Effect of spraying hatching eggs with quaternary ammonium on hatching traits of breeder broiler eggs

Hozan Ahmed Mustafa*

Animal Resource Department College of Agricultural Engineering Sciences, Salahaddin University , Erbil, Iraq.
***Corresponding author e-mail: hozan.mustafa@su.edu.krd**
https://doi.org/ 10.59658/jkas.v12i2.3791

Received: Jan. 24, 2025

Accepted: Mar. 25, 2025

Published: June 20, 2025

Abstract

This study examined the effectiveness of quaternary ammonium compound (QAC) as a sanitizer for controlling microbial activity on fertile eggshells and its impact on hatchability. A total of 660 fertile eggs from 60-week-old Lohmann White breeder hens were treated with QAC at concentrations of 1/100, 1/200, and 1/300 ml/L or left unsensitized. Results showed that QAC significantly reduced total aerobic bacteria on eggshells before incubation and at day 18, compared to non-sanitized eggs. Hatchability was higher, and embryo mortality was lower in eggs treated with QAC, particularly at the 1/300 ml/L concentration, compared to the non-sanitized group. Egg weight decreased across all treatments during incubation, with the highest weight loss observed at the 1/100 ml/L concentration. Overall, QAC demonstrated efficacy in reducing microbial contamination and enhancing hatchability, though its effects on egg weight loss varied.

Keywords: Quaternary ammonium, fumigation, Hatchability.

Introduction

Over time, the size of poultry hatchery facilities has increased. This change may lead to improved avian performance, reduced chick mortality, and greater hatchability. Incubation was not as well understood as other chicken production chain processes until recently. The knowledge that has been collected in recent years on the environment, health, management, and nutrition of chicken has not been beneficial to the incubation discipline. It has only been recently recognized that incubation factors play a significant role in chicken performance. A robust hatchery cleanliness program is necessary to maximize hatchability and generate chicks of the best caliber [1].

The foundation of the management of hygienic-sanitary eggs on farms, including the hatchery, is the sanitization of hatching eggs. Gains in poultry productivity are dependent on sanitizer benefits [2]. It is well-established that a high degree of hatchability and the development of superior chicks depend on an efficient sanitation program in the hatchery. Microorganisms can infiltrate a chick hatchery's surroundings and surrounding environment from a variety of sources. Contaminated eggs can introduce pathogens, causing embryonic mortality. Regular sanitation practices, such as washing or treating eggs with disinfectants, are essential to mitigate microbial risks. A clean hatchery environment reduces the chances of contamination during incubation [3]. The cleanliness of viable eggs is one of the most important strategic areas where the chicken industry can optimize productivity. In addition to lowering the frequency of pathogenic bacteria, which are extremely detrimental to embryonic development, it is necessary to reduce the eggshell microbial burden in order to increase hatchability and chick quality (3). Farms and hatcheries can successfully maintain low levels of eggshell contamination by using paraformaldehyde, a sanitizing agent [7,5]. Nonetheless, paraformaldehyde is extremely dangerous for the environment, handling personnel's health, and chick embryo development [7 ,8 ,9]. [9] discovered, for example, that paraformaldehyde can cause anomalies in developing chick embryos.

Because of its affordability, effectiveness, and straightforward process, quaternary ammonium compound sanitizer is also a recent technique [11,12]. In a study, [13] found that after quaternary ammonium washing, *Salmonella enteritidis* only penetrated 3.4 and 6.7% of the eggs. The effectiveness of quaternary ammonium sanitizer against the microorganisms on eggshells has been called into question by a few researches. However, the impact of quaternary ammonium on eggshell integrity during storage was not explored in these research [14,15]. The quaternary ammonium sanitizer successfully decreased the microbial population on eggshells without encouraging interior microbial activity, according to the current study findings by[16].

Removing dangerous microorganisms and sterilizing eggs for human use is believed to be achievable by the process of decontaminating eggshells. Eggshell sanitization techniques have included treatments of radiation and chemicals [17].

The objective of the current study was to determine how well quaternary ammonium at various concentrations regulates microbial activity on eggshells and evaluate the impact on the hatchability of viable eggs.

Materials and Methods

Ethics Committee Approval

The current study was approved by Salahaddin University's College of Agricultural Engineering Sciences' Animal Resources Scientific Ethical Committee on May 14, 2023 (No.: 3/5/5756).

Experimental design

The Vano commercial hatchery in the Kurdistan Region of Iraq's Erbil Governorate served as the research site for this study. The 60-week-old Lohmann White breeder hens produced 660 white fertile eggs weighing an average of 67.930±0.630 g. Prior to incubation, the eggs were weighed and randomly assigned to four treatments after being collected under aseptic circumstances. On the first and tenth days of incubation, they were sanitized. Commercial sanitizer Baysan® is a quaternary ammonium chemical.

Table (1): Description of treatments, chemical amounts, and application methods for eggs.

Treatment	Concentration (ml/L of water)	Application	Number of eggs
Non-sanitized	--	--	165
Quaternary ammonium	1:100	Spraying	165
Quaternary ammonium	1:200	Spraying	165
Quaternary ammonium	1:300	Spraying	165

Eggshell microbial count

According [18], the swab samples were taken using a sterile swab on an area of 2 cm^2 for each egg per treatment. On the first day of incubation and eighteen days prior to placing in the hatcher, each treatment was placed under sterile conditions on the eggshell surface. The samples were subsequently sent right away to the microbiology lab at Salahaddin University's College of Education. After serial dilution from an initial 10-1 dilution to 10-7, a 100 um aliquot of each dilution was plated on MacConkey agar (Sigma-Aldrich, UK) and SS agar (Oxoid, England) to count total aerobic bacteria and Salmonella, respectively. Counts of the colonies that formed throughout the incubation period were made, and the results were expressed in log10 CFU/mL.

Incubation and hatching

After being weighted and serially labelled, the eggs were put in an incubator that was adjusted to 37.5°C and 55% relative humidity. On the eighteenth day of incubation, the eggs were moved into hatcher baskets setter and left in a hatcher for the last three days of incubation, where they were incubated at 37.2°C and 65% relative humidity. The effects of hatching egg disinfection on growth parameters, including fertile eggs , egg weight loss percentages, and fertile egg weight, were assessed during the incubation period.

Equations 1 to5 were usedby [19] to determine the percentages of egg weight loss (%), fertility (%), hatchability of set eggs (%), hatchability of fertile eggs (%), and embryo death (%) following the conclusion of the incubation procedure.

1. Egg weight loss (%) = [(initial egg weight 2 egg weight recorded on the transfer day)/initial egg weight] × 100.
2. Fertility (%) = (number of fertilized eggs/number of eggs set) × 100.
3. Hatchability of total eggs (%) = (No. of hatched chicks/total No. of eggs) × 100.
4. Hatchability of fertile eggs (%) = (No. of hatched chicks/No. of fertile eggs) × 100.
5. Embryonic Mortality (%) = (No.of dead embryos during incubation period /No. of fertile eggs) × 100.
6. Embryo Weight (g) = ($EIW \times e^{(K \times D)}$). [22]

Where:

EIW = Initial Egg Weight (g)

K = Growth Constant (species-specific)
D = Incubation Day
e = Euler's number (approx. 2.718)

Statistical analysis

The data was computed using SPSS version 27 software using a one-way ANOVA technique (20). The summary statistics data comprised means and standard error. It was possible to indicate significant differences among the various parameters at the 0.05 levels using Duncan's test [21].

Results and Discussion

The Table (2) indicates the egg weight at the beginning of incubation as well as on days 18 and 21. Non- significant differences among the treatments at zero day and 18th day in this study., At the 21st day of incubation, the egg weight was reduced in all treatments compared to the control group. While the percentage of egg weight loss during incubation recorded significant differences among treatments in this study at 18th and the end day of incubation (Table 3). The higher egg weight loss during the whole period of incubations (0-21 days) was recorded for quaternary ammonium (1/100) compared to both other concentrations. Both concentrations of quaternary ammonium (1/200 and 1/300) were not significantly affected on the egg weight loss that compared with control group. Temperature and relative humidity, two physical parameters that are critical for incubation, have a greater impact on this parameter [22, 23]. Since the eggs in this experiment were exposed to identical incubation conditions, it was not anticipated that the treatments would differ. Furthermore, we were able to quantify the degree of sanitizing damage to the cuticle and, in turn, the development of the embryo by measuring the weight loss of the eggs throughout incubation [24]. Our results show that the cuticle was not damaged by any of the sanitation methods.

Table (2): Influence of quaternary ammonium disinfectant on fertile egg weight during incubation (Mean±SE).

Treatment	Egg weight (g)		
	Zero day	18th day	21st day
Non-sanitized	69.67±0.80	62.17±0.49	49.20±0.98 a
QA1/100	69.10±1.45	60.80±0.46	44.50±0.49 c
QA1/200	68.00±0.45	62.30±0.20	46.73±-.46 b
QA1/300	67.86±0.40	61.60±0.00	46.73±0.46 b
***P*. value**	0.455	0.059	0.006

a,b,c The means with various superscripts in the same column differ (P<0.05).

There was a significant (P≤0.05) difference in fertility according to total and fertile eggs (Table 3). The mean fertility was 69.7% in non-sanitized with total eggs compared to all sanitized treatments. Still, according to the fertile eggs, the quaternary ammonium (1/300) group recorded higher hatchability than other treatments. This result may return to the eggs were obtained from breeder hens aged 50 weeks old with similar management at the poultry field. Also, table (4) showed eembryonic mortality during the hatching period significantly (P<0.05) were reduced in quaternary ammonium (1/300) treatment compared with both concentrations of quaternary ammonium (1/100 and 1/200) and non-sanitized groups.

[25] tested the effects of quaternary ammonium on the cleanliness of eggs intended for incubation at two concentrations (1.5% and 3%). The hatchability of viable eggs in the 32-week-old flock was enhanced by 1.5% or 3.0% quaternary ammonium applications, but this impact was not statistically significant at the other flock ages (36, 42, 46, and 62 weeks). The youngest flock's increased hatchability seems to be mostly the result of a decrease in early embryonic mortality. It has been shown that higher water loss increases hatchability by lowering early embryonic mortality in eggs from young broiler-breeder flocks, but not from older flocks [26].

Table (3): Effect of quaternary ammonium disinfectant on weight loss of egg weight during incubation (Mean±SE).

Treatment	Egg weight loss (%)	
	0-18th day	0-21st day
Non-sanitized	7.50±0.40 ^{ab}	20.46±0.80 ^{ab}
QA1/100	8.30±1.87 ^{a}	24.60±1.06 ^{b}
QA1/200	5.7±0.60 ^{c}	21.26±0.92 ^{a}
QA1/300	6.26±0.40 ^{b}	21.13±0.44 ^{a}
***P*. value**	0.036	0.033

a,b,c The means with various superscripts in the same column differ (P<0.05).

Table (4): Effect of quaternary ammonium disinfectant on hatchability percentages and dead embryo (Mean±SE).

Treatment	Hatchability (%)		Embryotic Mortality
	Hatchability of total eggs (%)	Hatchability of fertile eggs (%)	
Non-sanitized	69.70±3.03 ^{a}	88.42±0.46 ^{ab}	13.10±0.60 ^{c}
QA1/100	55.76±3.03 ^{b}	79.27±1.48 ^{b}	26.20±1.24 ^{a}
QA1/200	52.12±3.41 ^{b}	86.52±2.92 ^{ab}	17.16±0.92 ^{b}
QA1/300	51.51±2.32 ^{b}	94.94±2.03 ^{a}	5.43±0.29 ^{d}
***P*. value**	0.012	0.032	<0.001

a,b,c,d The means with various superscripts in the same column differ (P<0.05).

The Results in table (5) shows the effect of disinfecting hatching eggs on the surface's total aerobic bacterial count. Table results show that utilizing different quantities of quaternary ammonium (1/100, 1/200, and 1/300) compared to non-sanitized considerably (P<0.05) reduced total aerobic bacterial counts on hatching eggshell surfaces. The total bacterial counts reduced from 5.72 log in non-sanitized to 4.85, 4.53, and 4.65 log in quaternary ammonium (1/100, 1/200, and 1/300), respectively. Also. at 18th day of hatching eggs, the total aerobic bacteria counting was significantly (P<0.05) lower after spraying with quaternary ammonium (1/200 and 1/300) than in the control group. At various points during the incubation process, no *salmonella* spp. was found in any of the treatments.

The microbial population on eggshells was effectively reduced by using different levels of quaternary ammonium (200 ppm and 100 ppm) as a sanitizer without encouraging internal microbial growth of approximately 4 log10 CFU/cm2 of the aerobic mesophilic bacteria, 1.5 to 2.5 log10 CFU/cm^2 of the mild population, and 1.5 to 2 log10 CFU/cm2 of the yeast population, as [16] showed. The 12–14 alkyl chain length of quaternary ammonium sanitizer contributes to its antibacterial qualities. One property of quaternary ammonium is that it inhibits the growth of pathogenic bacterial microbiota. Less total aerobic bacteria were found to be pathogens in this experiment because of the high concentration of alkyl chain length in quaternary ammonium, which has antibacterial activity [27]. Findings by [28] emphasized that the interaction between eggshell quality and incubation conditions, such as temperature and humidity, plays a critical role in the survival of embryos and overall hatchability. Thicker eggshells have been associated with better hatchability, with some studies suggesting that hatching results from eggs with thicker shells can be up to 9% [29].

Table (5): Effect of quaternary ammonium disinfectant on total aerobic bacterial count (Log_{10} CFU per egg) on eggshell at different stages of incubation (Mean±SE).

Treatment	Total aerobic bacteria	
	Before setting in a setter	Before setting in Hatcher
Non-sanitized	5.72 ± 0.04^{a}	8.04 ± 0.005^{a}
QA1/100	4.85 ± 0.51^{b}	8.04 ± 0.008^{a}
QA1/200	4.53 ± 0.11^{b}	7.96 ± 0.01^{b}
QA1/300	4.65 ± 0.09^{ab}	7.46 ± 0.01^{c}
***P.* value**	<0.001	<0.001

a,b,c The means with various superscripts in the same column differ (P<0.05).

The total population of aerobic microorganisms on the surface of viable eggshells was dramatically reduced by spraying hatching eggs with quaternary ammonium. Thus, its application is a substitute for formaldehyde and other chemicals in the sterilization of fertile eggs.

References

1) Park JA, Sohn SH. 2018. The influence of hen aging on eggshell ultrastructure and shell mineral components. *Korean J Food Sci Anim Resour*. 38(5):1080-1091. https://doi.org/10.5851/kosfa.2018.e41
2) Oliveira, G. S. , Concepta M. , Cristiane B. S. and Vinícius M. S. 2022. Effects of Sanitizers on Microbiological Control of Hatching Eggshells and Poultry Health

during Embryogenesis and Early Stages after Hatching in the Last Decade. *Animals,*12(2826):2-15. https://doi.org/10.3390/ani12202826

3) Hassan, W.H. and Tayeb, I.T., 2021. Effect of different administration methods of Vitamin AD3E to hatching eggs and supplement it with drinking water on hatchability traits and post hatch performance and biochemical parameter of broiler. *Iraqi Journal of Agricultural Sciences*, 52(2), pp.322-335. https://doi.org/10.36103/ijas.v52i2.1294
4) Shahein, E., and E. K. Sedeek. 2014. Role of spraying hatching eggs withnatural disinfectants on hatching characteristics and eggshell bacterial counts. *Egypt. Poult. Sci. J.* 34:211-228. https://doi.org/10.21608/epsj.2014.5313
5) Williams, J. E. 1970. Effect of high-level formaldehyde fumigation on bacterial populations on the surface of chicken hatching eggs. Avian Dis., 14:386-392. https://doi.org/10.2307/1588482
6) Whistler, P. E., and B. W. Sheldon. 1989. Biocidal activity of ozone versus formaldehyde against poultry pathogens inoculated in a prototype setter. *Poult. Sci.* 68:1068-1073. https://doi.org/10.3382/ps.0681068
7) Cadirci, S. 2009. Disinfection of hatching eggs by formaldehyde fumigation- a review. Arch. Geflugelk. 73:116-123.
8) Unsaldi, E., and M. K. Ciftci. 2010. Formaldehyde and it using areas, risk group, harmful effects and protective precautions against it. *Van Vet. J.* 21:71-75.
9) Rhomberg, L. R. 2015. Contrasting directions and directives on hazard identification for formaldehyde carcinogenicity. *Regul. Toxicol. Pharm*. 73:829-833. https://doi.org/10.1016/j.yrtph.2015.10.012
10) Zeweil, H. S., R. E. Rizk, G. M. Bekhet, and M. R. Ahmed. 2015. Comparing the effectiveness of egg disinfectants against bacteria and mitotic indices of developing chick embryos. J. Basic Appl. Zool. 70:1-15. https://doi.org/10.1016/j.jobaz.2014.12.005
11) Achiwa, N.; Nishio, T. 2003. The use of electrolyzed water for sanitation control of eggshells and GP center. Food Sci. Technol. Res.. 9, 100-103. https://doi.org/10.3136/fstr.9.100
12) Favier, G.I.; Escudero, M.E.; Mattar, M.A.; De Guzmán, A.M.S. 2000. Survival of Yersinia enterocolitica and mesophilic aerobic bacteria on eggshell after washing with hypochlorite and organic acid solutions. J. Food Prot., 63, 1053-1057. https://doi.org/10.4315/0362-028x-63.8.1053
13) 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. https://doi.org/10.4315/0362-028x-61.3.276
14) Berrang, M.E.; Frank, J.F.; Buhr, R.J.; Bailey, J.S.; Cox, N.A.; Mauldin, J.M. 1997. Microbiology of sanitized broiler hatching eggs through the egg production period. J. Appl. Poult. Res., 6, 298–305. https://doi.org/10.1093/japr/6.3.298

15) Al-Ajeeli, M.N.; Taylor, T.M.; Alvarado, C.Z.; Coufal, C.D. 2016. Comparison of eggshell surface sanitization technologies and impacts on consumer acceptability. Poult. Sci., 95, 1191–1197. https://doi.org/10.3382/ps/pew014

16) Chan, H. Y., Anis Shobirin M.H., Nurul H. A., Yaya R. and Abd-El-Aziem F. 2021. Effectiveness of Quaternary Ammonium in Reducing Microbial Load on Eggs. 30;26(17):5259. https://doi.org/10.3390/molecules26175259

17) Chousalkar KK, Khan S., McWhorter AR. 2021. Microbial quality, safety and torage of eggs. Curr Res Food Sci. 38:91-95. https://doi.org/10.1016/j.cofs.2020.10.022

18) Şimşek B, Sagdic O, Ozcelik S. 2007. Survival of Escherichia coli O157: H7 during the storage of Ayran produced with different spices. J Food Eng. 78(2):676-680. https://doi.org/10.1016/j.jfoodeng.2005.11.005

19) Baylan, M., G. C. Akpınar, S. D. Canogullari, and T. Ayasan. 2018. The effects of using garlic extract for quail hatching egg disinfection on hatching results and performance. Braz. J. Poult. Sci. 20:343–350. https://doi.org/10.1590/1806-9061-2017-0693

20) SPSS, IBM Corp. 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp.

21) Duncan, D. B. (1955). Multiple range and multiple “F” test. Biometrics.11,1-42.

22) Tullet, S. G., and F. G. Burton. 1982. Factors affecting the weight and water status of the chick at hatch. British Poult. Sci. 23:361-369. https://doi.org/10.1080/00071688208447969

23) Meijerhof, R., and G. van Beek. 1993. Mathematical modelling of temperature and moisture loss of hatching eggs. J. Theor. Biol. 165:27-41. https://doi.org/10.1006/jtbi.1993.1175

24) Peebles, E. D., T. Pansky, S. M. Doyle, C. R. Boyle, T. W. Smith, M. A. Latour, and P. D. Gerard. 1998. Effects of dietary fat and eggshell cuticle removal on egg water loss and embryo growth in broiler hatching eggs. Poult. Sci. 77:1522-1530. https://doi.org/10.1093/ps/77.10.1522

25) Brake, J. and Sheldon B. W. (1990). Effect of a Quaternary Ammonium Sanitizer for Hatching Eggs on Their Contamination, Permeability, Water Loss, and Hatchability. Poult. Sci. 69:517-525. https://doi.org/10.3382/ps.0690517

26) Vick, S. V., and Brake J. 1986. Effect of incubation humidity on hatchability with respect to egg weight and flock age. Poult. Sci. 65(Suppl. 1):130. (Abstr.).

27) Bundgaard-Nielsen, K.; Nielsen, P.V. 1996. Fungicidal effect of 15 disinfectants against 25 fungal contaminants commonly found in bread and cheese manufacturing. J. Food Prot., 59, 268–275. https://doi.org/10.4315/0362-028x-59.3.268

28) Ahmed, L.S. and Al-Barzinji, Y.M.S., 2020. Comparative study of hatchability and fertility rate among local quails. Iraqi Journal of Agricultural Sciences, 51(3). https://doi.org/10.36103/ijas.v51i3.1028

29) Ahmed, L.S., 2022. Impact of egg shell and spots colour on the quality of hatching eggs derived from three lines of local quail. Iraqi Journal of Agricultural Sciences, 53(6), pp.1256-1269. https://doi.org/10.36103/ijas.v53i6.1640