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Rearing system affects prevalence of keel bone damage in laying hens: a longitudinal study of four consecutive flocks

T.M. Casey-Trott, M.T. Guerin, V.Sandilands, S.Torrey, T.M. Widowski

INTRODUCTION

Over the last two decades, numerous studies have documented fractures and deformations of the keel bone in laying hens, referencing prevalence ranges between 5-97% depending on housing system and age (Fleming et al., 2004, Rodenburg et al., 2008, Wilkins et al., 2011, Petrik et al., 2015, Riber and Hinrichsen, 2016). More recent research has been dedicated to addressing the negative impact that keel damage has on the welfare of the hen in terms of pain (Nasr et al., 2012), restricted mobility (Nasr et al., 2012b), affective state (Nasr et al., 2013), and behavioural changes (Casey-Trott et al., 2016). The alarmingly high prevalence rates combined with the concern for hen welfare has brought the issue of keel bone damage to the forefront of laying hen research.

The keel bone is an extension of the ventral surface of the sternum progressing along the midline of the sagittal plane. In avian species, the keel serves as an anchor to flight muscles and also plays a pivotal role in expanding and contracting the thoracic cavity during inhalation and exhalation (Codd et al., 2005; Claessens, 2009; Lambertx and Perry, 2015). The keel spans from the cranial, *Carina apex* to the caudal tip, with the spine of the keel tapering off as it approaches the caudal portion of the keel (*as described in Casey-Trott et al., 2015*). The growth and ossification of the keel is a process that initiates in the cranial region of the keel progressing gradually toward the caudal tip. Ossification of the keel continues into the early stages of egg

laying until approximately 28-40 weeks of age (Buckner, 1949), well beyond the growth of the long bones which ceases at the onset of lay (Hurwitz, 1965; Hudson et al., 1993). Due to the slow ossification of the keel, the caudal portion of the keel is often still cartilaginous at the onset of lay (Casey-Trott, unpublished: Chapter 2).

The high rate of keel bone damage has led to an international movement to develop prevention strategies for this prevalent problem (Harlander-Matauschek et al., 2015). Keel bone damage typically occurs in the form of fractures and deformations along the spine of the keel as well as at the caudal tip (Casey-Trott et al., 2015). Damage has been shown to increase with age (Weitzenburger et al., 2006; Scholz et al., 2008; Kappeli et al 2011; Petrik et al., 2015; Stratmann et al., 2015) across a variety of system types, and is most prevalent in non-cage systems due to the greater opportunity for damage from high impact crashes and falls (Rodenburg et al., 2008; Wilkins et al., 2011; Kappeli et al 2011). Outfitting aviary systems with ramps (Stratmann et al., 2015) and reducing perch obstruction and adjusting perch placement (Moinard et al., 2005) have reported positive results, reducing crashes and falls in non-cage systems. However, keel bone damage also occurs in low-impact systems such as furnished (Weitzenburger et al., 2006; Rodenberg et al., 2008; Scholz et al., 2008) and conventional (Hester et al., 2012; Petrik et al., 2014) cages. The causes and prevalence rates within these low-impact systems is less understood.

As discussed by Harlander-Matauschek et al. (2015), some possible causes of keel bone damage other than high-impact injuries include unequal wing-loading during wing-flapping, perch use (Sandilands et al., 2009; Pickle et al., 2011; Hester et al., 2013), compression fractures due to osteoporosis as seen in humans (Kondo, 2008), early onset of egg production (Gebhardt-Henrich and Frohlich, 2015), nutritional inadequacies (Whitehead, 2004; Fleming et al., 2006),

or genetic factors (Whitehead, 2004; Stratmann et al., 2015). To combat the problem of non-impact keel injuries, strategies to assess genetic differences in keel bone composition (Bishop et al., 2000; Hocking et al., 2003) and genetic associations with keel damage (Vits et al., 2005; Fleming et al., 2004, 2006; Stratmann et al., 2016), as well as the development of nutritional strategies such as calcium particle size (Fleming et al., 1998) and administration of omega-3 fatty acids (Tarlton et al., 2013) have been explored.

Exercise to stimulate bone growth is another research avenue that has the potential to influence keel bone strength and composition. The beneficial effects of exercise on the long bones of adult laying hens has been demonstrated by comparing the bone characteristics of adult hens housed in a modified furnished cage system (Jendral et al., 2007), aviaries (Leyendecker et al., 2005), and free-range systems (Shipov et al., 2010) to the bones of hens housed in the relative confinement of conventional cages. Whereas direct effects of exercise on keel bone damage have not yet been addressed, improvement of the composition of the long bones (tibiae, humeri) has been shown to correlate with improvements to the composition of the keel bone (Hocking et al., 2003; Fleming et al., 2004). Unfortunately, the assessment of exercise on keel bone damage is often confounded by providing exercise in the form of increased space allowance, addition of furnishings, or housing in extensive systems -- increasing the risk for detrimental collisions or injuries (Fleming et al., 2006; Scholz et al., 2009).

Targeting the pullet rearing phase to improve bone health has yielded positive results improving muscle growth (Hester et al., 2013), breaking strength (Vits et al., 2005; Regmi et al., 2015; Casey-Trott, unpublished; Chapter 2), and bone composition and geometry (Regmi et al., 2015; Casey-Trott, unpublished; Chapter 2) of the long bones, with the beneficial effects shown to be sustained through the end of lay (Regmi et al., 2016; Casey-Trott, unpublished; Chapter 2).

Furthermore, preliminary evidence suggests that exercise during the pullet rearing period reduces keel damage scores (Vits et al., 2005) and influences the overall growth of the keel (Casey-Trott, unpublished: Chapter 2). Perhaps housing pullets in rearing systems that encourage regular, diverse forms of exercise can aid in the reduction of keel bone damage by improving motor skills within more complex systems and enhancing the strength and composition of the keel through loading exercise from wing flapping.

The objective of the current study was to quantify the development of keel bone damage over time in furnished and conventional cages, and assess whether exercise during the pullet rearing phase influences the prevalence of keel damage throughout the laying period. We hypothesized that keel bone damage would increase with age, that hens in conventional cages would have lower prevalence rates than hens in furnished cages, and that exercise during rearing would improve the overall keel bone status of adult hens in both adult housing systems. A secondary objective was to determine whether there was an association between the development of keel bone fractures and deviations using the Simplified Keel Assessment Protocol (SKAP). We hypothesized that a strong correlation would exist between both forms of keel damage in furnished and conventional cages.

METHODS

The effects of rearing system (standard cage (Conv) versus rearing aviary (Avi)) and adult housing environment (conventional cages (CC), 30-bird furnished cages (FC-S) or 60-bird furnished cages (FC-L)) were tested using a 2 x 3 factorial arrangement with rearing flock replicated in 4 blocks over time. Each of the 4 rearing flocks contributed 3 replicate cages to

each of the adult housing systems. Animal use was approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol #1947).

Pullet management

Four consecutive flocks of 540 Lohmann-selected Leghorn Lite laying hens were reared from one day of age to 16 weeks of age at the University of Guelph Arkell Poultry Research Station. Half of the pullets from each flock were reared in standard conventional cages (Conv: 16 pullets/cage week 0-6, Space allowance = $194 \text{ cm}^2/\text{pullet}$; 8 pullets/cage week 6-16, Space allowance = $387 \text{ cm}^2/\text{pullet}$; Total Cage Area = $3,096 \text{ cm}^2$) and half were reared in a Farmer Automatic Logia Pullet Portal (Clark Ag, Ontario, Canada; Avi: 756 pullets/aviary enclosure; System Space Allowance = $285 \text{ cm}^2/\text{hen}$, week 0-6; System + Litter Space Allowance = $770 \text{ cm}^2/\text{pullet}$, week 6-16). The aviary system was selected due to optimal opportunities for exercise starting at one day of age, allowing for access to the entire system floor area ($334,529 \text{ cm}^2$), perches, and a platform that was gradually raised vertically in accordance with the age of the pullet to encourage hopping and flight to access vertical space. At 6 weeks of age, additional space was added by opening the sides of the system to allow for access to the litter area and nine elevated terraces on the outer edge of the system, increasing the total area to $570,297 \text{ cm}^2$ for a total space allowance of $770 \text{ cm}^2/\text{pullet}$ (See Figure 1 from Casey-Trott, unpublished; Chapter 2).

Both the conventionally reared pullets and aviary reared pullets from all four flocks were fed identical, 21% starter diets (crumbles) from 0-6 week and identical 18% grower diets (crumbles) from 6-16 weeks with the addition of grit to the Avi diet in Flock 1, followed by the addition of grit to both the Conv and Avi diet for Flocks 2-4. Both treatments also followed

identical vaccination and lighting programs. Pullets were lit for 16 hours during weeks 1 and 2, alternating four hours on and two hours off. Starting at three weeks of age, lights were on continuously starting at 0500 for 14 hrs, and subsequently reduced by 1 hr/wk until maintaining eight hours of light from weeks 8-15. The lights were set at 40 LUX at placement, and reduced by 5 LUX until maintaining 10 LUX from weeks 4-16. For the Avi pullets, water lines were located in the middle of the enclosure with the chain feeder running along the perimeter. Water lines with nipple drinkers and chain feeders were suspended from the ceiling and could be raised vertically in accordance with pullet growth. For the Conv pullets, height-adjustable water lines with nipple drinkers were also located in the middle of the cage with the chain feeders running past the front of the cage. All chicks were beak trimmed at the hatchery using infrared treatment.

A sample of 100 chicks per flock in each of the rearing systems were weighed biweekly for comparison with target weights outlined in the Lohmann Tiertzucht breeder guidelines. In an attempt to manage variation in body weights between the Conv and Avi pullets to achieve equal and recommended target body weights at placement into adult housing, the room temperatures were increased slightly in the conventional room to reduce feeding behaviour when the biweekly weight of the Conv pullets exceeded the Avi pullets by more than 10%. Avi reared pullets from Flock 4 remained on the starter diet for one additional week in order to bring them up to a body weight matching that of the Conv reared pullets. All four flocks came into lay between 17-18 weeks, and achieved 50% production at during week 19.

Adult laying hen management

At 16 weeks of age, 294 pullets from each rearing system (Avi and Conv) from each flock (1-4) were placed into two rooms each holding 12 Farmer Automatic Enrichable

(Furnished) Cages (Clark Ag Systems, Ontario, Canada), and one room holding 90 standard conventional cages, with 12 standard, conventional cages included in the study. In all rooms, a randomly selected group of hens from a single rearing treatment (Avi or Conv) was placed into each cage, balancing both treatments equally within each room. Each furnished cage room contained six large furnished cages (FC-L: 60 hens, total area = 41,296 cm², 688 cm²/hen) and six small furnished cages (FC-S: 30 hens, total area = 20,880 cm², 696 cm²/hen). Each bank of six cages had three tier levels with one large and one small cage on each tier. The conventional room contained 12 standard conventional cages (CC) of equal size (total area = 2,346 cm², 293 cm²/hen), housing eight hens/cage, all on a single tier level. The same rearing and adult rooms were used for each consecutive flock.

All flocks were fed identical, standard commercial layer crumbled pellet diets with automatic feed chains running every three hours commencing at the start of a 14 hr light period from 0500-1900 with a 15-min sunrise and sunset starting at 0500 and 1845. The light intensity varied among tiers, with the highest intensity recorded on the top tiers measuring 10-15 lux and the lowest intensity at the bottom tiers measuring 4-5 lux. Each furnished cage provided a curtained nest area proportional to cage size, 10 cm high perches running parallel to the cage front throughout middle area, and a smooth plastic scratch area. Nipple drinkers with cups were located above the auger down the middle of the cage. The feed troughs were located on both outer sides of the cages. Conventional cages were equipped with a nipple drinker running down the middle of the cages, with the feed troughs on the outer side of the cage. All rooms were sealed and entirely lit with artificial light with no natural, external light sources present.

Egg production and mortality were recorded daily and will be reported elsewhere (Widowski et al., unpublished). All mortalities were sent for post mortem analysis and there

were no outbreaks of disease, feather pecking, or cannibalism throughout the duration of the study. The mean laying rate at 70 weeks was $93.2\% \pm 1.0$ SE for Flock 1, $90.6\% \pm 1.2$ SE for Flock 2, $93.2\% \pm 0.6$ SE for Flock 3, and $85.2\% \pm 1.5$ SE for Flock 4. The mean flock mortality was $3.3\% \pm 0.9$ SE for Flock 1, $4.7\% \pm 1.0$ SE for Flock 2, $5.5\% \pm 1.1$ SE for Flock 3, and $4.8\% \pm 0.7$ SE for Flock 4.

Keel bone scoring by palpation

All palpation scoring was completed by the same two observers for all data collection periods for all four flocks. Both observers underwent training and reliability assessment as part of a concurrent research project (Petrik et al., 2013). Previous assessment of the lead observer for the current study reported the accuracy, sensitivity, and specificity for detection of fractures and deviations. The accuracy for detection of fractures was 84% and 91% for deviations. The sensitivity for detection of fractures was 81% and 84% for deviations, and the specificity for detection of fractures was 87% and 97% for deviations (Casey-Trott et al., 2015). Prior to every scoring period, both observers completed consensus training by discussing scores together via palpation and inspection of excised keels. Each observer palpated exactly half of each experimental cage to ensure balanced scoring methods. Observers were blind to rearing treatment, but not adult housing system treatment.

During placement into adult housing at 16 weeks of age, all pullets were weighed and the keel bone status of each bird was scored for fractures and deviations using palpation. All subsequent keel bone scoring was completed at 30, 50, and 70 weeks on 20% of each cage using the same palpation technique. For ease of catching, the lights were dimmed in each room and the hens were caught randomly from multiple areas within each cage until 20% of each FC-L

(N=12) and FC-S (N= 6) were caught. For CC, 20% of each cage (N=2) were scored for Flock 1, but for Flocks 2-4 all hens in each CC (N=8) were scored to ensure a representative mean with a sample size comparable to the number scored from the furnished cages.

All keels were assessed for the presence of fractures and deviations. The hens were restrained in an inverted position by holding both legs, with the ventral surface of the keel facing away from the body of the person performing the palpation. The thumb and index finger were used to palpate the keel by running the fingers down the ventral surface of the keel. Keels were palpated from the cranial *Carina apex* all the way to the caudal tip of the keel bone. A keel was considered fractured (FR) if there was the presence of a sharp bend, one or more than one periosteal scar or callus, or if any detached or semi-detached bone fragments were present. The presence of a FR was a binomial score denoting only the presence or absence of a keel fracture as described by SKAP (Casey-Trott et al., 2015). In addition to the SKAP scoring, for Flock 2-4 the location of the fracture was also recorded. The fracture was classified as a tip fracture (FR-TIP) if a fracture was detected within the last 5 cm of the caudal tip of the keel. Only the ventral surface of the keel was palpated in this study. Pushing inward into the peritoneal cavity to palpate the dorsal surface of the caudal tip of the keel was not used. The fracture was classified as a spine fracture (FR-SPINE) if there was a fracture present anywhere on the spine of the keel from > 5cm from the caudal tip to the *Carina apex*.

Keel bone deviations were also scored. A keel was considered to be deviated (DEV) if it did not follow a normal, straight 180° line in the sagittal, frontal or transverse anatomical plane. The presence of a DEV was a binomial score as a part of the SKAP method (Casey-Trott et al., 2015). In addition to the SKAP method, the severity of the deviation was also scored for all four flocks. A mild deviation (DEV-MILD) was described as a deviation < 1cm from the normal,

180° line of the keel in any direction (sagittal, frontal or transverse). A severe deviation (DEV-SEV) was described as any deviation > 1cm from the 180° line, typically manifesting as a C- or S-shaped curve in the frontal plane, severe indentation in the sagittal plane, or a significant folding over of the keel in the transverse plane.

Statistical analyses

All statistical analyses were completed using SAS 9.4 (SAS Institute, Cary, North Carolina).

Analysis of age, rearing environment, adult housing, and flock effects on keel bone damage

To assess the effect of age (30, 50, and 70 wks), rearing environment (Avi vs Conv), adult housing (FC-L, FC-S, CC), and flock number (1-4) on keel bone damage (fractures or deviations), Proc Mixed analyses of variance were performed with age, rearing environment, adult housing, flock number, and the interactions (age*rearing, age*adult, rearing*adult) as fixed effects. Since cage was considered the experimental unit, the percentage of fractures present within each cage used in the analyses. Measurements from hens in each cage were repeated at 30, 50, and 70 weeks of age and thus age was a repeated measure within the analysis. The percentages of fractures and deviations were analysed using the same model. Although keel bone status was measured by palpation at 16 weeks, this measurement was meant to serve as a baseline value and was not included in any statistical since the values for both fractures and deviations were zero at this time point. The baseline keel scores at 16 weeks are presented in Figure 1. All data were tested for normality and normality of residuals using Proc Univariate prior to analyses of variance and no data required transformation.

Analysis of relationship between BW and keel damage

Body weight was not included in the main analyses as it was not independent from age ($P < 0.0001$) or adult housing system ($P < 0.0001$; Casey-Trott, Chapter 2). However, in order to assess the relationships between body weight and keel fractures or deviations, regression analyses (Proc Reg) were used on data from individual birds at each age (30, 50, and 70 wks).

Analysis of the association between keel fractures and deviations using the SKAP method

To test the level of association between keel bone fractures and deviations, a Chi Sq analysis in Proc Freq was used. An Odds Ratio, Relative Risk option was used to determine the direction of the relationship between fractures and deviations. The analysis was run at each age (30, 50, and 70 wks) to monitor changes in the relationship at different time points. Although palpation scoring was also completed at 16 weeks, a Chi Sq analysis could not be run due to the prevalence of zero values; fractures were absent and very few deviations were present at 16 weeks. The raw means for 16 weeks are reported in Figure 2.

RESULTS

Effect of age, rearing system, adult system, and flock on keel bone damage

Age had an effect on the percentage of fractures ($P < 0.0001$; Table 1 and Figure 1A). The mean percentage of fractures was $0.04\% \pm 0.002$ SE at 16 weeks, $35.2\% \pm 2.5$ SE at 30 weeks, $55.2\% \pm 2.8$ SE at 50 weeks, and $62.4\% \pm 2.6$ SE at 70 weeks. Rearing system also had an effect on the presence of fractures ($P < 0.0001$; Table 1 and Figure 1A). Hens raised in the Avi system had an overall lower percentage of fractures ($41.6\% \pm 2.8$ SE) compared to hens reared in

the Conv system ($60.3\% \pm 2.9\text{SE}$). Adult housing system did not affect the percentage of keel fractures ($P = 0.2227$; FC-L: $53.2\% \pm 2.8 \text{ SE}$; FC-S: $52.5\% \pm 2.7 \text{ SE}$; CC: $47.0\% \pm 2.7 \text{ SE}$). Flock had an effect on the overall mean percentage of keel fractures ($P = 0.0145$) occurring between 30 and 70 weeks, with Flock 4 having a lower percentage ($42.1\% \pm 3.2 \text{ SE}$) than Flock 1 ($51.4\% \pm 3.1 \text{ SE}$), Flock 2 ($54.6\% \pm 3.2 \text{ SE}$), and Flock 3 ($55.6\% \pm 3.2 \text{ SE}$). No interaction effects were significant.

Of the keel bones with fractures present, the majority of fractures were located at the tip of the keel at all ages: 30 wks: $76.9\% \pm 4.1 \text{ SE}$, 50 wks: $89.1\% \pm 2.9 \text{ SE}$, 70 wks: $89.5\% \pm 3.0 \text{ SE}$. Fractures occurring on the spine of the keel were less common at all ages: 30 wks: $36.8\% \pm 4.7 \text{ SE}$, 50 wks: $26.1\% \pm 3.4 \text{ SE}$, 70 wks: $31.0\% \pm 3.5 \text{ SE}$. Some keels had both a FR-TIP and a FR-SPINE present.

Age also affected the percentage of deviations ($P < 0.0001$; Table 1 and Figure 1B). The mean percentage of deviations was $0.2\% \pm 0.09$ at 16 weeks, $28.1\% \pm 2.6 \text{ SE}$ at 30 weeks, $40.0\% \pm 2.6\text{SE}$ at 50 weeks, and $51.6\% \pm 2.6 \text{ SE}$ at 70 weeks. Rearing system did not affect the percentage of deviations ($P = 0.2175$; Table 1 and Figure 1B). Adult housing system did not affect the percentage of deviations ($P = 0.5394$; FC-L: $39.7\% \pm 2.9 \text{ SE}$; FC-S: $42.4\% \pm 3.0 \text{ SE}$; CC: $37.3\% \pm 2.9 \text{ SE}$). Flock also affected the percentage of deviations ($P = 0.0100$), with Flock 1 ($31.0\% \pm 3.4 \text{ SE}$) differing from Flock 2 ($42.1\% \pm 3.3 \text{ SE}$) and Flock 3 ($47.7\% \pm 3.3\text{SE}$), but not Flock 4 ($38.8\% \pm 3.4\text{SE}$). No interaction effects were significant.

Of the keel bones with deviations present, the majority of deviations were mild at all ages: 30 wks: $76.3\% \pm 2.4 \text{ SE}$, 50 wks: $60.7\% \pm 2.4 \text{ SE}$, 70 wks: $59.9\% \pm 2.3 \text{ SE}$; however, there was a gradual increase of severe deviations with age: 30 wks: $23.7\% \pm 1.8 \text{ SE}$, 50 wks: $39.2\% \pm 1.8 \text{ SE}$, 70 wks: $40.1\% \pm 1.8 \text{ SE}$.

At 30 weeks of age, body weight had a minor but significant positive relationship with both the presence of fractures ($P = 0.0024$; Adj $R^2 = 0.1115$) and deviations ($P = 0.0040$; Adj $R^2 = 0.0997$). There was no relationship between body weight and fractures or deviations at either 50 (FR: $P = 0.1193$; DEV: $P = 0.5334$) or 70 weeks of age (FR: $P = 0.7513$; DEV: $P = 0.2649$). The mean body weights were $1380.9 \text{ g} \pm 19.8 \text{ SE}$ at 16 weeks, $1926.5 \text{ g} \pm 17.9 \text{ SE}$ at 30 weeks, $2040.6 \text{ g} \pm 17.9 \text{ SE}$ at 50 weeks, and $2136.2 \text{ g} \pm 17.8 \text{ SE}$ at 70 weeks.

Association between keel bone fractures and deviations using the SKAP method

Keel fractures and deviations were strongly associated at all ages: 30 wks: ($P < 0.0001$), 50 wks: ($P < 0.0001$), 70 wks: ($P < 0.0001$). The absence of both keel fractures and deviations was greatest at 30 weeks of age, steadily decreasing in favor of the occurrence of both fractures and deviations at 50 and 70 weeks of age (Figure 2).

At 30 weeks of age (N=515), 55.9% of keels had no fracture or deviation present, 17.9% had only a fracture present, 9.7% had only a deviation present, and 16.5% had both a fracture and a deviation present (Figure 2). The Odds Ratio at 30 weeks of age indicated that hens with deviations were 5.3 times more likely to have fractured keels compared to those without a deviation present (95% CI= 3.5-8.1). Based on the Relative Risk assessment, the likelihood of not developing a fracture was higher in hens with a non-deviated keel than when a deviation present (Relative Risk = 2.0; 95% CI = 1.6-2.6). Similarly, the Relative Risk of developing a fracture was lower in hens with a non-deviated keel than a hens with a deviation present (Relative Risk = 0.38; 95% CI = 0.31-0.48). At 50 weeks of age (N=550), 33.1% of keels had no fracture or deviation present, 28.0% had only a fracture present, 12.0% had only a deviation

present, and 26.9% had both a fracture and a deviation present (Figure 2). The Odds Ratio at 50 weeks of age indicated that hens with deviations were 2.6 times more likely to have a fracture compared to hens with no deviation present (95% CI = 1.8-3.8). Based on the Relative Risk assessment, the likelihood of not developing a fracture was higher in hens with a non-deviated keel than when hens with a deviation present (Relative Risk = 1.7; 95% CI = 1.4-2.2). Similarly, the Relative Risk of developing a fracture was lower in hens with a non-deviated keel than hens with a deviation (Relative Risk= 0.66; 95% CI = 0.57-0.77). At 70 weeks of age (N=559), 24.9% of keels had no fracture or deviation present, 22.9% had only a fracture present, 11.6% had only a deviation present, and 40.6% had both a fracture and a deviation present (Figure 2). The Odds Ratio at 70 weeks of age indicated that hens with a deviation were 3.8 times more likely to have a fracture compared to hens with no deviation present (95% CI = 2.6-5.5). Based on the Relative Risk assessment, the likelihood of not developing a fracture was higher in hens with a non-deviated keel than in hens with a deviation present (Relative Risk = 2.3; 95% CI = 1.8-3.0). Similarly, the Relative Risk of developing a fracture was lower in hens with a non-deviated keel than hens with a deviation present (Relative Risk = 0.62; 95% CI = 0.54-0.71).

DISCUSSION

Effect of age, rearing system, adult system, and flock on keel bone damage

This is the first experimental study to demonstrate that diverse load bearing exercise during the pullet rearing phase effectively alters keel bone growth in a manner that reduces keel bone fractures in adult laying hens housed in both furnished and conventional cages. It is also one of the few longitudinal studies to track prevalence rates in furnished and conventional cages

over the laying period of a hen. And it is the first paper to quantifiably identify an association between keel bone fractures and deviations at different age points.

The reduction of keel bone fractures in adult hens that were housed in an aviary rearing system highlights the role that diverse loading exercise, in the form of running, jumping, wing-flapping, and flight, has in the development of keel bones that are less susceptible to future damage. Unlike previous studies where increased exercise has been shown to have a positive effect on keel bone radiographic density, yet increase the risk of keel injuries from falls and collisions (Fleming et al, 2006), targeting the rearing phase takes a preventative approach by stimulating improved keel bone growth during the period of the greatest opportunity for increasing peak bone mass, before the period of greatest risk of fracture during mid to late lay. In humans, exercise prior to sexual maturity improves peak bone mass and has a protective effect on bones reducing the prevalence of osteoporotic fractures later in life (Bass, 2000). In addition to improved bone characteristics, routine, impact exercise during adolescence in humans also reduces the risk of fractures later in life by improving muscle tone, strength, and balance (Schmitt et al 2009; Body et al., 2011). Although changes specific to bone mineral density or breaking strength of the keel were not directly assessed here, the reduction of keel bone damage reported may be a result of the allowance of exercise in the aviary pullet rearing system encouraging improved keel bone growth and more controlled navigation of the housing systems as adults.

Although current information regarding keel bone growth in highly selected commercial lines is not readily available, preliminary evidence that the keel is not entirely ossified at 16 weeks of age (Casey-Trott unpublished, Chapter 2) agrees with previous research from the 1940's regarding the slow growth of the keel (Buckner et al., 1949). As discussed by Casey-

Trott (Chapter 2), exercise appears to have an effect on the overall growth and ossification of the keel at 16 weeks. The keels of aviary reared pullets were longer, had a greater area, and larger proportion of caudal cartilage than the conventionally reared pullets at 16 weeks of age. This suggests that exercise during rearing alters the growth of the keel bone, possibly by stimulating increased overall keel bone growth, or by slowing the progression of ossification. The detailed progression of keel bone growth using radiographic analyses throughout the life of currently available commercial hen is an area of research that is yet to be quantified. Understanding when the keel is completely ossified has the potential to shed light onto periods when the keel is especially susceptible to fractures either due to weak, newly calcified bone structure, or reduction in calcium allocation to the keel due to competition for calcium supply surrounding stages of peak lay or hormonal shifts.

The prevalence rates reported here for fractures, approximately 52.8% (± 2.2 SE) for furnished cages and 47.0% (± 2.2 SE) for conventional cages, are within the ranges of previously reported results for furnished cages (33-39% keel deformities: Weitzenburger et al., 2006; 62% keel fractures: Rodenburg et al., 2008) and conventional cages (83% keel fractures: Hester et al., 2013; 25% keel fractures: Petrik et al., 2015). The lack of adult housing effect parallels results in human medicine where exercise in adult women does not typically increase bone strength or bone mineral density, but rather continued exercise in adulthood can help preserve benefits accrued during childhood and adolescence (Kontulainen et al., 1999; Kontulainen et al., 2001).

Alternatively, the similarity between the fracture and deviation prevalence rates of conventional and furnished cages reported here is supportive of the notion that furnished cages provide an intermediate improvement to overall welfare by providing the benefit of added exercise and furnishings compared to conventional cages, without a dramatic increase in risk of

injuries as a result of the collisions and falls reported in non-cage systems (Lay et al., 2011). Although the increased exercise allowed by the FC-L or FC-S compared to the CC did not have any significant improvement in keel damage prevalence, the beneficial effect of exercise during rearing appears to be preserved as there is not a dramatic increase in damage with the allocation of increased space for movement and furnishings, as indicated in previous studies (Fleming et al., 2006). The prevention of a notable difference in fracture and deviation prevalence between the hens from furnished and conventional adult housing may be due to an increase in the overall bone quality of the hens housed in furnished cages compared to the hens in CC, a result reported for the same population of birds from a concurrent study with the same treatment design (Casey-Trott; Chapter 2). The greater tibiae and radii bone density of the FC-L compared to the FC-S and CC, greater bone mineral content of the FC-L and FC-S compared to the CC, and the greater breaking strength of the tibiae from the FC-L and FC-S compared to the CC indicates that adult hens in the furnished cages from the same population and treatment design had improved quality of the long bones (Casey-Trott; Chapter 2). Improvement to the bone quality of the long bones has been shown to parallel improved keel bone quality (Hocking et al., 2003; Fleming et al., 2004), suggesting the keels in the current study likely mirror the positive results report in Casey-Trott (Chapter 2). The result of comparable values for keel bone status between housing adult hens in conventional versus furnished cages was also reported by Widowski et al. (submitted?), with the conventional hens having slightly higher keel damage scores.

This is the first paper to provide detailed prevalence rates of keel bone deviations, as a separate measure from keel fractures, for hens housed in furnished (41.0 ± 2.3 SE) and conventional cages ($37.7\% \pm 2.4$ SE). As expected, age had an effect on deviations, increasing the prevalence at each time point; however, the lack of rearing environment effect on deviations

was somewhat unexpected. The slightly higher prevalence of deviations in the aviary reared hens may be related to an increased use of perches since rearing with perches typically increases perch use in adult housing (Roll et al., 2008; Brantsaeter et al., 2016). Although perching differences between the rearing treatments were not quantified in this study, a concurrent project on the same hens reported that hens with keel bone fractures present at 70 wks spent a greater proportion of time on the perches than hens with minimal to no keel damage present (Casey-Trott et al., 2016). Overall nighttime perching was low (< 10%; unpublished data). Alternatively, there may be a difference in the type of deviations incurred in different housing systems during the adult period that were not detected since only the presence or absence and severity of the deviations was assessed. Perhaps if the deviations were classified by the direction of deviation in each plane, differences may arise. The lack of adult housing effects on deviations may also be attributed to the limited benefit of exercise in adulthood as discussed above.

Several studies have reported an increase in keel bone damage with increasing age (Weitzenburger et al., 2006; Scholz et al., 2008; Tarlton et al., 2013; Petrik et al., 2015; Stratmann et al., 2015), which is in agreement with the results reported here. This pattern is likely attributable to several factors related to the skeletal growth and body composition. Considering that the ossification of the keel is not yet complete at the onset of lay (Casey-Trott, unpublished; Chapter 2), it is not possible for fractures of the tip, the most common type of fracture reported here, to form as the caudal portion of the keel is still cartilaginous. Similar to other studies (Petrik et al., 2015; Stratmann et al., 2015) fracture prevalence steadily increases approaching 30 weeks of age and generally continues to rise until 70 weeks of age. The rise in fractures at or just after 30 weeks of age may be a result of the caudal tip of keel no longer in a cartilaginous state, but still not completely ossified. This weak structure may be especially

susceptible to “greenstick” fractures. Greenstick fractures are commonly found in growing children and typically manifest as incomplete, bending fractures on the concave surface of a bone, with complete separation of the cortex on the convex bone surface (Hefti et al., 2007; Vernoji et al., 2012). It is possible that these incomplete greenstick fractures are a result of minor collisions with equipment or cage-mates, muscle contractions during wing movement or even increased muscle tension applied to the keel as the keel lowers ventrally to allow for egg production (Chapman, 1943). Understanding exactly how these incomplete fractures occur still needs further study and it is of utmost importance as this type of fracture is the most commonly reported. Unfortunately, even though these greenstick fractures appear to be minor (Casey-Trott et al., 2016), they are considered unstable and increase the risk for further fracture for several weeks after the initial incident (Randsborg et al., 2009). This may explain why multiple fractures of the tip are commonly seen by the end of lay, often increasing the severity of the damage by 70 weeks of age.

Increasing body weight may also play a role in greater keel damage reported late in life. Although hens housed in non-cage systems may be especially susceptible to keel damage related to body weight increases due to a now greater requirement for wing loading (Dunker, 2000), hens in cage systems are still susceptible to increased fracture rates related to higher body weights (Petrik et al., 2015). In the present study, the slight, but significant association between body weight and fractures and deviations at 30 weeks of age may be related to increased pressure loads on the keel while resting on perches or the cage floor. It may also be an artifact of earlier onset of sexual maturity due to increased body mass, initiating early onset of lay which has been shown to increase susceptibility to keel damage (Gebhardt-Henrich and Frohlich, 2013).

Flock variation also had an effect on the prevalence of fractures and deviations detected. The variation between flocks was an anticipated result as flock variation is commonly reported in commercial barns. The flock differences were not the main interest of the research study, and therefore are not discussed in detail. Repeating the experiment on each flock was meant to account for flock differences and increase both the internal and external validity of the results.

Association between keel bone fractures and deviations using the SKAP method

The relationship between keel bone fractures and deviations is not yet fully understood, although an association between the two has been previously suggested (Scholz et al., 2009; Casey-Trott et al., 2015). Especially in systems where impact injuries are less likely, such as conventional or furnished cages, the relationship between keel bone fractures and deviations is likely stronger since developing a fracture from a single, isolated impact is less common; whereas high impact injuries in a non-cage system may lead to severe keel fractures from a single event on an otherwise straight, non-deviated keel. The rise in both deviations and fractures with age, as reported here, supports the idea that keels become more susceptible to both forms of damage over time.

The strong association between keel fractures and deviations reported here, and the increased likelihood of non-deviated keels remaining free from fractures suggests that the relationship between the two may be related to the underlying bone physiology of the bird, or the bird's behavioural activities. In furnished cages, perching is one behaviour that is a likely cause of keel bone fractures and deviations (Casey-Trott et al., 2016) due to the long term pressure loading on the keel (Pickel et al, 2011). Stratmann et al. (2015) demonstrated that softening perch material effectively reduced both deviations and fractures, a result that may be particularly

useful to reducing keel damage in a furnished cage setting where perch use is a likely cause of keel deviations. However, it is also possible that the relationship between deviations and fractures is a result of the underlying bone physiology. Genetic differences between high bone index, low bone index, and commercial lines, have repeatedly demonstrated that genetic selection impacts the bone mineral density of the keel (Bishop et al., 2000; Fleming et al., 2006), and recent work by Stratmann et al. (2016) demonstrated that genetic selection using these same lines also influences the presence of both keel bone fractures and deviations. Perhaps if deviations and fractures are so closely related, then selection for more efficient calcium mobilization or improved bone characteristics can reduce the occurrence of both deviations and fractures with the same mechanism.

It is widely accepted that keel bone damage is multi-factorial as it manifests itself in a variety of ways throughout all housing systems. As such, a multi-faceted approach is likely required to reduce the prevalence of keel bone damage. Perhaps coupling genetic selection for improved keel bone characteristics with the allowance for exercise during rearing, the period with the greatest potential to develop peak bone mass, may have an additive effect on the underlying bone physiology, stimulating bone growth in a manner that substantially improves skeletal structure. These improvements may be further extended by improvements to housing design, namely in the form of perch placement and pliability of perch material.

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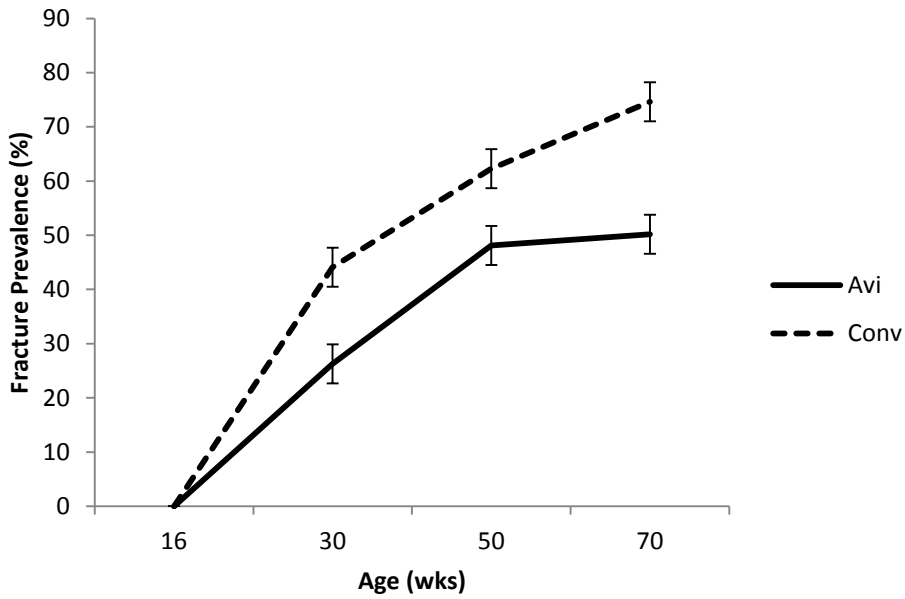
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Table 1. Main effects of age, rearing and adult housing environments on the percentages of fractures and deviations

	Fractures (%)	Deviations (%)
Age (wks)		
30	35.2 (\pm 2.5) ^a	28.1 (\pm 2.6) ^a
50	55.2 (\pm 2.8) ^b	40.0 (\pm 2.6) ^b
70	62.4 (\pm 2.6) ^c	51.6 (\pm 2.6) ^c
DF	136	136
F-Value	22.89	24.19
P-Value	<0.0001	<0.0001
Rearing		
Conv	60.3 (\pm 2.9)	37.8 (\pm 2.4)
Avi	41.5 (\pm 2.8)	42.1 (\pm 2.3)
DF	63	63
F-Value	35.39	1.55
P-Value	<0.0001	0.2175
Adult		
CC	47.0 (\pm 2.7)	37.7 (\pm 2.9)
FC-L	53.2 (\pm 2.7)	39.7 (\pm 2.7)
FC-S	52.5 (\pm 2.5)	42.4 (\pm 2.9)
DF	63	63
F-Value	1.54	0.62
P-Value	0.2227	0.5394

A.



B.

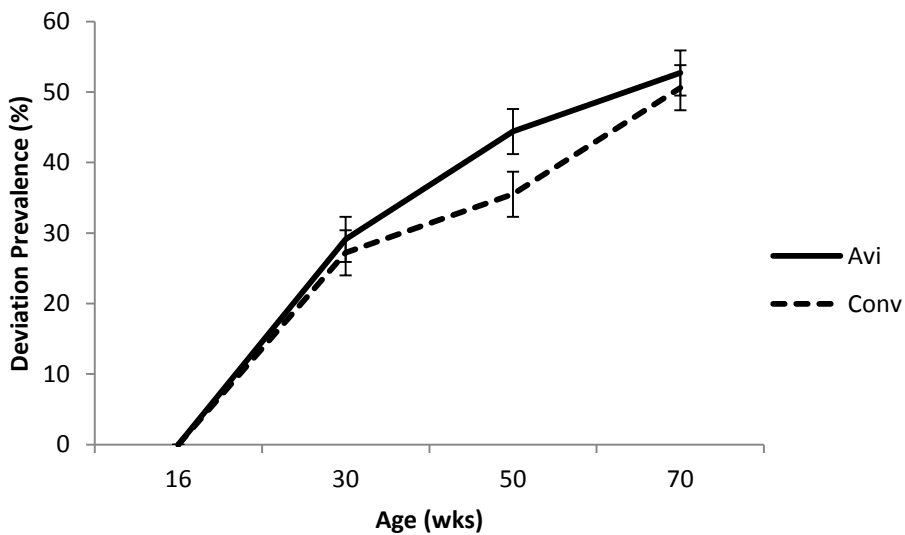


Figure 1. Effect of age and rearing environment on the percentage of fractures (A) and deviations (B). Age affected fracture ($P < 0.0001$) and deviation ($P < 0.0001$) prevalence and rearing environment (Aviary rearing: Avi; Standard rearing: Conv) affected fracture prevalence ($P < 0.0001$) but not on the prevalence of deviations ($P < 0.2175$).

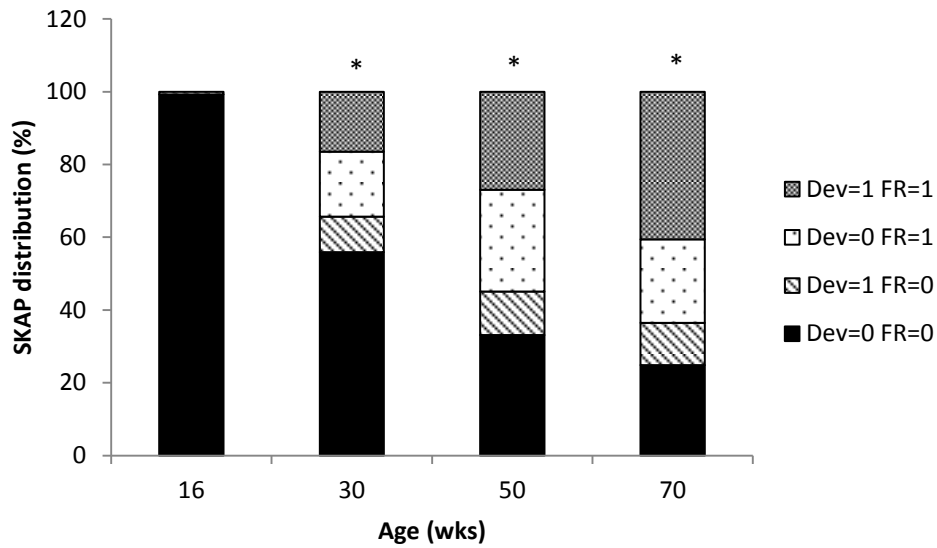


Figure 2. Distribution of fracture (FR) and deviation (DEV) prevalence as scored by the SKAP method. Significant association between fractures and deviations ($P < 0.0001$) is denoted by an * as determined by a Chi Sq analysis. Week 16 was not included in analysis as the majority of keels (99.3%) did not have the presence of a fracture or deviation.