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EFFECTS OF WASHING DIRTY EGGS OF GEESE WITH BORIC ACID AND VINEGAR ON HATCHABILITY AND MICROBIAL LOADS

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ABSTRACT

This study aimed to evaluate whether washing dirty goose eggs with tap water, vinegar, and boric acid solutions could improve hatching performance and reduce microbial load. A total of 3,360 eggs were used for hatching performance assessments, while 84 eggs were analyzed for microbiological parameters. The study consisted of seven treatment groups: physically clean eggs (PC), unwashed dirty eggs (NC), eggs washed in tap water (TW), eggs washed with a vinegar solution containing 2% acetic acid (S2), eggs washed with a vinegar solution containing 4% acetic acid (S4), eggs washed with a 2% boric acid solution (B2), and eggs washed with a 4% boric acid solution (B4). Hatchability of set eggs and hatch of fertile eggs improved significantly in the PC, S2, and B2 groups ($P<0.01$). Embryonic mortality was significantly low in the PC and B2 groups ($P<0.05$). Significant differences were observed between the groups for total mesophilic aerobic bacteria (TMAB) ($P<0.001$), total coliforms (TCN) ($P<0.01$), and *Escherichia coli* ($P<0.01$). The lowest TMAB counts were recorded in the S2 and B4 groups, while the lowest TCN counts were observed in the B4 group. Bacterial analysis of egg contents showed that all bacterial groups were below detectable limits. In conclusion, washing dirty goose eggs with tap water, vinegar, or boric acid solutions (2% and 4%) effectively reduced the microbial load on the eggshell for all examined microorganisms and significantly improved hatching parameters compared to dirty eggs.

Keywords: Egg, Microbial Load, Vinegar, Boric Acid, Washing.

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INTRODUCTION

Due to their high affinity for water, geese wet and soil their underpants more than other poultry. Since they often soil their bottoms and lay eggs on the ground, the rate of dirty eggs in geese is relatively high. Unsanitized dirty eggs are unsuitable for hatching due to their high bacterial load on the eggshell surface, contributing to reduced hatchability and increased mortality. (Karabulut *et al.,* 2017; Olsen *et al.,* 2017). Eggshells can be contaminated vertically and horizontally. Although ovary and oviduct infections are associated with vertical contamination, factors such as shell fractures and contaminated surfaces contribute to horizontal contamination. (Erol, 2007). Horizontal contamination in the egg starts when it separates from the oviduct and leaves the cloaca. The bacterial load of the egg changes rapidly depending on where it is laid, and eggs laid on the ground can be contaminated with thousands of bacteria. It can also contaminate other eggs by disrupting hatching hygiene. Poultry feces and other organic materials facilitate the attachment of pathogenic microorganisms such as *Salmonella spp.* and *E. coli* to the eggshell. To minimize pathogenic bacterial load on the eggshell, hatching eggs undergo various disinfection processes, which differ depending on the hatchery (Wales and Davies, 2020). Mechanical egg cleaners can be used to clean contaminated eggs, as well as manual washing processes. However, the high cost of mechanical egg cleaners limits the use of these devices in hatcheries. In a study conducted to determine the appropriate cleaning and disinfection methods, hatchability rates were higher

in dirty eggs wiped with a wet cloth and sanded with an abrasive pad than in dirty eggs (Yoho *et al.,* 2008). In contrast, Van den Brand *et al.* (2016) have suggested that washing eggs may damage the cuticle of the eggshell, which could increase the risk of bacterial contamination and negatively impact hatchability.

Unlike other poultry species, geese eggs are wetted during incubation. This allows pre-hatching washing to be considered as an alternative cleaning and disinfection method for goose eggs. However, egg washing must be performed quickly, as it can adversely affect hatchability by causing the shell membranes to become rubbery, which makes pipping more difficult (Salamon, 2020). One of the factors contributing to hatching is the application of appropriate disinfection programs with effective disinfectants. To reduce the adverse effects of bacterial contamination on hatching performance, eggs must be disinfected before being placed in the incubator. Chemicals including formaldehyde, oxidized water, quaternary ammonium compounds, sodium hydroxide, phenols, antibiotic luminol, hydrogen peroxide, thymene, and polyhexamethylenebiguanide hydrochloride are used during disinfection. However, many chemicals are not recommended for use in current applications due to their harmful effects on living organisms. Research on identifying alternative natural products has increased in recent years to control microbial contamination and reduce or eliminate reliance on synthetic pesticides (Baylan *et al.,* 2015). The primary benefits of using natural disinfectants and antimicrobials include their biodegradability, broad-spectrum efficacy, high biosecurity levels, and absence of residue. (Hrnčár *et al.,* 2021). For this purpose, substances such as grain alcohol, propolis solutions, oregano oil solutions, ethyl alcohol, peracetic acid, clove oil solution, and ozone have been used in the form of spraying or fumigation (ShaheIn and Sedeek, 2014; Melo *et al.,* 2019; Wlazlo *et al.,* 2020; Oliveira *et al.,* 2020).

Vinegar, used as a natural preservative in foods, is obtained from the oxidation of fermentable sugars into ethanol by yeasts and then oxidation of this ethanol by acetic acid bacteria. Organic acids in vinegar (e.g., tartaric acid, malic acid, and other nonvolatile organic acids), mainly acetic acid, penetrate the cell membranes of microorganisms and cause bacterial cell death. Parameters such as bacterial strains, temperature, pH, acid concentration, and ionic resistance affect the antimicrobial activity of organic acids in vinegar (Chen *et al.,* 2016). The study conducted by Yagnik *et al.* (2018) concluded that commercial apple cider vinegar has an antimicrobial effect on *E. coli, S. aureus*, and *C. albicans*. In the study by He *et al.* (2020), quail eggs were washed with solutions containing 2% and 4% vinegar, and it was found that there was higher hatchability in these groups compared to the control group (unwashed eggs); however, there was no significant statistical effect. Boron is also used in medicine as an antibacterial and disinfectant in toothpaste, perfumes, shampoos, and lens solutions (Yakıncı and Kök, 2016). Raimondi *et al.* (2006) reported that different derivatives of boron have bactericidal and fungicidal effects on *S. aureus* ATCC 25923, E. coli ATCC 25922, and *C. albicans* ATCC 10231 under in vitro conditions. Eroglu *et al.* (2024) conducted a study to determine whether the disinfection of soiled goose eggs by spraying with tap water, acetic acid, and boric acid. While no statistically significant effects were observed, the boric acid group demonstrated the highest hatchability.

This study aimed to explore the potential benefits of washing contaminated goose eggs with tap water, vinegar, and boric acid solutions (2% and 4%) to enhance hatching performance and reduce microbial contamination.

MATERIALS AND METHODS

Fırat University's Animals Experiments Local Ethics Committee (FÜHADYEK) (protocol no: 2021/21) had approved the study.

Animals and Experimental Design: The study was conducted on a private farm in Elazığ, Türkiye. Eggs were collected from two- and three-year-old breeders in a breeding flock of 1,000 geese, with a male-to-female ratio 1:4. All breeding geese were housed in closed sheds with straw mats, and the stocking density was one bird/m^2. The geese were fed a diet containing 16% crude protein and an energy content of 3000 kcal/kg. Incubation and microbiological analysis consisted of seven treatment groups with eight independent replicates each. The incubators (Yunfeng YFDF 17280, China) had trays with a capacity of 60 eggs, and each treatment group of 120 eggs was placed on two trays. The incubation groups in the study were arranged as follows: physically clean eggs (unwashed) as the positive control group (PC); unwashed dirty eggs as the negative control group (NC); dirty eggs washed in tap water as tap water group (TW); dirty eggs washed with vinegar solution that includes 2% acetic acid as S2 Group, dirty eggs washed with vinegar solution that includes 4% acetic acid as S4 Group; dirty eggs washed with 2% boric acid solution as B2 Group; dirty eggs washed with 4% boric acid solution as B4 Group. The vinegar solutions were prepared at the doses specified by He *et al.* (2020), while boric acid doses have been determined according to previous studies (Yılmaz, 2012; Eroglu *et al*., 2024).

According to the classification of Buhr *et al.* (1994), eggs with little or no blood, feces, yolk, albumin, or sawdust that cannot be easily removed by finger rubbing are classified as dirty eggs. In contrast, eggs with none of these characteristics are classified as clean eggs.

All solutions were freshly prepared using clean, potable tap water. The microbiological activity of the clean tap water was done by the Turkish Standards Institute for drinking water quality (TS 266 - Water intended for human consumption), and it does not contain coliform group bacteria and *Escherichia coli*. A commercially white vinegar product with an acidity of 4.5% acetic acid (Kühne, İzmir, Turkey) was used to prepare the vinegar solutions. Vinegar was diluted obtain to vinegar solutions, including 2% and 4% acetic acid. For vinegar solution (S2), 444 ml of vinegar was diluted with tap water to make a total volume of one liter. For vinegar solution (S4), 888 ml of vinegar was diluted with tap water to make a total volume of one liter. For the boric acid groups, 99% pure boric acid was used to prepare 2% and 4% solutions. For this purpose, 20 g/L (B2) and 40 g/L (B4) solutions were prepared. Each treatment group was immersed in the relevant solution bucket and then rubbed with a sponge. After the washing process, eggs were immediately dried with a clean paper towel. The washing process was carried out at room temperature, with the water temperature between 38°C and 40°C. The washing solutions were replaced after every 30 eggs to minimize contamination. After washing, the eggs were immediately dried using clean paper towels.

Hatching Characteristics: Before incubation, all eggs used in the study were stored for three days in a climate-controlled room (10-15°C and 70-75% humidity). During incubation, the temperature was 37.7°C with 55-60% humidity, and eggs were rotated every two hours. On the seventh day, infertile and early embryonic deaths were removed. From the 8th day, eggs were sprayed with 38°C water twice daily from the 20th day. On the 28th day, eggs were transferred to hatching baskets, maintaining 37.3°C and 75-80% humidity (Eroglu and Erisir, 2019). Group placement was designed to secure each group in the top two rows (60 eggs x 2), preventing group interference. Uniform environmental conditions for all groups were maintained by incubating all eggs in a single machine. Based on the data obtained from the study, the following formula (Onk and Kirmizibayrak, 2019) were used to calculate the values related to the incubation characteristics.

Hatchability %= (number of live goslings / total number of eggs set) x 100

Hatch of fertile % = (number of live goslings/number of fertile eggs) x 100

Embryonic mortality rate (%) = (number of embryos dead between day one and day 29/number of fertile eggs) x 100

Death-in-shell (%) = (number of chicks that died between day 30 and day 31/number of fertile eggs) x 100

Chicks whose growth and development performance are not suitable for commercial breeding due to their morphological and physiological characteristics are classified as non-commercial chicks (Şeremet, 2012).

Noncommercial chick rate = (number of noncommercial chicks hatched/total number of eggs) x 100

Viability: Since hatching-related mortality typically occurs in the first week (Yerpes *et al.,* 2020), groups were observed in separate compartments in a closed shelter with straw-bed compartments. The goslings were fed a diet containing 20% crude protein and 2900 ME kcal/kg energy, as detailed in Table 1, and had *ad libitum* access to water with nipple drinkers. The viability rates of each group were calculated at the end of the first week.

Microbiological Analysis: For the microbiological assessment, 84 eggs were sampled, with three samples from each of the seven groups and across four independent replicates. Egg collection followed aseptic conditions, both before and after cleaning. The eggs were transported to the laboratory without refrigeration and promptly analyzed. Eggs that were broken, damaged, or compromised were excluded from the study.

To sample the eggshells, sterile gauze soaked in 10 ml of 0.1% peptone water (PW) at room temperature was employed to cover the entire surface of each egg. Subsequently, the gauze was placed in a sterile sample bag, and 15 ml of 0.1% PW was added, reaching a total volume of 25 ml. This solution, obtained after one minute of homogenization in a stomacher, was utilized for the microbiological analysis of the eggshells.

The sampled eggs underwent a 10-15 second immersion in 70% ethanol for egg content sampling. The eggs were then left at room temperature for 5 minutes to allow the residual alcohol on the shells to evaporate. The aseptically broken egg contents (yolk and albumen) were homogenized in a stomacher for 120 seconds. Following these procedures, serial decimal dilutions were prepared from the homogenates of eggshells and egg contents, adhering to microbiological inoculation guidelines (Food and Drug Administration, 2021).

Determination of Total Mesophilic Aerobic Bacteria Count (TMAB): Plate count agar (PCA) medium was employed, and plates were incubated at 35°C for 24-48 hours. The colonies that developed after treatment were enumerated following the methodology outlined by Harrigan (1998).

Determination of Enterobacteriaceae Count: Violet Red Bile Glucose (VRBG) medium was incubated at 35°C for 24-48 hours, followed by colony enumeration. Following incubation, five presumptive Enterobacteriaceae colonies were biochemically confirmed via the oxidase test (ISO-21528-2, 2004).

Determination of Total Coliform Group Microorganisms Count: Violet Red Bile Lactose Agar (VRBL) was utilized, and petri dishes were incubated at

37±1°C for 24 hours. After the incubation period, dark red-colored specific colonies were assessed based on the ISO-4832 standard (2006).

Determination of Escherichia Coli Count: Harlequin Tryptone Bile X Glucuronide Agar (TBX) medium was used, with plates incubated at 30°C for four hours, followed by incubation at 44°C for 18 hours. Green-colored colonies were evaluated after incubation according to ISO-16649-2(2001).

Statistical Analyses: Firstly, the homogeneity of variances (Levene's test) and the normality of data distribution (Shapiro-Wilk test) were carried out to check the applicability of parametric tests. Parameters (Hatching characteristics, TMAB, and Enterobacteriaceae) that met the parametric test assumptions were analyzed using one-way analysis of variance (ANOVA), and group differences were further examined using Tukey's post hoc test. Conversely, for parameters (Coliform group and Escherichia coli) that did not satisfy the assumptions of parametric tests, non-parametric methods were used. The Kruskal-Wallis H test was used for overall group comparisons, and the Mann-Whitney U test was employed for pairwise analyses. All statistical analyses were performed using SPSS version 21, with the significance level at $P \leq 0.05$.

RESULTS

Hatching Characteristics: Table 2 shows the findings of hatching characteristics. The highest values for hatchablity and hatch of fertile were found in the PC, S2, and B2 groups, while the lowest values were found in the NK group (P<0.01). Similar values were found in the TW, S4, and B4 groups. Regarding embryonal mortality rates, the lowest values were recorded in the PC and B2 groups, while the highest values were found in the NC group. Similar values were observed in the TW, S2, S4, B2, and B4 groups.

Viability: Figure 1 shows the data, including viability. The highest survival rate was observed in the B4 group, with 98.41%, while the lowest survival rate was observed in the NC group, with 94.13%. The survival rates in the PC, TW, S2, S4, and B2 groups were similar to the other groups.

Microbial Analyses: Table 3 presents the bacterial counts and standard error of the eggshells. The differences between the groups were statistically significant for TMAB (P<0.001), TCN (P<0.01), and *E. coli* (P<0.01). The highest counts of TMAB, TCN, and *E. coli* were found in the NC group with 7.49, 2.66, and 1.62 $\log_{10}$ cfu/shell, respectively. The lowest values were observed in the S2 and B4 groups for TMAB and in the B4 group for total coliform group microorganisms. However, there was no significant difference among the S2, S4, B2, and B4 groups in TMAB counts (P>0.05). For the number of *Enterobacteriaceae*, the group differences were statistically insignificant (P>0.05). The coliform group bacteria counts showed that washing with tap water and the S2, S4, and B2 groups had no significant effect (P>0.05), while only the 4% boric acid (B4) treatment showed an effect (P<0.01). *E. coli* counts remained below the detectable limit in the S2, S4, and B4 groups, while the highest value was detected in the NC group with 1.62±0.21 $\log_{10}$ cfu/shell.

Table 4 shows the bacterial counts of the egg contents. As a result of the bacterial analyses of egg contents, all bacterial groups were found below the detection limit. Therefore, all bacterial groups analyzed in ml of egg content samples were less than 1 for all bacterial groups. There was no difference between the groups for any of the bacterial species analyzed in the study (P>0.05).

Table 1. Composition and calculated nutrient values of rations used to feed goslings

Ingredients	%	Nutrients	%
Corn	59.07	Dry matter	89.40
Wheataaw	1.00	Crude protein	20.00
Wheat bran	3.00	Crude cellulose	3.91
Corn bran	1.50	Crude oil	2.74
Corn gluten, 43% HP	4.75	Crude ash	5.16
Soya meal, 44% HP	27.35	Calcium	0.65
Vegetable oil	0.75	Phosphorus	0.30
Dicalcium phosphate	0.93	Sodium	0.18
Limestone	0.84	Lysine	1.00
L-Lysine hydrochloride	0.06	ME, kcal/kg	2900
DL-methionine	0.10		
Salt	0.30		
Sodium bicarbonate	0.10		
Vitamin-mineral mix*	0.25		
Total	**100**	**Total**	**100**

*Vitamin A: 10000 IU, Vitamin D3: 4000 IU, Iron: 30 mg, Iodine: 1.5 mg, Cobalt: 0.5 mg, Copper: 5 mg, Manganese: 80 mg, Zinc: 80 mg, Selenium: 0.3 mg

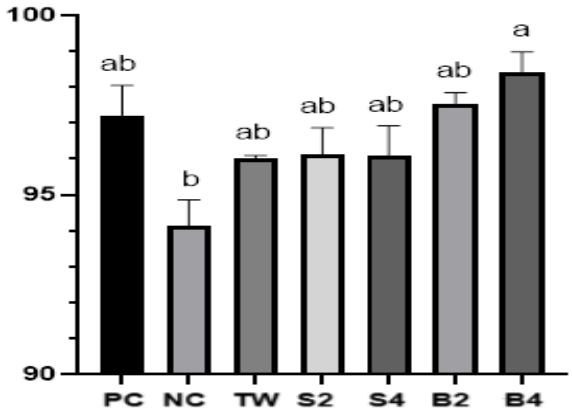

Figure 1. Viability rates of chicks during one week

[a, b]: Values not sharing a common superscript are significantly different at P≤0.05.

Table 2. Hatching characteristics of the groups.

Groups	Number of eggs (N)	Hatchability (%)	Hatch of fertile eggs (%)	Embryonic mortality (%)	Dead-in-shell (%)	Non-commercial chick rate (%)
PC (Positive control)	480	77.38±2.07[a]	82.04±1.62[a]	11.30±1.57[b]	2.57±0.73	5.15±0.46
NC (Negative control)	480	61.70±1.97[b]	68.58±1.64[b]	18.25±1.12[a]	4.76±1.19	2.97±0.83
TW (Tap water)	480	71.62±3.29[ab]	76.96±2.85[ab]	12.50±1.09[ab]	5.75±1.27	2.77±0.46
S2 (2% Vinegar)	480	73.80±2.30[a]	80.26±1.85[a]	12.89±1.70[ab]	2.38±0.59	3.17±0.39
S4 (4% Vinegar)	480	70.83±2.98[ab]	77.64±3.16[ab]	12.89±1.78[ab]	4.36±1.49	2.38±0.67
B2 (2% Boric acid)	480	76.19±3.17[a]	81.16±3.00[a]	11.90±1.67[b]	3.37±0.96	2.38±0.99
B4 (4% Boric acid)	480	72.61±2.35[ab]	76.84±2.12[ab]	14.48±1.42[ab]	4.96±1.81	5.15±0.51
***P* Value**		0.004	0.006	0.041	0.118	0.205

Mean ± standard error, [a, b]: Values within a column not sharing a common superscript are significantly different at P≤0.05.

Table 3. Bacterial counts of eggshells (log_{10} cfu/shell) after washing.

Groups	Number of eggs (N)	Total mesophilic aerobic bacteria (TMAB)	Enterobacteriaceae	Total coliforms microorganisms (TCN)	Escherichia coli
PC (Positive control)	12	5.40±0.16[b]	2.02±0.23	1.76±0.23[bc]	1.17±0.03[b]
NC (Negative control)	12	7.49±0.14[a]	2.76±0.27	2.66±0.28[a]	1.62±0.21[a]
TW (Tap water)	12	5.05±0.15[bc]	2.43±0.24	1.93±0.27[bc]	1.12±0.02[b]
S2 (2% Vinegar)	12	4.24±0.18[d]	2.03±0.19	1.52±0.15[bc]	1.10±0.00[b]
S4 (4% Vinegar)	12	4.43±0.11[cd]	2.00±0.21	1.44±0.16[bc]	1.10±0.00[b]
B2 (2% Boric acid)	12	4.54±0.22[cd]	2.27±0.32	1.97±0.28[ab]	1.12±0.02[b]
B4 (4% Boric acid)	12	4.25±0.20[d]	1.72±0.19	1.30±0.10[c]	1.10±0.00[b]
***P* Value**		0.000	0.084	0.002	0.002

Mean ± standard error, [a, b, c, d:] Values within a column not sharing a common superscript are significantly different at P≤0.05.

Table 4. Bacterial counts of egg contents (log_{10} cfu/ml) after washing.

Groups	Number of eggs (N)	Total mesophilic aerobic bacteria (TMAB)	Enterobacteriaceae	Total coliforms microorganisms (TCN)	Escherichia coli
PC (Positive control)	12	<1	<1	<1	<1
NC (Negative control)	12	<1	<1	<1	<1
TW (Tap water)	12	<1	<1	<1	<1
S2 (2% Vinegar)	12	<1	<1	<1	<1
S4 (4% Vinegar)	12	<1	<1	<1	<1
B2 (2% Boric acid)	12	<1	<1	<1	<1
B4 (4% Boric acid)	12	<1	<1	<1	<1
***P* Value**		1.000	1.000	1.000	1.000

DISCUSSION

Hatching Characteristics: Embryonic development is a complex physiological process characterized by intricate interactions between the internal and external structures of the egg, which are critical for the proper growth and maturation of the embryo. However, the eggshell, the interface between the embryo and the external environment, is susceptible to contamination by various microorganisms, including pathogenic bacteria. Such microbiological contamination could compromise the embryo's integrity and adversely affect its development. Effective sanitation practices, including the disinfection of hatching eggs, are essential for reducing bacterial contamination within hatcheries and associated poultry facilities (Oliveira *et al*., 2024). Reflecting this, the highest hatchability rates in this study were observed in the positive control, S2, and B2 groups, likely attributable to their lower bacterial loads. Notably, the S2 and B2 groups significantly reduced bacterial load compared to the unwashed negative control group. These reductions are attributed to the combined antibacterial properties of

vinegar and boric acid, as well as the mechanical effects of washing, which facilitate the removal of microorganisms from the eggshells (Raimondi *et al.,* 2006; Leleu *et al.,* 2011; Yagnik *et al.,* 2018). The acetic acid present in vinegar and boric acid has been shown to induce alterations in microbial cell membrane permeability and lead to microbial death (Budak *et al.,* 2014; Sengun *et al.,* 2019; Liu *et al.,* 2021). Reducing the microbial load on the eggshell effectively decreases the risk of trans-shell contamination. This procedure is important for non-caged egg production systems, where hens are more frequently exposed to environmental microorganisms, including those in litter and manure (Messens *et al.* 2005; De Reu *et al.* 2006). It is also significant for poultry species such as geese, ducks, and turkeys, which face similar challenges in maintaining eggshell hygiene.

Similar to this study, He *et al.* (2020) found that washing quail eggs with acetic acid and vinegar significantly increased hatchability, while Fouad *et al*. (2019) reported that using vinegar as a disinfectant in chicken eggs improved both hatchability and embryonic development. Eroğlu *et al.* (2024) found that spraying goose eggs with 3% acetic acid and boric acid solutions effectively reduced microbial contamination without adversely impacting hatch results. Tan *et al.* (2022) also recommended washing duck eggs to enhance microbial safety and preserve quality. The findings of this study differ from those of Van den Branden *et al*. (2016), who reported that incubation of floor eggs or washed floor eggs resulted in decreased hatchability due to contamination compared to clean nest eggs. Similarly, Gole *et al.* (2014) observed that washing dirty eggs with warm water may reduce hatchability by compromising the ultrastructural features of the eggshell, such as cap quality and alignment, causing erosion and confluence, eliminating type B bodies, and removing the cuticle cover.

An additional factor contributing to the improved performance of the S2 and B2 groups among the washed groups may be the thinning of the cuticle layer, the outermost layer of the eggshell, during the washing process. The cuticle layer, which is thicker in certain poultry species such as geese, ducks, and turkeys, plays a crucial role in regulating the permeability of the eggshell to gases and heat. Its partial removal or thinning may improve the eggshell's permeability, promoting enhanced embryo development (He *et al.,* 2020). However, excessive thinning or complete removal of the cuticle layer is significant as it may increase water evaporation from the egg, leading to higher albumen pH and a reduction in the activity of antimicrobial enzymes like lysozyme and ovotransferrin. These enzymes are essential for providing antibacterial protection within the egg (Deeming, 1987; Legros *et al.,* 2021). The comparatively lower performance observed in the S4 and B4 groups might be attributed to this effect. The present study's findings are in agreement with those of He *et al.* (2020), who found that higher concentrations of acids overreact with the eggshell, significantly reducing hatchability.

Embryonic mortality was highest in the negative control group and lowest in the clean, positive control group, with these findings mirroring the bacterial load on the eggs. The high embryonic mortality observed in the unwashed group is attributed to bacterial penetration through the eggshell's pores, followed by bacterial proliferation within the egg, which ultimately causes the death of the developing embryo. Furthermore, since the eggs in this group were not washed, the cuticle layer remained intact. This may have resulted in a reduced rate of water loss, causing it to fall below the optimal range required for embryo growth and survival during the final stages of incubation. Insufficient water loss limits embryo development, leading to an inadequate air cell and consequently impairing embryonic lung ventilation (He *et al.,* 2020).

Although differences in dead-in-shell rates and noncommercial chick rates were not statistically significant, ensuring high-quality chicks remains crucial for maximizing production efficiency and minimizing losses (Durmus and Kutlu, 2019). In this study, the highest rate of noncommercial chicks was in the PC and B4 groups at 5.15%, while the lowest rate was in the A4 and B2 groups at 2.38%.

Viability: The lowest survival rate was found in the NC group at 94.13%. The main reason for this may be the bacterial load carried by the eggs in this group, as shown in Table 3. Horizontal contamination of eggs starts with separating the egg from the oviduct. Depending on the laying site, the bacterial load of the egg changes rapidly. If hatching eggs are not disinfected before hatching, the chicks can be contaminated with various bacteria. Due to this contamination, the physiological and immune systems of chicks are not fully functional after hatching, and the highest mortality rates are generally observed during this period (Yerpes *et al.,* 2020).

Microbiological Analyses: Reducing microbial contamination on eggshells can help prevent bacterial infections in embryos and newly hatched chicks (Fasenko *et al.,* 2009). Consistent with the findings of this study, Wang *et al.* (2019) explored the impact of specially designed goose nest boxes on egg quality and found contamination levels of 4.95±0.98 log cfu/g and 6.70±0.43 $\log_{10}$ cfu/g in goose eggshells. Similar studies have reported contamination levels ranging from 3.0 $\log_{10}$ cfu/shell to 7.0 $\log_{10}$ cfu/shell for chicken eggs that were not subjected to disinfection processes (Fasenko *et al.,* 2009; Clímaco *et al.,* 2018; Sözcü and İpek, 2020). He *et al.* (2020) observed 4.30±0.47 $\log_{10}$ cfu/egg contamination levels in quail eggs. In the current study,

washing with tap water (TW group) reduced the total microbial aerobic bacteria (TMABs) by 2.44 $\log_{10}$ cfu/shell compared to the unwashed negative control (NC) group. In addition, vinegar and boric acid treatments significantly decreased TMABs on eggshells ($P<0.001$). In many countries, antibacterial agents are used to hatching eggs to prevent embryo mortality caused by pathogenic microorganisms. Various disinfectants such as formaldehyde, ozone, hydrogen peroxide, Virkon S, essential oils, propolis, and garlic have been tested to minimize bacterial penetration and improve hatchability in chicken eggs (Shahein *et al.,* 2014; Oliveira *et al.,* 2022).

Disinfectants must be safe for embryos, non-harmful to humans, affordable, readily available, and capable of delivering maximum efficacy with minimal concentrations and contact time (Oliveira *et al.,* 2022). Along with their antimicrobial properties, enhancing the viability and hatchability of the embryos is the primary goal. This study, compared to the manure-contaminated NC group, found that 4% boric acid (B4) significantly reduced the microbial load of coliforms and Enterobacteriaceae (Table 3). Additionally, the impact of vinegar and boric acid treatments on E. coli in the dirty eggs (NK) ($P<0.01$) aligns with the improvement in other hatching parameters, particularly embryo viability.

All bacteria detected in the egg contents during this study were below the detection threshold. This result can be attributed to the chemical defense mechanisms present in the albumen and yolk. Microbial agents that penetrate the eggshell, which acts as a physical barrier, encounter these chemical defenses. The presence of lysozyme, ovalbumin, ovotransferrin, and haptoglobin in goose egg albumen helps explain why the microbial load in the egg content remained below detectable levels (Chen *et al.,* 2019). Lysozyme, ovalbumin, and ovotransferrin are well-established antimicrobial agents (Sun *et al.,* 2017).

Additionally, trimethylamine (TMA), found in low concentrations in egg yolk, may also possess antibacterial properties (Shi *et al.,* 2022). The use of fresh hatching eggs, not older than three days, also indicates that the antibacterial activity of egg albumin was ineffective in preventing bacterial invasion and adaptation to the egg contents, regardless of whether tap water, boric acid, or vinegar was applied (Dang *et al.,* 2022). The use of fresh eggs from healthy geese, stored for a short period or had no transovarial transmission of bacteria, supports this conclusion (Sun *et al.,* 2017; Dang *et al.,* 2022).

Conclusion: The study's findings demonstrated that washing dirty goose eggs with tap water, vinegar, and boric acid solutions (2% and 4%) effectively reduced the microbial load on the eggshell, particularly for TMAB and total coliform group microorganisms. Furthermore, washing dirty goose eggs with these solutions had a positive impact on all microorganisms examined in terms of reducing the microbial load in the egg. Higher hatchability rates were achieved in all treatments than in dirty eggs, which is a natural reflection of these antimicrobial effects. Vinegar and boric acid solutions are cost-effective, easy to use, and do not adversely affect viability, making them safe for use by all breeders.

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