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# Effect of Chemical Treatments on Microflora Species on Eggshell and Hatchability of Broiler Eggs

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## Abstract

This study was conducted to determine the effect of three disinfectants on the microbes and hatchability of broiler eggs. One hundred and ninety-two medium-sized hatchable broiler eggs were divided into four groups with three replications of sixteen eggs each. Each group of the eggs was assigned to each of the following treatments, untreated, NaOCl, $H_2O_2$ and $KMnO_4$:HCHO combination (1:2). The eggs were incubated for 21 days. Data were analyzed using one-way analysis of variance in a completely randomized design. The bacteria isolated were *Escherichia coli* (225-390cfu/ml), *Pseudomonas aeruginosa* (78-139cfu/ml), *Staphylococcus aureus* and *Proteus mirabilis* (210-368cfu/ml), *Salmonella* spp (258-313cfu/ml) and fungi (63-101cfu/ml). Significant ($P<0.05$) variation was observed in the population of microflora species on egg shell. *Escherichia coli* were the pre-dominant bacteria recovered from all the samples before treatment. Effect of disinfectants on the microorganisms varied significantly ($P<0.05$) with $KMnO_4$ + formaldehyde combination having the highest (37.36%) effect on *Salmonella* spp. Similar effect of NaOCl and $H_2O_2$ was observed on *S. aureus* and *Proteus mirabilis*, *E. coli*, *P. aeruginosa* and fungi. Eggs treated with $KMnO_4$ + formaldehyde combination had the least significant ($P<0.05$) incubation weight losses value (14.63%), while eggs treated with $H_2O_2$ had the highest value (17.00%). The results further showed that hatchability, chick hatching weight and early embryonic mortality were not significantly ($P>0.05$) affected by the treatments unlike the late embryonic mortality which was significant ($P<0.05$). Although, $KMnO_4$ + formaldehyde combination is commonly used in the hatchery, in this experiment NaOCl and $H_2O_2$ compared favourably with formaldehyde as hatching disinfectants without adversely affecting hatching potentials.

**Key words:** Disinfectant, microbes, egg shell, hatchability, broiler

## Introduction

Hatchability as well as fertility are affected by genetic factors, but management transcends genetics in its effect (Kuehler and Good, 1990; Kuehler and Loomis, 1992). It is vital that hygienic standards in the breeder house are impeccable to avoid infections entering the incubator either within or on the shell of the egg. Inoculation studies in hatching eggs have shown that some bacteria are more potent in reducing hatchability than others. Bruce and Drysdale (1991) reported that *Proteus mirabilis*, *Staphylococcus aureus* and Group D *Streptococcus* Spp. reduced hatchability to zero, whereas, other organisms such as *Micrococcus* Spp. had a less dramatic effect on hatchability. In this connection, it is noteworthy that both *Proteus mirabilis* and *Staphylococcus aureus* are lecithinase positive. In addition to hatching and spoilage problems, the egg can also act as a vector in the transmission of food-poisoning organisms. Several other factors that can decrease hatchability are diseases, behavioural anomalies, improper nutrition, inbreeding and other genetic defects (Kuehler and Good, 1990; Kuehler and Loomis, 1992).

Avian eggs have evolved to protect embryos and allow their development to the point at which they are able to hatch. Non-domesticated egg incubation environments are frequently contaminated not only with microbes, but also physical hazards such as mud, faeces and water. In spite of these challenges, the successful hatch rates for eggs incubated in the wild are remarkably high (Sparks, 1985). This success is due in part to the complex chemical and physical defence systems that the egg has developed that either prevents or hinders the movement of bacteria from the shell into the contents of the egg (Hutchison *et al.*, 2003). Penetration of the hatching egg shell by microorganisms results in embryonic mortality, weak chicks, high chick mortality and poor chick growth. The most effective sanitation system involves treating the eggs as soon as they are collected from the nest and before microorganisms penetrate the shell (Henry, 2001). Therefore, this project was designed to determine the effect of three different sanitizers on hatchability of broiler eggs.

## Materials and Methods

*Location of the Experiment:* The first phase of the experiment; culturing and identification of microflora species on hatchable broiler eggs was carried out at the Department of Microbiology, University of Agriculture, Abeokuta, Ogun State. The second phase; hatching of the eggs was carried out at UNAAB-LFN Agro Allied hatchery, Kotopo, Abeokuta, Ogun State.

*Source of Materials:* One hundred and ninety two (192) hatchable broiler eggs (Ross breed) were bought from Nu-Breed Farm Apakila Camp, Abeokuta, Ogun State. The eggs were treated chemically with potassium permanganate ($KMnO_4$), formalin, sodium hypochlorite (NaOCl) and hydrogen peroxide ($H_2O_2$) before setting in the incubator.

*Experimental Design:* The eggs were randomly allotted into four (4) groups of forty-eight eggs per treatment. Each treatment was sub-divided into three (3) replicates of sixteen (16) eggs each. The first group was not treated with any chemical (control), second group was treated with sodium hypochlorite, third group with hydrogen peroxide while the fourth group was treated with potassium permanganate and formalin combination (1:2).

*Preparation of media and sample Collection:* The media used in this study were nutrient agar, salmonella shigella agar, pseudomonas agar, eosine methylene blue agar and potatoes dextrose agar. These agar media were prepared based on the manufacturer's prescription. An Evenron sterile swab was used to rub the egg shell surface of the eggs and put inside a bacteriological peptone that was prepared according to the manufacturer's instruction. The swab was used as a transport medium.

*Cultivation and Isolation of Pure Cultures using Pour Plate and colony count.* One ml of sample from peptone water was pipetted into 9ml of sterile distilled water in a test tube and appropriate serial dilutions were carried out to $10^{-5}$. This method was used for the total viable bacterial counts on the prepared media. All cultures for bacteria were incubated at 37°C for 24hours, while the plates for fungal counts were incubated at 25°C for three days. The samples were serially diluted, plated and incubated and the colonies which subsequently developed were counted. The numbers of colonies were then multiplied by the degree of dilution ($10^{-5}$) to obtain the number of bacteria in the original samples (Cowan and Steel, 1974).

*Determination of Incubation weight loss:* The eggs were weighed before they were set into the incubator and on the 18th day, incubation weight loss was determined using the formula below:

Incubation weight loss (g) = Initial weight$_{(g)}$ − Final weight$_{(g)}$

%Incubation weight loss =

$$\frac{\text{Weight loss (g)}}{\text{Initial weight (g)}} \times 100$$

*Determination of type of Embryonic Mortality:* This was done in the laboratory. The unhatched incubator eggs were broken gently to observe the stage of embryonic mortality. This was categorized into two:

Dead-in-germ (early embryonic) and Dead-in-shell (late embryonic) mortality.

*Statistical analysis:* All data collected were subjected to Analysis of Variance (ANOVA) in a Completely Randomized Design (CRD) using SAS Institute (1999). Significant ($P<0.05$) means were compared using Duncan's Multiple Range Test (Duncan, 1955).

## Results and Discussion

Tables 1 - 5 showed the microflora count on the hatchable broiler eggs before and after chemical treatments respectively. Generally, results obtained for all the organisms were statistically ($P<0.05$) different from one another. The *Escherichia coli* population was the highest; this might be due to the fact that the organisms are ubiquitous pathogens distributed throughout the world causing significant economic losses to commercially produced poultry (White *et al.*, 1993; Skyberg *et al.*, 2003), while fungi were the least. The result obtained for the microflora present on the hatchable broiler eggs showed the

Table 1: *Microflora count (cfu/ml) on Broiler egg shells before Chemical Treatments*

| | Egg categories | | | |
|---|---|---|---|---|
| Organisms | Untreated±SE | NaOCl±SE | $H_2O_2$±SE | HCHO+$KMnO_4$±SE |
| *Escherichia coli* | 335.00±0.58$^{a}$ | 419.00±0.58$^{a}$ | 225.00±2.00$^{c}$ | 390.00±3.46$^{a}$ |
| *Salmonella spp* | 288.00±2.00$^{b}$ | 255.00±1.57$^{b}$ | 312.00±2.00$^{b}$ | 269.00±2.60$^{b}$ |
| *Pseudomonas aeruginosa* | 100.00±0.87$^{c}$ | 139.00±[illegible] | 78.00±1.15$^{c}$ | 0.00±0.00$^{d}$ |
| *Staphylococcus aureus & Proteus mirabilis* | 356.00±1.14$^{d}$ | 210.00±2.31$^{d}$ | 355.00±2.31$^{a}$ | 246.00±1.[illegible] |
| Fungi | 81.00±1.32 | 63.00±1.15$^{d}$ | 72.00±2.03 | 101.00±3.18 |

abcd - Means in the same row with different superscripts differ significantly (P<0.05)
cfu - Colony forming unit
NaOCl = Sodium hypochlorite
$H_2O_2$ = Hydrogen peroxides
HCHO = formaldehyde
$KMnO_4$ = Potassium permanganate
SE - Standard Error

Table 2: *Microflora count (cfu/ml) on Broiler egg shells after Chemical Treatments*

| | Treatments | | | |
|---|---|---|---|---|
| Organisms | Untreated±SE | NaOCl±SE | $H_2O_2$±SE | HCHO+$KMnO_4$±SE |
| *Escherichia coli* | 312.00±1.73$^{a}$ | 167.00±0.87$^{c}$ | 94.00±2.60$^{d}$ | 218.00±2.02$^{b}$ |
| *Salmonella spp* | 174.00±8.37$^{a}$ | 118.00±1.15$^{c}$ | 162.00±1.44$^{b}$ | 114.00±1.15$^{c}$ |
| *Pseudomonas aeruginosa* | 87.00±0.58$^{a}$ | 66.00±0.87$^{b}$ | 28.00±0.87$^{c}$ | 0.00±0.00$^{d}$ |
| *Staphylococcus aureus & Proteus mirabilis* | 213.00±2.60$^{a}$ | 80.00±0.29$^{d}$ | 160.00±1.18$^{b}$ | 131.00±1.44$^{c}$ |
| Fungi | 67.00±1.[illegible] | 14.00±0.[illegible] | 16.00±3.[illegible] | 38.00±1.17$^{d}$ |

Superscripts differ significantly (P<0.05)
cfu - Colony forming unit
NaOCl = Sodium hypochlorite
$H_2O_2$ = Hydrogen peroxides
HCHO = Formaldehyde
$KMnO_4$ = Potassium permanganate
SE - Standard Error

Table 3: *Population (%) of microbial flora removed from broiler egg shell by chemical treatments.*

| Organisms | Treatments | | | |
|---|---|---|---|---|
| | Untreated±SE | NaOCl±SE | $H_2O_2$±SE | HCHO+$KMnO_4$±SE |
| *Escherichia coli* | 15.22±2.31$^{c}$ | 49.69±0.29$^{b}$ | 58.35±5.29$^{a}$ | 41.23±2.02$^{c}$ |
| *Salmonella* spp | 22.26±10.39$^{c}$ | 54.17±0.29$^{a}$ | 48.16±1.44$^{b}$ | 57.36±1.15$^{a}$ |
| *Pseudomonas aeruginosa* | 12.55±0.29$^{c}$ | 52.71±0.58$^{a}$ | 54.71±0.29$^{a}$ | 0.00±0.00$^{d}$ |
| *Staphylococcus Aureus & Proteus mirabilis* | 28.14±3.46$^{c}$ | 57.86±2.03$^{b}$ | 54.21±5.48$^{a}$ | 45.62±1.62$^{b}$ |
| Fungi | 23.60±5.77$^{c}$ | 78.57±0.87$^{a}$ | 80.42±0.87$^{b}$ | 63.18±2.60$^{d}$ |

abcd: Means in the same row with different superscripts differ significantly (P<0.05)
NaOCl= Sodium hypocholrite
H2O2= Hydrogen peroxides
HCHO= formaldehyde
KMnO4= Potassium permanganate
SE= Standard Error

Table 4: *Effect of chemical treatments of broiler eggs on incubation weight losses*

| Parameters | Treatments | | | |
|---|---|---|---|---|
| | Untreated±SE | NaOCl±SE | $H_2O_2$±SE | HCHO+$KMnO_4$±SE |
| Av. Initial Egg Weight (g) | 66.94±0.68 | 67.11±2.22 | 66.64±0.07 | 66.38±1.44 |
| Av. Final Egg Weight (g) | 56.70±0.63 | 56.10±2.31 | 55.23±0.30 | 56.67±2.73 |
| Egg Weight Loss (g) | 10.24±0.06$^{ab}$ | 11.01±0.32$^{ab}$ | 11.41±0.29$^{a}$ | 9.71±0.40$^{c}$ |
| Egg Weight Loss (%) | 15.30±0.09$^{b}$ | 16.45±0.42$^{a}$ | 17.00±0.44$^{a}$ | 14.63±0.51$^{c}$ |

abc: Means in the same row with different superscripts differ significantly (P<0.05)
NaOCl= Sodium hypocholrite
H2O2= Hydrogen peroxides
HCHO= formaldehyde
KMnO4= Potassium permanganate
SE= Standard Error

hatchable eggs need to be treated with a fumigant or other type of disinfectant to reduce the microorganism population on the shell surface. Contamination can be caused by lack of hygiene in the nests and presence of eggs on the floor (Cecilia *et al.*, 2004). The variation in the result of most common bacterium on fertile eggs might be due to the sources of the eggs and the management practices on the breeder farm.

The immersion of the eggs in the chemicals caused the reduction of the microflora population before setting into the incubator. All the chemicals: NaOCl, $H_2O_2$ and formaldehyde showed a general ability to reduce microorganisms on eggshells. The effect of $H_2O_2$ which reduced the *salmonella* spp population by 48.16% supported earlier researchers (Baily *et al.*, 1996) who reported that 7.5 % of $H_2O_2$ administered using 100 or 500ml/h reduced *Salmonella* spp on eggshells by 55%.

The slight reduction in microflora count in untreated eggs could be attributed to the storage inside the cold room at a temperature of 18°C for a day before setting into incubator. Board *et al.* (1989) reported that if infected eggs are kept at room temperature, microorganisms such as salmonella can multiply rapidly and reach high levels within the egg, whereas cold storage completely inhibit the growth of salmonellas.

Egg shell permeability (Table 4), as measured by egg moisture loss in the incubator was significantly affected by $H_2O_2$ (17.00%) when compared with other treatments. This observation is in agreement with the findings of Henry (2003) and Sheldon and Brake (1991) who reported that peroxide caused an increased loss of moisture from eggs during incubation.

The high hatchability of chicks from untreated eggs (Table 5) could be due to the formaldehyde fumigation of the incubator and hatcher before setting the eggs. It could also be as a result of matured cuticle on the eggshell which is a very effective barrier against water penetration and bacterial invasion by closing the pores, hence avoiding flooding of the egg (Berrang *et al.*, 1999). The hatchability result from untreated group in this present study was higher than the findings of Okoli and Udedibie (2001) who

Table 5: *Effect of Chemical Treatments of broiler eggs on hatchability of chicks*

| Parameters | Treatments | | | |
|---|---|---|---|---|
| | Untreated±SE | NaOCl±SE | $H_2O_2$±SE | HCHO+$KMnO_4$±SE |
| Number of Eggs set | 48 | 48 | 48 | 48 |
| Av. Egg Weight (g) | 66.94±0.68 | 67.11±2.23 | 66.64±0.07 | 66.38±1.44 |
| Fertility (%) | 78.61±8.06 | 81.86±6.09 | 87.50±7.21 | 91.67±2.08 |
| Hatchability (%) | 93.75±4.51 | 75.00±1.71 | 85.42±2.58 | 87.50±10.13 |
| Av. Chick Hatching Weight (g) | 44.42±0.79 | [illegible].34±3.56 | 45.42±0.72 | 45.30±0.35 |
| Embryonic Mortality (%) | 6.25±3.61 | 25.00±[illegible].61 | 14.58±2.08 | 12.50±9.55 |

NaOCl= Sodium hypochlorite
H2O2= Hydrogen peroxides
HCHO= formaldehyde
KMnO4= Potassium permanganate
SE= Standard Error

Table 6. *Embryonic Mortality in Broiler Eggs Treated with Different Chemicals*

| Parameters | Treatments | | | |
|---|---|---|---|---|
| | Untreated±SE | NaOCl±SE | $H_2O_2$±SE | HCHO+$KMnO_4$±SE |
| Early Embryonic Mortality (Dead-in-Germ) (%) | 2.08±2.08 | 2.08±2.08 | 0.00±0.00 | 4.17±4.17 |
| Late Embryonic Mortality (Dead-in-Shell) (%) | [illegible]7±4.17 | 22.92±2.08[a] | 14.58±2.08[b] | 8.33±3.51 |

a,b: Means in the same row with different superscripts differ significantly (P<0.05)
NaOCl = Sodium hypochlorite
H2O2= Hydrogen peroxides
HCHO= formaldehyde
KMnO4= Potassium permanganate
SE= Standard Error

reported hatchability of 80% in 60 untreated broiler eggs. Cason *et al.* (1994) reported that, although fertile hatching eggs were contaminated with high levels of *Salmonella* spp, they were still able to hatch. Hatchability of chicks from eggs treated with NaOCl which was 72.52% might be as a result of the chemical concentration and timing of immersion. This is in agreement with Patterson *et al.* (1990) who reported that hatchability of chicken eggs reduced when the eggs were dipped in chlorine dioxide ($ClO_2$) solutions for more than 5minutes or in concentrations greater than 100ppmCl. However, treating eggs with $ClO_2$ foam or fumigating with formaldehyde had no adverse effect on hatchability. The high hatchability recorded in all the treatments could be due to the fact that medium sized eggs were set. Average weight eggs are preferable to achieve good hatchability as far as chicken is concerned (Brah *et al.*, 1999; Gonzales *et al.*, 1999).

The result obtained for hatching weight in relation with egg weight was not in agreement with the findings of Burke (1992) who observed that chick weight was strongly influenced by the egg weight from where the chick hatched. Several factors are known to affect chick weight. Hager and Beane (1983) indicated that early-hatching chicks lose weight (10-12%) when held in hatcher for prolonged periods.

$H_2O_2$ treated eggs recorded no early embryonic mortality (Table 6). This result is similar to the findings of Sheldon and Brake (1991) who reported that $H_2O_2$ reduced level of early embryonic mortality. The values obtained for dead-in-shell (DIS) in this study were higher than those recorded for dead-in-germ (DIG). This is contrary to the reports of Krueger (1990) that ratio of DIG in poultry is relatively high compared to DIS. The results of the present study indicated that either NaOCl or $H_2O_2$ could be used as alternative to formaldehyde fumigation in the treatment of eggshell of hatchable broiler eggs.

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