EFFECT OF O2XY-WASH ON MOISTURE LOSS
AND HATCHABILITY OF BROILER EGGS

NOTE: [O2XY-WASH is a analog of AQUATIZE® (about 67% as concentrated).]

9 February 1996
Investigator: Dr. Michael J. Wineland
Extension Specialist (Broiler Breeders & Hatcheries)
Department of Poultry Science
North Carolina State University
Raleigh, North Carolina 27695
USA
Location: Piedmont Research Station
8350 Sherrill’s Ford Road
Salisbury, North Carolina 28144
USA
Sponsor of Study: Bioxy, Inc.
3733 National Drive
Suite 120
Raleigh, North Carolina 27612
USA

SUMMARY

The trial indicates that O2XY-WASH is an effective biocidal agent in decreasing shell surface microbial load at all of the concentrations used. The ability to decrease the microbial load on the shell surface will help to minimize microbial invasion into the egg interior and subsequently infection of the chick. Concentrations of 1:500, or greater, of O2XY-WASH demonstrated no detrimental effects upon hatchability. Additionally, unlike some shell surface sanitizers the use of O2 resulted in no apparent residue left on the shell surface as indicated by the increased moisture loss from the eggs during incubation. The increase in moisture loss would be indicative that there is no decrease in the passage of respiratory gases across the shell. The use of 1:500 or 1:1000 concentration of O2XY-WASH increased chick vitality, which was visually observed. This study indicates that O2XY-WASH is an effective egg sanitizer when used according to directions and at dilutions of 1:500 or greater.

O2XY-WASH:
Does Not Clog Egg Shell Pores  Is Not Affected by Warm or Cold Water
Effective in Presence of Organic Materials  User-Friendly & Odorless
Removes Contaminants from Shell Surface  Does Not Chlorinate Organic Compounds
Cost Effective

OBJECTIVES FOR THE PROJECT:

To determine the microbial counts on shell surfaces, moisture loss of the egg during incubation and hatchability in broiler eggs treated with O2XY-WASH.
SPECIFIC OBJECTIVES:

#1: To determine reduction of microbial load on the shell surface after treatment with O₂XY-WASH,
#2: To determine moisture loss during incubation after shell surfaces have been treated with O₂XY-WASH, and
#3: To determine the effect of O₂Y-WASH application upon hatchability of broiler breeder eggs.

PROJECT JUSTIFICATION OR RATIONALE:

The hatching egg is exposed to possible attack from many different microbes (bacteria, yeast and molds). Since human health concerns have reduced the use of formaldehyde, as an egg fumigant there is a need for effective, broad-spectrum egg sanitizers/cleaners. Use of some sanitizers such as quaternary ammonium compounds (depending upon their formulation) may alter the ability of the egg to exchange respiratory gases properly. Some hatcheries have experienced sanitizers occluding pores of the shell and causing reduced hatchability. Other sanitizers such as phenolics can be irritating to a person’s respiratory system when spraying the eggs.

MATERIALS AND METHODS:

Fresh, fertilized eggs from a 55-week-old broiler-breeder flock were randomized among 30 trays of 144 eggs per tray. There were 5 treatment groups, each consisting of six trays. The treatments were as follows:

Untreated Control
1:250 O₂XY-WASH
1:500 O₂XY-WASH
1:750 O₂XY-WASH
1:1000 O₂XY-WASH

The appropriate concentration of O₂XY-WASH was applied to the shell surface until thoroughly wet using a one-gallon garden type sprayer commonly used to apply sanitizer. The spray droplet was sufficiently large enough so as to effectively cover the shell surface and allow excess O₂XY-WASH to drip from the egg.

Two trays of eggs (288 eggs) for each treatment were individually numbered, weighed to the nearest hundredth of a gram prior to incubation using a mettler balance connected to a portable computer for recording the weight.

Thirty eggs of each treatment were placed in a sterile whirl pack bag immediately after treating, labeled and transported at 16 °C to Scott Hall, North Carolina State University, Raleigh, North Carolina, for determining shell surface microbial numbers.

Biocidal efficacy of eggs from each O₂XY-WASH dilution and control eggs were
processed according to a modified procedure of Gentry and Quarles to determine the extent of shell surface contamination. Twenty milliliters of sterile saline was added to each whirl Pack bag, shaken vigorously for one minute and an aliquot was spiral plated on a total aerobe type plate (tryptose soy agar). The plates were incubated for 24 hours at 37 °C. Efficacy of the spray treatment was calculated by determining percent reduction from the average number of microbial colonies found on the eggs of the control treatment.

Eggs were randomized in one compartment of a setter (Robbins Incubator Company) at the Piedmont Research Station. The setter was regulated to 99.9 °F, dry bulb, and 84 °F, wet bulb. On Day 18 of incubation the eggs were transferred to the hatchery set at 98.75 °F, dry bulb. Immediately before transfer to the hatching baskets the numbered eggs were reweighed to determine moisture loss of individual eggs in each treatment.

After incubation for 21 days (504 hours) the chicks were removed and counted. Hatching egg residue was removed from the tray to be examined macroscopically for determining stage of embryonic mortality.

**RESULTS AND DISCUSSION:**

**Microbial load determination:**

The biocidal efficacy of O₂XY-WASH is shown in the table (1) below. All dilutions demonstrated significant reduction of shell surface bacterial populations. The highest dilution of the O₂XY-WASH used did not show decreased efficacy, as the 1:1000 dilution was not statistically different from the 1:250 dilution. It is quite possible that the slightly increased bacterial load on the 1:750 dilution was due to chance alone as there was still a dramatic reduction in bacterial load.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1:250</th>
<th>1:500</th>
<th>1:750</th>
<th>1:1000</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFU/Egg</td>
<td>1200b</td>
<td>843b</td>
<td>2484a</td>
<td>1297ab</td>
<td>131,484c</td>
</tr>
<tr>
<td>Reduction (%)</td>
<td>99.1</td>
<td>99.4</td>
<td>98.2</td>
<td>99.0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Superscripts of the same letter indicate no significant difference.

**Moisture Loss Determinations:**

The effect of O₂XY-WASH upon moisture loss is shown below (table 2). Normal moisture loss during incubation will range from 7% to 17% with a normal bell curve distribution.
The moisture loss data in this trial demonstrated similar values. All concentrations increased the conductance of the shell although 1:750 increased the least compared to the controls. Dilutions of 1:250, 1:500 and 1:1000 were statistically different from the controls. The 1:750 dilution was not statistically different from the control, but this dilution did not impede the move entry of gases across the shell. The moisture loss demonstrated in this trial indicates that $O_2$XY-WASH does not interfere with movement of respiratory gases across the eggshell. This is similar to the effect of formaldehyde fumigation upon moisture loss (Wineland, Unpublished data). However, the use of formaldehyde has been reduced because of human health concerns. Additionally, care must be used when applying quaternary ammonium as some formulations of quaternary ammonium sanitizers have decreased moisture loss by occluding pores and thus interfering with respiratory gas exchange. Examination of the moisture loss and microbial load data demonstrates a coincidental relationship when examining data for the different concentrations. The relationship seen is that average percent moisture loss for a particular dilution correlates with shell surface microbial reduction.

Table 2: Moisture Loss

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1:250</th>
<th>1:500</th>
<th>1:750</th>
<th>1:1000</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight Loss (% Days 1-18)</td>
<td>11.18ab</td>
<td>11.47a</td>
<td>11.02bc</td>
<td>11.21ab</td>
<td>10.84c</td>
</tr>
</tbody>
</table>

A Superscripts of the same letter indicate no significant difference

Hatchability Determination:

The table below (3) demonstrates embryonic mortality patterns and hatchability from the five treatments. All calculations were based upon fertile eggs. Below is an explanation of abbreviations used in the data table.

% ED1 = Percent of embryonic mortality occurring during days 1 to 3 of incubation
% ED2 = Percent of embryonic mortality occurring during days 4 to 7 of incubation
% MD = Percent of embryonic mortality occurring during days 8 to 14 of incubation
% LD1 = Percent of embryonic mortality occurring during days 15 to 18.5 of incubation
% LD2 = Percent of embryonic mortality occurring during days 18.5 to 20 days of incubation
% Pip = Percent of embryonic mortality that is characterized by chicks making a hole in the shell (external pipping of the shell) but dying before hatching or failing to complete the hatching process before 21 days. In this project all hatch data is representative of status at 504 hours of incubation (21 days)
% “C” Chick = Percent of low grade chicks (chicks with unhealed navels, red hocks and chicks exhibiting severe unthrift ness)
% “A” Chick= Percent of remaining chicks not showing problems. This value is
an arbitrary evaluation of the investigator since no acceptable standard exists.

\% Chick (A+C) = Percent of total chicks that hatched.

The data below (Table 3) demonstrate that the 1:250 dilution had statistically more early dead embryos dying between 4 to 7 days of incubation. This could indicate a detrimental effect upon the developing embryo by the most concentrated treatment of O\textsubscript{2}XY-WASH. Also noted is that the 1:250 dilution demonstrated reduced \% “A” chicks and \% “A+C” chicks when compared to the control. These data indicate that the 1:250 concentration of O\textsubscript{2}XY-WASH should not be used. The O\textsubscript{2}XY-WASH treated eggs resulted in a lower percentage of low grade chicks. The data indicates that the 1:1000 dilution of O\textsubscript{2}XY-WASH exhibited the greatest percentage of “A” chicks. It should be noted that the control chicks, which while still classified as “A” chicks did demonstrate a decreased vitality when visually observed.

**Table 3: Hatchability Results**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1:250</th>
<th>1:500</th>
<th>1:750</th>
<th>1:1000</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>%ED1</td>
<td>3.18a</td>
<td>2.12a</td>
<td>3.30a</td>
<td>2.38a</td>
<td>3.26a</td>
</tr>
<tr>
<td>%ED2</td>
<td>5.08a</td>
<td>2.74b</td>
<td>2.98b</td>
<td>2.02b</td>
<td>1.64b</td>
</tr>
<tr>
<td>%MD</td>
<td>0</td>
<td>0.12</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>%LD1</td>
<td>4.19a</td>
<td>3.22a</td>
<td>2.86a</td>
<td>4.15a</td>
<td>2.64a</td>
</tr>
<tr>
<td>%LD2</td>
<td>0.89a</td>
<td>0.74a</td>
<td>0.74a</td>
<td>0.62a</td>
<td>0.25a</td>
</tr>
<tr>
<td>%Pip</td>
<td>3.65a</td>
<td>4.09a</td>
<td>4.05a</td>
<td>2.50a</td>
<td>3.95a</td>
</tr>
<tr>
<td>%”C”</td>
<td>1.75a</td>
<td>1.22a</td>
<td>1.85a</td>
<td>1.49a</td>
<td>2.14a</td>
</tr>
<tr>
<td>%”A”</td>
<td>81.27b</td>
<td>85.75a</td>
<td>84.23ab</td>
<td>86.83a</td>
<td>86.11a</td>
</tr>
<tr>
<td>%”A+C”</td>
<td>83.02b</td>
<td>86.97ab</td>
<td>86.08ab</td>
<td>88.33a</td>
<td>88.25a</td>
</tr>
</tbody>
</table>

\textsuperscript{A} Superscripts with the same letter indicate no significant difference