The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM)
ISSN: 2602-3199

The Eurasia Proceedings of Science, Technology, Engineering & Mathematics (EPSTEM), 2021

Volume 12, Pages 1-6

ICRETS 2021: International Conference on Research in Engineering, Technology and Science

Treatment of Egg Shells with Hydrogen Peroxide and Sodium Carbonate as a Disinfectant with Bactericidal Effect to Eliminate *Salmonella Entereditis*

Nora RRAHIMI HASANI
University of Pristina

Abstract: The objective of this study was to discover the effects of applying two antimicrobial drugs (sodium carbonate and hydrogen peroxide) on chicken eggshell on a poultry farm in Kosovo. Salmonella infections in egg contents may be related to external contamination of the eggshell. The first study was performed by applying sodium carbonate to the eggshell at different concentrations and pH of 10, 11 and 12 versus some Salmonella concentrations found in the analyzed eggs. A high amount of total bacteria was observed in all samples, but after the application of disinfectants we saw a decrease of these bacteria. The second study was performed to determine which concentration of H_2O_2 would give the maximum reduction of bacteria. In work 2, treatments were performed on 1) dry sample, 2) 0.5% H_2O_2, 3) 1% H_2O_2, 4) 1.5% H_2O_2, 5) 2% H_2O_2, 6) 2.5% H_2O_2 and 7) 3% H_2O_2.For shell tests, inactivation of Salmonella Enteritidis occurred at lower concentrations at pH 12 than at pH 11 and pH 10. The time of contact between the chemicals and Salmonella apparently results in accelerated bacterial inactivation.

Keywords: Egg shell, Antimicrobial, Sodium carbonate, Hydrogen peroxide.

Introduction

Eggs are one of the important sources of human food. In recent years, Salmonella enteritidis infections in humans have been traced to contaminated eggs(Coufal et al.2003, Gates, 1930). Salmonella is easily capable of contaminating bird eggs through vertical and horizontal transmission. Vertically transmitted salmonella contaminate the eggs during the laying process(Turnbull & Snoevenbos, 1974), while horizontal transmission occurs when the eggshell is contaminated by external sources such as incubators, the environment, or other infected birds (Smeltzer et al.1979). The calcareous skin surrounding the egg is porous and permeable to bacteria(Solomon, 2010) (Figure 1). The cuticle is a protein film covering the egg shell that provides a natural barrier to help prevent internal bacterial contamination (Peebles &Brake, 1986), however, defects in the shell or thinning of the cuticle can lead to invasion of the egg shell by bacteria in surface (Mayes &Takeballi, 1983). Salmonella can easily penetrate the egg cuticle and contaminate the internal contents (Williams et al, 1968, Wang & Slavik, 1998).

In egg shells, the total number of aerobic mesophilic bacteria can reach 3.75 to 7.07 $\log_{10}$ colony forming units (CFU) per egg. Therefore, reducing the microbial load of the egg shell through disinfection procedures would improve the quality of the egg to be incubated and reduce the incidence of bacterial infections in newborn embryos and hens. The salmonella enterica serotype is one of the most common serotypes associated with human salmonellosis (Rodriges et al, 1990). Studies have shown that contamination of egg contents can occur in the reproductive tract during the egg formation process. This microorganism, which is present in the feces, can also infect the contents of the egg by penetrating the shell through the shell pores or damaged areas (Humphrey, 1994, Protais et al.1989). Emphasis is placed on HACCP-based programs for identifying and preventing

- Selection and peer-review under responsibility of the Organizing Committee of the Conference

potential microbiological hazards that may arise from raw material, processing stages, product, and food plants (De Reu et al.2006, Kinner & Moats, 1981).

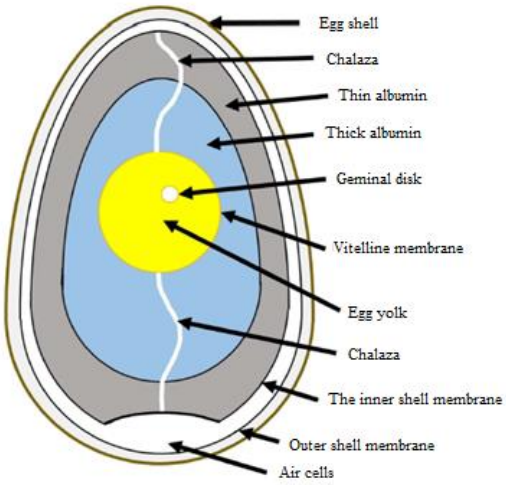

Figure 1. Chicken egg diagram

Hydrogen peroxide (H_2O_2) is a very strong oxidizer that forms free radicals exerting a destructive effect on cell membranes. As a result, it has found wide application as a biocidal (Linley et al. 2012). When O_3 is exposed to UV the net reaction results in the formation of hydrogen peroxide (H_2O_2) (Peyton & Glanze, 1988) and any hydroxyl radicals formed when O_3 reacts with UV are unable to escape this solvent cage(Peyton & Glanze, 1988). Although the advanced oxidation process (PAO) O_3 / UV is an effective disinfectant, the bactericidal properties are the result of the production of hydrogen peroxide instead of the hydroxyl radicals formed by the initial O_3 molecule.

The net photolysis of H_2O_2 yields 2 hydroxyl radicals, per quantum of absorbed radiation, which can continue to form peroxyl radicals leading to secondary oxidation reactions (Legrini et al. 1993). The H_2O_2 / UV photolytic reaction is one of the most widely used PAOs (Bustillo-Lecompte & Mehrvan, 2015), and has been demonstrated to effectively inactivate vegetative bacteria, bacterial spores, and viruses. be equal to or better than formaldehyde in hatching eggs for hatching (Sheldon & Brake, 1991). Hydrogen peroxide has previously been demonstrated to effectively reduce Salmonella contamination from experimentally contaminated eggs (Cox et al. 2000). After egg treatment, H_2O_2 evaporates easily without leaving any chemical residue and poses minimal safety issues for workers or embryo development (Sheldon & Brake, 1991, Cox et al.2000, Scott & Swetman 1993a, Padron, 1995, Keita et al. 2016). The bactericidal effects of H_2O_2 increase after UV photolysis (Ikai et al.2010).

Other benefits of using this system include the commercial availability of H_2O_2, its endless water solubility, and its lower health risk than O_3 for workers (Legrini et al.1993, Scott & Swetman 1993a). These benefits together with its effectiveness as a cleaner make the H_2O_2 / UV PAO system an attractive method of disinfection for eggs. These advanced oxidation processes are proving to be a new approach that effectively and safely reduces Salmonella contamination in egg shells. At the same time, a large variety of chemical agents have been developed, marketed as detergents, detergent-cleaners or detergents for cleaning and disinfecting eggs.

In practice, the cleaning and disinfection efficiency of agents is often determined by standard laboratory tests based on bacterial suspension tests, which are used to determine recommended concentrations (Best et el.1988). However, the applicability of these recommendations in harsh conditions is sometimes problematic because effective concentrations in suspension may be less active against bacteria sticking to a surface, especially a porous surface such as the eggshell.

Several other factors such as the number of bacteria present, the time of contact between the chemicals and the cells, and the pH of the microbial solutions also affect the effectiveness of the cleaning and cleaning agents. Commercial egg cleaners typically use alkaline ingredients to clean eggs, such as sodium carbonate and chlorine cleaners that operate at a pH ranging from 9 to 12 (Moats, 1978). However some authors have suggested that pH values> 11 be used to minimize the bacterial load on the eggshell and bath water (Cox et al. 2007, Frank & Wright, 1956).

The main objective of this study was to evaluate the effects of the application of two egg cleansing compounds contaminated with Salmonella Enteritidis and to determine the influence of pH and contact time on the activity of these antimicrobial compounds (sodium carbonate) and H_2O_2, as well as the amount the lowest chemical product needed to eliminate organisms on egg shell surfaces on a poultry farm in Kosovo.

Materials and Methods

Bacterial Registration Procedure

For the following experiments, bacterial counting was performed in the same manner. After individual eggs from the treated and dry sample groups were placed in Whirl-Pak bags, the bags were then filled with sterile 50 ml PBS (Phosphate-buffered saline). Each egg was massaged by hand into the bag for 1 min to remove bacteria located on the outer shell of the egg. Upon completion of the massage, the bags were opened and 10 ml of rinsing solution was aseptically collected in an empty tube of sterile culture. The rinsing solutions collected from all eggs receiving any treatment in the following 4 works were not serially diluted. However, 2 types of dilutions were required for all control eggs due to high bacterial loads. Once the dilutions were complete, 0.5 ml of all the rinsing samples and dilutions were spread in a non-selective medium showing the total bacterial count (agar plates). All samples were collected in duplicate. The plates were then incubated for 48 hours at 37°C. After the incubation period, the plates were removed and the colonies were counted. All results are reported as $\log_{10}$ cfu / egg.

During the experiment, 110 chicken eggs were used for microbiological evaluation. The eggs were bought from a commercial poultry farm in Kosovo, which uses brown chickens raised on the floor. For microbiological evaluation, 110 eggs were divided into 3 groups: 1) 10 eggs without disinfection; 2) 50 contaminated eggs which are then treated with sodium carbonate (30, 50 and 60ppm) in 2-12 minutes; 3) 50 eggs contaminated and then disinfected with H_2O_2 in different percentages and at different pH. A total of 110 eggs were randomly selected, cracked eggs were discarded and dispersed in disinfection treatments.

Realized works

A total of 110 apparently clean eggs were collected from chickens of a poultry farm in Kosovo and divided into 3 groups: 1) 10 eggs without disinfection; 2) 50 contaminated eggs which are then treated with sodium carbonate (40, 50 and 60ppm) in 2, 5 and 10 minutes; 3) 50 eggs contaminated and then disinfected with H_2O_2. 10 eggs tested for Salmonella positive served us as a negative control.For the egg procedure without disinfection, the egg rows were kept in the same room where the other treatments were performed, but the eggs did not undergo any disinfection procedure. Room temperature and humidity were recorded, from 26.7 to 30.5^{o}C and from 49 to 53%, respectively. The second experiment was a combination of three concentrations 40, 50 and 60ppm, three pH (10, 11 and 12), and three exposure periods (2, 5 and 10 min).

The third experiment was performed to determine which concentration of H_2O_2 would give the maximum reduction of bacteria in the eggshells. In work 3, treatments were done in 1) in the sample without treatment, 2) 0.5% H_2O_2, 3) 1% H_2O_2, 4) 1.5% H_2O_2, 5) 2% H_2O_2, 6) 2.5% H_2O_2 and 7) 3% H_2O_2 . 50 ml of disinfectant solution was sprayed on 50 eggs at the same time using a hand sprayer. The temperature of the solution was measured with a thermometer and ranged from 26 to 29ºC. To reach the entire surface of the eggs with the solution, the egg boxes were placed on a horizontal surface and the spraying was carried out in two steps: 25 ml of the solution was dispersed on one side of the eggs; and after we turned them over and 25 ml were dispensed on the other side. An average of 1 mL of hydrogen peroxide was sprayed on each egg, and 9 to 12 min were spent for this procedure. After each collection, shortly before and 1 hour after disinfection, the eggs from each treatment were selected to count the microbes in the egg shell. The eggs, collected with disposable gloves, were placed in groups in autoclaved bags, which were properly identified according to each treatment and then refrigerated at 4ºC. The samples were transported to the laboratory, where microbiological analyzes were performed 24 hours after cooling. Each bag was opened, and the eggs were transferred to another autoclaved bag, to which 50 ml PBS (Phosphate-buffered saline) solution was added. The eggs were massaged for 5 min to remove bacterial cells from their surfaces. Then, a 1.0 ml sample of PBS was taken from each bag, the plate was planted on agar, in order to obtain the Salmonella count. The plates were incubated at 37°C for 24 to 48 hours and, subsequently, bacterial colonies were counted and recorded. The microbial count was expressed as $\log_{10}$ CFU 1.0 mL^{-1} batch of eggs.

Results and Discussion

The purpose of this study was to evaluate the efficacy of chemicals used as disinfectants in chicken eggs. Salmonella contamination is considered an important hygienic issue, especially on small-scale farms that are not controlled by an authorized agency or when a Critical Risk Analysis (HACCP (Moats, 1978)), plan is not available. In this study, two commercial egg washings with sanitizing ingredients such as: sodium carbonate and hydrogen peroxide, in different concentrations, at pH values of 10, 11 and 12 were examined for their effectiveness in inactivating Salmonella Enteritidis in the shells of contaminated eggs.Compounds that do not demonstrate microbial effect at these concentrations were then examined until one found the effective amount of each cleaning agent. Figure 1 shows the bacterial count of Salmonella enterica calculated as $\log_{10}$ cfu / egg which will serve as a negative control. As seen in the picture we have a number of bacteria starting from 0.98$\log_{10}$ cfu/egg to 2.89$\log_{10}$ cfu/egg.

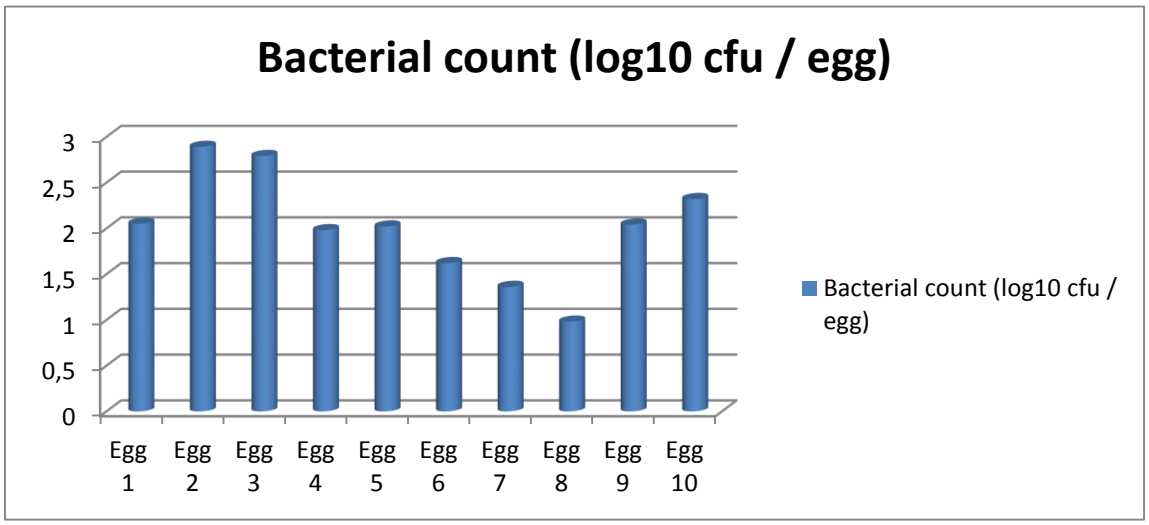

Figure 2. Bacterial counting at these sample eggs

In the second work on sodium carbonate, the effective concentrations were similar to those of other chemicals and were 5 to 6 times higher than the concentrations used in this work. The initial level of contamination of the eggshell can play an important role in the effectiveness of chemical agents. Because the numbers of bacteria present in the shell can affect the bactericidal activity of egg cleaning compounds, heavily contaminated eggs should not be placed in the egg washing machine. Moreover, before the cleaning operation they should be kept inside an environment that prevents the multiplication of bacteria in the husk.

Table 1. Bacterial count (log10 cfu/egg) in Na Carbonate treated eggs at different pH and time

		Bacterial count ($\log_{10}$ cfu/egg)								
		pH 10			pH 11			pH 12		
Product		2 min	5 min	10 min	2 min	5 min	10 min	2 min	5 min	10 min
Na	100ppm	2.02	1.89	1.56	1.85	1.65	1.41	1.52	1.36	1.12
Carbonate	150ppm	1.84	1.63	1.33	1.65	1.52	1.20	1.41	1.32	1.03
	200ppm	1.51	1.36	1.20	1.40	1.21	1.11	1.36	1.21	0.85

In work 3, all treatment groups gave significantly lower counts of bark bacteria compared to the dry sample (Figure 2). It is clearly seen from the diagram that with the increase of % of H_2O_2, the bacterial reduction calculated with $\log_{10}$ cfu/egg also increases.Bactericidal activities of any chemical treatment were determined against Salmonella Enteritidis in this paper. In this evidence, eggs with Salmonella were taken as experimental eggs, two disinfectant ingredients, such as sodium carbonate, at pH values of 10, 11, and 12 and for a contact time of 2, 5 or 10 min, and hydrogen peroxide at different concentrations were reviewed for their effectiveness in inactivating bacteria.

As shown in the results the analyzes showed differences between the values of Salmonella for both products; hydrogen peroxide showed a lower number of bacteria than Na carbonate. Bacterial values decreased significantly with an increase in pH or contact time: the number of bacteria at pH 10 was significantly higher than at pH 11, and the number of bacteria at pH 11 was significantly higher than at pH 12; the number of

bacteria for the time 2 min was not much higher than that for the time 5 min, although the values of the number of bacteria for both times were very significant and higher than for the time 10 min.

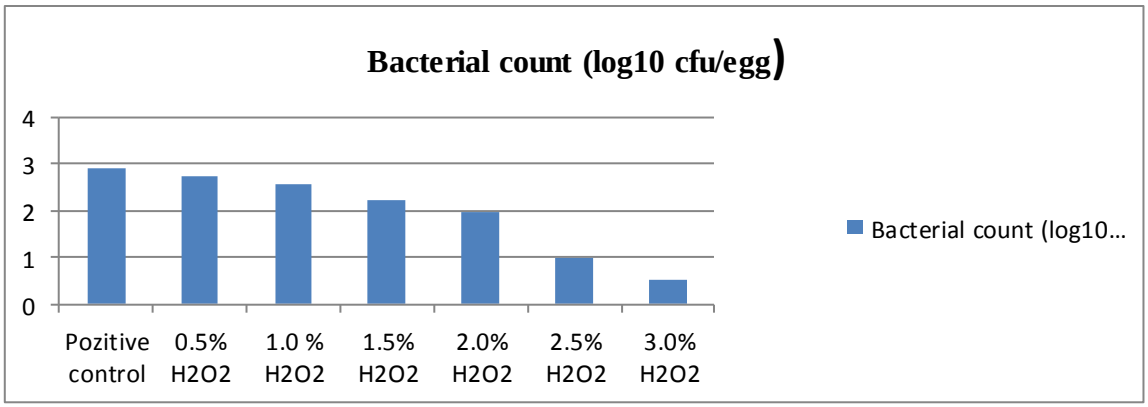

Figure 3. Comparison of different concentrations of H_2O_2 used alone in disinfection of egg shells N=50 eggs

Conclusion

This study was conducted to determine the minimum concentrations of the two chemicals needed to destroy Salmonella Enteritidis on egg surfaces. Our results indicate that very strong concentrations of these compounds were required, much higher than those commonly used in processing plants, were needed to inactivate Salmonella Enteritidis in contaminated shells, even when heat (48^0C) and alkaline environment (pH 10 to 12) were used as solutions. Therefore, preventive measures aimed at reducing or eliminating possible contamination of Salmonella peel should be a major concern when trying to control Salmonella. Further research in this area is needed to ensure egg industry with the most effective means of preventing and controlling Salmonella Enteritidis contamination. Furthermore, the use of these high concentrations of cleaning agents may be impractical for industrial application due to side effects on the eggshells and corrosive effect on equipment.As shown in the results the analyzes showed differences between the values of Salmonella for both products; hydrogen peroxide showed a lower number of bacteria than carbonate.

Scientific Ethics Declaration

The author declares that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the author.

References

Bayliss, C. E., & Waites, W.M., (1982). Effect of simultaneous high intensity ultraviolet irradiation and hydrogen peroxide on bacterial spores. *J. Food Technol. 17*, 467-470.

Best, M., Sattar, S.A., Springthorpe, V. S., & Kennedy, M. E.. (1988). Comparative mycobactericidal eficacy of chemical disinfectants in suspension and carrier tests. *Appl. Environ. Microbiol. 54*, 2856–2858.

Bustillo-Lecompte, C. F., & Mehrvar, M., (2015). Slaughterhouse wastewater characteristics, treatment, and management in the meat processing industry: A review on trends and advances. *J. Environ. Manag. 161*, 287 302.

Coufal, C.D., Chavez, C., Knape, K.D., & Carey, J. B.. (2003). Evaluation of a method of ultraviolet light sanitation of broiler hatching eggs. *Poult. Sci. 82*, 754-759.

Cox, N.A., Cason, J.A., & Berrang, M.E.. (2000). Salmonella penetration of egg shells and proliferation in broiler hatching eggs: A review. *Poult. Sci. 79*, 1571-1574.

Cox, N.A., Richardson, L.J., Buhr, R.J., Musgrove , M.T., Berrang, M.E., & Bright, W., (2007). Bactericidal effect of several chemicals on hatching eggs inoculated with Salmonella serovar Typhimurium. *J. Appl. Poult. Res. 16*, 623-627.

De Reu, K., Grijspeerdt, K., Herman, L., Heyndrickx, M., Uyttendaele, M., Debevere, J., Putirulan, F.F., & Bolder, N. M., (2006). The effect of a commercial UV disinfection system on the bacterial load of shell eggs. *Lett. Appl. Microbiol. 42*, 144-148.

Frank, J. F., & Wright, G. W.. (1956). The disinfection of eggs contaminated with *S. typhimurium. Can. J. Comp. Med. 20*, 406–410.

Gates, F. L., (1930). A study of the bactericidal action of ultra violet light: III. The absorption of ultra violet light by bacteria. *J. Gen. Physiol. 14*, 31-42.

Ikai, H., Nakamura, K., Shirato, M., Kanno, T., Iwasawa, A., Sasaki, K., Niwano, Y., & Kohno, M., (2010). Photolysis of hydrogen peroxide, an effective disinfection system via hydroxyl radical formation. Antimicrob. *Agents Chemother. 54*, 5086-5091.

Humphrey, T. J., (1994). Contamination of eggshell and contents with *Salmonella* Enteritidis: a review. *J. Food Prot. 21*, 31–40.

Keïta, A., Huneau-Salaün, A., Guillot, A., Galliot, P., Tavares, M., & Puterflam, J., (2016). A multi-pronged approach to the search for an alternative to formaldehyde as an egg disinfectant without affecting worker health, hatching, or broiler production parameters. *Poult. Sci. 95*, 1609-1616.

Kinner, J. A., & Moats, W. A.. (1981). Effect of temperature, pH and detergent on survival of bacteria associated with shell eggs. *Poult. Sci. 60*, 761–767.

Legrini, O., Oliveros, E., & Braun, A. M.. (1993). Photochemical processes for water treatment. *Chem. Rev. 93*, 671-698.

Linley, E., Denyer, S. P., McDonnell, G., Simons, C., & Maillard, J. Y.. (2012). Use of hydrogen peroxide as a biocide new consideration of its mechanisms of biocidal action. *J. Antimicrob. Chemother. 67*, 1589–1596.

Mayes, F. J., & Takeballi, M. A.. (1983). Microbial contamination of the hen's egg: A review. *J. Food Prot. 46*, 1092-1098.

Moats, W. A., (1978). Egg washing: a review. *J. Food Prot. 41*, 919– 925.

Padron, M., (1995). Egg dipping in hydrogen peroxide solution to eliminate Salmonella Typhimurium from eggshell membranes. *Avian Dis. 39*, 627-630.

Peebles, E. D., & Brake, J., (1986). The role of the cuticle in water vapor conductance by the eggshell of broiler breeders. *Poult. Sci. 65*, 1034-1039.

Peyton, G. R., & Glaze, W. H., (1988). Destruction of pollutants in water with ozone in combination with ultraviolet radiation. 3. Photolysis of aqueous ozone. *Environ. Sci. Technol. 22*, 761-767.

Protais, J., Lahellec, C., Bennejean, G., Morin, Y., Quintin, E., & CNEVA-SEA., (1989). Transmission verticale des salmonelles chez la poule: exemple de *Salmonella* Enteritidis. *Bull. Inf. Stn. Exp. Avic. Ploufragan 29*, 37–39.

Rodrigue, D. C., Tauxe, R. V., & Rowe, B., (1990). International increase in *Salmonella* Enteritidis: a new pandemic *Epidemiol. Infect. 105*, 21–27.

Scott, T. A., & Swetnam, C., (1993a). Screening sanitizing agents and methods of application for hatching eggs I. Environmental and user friendliness. *J. Appl. Poult. Res. 2*, 1-6.

Sheldon, B. W., & Brake, J., (1991). Hydrogen peroxide as an alternative hatching egg disinfectant. *Poult. Sci. 70*, 1092-1098.

Smeltzer, T. I., Runge, G., Peel, B., & Orange, K., (1979). Bacterial penetration in floor and nest box eggs from meat and layer birds. *Aust. Vet. J. 55*,592-593.

Solomon, S. E., (2010). The eggshell: Strength, structure and function. *Br. Poult. Sci. 51*, 52-59.

Turnbull, P. C. B., & Snoeyenbos, G. H., (1974). Experimental salmonellosis in the chicken. 1. Fate and host response in alimentary canal, liver, and spleen. *Avian Dis. 18*, 153-177.

Wang, H., & Slavik, M. F., (1998). Bacterial penetration into eggs washed with various chemicals and stored at different temperatures and times. *J. Food Prot. 61*, 276-279.

Williams, J. E., Dillard, L. H., & Hall, G. O., (1968). The penetration patterns of Salmonella Typhimurium through the outer structures of chicken eggs. *Avian Dis. 12*, 445-466.

Author Information

Nora RRAHIMI HASANI
University of Pristina-Departament of Mikrobiology
Pristina, Kosovo
Contact e-mail: *nora.rrahimi@hotmail.com*

To cite this article:

Rrahimi-Hasani, N. (2021). Treatment of egg shells with hydrogen peroxide and sodium carbonate as a disinfectant with bactericidal effect to eliminate Salmonella Entereditis. *The Eurasia Proceedings of Science, Technology,Engineering & Mathematics (EPSTEM), 12*, 1-6.