Slightly acidic electrolyzed water as an alternative disinfection technique for hatching eggs

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ABSTRACT Conventional chemical disinfectants used for egg disinfection could result in toxic residue and endanger hatchability, chick quality, and pullet growth performance. Slightly acidic electrolyzed water (**SAEW**) is known as a novel disinfectant for egg sterilization due to its high efficiency and no residue. In this study, a comprehensive assessment of slightly acidic electrolyzed water and benzalkonium bromide solution (**BBS**) used in the disinfection channel was conducted to assess the microbial count, eggshell quality, and hatchability concomitantly. The results show that the sterilization efficiency of SAEW increased with an increase in available chlorine concentration (**ACC**), spraying volume, and sterilization duration. SAEW with an ACC of 150 mg/L and 10,000 mg/L benzalkonium bromide solution had the same sterilization rates of approximately 86.2% at a spraying volume of 0.5 mL/egg and sterilization duration of 180 s. Neither had significant effect on eggshell strength or thickness. The eggshell cuticle quality in the benzalkonium bromide group was significantly higher than the control group (no disinfection) and the 150 mg/L SAEW group. The embryo weight, relative embryo weight, hatchability, and embryonic mortality in the SAEW group had no significant differences of those in the benzalkonium bromide group. SAEW should be more popular because of its simple preparation, low cost, and no residue. Our results indicate SAEW is an alternative disinfectant for the sterilization of hatching eggs instead of conventional chemical disinfectants, such as benzalkonium bromide, and give a recommendation is using SAEW as a disinfectant with 150 mg/L ACC, 0.5 mL/egg spray volume, and disinfection for 180 s in the novel disinfection channel.

Key words: hatching egg, alternative disinfectant, eggshell quality, egg incubation

2022 Poultry Science 101:101643
https://doi.org/10.1016/j.psj.2021.101643

INTRODUCTION

The surface of hatching eggs is often contaminated with quantities of microorganisms, including pathogens, due to contact with feces, dust, and other microbial-laden materials (De Reu et al., 2006; Park et al., 2017; Lei et al., 2020). Pathogens may invade hatching eggs through the eggshell and reproduce rapidly at the incubation temperature, which results in embryo deformities or even death (Zeweil et al., 2015; Olsen et al., 2017). Consequently, sterilization of hatching eggs before incubation is essential to improve quality of the hatch and chicks.

Various disinfectants were used to reduce the microorganisms on the surface of hatching eggs, experimentally and in practice. Formaldehyde is widely used as a conventional chemical disinfectant in the disinfection of eggs, which can result in toxic residue and endanger hatchability, chick quality, and pullet growth performance (Oliveira et al., 2020). Research on other alternative disinfectants, including peracetic acid, UV-C, O_3, hydrogen peroxide plus alcohol, essential oil, and benzalkonium bromide, has been conducted to assess the effects of disinfection and hatch (Wells et al., 2010; Al-Ajeeli et al., 2016; Gottselig et al., 2016; Suwannarach et al., 2017; Li et al., 2018; Motola et al., 2020 Tebrun et al., 2020). Among the research, benzalkonium bromide is a commonly used disinfectant in hatcheries, which could bind to an extracellular matrix with electrostatic interaction, thereby making biofilm removal easier (Huang et al., 2019). These aforementioned disinfectants have shown positive disinfection effects, but have caused variations in hatching traits. Some methods even reduce hatching traits, mainly due to

Received October 7, 2021.
Accepted November 25, 2021.

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the residual chemical disinfectant (Wlazlo et al., 2020). Therefore, it is necessary to find a disinfectant and disinfection method without residue.

Slightly acidic electrolyzed water (**SAEW**) is known as a novel disinfectant for egg sterilization due to its broad-spectrum, high-efficiency, and no-residue qualities (Huang et al., 2008; Rahman et al., 2010). The main effective form of chlorine compounds in SAEW is hypochlorous acid (HOCl), which has strong antimicrobial activity (Cao et al., 2009). SAEW has proven to be a promising disinfectant agent for eggshell washing without environmental pollution and shows a higher efficiency than NaClO solutions at the same ACCs of 80 or 100 mg/L against *E. coli O157: H7, Staphylococcus aureus*, and total aerobic bacteria on eggshells (Ni et al., 2014; Kim et al., 2016). Zang et al. (2019) found that a complete inactivation of *S. Enteritidis* and *E. coli* on the eggshell resulted from treatment with SAEW immersed at an ACC of 26 mg/L for 3 and 4 min but that it can cause damage to cuticles. Eggshell cuticle plays a protective function in trans-shell contamination with bacteria (Sheng et al., 2021). In addition to disinfection and incubation results, avoiding damage to the cuticle during disinfection is also necessary. However, the effect of spraying SAEW on eggshell quality and the effect of SAEW on embryonic development and hatching traits is still unknown. Previous studies of SAEW have not dealt with the combined effects of SAEW on the microbial count, eggshell quality, and hatchability concomitantly.

The process involved transferring hatching eggs to the hatchery after disinfection, knowing there was a low disinfection efficiency and that contamination may occur during the transfer process. A disinfection channel (supplementary material 1) was designed to disinfect the hatching eggs before entering the hatchery and after the egg storage. The hatching eggs were placed on the transport trolley, put through the spray area and ventilation area along the track before reaching the hatchery, which greatly improves the efficiency of disinfection and avoids contamination. However, the selection of disinfectants and disinfection parameters, including spray volume and disinfection duration, has not yet been determined.

Hence, the objective of this study was to comparatively assess the reduction of the eggshell microbial count, eggshell quality, and hatchability traits of slightly acidic electrolyzed water and benzalkonium bromide solution, and determine the disinfection protocol in the novel disinfection channel for hatching eggs.

MATERIALS AND METHODS

Experimental Platform and Hatching Eggs

In the novel disinfection channel, hatching eggs were firstly sprayed with disinfectant, and then kept in a closed and misted state. Finally, they were dried by dry air filtered by high efficiency particulate air (**HEPA**). Taking into account the transportation volume of the trolley, the demand for incubation and the habits of the workers after calculation, the time for the trolley to pass through the entire disinfection channel was not less than 3 min during the entire process, which meets the needs of the incubator. However, the allocation of spray time and closed time needed to be determined when the sterilization duration was 3 min. Thus, a self-made experimental spray apparatus (supplementary material 2) was made to simulate the disinfection channel in hatchery, including spray disinfection function and ventilation drying function, and take the total disinfection time of 180 s as the basis for the plan design in the laboratory.

The spray systems were made of hyperbaric spraying (JDT-12, Fog Machine, Chaoshan, China) and high-pressure nozzle (SAX00, Sasun white crystal, Taichung, China Taiwan), with a spray flow of 0.02 L/min. The ventilation systems were made of a blower (CZR-40, Shanghai Xin long, Shanghai, China), with the air volume of 1.07 m^3/min. A container was provided to load disinfectant.

A total of 7,752 hatching eggs weighing 60.20 ± 3.52 g, 60.75 ± 3.35 g, and 61.91 ± 3.56 g were purchased from Jing Hong No.1 breeders aged of 28 wk, 33 wk, and 42 wk of Beijing Huadu Yukou Poultry Industry Co., Ltd. And those eggs were stored at a storehouse (T: 17−23°C; RH: 65−75%) for no more than 2 d before disinfection.

Production of Disinfectants

The SAEW was produced using an electrolyzed water generator (JH-120, Shenzhen Jianghe Biotechnology Co., Ltd, Shenzhen, China). The pH values were measured by a pH meter (AZ8601, AZ Instrument Corp., Taichung, China Taiwan). ACC was measured by RC-3F (Kasahara Chemical Instruments Corp., Saitama, Japan). The pH meter was calibrated using commercial standard buffers with pH of 4.01 and 6.86 supplied by the manufacturer. BBS was prepared by dilution 5% of the original solution (Shanghai Yunjia Huangpu Pharmaceutical Co., Ltd, Shanghai, China). Both disinfectants were produced and use at room temperature (22°C −26°C).

Experiment Setup

The experiment design was divided into 4 trails to investigate the equivalent disinfection effect between SAEW and BBS, the eggshell quality, and hatchability traits concomitantly. Trial 1 was designed to compare the disinfection effect among different spray time and closed time at a total duration of 180 s. Eggs were divided into the following 4 groups, with 168 eggs per group: Control group (no disinfection), SAEW1 group (spray for 60 s and closed for 120 s, 0.33 mL/egg), SAEW2 group (spray for 90 s and closed for 90 s, 0.5 mL/egg), and SAEW3 group (spray for 120 s and closed for 60 s, 0.67 mL/egg). Trial 2 was designed to recommend disinfection concentration, while spray volume,

and closed time were based on the result of trial 1. Eggs were divided into the following 6 groups, with 168 eggs per group: Control group (no disinfection), Tap water group, 50 mg/L SAEW group, 100 mg/L SAEW group, 150 mg/L SAEW group, and 10,000 mg/L BBS group. After disinfection in trail 1 and trial 2, 12 eggs per group were randomly selected and sampled eggshell surface culturable bacteria to analysis sterilization rate. Then a disinfection technique including disinfection concentration, spray, and close time were recommended. Under the recommend technique, trial 3 was designed to analyze the eggshell quality and trial 4 was designed to analyze hatching traits. In trail 3, eggs were divided into the following 3 groups, with 168 eggs per group: Control group (no disinfection), SAEW group, and BBS group. After disinfection, 10 eggs per group were randomly selected and detected egg quality. In trial 4, a total of 400 eggs were divided into 2 groups, with 168 eggs per group: SAEW group and BBS group. After disinfection, all eggs were incubated and analysis hatching traits. Each trail was repeated 3 times under the same conditions.

Eggshell Surface Culturable Bacteria Sampling and Analysis

After disinfection, eggs were randomly selected from each group and moved into a sterile sampling bag with 50 mL of sterile normal saline. After shaking for 5 min, 1 mL of the sample solution was diluted serially with sterile normal saline. The samples were plated on plate count agar that was incubated at 37°C for 24 h prior to counting the resultant colonies. After incubation, colonies in the petri dishes with 30 to 300 colonies were enumerated. The total culturable bacteria on eggshell surface were calculated using Eq. (1):

$$C_{bacteria} = \frac{N_1 V_1}{V_2} \times 10^{a} \quad (1)$$

where $C_{bacteria}$ is the CB concentration, CFU/egg; N_1 is the average number of colonies in petri dishes with 30 to 300 colonies where 10^{-a} liquid samples are cultured, CFU; V_1 is the total volume of the original sample (50 mL); a is the dilution factor of the original sample; V_2 is the volume of 10^{-a} original sample plated on nutrient agar medium.

Eggshell Quality Detection

In this study, eggshell quality, including eggshell strength, eggshell thickness, and opacity of cuticle was detected. The eggshell strength was measured by an eggshell strength tester (Model-I, Robotmation Co. Ltd., Tokyo, Japan). The eggshell thickness was expressed by the mean thickness of the blunt end, medium end, and sharp end of the eggshell, which was measured by a helical micrometer (0−25 mm/0.001 mm, Mitutoy, Kawasaki, Japan) after stripping the inner shell membrane. The opacity of cuticle (α value) was calculated according to Chen et al. (2019) after the measurement of the eggshell color before and after dyeing with MST glue protective blue dye (M.S. Technology Co., Ltd, England) with color spectrophotometer (CM-700d, Konica Minolta Inc., Tokyo, Japan).

Incubation and Hatching Traits

For each experiment in trial 4, 400 eggs were randomly divided into 2 groups after disinfection, and placed in the 2 identical automatic incubators (OvaEasy 380 Advance EX Series II, Brinsea, Weston-Super-Mare, England). They were incubated under standard conditions with an air temperature of 37.6°C and a relative humidity of approximately 50% at E0-E18 and 60% at E18-E21. Egg turning was automatically performed in the incubator every 90 min at an angle of 90°. The eggs were candled at 18 d, and those with evidence of a living embryo were transferred from the turning trays to the hatcher baskets. The incubation was stopped at 512 h, and chicks were removed from the incubator.

Embryo weight and relative embryo weight:

On the 6th, 10th, 14th, and 18th d of incubation, 10 random eggs were taken from each group and weighed using an electronic scale (JCS, Wuxi Yingheng Electronics Co., Ltd, Wuxi, China) with an accuracy of 0.001 g. Egg and embryo weight was weighed. Eq. (2) was used to calculate relative embryo weight:

$$RW_{embryo} = \frac{W_{embryo}}{W_{egg}} \times 100\% \quad (2)$$

where RW_{embryo} is relative embryo weight, %; W_{embryo} is embryo weight, g; W_{egg} is embryo weight, g.

Hatchability:

After hatching, numbers of set eggs, unfertilized eggs, and chicks were counted. The hatchability was calculated by using Eq. (3):

$$\text{Hatchability} = \frac{N_{chicks}}{NE_{hatching} - NE_{sampled} - NE_{unfertilized}} \times 100\% \quad (3)$$

where Hatchability is the hatchability of hatching eggs, %; N_{chicks} is number of chicks after 21 d of incubation; $NE_{hatching}$ is hatching eggs number at 0th d of incubation; $NE_{sampled}$ is sampled egg number during incubation; $NE_{unfertilized}$ is unfertilized eggs number detected on the 18th d of incubation.

Embryonic mortality:

After 21 d of incubation, the unhatched eggs were counted, opened, and examined to determine the percentage of infertile eggs and the percentage of embryonic deaths. Embryonic mortality was calculated by using Eqs. (4), (5), and (6):

$$Dead_{early} = \frac{DN_{day1-7}}{N_{fertilized}} \times 100\% \quad (4)$$

$$Dead_{mid} = \frac{DN_{day8-14}}{N_{fertilized}} \times 100\% \quad (5)$$

$$\text{Dead}_{\text{late}} = \frac{\text{DN}_{\text{day15-21}}}{\text{N}_{\text{fertilized}}} \times 100\% \quad (6)$$

where, $\text{Dead}_{\text{early}}$ is early embryonic mortality of hatching eggs, %; Dead_{mid} is mid-embryonic mortality of hatching eggs, %; $\text{Dead}_{\text{late}}$ is late embryonic mortality of hatching eggs, %; $\text{DN}_{\text{day1-7}}$ is 1 to 7 d of incubation dead embryo numbers; $\text{DN}_{\text{day8-14}}$ is 8 to 14 d of incubation dead embryo numbers; $\text{DN}_{\text{day15-21}}$ is 15 to 21 d of incubation dead embryo numbers; $\text{N}_{\text{fertilized}}$ is fertilized eggs numbers.

Statistical Analysis

All experiments had 3 replications for each treatment and measurement. Mean values of all parameters were calculated from the independent triplicate trials. Statistical analysis was performed using SPSS 25.0 software (SPSS Inc., Chicago, IL). In the disinfection trials and egg quality detection trails, individual eggs served as the unit of replication for eggshell microbial counts and eggshell quality detection. Means were tested for significant differences by Tukey's test when the assumptions of normality and homoscedasticity were met. When the test for normality distribution and homogeneity of variance failed, the Kruskal−Wallis test was used. In hatching traits trial, analysis of the incubation parameters used 3 replications per treatment in which each batch of eggs constituted a replicate. A linear mixed model was used:

$$Y = \mu + T_j + B_k + CB_{jk} + I_m + \varepsilon \quad (7)$$

Where Y is the embryo weight, relative embryo weight, hatchability, and embryonic mortality, μ is the mean value, T is the disinfection treatment (j = SAEW group, BBS group), B is the batch factor (k = 1, 2 and 3), CB is interaction (treatment × batch), I is incubator (m = 1, 2), and ε = error influence. Statistical significance for all tests was considered at $P < 0.05$.

RESULTS

Determination of the Disinfection Technique and Disinfectant Parameters

Under the treatment of Control group, SAEW1, SAEW2, and SAEW3, the total culturable bacteria in the eggshells were 27.2×10^3 CFU/egg, 10.64×10^3 CFU/egg, 3.75×10^3 CFU/egg, and 10.60×10^3 CFU/egg, respectively (Figure 1). The sterilization rate of SAEW2 (spraying for 90s and closed for 90s) was the highest ($P < 0.05$). Spraying volumes were 0.5 mL/egg and sterilization durations were 180 s under the treatment of SAEW2.

In the comparison of different disinfectants, all treatments significantly reduced total culturable bacteria on the surface of eggshells. The total culturable bacteria in eggshells under the treatment of control group, tap water, 50 mg/L SAEW, 100 mg/L SAEW, 150 mg/L SAEW, and 10,000 mg/L BBS were 27.1×10^3 CFU/egg, 8.6×10^3 CFU/egg, 4.80×10^3 CFU/egg, 3.75×10^3 CFU/egg, 2.22×10^3 CFU/egg and 0.36×10^3 CFU/egg, respectively (Figure 1). The sterilization effect of 50 mg/L SAEW and 100 mg/L SAEW show no significant difference, while total culturable bacteria on the eggshell of them were significantly higher than that of 150 mg/L SAEW. Moreover, there was no significant difference between 150 mg/L SAEW and 10,000 mg/L BBS.

Effect of Slightly Acid Electrolyzed Water and Benzalkonium Bromide Disinfection on Eggshell Quality

The eggshell strength of the control group, SAEW and BBS, was 3.19 kg/cm^2, 2.90 kg/cm^2, and 2.84 kg/cm^2, respectively. The eggshell thickness of the control group, SAEW and BBS, was 0.324 mm, 0.325 mm, and 0.333 mm, respectively. Neither of those treatments had an effect on eggshell strength nor eggshell thickness (Figure 2).

There was no significant difference in the opacity of eggshell cuticle between the control group (26.07%) and the SAEW treatment group (21.37%) (Figure 2), but both groups were significantly smaller than the BBS treatment group (38.34%) ($P < 0.05$).

Effect of Slightly Acid Electrolyzed Water and Benzalkonium Bromide on Hatching Traits

The hatchability of the SAEW and BBS groups was 90.49% and 90.65%, respectively. The weight of the 1-day-old chicks in the SAEW and BBS groups were 41.75 g and 42.16 g, respectively. Moreover, there were no significant differences between the hatchability and the 1-day-old chicks' weight between 2 groups (Figure 3). Furthermore, the embryonic mortality rate of SAEW groups in early, mid and late stage was 3.17%, 1.10%, and 4.69%, while that of BBS groups was 4.00%, 0.70%, and 4.26%, respectively. There were no significant differences between the SAEW and BBS groups in early, mid and late embryonic mortality rate (Figure 3).

The embryo weight and relative embryo weight gradually increases with increasing embryo age during incubation. The embryo weight on d 6th, 10th, 14th, and 18th of SAEW group was 0.418 g, 2.622 g, 10.763 g, and 26.307 g, while that of BBS group was 0.419 g, 2.647 g, 9.777 g and 24.962 g. The relative embryo weight on d 6th, 10th, 14th, and 18th of SAEW group was 0.689%, 4.544%, 18.659%, and 48.456%, while that of BBS group was 0.699%, 4.632%, 17.341%, and 46.362%. The embryo weight of the SAEW group was significantly ($P < 0.05$) higher than the BBS group on d 14th and 18th, while the embryo weight on d 6 and 10, and the relative embryo weight of the whole sampling process between

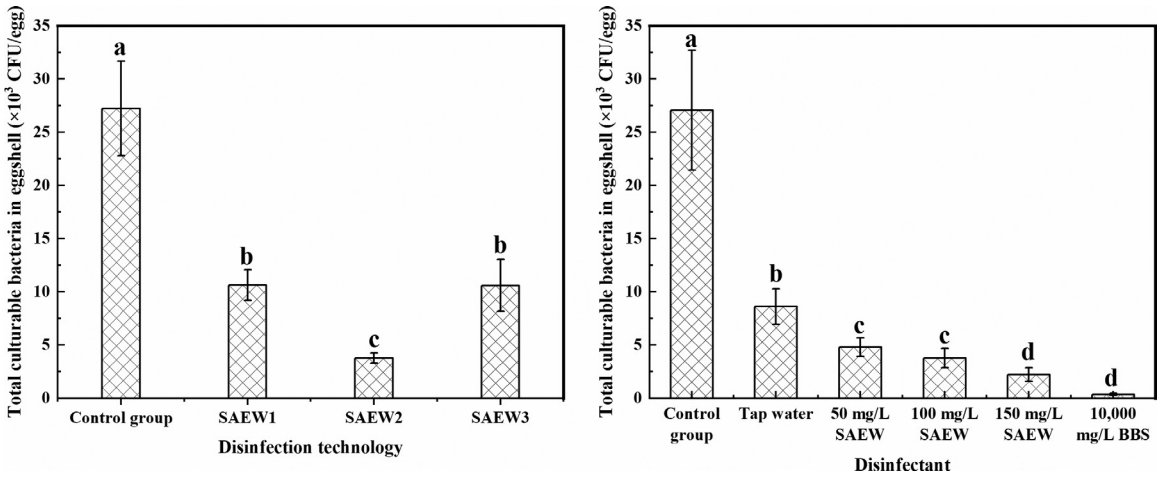

Figure 1. Effect of different spray time, closed time and disinfectants treatment on bactericidal effect.(A) Effect of different spray and closed time on bactericidal effect; (B) Effect of different disinfectant on bactericidal effect. Note: [a,b]Lowercase alphabets mean differences between mean values are significant at $P < 0.05$. Abbreviations: BBS, benzalkonium bromide solution; SAEW, slightly acid electrolyzed water; SAEW1, spray 60 s and closed for120 s; SAEW2, spray 90 s and closed for 90 s; SAEW3, spray 120 s and closed for 60 s.

the SAEW group and the BBS group showed no significant difference (Figure 3).

DISCUSSION

In this study, the combined effects on the microbial count, eggshell quality, and hatchability concomitantly of SAEW and BBS were elucidated. SAEW show its high disinfection efficacy and was proved to not affect the eggshell quality and hatching traits. The study on the effect of disinfection technique and disinfection parameter on eggshell culturable bacteria was first determined. In spray disinfection, spray time determined the disinfectant spray volumes, while closed time determined the contacting time for eggshell and disinfectants. SAEW2 which had larger spray time than SAEW1 and larger closed time than SAEW3 showed the highest sterilization rate in this experiment (Figure 1), indicating that greater spray volumes and longer contacting time are sufficient to achieve a positive disinfection effect. Similar results were reported by Ni et al. (2016), which showed that the bactericidal activity of SAEW increased with the increasing ACC and spraying duration. As for the disinfection efficacy, a microbial reduction of 23.48×10^3 CFU/egg was observed in the eggshells of hatching eggs after spraying with 100 mg/L SAEW for 180 s. By comparison, Wang et al. (2018) reported that a microbial reduction of almost 1.0 log CFU/cm^2 was observed in chicken carcasses after spraying with 30 mg/L SAEW for 15 s, which shown a higher sterilization rate per second. Different sterilization results between those studies may be due to the disinfectant concentration, droplet size, and disinfection objects (Zang et al., 2017). The sterilization effect of different disinfection techniques can provide a reference for the design of a novel disinfection channel to meet sprays at 90 s, closed for 90 s, and a disinfection duration for 180 s.

When comparing the sterilization effects of disinfectants, BBS and SAEW showed high sterilization rates. BBS is a kind of high efficiency disinfectant commonly used in hatchery, and the result in this experiment also shows its high sterilization rate. That may due to BBS is a type of quaternary ammonium salt disinfection, which took bactericidal action by inducing intracellular proteins and electrolyte leakage (Huang et al., 2019). By comparison, sterilization rate of SAEW increased with increasing ACC (Figure 1), and 150 mg/L SAEW showed the highest sterilization rate. This result shown that the sterilization rate of SAEW depends on the ACC, which was consistent with the observations of Zhang et al. (2021) who reported that there were

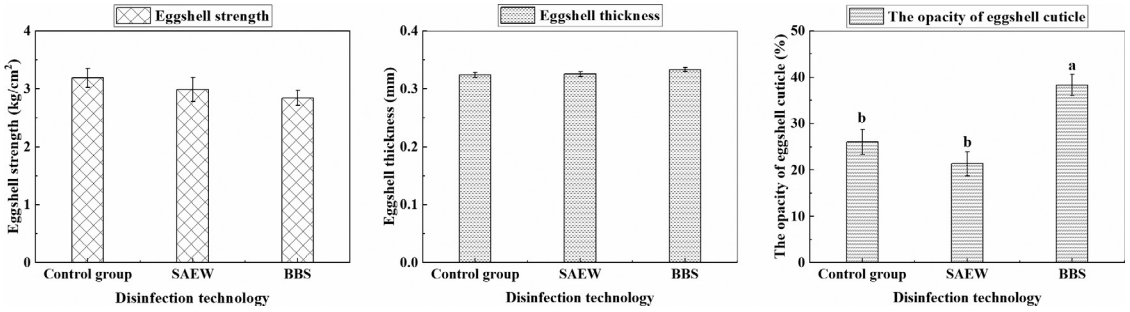

Figure 2. Effect of different disinfectant treatments on eggshell quality trait.(A) Eggshell strength; (B) Eggshell thickness; (C) The opacity of eggshell cuticle. Note: [a,b]Lowercase alphabets mean differences between mean values are significant at $P < 0.05$. Abbreviations: BBS, benzalkonium bromide solution; SAEW, slightly acid electrolyzed water.

significant ($P < 0.05$) differences among SAEW at different concentrations when the total bacteria of chickens was decreased. Zang et al. (2019) used the same disinfectant (SAEW) but found that an ACC of 26 mg/L SAEW spray disinfection for 3 and 4 min caused a complete inactivation of *S. Enteritidis* and *E. coli* on the eggshell. The disinfection technique used in this experiment was spray instead of immersion. The immersion method has more contact area than the spray method, which explained the difference in the sterilization effect between those experiments. Moreover, the sterilization rate of 50 mg/L and 100 mg/L of SAEW had no significant differences, while a higher concentration of SAEW (150 mg/L) had a significant difference ($P < 0.05$); this may be due to the existence of organic matter. Organic matter, such as feces and dust, on the surface of the eggshell may decrease the sterilization rate and make low concentration disinfection ineffective (Ni et al., 2015). However, Hao et al. (2013) reported that SAEW with an ACC of 250 mg/L, pH value of 6.19, and oxygen reduction potential of 974 mV, inactivated 100% of bacteria and fungi in solid materials (dusts, feces, feather, and feed). There may be organic matter, such as feces on the surface of eggs, which means that a higher concentration of SAEW should be used in the disinfection process. However, the preparation of too high a concentration of SAEW means higher cost, and this experiment proved that the disinfection effect of 150 mg/L SAEW was equivalent to that of 10,000 mg/L BBS, which means the ACC of 150 mg/L of SAEW is recommended.

The study on the eggshell quality trait after disinfection can reflect the destruction of the surface structure of the eggshell. The results of this experiment prove that the SAEW and BBS treatment did not affect the eggshell strength and eggshell thickness, which was consistent with the result of Melo et al. (2019), who found that hydrogen peroxide spraying, per acetic acid spraying, and water spraying did not affect eggshell quality. When the solution sprayed out, the mist covered the surface of the eggshell that had a smaller contact area and degree than immersion disinfection, and would not damage the strength and thickness of the eggshell. Moreover, the pH of SAEW is 5.0 to 6.5, and the slightly acidic solution was proven to be safe for use on metals and skin. However, the SAEW did not affect the opacity of eggshell cuticle while that of the BBS group was significantly higher (Figure 2). Zang et al. (2019) used the same disinfection but identified that SAEW may damage the egg cuticle, thus favoring trans-shell contamination with bacteria. This result may be due to immersed eggs in SAEW as found by Zang et al. (2019), while SAEW spraying was used in this experiment. Fasenko et al. (2009) also reported that there was no effect on cuticle structure by spray EO water. The reason why the opacity of cuticle in this group was higher than other groups may be due to more foam in the BBS solution, which forms a thin film on the eggshell and confuses the results. Moreover, the cuticle layer contains antibacterial proteins, which may be denatured by BBS.

As for the hatching traits, the weight of the embryo can reflect the development of the embryo. Higher embryo weights were found in the eggs treated with SAEW on d 14 and 18, respectively (Figure 3). However, the results could not draw the conclusion that SAEW treatment was better than BBS treatment in embryo development. High embryonic weight may be related to egg weight, and it is more accurate to use relative embryonic weight as a developmental evaluation standard. Former studies also explore disinfectant effect on

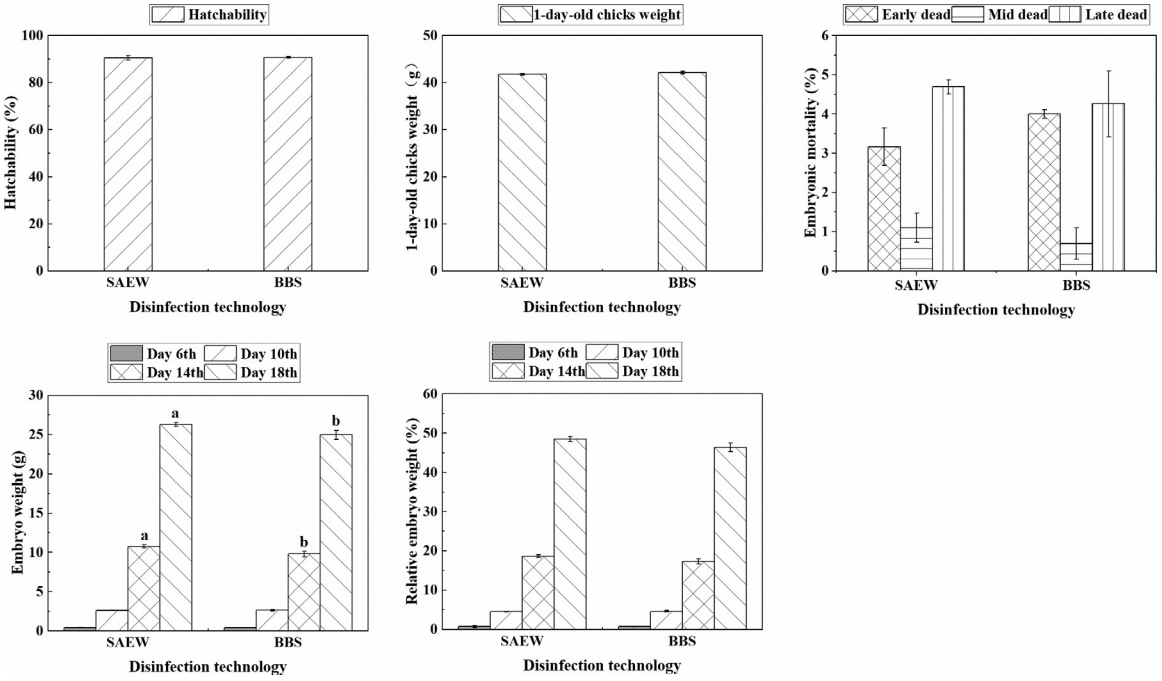

Figure 3. Effect of different disinfectant treatments on hatching traits.(A) Hatchability; (B) 1-day-old chicks weight; (C) Embryonic mortality; (D) Embryo weight; (E) Relative embryo weight. Note: [a,b]Lowercase alphabets mean differences between mean values are significant at $P < 0.05$. Abbreviations: BBS, benzalkonium bromide solution; SAEW, slightly acid electrolyzed water.

chick quality. Chick quality, as determined by visual assessment and broiler weight at the time of hatch, was also not affected by spray EO water (Fasenko et al., 2009). However, reduced hatching and significantly increased mortality occurred in the O_3 group (Wlazlo et al., 2020). Different disinfectants show different results because the chemical disinfectants may leave toxic residue and endanger embryos after disinfection. The 1-day-old chicks weight can reflect the chick quality of SAEW and BBS treatment group had no significance difference. The main content of SAEW is hypochlorous acid, which dissolves after disinfection (Cao et al., 2009). BBS has antibacterial and low toxic properties (Huang et al., 2019). Even if there was residue, it may have little effect on the development of the embryo. Embryonic mortality of early death is usually caused by microbial infection; there was no difference between SAEW and BBS treatment, proving their equivalent bactericidal effect. All in all, the characteristics and residual amount of the disinfectant are the key factors that affect the embryonic development during the egg incubation process. The use of low-toxic or no-residue disinfection methods can reduce the impact on the hatching traits.

We acknowledge several limitations in our study. First, although from the current concentration, 150 mg/L SAEW has the highest sterilization effect, it is unknown whether SAEW with a higher concentration will have a higher bactericidal effect or remain stable. Second, the morphology of eggshells was usually directly measured by electron microscopy (Melo et al., 2019), while we used a dyeing method reported by Chen et al. (2019). And the result of the opacity of cuticle in BBS treated groups were larger may be due to the limitations of the dyeing method. Therefore, it is necessary to further investigate if the eggshell cuticle could be affected by BBS by using electron microscopy.

This study also has strength. To our knowledge this is the first research to assess the combined effects on the microbial count, eggshell quality, and hatchability concomitantly of SAEW spray, which could contribute to the application of it. The guiding significance of the experimental results in this article for production is that it provides a novel disinfectant and disinfection technique in the novel disinfection channel for small breeders and those working in the field of incubation. Moreover, SAEW can be widely recommend for its advantages, including simple preparation, low cost, and no residue. The cost of generating SAEW includes electricity, water and electrolyte (sodium chloride and dilute hydrochloric acid) costs. The approximate cost of preparing 150 mg/L SAEW (CNY 0.46/USD 0.07 per 100 L) is the half of the cost of 10,000 mg/L BBS (CNY 0.88/USD 0.14 per 100 L).

CONCLUSIONS

In conclusion, SAEW can be an alternative disinfectant for the sterilization of hatching eggs compared to BBS. The recommended disinfection technique is to use SAEW as a disinfectant with 150 mg/L ACC, 0.5 mL/egg spray volume, and disinfection for 180 s in the novel disinfection channel. Although there were no differences in sterilization efficacy, eggshell quality, and hatching traits under the recommended disinfection procedures, SAEW is likely to be more popular because of its simple preparation, low cost, and lack of residue.

ACKNOWLEDGMENTS

The animal study proposal was approved by The Laboratory Animal Ethical Committee of China Agricultural University. The research team would like to acknowledge the financial support for this research project from National Natural Science Foundation of China (31802109) and open topic of Animal Disease Prevention and Food Safety Key Laboratory of Sichuan Province.

DISCLOSURES

There are no conflicts of interest to declare.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2021.101643.

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