

## Effects of housing systems on keel bone damage and egg quality of laying hens

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### Abstract

The aim of this study was to investigate whether keel bone damage is prevalent in laying hens in Greece. The study was conducted in three industrial farms using different housing systems: (a) enriched cages, (b) floor system, and (c) free-range system. One hundred hens per housing system, randomly selected, were evaluated for keel bone damages with the method of palpation. Complementarily, thirty eggs from each farm were selected for the measurement of egg weight, shape index, shell cleanness, shell color, shell breaking force, shell thickness, shell weight, egg yolk color, albumen height, and Haugh unit. The presence of keel bone damage was evident in all housing systems with the significantly highest occurrence being observed in the free-range system (50.00%), followed by enriched cages (24.00%) and floor system (7.00%). Eggs from all three systems had significant differences in all estimated egg quality parameters apart from shell color and Haugh unit.

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### Introduction

The term keel bone damage (KBD) refers to two types of lesions of the keel bone: deviations and fractures. It is a highly frequent problem of commercially raised laying hens reported by many countries mostly in Europe.<sup>1,2</sup> Its prevalence ranges between 1.00% and 97.00% depending, among others, on the housing system and hen's age.<sup>2-6</sup> Keel bone damage is a painful condition with a negative impact on the welfare of laying hens as it has been shown to reduce birds' mobility, increase time spent in the nest at egg laying and prevent the expression of natural behavior such as perching.<sup>7</sup> Moreover, KBD has been found to be linked with bumblefoot and poor feather cover<sup>7</sup> as well as with the consumption of more feed and water.<sup>8</sup> Additionally, there is evidence suggesting that KBD negatively affects productivity as it reduces the number of eggs laid<sup>8</sup> but also downgrade egg quality<sup>8-10</sup> posing financial concerns for producers.

So far, there are no reports of KBD incidence in Greece and the problem is unknown not only to the scientific and professional community but to the producers as well.

This study aimed to determine the prevalence of the KBD in laying hens in three different housing systems in Greece. A complementary purpose was to assess some egg quality traits among the three housing systems.

### Materials and Methods

The study was conducted in three farms of laying hens using different housing systems: (a) enriched cage system (EC), (b) floor system (FL), and (c) free-range system (FR). The enriched cage system consisted of three tiers of cages that were fully equipped and met the requirements of EU Directive 1999/74/EC. Floor system consisted of one level of plastic grade floor. At FR system, hens had access to a grass-covered run during the day and remained inside a barn similar to that of FL system during the night. All three farms had a similar rearing system. Hens from all production systems were fed with a standard commercial layer diet containing 17.50% crude protein, 2,750 kcal metabolizable energy per kg, 3.50% calcium, and 0.85% available phosphorus. Feed and water were offered *ad libitum*. In FR system feeders and drinkers were also available in the run.

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From a total of 32,600 farmed Isa Brown laying hens, 6,200 were housed in EC, 23,000 were housed in FL and 3,400 were housed in FR system. Hens age was ranged from 5.50 (FL system) to seven months old (EC and FR systems). A sample of 100 laying hens per housing system was randomly selected for the assessment of KBD.<sup>2</sup> Within FL system laying hens were fenced, while within EC system, they were taken from the different cages and levels again based on random sampling. The technique of palpation according to Wilkins *et al.* was used for the assessment of the prevalence of KBD.<sup>11</sup> Palpation was performed by running fingers alongside and over the keel bone. It was only determined whether KBD was present (fracture, deformation) or not (completely straight and flat keel bone). Hen day egg production (HDEP) for each housing system was recorded according to the following formula:

$$HDEP = \frac{\text{Total number of eggs produced on a day}}{\text{Number of hens present on that day}} \times 100$$

A total number of 90 eggs, 30 eggs from each housing system, were randomly selected on the same day of KBD evaluation, to determine some internal and external egg quality traits. External egg quality characteristics were: egg weight (EW), egg mass (EM), shape index (SI), shell cleanness (SCL), shell color (SC), shell breaking force (SBF), shell thickness (ST), shell weight (SW), while internal egg quality characteristics were: Egg yolk color (YC), albumen height (AH) and Haugh unit (HU). Haugh Index was determined according to the United States Department of Agriculture (USDA) guidelines.<sup>12</sup>

The EW and SW were measured by electronic digital balance. Egg mass was calculated from hen day egg production and egg mass (EM) as follows:

$$EM = \frac{HDEP \times EW}{100}$$

The SI was estimated using the following equation:<sup>13</sup>

$$SI = \frac{\text{Egg width}}{\text{Egg length}} \times 100$$

Shell cleanness was evaluated by a scoring system on a scale from 1 (very dirty shell) to 5 (completely clean).<sup>14</sup> Shell color was visually evaluated with points 1 (weight) – 5 (dark). Shell breaking force was determined by instrument Egg Force Reader, which measures the weight in kg necessary to break an egg shell (Orka Food Technology Ltd., Herzliya, Israel).

Shell thickness (mm) was measured using a dial gauge micrometer. Yolk color was determined according to Roche yolk color fan ranging from pale yellow (1) to deep orange (15). Albumen height was measured using a tripod micrometer. Based on EW and AH, the HU score was calculated. The HU values were calculated for individual eggs according to the following formula:

$$HU = 100 \log (H + 7.57 - 1.70M^{0.37})$$

where, *H* is albumen height (mm) and *M* is egg weight (g).

**Statistical analysis.** The data were analyzed using the statistical software IBM SPSS Statistics (Version 25.0; IBM Corp. Armonk, USA). A Chi-square test was used to compare the incidence of KBD among the three farming systems evaluated. For the analysis of the egg quality traits, the normality of the data was tested with the Kolmogorov-Smirnov test and the homogeneity of variances was tested with Levene's test. One-way ANOVA was used to compare the average values of the parameters evaluated among farming systems. Post hoc analysis was performed using Bonferroni or Tukey test according to homogeneity test results.<sup>15,16</sup> In cases that the distribution was not normal the comparisons were made with the non-parametric tests Kruskal-Wallis and Mann-Whitney.<sup>17,18</sup> All comparisons were made at a significance level of  $p \leq 0.05$ .

## Results

The incidence of KBD was significantly different among all groups: 50.00%, 24.00%, and 7.00% for FR, EC, and FL, respectively, ( $p < 0.01$ ). In Free-range system, 22.00% of the birds had deformation, 7.00% had only fractures and 21.00% had both deformation and fractures. In the other two systems, only deformation was detected and in FL the deformation was slight.

Hen day egg production, egg weight, and egg mass for each housing system are presented in Table 1. Hen day egg production was significantly higher in EC birds compared to that in the other two systems ( $p < 0.05$ ). However, birds from FR and FL systems had similar HDEP ( $p > 0.05$ ). Eggs from the FL system had significantly lower EW compared to eggs from EC and FR systems ( $p < 0.05$ ). The differences in EW between eggs from FR and EC system were not significant ( $p > 0.05$ ). Superior egg mass was observed in EC system, followed by that of FR and FL systems.

**Table 1.** The effect of housing system on hen day egg production, egg weight and egg mass.

Parameters	Housing system		
	Free-range	Enriched cage	Floor
HDEP (%)	80.49 <sup>b</sup>	89.52 <sup>a</sup>	81.13 <sup>b</sup>
Egg weight (g)*	59.70 ± 0.77 <sup>a</sup>	60.73 ± 0.74 <sup>a</sup>	49.40 ± 0.58 <sup>b</sup>
Egg mass (g)	48.05	54.36	40.08

\* Data are presented as mean ± SE.

<sup>abc</sup> Values within each row with different superscripts differ significantly at  $p < 0.05$ .

The effects of the production system on certain egg quality traits are given in Table 2. The results showed a significant effect of the housing system on most of the internal and external egg quality characteristics studied. Eggs from the FL system had significantly lower SW compared to eggs from EC and FR systems ( $p < 0.05$ ). Eggs from the EC system had similar SW with eggs from the FR system ( $p > 0.05$ ).

**Table 2.** The effect of the housing system on certain egg quality traits. Data are presented as mean  $\pm$  SE.

Parameters	Housing system		
	Free-range	Enriched cage	Floor
Shell color (points)	3.60 $\pm$ 0.13	3.40 $\pm$ 0.16	3.27 $\pm$ 0.13
Shell cleanness (points)	1.13 $\pm$ 0.06 <sup>a</sup>	1.00 $\pm$ 0.02 <sup>ab</sup>	1.03 $\pm$ 0.03 <sup>b</sup>
Shape index (%)	76.60 $\pm$ 0.32 <sup>a</sup>	74.03 $\pm$ 0.47 <sup>b</sup>	76.63 $\pm$ 0.31 <sup>a</sup>
Shell breaking force (kg per cm <sup>2</sup> )	4.90 $\pm$ 0.09 <sup>a</sup>	4.24 $\pm$ 0.25 <sup>b</sup>	4.35 $\pm$ 0.17 <sup>b</sup>
Shell thickness ( $\times 10^{-2}$ mm)	42.00 $\pm$ 0.46 <sup>a</sup>	41.90 $\pm$ 0.51 <sup>ab</sup>	40.37 $\pm$ 0.45 <sup>b</sup>
Shell weight (g)	5.99 $\pm$ 0.08 <sup>a</sup>	6.03 $\pm$ 0.10 <sup>a</sup>	5.16 $\pm$ 0.08 <sup>b</sup>
Albumen height (mm)	7.40 $\pm$ 0.19 <sup>ab</sup>	7.59 $\pm$ 0.19 <sup>a</sup>	6.87 $\pm$ 0.22 <sup>b</sup>
Haugh unit	85.68 $\pm$ 1.12	86.48 $\pm$ 1.11	85.48 $\pm$ 1.25
Yolk color	14.80 $\pm$ 0.09 <sup>a</sup>	14.07 $\pm$ 0.11 <sup>b</sup>	13.03 $\pm$ 0.11 <sup>c</sup>

<sup>abc</sup> Values within each row with different superscripts differ significantly  $p < 0.05$ .

The housing system significantly influenced the cleanness of eggs. Free-range eggshells were dirtier than shells from the other two production systems and this difference was significant between eggshells from FR and FL systems ( $p < 0.05$ ). On the other hand, eggs from the FR system had significantly higher SBF among all types of housing systems ( $p < 0.05$ ).

In the present study, eggs from EC system had significantly lower SI compared to FR and FL system eggs ( $p < 0.05$ ). Furthermore, eggs produced in the FL system had thinner eggshells than the eggs produced in EC and FR system and the difference was significant between eggs from FR and FL systems ( $p < 0.05$ ). Additionally, eggs from the FL system were characterized by a significantly lower AH compared with eggs from the EC system ( $p < 0.05$ ).

The yolk color of eggs was significantly different among all three types of production systems ( $p < 0.05$ ). The darker YC presented from eggs of FR system, followed by that of EC and FL systems.

In all three housing systems studied, significant differences in eggshell color, as well as HU values, were not detected ( $p > 0.05$ ).

## Discussion

The main objective of this study was to investigate whether KBD constitutes a welfare problem in the Greek laying hen industry. The three housing systems commonly applied in Greece have been selected.<sup>19</sup> It is well known that the incidence of KBD is dramatically increased at the age of 25-35 weeks, however, the rates of fractures and new deviations appear to flatten and possibly fall after 45 weeks of age.<sup>1</sup> For this reason it was decided to include hens aging from 5.5 (FL system) to seven-month-old (EC and FR systems) in this study.

The results of the present study confirmed previous reports that the housing system had a significant effect on the incidence of KBD. Stojčić *et al.* found the highest prevalence of KBD, at hens older than 45 weeks of age in fully equipped enriched cages (39.00%) compared to free-range (4.00%), conventional battery cages (1.00%), and enriched cages without equipment (3.00%).<sup>2</sup> Regmi *et al.* reported that 78-week old cage-free and free-range

hens were more likely to incur fractures and deformities during production than conventional-cage hens.<sup>4</sup> Similarly, Eusemann *et al.* stated that in the 72<sup>nd</sup> week of age laying hens housed in-floor system showed significantly more keel bone fractures than hens kept in cages.<sup>6</sup> According to the findings of Petrik *et al.* fracture prevalence of keel bone was 28.40% in cages and 48.30% in floor pens during the production cycle.<sup>3</sup> In particular, the percentage of keel bone fracture for hens aging between 20 and 35 weeks old ranged from 5.00 - 20.00% for cage housing system to 15.00-50.00% for the floor system, respectively.

On the other hand, there are reports in available literature indicating that the adult housing system (conventional vs. furnished cages) does not affect the percentage of keel bone fractures or deviations.<sup>5</sup> These authors, however, found that increased opportunities for exercise provided by an aviary rearing system reduced the prevalence of keel-bone fractures through the end of lay. Preliminary evidence has demonstrated that rearing in non-cage systems improves long bone quality characteristics of pullets lasting to the end of lay.<sup>20</sup> Furthermore, it has been shown that improvement in the composition of long bones (tibiae, humeri) correlates with the improvement of the keel bone.<sup>4</sup>

The differences in the prevalence of KBD observed in the present study compared to former reports could be attributed mainly to the young age of birds. Previous research evidence suggests a linear increase in rates of keel bone damage until hens age is that of 45-50 weeks old and then reaches a plateau.<sup>1,3</sup> Recent studies in bone properties of laying hens in different housing systems throughout the production cycle indicate that long bones of hens become brittle with age, requiring less energy to fracture.<sup>21</sup>

The highest prevalence of KBD detected in hens from FR housing system in the present study agreed with previous reports according to which even though KBD extends across all types of housing systems, 70.00-97.00% of keel bone fractures were reported in various non-cage systems.<sup>1,6,9</sup> It has been previously established that keel bone fractures were caused by high-impact collisions of hens with furnishings in their environment that led to fractures.<sup>19</sup> The highest incidence of KBD found in the EC

housing system compared to the FL housing system could be partly due to the older hens of the EC system and partly due to the lack of perches in the FL system. It seems that perches have a causal role in the occurrence of KBD due to prolonged pressure load on the keel during perching.<sup>9</sup>

In the present study egg production performance was affected by the housing system and better results were achieved in the EC system. These findings were in accordance with previous reports in which HDEP was higher in enriched cages compared to alternative housing systems.<sup>22,23</sup> Contrary to our results, Yilmaz Dikmen *et al.* recorded higher HDEP in the FR system compared to conventional or enriched cages.<sup>24</sup> On the other hand, Ahammed *et al.* found no remarkable differences in HDEP among conventional cage, barn, and aviary systems.<sup>25</sup>

A complementary objective of this study was to assess some egg quality traits among the three housing systems used. As noted in the results, the production system had a significant effect on EW and SW. The higher egg and shell weight observed in eggs from EC and FR system compared to eggs from the FL system were mainly attributed to the youngest age of birds (5.5 months old) from the FL system in comparison with that of EC and FR systems (seven months old). Previous research has shown that EW and by extension SW were increased with hens' age.<sup>26,27</sup> Heavier eggs and higher SW of eggs from the FR system compared to those of eggs from the EC system has been previously reported.<sup>22,26</sup> Other studies, however, indicated that EW was higher in cage systems than in FL or FR systems.<sup>28-30</sup>

In this investigation, the highest egg mass was observed in the EC system. This result is attributed to the highest HDEP of birds in EC as well as to the heaviest eggs produced in this system compared to the other two production systems. The lower EM recorded in FL compared to the FR system was due to similar differences observed in HDEP and EW between the two housing systems. According to the findings of Yilmaz Dikmen *et al.* EM was higher in the FR system compared to the caged housing system.<sup>24</sup> However, Ahammed *et al.* documented no effect of the farming system on EM.<sup>25</sup>

Previous investigations suggest that eggs produced in cage systems were cleaner when compared to those laid in the FL<sup>28</sup> or FR systems.<sup>22</sup> These findings are in agreement with our results according to which FR eggshells were dirtier than shells from the other two production systems with significant differences being observed between eggshells from FR and FL systems. In contrast, other researchers found no significant difference in the percentage of dirty eggs (overall dirt) between furnished cages and non-cage systems.<sup>31</sup>

Similar to our results, the housing system does not seem to significantly affect the eggshell color.<sup>28,29</sup> It has been documented, however, that the lightest colored eggshell was obtained from a deep litter system compared to that from cage systems.<sup>32</sup> Previous studies in alternative

housing systems have shown a tendency towards laying eggs with less intense shell color in FR and organic systems than in litter system.<sup>27</sup>

The egg shape index in this study was higher in FR and FL eggs than in the EC eggs. Similarly, Lewko and Gornowicz also noticed lower egg SI from the cage system than FR and litter system eggs.<sup>29</sup> Denli *et al.* also reported a significant influence of the rearing system (EC and FR) on egg SI at 30 weeks of age.<sup>22</sup> Other researchers also evidenced the impact of the rearing system on egg SI,<sup>23,26,27,32</sup> while Stojčić *et al.* established the opposite.<sup>28</sup>

The results concerning the assessment of the effect of the rearing system on eggshell traits in the available literature are inconsistent. Some reports support that the type of housing system affects ST and SBF,<sup>23,27,30</sup> whereas others claim the opposite.<sup>26,28,32</sup> The present study revealed that eggs from the FR system had significantly higher SBF among all types of housing systems. Moreover, eggs produced in the FL system had thinner eggshells than the eggs produced in EC and FR system, and significant differences were noted between eggs from FR and FL systems. Eggs with thicker shells produced in FR systems in comparison with those produced in EC, or litter systems has been previously demonstrated.<sup>22,29</sup>

In our study, the yolk color of eggs was significantly affected by the housing system. The darker YC was noticed in eggs of FR system, followed by that of EC and FL systems. The pigments in the feed affect yolk color, so the darker YC found in eggs of the FR system was expected due to FR hens' access to the grass. Lewko and Gornowicz also recorded that eggs laid by caged birds were characterized by yolks with the lightest color compared to those laid by hens reared in litter or FR system.<sup>29</sup> Additionally, Sokołowicz *et al.* observed more intense yolk color in eggs from FR and organic systems compared to eggs produced in a litter, at the beginning and end of the laying period.<sup>27</sup> This finding was linked with hens' access to the run where they could feed on green forage in autumn and spring. In other investigations, however, egg YC was not affected by the housing system.<sup>26,28,32</sup>

According to some reports the type of housing system has no impact on HU or AH.<sup>22,27,32</sup> On the other hand, numerous studies have demonstrated an interaction between various types of rearing systems and albumen quality.<sup>23,26,28,29</sup> The results of this study indicated an impact of the housing system on AH, like eggs from the FL system were characterized by a significantly lower AH compared to eggs from the EC system. However, no significant differences in HU values were observed in the eggs from all three production systems used in this study. Albumen height and HU are major determinants of internal egg quality. It is generally accepted that the higher the HU value than 70, the better freshness of eggs and the egg quality. According to USDA guidelines, eggs are graded and labeled as AA, A, and B. Grade AA are eggs of very good

quality. The whites are thick and firm and the yolks are free from any defects. An AA quality egg has a HU greater than 72. In our research, the HU value of eggs from all three housing systems remained above 85, and eggs were graded as AA.

To our knowledge, the present study was the first report on the prevalence of KBD in laying hens in Greece. This first monitoring was carried out at the three most commonly used housing systems currently existing in poultry production in Greece. The highest occurrence of KBD was noticed in the FR system. Moreover, it was shown that most of the estimated egg quality characteristics were different among the rearing systems used in this study. Further investigation is necessary to determine specific risk factors of KBD, its association with the egg quality traits, and strategies for reducing the occurrence and severity of this multifactorial disorder.

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### Conflicts of interest

The authors declare no conflict of interest.

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