Comparison of shell bacteria from unwashed and washed table eggs harvested from caged laying hens and cage-free floor-housed laying hens[1]

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ABSTRACT These studies evaluated the bacterial level of unwashed and washed shell eggs from caged and cage-free laying hens. Hy-Line W-36 White and Hy-Line Brown laying hens were housed on all wire slats or all shavings floor systems. On the sampling days for experiments 1, 2, and 3, 20 eggs were collected from each pen for bacterial analyses. Ten of the eggs collected from each pen were washed for 1 min with a commercial egg-washing solution, whereas the remaining 10 eggs were unwashed before sampling the eggshell and shell membranes for aerobic bacteria and coliforms (experiment 1 only). In experiment 1, the aerobic plate counts (APC) of unwashed eggs produced in the shavings, slats, and caged-housing systems were 4.0, 3.6, and 3.1 $\log_{10}$ cfu/mL of rinsate, respectively. Washing eggs significantly ($P < 0.05$) reduced APC by 1.6 $\log_{10}$ cfu/mL and reduced the prevalence of coliforms by 12%. In experiment 2, unwashed eggs produced by hens in triple-deck cages from 57 to 62 wk (previously housed on shavings, slats, and cages) did not differ, with APC ranging from 0.6 to 0.8 $\log_{10}$ cfu/mL. Washing eggs continued to significantly reduce APC to below 0.2 $\log_{10}$ cfu/mL. In experiment 3, the APC for unwashed eggs were within 0.4 log below the APC attained for unwashed eggs in experiment 1, although hen density was 28% of that used in experiment 1. Washing eggs further lowered the APC to 0.4 to 0.7 $\log_{10}$ cfu/mL, a 2.7-log reduction. These results indicate that shell bacterial levels are similar after washing for eggs from hens housed in these caged and cage-free environments. However, housing hens in cages with manure removal belts resulted in lower APC for both unwashed and washed eggs (compared with eggs from hens housed in a room with shavings, slats, and cages).

Key words: eggshell bacteria, egg washing, hen housing system, caged, cage-free

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INTRODUCTION

In the United States, commercial laying hens are primarily housed in conventional colony or battery wire cages because they offer lower production costs, improved egg hygiene, and greater hen livability compared with cage-free systems (De Reu et al., 2005; Singh et al., 2009). However, colony cage housing systems have recently been criticized by animal welfare and consumer groups for providing a barren, crowded, and confined environment for laying hens (Singh et al., 2009). Increasing concerns regarding hen welfare have prompted changes in the housing systems for table egg-laying hens. Many table egg producers are transitioning from conventional colony cages to either enriched environmental housing systems, which include a perch, nest, and shavings or litter area, or to cage-free housing systems, such as an aviary, litter-covered floor, paddock, or free range. California voters approved the implementation of Proposition 2, which will prohibit housing egg-laying hens in conventional colony cages beginning in 2015 (California Legislative Analyst's Office, 2008). Conventional colony cages for laying hens will be banned in the European Union by 2012 and replaced with either enriched environmental housing systems or cage-free systems (European Commission, 1999), which are predominantly aviaries. Increased consumer aversion to the use of conventional colony cages has also led to an increase in the demand for cage-free

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table eggs, although presently at less than 8% of the US table egg market (Savory, 2004; United Egg Producers, 2010).

The vast majority of eggs produced by healthy hens are clean at oviposition when passing through the vent (Mayes and Takeballi, 1983). However, regardless of the housing system, eggs are contaminated to some extent when they come in contact with environmental bacteria after being laid (Harry, 1963; Quarles et al., 1970; Wall et al., 2008). Studies have been conducted to compare the shell bacteria of eggs from hens housed in conventional colony cages with those from hens housed in noncage housing systems. Quarles et al. (1970) found that eggs obtained from hens housed on shavings-covered floors have 20 to 30 times more aerobic bacteria on the shell than eggs from hens on wire floors (eggs were collected daily and held for up to 14 d). Furthermore, eggs produced in conventional and furnished cages (enriched environmental housing systems) have been reported to harbor significantly fewer aerobic bacteria on the shell than eggs from aviary and free-range systems (De Reu et al., 2005, 2006a). However, eggs from these housing systems were reported to have similar levels of gram-negative bacteria (most human foodborne pathogens; De Reu et al., 2008). When comparing eggs from conventional and furnished cages, studies have shown that those from furnished cages have higher bacterial numbers on the shell (Mallet et al., 2006; Wall et al., 2008). A small number of studies have evaluated the effects of floor housing systems on eggshell bacterial contamination. However, no studies have evaluated the shell bacteria of eggs produced by pullets raised in the same housing system and then placed into caged and cage-free systems within the same environmental conditions (temperature and humidity ranges, photoperiod, and ventilation) during egg production.

Many genera of bacteria, including *Escherichia*, *Micrococcus*, *Salmonella*, *Streptococcus*, and *Staphylococcus*, have been recovered from the shells of naturally contaminated table eggs (Mayes and Takeballi, 1983; Musgrove et al., 2004). High levels of external shell contamination can adversely affect the shelf life and food safety of eggs. In an eggshell penetration study, De Reu et al. (2006b) reported a significant positive relationship between the level of shell contamination and the resulting internal egg contamination. Table eggs are routinely washed in the United States, Australia, Canada, and Japan to reduce shell contamination, thus reducing the potential for egg spoilage and human illnesses associated with the consumption of raw or undercooked eggs (Hutchison et al., 2004; De Reu et al., 2006c). However, washing class A table eggs is prohibited in the European Union and washed eggs cannot be sold as table eggs (European Commission, 2003, 2007). This practice is partially due to the historical perception that wetting or washing eggs before storage can increase egg spoilage rates (Brooks, 1951; Bagley and Christensen, 1991; Wang and Slavik, 1998; Hutchison et al., 2003) and reports that washing can damage the cuticle of the egg, a natural but temporary physical barrier that impedes bacterial penetration by covering the opening of each eggshell pore and reducing eggshell gas permeability.

With an increasing number of laying hens being housed in cage-free systems, shell bacterial levels of eggs from these systems will be a significant issue potentially affecting food safety policies. Limited published work is available on the shell bacteria of table eggs from cage-free hens, so additional research is needed to compare the shell bacterial numbers of eggs produced by hens in conventional cages with those produced by cage-free hens. The objective of this study was to evaluate the shell bacterial numbers of unwashed and washed eggs from caged and cage-free laying hens housed either on all wire slats or all litter floor systems. A single commingled flock of Hy-Line W-36 (White) and Hy-Line Brown (Brown) layer strains, reared and housed for laying in a single room, were used in 3 sequential experiments.

MATERIALS AND METHODS

Birds, Housing, and Management

Hy-Line International (West Des Moines, IA) provided 2 cases of hatching eggs from White and Brown layer strain flocks. White and Brown chicks were reared intermingled in an environmentally controlled facility from day of hatch through 15 wk of age. Pullets were reared on a concrete floor covered with new pine shavings in a single room [24 × 30 ft (7.32 × 9.14 m)] with access to a trough feeder line, nipple drinker lines, and perches (Hy-Line, 2006–2008). The photoperiod program followed the recommended Hy-Line management guide. At 15 wk of age, pullets were weighed and then selected within 1 SD of mean BW by strain, resulting in selection of 162 White (1.12 kg) and 153 Brown (1.38 kg) pullets.

Experiment 1. At 15 wk of age, pullets were placed by strain in the 3 housing systems: conventional cages [1 × 2 in. (2.54 × 5.08 cm) 16-gauge galvanized wire that were newly constructed], elevated wire slats [0.75 × 3 in. (1.90 × 7.62 cm) 12.5-gauge white polyvinyl chloride coated, sanitized, reused], and all new pine shavings-covered concrete floors. A total of 6 pens were used in experiment 1 with duplicate pens (1 for White and 1 for Brown pullets) of each housing system. For the conventional cages, each pen contained 9 colony cages [24 in. wide × 18 in. deep × 18 in. high (61 cm wide × 45.7 cm deep × 45.7 cm high)]. Six White hens were housed per cage [72 in.^{2}/hen (465 cm^2/hen)] or 5 Brown hens were housed per cage [86 in.^{2}/hen (555 cm^2/hen)]. Fifty-four White or Brown hens were housed in the all wire slat pens and the all shavings floor pens [1.8 ft^2/hen (0.16 m^2/hen)]. The cage and cage-free housing densities were compliant with United

Egg Producers recommendations (United Egg Producers, 2010). All hens in the 6 pens were housed in the same room [24 × 30 ft (7.32 × 9.14 m)], fed the same pelleted feed ad libitum from a central alley [6 × 24 ft (1.83 × 7.32 m)] accessing each pen, and subjected to the same environmental conditions (temperature and humidity ranges, ventilation, light intensity, and photoperiod program). Throughout the experiment, all birds were provided feed formulated to meet the nutritional requirements outlined in the Hy-Line Brown commercial layer management guide (8 diets; Hy-Line, 2006–2008). Trough feeders were used for hens in cages, whereas 2 tube or pan feeders [41.5 in. (105 cm) circumference, with 14 partitions] were used for hens housed in the wire slats and shavings pens. One-story front roll-out nest boxes with rubber finger nest pads were provided for hens housed on wire slats and shavings at a stocking density of 4.5 hens/nest (12 nests/54 hens). Perches providing 5.3 in./hen (13.5 cm/hen) were also placed in the wire slats and shavings pens. Eggs were collected by hand twice daily (1100 and 1500 h) and recorded for each pen. Hens were initially beak trimmed at 34 wk of age, and beaks were reblunted as needed at monthly intervals in an effort to control cannibalism. Egg production was recorded daily. All experimental bird procedures and protocols were approved by the Institutional Animal Care and Use Committee at the University of Georgia before placement of the chicks. Starting at 22 wk of age, at monthly intervals for 8 mo consecutively, eggs from the 6 pens were collected and the shells were sampled (n = 80 eggs for experiment 1).

Experiment 2. At 52 wk of age, all remaining hens were moved into triple-deck battery cage units and placed 2 hens/cage (1 White and 1 Brown hen/cage) that were 12 in. (30.5 cm) wide, 18 in. (45.7 cm) deep, and 18 in. (45.7 cm) high in the room containing the remainder of the hatch-mate hens that had been used for egg production in other research projects. The previous housing system (cages, slats, or shavings) designation of each hen was recorded during moving. After 5 wk in the triple-deck battery cages, eggs were collected and sampled weekly from 57 to 61 wk of age (n = 50 eggs for experiment 2).

Experiment 3. Both the remaining Hy-Line White and Hy-Line Brown hens used in experiments 1 and 2 were used in 4 sequential trials with a total of 45 hens/trial from 56 to 72 wk of age. White and Brown hens were commingled in either conventional colony cages [86 in.^{2}/hen (555 cm^2/hen), a total of 3 cages] on all wire slats [6.4 ft^2/hen (0.6 m^2/hen)] or on all shavings flooring systems [6.4 ft^2/hen (0.6 m^2/hen)]. They were placed back into the room in which they were housed from 15 to 51 wk of age in experiment 1, without cleanout. For each trial, a total of 15 hens were placed into each of the 3 housing systems. Commingled hens had access to the same feeding and watering systems, and at 12 d after reintroduction, eggs were collected, processed, and sampled at 59, 63, 67, and 71 wk of age, as described for experiment 2 (n = 36 eggs for experiment 3).

Egg Sampling and Washing

Experiment 1. On each of 8 replicate days (at 22, 25, 29, 34, 38, 42, 46, and 51 wk of age), up to 30 eggs were collected from each pen for bacterial analysis. On the day of sampling, starting at 0600 h, all eggs present in the pens were collected, recorded, and excluded from that day's sample to ensure that only freshly laid eggs (within 2 h) were sampled each replicate day. At 1000 h and again at 1400 h, all eggs present were aseptically collected from each pen, marked, and placed into new cardboard egg flats (1 egg flat/pen). Only visibly intact eggs that were laid in the nest boxes (for hens on slats or shavings) were selected for sampling. The differentiation of housing system (shavings, slats, or cages) and hen strain (White or Brown) was maintained. The collected eggs were then held uncovered overnight at 12°C and approximately 70% RH in an on-site egg cooler. The following morning (0800 h), each flat of eggs was placed into a clean plastic bag and the 6 flats of eggs were transported from the farm to the laboratory. Twelve representative eggs (of the 30 eggs from each pen) that were not to be washed were aseptically placed onto a sanitized plastic egg flat and remained in the laboratory. Twelve representative eggs from each pen remained on the egg flat (the remaining 6 eggs for each pen were discarded), were placed back into the plastic bag, and were transported to the egg processing facility (Jones et al., 2005). Groups of 6 eggs from each of the 6 pens were spray-washed together in a single batch using a commercial egg-washing solution (80 mL/26.5 L of Liquid Egg Wash 101, BioSentry, Stone Mountain, GA). The solution at pH 11 was heated to 50°C and sprayed onto the eggs for 1 min at 34.5 kPa from the heated recirculation washing solution tank while eggs were rotated in place on spindles identical to those used in commercial washing equipment. Eggs were aseptically removed from the rollers of the egg-spraying machine, placed into new foam egg cartons by pen, and dried for approximately 15 s with a handheld blower producing 124°C air. The second batch of 6 eggs from each of the 6 pens was then placed onto the rollers, sprayed together, removed, placed into the same foam egg carton with the first batch, and then dried. The cartons were then closed and placed into a cardboard egg box for transport back to the laboratory by 1000 h.

Ten of the 12 eggs (washed and unwashed groups for each pen) were sampled, including the eggshell and shell membranes, for aerobic plate counts (**APC**) of bacteria, *Escherichia coli*, and coliforms. If the remaining 2 eggs for each pen that were not selected for sampling were not needed as replacement eggs (for eggs found to have cracks in the shell or that were inadvertently cracked during handling), they were discarded. Each egg was cracked open on a sterile surface, using

a new latex glove each time, and the internal contents were discarded. The eggshell and shell membranes were then crushed by hand and forced into a sterile 50-mL centrifuge tube. Twenty milliliters of 0.85% saline was then added to the sample. Sterile glass rods were used to further crush the eggshell and shell membranes for 1 min and mix the sample with the saline solution (Berrang et al., 1991; Musgrove et al., 2005).

Experiments 2 and 3. Eggs sampled in experiments 2 and 3 were collected and selected as described for experiment 1, but were washed using a small-scale egg-washing unit (Model EEW-30-G-R, Modernmatic, Lancaster, PA) operating at 48°C, pH 11, at 68.9 kPa, for a wash time of 1 min. The egg-washing compound used in experiments 2 and 3 was 25 g/10 L of DBC-A Egg Wash Powder with 1 mL/6 L of Antifoam B (BioSentry), resulting in a 50 mg/L free chlorine solution in the 172-L heated recirculation tank. The main differences between the egg-spraying machines were as follows. For experiment 1, the eggs rotated in place while receiving a constant spray pattern, whereas for experiments 2 and 3, the eggs rotated while proceeding down the conveyer and therefore received a varied sanitizing spray pattern. In addition, the tank reservoir capacity for experiment 1 was 26.5 L and for experiments 2 and 3 was 172 L, and the spray pressure in experiment 1 was 34.5 kPa, which was increased to 68.9 kPa in experiments 2 and 3.

Shell Bacteriological Analysis

In experiments 1, 2, and 3, 1 mL of crushed eggshell rinsate was collected from each sample to prepare serial dilutions to 10^{-4}. For APC enumeration of unwashed eggs, 1 mL was transferred directly from the rinsate and the 10^{-2} and 10^{-4} dilutions to duplicate APC Petrifilm (3M Health Care, St. Paul, MN) plates. For APC enumeration of washed eggs, 1 mL was transferred directly from the rinsate and the 10^{-2} dilution to duplicate APC Petrifilm plates. In experiment 1 only, to enumerate *E. coli* or coliforms from both the unwashed and washed eggs, 1 mL was transferred directly from the rinsate and the 10^{-2} dilution to duplicate *E. coli* or coliform Petrifilm (3M Health Care) plates. All plates were incubated at 37°C for 24 to 48 h. Colonies on the APC and *E. coli* or coliform plates were enumerated following the manufacturer's directions, and counts were converted to $\log_{10}$ colony-forming units per milliliter of crushed eggshell rinsate.

Statistical Analysis

Analysis of variance according to the GLM procedure (SAS Institute, 2005) was used to test for differences in APC attributable to wash treatment (unwashed or washed) and laying hen strain (White or Brown). Tukey's honestly significant difference test was used to identify differences attributable to housing system (shavings, slats, or cages). Only positive rinsate samples were averaged. All differences reported as significant were evaluated at $P < 0.05$. The prevalence of *E. coli* and coliforms among White and Brown unwashed and washed eggs was insufficient for statistical testing. Dixon's Q test was applied once to identify and reject any individual egg outlier data within each housing system for unwashed and washed eggs for each sampling day (Dean and Dixon, 1951). Interactions between hen age at egg sampling and the recovered APC were not significant within each experiment, $P > 0.05$.

RESULTS AND DISCUSSION

Experiment 1

Hen-day egg production from 22 to 51 wk of age for White hens was 74, 74, and 77% for those housed on shavings, on slats, or in cages, respectively. Hen-day egg production for Brown hens during the same period was 77, 75, and 80% for those on shavings, on slats, or in cages, respectively.

Unwashed White eggs produced in the shavings pen had significantly higher APC (3.8 $\log_{10}$ cfu/mL of rinsate) than eggs produced on slats (3.2 $\log_{10}$ cfu/mL), which had levels similar to the eggs produced in cages (3.1 $\log_{10}$ cfu/mL; Table 1). Washing significantly reduced the APC of White eggs produced on shavings, on slats, or in cages to 2.2, 1.3, and 2.2 $\log_{10}$ cfu/mL, respectively. White eggs produced on slats that were washed had significantly lower APC than the eggs produced in cages or on shavings (Table 1). This low APC after washing eggs from hens on slats may be attributed to the restricted air flow over the eggs while the eggs sat in the roll-out nest egg tray, in contrast to the unrestricted air flow around the eggs from hens in cages located in front and below the feed troughs. The initial higher APC (Table 1) for unwashed eggs from the Brown hens on slats (4.1 $\log_{10}$ cfu/mL) or White (3.8 $\log_{10}$ cfu/mL) or Brown hens on shavings (4.2 $\log_{10}$ cfu/mL) may have overshadowed this benefit after washing.

Aerobic bacterial levels of unwashed Brown eggs produced on shavings (4.2 $\log_{10}$ cfu/mL) or on slats (4.1 $\log_{10}$ cfu/mL) were significantly higher than those produced by hens in cages (3.0 $\log_{10}$ cfu/mL; Table 1). Washing significantly reduced APC for Brown eggs produced on shavings, on slats, or in cages to 2.2, 2.5, and 1.3 $\log_{10}$ cfu/mL, respectively. Washed Brown eggs produced in cages had significantly lower APC than those eggs produced on slats and shavings (Table 1).

Unwashed Brown eggs produced on shavings (4.2 $\log_{10}$ cfu/mL) or on slats (4.1 $\log_{10}$ cfu/mL) had significantly higher APC than unwashed White eggs produced on shavings (3.8 $\log_{10}$ cfu/mL) or on slats (3.2 $\log_{10}$ cfu/mL; Table 1). Washed Brown eggs produced in cages (1.3 $\log_{10}$ cfu/mL) had significantly lower APC

Table 1. Eggshell aerobic plate counts (APC; mean ± SD) from unwashed and washed eggs produced by Hy-Line W-36 White and Hy-Line Brown hens housed in cages, on slats, or on shavings in experiments 1, 2, and 3

	APC ($\log_{10}$ cfu/mL of eggshell rinsate)		
Sample	Cages	Slats	Shavings
Experiment 1[1]			
White laying hens			
Unwashed eggs	$3.1^{b,Y} \pm 0.9$	$3.2^{b,Z} \pm 1.0$	$3.8^{a,Z} \pm 0.8$
Washed eggs	$2.2^{a,Y} \pm 1.6$	$1.3^{b,Z} \pm 1.0$	$2.2^{a,Y} \pm 1.1$
Prevalence[2] (%)	62	55	90
Brown laying hens			
Unwashed eggs	$3.0^{b,Y} \pm 0.9$	$4.1^{a,Y} \pm 0.9$	$4.2^{a,Y} \pm 0.9$
Washed eggs	$1.3^{b,Z} \pm 0.9$	$2.5^{a,Y} \pm 1.4$	$2.2^{a,Y} \pm 1.3$
Prevalence (%)	74	81	84
Experiment 2[3]			
White laying hens			
Unwashed eggs	$0.7^{ab,Y} \pm 0.4$	$1.0^{a,Y} \pm 0.8$	$0.6^{b,Y} \pm 0.6$
Washed eggs	$0.3^{a,Y} \pm 0.9$	$0.0^{a,Y} \pm 0.3$	$0.2^{a,Y} \pm 0.6$
Prevalence (%)	54	67	72
Brown laying hens			
Unwashed eggs	$0.8^{a,Y} \pm 0.6$	$0.7^{a,Y} \pm 0.6$	$0.8^{a,Y} \pm 0.6$
Washed eggs	$0.1^{a,Y} \pm 0.4$	$0.1^{a,Y} \pm 0.4$	$0.3^{a,Y} \pm 0.6$
Prevalence (%)	52	74	60
Experiment 3[4]			
White laying hens			
Unwashed eggs	$2.8^{b,Y} \pm 0.8$	$3.1^{b,Y} \pm 1.2$	$3.6^{a,Y} \pm 0.7$
Washed eggs	$0.5^{a,Y} \pm 0.8$	$0.4^{a,Y} \pm 0.7$	$0.7^{a,Y} \pm 0.7$
Prevalence (%)	80	69	88
Brown laying hens			
Unwashed eggs	$3.0^{b,Y} \pm 0.7$	$3.0^{b,Y} \pm 1.0$	$3.8^{a,Y} \pm 0.6$
Washed eggs	$0.4^{a,Y} \pm 0.6$	$0.6^{a,Y} \pm 0.8$	$0.7^{a,Y} \pm 0.7$
Prevalence (%)	86	86	82

[a,b]Means within a row with different letters differ significantly, $P < 0.05$.

[Y,Z]Means for White and Brown laying hens within a housing system for unwashed or washed eggs within an experiment with different letters differ significantly, $P < 0.05$.

[1]n = 80 eggs.

[2]Percentage of positive samples from total number of samples taken. Hen age during sampling: experiment 1 = 22 to 51 wk; experiment 2 = 57 to 61 wk; experiment 3 = 59 to 71 wk.

[3]n = 50 eggs (all hens 2/cage).

[4]n = 36 eggs.

than washed White eggs produced in cages (2.2 $\log_{10}$ cfu/mL).

The prevalence of *E. coli* and coliforms among White and Brown unwashed and washed eggs was insufficient for statistical testing. The prevalence of *E. coli* among unwashed White eggs was reduced from 15, 11.3, and 11.3% (shavings, slats, and cages, respectively) to 3.8% after washing for eggs from all 3 housing systems. Similarly, washing reduced the prevalence of coliforms among White eggs produced on shavings, on slats, or in cages from 16.3, 12.5, and 12.5% to 3.8, 8.8, and 3.8%, respectively. Overall, the prevalence of coliforms was slightly higher among unwashed Brown eggs produced on shavings (*E. coli* 25% and coliforms 28.8%) and on slats (*E. coli* 16.3% and coliforms 22.5%) than in cages (*E. coli* 6.3% and coliforms 12.5%). Once subjected to the spray-wash treatment, only 3.8% of Brown eggs (identical to the percentage for White eggs) produced in each housing type were positive for *E. coli*, whereas 6.3, 3.8, and 6.3% (shavings, slats, and cages, respectively) of the Brown eggs were positive for coliforms.

Experiment 2

After all hens were moved from the room with shavings, slats, and cages to the 2-hen cages in a separate room, the unwashed eggs had low APC, at 0.8 $\log_{10}$ cfu/mL. Levels ranging from 0.6 to 1.0 $\log_{10}$ cfu/mL (Table 1) did not differ between hen strains and were not influenced by previous housing system. The average APC for unwashed eggs (0.8 $\log_{10}$ cfu/mL) was more than 1.0 log less than the average APC attained for spray-washed eggs (1.9 $\log_{10}$ cfu/mL) in experiment 1. Furthermore, after washing, the APC for eggs in experiment 2 was further reduced to an average of 0.2 $\log_{10}$ cfu/mL, a 0.6 log reduction. The prevalence of APC in experiment 2 for eggs from the triple-deck caged hens after washing was 53% compared with 74% when hens were previously housed on shavings, slats, or cages in experiment 1. Housing hens in cages without shavings and with manure removal belts resulted in lower shell APC for both unwashed and washed eggs (compared with eggs from the same hens while housed in a room with shavings, slats, and cages).

Experiment 3

Moving hens from the triple-deck cages into the same room used in experiment 1 (which remained empty without clean-out from 52 to 56 wk) into the same shavings, slats, or cage pens resulted in unwashed eggs having APC similar to those reported in experiment 1. For caged hens, APC were 2.8 to 3.0 $\log_{10}$ cfu/mL (3.0 to 3.1 $\log_{10}$ cfu/mL in experiment 1); for hens on slats, APC were 3.0 to 3.1 $\log_{10}$ cfu/mL (3.2 to 4.1 $\log_{10}$ cfu/mL in experiment 1); and for hens on shavings, APC were 3.6 to 3.8 $\log_{10}$ cfu/mL (3.8 to 4.2 $\log_{10}$ cfu/mL in experiment 1). The APC differed by less than 0.2 $\log_{10}$ cfu/mL between hen strains (Table 1). The APC for unwashed eggs were within 0.4 log below the APC attained for unwashed eggs in experiment 1, although hens in experiment 3 were at 28% of the hen density used in experiment 1. In experiment 3, washing eggs lowered APC to 0.4 to 0.7 $\log_{10}$ cfu/mL, a 2.7 log reduction. The prevalence of APC in experiment 3 after washing was 80 to 86% for eggs from hens in cages (White and Brown), 69 to 86% for hens on slats, and 82 to 88% for hens on shavings. These percentages were similar to the results from experiment 1 for hens in cages (62 to 74%), hens on slats (55 to 81%), and hens on shavings (84 to 90%).

Although the density of hens per pen in experiment 3 was less than one-third (28%) of that used in experiment 1 (15 hens/pen compared with 54 hens/pen in experiment 1) and the resulting total room density was less than one-sixth (14%; 45 hens/room compared with 315 hens/room), eggs from hens in all 3 housing systems in experiment 3 had high APC (within 0.4 $\log_{10}$ cfu/mL) except for Brown hens on slats, which were 1 $\log_{10}$ cfu/mL lower. Washing eggs continued to significantly reduce APC to 0.4 to 0.7 $\log_{10}$ cfu/mL. The egg-washing machine and washing solutions used in experiments 2 and 3 continued to outperform the equipment and chemicals used in experiment 1 by 1.5 to 2.0 $\log_{10}$ cfu/mL. Aerobic plate counts for eggs in experiment 3 were 69 to 88%, comparable with the prevalence (55 to 90%) in experiment 1.

The influence of housing systems on shell bacterial contamination has been demonstrated in previous studies and, in general, eggs produced in systems such as furnished cages and aviaries have higher shell bacterial levels than eggs produced in conventional cages (Harry, 1963; Quarles et al., 1970; De Reu et al., 2005; Mallet et al., 2006; Wall et al., 2008). In the current study (experiment 1), unwashed White eggs produced on shavings had significantly higher APC (3.8 $\log_{10}$ cfu/mL) and a higher prevalence of *E. coli* and coliforms than eggs produced on slats (3.2 $\log_{10}$ cfu/mL) or in cages (3.1 $\log_{10}$ cfu/mL). The higher APC and prevalence of total coliforms (*E. coli* and coliforms combined) on the shells of White eggs produced in the all-shavings pen was likely due to the presence of excreta in the shavings and contact between the feet of the hen and the nest pad. Hens may transport fecal matter and other contaminants on their feet from the shavings area to the nest boxes and increase the potential for shell contamination within the nest during lay and while the egg rolls out of the nest into the covered egg tray area. Tauson et al. (1999) reported poorer foot hygiene among hens housed in systems with shavings or litter areas compared with those housed in conventional cages (no shavings area). Aerobic bacterial levels reported by Wall et al. (2008) for unwashed White eggs produced in conventional cages (2.7 $\log_{10}$ cfu/mL) or in furnished cages (3.0 $\log_{10}$ cfu/mL) were similar to the levels reported in this study in experiment 1 (3.1 $\log_{10}$ cfu/mL for hens in cages and 3.2 $\log_{10}$ cfu/mL for hen on slats). The similarity of these results could be influenced by the fact that hens housed on wire floors (i.e., cages and slats) are, for the most part, separated from their manure and shavings.

When comparing bacterial levels of Brown eggs, unwashed eggs produced on all wire slats and shavings had significantly higher APC than those produced in cages. Similarly, De Reu et al. (2005) reported higher aerobic shell bacteria on eggs from Brown hens housed in an aviary system compared with a conventional cage system. Total coliform prevalence was also higher among unwashed Brown eggs produced on shavings. Increased bacterial levels of unwashed Brown eggs produced on slats (4.1 $\log_{10}$ cfu/mL) and on shavings (4.2 $\log_{10}$ cfu/mL) may have been due to the presence of nest boxes in both housing systems. The Hy-Line Brown pullets were about 25% larger by BW than the Hy-Line W-36 (White) pullets at 15 wk of age. Hen size may influence shell bacterial levels because larger hens will consume more feed and water daily, eventually producing more manure daily, which can potentially contaminate the feet and eggs of the hens. Shell APC of Brown eggs (3.0 $\log_{10}$ cfu/mL) produced in cages were similar to those of White eggs (3.1 $\log_{10}$ cfu/mL) produced in cages. For the caged hens, hen size may not have been an important factor because the Brown hens were housed at a lower density than the White hens (5 vs. 6 hens). The prevalence of *E. coli* and coliforms was lower among White and Brown unwashed eggs produced in cages compared with those produced on slats and shavings. These results are similar to those of Singh et al. (2009), who reported lower *E. coli* and coliform contamination levels on eggs from hens in cages than on eggs from nest boxes for White and Brown laying hens.

Washing eggs significantly reduced the number of aerobic bacteria recovered from the shells of White and Brown eggs produced in all 3 housing systems. Washed White eggs produced on slats had significantly lower aerobic bacterial levels than those produced on shavings and in cages. Because the APC of unwashed White eggs produced on slats and in cages were statistically similar and eggs from both housing systems were subjected to the same washing procedures, this suggests that greater numbers of APC were removed from the eggs from hens

housed on slats than from eggs from hens housed in cages. Washing eggs significantly reduced the number of aerobic bacteria recovered from the surface of Brown eggs produced on slats and on shavings to comparable levels, which remained significantly higher than those of eggs produced in cages. This trend was also observed among unwashed Brown eggs. Overall, washing eggs reduced aerobic bacterial levels of White and Brown eggs by 1.5 and 1.8 $\log_{10}$ cfu/mL, respectively. Once subjected to the wash treatment, the prevalence of *E. coli* and total coliforms among White and Brown eggs were reduced to 3.8 and 5.4%. When identifying *Enterobacteriaceae* from unwashed and washed shell eggs, Musgrove et al. (2004, 2005) reported significantly fewer numbers of *Enterobacteriaceae* recovered from washed eggs. From a food safety perspective, if eggs are not going to be washed, it is important that they are produced in a housing system with as little contamination as possible and are collected frequently because eggs are susceptible to bacterial contamination before collection. Our results indicate that the housing system allowing for the least amount of shell aerobic bacteria for unwashed egg contamination would be the conventional cages, followed by the all wire slats, and then the all shavings floor pen.

In summary, the unwashed shells of eggs collected from hens housed in cages had lower levels of aerobic bacteria than eggs from hens housed on slats or shavings. In addition, washing eggs significantly lowered shell bacterial levels ($P < 0.01$), and after washing eggs from hens housed on shavings, on slats, or in cages, the level of bacteria recovered did not differ between housing environments in experiment 3. After moving hens back to the room with shavings, slats, and cages, the APC on the eggshells increased rapidly to the levels recovered in experiment 1, although the hen density was two-thirds lower in experiment 3. For unwashed eggs, APC are lowest in housing systems that separate hens from manure and shavings. After adequate washing of nest clean eggs, the resulting shell APC are comparable for eggs from White and Brown hens housed on shavings, on wire slats, or in cage housing systems.

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