Bacterial Contamination of Eggshells in Furnished and Conventional Cages

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Primary Audience: Advisors, Researchers, Egg Packers, Quality Assurance Personnel

SUMMARY

The study was composed of 480 hens housed in furnished 8-hen cages or in conventional 4-hen cages from 17 to 78 wk of age. Hens of 2 commercial genotypes, Hy-Line White and Hy-Line Brown, were used. Analyses for aerobic bacteria, *Enterococcus* and *Enterobacteriaceae*, on the shell of eggs were conducted when birds were 28 and 62 wk of age. No significant differences were found in proportions of dirty eggs between furnished and conventional cages or between the genotypes. Genotype did not affect bacterial contamination. Eggs produced in furnished cages had higher bacterial contamination than eggs in conventional cages, but levels could be considered as moderate in both housing systems. For some bacterial traits, an effect of sampling period (28 vs. 62 wk of age) was found, which needs to be further investigated before conclusions can be drawn.

Key words: egg quality, bacteria, hygiene, furnished cage, conventional cage, genotype

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DESCRIPTION OF PROBLEM

To improve the welfare of laying hens in the European Union, conventional battery cages will not be allowed after 2011 [1]. Instead, hens should be housed in alternative housing systems providing hens with nests, perches, and litter. The enrichments of furnished cages (i.e., nest, perches, and litter) make the environment more complex than conventional cages (for example, regarding hygiene [2]). Regardless of housing system, eggs will always come into contact with bacteria from the environment when laid and to a varying degree become contaminated with bacteria. In general, most eggs are sterile when passing through the vent and the main bacterial contamination occurs within a short period after being laid [3, 4]. Several studies have shown that, except for heavily soiled eggs, the correlation between visual shell contamination and bacterial contamination is poor and it is therefore not possible to rate the bacterial contamination of egg shells by visual examination [3, 5]. However, the economy of production is normally affected only by the visual contamination.

Washed eggs are not allowed to be sold as table eggs in the European Union [6]. Sweden, being the only member state with a long tradition of egg washing, has at present an exemption allowing some egg packers to continue to wash eggs for a limited period. Therefore, in general,

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bacteria from the birds' housing environment follow the eggs to the consumers and hence, for food safety it is important that eggs are produced in an environment generating as low bacterial contamination as possible.

The objective of the present study was to compare eggshell hygiene, including bacterial contamination, in conventional and furnished cages.

MATERIALS AND METHODS

Birds, Housing, and Management

The study was carried out from February 2003 until March 2004. The pullets were reared in conventional rearing cages and were not beak-trimmed (prohibited in Sweden). The experiment comprised 480 hens in total of which half were Hy-Line White 98 hens and half Hy-Line Brown [7]. There were 4 replicates per genotype in furnished and conventional cages, respectively. Each replicate consisted of 5 cages resulting in 40 hens per replicate in furnished cages and 20 in conventional. At 17 wk of age the pullets were transferred to the experimental building and housed in furnished or conventional cages. The furnished cage measured 120 × 50 cm (width × depth) and housed 8 hens (Figure 1). A nest box was positioned at one end of the cage, and on top of the nest was a litter box. The nest was separated from the cage area by a metal sheet partition and was lined with brown artificial AstroTurf. In accordance with standards of the European Union, the perch arrangement provided 15 cm perch per hen [1]. The conventional cage was a 4-hen metal cage measuring 48 × 50 cm (width × depth). All cages fulfilled the Swedish Animal Welfare Directives of a minimum of 600 cm^2 cage floor area per hen housed, excluding areas of litter baths and nests in furnished cages.

At the arrival to the experimental building and until slaughter, birds were fed a normal layer crumbled diet with a calculated content of 16.1% crude protein, 2,680 kcal of metabolizable energy/kg, 3.8% Ca, and 0.6% P. The birds in the present study were also used in a study comparing a conventional cage and furnished cages with different perch arrangements. More detailed descriptions of cages used and management of birds are given in a previous publication [8].

Recording and Statistical Analysis of Data

Eggs on which bacterial counts were conducted were collected at 28 wk of age (April 2003) and at 62 wk of age (November 2003). During each 7-d sampling period, 2 eggs were sampled daily from each replicate. To be sampled an egg must be regarded as clean at visual inspection and be positioned in the egg cradle of conventional cages or in the egg cradle outside the nest in furnished cages. Sampling was conducted at approximately 7 h after lights-on. Before lights-out in the afternoon, eggs laid after the ordinary egg collection were removed to ensure that eggs exposed to the environment during a whole night were not sampled.

Each egg was sampled in a sterile plastic bag with no direct contact with hands of the person collecting it. Eggs were transported to the laboratory in cold storage within 1.5 h from sampling of first egg. The measured temperature of eggs at arrival at the laboratory varied from 13 to 17°C. At the laboratory on the day of collection, 100 mL of saline peptone solution was added to each bag. Fluids were held at room temperature (i.e., 20 to 22°C). The surface of each egg was gently rubbed by fingers through the bag for 1 min. For the recovery of the total number of aerobic microorganisms, a sample of 1.0 mL of rinse was pour-plated in tryptone glucose extract agar and plates were incubated for 72 h at 25°C [9]. *Enterococcus* was enumerated by surface-plating a sample of 0.1 mL of rinse onto Slanetz and Bartley agar [10]. Plates were incubated for 48 h at 44°C. *Enterobacteriaceae* was determined by pour-plating a sample of 1.0 mL of rinse in violet red bile glucose agar [11]. Plates were incubated for 24 h at 37°C. *Enterococcus* and *Enterobacteriaceae* were confirmed by catalase and oxidase test, respectively.

Before the statistical analyses, the counts of colony forming units (cfu) were transformed to logarithms and thereafter expressed as log cfu/cm^2 by the following equation [12]:

$$S = 4.68\ P\ \exp(2/3),$$

where S = surface in cm^2, and P = egg weight in grams.

Before the statistical analyses, the mean of the 14 eggs analyzed per replicate at each age

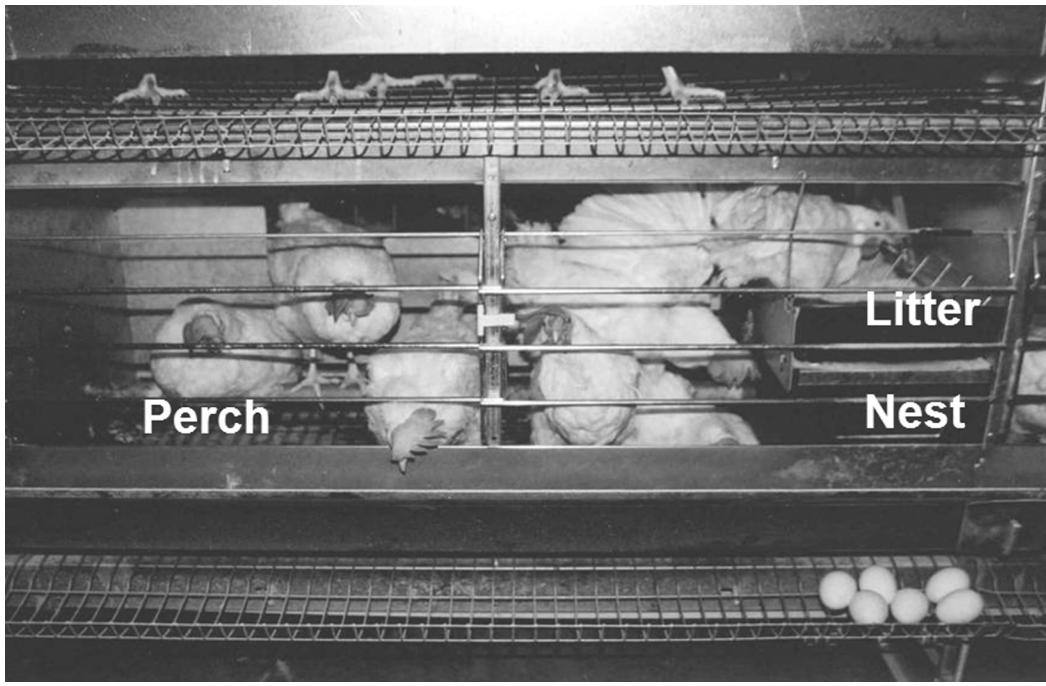

Figure 1. A furnished cage housing 8 hens. A litter box was positioned on top of a nest box, at the right end of the cage. There was a perch in the cage area. Water was provided by nipple drinkers positioned in the rear of the cage and feed was available in a trough at the cage front.

was calculated. There were 8 replicates of furnished cages and 8 of conventional cages. Statistical analyses were performed using the GLM procedure of SAS software [13]. Fisher's protected least-significant difference test was used to analyze individual differences between treatments. In the statistical model, genotype and cage model were considered fixed. Bird age and 2-way interactions were included in all analyses.

There were replicates in which the bacteria *Enterobacteriaceae* were not present on any of the 14 eggs analyzed, and the logarithmic transformation of cfu of *Enterobacteriaceae* did not result in a normal distribution. Therefore, the ANOVA was performed on the percentage of eggs in each replicate with *Enterobacteriaceae* present, instead of counts of cfu. To achieve a normal distribution, these proportions were subjected to arcsin transformation before statistical analysis [14]. *Enterococcus*, not present on all eggs but in all replicates, was analyzed both as cfu and as percentage of eggs with the bacteria present.

RESULTS AND DISCUSSION

One of the benefits of conventional battery cages is that birds are separated from their manure in a very efficient way. In furnished cages the presence of perches may impair birds' ability to efficiently trample the manure down through the cage floor [2]. Furthermore, how perches, litter areas, and nests are situated in relation to each other has impact on the hygiene of cage environment and eggs [15]. In the present study, the proportions of dirty eggs were 4.2 and 5.4% in furnished and conventional cages, respectively ($P < 0.24$). Our results and other recently published studies show that with well-designed furnished cages it is possible to achieve similar results regarding proportions of dirty eggs as in conventional cages [8, 15]. Because dirt is easier to detect on white egg shells than on brown, dirty spots are usually more frequently found on eggs with white shells [16, 17]. However, in the present study no differences in proportions of eggs with visible dirt were found between the genotypes.

Results of analyses of bacterial count are presented in Table 1. There were no significant differences between the genotypes in any of the bacteria measured. Eggs produced in furnished cages had higher counts of aerobic microorganisms and *Enterococcus* than eggs from conventional cages ($P < 0.001$). Also, there was a tendency ($P < 0.06$) of a higher percentage of eggs with *Enterococcus* present on the shell in the furnished cages as compared with the conventional cages. *Enterobacteriaceae* was found on a significantly higher proportion of eggs in furnished cages (12.3% on average) than in conventional (5.80% on average). These results indicate a difference in bacterial contamination of eggs produced in furnished and conventional cages. However, in relation to results in other studies also comparing furnished and conventional cages [18], the level of contamination of eggs in the furnished cage used in our study can be regarded as low. In contradiction to our results, De Rue et al. [18] found no systematic differences in bacterial contamination of egg shells between furnished and conventional cages but a significantly higher contamination of eggs produced in a floor housing system. As in the present study they analyzed only ostensibly clean eggs, and in the furnished cages only eggs laid in the nests were sampled [18]. Mallet et al. [15], also analyzing eggs visually clean, found that eggs laid in the nests of furnished cages had similar bacterial counts as eggs produced in conventional cages. In their study nests were only partly lined with artificial turf, leaving the wire mesh floor bare in the front part of the nest [19]. According to legislation of the European Union [1] nests must be lined in some way (e.g., with artificial turf). The turf can be a hygienic problem if the nest bottom becomes contaminated with manure from hens spending the night inside the nest instead of on the perch placed above the wire floor [8]. Covering only a part of nest bottoms in furnished cages with artificial turf was studied previously [16, 20]. In those studies, covering only 30 or 50% of the nest bottom area with artificial turf resulted in reduced proportions of eggs laid in the nest (i.e., those nests were perceived as less attractive than nests fully lined). Furthermore, in cages with partly covered nest bottoms, the proportions of dirty eggs were at similar levels or higher than

Table 1. Bacterial shell contamination of eggs from laying hens at 28 and 62 wk of age housed in furnished or conventional cages

		Cage model				Statistical significance	
		Furnished cage		Conventional cage		Cage model	Age
Trait	n	28 wk of age	62 wk of age	28 wk of age	62 wk of age		
No. of aerobic microorganisms[1]	32	3.04 ± 0.09	2.90 ± 0.10	2.70 ± 0.20	2.61 ± 0.20	0.001	0.05
No. of *Enterococcus*[1]	32	0.31 ± 0.12	0.34 ± 0.10	0.20 ± 0.09	0.16 ± 0.09	0.001	0.90
Presence of *Enterococcus*,[2] %	32	54.9 ± 15.9	58.8 ± 9.98	50.0 ± 15.7	41.1 ± 15.2	0.06	0.60
Presence of *Enterobacteriaceae*,[2] %	32	10.9 ± 6.53	13.7 ± 8.95	2.68 ± 5.31	8.93 ± 9.92	0.05	0.06

[1]Log cfu/cm^2 ± standard deviation.
[2]Percentage of eggs with the bacteria present ± standard deviation.

in cages with nests with full nest bottom lining [16, 20]. Also, in nests with artificial turf an egg laid generally stays on the turf long enough for its shell to dry, whereas an egg laid on wire mesh tends to roll out of the cage as soon as the hen stands up. Dirt on the wire mesh floor outside the nest attaches more easily to a moist shell than to a dry eggshell. Therefore, nests with artificial turf may in fact be a hygienic benefit.

The bacterial contamination of eggshells is affected by several factors such as the concentration of bacteria in the air of the poultry house [18] or birds' diet [21]. Diets increasing the moisture of birds' excreta not only lead to higher proportions of excreta-contaminated eggs but also increase the microbial contamination of ostensibly clean eggs [21]. In the present trial all hens were housed in the same building and were given the same feed, and differences found in bacterial load were not caused by those factors.

Based on the occurrence of dirty eggs, there was no indication of differences in hygiene between furnished and conventional cages in the present study, but according to the bacterial counts some hygienic differences did exist. In another study, in which hens and cage models in the present study were included, hygiene of birds' plumage and feet were compared [8]. Comparing the furnished and conventional cages, there was no differences in hygiene of birds' feet, but birds' plumage was significantly dirtier in the furnished cage. Furthermore, with the same cages as in the present study but with other genotypes, a scoring of cage floor hygiene showed that the cage floor was dirtier in furnished cages than in conventional cages [8]. Hence, although there may be hygienic differences between housing systems, these differences, if moderate, do not necessarily affect proportions of dirty eggs but may generate differences in bacterial contamination of eggshells.

Neither the number of cfu nor the presence of *Enterococcus* on eggshells was affected by the age of birds (i.e., by the interval since placing the hens in the cages). The number of aerobic microorganisms was higher at 28 wk of age, whereas the proportion of eggs with *Enterobacteriaceae* present on the shell tended to be higher ($P < 0.06$) at 62 wk of age than at 28 wk of age. De Rue et al. [5] found no effect of bird age on bacterial counts on eggs sampled at about 8-wk intervals during the production cycle. An effect of season with higher bacterial counts in the summer was found, but only in 1 of 2 experiments [5]. Because in our study eggs were collected and bacterial counts performed in April and November, effect of season due to large differences in temperature is not likely. It is possible that the contamination of *Enterobacteriaceae* in the cage environment increased with the time hens spent in the cage, but to draw such conclusions bacterial analyses need to be conducted more often during the production cycle.

CONCLUSIONS AND APPLICATIONS

1. Eggs produced in furnished cages have higher bacterial shell contamination compared with eggs from conventional cages, but the bacterial levels can be considered low in both housing systems.
2. Eggshell contamination was not affected by genotype.
3. With furnished cages of good design, proportions of dirty eggs are at similar levels as in conventional cages.

REFERENCES AND NOTES

1. Council Directive 1999/74/EC of 19 July 1999 laying down minimum standards for the protection of laying hens. P. 0053-0057. Official Journal L 203, 03/08/1999.

2. Abrahamsson, P., and R. Tauson. 1993. Effect of perches at different positions in conventional cages for laying hens of two different strains. Acta Agric. Scand., Sect. A Anim. Sci. 43:228–235.

3. Board, R. G., and H. S. Tranter. 1994. The microbiology of eggs. Pages 81–104 in Egg Science and Technology. W. J. Stadelman and O. J. Cotterill, ed. Food Products Press, New York.

4. Mayes, F. J., and M. A. Takeballi. 1983. Microbial contamination of the hen's egg: A review. J. Food Prot. 46:1092–1098.

5. De Reu, K., K. Grijspeerdt, M. Heyndrickx, M. Uyttendaele, and L. Herman. 2005. The use of total aerobic and Gram-negative flora for quality assurance in the production chain of consumption eggs. Food Contr. 16:147–155.

6. Commission of the European Communities 2003. COM(2003) 479 final. Report from the Commission to the Council with regard to developments in consumption, washing and marking of eggs. http://eur-lex.europa.eu/LexUriServ/site/en/com/2003/com2003_0479en01.pdf Accessed July 2006.

7. Hy-Line International, West Des Moines, IA.

8. Wall, H., and R. Tauson. 2007. Perch arrangements in small group furnished cages for laying hens. J. Appl. Poult. Res. 16:322–330.

9. Method 86. NMKL. Secretary General, % National Veterinary Institute, PO Box 8156, Dep. N-0033 Oslo, Norway.

10. Method 68. NMKL. Secretary General, % National Veterinary Institute, PO Box 8156, Dep. N-0033 Oslo, Norway.

11. Method 144. NMKL. Secretary General, % National Veterinary Institute, PO Box 8156, Dep. N-0033 Oslo, Norway.

12. Bonnet, Y., and P. Mongin. 1965. Mesure de la surface de l'œuf. Ann Zootech. 14:311–317.

13. SAS 9.1, 2002–2003. SAS Inst. Inc., Cary, NC.

14. Snedecor, G. W., and W. G. Cochran. 1989. Arc sine transformation for proportions. Pages 289–290 in Statistical Methods, 8th ed. Iowa State Univ. Press, Ames.

15. Mallet, S., V. Guesdon, A. M. H. Ahmed, and Y. Nys. 2006. Comparison of eggshell hygiene in two housing systems: Standard and furnished cages. Br. Poult. Sci. 47:30–35.

16. Wall, H., and R. Tauson. 2002. Egg quality in furnished cages for laying hens—Effects of crack reduction measures and hybrid. Poult. Sci. 81:340–348.

17. Vits, A., D. Weitzenbürger, H. Hamann, and O. Distl. 2005. Production, egg quality, bone strength, claw length, and keel bone deformities of laying hens housed in furnished cages with different group sizes. Poult. Sci. 84:1511–1519.

18. De Reu, K., K. Grijspeerdt, M. Heyndrickx, J. Zoons, K. De Baere, M. Uyttendaele, J. Debevere, and L. Herman. 2005. Bacterial eggshell contamination in conventional cages, furnished cages and aviary housing systems for laying hens. Br. Poult. Sci. 46:149–155.

19. Guesdon, V., A. M. H. Ahmed, S. Mallet, J. M. Faure, and Y. Nys. 2006. Effects of beak trimming and cage design on laying hen performance and egg quality. Br. Poult. Sci. 47:1–12.

20. Wall, H., R. Tauson, and K. Elwinger. 2002. Effect of nest design, passages, and hybrid on use of nest and production performance of layers in furnished cages. Poult. Sci. 81:333–339.

21. Smith, A., S. P. Rose, R. G. Wells, and V. Pirgozliev. 2000. The effect of changing the excreta moisture of caged laying hens on the excreta and microbial contamination of their egg shells. Br. Poult. Sci. 41:168–173.

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