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Determining Effects of Use of Various Disinfecting Materials on Hatching Results and Total Bacterial Count

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ABSTRACT

The study was conducted to identify the effects of the use of various disinfecting materials for disinfecting incubating eggs on the hatching results and total bacterial count. To this end, the disinfectants coded as A, B, C and D depending on their active ingredients. Disinfectants were compared according to their effects on the hatching results and total bacterial counts. No difference was found among groups with regard to hatchability, hatchability of fertile eggs, early, middle and late embryonic mortality, number of deformed chicks ($p>0.05$). As for the total bacterial count after the disinfection application, no significant difference was found among groups ($p>0.05$).

Key words: Hatching eggs, disinfectant, hatchability of fertile eggs, total bacterial count

INTRODUCTION

Bacteria and fungi cause early embryonic mortality at hatcheries. It was reported that in the hatcheries that do not comply with hygienic requirements, bacteria such as *E. coli*, *Staphylococcus* sp. and *Pseudomonas* sp. cause egg contamination which in turn lead to omphalitis (Yildiz, 2006).

Quality of fertile eggs is one of the main factors that affect the hatching results. Among the egg quality criteria are egg size, egg shape and abnormalities, air cell and eggshell quality. Structure, thickness and cleanliness of the shell are checked to ensure egg shell quality. Dirty eggs are a common source of bacteria and therefore, it is not advised to use them for hatching; however, after they are cleaned properly, they may be placed in the incubator. It is advised that the washing water to be used for disinfecting the dirty eggs for incubation should not be higher than that of eggs and it is reported that chlorine-based disinfectants are commonly used to this purpose (Ernst, 2010).

Given the fact that even a seemingly clean egg may contain up to 100,000 microorganisms (Ledoux, 2004), utmost care must be taken for cleaning and disinfecting hatcheries and eggs.

Fumigation, spraying, washing and applying ultraviolet ray are used in the disinfection of hatcheries and hatching eggs (Ernst, 2010). The 80-85% microorganisms can be eliminated by washing the hatcheries while the remaining microorganisms should be cleaned with disinfectants. As per the HACCP standards, disinfectants are supposed to reduce microbes by 99.99% (Ledoux, 2004).

Due to its advantages such as cheap price and ease of application compared to other disinfectants, formaldehyde is commonly used in many areas ranging from industrial applications to household materials and from the production of dental coatings to examination of cadavers in laboratories. Despite its widespread use, formaldehyde is considerably harmful to human health. Its harmful effect stems from its strong tendency toward bonding with proteins, nucleic acids and

unsaturated fatty acids which in turn causes denaturation of proteins, resulting in cytotoxicity, inflammatory reaction, necrosis, allergy and mutagenic effect (Unsaldi and Ciftci, 2010).

Disinfectants to be used should not have adverse effects on human and animal health as well as on environment and equipment. Several disinfectants have been produced as alternative to formaldehyde-based ones.

Yildiz (2006) studied the effects of formaldehyde and commercial products fumispore S and CID 2000 on hatching results. It was reported that the three disinfectants showed no difference in terms of their effects on the factors studied, but the disinfectant named CID 2000 was superior to the other two in terms of total bacterial count on eggs on the 18th day of incubation.

In a study conducted using 1.5 and 3% doses of the disinfectant containing quaternary ammonium, it was found that 1.3% dose killed 99.99% of microorganisms and the hatchability results of the eggs which were disinfected using both doses were higher by 6% than those of the control group (Brake and Shelton, 1990).

Copur *et al.* (2010) used oregano (*Origanum onites*) essential oil and formaldehyde to disinfect eggs. Essential oil fumigation lowered middle embryonic mortality and discarded chick rate but increased early and late embryonic mortalities compared to formaldehyde treatment. No difference was found between groups in terms of hatchability of fertile eggs.

Soliman *et al.* (2009) used five disinfectants TH4® (combination of quaternary ammonium compounds and gluteraldhyde), microzal® (combination of quaternary ammonium compounds and gluteraldhyde), incospect IC 22XA (combination of quaternary ammonium compounds, gluteraldhyde and formalin), povidone iodine® (iodophor) and formalin® (commercial formaldehyde 37%). They were tested in a laboratory trials against four bacterial isolates (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella oxytoca* and *Pseudomonas aeruginosa*) at concentration of ($\sim 10^5$) isolated during epidemiological surveillance. The results revealed that quaternary ammonium-gluteraldhyde combination (TH4®, Microzal® and Incospect IC 22XA) although they are not proven to be environmentally safe; they are the most powerful disinfectants because of the synergistic action of the quaternary ammonium and gluteraldhyde bases.

This study was designed and conducted to identify the effects of various commercially available disinfectants on the hatching results and total bacterial count.

MATERIALS AND METHODS

In the study, 3,000 hatching eggs obtained from commercially available ATAK-S layers and the disinfectants codes and active ingredients of which are given in Table 1 were used.

The hatching results were determined at the Poultry Research Station's hatchery and total bacterial counts were made at the Microbiology Department of the Veterinary Faculty of Ankara University. The hatching eggs were placed into 20 development machine trays, each with

Table 1: Active ingredients and codes of the disinfectants used in the study

Code	Active ingredients
A	37% formaldehyde (CH_2O)+potassium permanganate ($KMnO_2$)
B	Stabilized hydrogen peroxide: 210 g L^{-1}, peracetic acid: 55 g L^{-1}, acetic acid: 110 g L^{-1}
C	20% orthophenylphenol
D	Sodium hypochlorite: 0.035%, chlorine dioxide: 0.0011%, sodium chlorite: 0.0015%, ozone: 0.00002%, water: 99.96238%

a capacity of 150 eggs. The trays were divided into four groups and each group is disinfected using a different disinfectant. The study was conducted in five runs according to the randomized block experimental design. Each tray was considered as a run. A total of 3,000 hatching eggs were used 150×5 = 750 eggs total for each group and 750×4 = 3000. The eggs were disinfected using a different disinfectant for each group and in different rooms to avoid interaction as detailed below.

Disinfectant A: Twenty grams potassium permanganate ($KMnO_2$)+37% formaldehyde (CH_2O) for 1x dose and 2.8 m^3 volume were used for 20 min with a dose of 3x at a room with a temperature of 24°C and a relative humidity of 75%.

Disinfectant B: It was diluted by a rate of 1/5 and sprayed on eggs at a room with a temperature of 24°C and a relative humidity of 75%. Eggs were kept at the room for drying after the treatment.

Disinfectant C: Its dose was set as 16 g for a volume of 18 m^3 and it was at a room with a temperature of 24°C and a relative humidity of 75%. The eggs were kept in the room for a period of 2 h.

Disinfectant D: It was diluted by a rate of 1/1 and sprayed on eggs at a room with a temperature of 24°C and a relative humidity of 75%. Eggs were kept at the room for 1 h for drying after the treatment.

The eggs were removed from the disinfection rooms and kept at the pre-incubation rooms having a temperature of 26°C and a relative humidity of 65% for a period of 8 h before there were transferred to the development machines at a temperature of 37.8°C and a relative humidity 50%. After keeping the eggs here for 18 days and their fertility was checked and they were transferred to the hatching machines with a temperature of 36.5-37°C and a relative humidity of 60-78%. Eggs hatched after they were kept at the hatching machines for a period of 3 days. During this period, the following hatchability traits were identified:

Hatchability: The following formula is used to determine hatchability: (number of live chicks hatched/number of eggs incubated)×100.

Hatchability of fertile eggs: The following formula is used to determine hatchability of fertile eggs: (Number of live chicks hatched/Number of fertile eggs incubated)×100.

Early embryonic mortality: (Number of embryos that died between 0 and 6th days of incubation/Number of fertile eggs)×100.

Middle embryonic mortality: (Number of embryos that died between 7 and 18th days of incubation/Number of fertile eggs)×100.

Late embryonic mortality: (Number of embryos that died between 19th and 21st days of incubation/Number of fertile eggs)×100.

Discarded chick rate: (The number of eggs incubated/Number of discarded chicks hatched)×100.

Chick quality: The chick quality was determined according to Pasgar score chick quality assessment criteria, developed by Pas Reform company Boerjan (2006).

Microbiological analysis: Five eggs were randomly selected from each group and swap samples were taken to determine the total bacterial counts before and after the disinfection of eggs and on the 11th day of the incubation. In this method, sampling was performed before the disinfection application and at specified intervals after the eggs were disinfected according to the prospectus of the disinfecting material used. Sampling was performed by applying a sterile swap to randomly selected five eggs per each disinfectant. Samples were transferred to the laboratory in cold chain and at the shortest time. In the laboratory, the swaps were placed into sterile FTS (Filamentous Temperature Sensitive Proteins) and vortexed. One milliliter of the vortexed sample was mixed with 9 mL of FTS in another tube and the mixture was diluted by 10-fold with a ratio of 10:9. A new pipette was used for each run of dilution.

After the dilution, each dilution was cultured in 2 nutrient agars for determining the total bacterial count. After a 24 h incubation at a temperature of 37°C, bacterial growth was assessed. Arithmetic mean of the results was calculated according to the last dilution ratio at which growth was observed.

Statistical analysis: The data obtain was analyzed using the Minitab 14 statistical software package and incubation results were assessed according to the randomized block method while total bacterial counts were evaluated using the repeated measures analysis of variance (ANOVA) method.

RESULTS

The incubation results obtained from the assessment of the study findings are given in Table 2 while the discarded chick rate and chick quality values are given in Table 3. No difference was found among disinfectant groups with regard to discarded chick rate, hatchability, hatchability of fertile eggs and early, middle and late embryonic mortality ($p>0.05$). It was found that the disinfectants have similar effects on the hatching results and discarded chick rates. With regard to chick quality, the chicks obtained from all disinfectant groups were found to be quality chicks score 9 and above according to the Pasgar score evaluation.

Total bacterial counts of the disinfectant groups are given in Table 4. No significant difference was found among disinfectant groups with regard to their effect on the total bacterial count ($p>0.05$). The difference with respect to the bacterial counts taken before and after disinfectant

Table 2: Incubation results obtained from disinfectant groups

Disinfectant groups	Early embryonic mortality rate (%)	Middle embryonic mortality rate (%)	Late embryonic mortality rate (%)	Hatchability of fertile eggs (%)	Hatchability (%)
A	4.35±0.71	0.28±0.17	6.95±0.75	88.42±0.44	83.47±0.90
B	4.65±0.49	0.37±0.37	6.99±1.18	87.81±1.42	82.00±2.45
C	5.45±0.84	0.15±0.15	9.31±1.45	84.81±1.96	79.07±2.33
D	5.11±0.53	0.00±0.00	7.46±1.14	89.08±0.86	81.50±0.63

Table 3: Discarded chick rates and Pasgar scores obtained from disinfectant groups

Disinfectant groups	Discarded chick rate (%)	Pasgar score
A	0.00±0.00	9.97
B	0.19±0.19	9.86
C	0.33±0.20	9.80
D	0.20±0.20	9.86

Table 4: Total bacterial counts of the disinfectant groups

Groups	Total bacterial count before application (CFU mL^{-1})	Total bacterial count after application (CFU mL^{-1})	Total bacterial count on the 11th day of incubation (CFU mL^{-1})
A	6.87×10^{3}	0.5×10^{1}	1×10^{2}
B	4.82×10^{5}	3.8×10^{1}	1.5×10^{2}
C	7.87×10^{4}	3×10^{1}	5×10^{1}
D	4.28×10^{5}	0	0

*CFU: colony forming unit

application and on the 11th day was significant ($p<0.05$). However, no difference could be found among disinfectants with respect to applications ($p>0.05$). Thus, the total bacterial counts taken before the disinfectant application significantly dropped after the application. In this respect, disinfectants had similar effects. No significant change was observed in the bacterial counts taken after the disinfectant application and on the 11th day of incubation.

DISCUSSION

Disinfecting hatching eggs using the gas obtained from formaldehyde and potassium permanganate is a commonly employed disinfection method as it is cheaper, easily applicable and affects on all surfaces. Despite these advantages, this method has some negative effects on human and animal health. Therefore, studies are being conducted on alternative disinfectants that can be used to disinfect eggs and hatcheries. In this study, it was found that the disinfectant materials studies can be used without causing any loss with respect to the incubation results and total bacterial counts. However, the use of disinfectants which are applied in the form of spray is accompanied by several hardships. To defy these challenges, producers have developed and are marketing various equipments.

Researched was performed on the disinfection of hatching eggs using various disinfectants. Congruously with the findings of this study, some studies did not find any difference among disinfectants used with respect to incubation results (Yildiz, 2006; Copur *et al*., 2010; Wells *et al*., 2011). However, Kustura *et al*. (2009) reported that the hatchability of the eggs disinfected using formaldehyde is lower than those disinfected using ultraviolet light, negative ion and a combination of both while Cony *et al*. (2008) found that early embryonic mortality rate of the eggs disinfected with formaldehyde is higher compared to other disinfectant groups. Brake and Shelton (1990) stated that the hatchability of the eggs which were disinfected is 6% higher than those which were not disinfected.

This study found that the application of disinfectants significantly dropped the total bacterial count. Similar results were reported by previous studies in this respect (Yildiz, 2006; Copur *et al*., 2010; Wells *et al*., 2011; Cony *et al*., 2008; Brake and Shelton, 1990; Ledoux, 2004; Soliman *et al*., 2009). The finding that there is no difference among disinfectants with respect to the total bacterial counts in the late periods of incubation is no agreement with Yildiz (2006).

Pathogen microorganisms cause many diseases in living beings. Various disinfectants are used in the health and production sectors to minimize the harmful effects of pathogen microorganisms. Disinfectants with various active ingredients are being marketed under various commercial names.

Disinfecting substances generally have biocidal effects on bacteria, fungi and viruses. Those disinfectants whose biocidal effects are swifter and longer and which are more ecological, less odorless, easier to use, non-corrosive, economic and do not have harmful effects on human and animal health are considered as good disinfectants.

Some disinfecting substances studied have similar effects with respect to total bacterial counts and incubation results and therefore, it is concluded that their use may lead to favorable results. Yet, in addition to their effects on microorganisms, disinfecting substances should also have the above-mentioned qualities of a good disinfectant. Therefore, it is advised that in choosing a disinfectant, these qualities should be taken into consideration as well as their effects on microorganisms.

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