

Eggshell bacteria levels of non-washed and washed eggs from caged and cage-free hens

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Abbreviated title: Eggshell bacteria hen housing environment

Summary

The objective of the experiments was to evaluate the bacteria levels of non-washed and washed eggs obtained from caged and cage-free laying hens housed on either all shavings or all wire slat floors. From 22 to 52 weeks-of-age (at 4 week intervals), 20 eggs were collected from each pen and 10 eggs/pen were washed for 1 min with a commercial egg washing solution (50 C, pH 11), while the remaining 10 eggs were not washed prior to sampling the eggshell and membranes (crush-and-rub) for aerobic bacteria (APC) and coliforms. Non-washed eggs produced in an all shavings environment had slightly higher bacteria numbers (APC 4.0 and coliforms 1.1 log₁₀cfu/mL of rinsate) than eggs produced on slats (APC 3.6 and coliforms 1.0 log₁₀cfu/mL of rinsate), which had significantly higher bacteria numbers than eggs produced in cages (APC 3.1 and coliforms 0.9 log₁₀cfu/mL of rinsate). Washing significantly reduced APC by 1.8 log₁₀cfu/mL (49%), reduced coliform counts by only 0.5 log₁₀cfu/mL and coliform prevalence was reduced from 22.5, 17.5, 12.5% (shavings, slats, and cages, respectively) to 6%. No significant differences were found in APC and coliform counts on eggs from the three housing types following washing. When all hens were relocated into 2-hen triple deck cages in a separate room, eggshell APC levels (from 57 to 61 weeks-of-age) for non-washed eggs was 0.7 log₁₀cfu/mL and for washed eggs was 0.1 log₁₀cfu/mL, a 89% reduction. Housing hens in cage units with manure

removal and the absence of litter resulted in lower eggshell APC levels for both non-washed (by 2 log₁₀cfu/mL) and washed eggs (by 1 log₁₀cfu/mL).

Keywords: egg laying hens; caged; cage-free; eggshell bacteria

Introduction

With the increasing production of organic eggs for table consumption a greater number of laying hens are being housed in cage-free aviaries (litter area, slats, and nest boxes) and free-range (outdoor pasture access) conditions. The European Commission has required conventional colony cage use to be discontinued in the EU by 2012 (Anonymous, 1999). Several studies have compared conventional cages to these alternative systems in the areas of hen performance, welfare, and egg quality (Abrahamsson and Tauson 1995; Tauson et al., 1999; Mertens et al., 2006; Voslarova et al., 2006). However, few studies have examined the bacterial implications of housing laying hens in floor environments, and no studies have compared hens reared in the same housing system and then placing them into alternative housing systems (caged and cage-free) prior to the onset of egg production.

The levels of bacteria contamination from alternative housing systems compared to conventional cage systems will be an important factor influencing future food safety issues and regulations. Eggshell contamination level at the time of egg collection has previously been shown to be directly related to the final contamination of egg products (Pettrak et al., 1999). Eggs from hens housed on litter were shown to have 20 to 30 times more aerobic bacteria on the eggshells than eggs from hens housed in wire cages (Quarles et al., 1970). Eggs collected from conventionally caged laying hens compared with eggs collected from organic raised and housed laying hens (floor raised with outdoor access) and barn raised laying hens (indoor only) have been demonstrated to harbor fewer aerobic bacteria (Protais et al., 2003; De Reu et al., 2005). However, the levels of Gram-negative bacteria (coliforms and *E. coli*) did not differ between housing types (De Reu et al., 2005).

Increased levels of aerobic bacterial eggshell contamination may also be due to the increased levels of bacteria circulating in the air of litter-covered housing environments. Ellen et al. (2000) sampled the air of cage and aviary systems and found that aviaries had four to five times more dust in the air than found in conventional cage systems. Furthermore, air and eggshell contamination have been positively correlated, $r^2=0.66$ (De Reu et al., 2005).

Materials and Methods

Hatching eggs were obtained from Hy-Line International (2006) for White and Brown layer strains from flocks of hens at 56 weeks-of-age and set and hatched at the University of Georgia. Pullets were reared intermingled in a single environmentally controlled closed brood/grow facility on litter with access to perches, trough feeder, nipple drinker lines, and under photoperiod restriction until 15 weeks-of-age and provided diets formulated to meet the requirements set in the Hy-Line Brown layer commercial management guide.

Duplicate pens (one for White and one for Brown pullets) for housing 54 or 45 laying hens in 9-colony cages (6 White hens per cage 465 sq cm (72 sq inches)/hen or 5 Brown hens per cage at 555 sq cm (86 sq inches)/hen), 54 White and Brown hens housed on elevated wire slats, or 54 White and Brown hens housed on wood shavings. All hens were housed within the same room, fed *ad libitum* the same feed, water provided by enclosed nipple drinkers, and exposed to the same circulating air flow and subjected to the same environmental temperature and relative humidity profiles. Hens housed on shavings or slats were provided roll-out nest boxes at a density of 4.5 hens per nest (12 nests / 54 hens). Hens housed in cages were provided trough feeders while hens on slats or shavings were provided tube/pan feeders. Access to perches was provided for hens on slats or shavings (13.5 cm / hen). Eggs were collected and recorded by pen three times daily.

On each of eight replication sample days (from 22 to 52 weeks-of-age at approximate 4 week intervals) 20 eggs were collected from each pen for bacteria analysis (n=120). Eggs were collected and held overnight in an egg cooler at 12°C. The following day, 10 of the eggs collected from each pen were washed for 1 min with a commercial egg washing solution (50°C, pH 11, 34.5 KPa), while the remaining 10 eggs were not washed prior to sampling the eggshell and membranes (modified crush-and-rub; Berrang et al., 1991) for aerobic bacteria (APC), *Escherichia coli* (*E. coli*), and coliforms. Eggs were aseptically opened and the internal contents were discarded. The eggshell and membranes

were crushed and forced into a sterile 50 mL centrifuge tube to which 20 mL of sterile 0.85% saline solution was added. The eggshells were further crushed into fine pieces through the use of sterilized glass rods for 1 min. One mL of solution was collected from each eggshell sample for serial dilutions. From the dilutions for APC, 1.0 mL was spread onto 3M APC Petrifilm™ (in duplicate) and incubated at 37°C for 24-48 h. For *E. coli* /coliforms an additional 1 mL was transferred to 3M EC Petrifilm™ plates (in duplicate) and incubated at 37°C for 24-48 h. Coliforms were identified as red gas producing colonies and *E. coli* were identified as blue gas producing colonies. All counts were converted to log₁₀cfu/mL of rinsate. ANOVA according to GLM procedure (SAS, 2001) was used to test for differences due to hen housing type (cage, slats, and shavings), strain (White and Brown), non-washed vs. washed eggs, and differences were considered significant at P<0.05. Tukey's HSD test was used to identify differences due to hen housing type.

At 52 weeks-of-age all hens were moved to triple-deck battery units (2 hens/cage) into the same room containing replacement hatch mate hens of the same age and their previous housing (cages, slats, or shavings) designation was recorded. Eggs were collected and sampled weekly from 57 to 61 weeks-of-age. Eggs were washed using a small scale egg washing unit operating at 48°C, pH 11, at 68.9 KPa, for a wash time of 1 min. Only APC was determined for both washed and non-washed eggshells.

Results and discussion

Daily egg production per hen, from 22 to 52 weeks of age was 75% for the White hens and 77% for Brown hens. Daily egg production for hens housed in cages (combined for White and Brown hens) averaged 78%, for hens housed on shavings 76%, and for hens housed on wire slats 75%. Higher egg production for hens in cages by 29%, compared to hens on deep litter, over a nine month production period was reported by Voslarova *et al.*, (2006).

Non-washed eggs produced in the all shavings environment had slightly higher APC levels (Brown 4.2 and White 3.8 log₁₀cfu/mL of rinsate; Figure 1) than eggs produced on slats (Brown 4.1 and White 3.2 log₁₀cfu/mL; the only significant difference detected between eggs from White and Brown hens), which had significantly higher bacteria

numbers than eggs produced in cages (APC Brown 3.0 and White 3.1 log₁₀cfu/mL). Washing significantly reduced APC levels for Brown eggs to 2.2 log₁₀cfu/mL and for White eggs to 2.2 log₁₀cfu/mL from hens housed on shavings, to 2.4 log₁₀cfu/mL for Brown and to 1.2 log₁₀cfu/mL for White eggs from hens on slats, and to 1.2 log₁₀cfu/mL for Brown and 1.8 log₁₀cfu/mL for White eggs from hens in cages. Overall washing eggs lowered APC levels by 49%. Washing reduced coliform counts by only 0.5 log₁₀cfu/mL of rinsate and the prevalence was reduced from 22.5, 17.5, 12.5% (shavings, slats, and cages, respectively) to 6%. There were no significant differences found in APC, *E. coli*, and coliform levels on eggs from the three housing types following washing. Higher aerobic bacteria levels were reported for eggshells from aviary housed hens, by more than 1 log, compared to eggs from hens housed in conventional and furnished cages (De Reu *et al.*, 2005). In our experiments, beak trimming at monthly intervals of all hens was required to control cannibalism, especially for Brown hens housed on wire slats.

After all hens were moved from the cages, slats, and shavings room to 2-hen cages in a separate room, the non-washed eggs had lower APC levels at 0.7 log₁₀cfu/mL (Figure 2) and the washed eggs had lower APC levels at 0.1 log₁₀cfu/mL, an 89% reduction. Housing hens in cage units without litter and with manure removal three times weekly resulted in lower eggshell APC levels from both non-washed and washed eggs.

In summary, the eggshells of eggs collected from hens housed in cages had lower levels of bacteria than eggs from hens housed on slats or shavings. In addition, washing eggs significantly lowered eggshell bacteria levels and after washing eggs from hens housed in cages, on slats, or on litter the level of bacteria recovered did not differ. When all hens were moved to triple-deck cage units in a separate room, with manure removal and absence of shavings, there were subsequently lower levels of aerobic bacteria recovered from both non-washed and washed eggs for both White and Brown hens.

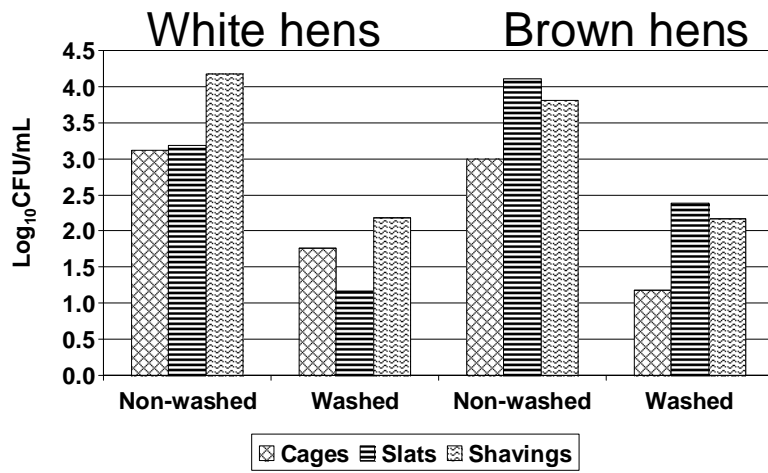


Fig. 1 Eggshell aerobic bacteria recovery from White and Brown hens housed in cages, on slats, or on shavings in the same animal room from 22 to 52 weeks-of-age

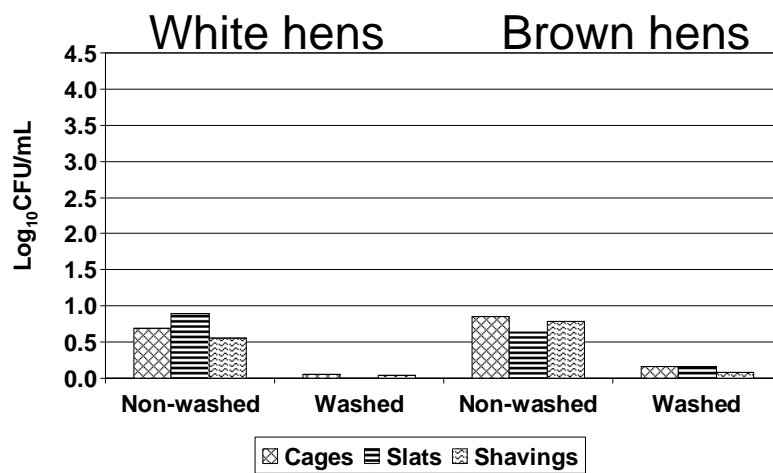


Fig. 2 Eggshell aerobic bacteria recovery from White and Brown hens housed in triple deck cages in the same animal room from 57 to 61 weeks-of-age (previously housed in cages, on slats, or on litter)

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