 20 G-unit range were typically associated with general hen movements, such as wing stretching and preening events, rather than behaviors that would be likely to impact keel integrity. Of all grooming events 94.6% were categorized into the <20 G grooming variable.

The PCA variable for Sum AUC grouped mainly with the collision component (0.65 loading value), which highlights our previous finding that acceleration events associated with collisions are associated with the largest number of acceleration peaks and, therefore, higher AUC values than those associated with other behaviors (Baker et al., 2020). The collision component also had the highest eigenvalue, meaning it explained the most variance among factors. Overall, the collision data offered the largest and most varied sample set.

Hens prone to collisions had more additional fractures at the end of the 3 wk (*ChangeF* +2 vs. +1; $P = 0.03$). Since acceleration peak categories of collision events loaded together, it seems that collision behavior itself is a risk to keel damage, not dependent on the acceleration peak of each collision. Several studies have reported that the presence of perches is correlated with higher flock-level prevalence of keel bone fractures (e.g., Abrahamsson and Tauson, 1993; Appleby et al., 1993; Abrahamsson et al., 1996), and that navigating the furnished cage space to use perches is a likely risk factor for increased keel bone fractures (Appleby et al., 1998; Moinard et al., 2004; Stratmann et al., 2015a,b; Baker et al., 2020). The current study delivers more direct evidence of a relationship between the number of collisions an individual hen experiences and her susceptibility to sustaining fractures, independent of acceleration experienced at the keel. Our finding aligns with Thøfner et al. (2020), who concluded that external trauma caused by high impact collisions may not be a primary cause of keel fracture formation based on a histological evaluation of keel bone fractures. It is likely that, as collisions occur, the bone may become gradually compromised, and prone to breaking. Collisions acting on a weakened bone has previously been noted as a possible explanation for keel fracture formation (Toscano et al., 2020). As we previously reported, the collisions experienced by our focal birds were most commonly associated with movement onto, off of, or between perches (Baker et al., 2020). We deduce that perch collisions seem to be a main factor contributing to the development of bone fractures in furnished cage systems. Perch shape, material, height, orientation, and location within the cage structure (e.g., in relation to the cage top) have all been shows to influence keel fracture prevalence (Struelens and Tuyttens, 2009). A redesign of the perch and cage systems is, therefore, a possible strategy for reducing keel bone fractures in furnished cage systems.

Hens that engaged in more aggressive interactions were more likely to sustain multiple fractures. Aggressive interactions were the second most common behavior associated with acceleration events at the keel. During aggressive interactions hens sustained impacts at the keel when they were pushed into cage objects and opposing bird(s). In other words, aggressive interactions led to collisions that were caused by the aggressor (vs. the hen's own failed movements). Hen aggression has been linked to group size. For example, Nicol et al. (1999) reported more aggression among hens housed in groups of 72 than in larger group sizes (over 168 hens). Whereas hens in smaller groups establish and maintain social hierarchies through aggression, hens in large groups may not form social hierarchies (Hughes et al., 1997) or use cues such as body or comb size rather than aggressive interactions to establish dominance rank (D'Eath and Keeling, 2003). Additional research is warranted to explore the impacts of group size on aggression and keel bone damage in relation to perch and cage design.

In the current study, previous injury to the keel bone did not make hens more susceptible to further fracture formation via collisions or aggressive interactions as none of the components were associated with *ChangeF* +0 to +1. However, it is possible that the effect of initial bone integrity was masked by the relatively small sample sizes of each damage type and severity combination observed. It is also possible that hens with keel bone fractures may have changed their behavior to limit further damage potential. Hens with keel bone fractures are known to behave differently than those without fractures, with the presence of fractures associated with decreased frequency and duration of perch use (Nasr et al., 2012b), increase time spent sleeping on floors vs. perches (Casey-Trott and Widowski, 2016), and hesitancy to move down from perches (Nasr et al., 2012b) or higher levels of aviary systems (Rufener et al., 2019a).

None of the 4 identified PCA components were associated with changes to keel bone deviation scores. Changes in keel bone deviation severity were affected only by the presence or absence of deviations, with or without fractures, at the start of a 3 wk trial. This finding supports previous postulations that the causal mechanisms underlying the development of keel bone deviations are separate from those underlying the development of fractures. Unlike fractures, deviations are believed to be associated with prolonged pressure at the keel (Pickel et al., 2011; Harlander-Matauschek et al., 2015; Stratmann et al., 2015b), although more research is needed to confirm this mechanism. In the current study, most hens did not sustain any new deviations during the 3-wk trials, possibly a reflection of the relatively short time frame over which change in damage was measured. It is also possible that the subjective way in which deviation degrees were evaluated may have impacted the results. Keel bone deviations can present as deviation of the bone away from the theoretically ideal 2-dimensional traverse and/or sagittal planes, as well as indentations along the ventral crest of the bone (Casey-Trott et al., 2015). A number of methods have been used to quantify this type of damage, including scoring the distance (Heerkens et al., 2016; Chargo et al., 2019a) or angle (Regmi et al., 2016) of the bone's crest (carnia sterni) relative to a straight line, or calculating the proportion of bone affected by this type of damage (Eusemann et al., 2018). More recently, Jung et al. (2022) proposed using a tagged visual analog scale to quantify keel deviation severity. Although the relative sensitivity of these methods is yet to be confirmed, it is possible that using a different keel bone deviation scoring system could have yielded different study results.

In conclusion, our data support that collisions are the main source of fractures in furnished cage systems. We found a higher than average prevalence of keel damage in furnished cages, but we also saw a clear deterioration of the integrity of the keel bones and an increase in fractures over time. A higher number of collisions and a higher number of aggressive interactions affected the likelihood that a hen received one or more additional fractures within a 3 wk time span, independent of summed acceleration associated with the event. Deviations were not affected by any identified components, evidence that deviations likely occur through a different mechanism than direct impacts at the keel. A reduction of collision events and a decrease in aggressive tendencies could decrease keel bone fracture prevalence in furnished cage systems. Additional work is warranted to optimize perch and cage design, and to further explore causes and consequences of aggressive interactions in furnished cages. Future research should include strain comparisons as hen strain can influence the performance and keel bone damage prevalence of hens in furnished cages (Vits et al., 2005).

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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 Egg Industry Center. Poultry Science Animal Well-being and Behavior Section Editor (MT) Poultry Science Animal Well-being and Behavior Associate Editor (MM) 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 the present study.

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