

Causes of keel bone damage and their solutions in laying hens

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Keel bone damage (KBD) is a critical issue facing the contemporary laying hen industry due to the likely pain leading to compromised welfare and reduced productivity. Recent reports suggest that KBD, while highly variable and likely dependent on a host of factors, extends to all housing systems (including traditional battery cages, furnished cages and non-cage systems), genetic lines, and management styles. Despite the extent of the problem, the research community remains uncertain as to the causes and influencing factors of KBD. To combat these issues, the current review was produced following discussions from the 1st International Keel Bone Damage Workshop held in Switzerland in April 2014. This exercise sought to assess current knowledge, foster novel collaborations, propose unique methodologies and highlight the key areas where innovative research is needed. The following paper is based on the content of those discussions and presents nine recommendations for future research efforts.

Keywords: keel; fracture; damage; welfare; bone; laying hen

Introduction

The high frequency of damage seen in the keel bone (a bone extending from the sternum) of laying hens within commercial systems represents one of the greatest welfare problems facing the industry (FAWC, 2010; 2013). Beyond the obvious welfare issue of gross skeletal deformity, concern stems from the likely associated pain indicated by the decreased latency to descend from a perch (Nasr *et al.*, 2012a; 2012c, 2014). One type of KBD, keel fractures (KF), sometimes referred to as 'old breaks', can be

defined as breaks in the bone that will typically manifest as a callus around the fracture site after a few days; KF may also involve sharp, unnatural deviations, or bending of the bone (Wilkins *et al.*, 2004). The problem of keel damage is clearly widespread with similar yet highly variable levels reported in various countries, such as Switzerland (Kappeli *et al.*, 2011); UK (Wilkins *et al.*, 2011); The Netherlands, Belgium, Germany (Rodenburg *et al.*, 2008; Heerkens *et al.*, 2013) and Canada (Petrik *et al.*, 2015). Keel bone damage (KBD) extends across genetic lines (Kappeli *et al.*, 2011) and all types of housing systems (Wilkins *et al.*, 2011; Petrik *et al.*, 2015) including organic production systems (Bestman and Wagenaar, 2014). Despite their ubiquity, the causes and influencing factors of KBD remain largely unknown to the research community, a circumstance that severely handicaps the development of effective strategies to reduce their occurrence and severity.

To identify critical areas where research is needed and coordinate the multiple ongoing and future research efforts of laboratories, the International Keel Bone Damage Workshop was organised by the University of Bern in April 2014. This manuscript serves to highlight the workshop dialogue and harness the collective energies of the research community towards reducing KBD in laying hens by formulating key recommendations for forthcoming research.

Recommendation 1 - A uniform method of evaluating KBD should be employed with relevant criteria to ensure reliability of results

Currently, evaluating KBD in live birds is principally done by palpation; a method that originated primarily from two papers (Wilkins *et al.*, 2004; Scholz *et al.*, 2008) which have since been adopted by a variety of labs. While broadly similar in their classification of damage, key differences exist and require resolution. Most critically, Wilkins *et al.* (2004) only discussed KF while Scholz *et al.* (2008) included deviations of the keel (deformations from a theoretically flat, two dimensional plane; also referred to as curving or bending). Given that KF and deviations likely result from different causal factors (*i.e.* sudden impacts causing large forces versus sustained and small forces, respectively), this lack of clarity represents a major obstacle in combining results to allow for comprehensive interpretation of the problem. The multiple methods in use are a challenge in resolving KBD, hindering our abilities to compare findings and resulting in needless and inefficient replication. We recommend development of a uniform assessment of KBD that assesses KF and deviations where each uses a binary scale of whether damage is present. The system should be developed in concert with an associated scoring sheet which should be made publicly available to facilitate the amalgamation of data produced by the labs conducting related research.

Beyond defining the presence or absence of damage, identifying grades of severity is needed to determine the types of KBD that are a concern. Research that can provide reliable and consistent markers to classify KBD in grades of severity that are grounded in meaningful criteria relevant to animal welfare (*e.g.*, pain caused by fracture) and/or other spheres of concern (*e.g.* productivity) is essential. We also recommend adoption of training criteria for persons assessing KBD (particularly by palpation which is more subjective than dissection) to ensure greater reliability of results. A system employed by assessors with appropriate training can produce results that are more useful in determining true occurrence of damage and evaluating interventions. The method of assessment, including free access to a developed scoring form, the described definitions for the various forms of damage, as well as criteria and training for palpation, is described elsewhere (Casey-Trott *et al.*, in press).

Recommendation 2 - Investigate low energy, non-collision events as a source of KF

High energy events within non-cage systems provide a likely mechanism for KF that have been suggested to result from collisions with elements of animal housing such as perches (Scott *et al.*, 1997; Moinard *et al.*, 2004a; Sandilands *et al.*, 2009; Wilkins *et al.*, 2011). Counter-intuitively, KF frequently occur in cage systems where there exists seemingly limited opportunities for dynamic loading as might occur during collision. Thus, improving our understanding of the sources of KF will require insight of dynamic as well as static events. The mechanisms of KF in cage systems are likely not exclusive to this environment, *i.e.* static mechanisms are likely to cause damage in non-cage systems as well, and thus deserve investigation to provide a more comprehensive understanding of the sources of KBD.

Although commercial laying hens kept in enriched cages and non-cage systems have opportunities for weight-bearing activities, birds' skeletons remain fragile because of a combination of nutritional, environmental and genetic factors (Whitehead, 2004a). It would be worthwhile to test whether seemingly innocuous, quasi-static activities can induce KF. While perching, hens place considerable forces on their keels which is in contact with the perch (Pickel *et al.*, 2011) and certain perch designs or material may result in severe keel bone deviations and perhaps also KF (Pickel *et al.*, 2010). This mechanism would be similar to compression fractures in osteoporotic bones of humans, which have been shown to occur spontaneously or with normally innocuous activities, such as sneezing or twisting (Kondo, 2008). Furthermore, application of modeling techniques, including finite-element models (Tomaszewski *et al.*, 2010) that represent the keel bone, would be useful to provide an understanding of the static and dynamic loading patterns in the bone.

More generally, a broader understanding of the keel, using a comparison of relevant bird phylogenies, could be helpful to establish the morphological capacity of the keel and how modern housing conditions exceed that capacity. It would be useful to assess keel integrity in relation to the functional morphology of diverse avian clades that exhibit significant variation in hindlimb/forelimb modules, keel morphology, flight style, and use of natural perches (Heers and Dial, 2012).

Recommendation 3 - Investigate the relationship between deviations and KF

A keel with deviations may lead to unequal bone loading during wing-flapping and concentration of strain energy in ways that increase the risk of fracture. The paired *pectoralis* muscles are capable of generating enormous force, work and power output during normal flight (Tobalske *et al.*, 2003) and these outputs increase dramatically during vigorous wing-flapping (Tobalske and Dial, 2000; Jackson and Dial, 2011). Comparable wing-flapping is often observed in commercial laying hens that slip from a perch or aviary tier and try to regain their footing (personal observation, M Toscano). Three-dimensional force-balance calculations (Hutchinson *et al.*, 2005; Baier *et al.*, 2006) should be used to test for effects of keel deformity upon bone loading during *pectoralis* muscle contraction; particularly those observed during episodes where balance is lost or panics (see Recommendation #5). Deviated keels may lead to KF indirectly by complicating balance maneuvers, an additional topic which deserves investigation.

Recommendation 4 - Investigate the role of bird development in KBD susceptibility

It is of prime importance to learn more about development of locomotor and cognitive skills as these contribute towards the ability of hens to navigate within the home system. It is doubtful that the traditional aviary rearing system, where chicks are kept confined to platforms for the first four weeks after which the sides are opened, is the best system to prepare birds for aviary housing (Kozak *et al.*, 2015). Under natural conditions, locomotor capacity is critical in Galliformes as they are confronted with immediate challenges to escape predators, search for food and seek shelter (Dial and Jackson, 2011). Beginning at six days post-hatching, wild Galliformes will readily flap their wings to produce aerodynamic forces that enhance hindlimb function while moving up inclines, a behavior called wing-assisted incline running (WAIR) (Dial, 2003; Tobalske and Dial, 2007; Dial *et al.*, 2008). Adult Galliformes may prefer WAIR rather than flight to reach an elevated area in a complex, natural habitat (Dial and Jackson, 2011). A better understanding of this process in commercial strains could be helpful to design juvenile and/or adult hen housing systems that improve locomotor abilities (Leblanc *et al.*, 2015). For instance, variable-engineered systems would be suitable for accommodating the birds as they develop, using more ramps and adjustable angles with increasing age. A potential advantage of WAIR compared with flight may be that whole-body kinetic energy is less during WAIR (Tobalske and Dial, 2000; 2007), a benefit that could reduce the risk of KBD due to accidental impact with the housing environment. Additionally, increased wing-flapping during development may assist in improving balancing abilities (Filipa *et al.*, 2010) while greater activity is known to correlate with bone strength (Rath *et al.*, 2000). Beyond musculo-skeletal development, juvenile birds using WAIR will likely learn neuromuscular coordination that will be useful for negotiating three-dimensional structures in adulthood, an impairment suggested by Gunnarsson *et al.* (2000). Research in this area will help to identify optimum rearing conditions likely to protect birds from cognitive impairment in adulthood.

Recommendation 5 - Investigate the role of escape reactions as a source of KF

Efforts should be made to understand the damage resulting from sudden escape reactions, or during panic. Escape is normally triggered by a situation that is, or is perceived to be, life-threatening and thus is not 'normal' in the context of commercial poultry husbandry, though has been documented (Richards *et al.*, 2012). 'Escapes' resemble the pattern seen in Galliformes involving take-off using high-frequency, high-amplitude wing beats that feature enormous power output (Tobalske and Dial, 2000; Tobalske *et al.*, 2003), a quick return to the ground using a glide, and then resumption of walking or running. Given that the response is one of last resort, its manifestation is likely to exceed the keel's morphological capacity. Additionally, escape flights will not allow for the precise navigation required in housing systems. A more thorough understanding of the causes of escapes is necessary, including the roles of genetic selection and stockmanship, and the effects of dim lighting conditions and intra-bird spacing (Tillmann, 2009).

A comparative evolutionary approach could further aid investigation regarding the role of escape reactions as a source of damage. A museum survey of wild birds of different species showed that 4.5% out of a sample size of 6,212 specimens had sustained and survived bone injuries of which clavicle injuries were the greatest in number, especially

in smaller birds, and were attributed to collisions with solid objects (Tiemeier, 1941). The sample included 45 birds in the *Phasianidae*, the family that includes the chicken, with an incidence rate of 10% in this family. These percentages are far lower than incidence rates of KBD in layer hens, but they do suggest that the escape flight of birds in the *Phasianidae* (Tobalske and Dial, 2000) may be correlated with bone damage. An alternative explanation, however, is that some damage reported by Tiemeier (1941) was due to gunshot, as many species in the *Phasianidae* are gamebirds.

Recommendation 6 - Investigate genetic capacity to reduce KBD

The role of genetics as a contributing factor in KBD dates back to work by Hyre (1955) who showed that the tendency to develop keel deformities was heritable by successfully selecting for and against KBD over six generations. Even earlier, Warren (1937) showed that crooked keel bones (in comparison to straight keels) had a reduced ash content which he suggested was a causal factor. In considering the scope for genetic selection against KBD, we must first determine what traits should be selected where possibilities include: stronger bones, improved physical ability, and increased docility. Alternatively, as KBD is a relatively complex trait with a number of genetic and environmental factors playing a role, genomic selection should be considered (Fulton, 2012) by carefully monitoring the incidence of KBD in a large population of laying hens and then comparing genomic information of hens with and hens without KBD. One of the strengths of this approach is that it does not target a single factor, *e.g.* bone strength, but focuses on the actual presence or absence of damage. In theory, the associated mechanism(s) is (are) selected in the process of identifying birds with the desired trait. A challenge with the genomic approach is that it requires a large sampling population (>5,000) of which a clear KBD phenotype is needed. However, once the genomic fingerprint of a hen with no KBD is acquired, no further phenotypic measurements are required for the selection program (Eggen, 2012). Although breeding for reduced KBD is attractive, one should be aware of possible linkages and trade-offs with other traits, *e.g.*, reduced egg shell thickness and egg breaking strength (Stratmann *et al.*, *in press*). Whitehead (2004b) provides an excellent review of the relationship between skeletal integrity and egg quality.

Recommendation 7 - Investigate housing adaptations that affect frequency of KBD

Large differences between housing systems in the incidence KF indicate that housing design and/or management plays a key-role (Rodenburg *et al.*, 2008; Wilkins *et al.*, 2011). Perches have received particular attention. As an indication that perches have a causal role in the occurrence of KBD, higher rates were reported at end of lay for hens in conventional cages with (92%) compared to those without (83%) metal perches (Hester *et al.*, 2013). Similarly, Wilkins *et al.* (2011) reported a 10-34% increase in KF when perches were added in an organic mobile system. Often, round metal perches are used, which may not offer hens adequate support for their grip. Perches with slightly larger diameters and those made from more flexible materials (wood, rubber) have been suggested to be more capable of absorbing forces during impact and preventing KBD (Pickel *et al.*, 2010; 2011). Perches covered with a soft rubber layer were successful in reducing the number of keel damage within a commercial aviary suggesting the benefit of this option (Stratmann *et al.*, 2015b), possibly not only by reducing the pressure on the

keel, but also by providing a cushion and improved grip when landing (Scholz *et al.*, 2014). Perches as a source of fracture is discussed in more detail by Sandilands *et al.* (2009).

Apart from perches, the three-dimensional environment of the hens has to be designed so that it allows the hens to navigate between the different parts of the system. In most commercial aviary systems, improvements are possible that would improve hens' possibilities for safely navigating through the system. One option is to add ramps to aid transition between tiers. Stratmann *et al.* (2015a) showed that adding ramps to a commercial aviary system reduced falls by 55%, collisions by 41% and keel fractures by 24%, while movements between tiers increased by 44%. The width of the corridors between the different rows within systems also needs further attention. If the corridor width is at the limit of the birds' navigational ability, it may cause increased collisions due to misjudged jumps (Heerkens *et al.*, 2014).

Another factor to be considered when determining optimum housing is lighting. In many commercial laying hen operations, light intensity is kept at a relatively low level, especially in flocks that are prone to develop feather pecking. Birds need sufficient light and contrast to make an appropriate jump and safe landing (Moinard *et al.*, 2004b). The timing of the light transition also seems important: Stratmann *et al.* (2013) reported that vertical movements occurred mainly during a dusk phase when hens usually move to a perching spot for the night. A sudden switch from light to dark periods may increase the risk of KF during this time. Hence, a gradual dawn- and dusk should be investigated as a possible means of reducing KF. Lastly, Heerkens *et al.* (2014) found flooring type to also be a factor where wire flooring had greater frequency of KF compared with plastic flooring, though the underlying causes need to be explored.

Recommendation 8 - Investigate nutritional solutions to reduce KBD

Solutions should include changes in bird management and nutrition. The high incidence of KF in cages (Hester *et al.*, 2013) could point to a calcium shortage. Egg shell formation takes place during the night and hens need a large amount of calcium at this time to produce an eggshell each day. Hens can mobilise this calcium partly from their bones, but they need to restore their supply, otherwise the risk osteoporosis (Whitehead and Fleming, 2000). One way to supply hens with calcium during the night is to give them daytime access to calcium sources with larger particle sizes, such as grit or shells, which will then be digested during the night. Larger particle sizes of calcium has been shown to benefit skeletal health (Cheng and Coon, 1990; Guinotte *et al.*, 1995) including that of the keel (Fleming *et al.*, 1998), though usage of this technique varies due to multiple factors including damage to feeding equipment and birds selectively eating the larger particles. Thus, there may be a benefit in supplying grit separately, or developing other sources of calcium that help the hens to restore their supplies and prevent bone weakness. Other nutritional changes could involve incorporation of omega-3 content into the diet which has been shown to result in reduced fracture incidence (Toscano *et al.*, in press; Tarlton *et al.*, 2013) possibly by modulating bone metabolism and modeling (Liu *et al.*, 2003; Watkins *et al.*, 2003; Baird *et al.*, 2008).

Recommendation 9 - Investigate and quantify KBD and production losses

Physical conditions that are associated with pain can, if severe enough, induce redistribution of endogenous resources and derail physiological processes that ensure long-term survival, a classical criterion for compromised welfare (Moberg, 1985; Broom, 1991). More specifically, Prunier *et al.* (2013) advocated changes in productivity as an indication of pain and potentially for compromised welfare. As mentioned before, recent work has shown that individual birds with fractures housed in large groups (~350 birds/group) produced eggs that were characterised with reduced breaking strength and thinner shells (Toscano, *in press*). This response may represent a diversion of resources where minerals (*e.g.* calcium) and energy, normally directed towards egg production, must consequently be reallocated to the process of healing bone (Thiruvankadan *et al.*, 2010). Similar results for altered egg production in individual birds were found by Nasr *et al.* (2012b; 2013), although this was assessed in non-commercial conditions (*i.e.*, individual hens isolated in separate cages) in order to link the egg and hen. More critically, work by Nasr *et al.* (2012b; 2013), as well as that by Toscano (*in press*), did not control for natural variation in bird laying capacity. The lack of pre-KF data leaves open the possibility that birds prone to KF may produce less and weaker eggs independent of whether KFs occurred. Differences in egg character after fracture must be shown to be absent beforehand if the measure is to be a valid indicator of welfare. Interestingly, others have been unable to demonstrate a link between egg production and keel fracture at the flock level (Heerkens *et al.*, 2013). The lack of a relationship could be due to high flock-level variance rather than the absence of an effect, indicating the need for research at bird level responses. Alternatively, Whitehead (2004b), reviewing several studies that examined individually housed birds bred for different bone qualities, suggested that little correlation existed between egg production and bone quality. The finding was supported by Gebhardt-Henrich and Fröhlich (2012) who reported more fractures in hens which laid their first egg earlier. As an additional complication in linking the occurrence of fractures with production data, the period in which fractures are seen to most dramatically increase (25 -35 weeks of age) is the one in which birds are coming off peak of lay, thus a drop in production is expected independent of fractures. Therefore, the predicted falloff in egg production resulting from KBD may be subsumed by the drop in egg production as the hen exits the peak of lay period.

Quantification of production endpoints and the loss of productivity associated with KBD can be used as a powerful means to assess changes in animal welfare. More so, because concern for animal welfare is not globally consistent (Lopez, 2007) with the strongest interest in Europe and North America, framing the problems of KBD in terms of productivity losses and compromised profit could provide alternative motivations that move towards an ultimate goal of reducing KBD. This particular argument is powerful as it does not diminish the reality that action on the grounds of compromised welfare is necessary, but rather adds a supplementary dimension that will drive stakeholders to effect change.

Interestingly, it is often suggested that KF result from bone that is weakened by the process of demineralisation to provide adequate amounts of calcium for egg shell formation. If correct, continued egg production should associate with a decrease in bone strength and an increase in the occurrence of KF. While this appears to be the case for the first 20 weeks of egg production, recent comparisons of several studies suggest that rates of fractures actually appear to flatten and possibly fall after 45 wks of age (Toscano *et al.*, 2015; Tarlton *et al.*, 2013; Stratmann *et al.*, 2015b; Petrik *et al.*, 2015). It is possible that this decrease could be attributed to altered behaviour, though use

of an *ex vivo* impact testing protocol with dead hens (Toscano *et al.*, 2013) identified a pattern of decreased susceptibility to fracture (Toscano *et al.*, 2014) that mirrored the on-farm observations of live hens. Further research is needed to determine how this change in fracture occurrence relates to altered bone physiology and egg production during this period.

Conclusions

Keel bone damage represents a welfare and productivity problem for the laying hen industry and, while achievements have been made in understanding the nature and cause of occurrence, we remain far from resolving the issue. The current paper highlights areas of research that would achieve the goal of reducing KBD, encourage adoption of methods to improve the accuracy and reliability of reporting, and provide technical changes that could be adopted.

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