

Effects of laying hens housing system on laying performance, egg quality characteristics, and egg microbial contamination

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ABSTRACT: The objective of this study was to compare the performance, egg quality, and microbial contamination of egg shells from hens maintained in different housing systems, such as conventional and enriched cages, litter, and aviaries. The housing system significantly ($P < 0.001$) influenced the performance characteristics. The highest egg production, lowest daily feed consumption, and feed conversion ratio were measured in conventional cages compared to litter and aviaries. Higher egg shell and albumen qualities were observed in conventional cages, whereas hens housed in enriched cages and aviaries laid eggs with a higher yolk index ($P < 0.001$). The housing system significantly ($P < 0.001$) influenced the total count of bacteria on the egg surface and the microbial contamination of *Enterococcus* and *Escherichia coli*. The lowest values for the total count of bacterial contamination ($P < 0.001$) were found in eggs from conventional cages (4.05 log colony-forming units (CFU)/egg) and enriched cages (3.98 log CFU/egg). Eggs from aviaries had 5.49 log CFU per egg, and the highest level of contamination was observed in eggs that were laid on litter (6.24 log CFU/egg). The level of the microbial contamination of egg shells from litter and aviaries was by 2 log CFU higher than in eggs from cages. It could be concluded, from the viewpoint of egg safety, a more suitable substitute for conventional cages are enriched cages and aviaries than litter.

Keywords: cage; aviary; litter; egg production; egg shell; bacterial contamination

INTRODUCTION

The housing system is an external factor that influences both the performance of hens and the egg quality characteristics. Conventional cages have been banned in the European Union since 2012, and the housing of laying hens is permitted only in enriched cages or in alternative systems, such as litter housings, aviaries or free range, to improve the welfare of the hens. Better performance of layers is achieved in conventional cage systems (Tauson et al. 1999; Leyendecker et al. 2001a; Tumova and Ebeid 2003; Hulzebosch 2006; Voslarova et al. 2006; Valkonen et al. 2010), including more eggs, improved

feed consumption and feed conversion ratios, and lower mortality. Tauson (2005) reported that the most developed models of furnished cages provide production results similar to those of conventional cages. More eggs per hen were collected when small groups of hens were housed in enriched cages, but feed consumption was higher (Appleby et al. 2002). The results of Tanaka and Hurnik (1992) indicated that production performance of hens is similar, and relatively high, in both battery cages and aviary systems, but aviaries provide a more comfortable environment for birds than cages.

The egg quality characteristics are better in eggs produced in cages when compared to alternative

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systems. Eggs from cage systems had higher values of Haugh units, albumen and yolk indices (Anderson and Adams 1994; Tumova and Ebeid 2003). Opinions on egg weights are ambiguous. Some investigators (Anderson and Adams 1994; Leyendecker et al. 2001b) observed higher egg weights from hens that were housed in cages, whereas others (Tumova and Ebeid 2005; Pistekova et al. 2006) reported heavier eggs from litter systems.

Egg quality could also be evaluated by the level of microbial contamination. The level of egg shell contamination with bacteria depends on the housing system and is related to temperature and humidity. A higher number of microorganisms was recorded on eggshells from alternative housing systems when compared to cages (Quarles et al. 1970; De Reu et al. 2005, 2006). The microflora of the egg shell is dominated by Gram-positive bacteria, which may originate from dust, soil or faeces, most likely due to their tolerance to dry conditions. The contamination of egg surfaces ranges from 2 to 7 log colony-forming units (CFU) per egg shell (Board and Tranter 1995).

The ban on housing hens in conventional cages has led to a search for suitable housing systems. In terms of the welfare of hens, alternative housing systems are preferable to cages. However, the influence of the housing system on egg quality still needs more investigations. Therefore, this study was conducted to compare the performance of hens and the quality of eggs, expressed as the physical egg quality characteristics and microbial contamination of egg shells, from conventional and enriched cages, housings with litter, and aviaries to find a suitable substitute for conventional cages.

MATERIAL AND METHODS

The experiment lasting for 40 weeks was conducted with 232 Hisex Brown hens, 20 weeks old. The laying hens were housed in conventional cages Eurovent (72 hens, 3 hens per cage, 550 cm² per hen), enriched cages SKN-O 30-60 (Kovobel, Domažlice, Czech Republic) (60 hens, 10 hens per cage, 750 cm² per hen), aviaries (40 hens, 15 birds per m²), and on litter (60 hens, 10 hens per box, 7 birds per m²). They were fed two feed mixtures according to the age of hens (i.e. from 20 to 40 weeks of age and from 41 to 60 weeks of age). The ingredients of the feed mixtures are listed in Table 1. Feed and water were supplied *ad libitum*. The daily photoperiod was set at 15 h light : 9 h

Table 1. Feed mixture composition for laying hens¹

Component (g/kg)	Weeks	
	20–40	41–60
Wheat	343.8	355
Maize	283	303
Soya extracted meal	175	155
Fish meal	15	15
Yeast	15	0
Wheat bran	20	25
Lucerne meal	20	20
Rapeseed oil	30	30
Limestone	80	80
Dicalcium phosphate	10	10
Sodium chloride	2	2
Vitamin-mineral premix ²	5	5
Methionine 50	1.2	0
Calculated content of nutrients (g/kg)		
Crude protein	166.6	153.7
AME _N (MJ/kg)	11.4	11.48
Calcium	34.8	34.8
Phosphorus	5.6	5.6

AME_N = apparent metabolizable energy value

¹laying hens were fed two feed mixtures according to age: from 20 to 40 weeks and from 41 to 60 weeks

²vitamin-mineral premix provided per kg of diet: retinyl acetate 8000 IU, vitamin D₃ 2250 IU, vitamin E 15 mg, menadione 1.5 mg, thiamine 1.5 mg, riboflavin 4 mg, pyridoxine 2 mg, cobalamine 0.01 mg, niacinamide 20 mg, Ca pantothenate 6 mg, biotin 0.06 mg, folic acid 0.4 mg, choline chloride 250 mg, betaine 50 mg, DL-methionine 0.3 g, Co 0.3 mg, Cu 6 mg, Fe 30 mg, I 0.7 mg, Mn 60 mg, Zn 50 mg, Se 0.2 mg

darkness. The light intensity was ca. 10 lux. The room temperature was kept at 20–22°C.

The performance of the hens was recorded with the daily number of hens and eggs, as well as the feed intake per cage, box, and/or aviary. The health of the hens was assessed daily.

Eggs for physical quality determination were collected at 7 h at 28-day intervals for two consecutive days following the procedure of Tumova and Gous (2012) or Skrivan et al. (2013). All laid eggs were examined. A total of 1509 eggs were analyzed. The egg shape index (ESI; %) was determined using the formula:

$$\text{ESI} = (\text{egg width/egg length}) \times 100$$

The albumen, yolk, and shell percentages were calculated using the individual weight of each egg and the weight of its components. The shell weight was measured after drying at 50°C for 2 h. The shell strength (g/cm²) was measured using a destructive method that was performed with a QC-SPA apparatus (TSS, York, UK). The shell thickness (mm) at the equatorial plane was evaluated using a QCT micrometer (TSS) after removing the inner and outer egg shell membranes. The egg shell index was calculated after Ahmed et al. 2005:

$$SI = (SW/S) \times 100; S = 4.68 \times EW^{2/3}$$

where:

SI = egg shell index (g/100 cm²)

SW = shell weight (g)

S = shell surface (cm²)

EW = egg weight (g)

The albumen height and Haugh units (Haugh 1937) were evaluated using a QCH device (TSS). The yolk height (mm) was measured using a digital micrometer head (Mitutoyo, Kawasaki, Japan). The formula for the albumen index (AI; %) calculation is as follows:

$$AI = \{ \text{albumen height} / [(\text{long diameter of albumen} + \text{short diameter of albumen}) / 2] \} \times 100$$

The yolk index (YI; %) was calculated using the formula:

$$YI = (\text{yolk height} / \text{yolk diameter}) \times 100$$

The yolk to albumen ratio (YAR; %) was calculated as:

$$YAR = (\text{yolk weight} / \text{albumen weight}) \times 100$$

Eggs for the measurement of microbial egg shell contamination were collected at 7 h every four weeks, nine times per experiment (from July to March), four eggs from each treatment; 36 eggs per treatment (9 × 4) and 144 eggs per experiment (9 × 4 × 4) in total. Eggs were randomly collected

from middle floor of cages or from nests. The microbiological analysis of the egg shells consisted of counting the *Escherichia coli*, *Enterococci*, and total bacteria using the standard plate count method. Eggs were individually placed into sterile plastic bags with 10 ml of sterile saline with peptone (SSP) and were gently rubbed for 3 min. Next, 1 ml of neat (10⁰) or diluted (10⁻¹–10⁻⁵) SSP was pipetted into Petri dishes, and standard plate count agar (Oxoid, Basingstokes, UK) was added for the total count of bacteria. Agar with a diluted sample was mixed by rotating the plates, and the plates were incubated at 30°C for 5 days after solidification. In addition, 0.1 ml of neat (10⁰) or diluted (10⁻¹–10⁻³) SSP was spread on different media: *Escherichia coli* on MacConkey agar (Oxoid) and *Enterococcus* on Slanetz-Bartley agar (Oxoid), both incubated at 37°C for 48 h. All samples were tested in duplicate. The number of typical colonies on Petri dishes was counted after incubation, after which the colony-forming units (CFU) per egg were calculated and log transformed before performing the statistical analysis.

Statistical analysis. The data were statistically evaluated using One-Way Analyses of Variance (ANOVAs), the General Linear Models (GLM) procedure of the SAS software (Statistical Analysis System, Version 9.1.3., 2003). All differences were considered significant at $P < 0.05$. The results in the tables are presented as the means and standard error of the mean (SEM).

RESULTS

The performance characteristics (Table 2) were significantly ($P < 0.001$) influenced by the housing system. The highest hen-day egg production was recorded from the enriched (92.2%) and conventional (91.3%) cages, compared with the litter housings (79.8%) and aviaries (71.8%). The housing in the enriched cage and alternative systems (aviary and litter) increased the feed conversion ratio

Table 2. Effect of housing system on performance characteristics in laying hens

Characteristic	Housing system				SEM	Significance
	conventional cage	enriched cage	aviary	litter		
Hen-day egg production (%)	91.3 ^a	92.2 ^a	71.8 ^c	79.8 ^b	0.69	< 0.001
Daily feed consumption (g/hen)	121 ^b	137 ^a	131 ^a	136 ^a	1.1	< 0.001
Feed conversion ratio (feed/egg mass)	2.24 ^c	2.38 ^b	2.92 ^a	2.87 ^b	0.029	< 0.001

^{a-c}means followed by different letters in the same row are significantly different

Table 3. Effect of housing system on physical egg quality characteristics in laying hens

Characteristic	Housing system				SEM	Significance
	conventional cage	enriched cage	aviary	litter		
Egg weight (g)	60.1 ^b	61.8 ^a	62.2 ^a	58.9 ^c	0.16	< 0.001
Egg shape index (%)	76.0 ^c	77.2 ^{ab}	77.6 ^a	76.8 ^b	0.08	< 0.001
Albumen index (%)	8.6 ^c	10.0 ^a	10.2 ^a	9.2 ^b	0.08	< 0.001
Albumen percentage (%)	62.6 ^b	64.0 ^a	64.2 ^a	62.8 ^b	0.08	< 0.001
Haugh units	88.5 ^a	81.3 ^b	78.2 ^c	83.0 ^b	0.40	< 0.001
Yolk index (%)	44.6 ^b	46.2 ^a	46.2 ^a	45.2 ^b	0.11	< 0.001
Yolk percentage (%)	26.7 ^a	25.5 ^b	25.0 ^c	26.6 ^a	0.07	< 0.001
Shell thickness (mm)	0.377 ^b	0.379 ^b	0.387 ^a	0.376 ^b	0.0008	< 0.001
Shell strength (g/cm ²)	4930 ^a	4743 ^b	4665 ^b	4794 ^{ab}	25.4	0.002
Shell percentage (%)	10.6 ^a	10.5 ^b	10.7 ^a	10.7 ^a	0.02	0.024
Egg shell surface (cm ²)	71.6 ^b	72.9 ^a	73.2 ^a	70.6 ^c	0.13	< 0.001
Shell index (g/100 cm ²)	8.9 ^b	8.9 ^b	9.1 ^a	8.9 ^b	0.02	< 0.001
Yolk and albumen ratio (%)	42.9 ^a	40.1 ^b	39.2 ^b	42.7 ^a	0.17	< 0.001

^{a–c}means followed by different letters in the same row are significantly different

(2.38, 2.92, and 2.87) and daily feed consumption (137, 131, and 136 g). The lowest values of these characteristics were recorded from conventional cages (2.24 and 121 g).

Results of egg quality characteristics are provided in Table 3, and it is clear that all of the internal and external egg quality characteristics were influenced by the housing system. Significantly ($P < 0.001$) heavier eggs were laid by the hens from the aviaries (62.2 g) and enriched cages (61.8 g), while the lightest eggs were from the hens housed on litter (58.9 g). Table 3 provides the percentage of egg components related to egg weight. Eggs with lower weights (litter and conventional cages) had lower albumen percentages ($P < 0.001$; 62.8%, 62.6%, respectively), while the opposite condition occurred with heavier eggs from enriched cages (64.0%) and aviaries (64.2%). Accordingly, the yolk and albumen ratios were significantly ($P < 0.001$) higher in eggs from the hens that were housed in

conventional cages and on litter. The interior egg quality, expressed by the albumen and yolk indices, was significantly ($P < 0.001$) higher in eggs from enriched cages (10.0 and 46.2%) and aviaries (10.2 and 46.2%). However, the highest value of Haugh units ($P < 0.001$) came from eggs laid in conventional cages (88.5), while the lowest value was from eggs laid in aviaries (78.2). A significant effect of the housing system was found for the egg shell quality parameters. Although thicker egg shells ($P < 0.001$) and higher shell percentages ($P = 0.024$) were detected in eggs from aviaries (0.387 mm and 10.7%), eggs laid in conventional cages had significantly ($P = 0.002$) higher eggshell strength (4930 g/cm²).

Table 4 provides the results of the microbial contamination of egg shells from the four housing systems. A significant effect of the housing system was found for the total count of bacteria on the egg surface and in the microbial contamination

Table 4. Effect of housing system on microbial contamination of egg shells

Characteristic of egg shell contamination (log CFU/egg shell)	Housing system				SEM	Significance
	conventional cage	enriched cage	aviary	litter		
Total count of bacteria	4.05 ^c	3.98 ^c	5.49 ^b	6.24 ^a	0.101	< 0.001
<i>Escherichia coli</i>	3.40 ^c	3.50 ^c	5.22 ^b	5.68 ^a	0.108	< 0.001
<i>Enterococcus</i>	1.50 ^b	1.46 ^b	3.33 ^a	3.58 ^a	0.129	< 0.001

CFU = colony-forming units

^{a–c}means followed by different letters in the same row are significantly different

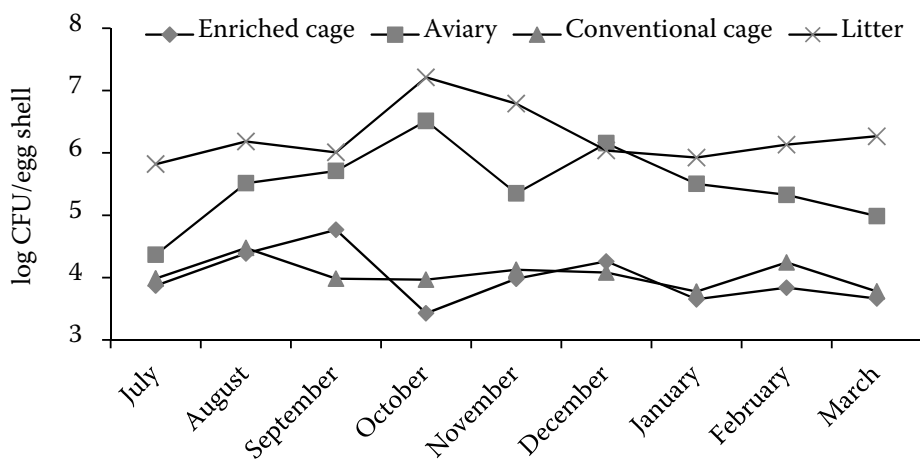


Figure 1. Effect of housing system on total count of bacteria on shells during experiment

of *Enterococcus* and *Escherichia coli*. Eggs from both cage systems had significantly ($P < 0.001$) lower values of egg shell contamination for both the total count of bacteria and *Escherichia coli*; conventional cages had 4.05 and 3.40 log CFU/egg, respectively, and enriched cages had 3.98 and 3.50 log CFU/egg, respectively. The highest contamination was discovered in eggs from litter housings (6.24 and 5.68 log CFU/egg). In addition, the results indicate that eggs from conventional cages (1.50 log CFU/egg) and enriched cages (1.46 log CFU/egg) had significantly ($P < 0.001$) lower numbers of *Enterococci* on the egg shell surface. The egg shells from alternative housing systems (litter housings and aviaries) were 100 times more contaminated than eggs from cages.

Changes in total count of bacteria on shells from different housing systems during the whole experimental period are evident from Figure 1. In alternative housing systems, there was recorded a gradual increase of contamination in the first months of observation. The peak was observed in October and then the values slowly decreased in winter season. Conversely, microbial contamination of shells from cages systems was without substantial changes and fluctuated around 4 log CFU/shell.

DISCUSSION

The housing system influenced the performance characteristics of laying hens. Better results were achieved in cages (e.g. lower feed consumption and conversion and higher egg production) compared to alternative systems. These performance results are in agreement with studies by Leyendecker et al. (2001a) and Tumova and Ebeid (2003). Addi-

tionally, Voslarova et al. (2006) obtained a higher number of eggs, a higher number of eggs per hen per day, and a higher egg mass weight per hen per day in a cage system. In addition, Hulzebosch (2006) reported that hens housed in cages had higher egg production than hens housed in litter housings and aviaries, whereas a higher number of eggs were laid in aviaries due to the ability to lay eggs longer. Michel and Huonnic (2003) also showed lower egg production in aviaries than in cages, but the difference was smaller than in the present study. Lower egg production in aviaries could be caused by the fact that some eggs were laid on the floor, perhaps eaten, and not counted. The hens that were housed on litter had by approximately 10% higher feed consumption per day than hens from cages (Tauson et al. 1999). Conversely, Tactacan et al. (2009) compared conventional battery cages and enriched cages and found that the cage design did not affect the hen-day egg production, feed consumption or egg weight. The size of the group of hens is also important. A high level of productivity and good egg quality can be obtained in large, furnished cages (Huneau-Salaun et al. 2011).

In the present study, heavier eggs were laid in enriched cages and aviaries. These results are in agreement with Anderson and Adams (1994) and Leyendecker et al. (2001b), who observed higher egg weights from hens housed in cages, whereas Tumova and Ebeid (2005) and Pistekova et al. (2006) recorded heavier eggs on litter. Furthermore, the lower weight of eggs from hens housed in conventional cages is likely related to greater egg production.

A higher quality of egg shell and albumen was found in cages. Accordingly, Anderson and Adams

(1994) and Tumova and Ebeid (2003) reported higher values of Haugh units and albumen indices, as well as yolk indices in eggs from cage systems. In the present experiment, eggs with a higher yolk index were laid in aviaries and conventional cages. In addition, numerous studies focused on egg shell quality indicated a higher quality of eggs from cages (Tumova and Ebeid 2005; Lichovnikova and Zeman 2008; Tumova et al. 2009). Although eggshell thickness was lower in eggs that were produced in cages, the eggshell strength was higher (Tumova et al. 2011). On the other hand, other authors reported a higher number of cracked eggs from cage systems compared to aviaries (Tauson et al. 1999), litter systems (Voslarova et al. 2006), and free range and organic systems (14% vs 10 and 5%; Hidalgo et al. 2008). Contrary to the results of our experiment, Platz et al. (2009) reported that the egg quality and health of hens housed in furnished cages did not exhibit any significant advantage over those housed in aviaries.

Lower bacterial contamination on egg shells was observed on eggs from conventional and enriched cage systems compared to alternative housing systems, such as aviaries and litter. Numerous authors also mention that eggs from alternative housing systems are more contaminated by microorganisms on their surfaces (Quarles et al. 1970; De Reu et al. 2005, 2006; Huneau-Salaun et al. 2010; Mallet et al. 2010). The higher bacterial contamination in eggs from alternative housing systems was caused by a higher probability of eggs contact with faeces or bedding material. This same result was reported by Singh et al. (2009), who determined that eggs from cages had lower *Escherichia coli* and coliform contamination than those from nest-boxes and the floor. The percentage of eggs laid in the nest and the disposition of the equipment (nest, perch, and scratching area) may significantly affect the bacterial load of the egg shell. Many other factors, such as cracks or dirt on the shell, dust concentration in the rooms or season, may also influence egg shell bacterial contamination (Mallet et al. 2010). The initial egg shell contamination was correlated with the concentration of aerobic bacteria in the air of the poultry houses (Quarles et al. 1970; De Reu et al. 2005, 2006). Moreover, *Staphylococcus* spp. dominates on the shells of table eggs and also appears to be the most dominating species in the air in the poultry houses (De Reu et al. 2006). Higher bacterial contamination of egg shells can increase the

penetration of microorganisms into egg content. Furthermore, the penetration depends on other factors such as e.g. presence of cuticle, egg shell and membrane quality or storage conditions. De Reu et al. (2008) stated a limit of 5 log CFU/egg which could be considered to refer to egg shells with an acceptable hygienic quality. From this point of view, especially the litter housing seems to be the most risky.

A decrease of bacterial contamination on egg shells in the winter period was probably caused by low temperature. Seasonal influence on the egg shell contamination is also evident from results of experiment of Mallet et al. (2006). Significantly ($P < 0.05$) lower total counts of aerobic bacteria and *Enterococci* on the egg shells were ascertained in the winter period than in summer. Also in one of the experiments performed by De Reu et al. (2005) a possible seasonal influence on the eggshell contamination was found with a decrease in the winter period (up to > 0.5 log CFU/eggshell) for total count of aerobic and Gram-negative bacteria. Egg shell contamination correlates with concentration of bacteria in hall (Quarles et al. 1970; De Reu et al. 2005, 2006). Saleh et al. (2003) showed that air in conventional cage, enriched cage, and aviary contained 5.4, 5.6, and 6.3 log CFU/m³ of bacteria in summer and 5.6, 5.1, and 5.7 log CFU/m³ of bacteria in winter. On the other hand, Quarles et al. (1970) could not always confirm their supposition of the influence of the season on the shell contamination. But some results of Quarles et al. (1970) suspected that high temperatures might lead to an increased eggshell contamination.

CONCLUSION

A higher quality was found in eggs laid in cage housing systems and aviaries than in those on litter. In alternative housing systems, bacterial contamination of egg shells fluctuates more than in cages. From the viewpoint of performance of laying hens and egg quality, a suitable substitute for conventional cages are enriched cages and aviaries.

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