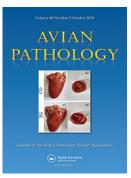


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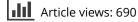
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Influence of different housing systems on prevalence of keel bone lesions in laying hens

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ABSTRACT

The present study aimed to investigate the effect of three housing systems (furnished cages – FC, barns – B, and free-range – FR) on the prevalence and severity of keel bone protrusion and deformations. These health and welfare indicators were measured at the slaughterhouse, using a 4-point scale (0 = absence, 1 = slight, 2 = moderate and 3 = severe). Keel bone deformation was also categorized in relation to the presence of compression over the ventral surface, deviation from a 2D straight plane and deviation from the transverse (C-shaped) or median sagittal (S-shaped) plane. The housing system had a significant effect on prevalence of keel bone deformation, followed by 54.2% in FC and 53.5% in B; however, higher scores for keel bone deformations were more frequent in B systems. Although keel bone protrusion was observed in all laying hen systems, the majority of hens only presented a slight degree (score 1) of protrusion. A positive correlation was obtained for keel bone protrusion and emaciation. The results could be used to initiate detailed investigations into problematic issues that occur during the laying period to improve the health and welfare conditions on farms.

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Bird welfare; housing system; laying hen; keel bone deformation; slaughterhouse

Introduction

The keel bone is an extension of the ventral surface of the sternum, progressing along the midline of the sagittal plane. The keel spans from the cranial *Carina apex* to the caudal tip, with the keel spine tapering off as it approaches the caudal portion of the keel (Casey-Trott *et al.*, 2015). Due to its exposed anatomical location, the keel bone is usually the first point of contact when collisions occur (Scholz *et al.*, 2008), and therefore appears prone to damage (Donaldson *et al.*, 2012). In avian species, the keel serves as an anchor for wing muscle attachment, thereby providing adequate leverage for flight (Claessens, 2009).

In domestic fowl, the flight is not sustained over great distances. Instead, fowl focus flight is associated with their anti-predator survival techniques by short bursts of direct-lifting flight, the ratio between the body weight and pectoral muscle mass being essential for flight (Duncker, 2000). The keel bone also plays an essential role in expanding and contracting the thoracic cavity during the respiratory process. Therefore, it is important to the successful daily function of birds, in both flight and respiratory efficiency (Claessens, 2009). Furthermore, due to their genetic selection for high egg production, laying hens are at risk of rapid depletion of body reserves. Thus, the modern breast conformation of laying hens with a prominent keel bone may be a predisposition factor for its damage (Fleming *et al.*, 2004; Sherwin *et al.*, 2010). Gregory and Robins (1998) demonstrated that scoring the body condition of hens, according to the keel protuberance and breast muscle size, was well correlated with fat and muscle development. Visual assessment of the body condition directly in the slaughter line is straightforward and it is regularly used during meat inspection for condemnation of emaciated carcasses (Graft *et al.*, 2017).

The European Union (EU) Council Directive 1999/ 74/EC established that laying hens may only be housed in either furnished cages or alternative systems, from 1st January 2012. Within the adoption of this regulation, the provision of perches in all types of housing systems became mandatory (European Union, 1999).

The presence of perches has been associated with a higher incidence of damage in the keel bone (Scholz *et al.*, 2008; Sandilands *et al.*, 2009; Hester *et al.*, 2013; Ali *et al.*, 2016). High-impact injuries, unequal wing-loading during wing-flapping, perch use and compression fractures due to osteoporosis are the main causes of keel bone damage which can take different forms, including fractures, deformations, or indentations along the ventral edge of the bone (Pickel *et al.*, 2015). Hester *et al.*, 2013; Harlander-Matauschek *et al.*, 2015).

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Soft perches increase the spread of pressure on the keel bone during perching, reducing keel bone deviations (Stratmann et al., 2015). However, in some countries, raised slatted floors are currently used as perches and, in these cases, the pressure on the keel bone during perching is reduced (Donaldson et al., 2012). Recent research confirms that the keel bone of laying hens is particularly susceptible to fractures (Casey-Trott and Widowski, 2016). The role of collisions as a cause of fractures was investigated by Toscano et al. (2013) using an *ex vivo* protocol to model bone fracture in laying hens, and this showed that greater collision energies resulted in an increased likelihood of fractures and of greater fracture severity. Harlander-Matauschek et al. (2015) also emphasized that more research should be addressed to the relationship between keel bone deformations and keel bone fractures. Impact injuries leading to fractures of keel bone can cause acute and/ or chronic pain, which, in turn, may depress behaviour of laying hens and reduce their productivity, ensuing economic losses (Fleming et al., 2004; Nasr et al., 2012; 2013).

Previous studies have reported differences in prevalence of keel deformations which seem to depend on housing systems. In general, a range of 25–36% was previously reported for commercial layer cage flocks (Sherwin *et al.*, 2010; Wilkins *et al.*, 2011; Petrik *et al.*, 2015), and approximately 48–90% in non-cage flocks in alternative housing systems, such as barn and free-range (Sherwin *et al.*, 2010; Käppeli *et al.*, 2011; Wilkins *et al.*, 2011; Stratmann *et al.*, 2015; Regmi *et al.*, 2016).

Some risk factors closely linked to keel bone deformations, such as different housing designs (Wilkins et al., 2011; Stratmann et al., 2015), perch materials and designs (Pickel et al., 2011; Scholz et al., 2014; Stratmann et al., 2015), nutrition (Riber et al., 2018), reduced breast muscle mass of modern layers (Fleming et al., 2004) or genetic factors (Whitehead, 2004; Stratmann et al., 2016) have been investigated. Sandilands et al. (2009) suggest that the risk of keel bone damage can be reduced by preventively assessing each new housing system. Osteoporosis is also prevalent in hens from cages due to lack of exercise (Lay et al., 2011). However, genetic improvements of hens may influence health and bone strength (Whitehead, 2004; Fleming et al., 2006). To the best of our knowledge, this is the first study measuring keel bone deformations and protrusion through a visual assessment at the slaughterhouse, using a 4-point scoring system. Previous investigations have shown that the prevalence of keel bone deformations within flocks increases throughout the laying period until the end-of-lay. In this context, it is important to collect this health and welfare information in slaughterhouses for laying hens.

The present study aimed to investigate the effect of three housing systems (furnished cages – FC, barns –

B and free range - FR) on the prevalence, severity and morphology of keel bone lesions.

Materials and methods

Collection of data

This study was conducted in a Portuguese poultry slaughterhouse. Sixteen batches of end-of-lay hens were assessed with regard to the condition and integrity of the keel bone. From a total of 41,435 slaughtered hens, 18,920 were from furnished cages (FC); 12,125 from barns (B) and 10,390 from free-range (FR). On average, FC hens were 92 weeks of age (83–102 weeks), B hens were 78 weeks (75–84 weeks) and FR hens were 86 weeks (83–87 weeks), corresponding to body weights of 1.91, 1.88 and 1.89 kg, respectively.

Breeds used in this study were HN brown, Hyline brown, ISA brown, Lohmann brown and Novogen brown which were reared identically in conventional aviary systems until 17 weeks of age. Thereafter, birds were transferred to 16 adult egg production systems, namely six FC (two ISA and four Lohmann), five B systems (three Novogen and two Lohmann) and five FR systems (three Lohmann and two HN).

The B system consisted of a traditional floor system with litter, slats over a manure pit and equipped with perches at different levels. Perches were composed of a circular metal pipe with a diameter of 5 cm. FR systems were characterized by having multi-levels with perches, nest boxes and feeders on each level. Birds were provided with continuous daytime access to land, mainly covered with vegetation, and access to outside via popholes. Perches were of the same material as used in barns.

Data were collected by inspection on the slaughter line immediately after defeathering. From each flock, one hundred hens were randomly assessed for keel bone protrusion, deformations and morphology of damage. Detailed descriptions were used to standardize the lesions as presented in the assessment protocol (Table 1). Deformation scoring was conducted using a 4-point scoring scheme, adapted from Scholz *et al.* (2008), indicating the severity of keel bone damage. Keel bone protrusion was scored using a 4-point scoring system adapted from that described by Gregory and Robins (1998).

Statistical analysis

Pearson's chi-squared test (χ^2) was used to test the differences between the observed and expected frequencies of keel bone deformations and keel bone protrusion with regards to the housing system (furnished cages – FC, barns – B and free range – FR). Data followed a normal distribution and the *P*-value was set at 0.05.

Table 1. Summary of assessment protocol conducte	d directly
at the slaughter line.	

Health and welfare variables	Measures for scoring
Keel bone protrusion	Score 0 = no protrusion (well-developed relatively round breast muscle with limited protuberance at the keel bone) Score 1 = keel less prominent (relatively well-developed breast muscle) Score 2 = keel prominent (moderate development of breast muscle) Score 3 = prominent ridge on the keel (scarce overall breast muscle)
Keel bone deformations	Score 0 = no deformation Score 1 = slight deformation Score 2 = moderate deformation Score 3 = severe deformation
Morphology of deformation	Compression over the ventral surface of the keel Deviation (moderate) from a theoretical 2D straight or a transverse (C-shaped) plane Deviation (severe) from a transverse (C- shaped) or a median sagittal (S-shaped) plane
Ascites, septicaemia and emaciation	Score 0 = absence Score 1 = presence

Spearman's correlation coefficients (P < 0.01) were calculated to determine the relationship between keel bone deformations, keel bone protrusion, emaciation, septicaemia and ascites. Data analysis was carried out using XLStat (release 2011, Addinsoft).

Results

Table 2 shows the frequencies of keel bone deformations (4-point) according to the type of housing system (FC, B and FR).

Other investigators reported similar results and concluded that almost all moderate and severe keel bone deformities were associated with callus formation and most likely resulted from traumatic bone fractures (Fleming *et al.*, 2004; Scholz *et al.*, 2008).

The frequencies of keel bone deformations differed between the housing systems ($\chi^2 = 45.465$, df = 6, P < 0.001). The prevalence of keel bone deformation was significantly higher in FR (60.4%), followed by FC (54.2%) and a lower prevalence was observed in B (53.5%) system. However, the majority of the keel bone deformations were of slight degree (score 1). Moderate (score 2) and severe (score 3) deformations

Table 2. Pearson's chi-square value (χ^2) , the number of degrees of freedom (df) and frequencies of keel bone deformations (4-point) according to housing system (FC, B and FR).

Production system	Keel bone deformations (%)			
	Score 0	Score 1	Score 2	Score 3
Furnished cages (FC)	45.8ª 46.5ª	45.6ª 35.5ª	7.8 ^a 13.0 ^b	0.8 ^a 5.0 ^b
Barns (B) Free-range (FR)	39.6 ^b	35.5 49.1 ^a	13.0 8.7 ^a	5.0 2.6 ^a
$\chi^2 = 45.465$, df = 6, P < 0	0.000			

Note: In each row, different superscript letters indicate statistically significant differences between systems (P < 0.05). were more frequent in B flocks (18.0%), followed by FR (10.3%) and FC (8.6%).

The frequencies of different morphologies (compression, moderate deviation and severe deviation) in relation to the type of housing system (FC, B and FR) are presented in Table 3.

The frequencies of keel bone morphology differed between the housing systems ($\chi^2 = 77.212$; *P* < 0.001). In hens from B and FR systems, the compressive morphology was less frequently observed, differing significantly from FC hens (48.3%). In contrast, hens from B systems showed most frequently a severe deviation of the keel bone (39.2%).

Table 4 shows the frequencies of keel bone protrusion (4-point) according to the type of housing system (FC, B and FR).

Keel bone protrusion was more frequent in hens from B with 93.6%, followed by 88.0% in FR hens and 82.0% in FC hens. However, the majority of hens presented only a slight degree of keel protrusion, ranging from 68.2–73.5% per housing system. Higher scores for keel bone protrusion were observed in hens from B with 26.4% (score 2) and 1.8% (score 3).

Keel bone deformations were positively correlated with keel protrusion (r = 0.590; P < 0.001). Keel protrusion was also positively correlated with emaciation (r = 0.359; P < 0.001) and with septicaemia (r = 0.251; P < 0.001).

Discussion

This study demonstrates that keel bone deformations remain a prevalent welfare problem in all housing systems. A prevalence of 60.4% was observed in FR, 54.2% in FC and a lower prevalence of 53.5% was observed in B systems.

Nicol *et al.* (2006), in a study with 36 barn flocks, obtained a prevalence of 60% for keel bone deformations and fractures. Similar or even higher prevalence was found by Freire *et al.* (2003), Rodenburg *et al.* (2008), and Käppeli *et al.* (2011) in alternative (non-cage) systems. In accordance with Blatchford *et al.* (2016) the prevalence of keel bone deformations within flocks would increase throughout the laying

Table 3. Pearson's chi-square value (χ^2) , the number of degrees of freedom (df) and frequencies of different keel bone shapes (compression, moderate deviation and severe deviation) according to housing system (FC, B and FR).

	Morpho	Morphology of damage (%)			
Production system	Compression	Moderate deviation	Severe deviation		
Furnished cages (FC)	48.3ª	24.9 ^ª	26.8 ^a		
Barns (B)	32.4 ^b	28.5 ^b	39.2 ^b		
Free-range (FR) $x^2 = 77.212$, df = 4, P <	28.7 ^b 0.000	47.3 ^b	24.1 ^c		

Note: In each row, different superscript letters indicate statistically significant differences between systems (P < 0.05).

Table 4. Pearson's chi-square value (χ^2) , the number of degrees of freedom (df) and frequencies of keel bone protrusion (4-point) according to housing system (FC, B and FR).

Production system	Keel bone protrusion (%)			
	Score 0	Score 1	Score 2	Score 3
Furnished cages (FC)	18.0 ^c	68.2 ^{a,b}	13.2ª	0.6ª
Barns (B)	12.0 ^a	73.5ª	13.0 ^a	1.5 ^a
Free-range (FR)	6.4 ^b	65.4 ^b	26.4 ^b	1.8 ^a
$\chi^2 = 68.77$, df = 6, P < 0.	.001			

Note: In each row, different superscript letters indicate statistically significant differences between systems (P < 0.05).

period, reaching a higher incidence (40-78%) in endof-lay hens from alternative husbandry systems (Wilkins et al., 2004; Petrik et al., 2015; Blatchford et al., 2016). However, the most surprising result obtained in the present study was the prevalence of keel bone deformations in FC. It was higher than those obtained in the most recent research conducted in laying hens from caged systems (Sherwin et al., 2010; Wilkins et al., 2011; Petrik et al., 2015). According to Käppeli et al. (2011) current levels of keel bone deformations in cages may raise some concerns about the imminent introduction of higher activity husbandry systems. In agreement with Fleming et al. (2006), more active housing systems do improve bone strength, but do not necessarily result in lower fracture incidences due to the higher probability of traumatic accidents. In addition, Käppeli et al. (2011) considered the rules imposed by EU legislation, which banned the use of conventional battery cages, an unexpected challenge for the laying hen industry, leading to an increased prevalence of keel bone damage. Earlier, Vits et al. (2005) also questioned the intensive use of perches in furnished cages that seemingly increases the occurrence of keel bone deformations. However, various studies indicate that keel bone deformations are more likely to arise in laying hens with weaker bones. In this context, the findings from this study can be related to the current layer hen genotypes which probably are not sufficiently robust to withstand production demands. Osteoporosis occurs with the decrease of the amount of structural mineralized bone tissues leading to bone fragility and higher susceptibility to fracture (Whitehead, 2004). In agreement with Riber et al. (2018), because of the large amounts of calcium required for eggshell production, starting at the onset of lay, it is possible that, for high-producing layers, the cartilaginous keel bone receives less than adequate calcium for proper ossification during the early laying period which continues until approximately 40 weeks of age. Genetic selection is referred to by Fleming et al. (2004) as a means for improving the skeletal characteristics of hens.

In relation to severity of lesions, moderate and severe keel bone deformations were more frequent in hens from B (18.0%), followed by FR (10.3%) and,

finally, FC (8.6%). Several reports concluded that almost all moderate and severe keel bone deformations resulted from traumatic bone fractures and callus formation which are associated with chronic pain (Fleming et al., 2004; Scholz et al., 2008; Nasr et al., 2013; Petrik et al., 2015). These reports showed a high prevalence of moderate and severe keel bone deformations in alternative systems, suggesting that painful fractures are more probable in B and FR systems. These fractures may often be intensified due to the action of the breast musculature which causes additional movement and discomfort. Furthermore, a fractured keel is unlikely to be detected in a commercial laying hen house as easily as a long-bone fracture and the bird may experience prolonged unnecessary suffering as a result of this (Fleming et al., 2004). A strong correlation between keel bone deformation and protrusion (r = 0.590; P <0.001) was found, suggesting that a higher protuberance of the keel bone can be a predisposition factor for the occurrence of keel deformations/fractures. In this respect, a high prevalence of keel bone protrusion was identified in all housing systems. Nevertheless, 68.2-73.5% of laying hens per housing systems presented only a slight degree of keel bone protrusion (score 1). A higher protuberance of the keel bone can be also a consequence of the reduced mobility and therefore the access to resources (feed, water, and nest boxes) (Riber et al., 2018). These negative consequences are likely to differ among housing systems, too. For example, caged hens live in a highly restricted area, and therefore the vital resources are more accessible.

Hens from B presented a significantly higher frequency of moderate protrusion with 26.4% compared with hens from FR (13.2%) and FC (13.0%). In addition, a positive correlation was found between keel bone protrusion and emaciation (r = 0.359; P <0.001), showing that keel protrusion is an indicator of possible emaciation. These findings are in agreement with Sherwin et al. (2010) showing that B hens were the lightest at post mortem and had the greatest prevalence of severe keel protrusion. The type of housing system had a large effect on emaciation prevalence, but all housing systems produced hens that had protruding keel bones (Sherwin et al., 2010). Recently, Grafl et al. (2017), in an experiment assessing health and welfare at the slaughterhouse, showed that hens with better body condition are correlated with significantly higher body weight.

The housing system has a significant effect on the morphology of the keel bone damage ($\chi^2 = 77.212$, *P* < 0.001). Hens from FC presented a significantly higher compressive keel deformation (48.3%) than B hens with 32.4% and FR hens with 28.7%. This could be due to osteoporosis which occurs normally in cage systems and is also characterized by causing more frequent compressive lesion of the keel bone. On the

contrary, B hens showed more frequent severe deviations including D-deviation or S-deviation of the keel bone (score 2 or 3) (39.2%). Significantly more deformations occur in pens equipped with metal perches than in those equipped with plastic perches (Käppeli *et al.*, 2011). In the case of a collision, harder materials such as metal cause more injuries compared with plastic perches. The high frequencies of keel deformations observed in the present study may be related to the fact that all perches were made of metal. On the other hand, the severe deviations which occur more frequently in B may be a result of high impact trauma from high distances. In B system, the resources were on the floor and the perches were at different levels. In FR systems, each level had nest boxes and feeders without the need for hens to move between different levels to access resources; however, the perches were at different levels. For this reason, multilevel perches potentially place laying hens at risk of bone breakage, due to crash landings or impacts with the environment due to movements between different levels to access resources (Scholz et al., 2008; Banerjee et al., 2014).

Selection of specific bone traits associated with bone strength, as well as the related differences in body morphology (i.e. lower index of wing loading), has potential to reduce keel bone damage in commercial settings. Also, the housing environment (i.e. aviary design) may have additive effects (Stratmann *et al.*, 2016). Käppeli *et al.* (2011) recommend bone strength to be considered in genetic selection of modern laying hybrids in order to reduce the prevalence of broken keel bones. Bishop *et al.* (2000) showed that it is possible to select laying hens with stronger bones without compromising laying performance. Further research should be conducted to improve recommendations for aviary design, perch type and the array of perches within the system (Käppeli *et al.*, 2011).

Good keel quality should be a prerequisite for all housing systems, since the keel appears particularly vulnerable to fracture. In future design of aviaries, efforts should be made to include conditions that help prevent accidents and keel bone deformations. It is also important to study the effects of keel bone damage on the affective states of laying hens especially on highly motivated natural behaviour such as perching.

Conclusion

A meaningful overview of keel bone integrity for laying hens can be obtained by using simple scoring scales at the slaughterhouse, highlighting the importance of using simple welfare indicators to be collected at the slaughterhouse. It is crucial to identify several areas of action to implement minimum standards for the protection and welfare of laying hens which can highlight specific problems that must be checked at the slaughterhouse, such as those proposed in the present study.

Disclosure statement

No potential conflict of interest was reported by the authors.

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