

Influence of Eggshell Condensation on Eggshell Penetration and Whole Egg Contamination with *Salmonella enterica* Serovar Enteritidis

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ABSTRACT

Shells of agar-filled and whole eggs were inoculated with 10^3 to 10^4 CFU of *Salmonella enterica* serovar Enteritidis per eggshell. The agar-filled eggs were used to study bacterial eggshell penetration, and the whole egg results were used to characterize contamination of the egg contents. In each group, half of the eggs were stored for 21 days at 20°C and 60% relative humidity (RH), and the other half was stored for 24 h at 6°C and then for 20 days at 20°C. The latter conditions resulted in condensation on the eggshell for 30 min from the moment the eggs were placed in the 20°C chamber. Taking into account the ages at which hens were studied (39, 53, and 67 weeks), an average of 62% of the eggshells with condensate were penetrated compared with 43% for the control group; this difference was significant ($P < 0.01$). No significant difference in whole egg contamination was found; 18% of the control eggs were contaminated compared with 22% of the condensate eggs. Whole egg contamination was significantly higher for eggs from the hens at an older age (67 weeks). This difference probably was not due to a higher penetration potential because differences were not observed for the corresponding agar-filled eggs. Condensation on the eggshell seemed to encourage bacterial penetration of the eggshell but had a smaller impact on whole egg contamination.

Salmonella infection resulting from the consumption of contaminated eggs is still a major public health problem. *Salmonella enterica* serovar Enteritidis (SE) is responsible for the majority of egg-associated infections. Two possible routes of *Salmonella* contamination of intact eggs have been considered: transovarian (vertical) transmission of SE occurs when eggs are infected during their formation in the hen's ovaries, and horizontal transmission occurs when freshly laid eggs are subsequently exposed to an environment contaminated with SE and the microorganism penetrates the eggshell. Sauter and Petersen (27), Nascimento and Solomon (24), and De Reu et al. (8) suggested a relationship between eggshell quality and bacterial penetration of eggshell and/or whole egg contamination (horizontal transmission). Harry (15), Smeltzer et al. (30), and De Reu et al. (8) also reported a correlation between the degree of eggshell bacterial contamination and egg infection. Limited data are available on the prevalence of *Salmonella*-contaminated eggshells. In a laying house in which *Salmonella* was isolated from 72% of the environmental samples, 7.8% of the eggshells were contaminated (19). A lower prevalence was observed by Perales and Audicana (25) in flocks implicated in an outbreak of foodborne disease; SE phage type 4 was found on 0.8% of the shells. Musgrove et al. (22)

identified 1 of 105 *Enterobacteriaceae* isolates obtained from 84 eggshell surfaces as *Salmonella*.

Fromm and Margolf (12) reported that sweating of the eggshell resulted in increased bacterial contamination of the egg contents. In a more recent study, Ernst et al. (10) reported no increase in the fraction of SE-positive eggs or the numbers of SE present in the egg contents after eggshells had sweated for 30 min. These authors also mentioned that additional research was needed to determine the relationship between sweating or condensation on eggshells and bacterial penetration of the shell. In the present study, the influence of condensate on bacterial penetration of the eggshell and on whole egg contamination was studied.

MATERIALS AND METHODS

Eggs. Eggs from ISA-Brown laying hens in a commercial conventional housing system were collected the day of lay from hens at 39, 53, and 67 weeks of age, respectively. The next day (after storage under ambient conditions), eggs were visually inspected by candling, and only intact eggs (no cracks or pinholes) were included in further analyses.

Agar method for the assessment of eggshell penetration. An agar method described by Berrang et al. (2) was adapted to study and visualize bacterial penetration of the eggshell as described in detail by De Reu et al. (8). This adapted method consisted of replacing the egg contents with sterile molten nutrient agar (Oxoid, Basingstoke, UK) containing streptomycin (Sigma-Aldrich, Bornem, Belgium), cycloheximide (Sigma-Aldrich) to

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prevent yeast and mold growth, and the indicator 2,3,5-triphenyl-tetrazolium-chloride (TTC; Sigma-Aldrich). The addition of streptomycin to the agar assured that only the inoculated streptomycin-resistant SE strain was able to grow on the agar. When bacterial penetration of the eggshell occurred, SE grew on the agar and reduced the TTC to formazan (with a red color). Penetration was recorded when red colonies on the agar were visible by candling. Candling was performed daily during the first week and then three times each week for the next 2 weeks.

Inoculation and storage. Agar-filled and whole eggs were inoculated by immersion for 1 min in phosphate-buffered saline (Oxoid) containing a streptomycin-resistant strain of SE (MB 1409, isolated from egg contents at our laboratory) at 10^5 to 10^6 CFU/ml. This inoculation resulted in 10^3 to 10^4 CFU of SE on the eggshell. After drying at ambient conditions for 2 h, the eggs were stored for up to 21 days, which is the common sell-by date in Belgium (8).

Determination of the eggshell contamination. At the day of inoculation (day 0) and 21 days later, contamination of the eggshell with the selected SE strain was quantified (detection limit of 10 CFU per eggshell) by adding 10 ml of $0.25\times$ Ringer's solution (Oxoid) to an agar-filled egg or to a whole egg in a plastic bag and rubbing the eggshell through the bag for 1 min to detach the bacteria (7). The diluent was next plated with a spiral plater (Eddy Jet, IUL Instruments, Barcelona, Spain) on nutrient agar with 25 ppm streptomycin. Plates were incubated at 30°C for 72 h.

Determination of contamination of the contents of whole eggs. To remove the contents of whole eggs aseptically, a modification of the method described by Himathongkham et al. (17) was used. Each egg was placed in a petri dish and sprinkled with 75% ethanol. After rolling the egg in the dish with tweezers, the alcohol was burned off for ca. 5 s. After a second successive short flaming, the disinfected egg was broken by hand with a sterile blade and sanitized plastic gloves. The whole egg was separated into two fractions: the albumen with yolk and the burned-off eggshell with the membranes. Both fractions were diluted in 25 ml of buffered peptone water (Oxoid), stomached (albumen and yolk) or shaken and softly rubbed (eggshells with membranes), and incubated for 24 h at 30°C. The enrichment broth was plated on nutrient agar with 25 ppm streptomycin, and plates were incubated at 30°C for 72 h (8).

Eggshell characteristics. The surface area, dynamic stiffness (k_{dyn}), damping of vibration, and resonance frequency were studied on the fresh eggs immediately after candling (crack detection), i.e., before the penetration experiment. When the eggshell penetration (agar-filled eggs) experiment was completed, the following eggshell characteristics were determined: shell thickness, number of pores, and cuticle score. The weight (W) of the fresh eggs was determined and used to calculate the shell surface area (S) using the formula $S = 4.67W^{0.75}$ (31). The dynamic stiffness, damping of vibration, and resonance frequency were measured with a desktop unit that detects eggshell breakage and strength based on vibration measurements (5, 6). The shell thickness was determined at three places with a micrometer. To determine the number of pores, pieces of eggshell were immersed for 25 s in 65% nitric acid solution and rinsed with distilled water, the membranes were removed, and pores were counted by microscopic examination (ocular of $\times 8$ and objective of $\times 4$; Olympus BH2-RFCA, Tokyo, Japan) (31). The cuticle score was determined by dyeing the eggshell with Edicol Pea Green, which is an aqueous mixture of 7.2 g/liter tartrazine and 28 g/liter green S (Barentz N.V., Zaventem, Belgium) (3). The remaining red color, i.e., the color at places where the

dye did not bind, was analyzed with Paint Shop Pro version 8 (Jasc Software, Eden Prairie, Minn.) using the histogram function. With this method, the red score or cuticle score is inversely correlated with cuticle deposition.

Because the determination of whole egg contamination is a destructive method, only the eggshell characteristics surface area, dynamic stiffness, damping of vibration, and resonance frequency could be measured.

Condensation experiment. At each hen age of 39, 53, and 67 weeks, 105 agar-filled eggs and 105 whole eggs were inoculated. The agar-filled eggs were used to study bacterial penetration of the eggshell (eggshell and membranes), and the whole eggs were used to study the contamination of the egg contents. On the day of inoculation (day 0), five agar-filled eggs and five whole eggs were randomly selected to determine the inoculation dose (10^3 to 10^4 CFU per eggshell). After inoculation, half of the remaining eggs in each group (50) were stored for 21 days in a climate chamber (Termaks KBP 6395 F, Solheimsviken, Norway) at 20°C and 60% relative humidity (RH). The other half (50) were first stored for 24 h in a refrigerator at 6°C and 70 to 85% RH and then stored for 20 days at 20°C and 60% RH. After placing the 6°C eggs into the climate chamber, condensation on the eggshell was observed for 30 min. The temperature-RH combination (20°C and 60% RH) resembles the environmental conditions the eggs are exposed to most of the year at the packaging station and the store (8).

Statistical analysis. The penetration and contamination data were analyzed using a generalized linear regression model, with penetration or contamination as binomial (yes or no) dependent variables and the presence of condensate, the number of bacteria on the eggshell, and the eggshell characteristics (thickness, surface area, number of pores, cuticle score, dynamic stiffness, damping of vibration, and resonance frequency) as independent variables. Differences in eggshell characteristics as a function of the presence of condensate or penetration were assessed with an analysis of variance. Interval estimation in the Power analysis module was used to calculate the confidence intervals (at the 95% confidence level) of the percentage of penetrated eggshells and contaminated whole eggs. A simple linear regression was carried out to determine the influence of hen age on eggshell penetration, whole egg contamination, and eggshell characteristics. All analyses were done with Statistica 7 (Statsoft, Tulsa, Okla.).

For the data on eggshell contamination with SE on day 21, there were left and right censored data simultaneously because a fraction of the data consisted of values <10 and $>3,000$. However, there also were bacterial counts larger than 3,000. Hence, we took a different approach for the left and right censored part. For the agar-filled eggs, we assumed that the data that were present are the best guess for the data that were to be reconstructed. We constructed distributions, derived from the available data, from which we sampled in a bootstrap procedure. Because there were actual data available above 3,000, we constructed an empirical cumulative distribution based on these data. This approach is equivalent to supposing that the censored data had the same distribution as the available data. This reconstruction was done separately for condensate positive and negative data. The values $>3,000$ were then each replaced with a random sample from the corresponding distribution. Because there are no measured data available for counts <10 , we fitted a distribution to all the data (excluding the censored values) and extrapolated to the <10 zone. For the combined condensate and control groups, a normal distribution was fitted to the log-transformed data and then truncated between 0 and 1. The values <10 were then replaced with random

samples from this distribution. A 10,000-iteration bootstrap was done on the averages of the condensate and control groups of agar-filled eggs, where the censored data were sampled from the constructed distributions as outlined above (20).

RESULTS

Eggshell characteristics. Table 1 presents the mean values with standard deviations for each analyzed eggshell characteristic for eggs with and without condensate (all weeks; agar-filled eggs). Although the eggs of both groups came from the same lot of sampled eggs (same henhouse, hen breed, hen age, etc.), evaluation of the data revealed a significant difference ($P < 0.05$) in the shell thickness and a highly significant difference ($P < 0.01$) in cuticle score (Table 1, both groups). Because cuticle score was inversely correlated with cuticle deposition; eggs without condensate had significantly less cuticle deposition. This difference in cuticle score was systematic, i.e., the difference was found at each sampled week (data not shown). Table 1 also provides a comparison of the eggshell characteristics between the penetrated eggs and the nonpenetrated eggs for both the control group and the condensate group. For both individual groups, penetrated eggshells contained significantly more pores ($P < 0.05$) than did nonpenetrated eggshells. A significantly higher cuticle score ($P = 0.0125$) was found for the penetrated eggs than for the nonpenetrated eggs from the control group; for the condensate group, this difference was not observed. Thus, the average cuticle deposition for penetrated eggs from the control group was less than that of the nonpenetrated eggs in that group. The number of pores and cuticle score were significantly influenced by hen age (Fig. 1). The number of pores decreased significantly ($P < 0.001$) and the cuticle score increased significantly ($P < 0.001$) with hen age, indicating that cuticle deposition decreased with hen age.

For the eggs in the whole egg (egg contamination) experiment (data not shown), no significant differences were found when comparing the same groups for surface area, dynamic stiffness, damping of vibration, and resonance frequency.

Eggshell penetration and whole egg contamination.

For eggs from the hens at 39 weeks of age, the bacterial penetration of the eggshell (agar-filled eggs) increased from 46% (23 of 50) for the control group to 64% (32 of 50) for the condensate group, but this increase was not significant. Contamination of whole eggs did not increase for the group of eggs with condensation; 12% (6 of 50) of the control eggs were contaminated compared with 10% (5 of 50) of the eggs that had condensate on the eggshell (Fig. 2).

When hens were 53 weeks of age, similar results were obtained (Fig. 2). Bacterial penetration of the eggshell (agar-filled eggs) increased (not significantly) from 53% for the control group to 69% for eggs with condensate on the shell. Contamination of whole eggs was similar for control eggs (10% contaminated) and condensate eggs (8% contaminated).

At the end of the laying period (week 67), less bacterial penetration of the eggshell (agar-filled eggs) was found;

TABLE 1. Eggshell characteristics of agar-filled eggs^a

Egg group/ treatment	<i>n</i>	Shell thickness (mm)	Surface area (cm ²)	No. of pores per shell	Cuticle score	<i>k</i> _{dyn} × 100 (N/m)	Damping (%)	Resonance frequency (Hz)
Both groups								
Control	150	0.408 ± 0.034	68.2 ± 3.8	5,700 ± 2,300	69 ± 24	14,400 ± 4,800	3.05 ± 1.05	5,070 ± 710
Condensate	149	0.417 ± 0.034	68.3 ± 3.9	5,700 ± 2,300	61 ± 23	13,900 ± 3,800	3.27 ± 1.18	4,960 ± 680
<i>P</i>		<0.05	NS	NS	<0.01	NS	NS	NS
Control group								
Penetrated	65	0.407 ± 0.033	68.3 ± 4.1	6,300 ± 2,100	74 ± 27	14,700 ± 6,500	2.95 ± 1.09	5,120 ± 910
Not penetrated	85	0.409 ± 0.035	68.2 ± 3.6	5,300 ± 2,300	64 ± 20	14,200 ± 3,000	3.13 ± 1.02	5,040 ± 510
<i>P</i>		NS	NS	<0.05	<0.05	NS	NS	NS
Condensate group								
Penetrated	93	0.419 ± 0.036	68.1 ± 3.8	6,100 ± 2,200	61 ± 25	14,100 ± 4,600	3.19 ± 1.17	4,970 ± 830
Not penetrated	56	0.414 ± 0.031	68.6 ± 4.0	5,100 ± 2,200	60 ± 21	13,600 ± 2,000	3.41 ± 1.18	4,940 ± 340
<i>P</i>		NS	NS	<0.05	NS	NS	NS	NS

^a Values are means ± standard deviation. *n*, number of eggs; NS, not significant.

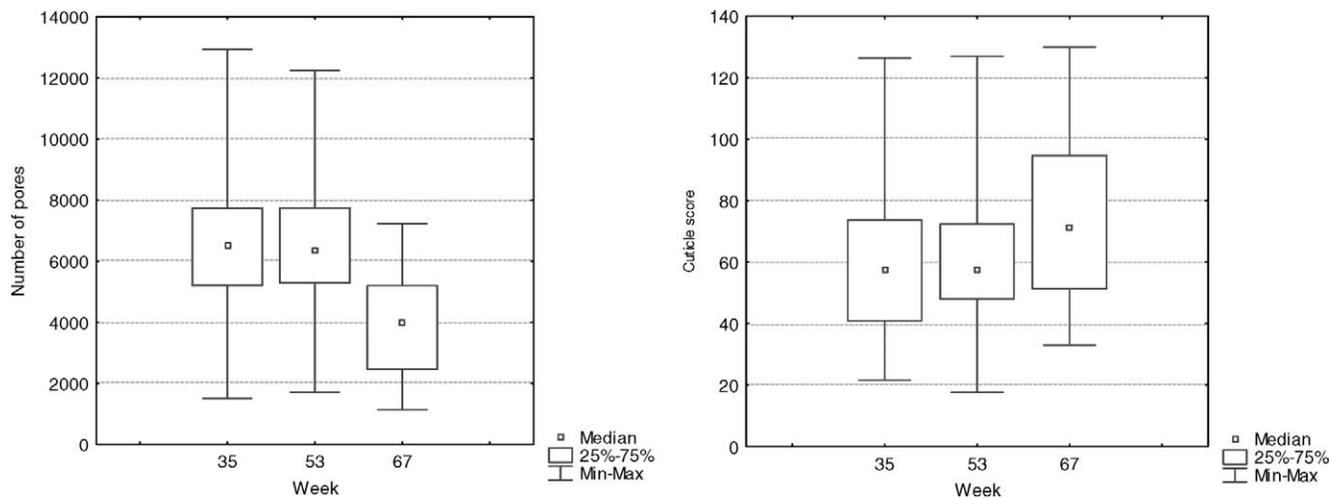


FIGURE 1. Influence of hen age on eggshell characteristics: number of pores and cuticle score.

48% of the eggshells with condensate were penetrated compared with only 29% for the control group (no significant difference) (Fig. 2). In contrast, whole egg contamination was higher than that for eggs from the same hens at an earlier age; 31% of the control eggs were contaminated compared with 48% of the condensate eggs (no significant difference) (Fig. 2). The increase in contamination of the whole eggs with condensate from 10% and 8%, respectively, for week 39 and week 53 eggs to 48% for week 67 eggs was highly significant ($P < 0.001$).

Taking into account the three hen ages, 62% (93 of 149) of the eggs with condensate were penetrated compared with 43% (65 of 150) of eggs in the control group; this difference was significant ($P < 0.01$) (Fig. 2). No significant difference in whole egg contamination was found; 18% (27 of 150) of control eggs were contaminated compared with 22% (33 of 150) of the whole eggs with condensate on the shell.

Effects of storage time on bacterial penetration of the eggshell (agar-filled eggs). The day of eggshell penetration was not significantly influenced by condensation; both groups (control and eggs with condensate) were on average (weeks 39, 53, and 67) penetrated on approximately day 4 (days 3.6 and 4.2, respectively; Fig. 3).

Survival of bacteria on the shells of agar-filled eggs and whole eggs. Figure 4 illustrates the significantly higher average count of the inoculated SE strain present on the shells of agar-filled eggs in the condensate group at day 21 compared with the control group (means of 2.59 and 1.95 log CFU per eggshell, respectively, for 149 and 150 agar-filled eggs, respectively).

No difference in shell contamination on whole eggs was found between both groups at day 21. Of the 150 whole eggs in the control group, 135 eggs were contaminated with <10 CFU per eggshell (detection limit), whereas 134 of the 150 whole eggs with condensate were contaminated with <10 CFU per eggshell. The 15 remaining whole eggs (control group) had an average contamination of 1.96 log CFU per eggshell (standard deviation of 1.00 log CFU per eggshell) compared with an average of 2.47

log CFU per eggshell (standard deviation of 1.00 log CFU per eggshell) for the condensate group ($n = 16$). Because of the high standard deviations, this difference was not significant ($P = 0.16$).

Comparing the SE counts on eggshells at day 21 between the agar-filled eggs and the whole eggs, 34% (103 of 299) and 90% (269 of 300) of the eggs, respectively, had counts of <10 CFU per eggshell.

DISCUSSION

The cuticle on the eggshell serves as waterproofing and as a barrier of primary importance for particle, bacterial, and fungal invasion (3). Alls et al. (1), Drysdale (9), and De Reu et al. (8) found that major cuticle deposition resulted in less bacterial penetration and contamination. In the present study, this result was obtained for the control eggs. Notwithstanding the major cuticle deposition of the eggshells with condensate, greater bacterial penetration of the eggshell was found for the agar-filled eggs with condensate than for the control group. These results indicate that the major cuticle deposition was less effective as a barrier, possibly because of the presence of condensate. Although the eggs of both groups came from the same lot of sampled eggs, a systematic difference in cuticle deposition between the control and condensate eggs was found. Because the cuticle deposition was examined when the penetration (agar-filled eggs) experiment was completed, the increased cuticle deposition (lower cuticle score) of the condensate eggs could be due to the absorption of water from the condensate, less digestion of the cuticle because of inhibition of bacteria during incubation for 24 h at 6°C, or other unknown reasons. Simons and Wiertz (29) observed that the cuticle thinned during egg storage as a result of dehydration. Because shell thickness does not affect penetration (8, 21, 30, 32), the minor difference in shell thickness between control and condensate eggs did not influence the results of this study.

The significantly higher SE contamination on day 21 of shells of the agar-filled eggs that had developed condensate for 30 min was striking. No significant difference in shell contamination of agar-filled eggs stored for 24 h at

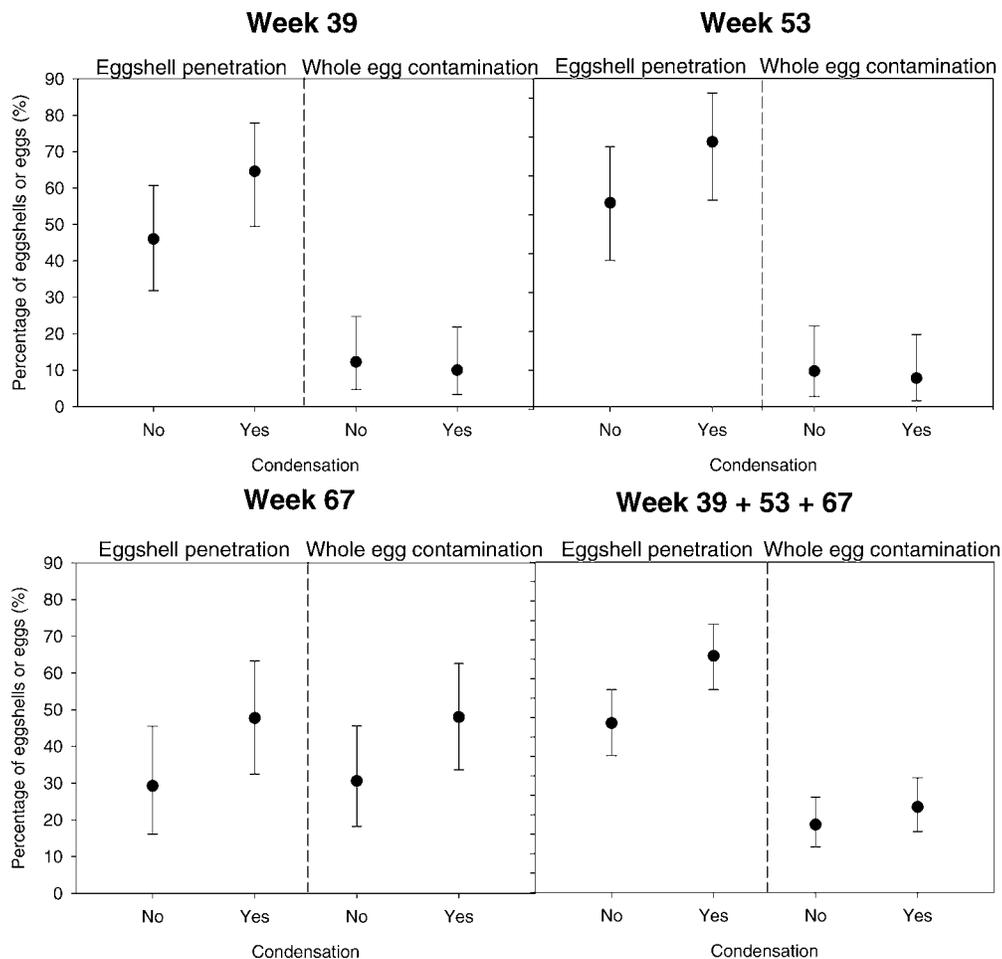


FIGURE 2. Percentage of penetrated eggshells and contaminated whole eggs with shells without and with condensate as a function of hen age. Vertical bars denote 0.95 confidence intervals; n = 50 in each group of eggs for weeks 39, 53 and 67, and n = 150 in each group of eggs for weeks 39 + 53 + 67.

20°C and 60% RH compared with 24 h at 6°C and 85% RH was found (data not shown). The presence of condensate on the eggshell after cold storage must have positively influenced bacterial survival and growth on the eggshell and indirectly affected eggshell penetration. This finding is

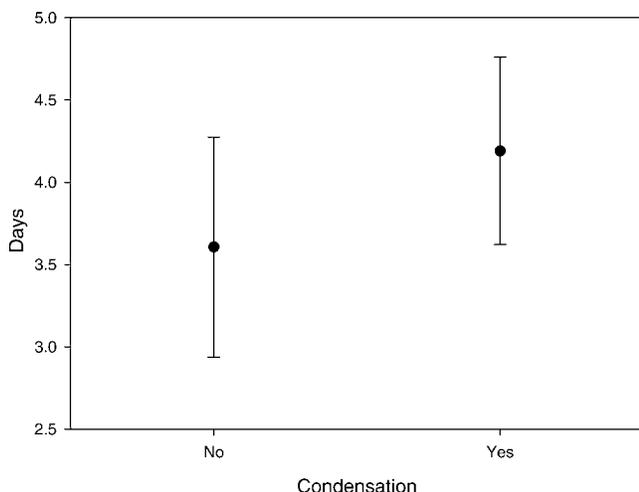


FIGURE 3. Moment of observing SE penetration of the eggshell for control eggs versus eggs with condensation on the shells (agar-filled eggs). Vertical bars denote 0.95 confidence intervals.

consistent with ample evidence in the literature that eggshell penetration is related to the degree of bacterial contamination on the eggshell. Messens et al. (21) found a high correlation between SE contamination of eggshells and eggshell penetration. For each of seven bacterial species originating from egg contents, De Reu et al. (8) found a correlation between eggshell bacterial contamination and the eggshell penetration by these bacteria.

The moment of eggshell penetration was not significantly influenced by cold storage (6°C and higher RH of 70 to 85%) of the agar-filled eggs for 24 h (condensate group). Only a slightly earlier penetration time (day 3.6) for the control eggs was found, which can be due to the faster growth of SE on the agar at 20°C than at 6°C during the first 24 h after inoculation.

A comparison of eggshell characteristics between penetrated eggs and nonpenetrated eggs for both the control group and the condensate group revealed that penetrated eggshells contained significantly more pores ($P < 0.05$) than did nonpenetrated eggshells, which indicates that porosity is correlated with bacterial penetration, as shown previously (3, 13). In contrast, Reinke and Baker (26), Hartung and Stadelman (16), Nascimento et al. (23), Messens et al. (21), and De Reu et al. (8) found that bacterial penetration

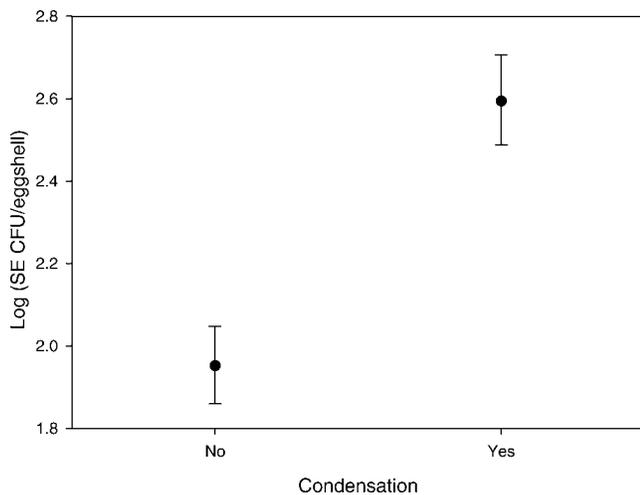


FIGURE 4. Correlation in agar-filled eggs between the presence of condensate and SE contamination of the eggshell at the end of storage (21 days). Vertical bars denote 0.95 confidence intervals; $n = 150$ and 149 for control and condensate groups, respectively.

of the eggshell was not pore dependent. These conflicting opinions may be related to the fact that some pores do not extend through the thickness of the shell but end abruptly (28) and cuticular capping and plugs often are present on or in pores, preventing microbial penetration (3). In the absence of condensate, the cuticle remains an important barrier; significantly higher cuticle deposition (lower cuticle score) ($P < 0.05$) was found for the nonpenetrated eggs than for the penetrated eggs. For the condensate group, however, no difference in cuticle score between penetrated and nonpenetrated eggs was found.

In agreement with our results, De Reu et al. (8) and Messens et al. (21) also did not find a significant influence of flock age on eggshell penetration. The trend found by Messens et al. (21) toward reduced SE penetration of eggshells (for agar-filled eggs) as the flock aged (45.0% of eggs were penetrated at the beginning of the laying period versus 31.6% at the end) was confirmed by our results: 46% penetration at week 39 to 29% penetration at week 67 (agar-filled control eggs). However, Nascimento et al. (23), also using agar-filled eggs, reported increasing SE penetration of the eggshell from 12.9% of the eggs (beginning of lay) to 25.0% (end of lay). In our study, cuticle deposition and number of pores on the eggshells decreased significantly with flock age. The lower number of pores could explain the lower penetration, whereas less cuticle deposition would encourage eggshell penetration.

In contrast to the results for the agar-filled eggs, the shells of whole eggs in the condensate group were not significantly more often contaminated with SE than were the shells of whole eggs in the control group. When the results of all weeks were analyzed together, no significant difference in whole egg contamination was found between the two groups of eggs. The higher potential for eggshell penetration observed for agar-filled eggs with condensate did not result in greater contamination of the egg contents. At day 21, the SE counts for the shells of whole eggs were significantly lower than those for the shells of agar-filled

eggs. This finding suggests that survival and growth of SE on the eggshell was favored by nutrients available from the agar of the agar-filled eggs and/or not stimulated by antimicrobial components of the contents of whole eggs, as suggested by De Reu et al. (8). Taking into account the data for all weeks, whole egg contamination was significantly less prevalent than bacterial penetration of the eggshell. De Reu et al. (8) suggested that the reduced survival of SE on the eggshell and the antimicrobial defenses of the albumen inside the whole eggs must have prevented whole egg contamination. The importance of the antimicrobial properties of the albumen was also revealed by Jones et al. (19), who reported that despite a *Salmonella* prevalence of 7.8% (7 of 90 eggshells), no *Salmonella* was found in 180 contents from eggs of the same sample.

Higher rates of whole egg contamination were found in eggs from the end of the laying period than in eggs from the previous two hen ages. This higher contamination probably was not due to a higher penetration potential because it was not observed in the agar-filled eggs. Bruce and Johnson (4) reported a similar increase in contamination of hatching eggs as flocks became older. Data from Jones et al. (18), who studied whole eggs artificially contaminated on the shell with SE and *Pseudomonas fluorescens*, suggest also that bacterial contamination of air cells, shell membranes, and egg contents is more easily achieved in eggs from older hens than in eggs from younger hens. In that study, SE contamination increased from 30% in eggs from week 34 hens to 50% in eggs from week 74 hens. Fajardo et al. (11) reported that 43% of whole eggs from 72-week-old hens were positive for inoculated SE after incubation for 48 h at 32°C. De Reu et al. (8) found that whole egg SE contamination also slightly increased from 13, 13, and 15% in eggs from hens at weeks 34, 46, and 60, respectively, to 26 and 20% in eggs from hens at weeks 69 and 74, respectively. According to Jones et al. (18), shell and egg quality decreases as hens age, making it easier for microorganisms to infect the egg. During our study, no significant decrease in shell quality (dynamic stiffness, damping of vibration, and resonance frequency) of the whole eggs was observed.

Ernst et al. (10) evaluated intact eggs (hen age not reported) that had been stored at 4°C for 32 days and found no significant difference in SE contamination of egg contents due to sweating: 2.8% (1 of 36) of nonsweated eggs and 5.7% (2 of 35) of sweated eggs were contaminated. This prevalence of contamination approaches ours in eggs from the hens at 39 and 53 weeks of age. For cracked eggs (small line checks), a similar result was obtained: 77% of nonsweated cracked eggs were contaminated versus 64% of sweated cracked eggs. In that study, moisture on the eggshell was created by placing inoculated eggs in sterile plastic bags, which were stored overnight at 2 to 4°C and then at 32°C and about 95% RH. Under this protocol, eggs sweated continuously for 3 h. In an earlier study, Fromm and Margolf (12) found that bacteria were more likely to be present in albumen or yolk of eggs allowed to sweat for 1, 3, or 5 h. They used four groups of eggs: clean unwashed, dirty unwashed, clean washed, and dirty washed.

The procedure to obtain sweating differed from that in our study. Eggs were stored for 0, 1, 4, 8, or 12 days at 10 to 12°C and 80% RH, moved to 22 to 24°C and 80 to 85% RH for 1, 3, or 5 h, and then returned to storage in the refrigerator (10 to 12°C and 80% RH) until day 12. All eggs were analyzed for bacterial contamination at day 12. The higher incidence of contamination of the sweated eggs could be due to the negative pressure in the eggs; placement of the sweating eggs in the refrigerator could allow bacteria-loaded moisture to be drawn through the shell pores, resulting in contamination of the egg contents (14).

Condensation on the eggshell encouraged SE penetration of the shell but had less of an impact on whole egg contamination. The poorer survival of SE on the shell of whole eggs and the antimicrobial defenses of the albumen inside whole eggs must have discouraged whole egg contamination. The better survival of SE on the shells of agar-filled eggs with condensate might explain the higher rate of SE penetration of those eggshells.

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