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Experimental studies on the influence of washing of table eggs on their microbial quality*

*Experimentelle Studien über den Einfluss des Waschens von Hühnereiern auf deren mikrobiologische Beschaffenheit**

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** Dedicated to Prof. Dr. Karsten Fehlhaber on the occasion of his 65th birthday*
Herrn Prof. Dr. Karsten Fehlhaber zum 65sten Geburtstag gewidmet

Summary

Considering the EC-regulations 1234/2007 and 589/2008 the sale of washed table eggs is not allowed in general, although exceptions are possible. Nevertheless, washing of eggs has been practiced in non-EC-states like US and Japan since years and the eggs are classified as nearly riskless. The concerns are a damage of the cuticle caused by the washing procedure followed by an increasing penetration of microorganisms, pathogens such as salmonellae or spoilage organisms in particular.

The aim of this study in lab scale was therefore to analyze the effect of washing of table eggs on their microbiological quality during storage. Experimental washing trials (n=2,220 eggs) were carried out. Parameters such as intactness of the cuticle by staining with Evans Blue, total viable count on egg shell before and after washing and in the egg content as well as the development of air cell size during a 28 days storage (end of the best before date) at room temperature were tested.

It could be proved that the egg shell aerobic plate count was degraded significantly by the experimental washing in clean as well as in dirty eggs by 2.40–2.43 log grades ($p \leq 0.001$). There was no significant difference in total aerobic plate count of the egg content between tap water washed and unwashed eggs after storage although a cuticle damage of the washed eggs was determined by shell staining. The results showed further a slightly higher air cell in washed eggs due to the cuticle damages.

Keywords: Penetration, cuticle, *Salmonella*

Zusammenfassung

Unter Berücksichtigung der EU-Verordnungen 1234/2007 und 589/2008 ist das Waschen von Konsumeiern nur in Ausnahmefällen gestattet. In Nicht-EU-Staaten wie Japan und den USA wird das Waschen hingegen seit Jahren praktiziert, und die Eier werden als risikoarm eingestuft. Kritisch wird die mögliche Zerstörung der Kutikula durch den Waschvorgang diskutiert, was zu einer verstärkten Penetration von pathogenen Keimen wie Salmonellen oder Verderbniserregern in das Eiinnere führen könnte. Das Ziel dieser Studie war daher, den Einfluss des Waschens auf die mikrobiologische Beschaffenheit der Hühnereier bis zum Ende des Mindesthaltbarkeitsdatums zu prüfen. Das experimentelle Waschen wurde an 2220 Eiern eingesetzt; die Lagerung der Eier erfolgte anschließend bis zu 28 Tage bei Raumtemperatur. Der Schalenkeimgehalt wurde vor und nach dem Waschen bestimmt. Nach 28 Tagen wurde auch die Gesamtkeimzahl im Eiinhalt ermittelt. Die Intaktheit der Kutikula wurde durch die Färbung mit Evans Blue geprüft. Zusätzlich wurde auch die Entwicklung der Luftkammerhöhe gemessen.

Es konnte nachgewiesen werden, dass der aerobe Schalenkeimgehalt durch den Waschvorgang signifikant um 2.40–2.43 log Einheiten ($p \leq 0{,}001$) sowohl bei optisch sauberen als auch verschmutzten Eiern gesenkt wurde. Im Eiinhalt gewaschener und ungewaschener Eier konnte am Ende der Haltbarkeit kein signifikanter Unterschied festgestellt werden, obwohl Kutikulabeschädigungen bei den gewaschenen Eiern nachgewiesen werden konnten. Allerdings konnten bei den gewaschenen Eiern als Folge des Kutikulaabriebs leicht erhöhte Luftkammerhöhen gemessen werden.

Schlüsselwörter: Penetration, Kutikula, *Salmonella*

Introduction

Concerning the current marketing standards for eggs, i. e. EC-Regulation 589/2008, define only two grades of eggs (A and B) according to physical characteristics. Grade A eggs ("fresh eggs" or "table eggs") shall have a normal, clean and undamaged shell and cuticle and the air space height shall not exceed 6 mm. Further they shall not be washed or cleaned before or after grading, and will be not chilled or treated for preservation. However, grade B eggs do not meet requirements applicable to eggs in grade A and may be passed only to the food or non-food industries.

Due to the changing of holding systems of hens to free range or deep litter systems with a higher percentage of dirty eggs the practice of washing of eggs becomes of more interest.

Modern in-line egg washing procedures involves normally three stages (AECL, 2005; Hutchison et al., 2003). 1) The pre-washing or wetting stage is typically done with a gentle spray of 40 °C warm water to soften debris. 2.) The main wash usually involves rubbing the eggs with brushes and/or spraying them with jets of potable water containing several chemicals. 3.) The final stage contains rinsing with about 60 °C hot water to remove any loose debris that the egg has picked up during the washing, to remove the residues of any chemicals or other dissolved matter and to accelerate the drying process. An oiling and/or cooling can be added as post-washing treatment. In a two-step process done by some users the prewashing is eliminated (AECL, 2005).

The washing procedure leads to a reduction of bacterial load on the surface ("sanitizing") and thereby preventing the contamination of eggs by horizontal transmission of bacteria such as *Salmonella* or potential spoilage organisms. Data regarding current egg washing practices under optimum conditions indicate a *Salmonella* reduction of more than 5 log10 units (Hutchison et al., 2004).

The major discussed disadvantage of egg washing is the potential damage of the cuticle, which serves as a competent barrier to bacterial ingress (Board, 1975; Board, 1982; Haigh and Betts, 1991; Kim and Slavik, 1996; Sparks and Board, 1985). In dependence of sub-optimal storage those damages may favor trans-shell contamination with pathogens and spoilage bacteria and thereby increase the risk to consumers. Additionally the moisture loss may result in higher air cells and lower weight which could cause difficulties with grading at the end of the best before date.

However, in countries like US, Australia and Sweden the authorities established strict rules concerning the washing process, the parameters washing temperatures, additives, water recycling, drying, cleaning and disinfection and storage of eggs. According to the information provided by countries where washing is permitted, washed eggs had lower microbial counts on the shell surface and no increased penetration was observed (EFSA, 2005). However, less data are available about particular cuticle damages or ability of storage, etc.

The aim of these investigations was therefore to analyze the effect of washing of table eggs on their quality. Parameters such as intactness of the cuticle, development of air space in combination with the determination of the total viable count on egg shell before and after washing and in the egg content during storage were measured.

TABLE 1: *Parameters measured using washed and unwashed eggs.*

Day of storage	No. of eggs before/after washing 0.	18.	28.
TVC shell – clean eggs stored at 20 °C, 70 % rh	10/10	10/10	10/10
TVC shell – dirty eggs stored at 20 °C, 70 % rh	10/10	10/10	10/10
TVC egg content – dirty eggs stored at 20 °C, 70 % rh	–/60	150/150	150/150
Air space height – dirty eggs stored at 20 °C, 70 % rh	–/60	–/60	150/150
85 % rh	150/150	150/150	150/150
Cuticle staining – clean eggs	40/40*	–/–	–/–

TVC = total viable count; * = another 40 eggs were washed with hypochlorite acid to remove cuticle completely

Material and methods

Eggs

To generate the data, freshly laid eggs (visibly clean and dirty) of laying hen breeds (Lohmann White LSL) were obtained from a local hatchery. The age of the breeder flokks at the time of the experiments was between week 42 and 68 of life. Only eggs with intact shells were used for the washing procedure.

Washing procedure

To imitate a commercial washing process in the lab scale experiments the eggs were treated with an autoclavable hand brush (Heiland, article number 58-018) using tap water (temperature 38–42 °C) for one minute. Afterwards eggs were dried for about two min by 40 °C warm streaming air and stored on egg trays for 28 days at 20 °C at 70 and 85 % relative humidity. The following parameters (Tab. 1) were investigated immediately after washing and after 18 and 28 days of storage.

The height (in mm) of the air space was measured during candling.

For determining the total viable count (TVC as egg aerobic plate count) on the shell the eggs were rinsed with 25 ml buffered peptone water (BPW, Roth, 9265.1; casein peptone, Merck, 1.07216.1000) in a bag for one minute. Afterwards the rinsing fluid was decimally diluted and plated on Plate Count-Agar (Sifin, TN 1189) and incubated aerobically at 30 °C for 48 hours.

The air cell and the shell membrane were opened after disinfection the egg shell with 95 % ethanol for determining the total viable count (TVC) of the egg content. 45 ml of the egg content were taken and mixed. 36 ml were enriched immediately in 100 ml BPW for 48 h at 30 °C, afterwards plated on Plate Count Agar and incubated again at the same conditions. 1 ml of the residual 9 ml (stored at 4 °C) was used for the quantitative determination using the procedure described above if colonies were grown.

Cuticle staining

The cuticle was stained 5 min with 1 % Evans Blue (Fluka, 46160) using the method of Mamadou (1990). After rinsing of 10 sec with tap water, the eggs were dried and the intensity of the blue color was evaluated using a self made index (0=nearly no stain; 7=maximal stain). The staining was

TABLE 2: *Air space height (mm) at day of laying, 18th and 28th day of storage (20 °C, 70 % relative humidity); (n = 660).*

Air space height	– Without storage – control (n=60)			– Storage: 18 days – control (n=150)			washed (n=150)			– Storage: 28 days – control (n=150)			washed (n=150)		
	Med*	Min	Max	Med	Min	Max	Med	Min	Max	Med	Min	Max	Med	Min	Max
day of laying	3.0	3	3	3.0	2	3	3.0	2	3	3.0	2	3	3.0	2	3
after storage	–	–	–	5.0	4	7	5.0	3	7	6.0	5	8	6.0	4	11
increase	–	–	–	2.0	1	5	2.0	0	4	3.0	2	5	3.5	1	8

*Med = median value

TABLE 3: *Air space height (mm) at day of laying, 18th and 28th day of storage (20 °C, 85 % relative humidity); (n = 660).*

Air space height	– Without storage – control (n=60)			– Storage: 18 days – control (n=150)			washed (n=150)			– Storage: 28 days – control (n=150)			washed (n=150)		
	Med*	Min	Max	Med	Min	Max	Med	Min	Max	Med	Min	Max	Med	Min	Max
day of laying	2.0	2	2	2.0	2	3	2.0	2	3	2.0	2	3	2.0	2	3
after storage	–	–	–	4.0	3	7	4.0	2	7	5.0	3	7	4.0	3	7
increase	–	–	–	2.0	0	5	1.0	0	5	2.0	0	5	2.0	0	5

done immediately after washing of visible clean eggs (40 control eggs and 40 washed eggs). As a positive control eggs (n=40) were washed with hypochlorite acid (20–24 ppm free available chlorine) to remove the cuticle completely.

Statistical Analysis

The results were evaluated by the statistical program SPSS 11.5, using the Mann-Whitney-U-test and the FISHER-Test. Values P<0.05 were considered significant.

Results/Discussion

Cuticle staining

The index of the cuticle staining of the unwashed control group was significantly higher than the one of the washed eggs (Fig. 1; p<0.001) indicating a mechanical impact of the brushes as extraneous cause. Similar results were reported by Ball et al. (1975), Hutchison et al. (2003) and Zurek (1938). Another reason for abrasion could be the induced swelling of the cuticle caused by treatment with tap water. Washing the eggs with hypochlorite acid to remove cuticle leads, as expected, to a significant lower index (Fig. 1; p<0.001).

Air space height

An increase of air space height was of special interest for the evaluation of possible cuticle damages. A slight increase of the air space height was measured only at day 28 after washing the eggs with tap water and storage at 20 °C/70 % relative humidity compared to the control group (Tab. 2, not significant). Eggs storaged at 20 °C/85 % relative humidity showed a lower air space height (Tab. 3, not significant). In general, storage of eggs at the higher relative humidity led to a slower and lower increase of the air cell confirming data from Demming (1993) and Peebles et al. (1987). Eggs treated with hypochlorite acid also did not show significant higher air cells. However, the results show that at the end of the best before dates (= 28 days) the air cells of a part of the eggs storaged at 70 % rh were in both groups higher than 6 mm (up to 21.3 % for control, up to 33.3 % for washed eggs). This was a violation against EC-regulation 589/2008 (maximal air size < 6mm). Therefore, a shortened best before date and sell-by date respectively should be discussed since relative humidity in supermarkets and at home is much lower resulting in much higher air cell heights.

Eggshell aerobic plate count

TVC (egg aerobic plate count) was degraded significantly by the experimental washing in clean eggs by 2.40-log grades and in dirty eggs by 2.43 log grades (Tab. 4; p< 0.001). This positive effect of egg washing was present until the end

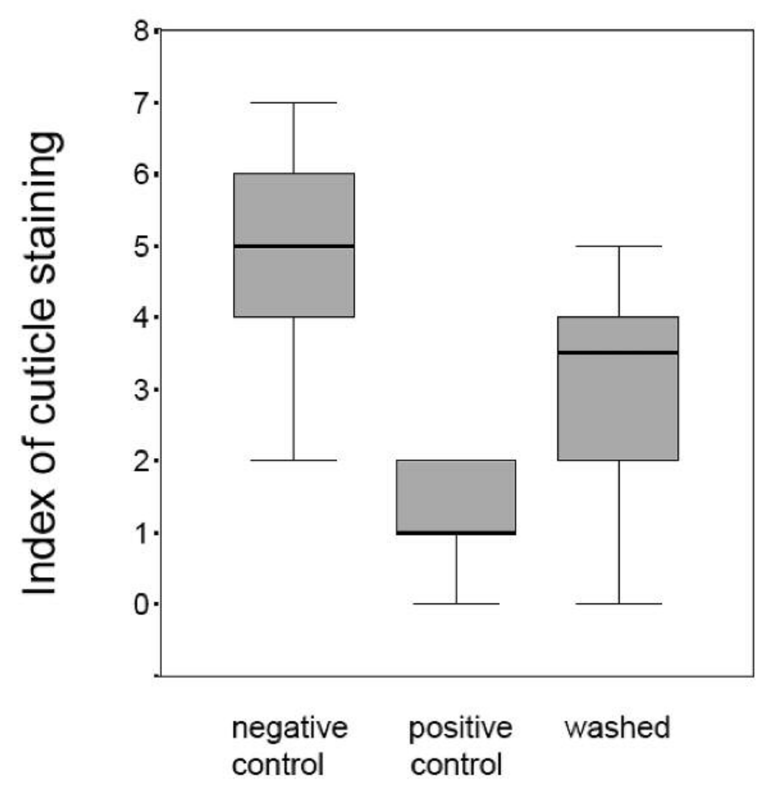

FIGURE 1: *Index of cuticle staining of eggs (washed eggs, negative and positive control eggs, n = 40 each).*

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TABLE 4: *TVC on the egg shell after washing and storage of 28 days storage at 20 °C, 70 % rh (log cfu/shell); (n = 60).*

Day of storage	Eggs	TVC (log cfu/shell) clean eggs			TVC (log cfu/shell) dirty eggs		
		Med	Min	Max	Med	Min	Max
0	control eggs (n=10)	4.80	4.40	5.66	4.83	4.51	5.62
	washed eggs (n=10)	2.40	2.40	2.70	2.40	2.40	3.00
18	control eggs (n=10)	4.06	3.52	5.04	6.72	4.68	7.36
	washed eggs (n=10)	2.40	2.40	3.40	2.40	2.40	5.76
28	control eggs (n=10)	4.47	3.77	4.87	5.87	3.88	6.72
	washed eggs (n=10)	2.40	2.40	2.40	2.40	2.40	5.08

Detection limit: 2.40 log cfu/shell

TABLE 5: *Results of enrichment and direct plating and TVC of the egg content after storage at 20 °C, 70 % relative humidity (n = 650; EN= enrichment, DP Directly plated).*

Day	Eggs		En pos.	DP pos.	TVC (log cfu/ml) Med	Min	Max
0.	control	n	1	0	–	–	–
	(n = 60)	%	1.7	0.0			
18.	control	n	11	6	3.32	0.00	6.34
	(n = 150)	%	7.3	4.0			
	washed	n	11	3	3.08	0.86	4.49
	(n= 150)	%	7.3	2.0			
28.	control	n	10	7	3.90	1.70	8.15
	(n = 150)	%	6.7	4.7			
	washed	n	9	7	3.66	0.00	8.56
	(n= 150)	%	6.0	4.7			

of the best before date and confirmed former results (Favier et al. 2000a, b; Hutchison et al., 2004; Jones et al., 2004; Moats, 1979; Moats, 1981; Musgrove et al., 2004).

Egg content aerobic plate count

The egg content was investigated immediately after washing and storage to evaluate the penetration rate of microorganisms through the shell (Tab. 5). In both groups (washed, unwashed eggs) colonization of the egg content was measured. However, the penetration rate in washed eggs was not increased although a slight damage of the cuticle was detected by staining. Comparing these data with the eggs where the cuticle was almost completely removed (positive control) a significant higher contamination of the egg content could be observed at day 28 of storage (13 % were positive in the enrichment).

In conclusion, a proper wash procedure led to a significant increase in the quality of eggs measured by reduction of the eggshell aerobic plate count. However, a slight damage of the cuticle could be induced by the wash procedure resulting in a marginal water loss and heightened air space. However, also parts of the untreated control group had air space heights > 6 mm confirming former investigations (Braun, 2006). Therefore, a reduction of best before date should be discussed. A further penetration of microorganisms into the egg content could not be detected since the cuticle is only slightly damaged, however a complete removal of cuticle induced by hypochlorite acid resulted in a significant higher penetration rate at day 28 of storage. It is likely, that longer storage periods as used in former times would lead to a significant higher penetration and spoilage of washed eggs.

Summary/Conclusion

The evaluation of the main advantage of egg washing such as reduction of microbial load and disadvantages of egg washing like cuticle damages and increased penetration of pathogens or spoilage bacteria need to be related to the particular washing procedure. The possible risks associated with egg washing can be reduced by the adoption of defined best washing practice procedures such as defined by AECL (2005) for Australia, SVENSSKAAG (2007) for Sweden and USDA (2004) for the US. In those countries where the washing of Class A eggs is permitted, the process is carried out using on-line systems whereby all eggs destined for Class A are treated. The process is undertaken immediately prior to grading and packing followed by a prompt and thorough drying of eggs after washing and before packing to avoid mould growth and bacterial trans-shell penetration. If properly done under hygienic conditions and without any chemicals removing the cuticle completely, there are clear advantages to egg washing. In contrast, poor practices could increase the risk as shown in own investigations (Wittmann et al., 2006).

Additionally, the primary layer production needs to reduce the occurrence of salmonellae on and in eggs to prevent *Salmonella* infection. This will reduce the risks associated with egg washing. The new target for reduction of *Salmonella* is set within the EU for laying hen flocks being 2 % or less remaining positive for all *Salmonella* serovars with public health significance. Further, a storage of eggs below 8 °C is another option to prevent growth of *Salmonella* spp. present in the egg and reduce the risk of a possible trans-shell penetration and migration of *Salmonella* from the albumen into the egg yolk (Braun and Fehlhaber, 1995; Greiner et al., 2011).

References

AECL (2005): Code of practice for shell egg, production, grading, packing and distribution. 19-9-0006.

Anon. Verordnung (EG) Nr.589/2008 der Kommission vom 23. Juni 2008 mit Durchführungsbestimmungen zur Verordnung (EG) Nr. 1234/2007 des Rates hinsichtlich der Vermarktungsnormen für Eier (ABl. L 163, S. 6–23).

Anon. Verordnung (EG) Nr.1234/2007 des Rates vom 22. Oktober 2007 über eine gemeinsame Organisation der Agrarmärkte und mit Sondervorschriften für bestimmte landwirtschaftliche Erzeugnisse (Verordnung über die einheitliche GMO) (ABl. L 299, S. 1–149).

Ball RF, Logan V, Hill JF (1975): Factors affecting cuticle of egg as measured by intensity of staining. Poult Sci 54: 1479–1484.

Board RG (1975): The microstructure of the cuticle-less shell of the eggs of the domestic hen. British Poult Sci 16: 89–91.

Board RG (1982): Properties of avian eggshells and their adaptive value. Biol Rev Camb Philos Soc 57: 1–28.

Braun P (2006): Untersuchungen zum Frischegrad von Hühnereiern bis zum Ablauf des Mindesthaltbarkeitsdatums unter Berücksichtigung verschiedener Lagerungsbedingungen. Tagungsband der 46. Arbeitstagung des Arbeitsgebietes Lebensmittelhygiene der DVG, Garmisch-Partenkirchen, 27. 9.–30. 9. 2005, 337–339.

Braun P, Fehlhaber K (1995): Migration of *Salmonella enteritidis* from albumen into egg yolk. Int J Food Microbiol 25: 95–99.

Deeming C (1993): Relative humidity affects water loss from eggs. World Poultry 9: 34–37.

EFSA (2005): Opinion of the Scientific Panel on Biological Hazards on the request from the Commission related to the Microbiological Risks on Washing of Table Eggs. The EFSA Journal 269: 1–39.

Favier GI, Escudero ME, Mattar MA, de Guzman AMS (2000a): Survival of *Yersinia enterocolitica* and mesophilic aerobic bacteria on eggshell after washing with hypochlorite and organic acid solutions. J Food Prot 63: 1053–1057.

Favier GI, Escudero ME, Velazquez L, de Guzman AMS (2000b): Reduction of *Yersinia enterocolitica* and mesophilic aerobic bacteria in egg-shell by washing with surfactants and their effect on the shell microstructure. Food Microbio 17: 73–81.

Greiner M, Alter T, Johne A, Schlichting D, Knobloch S, Braun P, Appel B (2011): Studies on the growth of *Salmonella* in table eggs. J Food Safety Food Qual 62: 6–10.

Haigh T, Betts WB (1991): Microbial barrier properties of hen eggshells. Microbios 68: 137–46.

Hutchison ML, Gittins J, Walker A, Moore A, Burton C, Sparks N (2003): Washing table eggs: a review of the scientific and engineering issues. Worlds Poult Sci J 59: 233–48.

Hutchison ML, Gittins J, Walker A, Sparks N, Humphrey TJ, Burton C, Moore A (2004): An assessment of the microbiological risks involved with egg washing under commercial conditions. J Food Prot 67: 4–11.

Jones DR, Musgrove MT, Northcutt JK (2004): Variations in external and internal microbial populations in shell eggs during extended storage. J Food Prot 67: 2657–60.

Kim JW, Slavik MF (1996): Changes in eggshell surface microstructure after washing with cetylpyridinium chloride or trisodium phosphate. J Food Prot 59: 859–863.

Mamadou D (1990): Nachweis der Reinigung der Eier. Universität Leipzig, Veterinärmed. Fak., Diplomarbeit.

Moats WA (1979): The effect of washing eggs under commercial conditions on bacterial loads on egg shells. Poult Sci 58: 1228–1233.

Moats WA (1981): Factors affecting bacterial loads on shells of commercially washed eggs. Poult Sci 60: 2084–2090.

Musgrove MT, Jones DR, Northcutt JK, Cox NA, Harrison MA (2004): Identification of Enterobacteriaceae from washed and unwashed commercial shell eggs. J Food Prot 67: 2613–2616.

Peebles ED, Brake J, Gildersleeve RP (1987): Effects of Eggshell Cuticle Removal and Incubation Humidity on Embryonic-Development and Hatchability of Broilers. Poult Sci 66: 834–840.

Sparks NHC, Board RG (1985): Bacterial penetration of the recently oviposited shell of hen's eggs. Aust Vet J 62: 169–170.

Svenskaag (2007): Nationella riktlinjer för industreill tvattning av ägg (zitiert vom 9.7.2007; 1-7, http://www.svenskaagg.se/bransch/branschinformation/agg/branschvagledning/3-1_Tvattning_av_agg.pdf)

USDA (2004): Regulations governing the voluntary grading of shell eggs. effective January 1, 2004.

Wittmann C, Braun PG, Fehlhaber K (2006): Untersuchungen zur mikrobiellen Qualität von maschinell gewaschenen Eiern. Amtstierärztlicher Dienst und Lebensmittelkontrolle, Sonderausgabe 26.–29. 9. 2006, 47. Arbeitstagung des Arbeitsgebietes Lebensmittelhygiene der DVG, Garmisch-Partenkirchen 27. 9.–30. 9. 2006: 66.

Zurek F (1938): Vergleichende Untersuchungen über Reinigung von Schmutzeiern mit besonderer Berücksichtigung der mechanischen Reinigung. Zeitschrift für Fleisch- und Milchhygiene 48: 147–150, 164–168.

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