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Comparison of eggshell hygiene in two housing systems: Standard and furnished cages

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Abstract 1. The hygienic properties of eggs produced in two designs of furnished cage were investigated and compared to two standard cage designs.
2. At 28, 37, 47 and 58 weeks of age, the proportion of dirty eggs was higher in one of the furnished cage designs while, in the other, it was similar to standard cages.
3. At 27, 33 and 60 weeks, the bacterial load on the eggshell (total aerobic bacteria and enterococci) was higher in furnished cage designs. A seasonal effect was observed with lower counts at 60 weeks (winter) than at 27 weeks (summer).
4. More dirty eggs and a higher bacterial load were observed in eggs laid outside the nests, which suggests egg hygiene in furnished cages could be similar to standard cages if the equipment in furnished cages was improved to enhance nest laying.

INTRODUCTION

The 1999 European Directive (European Commission, 1999), to improve animal welfare, has specified that, if cages have to be used after 2012 for laying hens, they should be a furnished cage design with nests, perches and dust bath. Nevertheless, furnished cages may have consequences for egg hygiene by increasing the percentage of cracked and dirty eggs (Duncan *et al*., 1992; Carey *et al*., 1995; Abrahamsson and Tauson, 1998; Wall and Tauson, 2002; Michel *et al*., 2003) or eggshell bacterial contamination. Just after laying, the content of the egg from a healthy hen is generally sterile (Mayes and Takeballi, 1983); nevertheless, the eggshell surface will be rapidly contaminated by environmental bacteria present in faeces, dust or breeding material and some of these bacteria may be pathogenic for humans and able to contaminate the egg content (Board and Tranter, 1994). The incidence of egg contamination may be correlated with the bacteria present in the environment where the eggs are laid (Harry, 1963; Protais *et al*., 2003*b*, *c*).

The objective of our work was to analyse the hygiene (percentage of dirty eggs and eggshell bacterial contamination) of the eggs produced in two furnished cage designs available in France from two manufacturers, to compare them to standard cages, and to explore these variables at different egg laying locations in the furnished cages. Performance and egg quality in these various designs are described in a companion article (Guesdon *et al*., 2006).

MATERIALS AND METHODS

Animals and housing conditions

Laying hens (2028, ISA Brown strain) were housed in 4 different cage designs. The cage units were of two categories: two standard designs (S), with access only to feeders and drinking nipples and differing in number of hens per cage, 5 hens/cage for S5 and 6 hens/cage for S6; two furnished cage designs (F), with 15 hens per cage (F15M and F15P) differing mainly in the arrangement of the equipment (perch, nest and dust box). All were in accordance with European standards. The hens were housed between 18 and 70 weeks. The two furnished cage designs as well as housing conditions are described in detail in a companion paper (Guesdon *et al*., 2006). Both standard cage units were housed in the same room and both furnished cage units in another

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room in the same building with similar housing conditions. Each room contained two batteries (one battery for each cage design). In half the cages of each design, hens were beak trimmed whilst in the other halves they were not. Each category was housed alternately in adjacent cages.

Dirty eggs

When the hens were 28, 37, 47 and 58 weeks of age, the percentage of dirty eggs for each cage was estimated daily over 4 d (Tuesday to Friday) by visual examination. In furnished cages, the eggs were collected by egg laying location and the percentage of dirty eggs at each location was determined.

Microflora analysis

When the hens were 27 and 60 weeks of age, 20 batches of three eggs (only visually clean eggs suitable for consumption) from each cage design were analysed. To estimate accurately the actual bacterial load, the eggs were randomly sorted by hand in the carton filler-flats from the total daily egg production and represented about 10 to 20% of the egg production of one cage unit. To prevent cross-contamination, gloves were worn and changed between each experimental unit. Each batch of three eggs was placed in a sterile plastic bag and stored for 24 h at 4°C. Then, 200 ml of peptone water was added and the eggs gently rubbed through the bag for 3 min manually (one minute for each egg) to remove the bacteria from the egg surface. At 33 weeks of age, the eggs were collected from furnished cages at different laying locations (nest, dust bath or cage level); the procedure was similar, but as fewer eggs were available from dust bath and cage floor, only 10 batches of two eggs were picked from each egg laying location and cage design. The protocol was modified by adding 125 ml in the sterile bag before rubbing the eggs for 2 min. The peptone solution was thus considered as the first dilution and used immediately for bacterial analysis. The total number of mesophilic aerobic bacteria was estimated by surface plating of 0·1 ml sample on PCA agar (AES ref. AEB 150702). Enterococci were estimated by surface plating of 0·1 ml sample on m-Enterococcus agar (DIFCO ref. A5074617). The plates were incubated for 48 h at 30°C for colony counting. The results were expressed as the log of colony forming units (CFU) per cm^2. The eggshell surface (S) was calculated from the mean egg weight (W) at the different periods using the Bonnet and Mongin (1965) equation ($S = 4{\cdot}68W^{0{\cdot}66}$).

Statistics

Statistical analyses were carried out using an analysis of variance (ANOVA) with the Statview software 5·0 for Windows (SAS Institute Inc., 1992–1998). Two-way ANOVA was used for studying the combining effect of cage design and different age periods (28, 37, 47 and 58 weeks for the percentage of dirty eggs; 27, 33 and 60 weeks for bacterial counts). PLSD Fisher tests for multiple comparisons were carried out when significant differences were detected. The number of dirty eggs at the three laying locations were analysed in each furnished cage design by a test of χ^2 using a two-way table of counts cross classified as dirty/not dirty eggs × location and followed by a χ^2 pair comparison when overall comparison was significant ($P < 0{\cdot}05$). Because of high mortality in non-beak trimmed hens, which meant that too few eggs were laid in some of the cages, only beak trimmed hens were statistically analysed for dirty eggs. The eggs were randomly collected by row from the overall egg production in each cage design. For bacterial counts, therefore, eggs from both beak trimmed and non-beak trimmed hens were sampled for bacterial counting.

RESULTS

Dirty eggs

The percentage of dirty eggs (Table 1) differed between cage designs ($P < 0{\cdot}001$), but was not affected by hen age ($P = 0{\cdot}61$). The interaction between time and cage design was not significant ($P = 0{\cdot}92$). The mean percentages in S6 (4·9%), S5 (4·9%) and F15M (3%) were similar ($P = 0{\cdot}77$, 0·29 and 0·39 for S6 *vs* S5, F15M *vs* S6 and S5, respectively) and significantly lower than the percentage in F15P (7·1%) ($P < 0{\cdot}001$ for the three comparisons).

In furnished cages, the percentages of dirty eggs for the three egg laying locations (nest, dust bath and cage) were not affected by hen age ($P = 0{\cdot}15$, 0·21 and 0·67, respectively); no interaction was observed between time and cage design ($P = 0{\cdot}55$, 0·67 and 0·20). The percentages of dirty eggs amongst the eggs laid at each location were significantly higher in F15P than in F15M when the eggs were laid in the nest or in the dust bath ($P < 0{\cdot}001$ and $P = 0{\cdot}004$, respectively). Nevertheless, when the eggs were laid in the rest of the cage, the percentage of dirty eggs tend to differ inconsistently between the two furnished cage designs depending on age of sampling (cage × age interaction, $P = 0{\cdot}2$).

When comparing the three egg laying locations, the percentage of dirty eggs was different at each one for F15P and F15M

Table 1. *Mean percentage (±SE) of dirty eggs laid at different locations of 4 cage designs (two conventional ones: S5 and S6 and two furnished ones: F15P and F15M) at 4 ages (28, 37, 47 and 58 weeks). The percentage of dirty eggs was calculated amongst all laid eggs when comparing cage designs. When comparing locations in the two furnished cage models, it was calculated on the basis of the eggs laid at each location*

Cage design	Location			
	Whole	Nest	Dust bath	Other parts
At 28 weeks of age[1]				
S5	5·6 ± 0·8			
S6	5·1 ± 0·7			
F15P	6·3 ± 1·1	3·6 ± 0·9	25·0 ± 7·2	2·9 ± 1·6
F15M	3·8 ± 0·9	1·5 ± 0·5	7·8 ± 2·5	14·3 ± 6·8
Means	5·2 ± 0·9	2·6 ± 0·7	16·4 ± 4·9	8·6 ± 4·2
At 37 weeks of age[1]				
S5	4·7 ± 0·7			
S6	5·5 ± 0·9			
F15P	6·4 ± 1·0	5·1 ± 1·3	13·6 ± 5·0	4·6 ± 3·2
F15M	2·4 ± 0·5	1·8 ± 0·6	6·1 ± 3·3	0·5 ± 0·5
Means	4·8 ± 0·8	3·5 ± 0·9	9·9 ± 4·2	2·6 ± 1·9
At 47 weeks of age[1]				
S5	4·3 ± 0·8			
S6	4·0 ± 0·7			
F15P	6·7 ± 1·3	6·2 ± 1·5	11·6 ± 4·3	2·9 ± 2·2
F15M	3·0 ± 1·1	2·4 ± 1·1	6·7 ± 3·8	10·7 ± 8·5
Means	4·5 ± 1.0	4·3 ± 1·3	9·2 ± 4·1	6·8 ± 5·4
At 58 weeks of age[1]				
S5	4·7 ± 0·9			
S6	5·1 ± 0·7			
F15P	9·1 ± 1·4	7·4 ± 1·3	28·9 ± 7·4	9·9 ± 6·5
F15M	2·7 ± 0·9	2·3 ± 0·9	12·7 ± 6·8	1·4 ± 1·4
Means	5·4 ± 1·0	4·9 ± 1·1	20·8 ± 7·1	5·7 ± 3·9
Probability[2]				
Cage	<0·001	<0·001	<0·01	0·71
Age	0·61	0·15	0·21	0·67
Cage × age	0·92	0·55	0·67	0·20

[1] Number of observations: S5, $n = 51$; S6, $n = 48$; F15P, $n = 16$; F15M, $n = 12$.
[2] Although means and SE are presented on the original scale, results of ANOVA were obtained for repeated measurements after angular transformation of the data.

($P < 0·001$ for both designs). In F15P, the percentage of dirty eggs was higher when laid in the dust bath than in the nest or the rest of the cage ($P_{dust/nest}$ and $P_{dust/cage} < 0·001$). Nevertheless, there was no difference between the eggs laid in the nest and in the rest of the cage ($P_{nest/cage} = 0·20$). In F15M, the percentage of dirty eggs amongst the eggs laid at each location was higher when laid in the rest of the cage than in the dust bath or the nest ($P_{cage/dust} = 0·02$ and $P_{cage/nest} < 0·001$). Moreover, eggs laid in the dust bath were more often dirty than those laid in the nest ($P < 0·001$).

When taking into account the percentage of eggs laid at each location, the overall percentage of dirty eggs was 4% in the nest, 14·1% in the dust bath and 5·9% in the rest of the cage.

Bacterial load

The mean egg weight was 63 g at 27 weeks, 66 g at 33 weeks and 68 g at 60 weeks. Bonnet and Mongin's equation (1965), yields eggshell surfaces of 74, 76 and 78 cm^2, respectively, which were used to estimate the log CFU/cm^2.

Cage design and hen age similarly affected the bacterial load on the egg, which was evaluated either as total mesophilic aerobic bacteria or enterococci (Table 2). Interaction between the two variables was also significant. At 27 weeks, the total bacterial count was lowest in S5 cages and highest in F15P and S6, which both had similar bacterial loads on eggs; the bacterial count in F15M was intermediate and different from those observed in S5 and S6–F15P. At 60 weeks, bacterial counts were similar in S5 and S6 and lower than in F15M and F15P. At 33 weeks, an intermediate pattern was observed,

Table 2. *Evolution of total mesophilic aerobic microflora and enterococci on the egg shell at three ages (27, 33 and 60 weeks) in 4 cage models (two conventional ones: S5 and S6 and two furnished ones: F15P and F15M)*

Cage design	Age (weeks)			Probability		
	27	33	60	Design	Age	Design × age
Total bacteria[1]						
S5	$2·44^{c} \pm 0·08$	$2·28^{c} \pm 0·06$	$2·29^{c} \pm 0·06$	<0·0001	<0·0001	<0·0001
S6	$3·15^{a} \pm 0·04$	$2·80^{ab} \pm 0·07$	$2·18^{c} \pm 0·09$			
F15P	$3·22^{a} \pm 0·04$	$2·97^{a} \pm 0·06$	$3·06^{a} \pm 0·06$			
F15M	$2·74^{b} \pm 0·03$	$2·69^{b} \pm 0·05$	$2·54^{b} \pm 0·07$			
Means	$2·89^{A} \pm 0·05$	$2·75^{B} \pm 0·06$	$2·51^{C} \pm 0·07$			
Enterococci[1]						
S5	$0·56^{c} \pm 0·13$	$0·67 \pm 0·15$	$0·36 \pm 0·11$	<0·0001	<0·0001	0·007
S6	$1·46^{a} \pm 0·08$	$1·51 \pm 0·16$	$0·40 \pm 0·13$			
F15P	$1·47^{a} \pm 0·07$	$1·29 \pm 0·08$	$0·69 \pm 0·16$			
F15M	$1·00^{b} \pm 0·11$	$1·20 \pm 0·12$	$0·51 \pm 0·14$			
Means	$1·12^{A} \pm 0·10$	$1·20^{A} \pm 0·13$	$0·49^{B} \pm 0·14$			

[1] Log CFU/cm^2, mean ± standard error for 20 batches of three eggs (27 and 60 weeks), 30 batches of two eggs (F cages at 33 weeks) or 10 batches of two eggs (S cages at 33 weeks).
[a–c] Means in the same column with no common superscripts differ significantly ($P < 0·05$).
[A–C] Means in the same line with no common superscripts differ significantly ($P < 0·05$).

particularly due to S6 bacterial counts. For enterococci, lower counts were found compared to the total bacterial load. However, cage design and age affected enterococci in a similar way to total bacteria loads, the effect being statistically significant only at 27 weeks. For both bacterial populations, the mean eggshell contamination in the 4 cage designs was lower at 60 weeks ($2{\cdot}51 \pm 0{\cdot}05$ log CFU/cm^2 for total bacteria and $0{\cdot}49 \pm 0{\cdot}07$ log CFU/cm^2 for enterococci) than at 27 weeks ($2{\cdot}89 \pm 0{\cdot}04$ log CFU/cm^2 for total bacteria and $1{\cdot}12 \pm 0{\cdot}07$ log CFU/cm^2 for enterococci). At 33 weeks, total bacteria counts were intermediate ($2{\cdot}75 \pm 0{\cdot}04$ log CFU/cm^2) and different from 27- and 60-week counts, but enterococci counts ($1{\cdot}2 \pm 0{\cdot}07$ log CFU/cm^2) were similar to those at 27 weeks and different from those at 60 weeks.

When data were analysed with regard to cage systems (standard or furnished), significantly higher bacterial counts were observed in furnished cages ($2{\cdot}86 \pm 0{\cdot}03$ log CFU/cm^2 for total bacteria and $1{\cdot}06 \pm 0{\cdot}05$ log CFU/cm^2 for enterococci) compared to standard cages ($2{\cdot}52 \pm 0{\cdot}04$ log CFU/cm^2 for total bacteria and $0{\cdot}77 \pm 0{\cdot}07$ log CFU/cm^2 for enterococci). Expressed as the number of bacteria per egg, this represents $41{\cdot}86 \pm 4\,\mathrm{E}+03$ CFU/egg for total bacteria and $1{\cdot}39 \pm 0{\cdot}3\,\mathrm{E}+03$ CFU/egg for enterococci in standard cages; and $74{\cdot}09 \pm 5\mathrm{E}+03$ CFU/egg for total bacteria and $1{\cdot}88 \pm 0{\cdot}3\mathrm{E}+03$ CFU/egg for enterococci in furnished cages.

For both total bacteria and enterococci, more bacteria (Table 3) were counted on eggs laid outside the nest regardless of furnished cage design.

DISCUSSION

Two zoonoses, *Salmonella* and *Campylobacter*, are responsible for the majority of cases of food-borne outbreaks in Europe (Cavitte, 2003). Eggs are often implicated in salmonellosis because of their consumption as a raw product. Egg contamination can occur through the vertical or horizontal route; recently, the latter has been more common in Europe (Messens *et al*., 2005). The bacterial load on the eggs and, therefore, the extent of contamination of their surface, influences the prevalence of trans-shell penetration (Schoeni *et al*., 1995; Braun *et al*., 1999). In our study, the percentage of dirty eggs differed markedly in the two furnished cage designs. One of the designs (F15M) had, like the standard cages, a low incidence of dirty eggs, in contrast to the other design (F15P). Smith *et al*. (1993) found the percentage of dirty eggs tended to be higher when no door was present on the nest and dust bath. The contamination of the nest and dust bath was consistent with this trend because the nests and dust bath without doors were significantly dirtier than those with doors. Appleby *et al*. (2002) also noticed that eggs laid in the dust bath were dirtier. In the present experiment, the eggs laid in the F15P design dust bath were also frequently more dirty than those laid in the F15M dust bath. Nevertheless, the dust baths in both designs were similar and composed of an Astroturf carpet. They differed in the location of the Astroturf carpet, situated at the rear of the cage in F15M, whereas in F15P, it was in the front part of the cage with a perch placed parallel and very close to it. The hens perched on it, dropped their excreta on the carpet or on the part between the carpet and the back of the cage with an accumulation of excreta at both locations. Thus, the eggs laid at this location were more likely to become dirty than anywhere else. The part of the cage just behind the longitudinal perch, narrow and less accessible, was definitely less visited by the hens, thus allowing a higher accumulation of the excreta. Appleby *et al*. (2002) reported a similar drawback in their furnished cage designs. Therefore, the higher percentage of dirty eggs in F15P is partly explained by the location of the dust bath. In addition, the percentage of dirty eggs laid in the nest was higher in F15P than in F15M and contributed to the overall higher incidence of dirty eggs in this cage design compared to the other because a high percentage of eggs

Table 3. *Mesophilic aerobic microflora and enterococci on the shell of eggs laid at 33 weeks of age at different locations in furnished cages (nest, dust bath and the rest of the cage)*

Cage design	Location			Probability		
	Nest	Dust bath	Other parts	Location	Design	L × D
Total bacteria[1]						
F15P	$2{\cdot}65 \pm 0{\cdot}05$	$3{\cdot}18 \pm 0{\cdot}08$	$3{\cdot}11 \pm 0{\cdot}10$	<0·0001	<0·0001	0·21
F15M	$2{\cdot}50 \pm 0{\cdot}07$	$2{\cdot}78 \pm 0{\cdot}06$	$2{\cdot}83 \pm 0{\cdot}09$			
Enterococci[1]						
F15P	$0{\cdot}99 \pm 0{\cdot}12$	$1{\cdot}48 \pm 0{\cdot}08$	$1{\cdot}45 \pm 0{\cdot}14$	0·013	0·52	0·66
F15M	$0{\cdot}95 \pm 0{\cdot}18$	$1{\cdot}22 \pm 0{\cdot}25$	$1{\cdot}48 \pm 0{\cdot}18$			

[1] Log CFU/cm^2, mean ± standard error for 10 batches of two eggs.

were laid in the nest. Sherwin and Nicol (1992) observed that the hens did not take just any position when settling in the nest, but that it depended on the orientation of the large and small sides of the nest in the cage. In F15M, the Astroturf rectangle in the nest was positioned with its long side parallel to the back of the cage while in F15M, it was positioned with its short side parallel to the back of the cage. In both cage designs, the hens were often observed on the Astroturf in the nest with the head positioned backward. Consequently, when laying eggs, the hens in F15M had their back turned toward the front of the cage, close to the plastic wire netting allowing the excreta to pass through, in contrast to what occurred in F15P.

The bacterial load of the eggs laid in standard cages was similar to what has previously been observed (Chavez *et al*., 2002; Protais *et al*., 2003*a*,*c*). A slight but significantly lower bacterial count was observed for all the caging systems at 60 weeks compared to 27 weeks, in contrast to the absence of age effect observed by Protais *et al*. (2003*a*). However, Madelin and Wathes (1989) reported greater bacterial contamination of the air with time in a broiler poultry house. The lower bacterial count we observed at 60 weeks most probably results from differences in environmental factors or an effect of season, because the higher bacterial load (27 weeks) coincided with summer and the lower (60 weeks) with winter. In the summer, higher temperatures, greater ventilation of the poultry house and use of a cooling system favouring misting water, may have increased the number of airborne bacteria in the poultry house as well as the bacterial proliferation in the different parts of the cage in contact with eggs after laying. Indeed, there is much evidence to support of the hypothesis that the number of bacteria on the egg results from contamination by the bacteria present in the environment where the eggs are laid (Harry, 1963; Protais *et al*., 2003*a*, *c*), due to dust and excreta present on the cage structure and airborne bacteria. Whatever the age of the hens, the bacterial count was higher on the eggs laid in furnished cages, except at 27 weeks in the standard C6B which had a high bacterial load similar to that of F15M. The bacterial load only partly reflected the changes observed in the percentage of dirty eggs, with higher percentages recorded only for the F15P design. This is explained by the fact that bacterial counts were performed only on clean eggs selected by visual absence of dust and excreta contamination. Therefore, the bacterial load reflected the general contamination of the cage equipment and the air, while the percentage of dirty eggs was more a consequence of faecal contamination. This can be also explained by the difference in sampling methods between the two variables; analysing the eggs for bacterial load on batches of three eggs selected at random from the total egg production might have overstated significance for this variable. Similar experimental approaches have been used to compare bacterial loads of eggs in different housing systems. Tauson *et al*. (personal communication reported in the final report of QLRT-2001-01606 of the European Project Egg Defence) found a greater bacterial load on eggs from furnished cages compared to conventional ones and Protais *et al*. (2003*a*) demonstrated a higher bacterial contamination in an aviary compared to standard cages. De Reu *et al*. (2003) reported a greater bacterial load in perchery systems compared to standard cages, but no difference with furnished cages. This clearly demonstrates that differences exist depending on the design of the furnished cages.

As for dirty eggs, a greater bacterial load was observed on the eggs laid outside the nest but, in the nest, the bacterial load was similar to that recorded in standard cages.

Thus, it can be concluded that increasing the number of eggs laid in the nest by improving nest design should greatly reduce the overall number of dirty eggs and bacterial contamination in furnished cages, to an incidence similar to or even better than that of standard cages.

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