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Review

Skeletal health of layers across all housing systems and future research directions for Australia

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Abstract. Modern laying hens have been selected for an astounding rate of egg production, but the physiological calcium demand takes a significant toll on their skeletal health. Bones can be assessed both *in vivo* and *ex vivo*, using a combination of different structural and mechanical analysis methods. Typically, the properties of leg, wing and keel bones are measured. Conventional caged layers are restricted in movement, which imbalances structural bone resorption and new bone formation, resulting in osteoporosis. Hens within alternative housing systems have opportunities to exercise for strengthening bones, but they can also suffer from higher rates of keel fractures and/or deviations that are likely to have resulted from collisions or pressure force. Limited research has been conducted within Australian commercial housing systems to assess hen skeletal health, including prevalence of keel damage across different system types. Research conducted on both brown and white hen strains approximately within the past decade internationally (2009 onward) has shown that skeletal health is impaired across all housing systems. Keel-bone damage is of specific concern as it occurs at high rates, particularly in multi-tiered systems, is painful, can alter hen behaviour, and reduce both production and egg quality. Management strategies such as the provision of ramps to access perches and tiers can reduce the incidence of keel-bone damage to a degree. Bone strength can be improved through exercise opportunities, particularly when available during pullet rearing. Genetic selection for high bone strength may be necessary for hens to adequately adapt to loose-housed systems, but the best strategy for improving skeletal health is likely to be multifactorial.

Additional keywords: damage, density, fracture, humerus, keel, tibia, welfare.

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Introduction

Modern laying hens have been selected to lay eggs almost daily for consecutive months, which requires significant amounts of calcium. Unsurprisingly, this places physiological strain on the hen's skeleton (Whitehead and Fleming 2000; Whitehead 2004). Layer skeletons are made of cortical (dense outer bone surface), and trabecular (internal woven bone) bone types that form during development (Whitehead 2004). A third bone type, namely, medullary bone, is a spongy bone formed only at sexual maturity and acts as a readily available source of calcium (Whitehead and Fleming 2000). Approximately 10% of a hen's total body calcium volume daily is used in producing egg shells, of which half is sourced from the diet, and half is sourced from medullary bone (Kerschnitzki *et al*. 2014). An imbalance between structural bone resorption and regeneration results in osteoporosis, that is, brittle and weak bones (Whitehead and Fleming 2000). For several decades, osteoporosis in laying hens has been a recognised problem that results in bone fractures, layer fatigue, production loss, and is a significant welfare concern (Whitehead and Fleming 2000). Historically, layers within caged housing systems have come under increased scrutiny because of the significant impacts that exercise restriction can have on bone strength (Whitehead and Fleming 2000; Webster 2004; Shipov *et al*. 2010; Aguado *et al*. 2015). Physiologically, across all animals, bones develop through loading or biomechanical stress placed on the bones, which stimulates cell development in the stressed area (Burr *et al*. 2002). Restriction of movement hinders bone development, increasing the calcium that is reabsorbed for egg laying, which reduces strengthening bone formation.

Exercise opportunities within alternative loose-housed systems (e.g. barn, aviary or free-range systems) in comparison to conventional cages will increase bone strength (Silversides *et al*. 2012; Habig *et al*. 2017; Regmi *et al*. 2017*a*). However, physically, laying hens are not of the same design as their ancestors. Genetic selection has increased their wing loading, resulting in a smaller wing size to body ratio than for jungle fowl (Moinard *et al*. 2004; Stratmann *et al*. 2016). Subsequently, the modern layer is not as physically adept at navigating housing systems, leading to collisions with other birds or solid structures, potentially resulting in bone fractures (Moinard *et al*. 2004; Campbell *et al*. 2016;

Gebhardt-Henrich *et al*. 2017; Baker *et al*. 2020). However, Thøfner *et al*. (2020) recently used computed tomography (CT) scans and histology to show an absence of associated soft-tissue damage around fractures and, thus, suggested that these fractures may possibly result from internal pressures of the egg-laying process rather than high-energy collisions. A hen's larger body size also places increased pressure on their keel bone when resting on solid surfaces (Pickel *et al*. 2011), which may cause keel-bone deviations (Scholz *et al*. 2008). Thus, perches represent a welfare conundrum where their presence can increase bone strength and fulfil the behavioural need to roost but can also increase the incidence of keel-bone damage (Sandilands *et al*. 2009; Scholz *et al*. 2009; Hester 2014). Keel-bone damage is currently a global layer welfare concern, where up to 95% of birds at the end of the laying cycle may have some type of damage present (Wilkins *et al*. 2011). Research into keel-bone damage and ways to prevent it is at the forefront of current layer health and welfare science.

Modern laying hens in both caged and alternative housing systems currently suffer from significant skeletal health issues of different forms. Optimal skeletal health is likely to be achieved through an integrated approach involving multiple factors including nutrition, genetics and rearing environments. Most of the available research has been conducted internationally, but it is likely that this information will also apply to Australian conditions as the global patterns are consistent across countries. This review focusses on international research conducted within approximately the past decade (2009 onward), predominantly during the laying period. Information is included for white strains of hens where there is a lack of any research on brown layer strains or if the studies provide information on multiple hybrids. Discussion of methods of skeletal-health assessment, keel-bone damage, impacts of different housing systems, and effects of rearing, management and genetic selection on skeletal health are included. Recommendations for areas of future research focussing on birds in Australian housing systems, which are predominantly brown strains, are detailed.

Skeletal health-assessment methods

The skeletal health of layers can be assessed in a multitude of ways that incorporate both *in vivo* and *ex vivo* bone-health measurement techniques (reviewed in Donnelly 2011). The measures all quantify slightly different parameters and, typically, a combination of techniques is applied within a research trial, or applied to the same bones to achieve a comprehensive bone analysis. For detailed measurements on bone structural integrity, hens are often euthanised and the bones of the legs, wings, and keel are excised. Typically, the long bones of the wings and legs, that is, the tibia and humerus, are sampled, but radius and femur may also be included. Both the legs and wings are often analysed as these bones respond differently to exercise or increased physical activity (e.g. Regmi *et al*. 2016). Perching, for example, may improve leg-bone parameters, but wing-assisted jumping improves wing-bone parameters and, thus, the impact of different types of housing systems will be reflected accordingly. These extracted bones can be scanned using CT or micro-CT and the reconstructed bone images analysed with image software. Precise image measurements can be taken for bone structural properties, including length, width, cortical thickness, cortical density, trabecular density (includes medullary bone in mature layers), and total bone mineral density (Korver *et al*. 2004). Of note here, CT scans can also be conducted on the whole bird, including immobilised live birds, but the resolution and accuracy of measurements from the micro-CT scan on extracted bones is superior (Regmi *et al*. 2017*b*). Other types of X-ray or laboratory analyses can also be applied, such as DEXA technology, X-ray fluorescence or Raman spectroscopy (Kerschnitzki *et al*. 2014; Li *et al*. 2016; Toscano *et al*. 2018) and, in the absence of expensive scanning equipment, bones are ashed to provide a measure of mineral content (e.g. Regmi *et al*. 2016). Recent estimations of bone mineral density using quantitative computed tomography were highly correlated with analytical bone calcium and bone ash, indicating that this scanning technique could also be used to examine live birds (Robison and Karcher 2019). Cortical geometry can also be measured manually with callipers in *ex vivo* bone samples. To determine mechanical properties such as breaking strength, bending tests are applied to cleaned bones until the point of fracture (e.g. Regmi *et al*. 2016).

In vivo, keel-bone damage is assessed via palpation, which is less accurate than damage assessment via dissection, but may provide a reliable assessment in a live bird (Petrik *et al*. 2013), dependent on fracture location (Buijs *et al*. 2019). More recently, keel-bone health has been measured in live birds via radiographs and CT scans of restrained birds, which allows accurate tracking of damage and healing across time (Richards *et al*. 2011; Eusemann *et al*. 2018; Rufener *et al*. 2018; Chargo *et al*. 2019*a*, 2019*b*; Baur *et al*. 2020). Development of virtual biomechanical analyses could enable more studies on live birds and quantification of bone properties *in vivo* (Vaughan *et al*. 2016). Finally, blood samples can be taken throughout pullet development or layer production to quantify serum markers of bone formation and resorption (e.g. Kerschnitzki *et al*. 2014; Regmi *et al*. 2015) and gain insight into the physiological processes of skeletal formation. The impacts of development, exercise, nutrition, housing and genetics on the layer skeletal system are complex and are an area of high research interest as methods for minimising damage and optimising skeletal integrity are still needed.

Keel bones

In comparison with conventional caged housing systems, osteoporosis is not as prevalent in hens that are loose-housed, due to their increased exercise opportunities. Instead, loose-housed hens do suffer from increased fractures, typically believed to be caused by isolated collision or fall incidences (although see Thøfner *et al*. (2020) for new evidence that challenges this common interpretation), and deviations (bent keels), which are believed to be pressure-related (Scholz *et al*. 2008; Pickel *et al*. 2011). Keel damage can be found in pullets at the end of the rearing period (Blatchford *et al*. 2016), but generally occurs in mature layers and steadily increases in prevalence across the laying cycle (Käppeli *et al*. 2011*a*; Habig and Distl 2013; Blatchford *et al*. 2016; Heerkens *et al*. 2016*a*; Eusemann *et al*. 2018), with some research indicating a peak ~50 weeks of age, and a decrease thereafter (Petrik *et al*. 2015; Toscano *et al*. 2018). Keel fractures will heal across a period of several weeks but birds can sustain multiple fractures across the flock cycle, with calluses forming on the bone (Richards *et al*. 2011; Baur *et al*. 2020). Prevalence of hens with some form of damage can be up to 95% by the end of production (Wilkins *et al*. 2011). Hens with keel fractures experience pain (Nasr *et al*. 2012*a*, 2013*a*), reduced egg production, egg size and egg quality, along with increased feed and water consumption (Nasr *et al*. 2012*b*, 2013*b*; Heerkens *et al*. 2016*b*). In contrast, Gebhardt-Henrich and Fröhlich (2015) observed no correlation between keel damage and egg production. Hens that are suppressed from egg laying show increased keel density and a lower risk of fractures than do hens still producing eggs, but a similar relationship has not been observed for keel deviations (Eusemann *et al*. 2020). The impact of fractures on egg production may be greater towards the end of the laying cycle when hens are physiologically weaker (Rufener *et al*. 2019*a*). Hens with fractures also show a reduced ability to perch and fly (Nasr *et al*. 2012*b*). Hens with severe fractures will spend less time resting on the floor and standing, more time perching (Casey-Trott and Widowski 2016), and less time inactive, suggesting that the birds may be uncomfortable leading to restlessness (Casey-Trott and Widowski 2018). Recently, hens that sustained fractures showed reduced hippocampal neurogenesis, indicating that a negative affective state was experienced while suffering from fractures (Armstrong *et al*. 2020). Physiologically, keel fractures induce an inflammatory response and cause stress, inhibiting the orexin system, which can affect metabolism (Wei *et al*. 2019). Commercial free-range birds will show reduced use of pop holes when suffering from keel damage (Richards *et al*. 2012) and movement patterns within the tiers of an aviary system change across time relative to the hen's fracture severity (Rufener *et al*. 2019*b*). Thus, keel fractures are a documented significant management and welfare concern (reviewed in Riber *et al*. 2018) for which solutions are likely to be multifactorial (Harlander-Matauschek *et al*. 2015; Hardin *et al*. 2019). Further research to understand the precise causes of keel damage and resulting impacts on physiological processes may provide further insight into the welfare impacts of fractures and deviations and how to mitigate the damage occurrences.

Keel damage can occur in all types of housing systems and in both brown and white strains of hens. Examples of research across commercial farms have shown that, in 79 Austrian barn, conventional, and free-range flocks, 27% of palpated brown and white birds sustained damage (Grafl *et al*. 2017), and over 85% of sampled brown and white hens had sustained a fracture within 47 commercial aviary and free-range flocks in Belgium (Heerkens *et al*. 2016*b*). Across 31 commercial flocks in Denmark, 25% of sampled birds in multi-tiered systems showed keel abnormalities as assessed via palpation (Riber and Hinrichsen 2016). Up to 83% of palpated brown and white birds within 39 commercial aviary, barn and free-range flocks in Switzerland sustained some type of keel deformity (Käppeli *et al*. 2011*b*). Finally, up to 95% of birds in 15 free-range, barn and furnished commercial housing systems across the UK had suffered damage when assessed by dissection at the end of lay (Wilkins *et al*. 2011). Prevalence of 25–95% across all commercial systems indicates that this is a significant problem in laying hen production.

The identification of contributing factors towards damage may help mitigate the potential occurrences. Risk factors for keel damage may include the presence of bumble foot, which can hinder the ability to grasp perches or surfaces properly, and fractures may be more likely to occur at the onset of lay, and during peak egg production (Gebhardt-Henrich and Fröhlich 2015). Individual hens that come into production earlier may have a greater risk of keel damage at the end of the production cycle (Gebhardt-Henrich and Fröhlich 2015). A survey across 47 commercial aviary and aviary free-range farms in Belgium showed that the specific aviary design, flooring material and genetic hybrid were all predictors of keel-bone damage (Heerkens *et al*. 2016*b*). Similarly, Käppeli *et al*. (2011*b*) found a higher prevalence of keel damage in birds from specific breeding companies over other suppliers when assessing damage on Swiss commercial farms using both brown and white strains. However, in post-mortem assessments of 79 Austrian flocks at the end of lay, strain, flock size, season at slaughter or whether the birds were barn or free-range had no effect on the incidence of keel-bone deformities (Grafl *et al*. 2017). Modelling risk factors across 50 sampled organic flocks in Europe found increased damage if more birds were underweight, produced more eggs, and if there was no natural daylight inside (Jung *et al*. 2019). Greater tibia and/or humerus bone strength may also result in a stronger keel bone and, thus, reduced breakage (Wilkins *et al*. 2011; Toscano *et al*. 2015, 2018), as seen in birds selected for higher bone strength (Stratmann *et al*. 2016). However, Gebhardt-Henrich *et al*. (2017) suggested that environmental factors such as perch design, housing system and collisions/falls may be more likely to contribute to incidence of fractures than are individual hen differences in skeletal health. In contrast, Donaldson *et al*. (2012) looked at HyLine Brown hens housed in free-range laying systems with or without access to aerial perches and concluded that individual variation in bone strength was a contributing factor to the likelihood of keel-bone injury rather than, specifically, the presence of perches. Keel-bone mineral density can mitigate the negative effects of minor collisions, but not of stronger impact force (Toscano *et al*. 2018). Additionally, if birds already have fractures present, then they will be more likely to sustain further fractures (Toscano *et al*. 2018).

Keel-bone damage is a significant welfare and production concern and it is prevalent globally. The frequency and types of keel-bone damage within Australian systems are currently poorly understood. A single recent study has shown severe keel damage in end of lay hens from a commercial aviary free-range system at frequencies similar to those found in other non-cage housing systems (Kolakshyapati *et al*. 2019). Risk factors for damage are multifactorial and may be related to individual-bird bone strength, rate of accidents and housing-system design. There is no single cause for damage and, thus, no single solution, but all producers should work towards reducing keel injuries.

Genetic effects

Genetic background plays a role in skeletal health, particularly in susceptibility to keel-bone damage, thus indicating that there is potential to select against it. This was highlighted by Käppeli *et al*. (2011*a*) who found fewer keel-bone deformities in Lohmann Brown parent stock hens than in their Lohmann Brown progeny. Selection specifically for bone strength has shown denser bones in a ‘high bone’ line than in both a ‘low bone’ line and a standard Lohmann Selected Leghorn commercial line, including reduced keel damage (Stratmann *et al*. 2016). But different aviary designs do also affect damage prevalence, indicating interactive effects of genetics and environments on skeletal health (Stratmann *et al*. 2016).

Behavioural research of brown and white hen strains in aviaries has shown different use of space and resources, suggesting that system-design variants may best fit specific strains (Ali *et al*. 2016). Strain susceptibility to damage could potentially factor into the system design and best-matched strain. Comparisons between brown and white strains in cages and floor housing by Eusemann *et al*. (2018) showed that brown hens had more fractured keels than did the white hens, and fewer deviations and fractures in the low-performing brown line than in a high-performing brown line (Eusemann *et al*. 2018). The frequency of deviations also differed between two low-performing white layer lines in comparison with a high-performing white line and both low-performing and high-performing brown layer lines, indicating that the genetic differences may be subtle but can still have an impact on keel-damage susceptibility (Eusemann *et al*. 2018). A further study within aviary systems in Switzerland showed a trend for brown hens to be more susceptible to developing keel fractures (Gebhardt-Henrich and Fröhlich 2015). Heerkens *et al*. (2016*a*) confirmed significantly higher prevalence of keel-bone fractures in ISA Brown hens than in Dekalb White hens housed in aviaries, but comparatively fewer keel-bone deviations. Whereas Baur *et al*. (2020) found that individual Lohmann Brown hens sustained a greater number of fractures per keel than did Lohmann Selected Leghorns. Additionally, more overall deformities (fractures and deviations) were found in Lohmann Brown layers than in Lohmann Selected Leghorns when housed in furnished cages (Habig and Distl 2013). But the Lohmann brown hens did have a higher humerus breaking strength (Habig and Distl 2013). Finally, Stratmann *et al*. (2015*a*) found that ISA Brown hens had more fractured keels and fewer undamaged keels at 18 weeks of age than did Dekalb White hens, but, at 64 weeks of age, the opposite pattern was observed. In other assessments, differences in impact damage between commercial brown and white strains could not be found (Candelotto *et al*. 2017). Experimental lines that had not been subject to the same production selection pressure as were commercial strains showed stronger keel bones (Candelotto *et al*. 2017). Birds that were more susceptible to keel breakage also showed lower egg-breaking strength and clear genetic variation in fracture susceptibility, with keels from the commercial strains showing the greatest likelihood of being fractured (Candelotto *et al*. 2017). This susceptibility was also related to egg production where strains with a higher likelihood of damage also had lower egg-breaking strength, highlighting the toll that high calcium turnover can take on the hen’s skeleton (Candelotto *et al*. 2017). Further to the influence of egg production, comparisons between low-performing and high-performing brown and white layer lines showed higher breaking strengths of the tibia and humerus in the low-performing lines and a higher tibial-bone mineral density (Habig *et al*. 2017). These studies on strain differences have shown that the heavier brown strains, and those that have a high calcium turnover due to prolific egg production, are more susceptible to breakages.

Inherent differences in movement behaviour of white and brown strains and wing loading may also play a role in their susceptibility to damage (LeBlanc *et al*. 2018). White strains have lower wing loading and show more wing-assisted locomotion (LeBlanc *et al*. 2018), but any specific relationship with impact damage would need to be confirmed. Similarly, differences in how brown and white strains utilise the tier and perching resources within alternative housing systems (Ali *et al*. 2016) may factor into prevalence of keel damage, which is an avenue to be explored. Differences in damage susceptibility are also present among strains within the same colour lines. Heerkens *et al*. (2016*b*) identified a significantly higher damage risk in ISA Brown hens than in Lohmann Brown Classic hens within commercial aviary and free-range systems.

Management methods to reduce skeletal problems

Producers may have limited ability to select for bone strength, particularly when using commercially available genetic lines, but system modifications could help minimise skeletal damage. Ramps are a beneficial addition for alternative systems that require hens to jump to access perches or different system levels, as they allow for controlled walking or wing-assisted incline running (LeBlanc *et al*. 2018). Heerkens *et al*. (2016*a*) showed that the addition of ramps between perches and the floor in an aviary system reduced the incidence of keel-bone fractures in both white and brown hen strains. Similarly, the addition of ramps in aviaries resulted in more controlled movements by Lohmann Selected Leghorns, with fewer collisions and fewer falls, culminating in reduced incidences of keel fractures when assessed at 60 weeks of age (Stratmann *et al*. 2015*b*). However, after slaughter at 66 weeks of age, the incidence of old fractures (i.e. did not occur during slaughter) were similar between control and ramp-aviary pens (Stratmann *et al*. 2015*b*). The specific design of the ramps used, such as incline and construction material are important for ensuring that the birds will use them (Pettersson *et al*. 2017; LeBlanc *et al*. 2018). Additionally, the birds will be more adept at using ramps if they are first provided during the rearing phase (Norman *et al*. 2018).

The material that systems are designed from, particularly perches, could also be modified to reduce keel damage. Pickel *et al*. (2011) showed a higher peak-pressure force of the keel in both Lohmann Selected Leghorns and Lohmann Brown hens when resting on commercially used perches than on prototypes of soft, round, polyurethane perches. The contact area with the polyurethane perch was also larger, suggesting that this type of perch may reduce keel-bone damage by absorbing more energy on impact and/or distributing the pressure force across a larger area (Pickel *et al*. 2011). Plastic perches in aviaries were also associated with fewer keel-bone deformities in Lohmann Brown hens and Lohmann Brown parent stock than were rubber-coated metal perches (Käppeli *et al*. 2011*a*). Similarly, Stratmann *et al*. (2015*a*) found that soft polyurethane perches in a commercial aviary system reduced both fractures and deviations in Dekalb White and ISA Brown hens, although the practicality and durability of using this material would need to be improved on, so as to be commercially sustainable (Stratmann *et al*. 2015*a*). The research has been conducted internationally and a survey of the current use and impacts of ramps and padded perches within Australian alternative systems would be valuable.

Approximately half of a hen's daily calcium needs are sourced directly from their diet, and, thus, adequate nutrition plays a role in skeletal health and is a management method able to be directly manipulated by producers. Research is particularly focussed on the provision of vitamin D, phosphorus and calcium, including calcium particle size (reviewed in Olgun and Aygun 2016; Li *et al*. 2017). Additional feed supplements such as melatonin and herb extracts have also been considered as methods for improving calcium retention in bones (Świątkiewicz and Arczewska-Włosek 2012; Taylor *et al*. 2013; Świątkiewicz *et al*. 2018), although there may be a resulting trade-off between skeletal health and egg quality (Taylor *et al*. 2013). Further information on the role of nutrition on skeletal health is provided in Olgun and Aygun (2016).

Rearing impacts

Exercise during the production period can have a significant impact on the skeletal health of layers, but the rearing period sets a critical foundation for strong bone (and muscle) development. There is a dramatic change in bone structure as birds come into sexual maturity and start egg production, but bone growth occurs throughout rearing, where biomechanical stress placed on the bones during the rearing period will increase their strength. Thus, pullet housing environments can have life-long impacts on skeletal quality. A more detailed physiological overview of bone growth and development has been given by Whitehead (2004). Layer chicks will begin perching after 1 week of age (Kozak *et al*. 2016) and are highly motivated to perch on any available structure as they develop. This can include water lines and feeders in the absence of perches. This rearing experience is important for the development of appropriate perching and jumping behaviours to enable full use of provided perch resources as adults (Heikkilä *et al*. 2006), and to develop the spatial skills to navigate three-dimensional environments (Gunnarsson *et al*. 2000), both of which may reduce injury risks as adults. Increasing numbers of studies have followed birds through rearing until the end of lay, but these have not been conducted within Australia and have been conducted primarily on different strains of white hens. The results of these studies as presented have consistently highlighted the impacts of rearing environments.

White Leghorn pullets reared in cages or aviaries (aviary birds were caged housed until given floor access at 6 weeks of age) showed greater cortical bone density in the aviary-reared humeri and tibiae when assessed at 16 weeks of age, and both bones had a thicker cortex and greater load-bearing capacity than did the conventional cage-reared pullet bones (Regmi *et al*. 2015). Pullets from these same flocks were then placed into cage, aviary or furnished systems during lay. Bone parameters assessed at the end of lay indicated that benefits acquired from aviary rearing were best maintained if exercise opportunities were still available throughout the production cycle (Regmi *et al*. 2016). However, the aviary-reared pullets showed more keel abnormalities (fractures and deviations combined) than did the caged-reared pullets at 19 weeks of age and continued to sustain more keel damage throughout the lay cycle than did the conventional cage-housed layers (Blatchford *et al*. 2016). In contrast, research across the flock cycle using Lohmann Selected Leghorn-Lite birds found significantly fewer keel-bone fractures in aviary-reared birds irrespective of subsequent housing in conventional or furnished cages, but no differences in the prevalence of deviations (Casey-Trott *et al*. 2017*a*). The aviary-reared pullets also showed improvements in multiple bone-development measures for the radius, humerus and tibia, including a higher total bone density, a higher total bone mineral concentration, and a higher breaking strength (Casey-Trott *et al*. 2017*b*). Measurements taken at the end of lay showed that aviary-reared hens had a greater bone mineral concentration, and a greater total and cortical cross-sectional area of the radius and tibia than did conventional cage-reared hens, but a lower total and cortical bone mineral density (Casey-Trott *et al*. 2017*c*). Overall, Casey-Trott *et al*. (2017*b*, 2017*c*) found that the aviary-reared hens maintained more life-long benefits of rearing exercise than did conventional cage-reared hens.

Conventional cages, in comparison to loose-housed systems, limit movement and there are clear differences in skeletal development when compared with aviary rearing. However, perches can be added to conventional cages, which can affect bone growth in comparison to just wire flooring. By 12 weeks of age, Enneking *et al*. (2012) found greater bone mineral content of the tibia, humerus, and sternum in White Leghorn pullets with access to perches. The effects of rearing with perches persisted through to 71 weeks of age in White Leghorns, with a greater shank width in perch-reared birds, even when subsequently housed with perches as adults (Yan *et al*. 2014). There were also some increases in keel-bone mineralisation density, but the presence of perches as adults increased keel damage and this was not prevented by rearing with perches (Hester *et al*. 2013).

Exercise opportunities during rearing are necessary for good bone and behavioural development, but these should aim to be provided in a manner that minimises any associated risks of damage, such as by inclusion of ramps, or softer materials.

Comparative studies

Laying hens in all types of housing systems are prone to skeletal issues. However, the types of issues vary among housing systems, specifically caged, versus floor, versus multi-tier, and are dependent on the behaviours that birds can perform within these systems. Comparative studies across system types have highlighted these differences. As might be expected, cage-housed birds have poorer limb bone quality than do loose-housed birds, particularly in their wings as there is limited opportunity to stretch and flap. This was demonstrated in assessments across commercial farms within the UK housing predominantly brown strains, which showed birds from furnished cages had a lower breaking strength in their tibiae and keels than did barn and free-range birds, and even more pronounced differentiation in their humeri (Wilkins *et al*. 2011). Lohmann white hens that were housed in commercial aviaries or furnished cages showed improved bone strength compared with conventional cage-housed hens, particularly within their humeri (Regmi *et al*. 2017*a*). Across various brown and white hen strains in a research setting in Canada, floor-housed hens showed heavier radii and a greater cross-sectional area than did cage-housed birds (Silversides *et al*. 2012). The differences between cage and floor housing were not as pronounced in the tibial bones because caged hens do spend time standing when other movements are restricted (Silversides *et al*. 2012). Similarly, comparisons of Lohmann White/Rhode Island Red crossbred hens housed in conventional, furnished, free-range and single-tier aviary systems showed that the density of the humerus was lower in caged than in non-cage systems, but no differences were found in tibial density and keel damage (Shimmura *et al*. 2010). However, Habig *et al*. (2017) found that birds from five different layer lines housed in floor pens had higher breaking strengths of both the tibia and humerus than did cage-housed birds. Specifically, in HyLine Brown hens, birds from furnished cages had a greater tibial strength than did conventional cage-housed birds, which may have been related to the higher frequencies of walking observed in birds within large furnished cages (Meng *et al*. 2017). However, no differences in tibial bone weight, length and density were found in these birds, emphasising the need for multiple bone parameters to be measured for accurate detection of potential differences (Meng *et al*. 2017). In contrast, Onbaşılar *et al*. (2016) found no differences in tibia and femur length, width, diameter and strength of Lohmann Brown Classic and Lohmann Selected Leghorn Classic hens from conventional and furnished cages. Collectively, these studies showed that systems where birds are given more opportunities to move will result in stronger bones, but the differences are less pronounced in leg than in wing bones where birds in both caged and loose-housed systems spend time standing.

Housing-system design can affect bone development, but also affects the degree of keel-bone abnormalities. Multi-tier systems result in a higher rate of damage than do single-tiered or caged systems. Postmortem assessment of hens at end of lay across 79 flocks throughout Austria housing predominantly Lohmann Brown-Classic and Lohmann Brown-Extra hens found that birds from aviary systems sustained more frequent or more severe keel-bone damage than did those from barn or free-range single-tier systems, although, overall, a high proportion of all sampled birds did have some form of damage (Grafl *et al*. 2017). This finding was confirmed across 37 Swiss commercial farms housing both white and brown strains where birds from indoor aviary systems had more frequent and more severe abnormalities than did hens from floor housing (Käppeli *et al*. 2011*b*). Additionally, palpations on commercial farms in Denmark for both white and brown strains also found that hens in multi-tiered systems were more likely to sustain keel bone fractures than were those in single-tiered systems (Riber and Hinrichsen 2016). Relative to floor housing, hens housed in aviaries displayed increased keel-bone damage across 50 organic flocks within Europe (Jung *et al*. 2019). Finally, assessments of old fractures in hens at the end of lay across commercial furnished, barn and varying free-range systems within the UK showed that birds from the furnished systems showed the least damage and birds from the aviary-style free-range systems had the highest damage prevalence (Wilkins *et al*. 2011). Incorporation of perches into the systems increased the severity of observed keel damage (Wilkins *et al*. 2011).

Differences in damage are also present when comparing caged to floor-housed systems. Eusemann *et al*. (2018) found the proportion of deviated keel bones was higher for caged white hens than for floor-housed white hens. But at 72 weeks of age, both brown and white layer hens had more fractures in the floor-housing system than did caged birds (Eusemann *et al*. 2018). Similarly, Petrik *et al*. (2015) visited 17 commercial conventional cage and floor farms housing various brown hen strains within Canada and found more keel fractures in the floor-housed than in cage-housed birds. However, Shimmura *et al*. (2010) found no differences in keel damage among White Leghorn/Rhode Island Red crossbred hens housed in conventional cages, furnished cages, single-tiered aviaries and free-range single-tiered systems. Overall, more complex systems result in higher rates of keel damage, but these types of studies are currently missing for Australian housing systems and would be beneficial to be able to benchmark system improvements.

Conclusions

Keel-bone damage is a significant welfare and production concern and it is prevalent globally. The frequency and types of keel-bone damage within Australian systems are currently poorly understood. Risk factors for damage are multifactorial and may be related to individual-bird bone strength, rate of accidents, and housing-system design. Keel fractures may be associated with collisions, whereas keel deviations may be associated with suboptimal perching structures. There is no single cause for damage and, thus, no single solution, but all producers should work towards reducing keel injuries. There is a genetic component to keel-damage susceptibility and a relationship with egg production. Higher egg production increases the risk for keel-bone damage. Strains selected for high bone strength will have reduced keel damage but impacts on other aspects of health and production still need to be assessed in commercial settings. Brown strains of hens may have greater susceptibility to damage, highlighting the need for assessments of damage prevalence within Australian housing systems. The addition of ramps can assist in safe locomotion among areas of different height to reduce accident-related keel-bone damage, but material and design must be considered to ensure optimal use by the birds. Softer material on perches can also reduce collision and pressure-related keel damage. Rearing environments have long-lasting impacts on bone quality. Exercise opportunities during rearing will increase bone strength and development, with these positive impacts persisting throughout lay, although the adult housing environment is still critical. Bone quality will decrease if exercise opportunities are limited during the lay cycle. However, increased exercise opportunities through perches or aviary housing do increase the risk of keel damage and other methods for preventing keel damage will be necessary (e.g. genetic selection, ramps, nutrition). Further study on rearing impacts with brown hen strains would be valuable. When hens are given the opportunity to exercise, they will show improvement in bone structural parameters and mechanical properties. These differences may be more prominent in the wing bones, as increased space will allow for wing flapping and wing-assisted locomotion. Multiple measures on each bone are recommended as not all measures will identify differences resulting from specific movements by the hen. Internationally, across multiple commercial studies, hens from multi-tier systems show a higher prevalence of keel-bone damage, but the prevalence of these injuries within Australian systems is currently unknown. The literature discussed in the present review is sourced internationally and, thus, the Australian industry would benefit from data collected on different types of commercial systems to provide a benchmark for any future improvements in the skeletal health of laying hens within Australia.

Conflicts of interest

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