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Effect of Egg Washing on the Cuticle Quality of Brown and White Table Eggs

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ABSTRACT

Egg washing is currently not permitted within the European Union, with few exceptions. This is mainly because there are concerns that cuticle damage could occur during or after the washing process, as a result of a suboptimal operation. In this study, the cuticle coverage levels of 400 washed or unwashed eggs, derived from either a brown or a white egg–laying flock at the end of lay, were compared. The eggs from older hens inherently have poorer cuticle coverage and as a result arguably constitute a greater risk to consumer safety if they are then washed. Thus, the effects of the washing procedure used in this study on cuticle quality were tested under the worst-case scenario. A standard Swedish egg washing process was used. The cuticle coverage of the eggs was assessed by a colorimeter by quantifying the color difference before and after staining with Tartrazine and Green S. The cuticle of an additional 30 eggs from each of the four groups was then visually assessed by scanning electron microscopy. The staining characteristics of the cuticle varied greatly within each group of eggs and showed that the washing process did not lead to cuticle damage. Scanning electron microscopy confirmed that there was no irreversible damage to the cuticle of the washed eggs and that it was not possible to correctly assign the treatment (washed or not) based on a visual assessment. In conclusion, no evidence could be found to suggest that the washing procedure used in this investigation irreversibly changed the quality of the cuticle.

In some countries, such as the United States, Australia, and Japan, egg washing has become a routine and established practice, is regarded as safe, and is perceived by consumers as an essential part of the hygienic production of eggs *(10)*. Washing of class A table eggs is currently prohibited within the European Union, as Regulation (EC) No 589/2008 *(4)* states that ''Grade 'A' eggs shall not be washed or cleaned, before or after grading, except as provided for in article 3.'' This article states that egg washing is only permitted in Sweden and in one plant in The Netherlands.

When part of a continuous in-line process, commercial egg washing must be carried out before the grading and packing operations. The egg washing process itself consists of four stages: wetting, washing, rinsing, and drying. Wetting has the intention of softening any debris by use of a water spray. While the eggs are being rotated, a water spray with detergent, possibly in combination with brushes, washes the eggs. During the rinsing stage, any loose debris, chemical residues, or other dissolved matter is removed. After rinsing, the eggs must be dried completely to avoid mold growth and to reduce the risk of moisture and bacteria being drawn into the egg *(16)*.

Egg washing was mainly developed to clean dirty eggs (class B). However, washing of eggs may also improve the visual appearance of eggs and their hygienic quality by decreasing the bacterial load on the shell (''sanitizing'') *(16)*. The latter is the major benefit of washing class A eggs. According to the European Food Safety Authority *(8)*, bacterial reductions of 1 to 6 log were found in current egg washing practices. Hutchison et al. *(10)* found a reduction of >5 log of *Salmonella* Enteritidis and *Salmonella* Typhimurium, artificially inoculated on the eggshells, as a result of spray jet washing (wash and rinse water at 44 and 48°C, respectively; Chlorwash wash agent). The lower shell contamination level can lead to a lower occurrence of cross-contamination during food preparation in the kitchen and of trans-shell penetration, potentially leading to egg content contamination *(7, 19)*. By definition, class A eggs

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should have a normal, clean, and undamaged shell and cuticle *(3)*. One of the major concerns of allowing the practice of egg washing within the European Union, however, is based on the potential damage to the cuticle as a result of a suboptimal operation *(8),* such as was demonstrated by Kuhl *(13)* and Overfield *(21).* Such damage may favor moisture loss and trans-shell penetration with bacteria and thereby increase the risk to consumers, particularly if subsequent drying and storage conditions are also suboptimal. Another concern is the possible use of washing to cover up the poor husbandry and hygiene standards on farms and in packing centers *(8),* as washing of eggs that are heavily contaminated with organic material will remain a hazard to the consumer. After all, it is likely that the contaminants will have penetrated the shell by the time the egg is washed and that egg washing will not prevent egg-related illnesses that are vertically transmitted *(16).*

The aim of this study was to compare the cuticle coverage, by means of color measurement (using a dye) and scanning electron microscopy (SEM), of washed and unwashed eggs derived from a flock of brown and a flock of white egg layers. In this study, a washing machine was used in which brush washing and jet washing were combined, and the washing procedure was carried out under optimum conditions as recommended by the manufacturer.

MATERIALS AND METHODS

Eggs. Two hundred sixty brown and 260 white eggs were collected at random at packing stations before grading, 2 and 3 days postlay, respectively. The brown eggs originated from a flock of Hisex Warren laying hens at 60 weeks of age that were kept in traditional cages in Belgium. The white eggs originated from a commercial free-range flock of Lohmann LSL Classic layers that were 55 weeks old in Sweden.

The eggs were kept at ambient conditions in cardboard trays in cardboard boxes during transport and storage until washing or testing. They were neither treated for external contamination nor candled.

Washing process. One-half of the brown eggs (130 eggs) were transported to Sweden at ambient conditions and washed 5 days postlay. One-half of the white eggs (130 eggs) were washed 3 days postlay. Washing was performed by a standard Swedish egg washing process at the packing station (Väst-Farm, Färgelanda, Sweden), approved by the Swedish authorities as packing station SE102. The eggs were loaded onto the conveyor belt of the egg washer (SB 80-100-6A, Kuhl Corporation, Flemington, NJ) and were washed at a temperature between 40 and 45°C. Washing occurred as a combination of brush washing and jet washing, as the jet washers spray a mix of potable water and detergents onto the brushes, which are oval. The eggs were then rinsed at 43 to 48°C and subsequently dried by a forced-air drying system. The entire washing process took approximately 55 s. After washing, the eggs were loaded onto new cardboard trays and placed into new cardboard boxes. The chemicals used during the washing process were Suma Nova L6 washing detergent and Suma Select A7 rinsing agent (both from Diversey [previously JohnsonDiversey], Stockholm, Sweden), at the manufacturer's recommended concentrations required to maintain a pH level between 10 and 11.5.

The eggs were transported at ambient conditions 1 or 7 days postwashing to the institutions for cuticle staining and ultrastructural assessment, respectively.

Cuticle staining. The cuticle coverage was assessed for 100 eggs from each group as follows: brown eggs, washed and unwashed; white eggs, washed and unwashed. Staining was achieved by immersion of each egg for 1 min in an aqueous solution containing, per liter of solution, 7.2 g of Tartrazine and 2.8 g of Green S (Barentz N.V., Zaventem, Belgium; also referred to as Edicol Pea Green). Then, the shell was rinsed in water to remove excess dye prior to drying. This method was developed by Board and Halls *(1)* and used by others including De Reu et al. *(6)* and Messens et al. *(18).* Quantification was done with a colorimeter (spectrophotometer CM-2600d, Konica Minolta) by measuring the color difference (as detailed below) of the eggshell at four points around the equator before and after staining of the cuticle. This uses the L*a*b* color space, measuring the degree of lightness, L*, and the chromaticity coordinates, a* and b*. Color difference can be expressed as a single numerical value, ΔE^*_{ab}, defined by the following formula:

$$\Delta E^*_{ab} = \sqrt{\left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\right]}$$

The average L*, a*, and b* values, before and after staining, were calculated per egg and used to calculate ΔE^*_{ab}. A higher ΔE^*_{ab} denotes a higher staining affinity and hence more cuticle coverage.

Ultrastructural assessment. Ultrastructural assessment of the cuticle was carried out for 30 eggs from each of the four groups. A 1-cm^2 section of eggshell was removed from the equator of each egg by use of a diamond-tipped circular saw. Each section was then mounted on an aluminium stub, coated with gold palladium, and viewed with a Hitachi 501B scanning electron microscope (SEM) at a standard working distance of 12 mm and an accelerating voltage of 15 kV. The gold palladium coating masked the color of the eggshell, which, in addition to the use of a coding system, ensured a nonbiased approach.

Each 1-cm^2 section was analyzed randomly (brown and white eggs mixed) with the SEM at a standard magnification of ×500 and then assigned a score by an experienced assessor for each of the following criteria:

(i) Cuticle coverage (even coverage = 0; occasionally patchy = 2; very patchy = 5; no cuticle = 8)
(ii) Mechanical damage (none = 0; occasionally present = 2; frequently observed = 6)
(iii) Presence of surface debris (absent = 0; isolated = 2; moderate = 5; heavy = 8)
(iv) Exposed pores (none visible = 0; occasional pore visible = 2; frequently observed = 5)

The scores for each of these criteria were weighted in terms of their perceived effect on the functionality of the cuticle such that the higher the total score, calculated as the sum of the scores for the four criteria, the more negative the effect. The weighted scoring system takes into account such factors as the age-related changes in cuticle coverage and the perceived role of the cuticle and pores in terms of facilitating the trans-shell route of bacterial ingress into the egg contents.

A representative image of each eggshell was also taken at a magnification of ×500. These images were collectively presented to a second independent assessor, who was asked to categorize the images as being representative of washed or unwashed eggs. In this particular assessment, the 60 brown eggs (washed and unwashed) were assessed independently of the 60 white eggs (washed and unwashed), but randomly within each group. The assigned category of each coded egg was then compared to its real treatment. The assessor was informed that there were 30 washed and 30 unwashed eggs in each presented group but did not know their origin (brown or white) *(5, 22)*.

Statistical analysis. A two-sample one-sided *t* test was performed to assess whether ΔE^*_{ab} was reduced by egg washing. This test was performed on the square-transformed variable for both the brown and white eggs independently in order to obtain a normal distribution. The significance level, α, was set at 0.05. All analyses were done in R version 2.7.2 (25 August 2008).

The total score for the cuticle quality of each egg as determined by SEM was calculated as the sum of the scores for the four criteria presented earlier. The average values of the total scores and their standard deviations (SD) were computed. To assess the influence of the treatment (unwashed versus washed for the brown eggs and again for the white eggs), a one-sided *t* test was applied. The significance level, α, was set at 0.05. All analyses were done in Statistica 8.0 (Statsoft Inc., Tulsa, OK).

A proportional odds logistic regression model for ordinal responses (*polr* function in *MASS* package in R) was used to assess whether the ordered scores of each of the four criteria were significantly different for the washed eggs compared to the unwashed eggs. The significance level, α, was set at 0.05. This analysis was performed using R, version 2.10.0 (26 October 2009).

RESULTS

Cuticle staining. The ΔE^*_{ab} values of both the brown (unwashed or washed) and white eggs (unwashed or washed) are depicted in Figure 1. The mean ΔE^*_{ab} values (and SD) of the unwashed and washed brown eggs were 35.5 ± 9.6 and 39.6 ± 9.7, respectively. For the unwashed and washed white eggs, the mean ΔE^*_{ab} values and SD were 42.8 ± 8.5 and 41.3 ± 12.1, respectively. For both the brown and white eggs, there was no statistical difference between the unwashed and washed eggs ($P > 0.05$).

Ultrastructural assessment. In Figure 2, images show examples of the types of observations that formed the basis of the cuticle assessment carried out by SEM. The total scores of the four criteria are presented in Figure 3. The mean total scores and SD of the unwashed and washed brown eggs were 7.7 ± 4.6 and 8.0 ± 5.0, respectively, while those of the unwashed and washed white eggs were 9.2 ± 4.3 and 9.1 ± 3.2, respectively. No significant difference ($P > 0.05$) was found between the unwashed and the washed eggs of either the brown or white eggs.

The individual scores for each of the four criteria listed above are presented in Table 1. An even cuticle coverage was present on only a few of the eggs tested (13 of 120 eggs), and these were mainly brown eggs. Between 56.6 and 83.3% of the eggs had an occasionally patchy or very patchy cuticle coverage, while about 10.0 to 23.3% of the eggs had no cuticle coverage. Of all the eggs tested (brown and white eggs), pores were visible in 63.3 to 76.7% of the eggs assessed. In our study, almost none of the eggs (0.0 to 10.0%) were heavily contaminated with surface debris. SEM allows a critical appraisal of mechanical damage to the shell, resulting from the washing process. In most cases

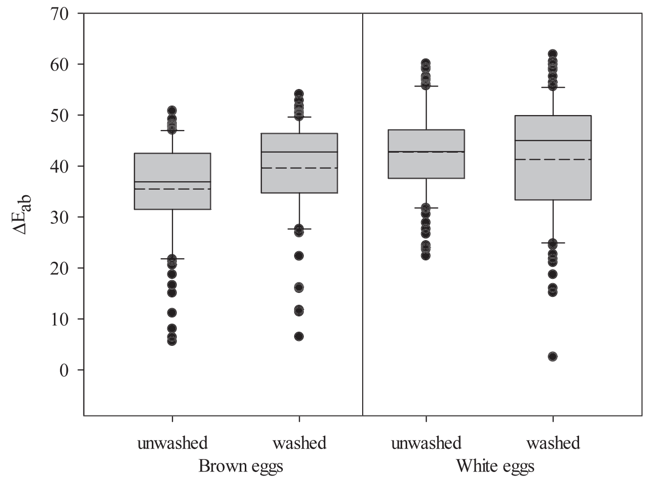

FIGURE 1. *Boxplot presenting* ΔE^*_{ab}*, a rate for cuticle coverage, for brown and white eggs that were either unwashed or washed. The line in the box marks the median, while the dashed line marks the mean. The boundaries of the box represent the 25th and 75th percentiles, while the whiskers above and below the box denote the 10th and 90th percentiles. The outliers are shown as dots.* n $= 100$ *samples in each group.*

(ranging from 63.3 to 86.7%), mechanical damage of the cuticle was not observed. In the proportional distribution of the brown or white eggs over the several categories of each criterion, no significant difference ($P > 0.05$) between the unwashed and washed eggs was shown.

The results of the second assessment are shown in Table 2. Of the brown eggs, only 18 and 17 eggs of 30 unwashed and washed eggs, respectively, were correctly identified. For the group of white eggs, only one-half were correctly evaluated. According to the McNemar's tests ($P > 0.05$) and the kappa values ($\kappa < 0.4$), the observations were discordant, meaning that no agreement could be found between the real treatment of the eggs and the assessor's choice.

DISCUSSION

For this study, eggs were collected from old laying hens that were at the end of lay. This was done on purpose, to ensure that we were working with eggs of the worst-case scenario, since cuticle thickness significantly decreases with increasing age of the hens *(8, 23)*.

The natural variability in cuticle coverage was reflected in the variability of the ΔE^*_{ab} values within the groups of brown and white unwashed eggs and in the proportional distribution (over the several categories) of each of the four criteria that were scored by the assessors of the SEM images. The cuticle coverage was considered poor, irrespective of treatment or origin. Messens et al. *(17)* also reported a low cuticle coverage for brown and white eggs from laying hens of 63 and 88 weeks of age, respectively, kept in conventional cages. Likewise, Nascimento *(20)* and Watt *(26)* reported that the cuticle coverage is variously present in eggs from commercial layers.

It has previously been shown that washing can result in the removal of the cuticle, thereby exposing the external

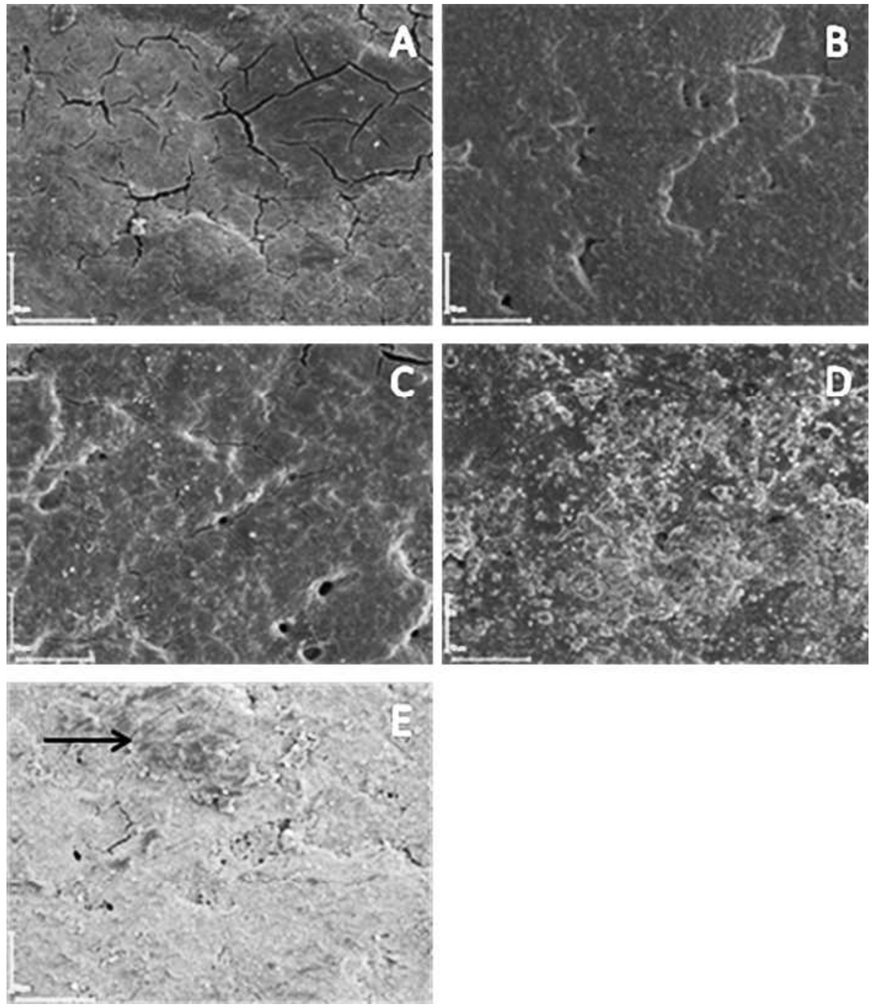

FIGURE 2. *Examples of the types of observation that formed the basis of the cuticle assessment carried out by SEM (×500). Scores for the criteria mentioned in ''Materials and Methods'' are given between brackets. (A) Typical SEM appearance of an eggshell with good cuticle coverage. The cuticle has a typical ''cracked mud'' appearance, which is induced as the cuticle dries just after oviposition (i: 0; ii: 0; iii: 0; iv: 0). (B) SEM image of an eggshell with no cuticle. The calcite columns of the palisade layer are completely exposed (i: 8; ii: 0; iii: 0; iv: 2). (C) SEM appearance of an eggshell with patchy cuticle coverage and exposed gaseous exchange pores (i: 5; ii: 0; iii: 2; iv: 5). (D) SEM appearance of an eggshell where the cuticle is covered with surface debris (i: 5; ii: 0; iii: 8; iv: 2). (E) SEM appearance of an eggshell with evidence of mechanical abrasion (see arrow) and with less cuticle (i: 5; ii: 2; iii: 2; iv: 2).*

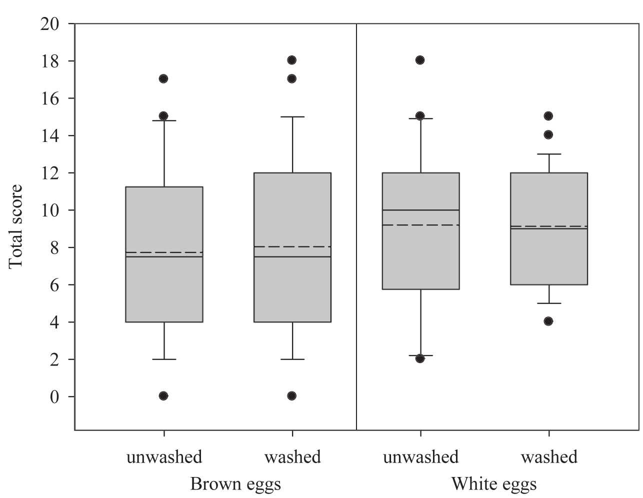

FIGURE 3. *The total scores relating to the general quality of the cuticle for the brown unwashed and washed eggs and for the white unwashed and washed eggs. A higher score presents a more negative perceived effect on the functionality of the cuticle. The line in the box marks the median, while the dashed line marks the mean. The boundaries of the box represent the 25th and 75th percentiles, while the whiskers above and below the box denote the 10th and 90th. The outliers are shown as dots.* n *= 30 samples in each group.*

openings of the gaseous exchange pores *(22, 24)*. The presence of exposed pores in this study, however, was not due to the washing procedure per se but was due to a general lack of cuticle in the first place; and even when the cuticle was present, the washing procedure did not appear to induce any further noticeable change.

The presence of debris, consistent with the use of sanitizers, on the surface of washed eggs has previously been reported *(5)*. In the present study, the debris, when present, resembled dust (originating from, e.g., the cardboard flats and packaging) rather than the mesh-like phosphorus-rich deposits reported by Cranstoun *(5)*. Moreover, the unwashed eggs did not have more surface debris than the washed eggs.

The type of mechanical damage on eggs observed after washing is usually reflective of the type of wash action employed; namely, brush versus jet washers, as found by Cranstoun *(5)* and through our personal experience (M. Bain). In the present study, the egg washer combined brush washing and jet washing. The jet washers sprayed a mixture of potable water and detergents onto the brushes in order to reduce mechanical damage. For the same purpose, the brushes were oval. The manufacturers' intentions to reduce

TABLE 1. *Results of cuticle assessment performed by visually assessing images obtained by scanning electron microscopy*[a]

Score	Category	Brown, unwashed	Brown, washed	White, unwashed	White, washed
Cuticle coverage					
0	Even	7 (23.3)	5 (16.7)	1 (3.3)	0 (0.0)
2	Occasionally patchy	13 (43.3)	12 (40.0)	9 (30.0)	8 (26.7)
5	Very patchy	4 (13.3)	10 (33.3)	16 (53.3)	15 (50.0)
8	No cuticle	6 (20.0)	3 (10.0)	4 (13.3)	7 (23.3)
Exposed pores					
0	None visible	8 (26.7)	11 (36.7)	7 (23.3)	10 (33.3)
2	Occasional pore visible	12 (40.0)	11 (36.7)	14 (46.7)	15 (50.0)
5	Frequently observed	10 (33.3)	8 (26.7)	9 (30.0)	5 (16.7)
Presence of surface debris					
0	Absent	8 (26.7)	10 (33.3)	12 (40.0)	11 (36.7)
2	Isolated	19 (63.3)	14 (46.7)	13 (43.3)	15 (50.0)
5	Moderate	2 (6.7)	3 (10.0)	5 (16.7)	4 (13.3)
8	Heavy	1 (3.3)	3 (10.0)	0 (0.0)	0 (0.0)
Mechanical damage					
0	None	26 (86.7)	23 (76.7)	21 (70.0)	19 (63.3)
2	Occasionally present	4 (13.3)	7 (23.3)	8 (26.7)	11 (36.7)
6	Frequently observed	0 (0.0)	0 (0.0)	1 (3.3)	0 (0.0)

[a] Values are numbers (percentages) of eggs from a total of 30 eggs per group.

mechanical damage by incorporating these features into the design of the washer are confirmed by the lack of mechanical damage reported in our results.

An alternative, more qualitative assessment of the cuticle quality of the eggs used in our study can be obtained by summing the scores obtained for each of the four criteria assessed by SEM. Again, the natural variability of the cuticle coverage can be observed by comparing the total scores for each group of eggs. More importantly, however, the egg washing procedure does not appear to have any modifying effect on the total score values for the washed versus the unwashed eggs.

After the first assessor had assigned scores to the cuticle of each egg, a second assessment was carried out to investigate whether it was possible to identify the treatment (unwashed or washed) by simply viewing a series of printed SEM images. Again it was not possible to distinguish unwashed from washed eggs based on a visual assessment of the cuticle.

TABLE 2. *Results of the second assessment of the cuticle SEM images to test whether the real treatment of the eggs (unwashed or washed) can be assessed by an independent assessor*[a]

	Real treatment		
Assessed treatment	Unwashed	Washed	Total
Brown eggs			
Unwashed	18	13	31
Washed	12	17	29
Total	30	30	60
White eggs			
Unwashed	15	15	30
Washed	15	15	30
Total	30	30	60

[a] Values are numbers of images (eggs).

Both the results from the cuticle staining and the ultrastructural assessment after SEM are in agreement that the cuticle was unaffected by the washing process applied in this study. According to Kuhl *(13)* and Overfield *(21)*, egg washers of the pressure spray type do not damage the cuticle under normal operating conditions. However, Sparks and Burgess *(24)* found that the cuticle was significantly eroded after sanitizing eggs from a broiler breeder flock with a continuous-spray sanitizing machine (the active ingredient of the sanitizer was sodium-*n*-chloro-*p*-toluene sulfonamide). Any damage to the cuticle is likely to lead to an increase in bacterial penetration *(12, 25)*. In their review, Hutchison et al. *(11)* discuss the negative effects that inappropriate use of washing chemicals and sanitizers can have on the cuticle *(9, 12, 25)*. However, the effects of low-temperature washing on the cuticle are less conclusive *(2, 14, 15)*. The washing procedure used in this study was carried out following standard operating procedures. It is beyond the scope of this study to comment on whether deviation from this might have induced damage to the cuticle, but this issue does emphasize the need for strict control to minimize risks of damage.

To conclude, we could find no evidence to suggest that the washing procedure used in this study irreversibly changed the cuticle quality of eggs from the end of lay, which inherently had poor cuticle coverage (worst-case scenario). This conclusion was valid for both brown and white eggs and was based on the assessment of the cuticle coverage, using a dye, and on the cuticle quality criteria as assessed by SEM. Moreover, within each group of eggs, a lot of variation in cuticle coverage and quality naturally occurred.

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