

Influence of Slat Material on Hatching Egg Sanitation and Slat Disinfection

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Primary Audience: Breeder Managers, Hatchery Managers, Veterinarians

SUMMARY

Where an egg is laid has significant influence on the degree of surface bacterial contamination. Bacterial counts on shell surfaces from the eggs of broiler breeder hens housed in partial slat pens revealed that the eggs laid in litter material ($1.75 \diamond 10^9$ cfu/mL) were significantly dirtier than eggs laid in the nest ($6.96 \diamond 10^4$ cfu/mL) or on the slats ($3.87 \diamond 10^5$ cfu/mL). The type of raised slat used in broiler breeder houses influenced the degree of bacterial contamination accumulating on the slats over the life of the flock. Wood and plastic slats harbored more bacteria than polyvinyl chloride (PVC) coated wire slats before and after the slats were washed and disinfected. But wood and plastic slat materials also had a greater surface area. There was no difference in effectiveness of bacterial reduction when a quaternary ammonium compound ($4.37 \diamond 10^7$ cfu/mL) or a phenolic compound ($6.43 \diamond 10^6$ cfu/mL) was used to disinfect the slats. Eggs laid on slats with square openings, regardless of surface area, were significantly cleaner than eggs laid on wooden slats.

Key words: bacteria, disinfection, hatching egg, slat

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DESCRIPTION OF PROBLEM

The majority of infections in animals with bacterial pathogens involve either direct spread from other animals or contact with their immediate environment, which becomes polluted by secretions or excretions of other animals. Intensive systems of animal management may influence the ecology of some pathogens by favoring their accumulation in the animals' immediate environments [1]. Survival by any bacteria in the environment is dependent on many

factors including the availability of nutrients, moisture, pH, and temperature [2].

In the husbandry of broiler breeder chickens, the use of raised slats was introduced to allow placement of birds at an increased density [3, 4, 5]. This configuration should result in a decreased contact with bacterial pathogens that could cause disease in the breeder flocks [4, 6, 7, 8]. Studies have found varying results in productivity and health of flocks housed with raised slat flooring [3, 4, 5, 9, 10, 11, 12, 13].

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The chief source of contamination of hatching eggs is contact of shells with dirty surfaces [1, 14, 15]. Most shell contamination occurs immediately after eggs are laid; thus, location and sanitation of the place of oviposition is critical to clean eggshells [1, 16]. Eggshells have 7,000 to 17,000 pores through which surface bacteria can pass to the inside [1, 17]. Studies have shown a significant difference in the percentage of bacterial penetration in eggs laid in a nest when compared with eggs laid on the floor [6, 15, 18]. The bacterial penetration is enhanced because the internal temperature of the egg at the time it is laid is 42°C. When a warm egg contacts a cool surface, the egg contents contract, allowing organisms on the surface of the egg to be sucked into the eggshell through the pores [1, 16, 19]. As a hen ages, the size of her egg increases, and the thickness of the shell decreases. The pore length is determined by shell thickness; thus, thinner shells are more prone to bacterial contamination caused by increased penetration of bacteria from the surface of the egg [1, 16]. Therefore, as a hen ages, her eggs may become more prone to contamination. Coliform and *Escherichia coli* densities remain fairly consistent in poultry litter throughout growout with about one-third of the coliforms being *E. coli* [2]. *Escherichia coli* is the most common contaminant of yolk sacs in chickens [20]. These enteric organisms can penetrate the shell, decreasing hatchability. Omphalitis may result in mushy chicks, high early mortality, poor chick quality, and perpetuation of infections in growing birds [21].

Historically, the material used in the manufacture of raised slat sections used in broiler breeder houses has been hardwood, a very porous material that can be difficult to properly clean and disinfect. This contamination could result in carry-over of pathogens from flock to flock. Moreover, during periods of decreased hatching-egg production, companies may use all eggs produced for hatching, including those laid in litter and on slats. Eggs that are laid on unsanitary surfaces may have a higher incidence of internal contamination. If these eggs are incubated, decreased hatchability and omphalitis and high chick mortality could result [6, 7, 8, 21, 22]. A variety of new plastic and

coated wire materials have been introduced to address this problem in hopes of improving slat sanitation.

The first objective of this study was to determine the degree of bacterial contamination found on the surface of hatching eggs laid on various types of slat materials over the life of the flock. The second objective was to evaluate the degree of bacterial contamination present on a selection of slat materials after being used for 45 wk in commercial broiler breeder houses. The final objective was to evaluate the ability to clean and disinfect these same slat materials using techniques and products common to the commercial poultry industry.

MATERIALS AND METHODS

Six hundred Arbor Acre [23] pullets and 60 Peterson [24] cockerels, 20 wk of age, were obtained from a poultry integrator. The pullets and cockerels were divided into 12 groups of 55 birds (50 pullets and 5 cockerels) resulting in three replicate pens per slat type (treatment). Each floor pen was 9 m² of which 62% was covered by one of the following four slat materials: typical hardwood (wood) slats with a 3.8-cm slat and 3.2-cm openings, black plastic (plastic) slats with a 0.8-cm slat and 2.5-cm square openings, white polyvinyl chloride (PVC) coated 12.5-gauge galvanized wire (white-rec) with a rectangular opening of 1.9 × 7.6 cm, and black PVC-coated 14-gauge galvanized wire (black-square) with a square opening measuring 2.5 × 2.5 cm (Figure 1). All slats were placed at a height of 50 cm. The rest of the floor pen was covered with clean pine shavings.

The hens ate from three tube feeders per pen placed on the slats each with 61 cm of feeder space available. The roosters ate from a straight feeder trough (61 cm long) placed in the scratch area. A plastic grill restricted males from eating from the tube feeders. All birds were fed a typical broiler breeder laying ration at levels to keep them at breeder-standard body weights. In addition, each pen had a 122-cm section of nipple drinkers (six nipples/pen) and a 152-cm section of side-belt mechanical nests (15 to 24 cm wide nests). The mechanical nests were equipped with plastic molded bottoms with rubber finger pads [25].

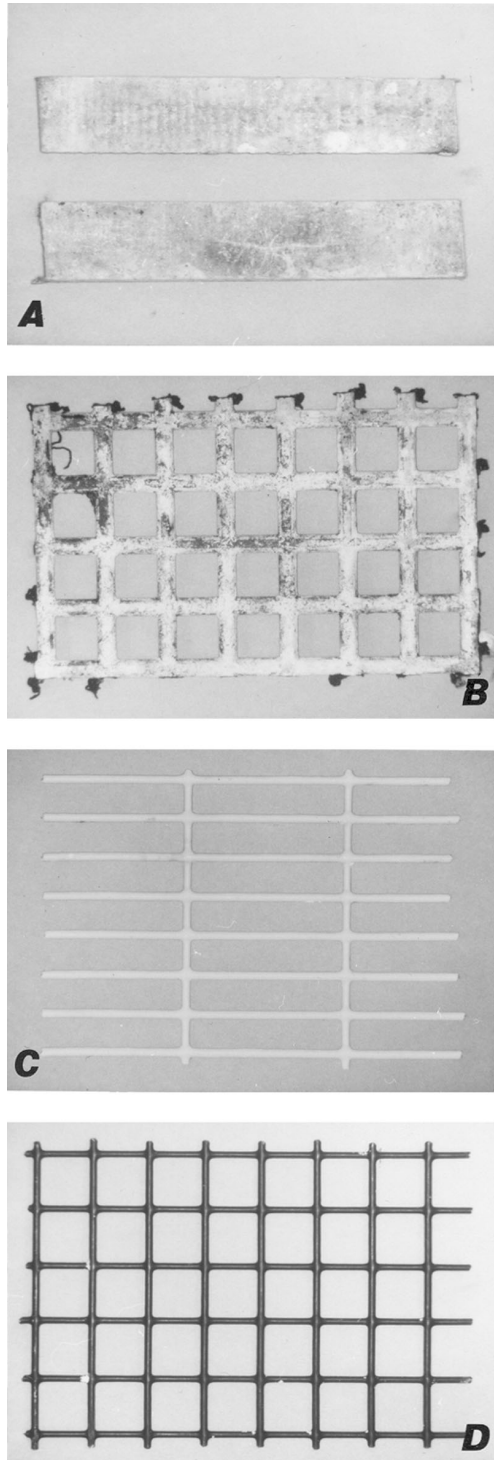


FIGURE 1. Slat types used in broiler breeder pens. A) wood (slat section); B) plastic (slat section); C) white-rec polyvinyl chloride (PVC-coated wire slat section); D) black-square (PVC-coated wire slat section).

Day length was increased from 8 to 16 h per day at 20 wk of age.

At 0600 h daily, all eggs in each pen were collected and discarded to prevent inclusion of eggs that might have been exposed to the environment for up to 12 h. All test eggs were individually collected with a freshly gloved hand and placed in sanitized flats every 60 to 90 min from 0600 to 1200 h. One final collection was made between 1600 and 1700 h daily, when eggs were discarded. When available, one egg was collected daily from each location: nest, slat, or litter, from three pens of each of the four slat types. Eggs were identified with pencil on the shell surface indicating pen number, date, and location. Eggs were collected over a 2-d period at each of the following hen ages: 28 wk, 31 wk, 45 wk, and 59 wk. In total 198 eggs were collected, 46 from slats, 85 from nest, and 67 from litter.

In the laboratory, eggs were placed in sterile plastic bags to which 50 mL of trypticase soy broth was added. The surface of each egg was scrubbed through the bag for 3 min, allowed to rest for 5 min, and scrubbed again for another 3 min. One-tenth of a milliliter was taken in duplicate from each rinsate and evenly spread using a sterile glass rod onto blood agar. Tenfold dilutions were also made in duplicate of the rinsate. All plates were incubated overnight, and colonies were counted. In order to provide the most accurate bacterial counts, only the plates with a count closest to 100 were used to calculate the level of surface contamination of the eggs using the following formula: colony-forming units (cfu)/mL = bacteria count from the plate \times dilution factor \times 10.

After the 45-wk laying period, two 15- \times 20-cm sections of each of the slat types were cut and removed from the pens for sampling. Each test section was removed from a similar area of each pen in relation to feeders, waterers, nest boxes, and pen walls. The plastic slats had jointed areas formed by overlapping two sections of slat material. Because of the increased potential for debris and bacterial retention in the seamed areas, jointed and nonjointed sections of the plastic slats were cut for separate sampling. All samples were placed in sealed bags and taken to the laboratory for microbiological analysis. Each section was

aseptically stored until testing 12 to 24 h after collection.

The level of bacterial contamination on the surface of the various slat materials was evaluated by placing each section in a sterile plastic pan containing 1,000 mL of PBS. Under a laminar flow hood, the slat-and-PBS mixture was manually agitated for 5 min and allowed to sit for 45 min. The mixture was agitated again for 30 s before sampling. Ten milliliters of the PBS rinsate was removed from the slat sample and placed in a 50-mL plastic tube. From this sample, 1 mL was removed in duplicate, and 10-fold serial dilutions were made. One-tenth of a milliliter was removed from each dilution and evenly spread onto blood agar plates and incubated at 37°C overnight. Plates were evaluated as described above. The total number of bacteria washed from the slat sections was recorded as colony-forming units per milliliter = bacterial count from plate \times dilution factor \times 10. Total bacterial counts were multiplied by 1,000 to determine colony-forming units per slat section.

After the dilutions were made, the slat sections were removed from the PBS and allowed to dry under the hood. Each slat section was then attached to a wire surface by plastic pull ties. All samples were rinsed with water for 30 s using a pressure washer at 1,000 pounds per square inch (psi) of water pressure. One-half of each type of the samples had a 20% quaternary ammonium disinfectant applied at 15 mL/3.78 L of water. The other half of the slat sections had a 21% phenolic disinfectant applied at the same rate. The sections were allowed to remain in contact with the disinfectant for 2 min before being rinsed for another 30 s as described above. All slat sections were allowed to air dry, and then the PBS washing procedure was repeated as described above.

All statistical analysis of the data was performed with SigmaStat software [26]. Analysis of the number of colony-forming units of bacteria per milliliter of rinsate per egg was performed based on the slat-type from which the egg was taken. Because of the unequal number of eggs per slat-type and nonnormal distribution of the data, a natural log (ln) transformation was performed on the number of colony-forming units per milliliter per egg before anal-

ysis. A one-way analysis of variance was performed, which was followed by the Tukey test for all pairwise multiple comparisons of the means. These results were converted back to colony-forming units and are listed in Table 1. The power of the performed test with $\alpha = 0.05$ was calculated.

Analysis of the number of colony-forming units of bacteria per 15- \times 20-cm section of slat was performed based on the slat type. Measurements were taken before and after disinfection. The data were transformed using the log transformation. A one-way analysis of variance was performed and this was followed by the Tukey test for all pairwise multiple comparisons of the means. These results were converted back to colony-forming units. The power of the performed test with $\alpha = 0.05$ was calculated.

RESULTS

Table 1 shows that the place of oviposition had a significant influence on the surface bacterial counts of the eggs. Those eggs laid in the litter material had higher bacterial counts on average than eggs laid in the nest box or on the slat material. However, as the flock aged, there was not a detectable increase in surface contamination of the eggs laid on the slats or in the nest. There were overall higher levels of contamination, which was most noticeable in the eggs laid in the litter at both 28 and 45 wk of age, which corresponds to periods when hens were flushing or their manure appeared to have a high moisture content. No litter moisture measurements were made, but additional litter had to be added because of the moisture problem.

Table 2 shows the level of bacterial contamination of eggs laid on the various slat material. When eggs were evaluated for surface bacterial contamination based solely on oviposition on different slat types, a statistically significant difference was observed. Eggs laid on plastic and black-square slats had significantly less surface bacteria than eggs laid on wood slats.

Significantly reduced bacterial counts were obtained after washing and disinfecting when compared with bacterial counts before, regardless of the material type (Table 3). In general,

TABLE 1. Bacterial counts of egg shells (cfu/mL) for each location as influenced by flock age and overall averages of egg surface contamination based on location of oviposition

Weeks of age	n ^A	Bacterial counts of eggs laid on litter ^B	Bacterial counts of eggs laid on slat	Bacterial counts of eggs laid in nest
28	52	6.02×10^7 ^a	1.12×10^4 ^b	2.91×10^4 ^b
31	51	7.36×10^4 ^b	2.46×10^4 ^b	1.17×10^4 ^b
45	49	6.94×10^9 ^a	1.35×10^6 ^b	2.20×10^5 ^b
59	46	5.41×10^5 ^b	1.62×10^5 ^b	1.76×10^4 ^b
Overall mean ^B	198	1.75×10^9 ^a	3.87×10^5 ^b	6.96×10^4 ^b

^{a,b}Values within a column for 28, 31, 45 and 59 wk of age followed by a different lowercase superscript differ significantly ($P \geq 0.05$).

^An = number of eggs tested at each age.

^BValues within row for overall means followed by a different lowercase superscript differ significantly ($P \geq 0.05$).

the wood and plastic slat sections had a significantly higher level of bacterial contamination than the white-rec and black-square sections. This finding was true before and after washing and disinfecting the slat sections. However, it must be noted the wood and plastic slats also had a greater surface area upon which to harbor the bacteria. We chose not to adjust these counts based on surface area because the contact surface of the slat type is a real variable in the poultry house.

Disinfectant type had no significant affect on bacterial reduction. Both phenol (6.43×10^6 cfu/mL) and quaternary ammonia (4.37×10^7 cfu/mL) had similar disinfecting potentials when applied to all slat products. The jointed area of the plastic slat had a similar bacterial level after disinfection (5.15×10^6 cfu/mL) to the nonjointed areas of the plastic slat (8.86×10^7 cfu/mL).

DISCUSSION

The microflora of the shell is almost certainly derived from dust, soil, and feces [1, 14, 15, 19]. A predominant type of bacteria in the

feces is *E. coli* [2], which is the most predominant cause of omphalitis in chicks [20]. Eggshells of hens are perforated with many pores of diameters from 9 to 35 μm [17]. The shell is most susceptible to bacterial penetration within a very short time after laying, based on the assumption that the yolk and albumen contract on cooling, thereby causing the surface organisms to be sucked through pores, which are still moist [1, 16, 19, 27]. It would stand to reason, therefore, that the level of bacterial contamination of the environment where the egg is laid could directly influence the chance of contamination [15]. In fact, a high incidence of rotting occurs during storage of eggs gathered from contaminated areas such as litter [19]. The results of this study agree with others in that surface bacterial contamination is highest in eggs laid in the litter when compared with eggs laid in nests or on slats [6, 15, 18].

The level of surface bacterial counts on eggs laid in various locations did not significantly increase over the life of the flock as the level of environmental contaminants would be

TABLE 2. Overall bacterial counts (cfu/mL) of the surface of eggs laid on various slats

Slat type	cfu/mL	Number of eggs
White-rec ^A	1.38×10^4 ^{ab}	10
Plastic	3.93×10^3 ^a	12
Wood	8.10×10^4 ^b	6
Black-square	4.39×10^3 ^a	18
Total eggs sampled (n)		46

^{a,b}Values within a column followed by a different lowercase superscript differ significantly ($P \geq 0.05$).

^APolyvinyl chloride coated wire slat.

TABLE 3. Bacterial contamination of various slat materials after 45 wk of use in a broiler breeder facility before and after cleaning and disinfection

Slat type	cfu per slat before disinfection ^A	cfu per slat after disinfection	Difference
White-rec ^B	5.56×10^8 ^b	6.80×10^6 ^a	5.50×10^8
Plastic	9.72×10^{12} ^a	1.59×10^{10} ^b	9.70×10^{12}
Wood	1.46×10^{13} ^a	4.06×10^9 ^b	1.45×10^{13}
Black-square	1.28×10^9 ^b	3.80×10^5 ^a	1.27×10^9

^{a,b}Values within a column followed by a different lower case superscript differ significantly ($P \geq 0.05$).

^APolyvinyl chloride coated wire slat.

expected to increase. However, eggs laid on wet litter did have significantly increased surface bacterial counts. Keeping the litter dry may keep the bacterial counts of eggs laid in the litter to the same level as eggs laid on the slats or in the nests. In this study, there was not a significant difference in the level of surface bacterial contamination of eggs laid on the slats when compared with eggs laid in the nests. This trial utilized mechanical nest boxes whose internal surface was more similar to the firm slat material than the loose litter material and might have had an impact on these results.

If eggs that are laid on the slats are used as hatching eggs, the degree of bacterial contamination on the surface of the egg could be an important factor in influencing the degree of internal contamination. The type of slat material upon which eggs are laid had a significant effect on the level of surface contamination of these eggs. Slat materials with square openings resulted in lower egg contamination levels when compared with eggs laid on wood slats that have a rectangular opening. The white-rec, which was coated wire with a rectangular opening, did not significantly differ from wood or slats with square openings. It did not appear that the surface area of the slat or the material type (white-rec and black-square were both

coated wire) had a significant effect on surface egg contamination. Although not evaluated in this study, it is possible that the shape of the opening of the slat material influenced the contact surface of slat to egg. Another possibility is that the shape of the slat opening affected the stability of the egg as it was laid, resulting in a greater or lesser likelihood of movement on the dirty surface.

The degree of bacterial contamination and potential disease spread from flock to flock is based on the ability to clean and disinfect the various types of slat materials. The two wire-type slats (white-rec and black-square) had the least surface area exposed and also resulted in the lowest bacterial counts before and after cleaning and disinfection. The degree to which each slat type could be cleaned and disinfected did not vary; however, the slat types with lesser visible surface area did result in the lowest overall bacterial counts and, therefore, would be less likely to transmit these upon contact.

The type of disinfectant used (quaternary ammonium compound versus phenolic compound) did not affect the ability to disinfect the cleaned slat material. Moreover, there was no adverse affect associated with the presence of seams in the slat material in the ability to disinfect the material.

CONCLUSIONS AND APPLICATIONS

1. When eggs were laid on the slats, the black coated wire and plastic slats, both with square openings, resulted in eggs with the lowest surface contamination.
2. The highest level of bacterial contamination on the slat surface before and after cleaning and disinfection occurred on the wood slats.

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