

## Effect of A Quaternary Ammonium Compounds on Hatching in Breeder Broiler Eggs

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### ABSTRACT

The aim of this study was to determine whether successfully different quaternary ammonium levels for sanitizing fertile eggs controlled microbial activity on eggshells and how that activity affected the hatchability of fertile eggs. Four treatments (non-sanitized the Quaternary ammonium compound at different levels 1/100, 1/200, and 1/300 ml/L of water) were randomly assigned to 660 white fertile eggs weighing an average of  $67.930 \pm 0.630$  gm, produced by 60-week-old Lohmann White breeder hens. before and at the tenth day of incubation. The findings demonstrated that, in comparison to quaternary ammonium at the (1/300) level, non-sanitized viable eggs had a lower hatchability and a lower percentage of dead embryos at the same level. There were no significant ( $P > 0.05$ ) differences recorded in egg weight among treatments at zero-day and 18<sup>th</sup> day in this study. But, at the 21<sup>st</sup> day of incubation, the egg weight was reduced in all concentrations of quaternary ammonium compared with the control group. The higher egg weight loss during the whole period of incubation (0-21 days) was recorded for quaternary ammonium (1/100) compared with both other concentrations of quaternary ammonium. Prior to being placed in the incubator, the hatching eggshell surface's total aerobic bacterial counts were considerably ( $P < 0.05$ ) lower when quaternary ammonium was used as opposed to non-sanitized. Additionally, at day eighteen of hatching, spraying with varying concentrations of quaternary ammonium (1/100, 1/200, and 1/300) resulted in a considerably ( $P < 0.05$ ) lower count of total aerobic bacteria when compared to non-sanitized.

**Key words:** fumigation, Hatchability, Quaternary ammonium.



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### INTRODUCTION

Poultry hatchery operations have grown in scale over time. 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 (Park and Sohn, 2018). 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 (Oliveira *et al.*, 2022). 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. 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

(Shahein and Sedeek, 2014). Farms and hatcheries can successfully maintain low levels of eggshell contamination by using paraformaldehyde, a sanitizing agent (Williams, 1970; Whistler and Sheldon, 1989). Nonetheless, paraformaldehyde is extremely dangerous for the environment, handling personnel's health, and chick embryo development (Cadirci, 2009; Unsaldi and Ciftci, 2010; Rhomberg, 2015). Zeweil *et al.* (2015) 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 (Achiwa and Nishio, 2003; Favier *et al.*, 2000). In a study, Wang and Slavik (1998) 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 research. However, the impact of quaternary ammonium on eggshell integrity during storage was not explored in these research (Berrang *et al.*, 1997; Al-Ajeeli *et al.*, 2016). The quaternary ammonium sanitizer successfully decreased the microbial population on eggshells without encouraging interior microbial activity, according to the current study findings by (Chen *et al.*, 2021). 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 (Chousalkar *et al.*, 2021). On the other hand, the environment, human health, and egg quality will all suffer by utilizing these approaches. It would be ideal to find a safe replacement method; using essential oils to clean eggshells is one option. 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 Scientific Ethical Committee of Salahaddin University's

Animal Resources Department, College of Agricultural Engineering Sciences, accepted the current study on May 14, 2023 (No.: 3/5/5756).

**The Design of Experiment:** 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  $67.930 \pm 0.630$  g. The eggs were collected under aseptic conditions, randomly divided into four treatments prior to incubation, and sanitized on the first and tenth days of incubation as per (Table 1). Commercial sanitizer Baysan<sup>®</sup> is a quaternary ammonium chemical.

**Eggshell Microbial Count:** According to Şimşek *et al.* (2007), the swab samples were taken using a sterile swab on an area of 2 cm<sup>2</sup> for each eggs per treatment. On the first day of incubation and eighteen days prior to placing in the incubator, 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 µm 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 log<sub>10</sub> CFU/mL. **2.4 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 hatching nests 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 metrics, including hatchability percentages, egg weight loss percentages, and fertile egg weight, were assessed during the incubation period. Equations 1 through 5 were utilized by Baylan *et al.* (2018) 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. Embryo dead (%) = (No. of dead embryos during incubation period /No. of fertile eggs) × 100

**Statistical Analysis:** The data was assessed using SPSS version 27 software using a one-way ANOVA technique (SPSS, 2020). The summary statistics data comprised means and standard error. It was possible to indicate significant differences between the various parameters at the 0.05 levels using Duncan's test (Duncan, 1955).

## RESULTS AND DISCUSSION

Table 2 indicates the egg weight at the beginning of incubation as well as on days 18 and 21. No significant differences ( $P>0.05$ ) between the treatments at zero day and 18<sup>th</sup> day in this study. But, at the 21<sup>st</sup> day of incubation, the egg weight was reduced in all concentrations of quaternary ammonium compared with the control group. While the percentage of egg weight loss during incubation recorded significant differences among treatments in this study at 18<sup>th</sup> and the end day of incubation (Table 3). The higher egg weight loss during the whole period of incubation (0-21 days) was recorded for quaternary ammonium (1/100) compared with both other concentrations of quaternary ammonium. While, both concentrations of quaternary ammonium (1/200 and 1/300) not significantly affect 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 (Tullet and Burton, 1982; Meijerhof and van Beek, 1993). 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 (Peebles *et al.*, 1998). Our results show that the cuticle was not damaged by any of the sanitation methods. There was a significant ( $P>0.05$ ) difference in fertility according to total and fertile eggs (Table 4). The mean fertility was 69.7% in non-sanitized with total eggs compared with all sanitized treatments. Still, according to the fertile eggs, the quaternary ammonium (1/300) group recorded higher hatchability than other treatments. This result was observed because the eggs were obtained from breeder hens aged 50 weeks old with the same management at the poultry house. Also, table 4 showed embryos dead 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. Brake and Sheldon (1990) 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 (Vick and Brake, 1986). Table 5 highlights the data that was obtained about 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 18<sup>th</sup> 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 log<sub>10</sub> CFU/cm<sup>2</sup> of the aerobic mesophilic bacteria, 1.5 to 2.5 log<sub>10</sub>

CFU/cm<sup>2</sup> of the mold population, and 1.5 to 2 log<sub>10</sub> CFU/cm<sup>2</sup> of the yeast population, as Chen *et al.* (2021) showed. The 12–14 alkyl chain length of quaternary ammonium sanitizer contributes to its antibacterial qualities (Bundgaard-Nielsen, 2012). 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 (Chen *et al.*, 2021).

**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

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

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

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

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

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

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

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

**Table 5.** Effect of quaternary ammonium disinfectant on total aerobic bacterial count (Log<sub>10</sub> CFU per egg) on eggshell at different stages of incubation (Mean±SE).

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

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

## CONCLUSION

In conclusion, 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.

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## CONFLICT OF INTEREST

The author declare that they have no conflicts of interest.

## AUTHOR/S DECLARATION

The author declare that this manuscript is original, has not been published previously, and is not currently under consideration by any other journal. All figures and tables are original and prepared by the author. Any material obtained from third parties has been included with the required permissions. The author has read and approved the final manuscript.

## AUTHOR'S CONTRIBUTION STATEMENT

The author made equal contributions to the study design, methodology, experimental work, data analysis, and manuscript writing. The author reviewed and approved the final version of the manuscript

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## تأثير استخدام تراكيز المختلفة من المركبات الأيونية الرباعية على صفات الفقس في دجاج امهات اللحم

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### المستخلص

الهدف من هذه الدراسة هي تحديد مستويات الأيونوم الرباعية المختلفة لتطهير البيض المخصب في النشاط الميكروبي على قشر البيض وكيفية تأنيهاً على قابلية البيض للمخصب للتفقيس . تم توزيع أربع معاملات (غير معقمة بمركب رباعي الأيونوم بمستويات مختلفة 100/1 و 200/1 و 300/1 مل/لتر ماء) بشكل عشوائي على 660 بيضة مخصبة بمتوسط وزن  $67.930 \pm$  0.630 غم، أنتجت دجاج امهات لوهمان وايت بعمر 60 أسبوعاً. باليوم العاشر من الحضانه. أظهرت النتائج أنه بالمقارنة مع رباعي الأيونوم عند مستوى (300/1)، فإن البيض القابل للفق غير المعقم كان له معدل فقس أقل ونسبة أقل من الأجنة الميتة عند نفس. لم تم تسجيل أي فروق ذات دلالة إحصائية) وزن البيض بين المعاملات في اليوم صفر واليوم الثامن عشر عند 10 يوم في هذه الدراسة. ولكن في اليوم الحادي والعشرين من الحضانه، انخفض وزن البيض في جميع تركيزات رباعي الأيونوم مقارنة بمجموعة السيطرة. تم تسجيل أعلى فقدان لوزن البيض خلال فترة الحضانه بأكملها (0-21 يوماً) للأيونوم الرباعي (100/1) مقارنة بكلا التركيزين الآخرين من الأيونوم الرباعي. قبل وضعها في الحضانه، كان إجمالي عدد البكتيريا الهوائية على سطح قشرة بيض الفقس أقل بشكل كبير ( $P < 0.05$ ) عند استخدام الأيونوم الرباعي مقارنة بمجموعة السيطرة. بالإضافة إلى ذلك، في اليوم الثامن عشر من الفقس، أدى الرش بتركيزات مختلفة من الأيونوم الرباعي (100/1، 200/1، و 300/1) إلى انخفاض كبير في إجمالي البكتيريا الهوائية عند مقارنتها بعدم التعقيم ( $P < 0.05$ ).

**الكلمات المفتاحية:** التبخير، قابلية الفقس، الأيونوم الرباعي.