Evaluation of a Method of Ultraviolet Light Sanitation of Broiler Hatching Eggs

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ABSTRACT  Sanitation of hatching eggs is an important area of research due to the need for an effective, economical, and safe method of egg sanitation. Improved hatching egg sanitation is an important part of an overall pathogen reduction program within integrated poultry operations. This must be accomplished without disturbing the cuticle of the egg, which can decrease hatchability. The ability of ultraviolet (UV) light to kill bacteria on eggshell surfaces has been well documented. To accomplish the task of treating the eggs in a method that could be commercially implemented, a cabinet was constructed in which ultraviolet lamps were placed. A conveyor system was used to carry a plastic hatching egg flat containing 42 eggs through the cabinet for a period of 3 or 4 min. Ultraviolet intensities within the cabinet reached a maximum of 14 mW/cm². Experiments were conducted to test the impact of UV light (254 nm) exposure of hatching eggs on aerobic plate counts (APC), inoculated Salmonella typhimurium and inoculated Escherichia coli. In the first three experiments, seven eggs were sampled from a flat passed through the UV chamber. Ultraviolet-treated eggs compared to untreated eggs had APC reductions of 1.3 log, S. typhimurium had a 4 log reduction, and E. coli had a 4 to 5 log reduction. Laboratory trials were also conducted to test the effects of UV irradiation on the cuticle of the egg and hatchability. No significant differences for eggshell conductance or hatchability were found between UV-treated and control eggs. From these trials, it can be concluded that UV irradiation of hatching eggs in a prototype irradiation cabinet can effectively reduce aerobic and pathogenic bacteria on eggshell surfaces without affecting eggshell conductance or hatchability.

(Key words: ultraviolet light, hatching egg, sanitation, hatchability, contamination)

INTRODUCTION

Hatchery sanitation is an imperative concern to integrated broiler producers and is an important step in an overall pathogen reduction program. However, hatchery sanitation and pathogen reduction in day-old chicks is difficult to achieve if the eggs entering the hatchery from the breeder farm are already heavily contaminated. Sanitation of broiler hatching eggs continues to be a problem in the broiler industry due to the lack of an economical, effective, and safe alternative sanitation method to replace formaldehyde fumigation. If hatching eggs are not sanitized prior to incubation, excessive bacterial contamination and subsequent growth can lead to decreased hatchability, poor chick quality, growth and performance (Scott and Swetnam, 1993), and increased mortality (Reid et al., 1961). Hatching egg sanitation is also imperative due to the potential for extensive cross-contamination of pathogenic bacteria, such as Salmonella during the hatching process (Cason et al., 1994). In order to increase survivability, the egg surface must remain free of contaminating microorganisms (Kuo et al., 1997a). Control of microorganisms on the shell surface of hatching eggs requires an effective method of killing bacteria, such as Salmonella and Escherichia coli, without injury to the live embryo and the cuticle of the egg. The cuticle of the egg is a proteinaceous layer that helps to seal the pores of the egg and acts as a natural barrier to bacterial penetration (Wang and Slavik, 1998). Therefore, it is preferable to leave the cuticle intact on hatching eggs. Removal of the cuticle from the egg also tends to increase shell porosity (conductance) and therefore increases moisture loss during incubation (Peebles and Brake, 1986). Eggshell sanitation methods that disturb the cuticle and increase shell permeability are therefore not preferable alternatives.

Ultraviolet (UV) radiation is produced by the sun and is therefore a natural component of our environment. Ultraviolet radiation at 254 nm is well known and documented for its use to kill various types of microorganisms, such as bacteria, yeasts, molds, fungi, and viruses. It can be generated at high intensities by the use of low-pressure mercury-vapor discharge lamps called germicidal lamps.

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Abbreviation Key: APC = aerobic plate counts; BGA = brilliant green agar; NO = novobiocin; TSB = tryptic soy broth; UV = ultraviolet.
Germicidal lamps are relatively easy and inexpensive to obtain and use and have been used in a variety of industries to sanitize air, water, dairy products, vegetables, meat, maple syrup, fresh cider, and packaging materials (Huang and Toledo, 1982). Ultraviolet light treatment of eggshells has been shown to be effective at reducing aerobic microbial counts, counts of yeasts and molds, and inoculated Salmonella typhimurium populations on eggshells (Kuo et al., 1997a,b). Chavez et al. (1999) showed a 3 log10 CFU/egg reduction in aerobic plate counts (APC) on the inside top, bottom, and two sides of the cabinet. Ultraviolet light treatment of meat, maple syrup, fresh cider, and packaging materials attempts to sanitize air, water, dairy products, vegetables, and metal surfaces.

Past research has utilized various methods to expose eggshell surfaces to UV light (Goerzen and Scott, 1995; Gao et al., 1997; Kuo et al., 1997a,b; Chavez, et al., 1999). For commercial application, we hypothesize that a UV treatment should be applied before hatching eggs reach the farm cooler. It has been reported that the egg is susceptible to bacterial penetration within a short time after laying (Zagaevsky and Lutivoka, 1944). As the freshly laid egg cools, the yolk and white contract more than the shell; thus, water and microorganisms are drawn into the pores. In most commercial broiler breeder operations, eggs are collected at the end of the house on belts and placed on plastic egg flats by hand. The most advantageous point to apply UV light sanitation is after the eggs are placed in the flats since the eggs will not be directly handled again. Therefore, recontamination of the shell by direct contact should not occur once the eggs are irradiated on the flats. In addition, the eggs will have experienced a minimal amount of cooling at this time.

The objectives of the current investigations were (a) to develop a method of high-intensity UV irradiation for hatching eggs suitable for use at a commercial broiler breeder facility, (b) to evaluate its effectiveness against aerobic microorganisms and pathogenic organisms, such as Salmonella and E. coli on the shells of broiler hatching eggs, and (c) to determine the effects of UV light on the cuticle of the egg and resulting effects on hatchability.

**MATERIALS AND METHODS**

**Development of UV Irradiation Method**

Any UV light delivery method to be used in a commercial setting would have to prevent exposure of workers to the light rays. An enclosed box or “cabinet” could accomplish this requirement. Therefore, a cabinet was constructed with UV lamps (254 nm wavelength) placed on the inside top, bottom, and two sides of the cabinet. A conveyor system was constructed to carry commercial-type plastic hatching egg flats of 42 eggs through the cabinet (Figure 1). The conveyor consisted of two tracks with a belt lying in the bottom of each track upon which the feet of the plastic egg flats rested. This system was employed to minimize shadows on the eggs, which would prevent the effectiveness of the UV light. Ten 91.5-cm germicidal lamps were placed in the bottom of the cabinet below the conveyor tracks. Two additional lamps were placed on either side of the cabinet at the level of the eggs on the flat. Eight 30.5-cm lamps were placed on the inside top of the cabinet perpendicularly over the conveyor. Three 25.4-cm pen lights were also added over the conveyor to irradiate the top of the eggs. Aluminum flashing was placed behind all of the UV light bulbs to reflect the light and achieve higher intensities within the cabinet. Ultraviolet intensities within the cabinet were measured by taping the sensor of a UV meter to a plastic egg flat (in place of eggs) and placing the flat on the conveyor. This effectively measured the intensities received by the eggs. Intensities ranged from 4 mW/cm² in the area over the conveyor where no UV lamps were placed to 14 mW/cm² directly under the three pen lights. The germicidal lamps were mounted in the cabinet to be as close to the eggs as possible without accidental contact. Approximately 1 to 2 cm of clearance was allowed on all sides between the lamps and the eggs on the flat when the flat was placed on the conveyor. Conveyor speed could be manipulated between 1 and 6 min by changing the drive pulley size. The use of 23 germicidal lamps in the small cabinet was found to generate enough heat to raise the air temperature within the cabinet to greater than 50°C. Therefore, a fan was used to blow filtered air into the bottom of the cabinet, and a vent was cut in the top to allow heated air to escape. The addition of the fan and vent allowed temperatures within the cabinet to remain below 38°C during egg irradiation.
Serially diluted rinse solution on plate count agar and 50 mL was removed and serially diluted. Aerobic microorganism counts were obtained by plating 1 mL of the serially diluted rinse solution on plate count agar and incubating the plates at 37°C for 48 h. Three trials were performed using seven untreated control eggs and seven UV-treated eggs in each trial. Eggs for all trials were visibly clean, unwashed, and collected at the Texas A&M University Poultry Research Farm immediately prior to UV treatment.

**Salmonella typhimurium Count Study**

In the second experiment, a primary poultry isolate of *S. typhimurium* (accession no. 87-26254) selected for resistance to novobiocin (NO) and nalidixic acid (NA) was obtained from National Veterinary Service Laboratory, Ames, IA. Cultures were grown and maintained in tryptic soy broth (TSB) containing 25 µg/mL each of NO and NA. To initiate the log growth of the organism, 0.5 mL of the refrigerated culture was added to 10 mL of TSB and incubated at 37°C for 12 h. After initiation, 5 mL of activated culture was added to 800 mL of TSB and then incubated at 37°C for 12 h. The final culture concentration was 10^8 to 10^9 cells/mL. Cultures were verified by plating dilutions on duplicate brilliant green agar (BGA) plates containing 25 µg/mL NO and NA, respectively.

Fourteen unwashed, clean eggs were inoculated with 50 mL of final *S. typhimurium* culture in a sterile plastic bag for 1 min. After the eggs were inoculated for 1 min in the culture, the eggs were aseptically removed from the plastic bags and placed in sterile paper egg cartons and incubated at 37°C for 30 min.

Seven eggs inoculated with *S. typhimurium* were placed in each column of a plastic egg flat and surrounded with other visibly clean, unwashed eggs. The flat was then passed through the UV light chamber on the conveyor system as previously described for APC experiments. Sample size and recovery methods were the same as previously described. Serial dilutions of the PBS were plated on BGA containing 25 µg/mL each of NO and NA. The BGA plates were incubated at 37°C for 24 h.

**Measurement of Eggshell Conductance**

In experiment 4, the effects of high intensity UV irradiation on the cuticle of the egg were evaluated by measuring eggshell conductance following UV treatment. Eggshell conductance can be measured by placing eggs in a desiccator to expedite moisture loss from the egg (adopted from Sparks and Board, 1984). Twenty untreated eggs were used as a control, and 20 eggs were treated using the prototype UV irradiation cabinet as previously described. Ultraviolet exposure time was reduced to 3 min. In addition, five eggs were dipped in a 7,000 ppm NaClO solution for 1 min. Sodium hypochlorite has been shown to remove the cuticle of the egg and increase shell porosity (Peebles and Brake, 1986). The NaClO group was used as a measure of moisture loss for eggs with cuticle damage. All eggs were individually numbered and weighed to the nearest 0.01 g prior to placement in the desiccator. The desiccator with eggs was then placed in an incubator at 37°C. In trial 1, eggs were reweighed after 2 d, and in trials 2 and 3 eggs were reweighed after 2 and 3 d to determine weight loss. Conductance was calculated as milligrams H₂O per day per torr (Ar et al., 1974).

**Hatchability Trials**

In experiment 5, three trials were conducted to determine if high intensity UV irradiation had any effects on embryo viability or hatchability. Ross × Ross broiler hatching eggs were obtained from a commercial broiler operation. All eggs were inspected for acceptable hatching egg characteristics, and eggs that were cracked, thin shelled, possessed body checks, or were excessively dirty or stained were discarded. Eggs were randomly assigned to control or treated groups. Untreated eggs were used as the control group and were placed into incubator trays with clean hands. The eggs for the UV-treated group were irradiated by allowing plastic hatching egg flats with 42 eggs on each flat to pass through the cabinet for 3 min as previously described. Ultraviolet-treated eggs were then placed on an egg flat and passed through the UV light chamber as previously described. Sample size and recovery methods were also as previously described. Three trials were conducted utilizing a total of 42 eggs (21 control and 21 treated). Serial dilutions of the PBS were plated on eosin methylene blue plates that contained 25 µg/mL each of NO and NA and incubated at 37°C for 24 h.

In the first experiment, 42 eggs were placed on an egg flat (six rows, seven columns of eggs) and passed through a UV chamber (4 to 14 mW/cm²) for 4 min. Seven eggs were collected, one from each column, and aseptically placed into sterile plastic bags (one egg per bag) containing 50 mL of sterile PBS pH 7.2. After 5 s of gentle hand massage to remove bacteria from the eggshell surface, 5 mL was removed and serially diluted. Aerobic microorganism counts were obtained by plating 1 mL of the serially diluted rinse solution on plate count agar and incubating the plates at 37°C for 48 h. After initiation, 5 mL of activated culture was added to 800 mL of TSB and then incubated at 37°C for 12 h. The final culture concentration was 10^8 to 10^9 cells per milliliter. This was verified by plating the dilutions of the culture on duplicate eosin methylene blue (EMB) plates containing 25 µg/mL each of NO and NA.

Seven eggs inoculated with *E. coli* were placed on an egg flat and passed through the UV light chamber as previously described. Sample size and recovery methods were also as previously described. Three trials were conducted utilizing a total of 42 eggs (21 control and 21 treated). Serial dilutions of the PBS were plated on eosin methylene blue plates that contained 25 µg/mL each of NO and NA and incubated at 37°C for 24 h.
as aseptically removed from the flats and placed into incubator trays. Equal numbers of eggs from each treatment group were placed in two incubators at 37.6°C dry-bulb and 30°C wet-bulb. At 10 d of incubation, all eggs were briefly removed from the incubators and candled to determine embryo viability. Those eggs appearing infertile, containing dead embryos, cracked, or otherwise nonviable were removed. All nondamaged eggs that had been removed were dipped in an iodine solution for 1 min to sterilize the outside of the shell and then dried of excess iodine by wiping with sterile gauze or cotton balls. The contents of the eggs were then broken into a sterile petri dish and classified as infertile or by stage of embryonic death. The contents were also cultured for bacterial contamination using a sterile cotton swab. Swabs were streaked on TSA II 5% sheep blood agar plates and MacConkey agar plates in duplicate. The plates were incubated 24 h at 37°C, and then inspected for bacterial growth. The same procedure was performed on d 18 prior to transfer of the eggs to hatcher units. Eggs containing viable embryos were transferred to hatcher units and maintained at 37.5°C dry-bulb and 31°C wet-bulb for d 19 to 21 of incubation. On d 21, hatched chicks, mortalities, and hatch residue were collected. Eggs remaining unpipped after 21 d were also broken out for classification and cultured for microbial contamination. After removal of infertile, cracked or broken eggs from all trials, 776 and 1,561 viable eggs were used over three trials for control and UV-treated groups, respectively.

**Statistical Analysis**

The colony counts for APC, *S. typhimurium*, and *E. coli* experiments were multiplied by 50 (50 mL PBS) to estimate the total microbial load recovered from the eggshell and transformed to log_{10} cfu/egg. The lowest detection level for this procedure was 1.7 log_{10} cfu/egg. Thus, counts of 0 mean < 1.7 log_{10} cfu/egg and 0.85 were used to represent <1.7 log_{10} cfu/egg. Counts were analyzed using the general linear models (GLM) procedure of SAS. Mean differences were separated by the PDFF option (pairwise t-tests) of the GLM procedure. Hatchability, embryonic mortality, and microbial contamination of control vs. UV-treated groups were compared using the test of binomial proportions. Statistical significance for all data was considered at the probability level of *P* < 0.05.

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6Model 1 Incubator, Petersime Incubator, Co., Gettysburg, OH.  
7Trypticase Soy Agar II with 5% sheep blood, Becton Dickinson and Company, Cocksleyville, MD.  
8Petersime Model H-145, Petersime Incubator Company, Gettysburg, OH.  
9SAS Institute Inc., Cary, NC.
Effects of UV Irradiation on Eggshell Conductance

No significant differences in eggshell conductance were found between treatments for all trials in experiment 4. After 2 d of incubation, conductance for control, UV-treated and NaClO-dipped eggs was 6.17, 6.26, and 6.66 mg H₂O per day per torr, respectively. After 3 d of incubation, conductance values were 5.69, 5.61, and 5.84 mg H₂O per day per torr for control, UV-treated, and NaClO-dipped eggs, respectively. These findings suggest that high-intensity UV irradiation has no effect on the cuticle or water vapor conductance of the egg for the first 3 d of incubation. These results agree with those of previous workers. Scott (1993) observed no difference in moisture loss from 1 to 18 d of incubation between eggs treated with formalin and eggs treated with UV light prior to incubation. Scott (1993) also observed no differences in early or late embryonic mortality with UV-treated and formalin-treated eggs. If UV light would have adversely affected the cuticle, an increase in early or late embryonic mortality or both would probably have been observed. Therefore, it would appear that high-intensity UV irradiation does not affect the cuticle of the egg, which would result in excessive moisture loss from the egg.

The lack of significant difference in eggshell conductance between control and sodium hypochlorite-treated eggs would suggest that the cuticle was not completely removed from the shell, as was also reported by Peebles and Brake (1986). These results could have occurred if the concentration of sodium hypochlorite in the solution in which the eggs were dipped or the dipping interval was insufficient. Wang and Slavik (1998) obtained similar results after 3.5 min of washing in a 100 ppm NaClO solution. Using electron microscopy, they observed very little damage to the cuticle. Such circumstances could explain the obtained results. However, the slight increase in conductance does show that some cuticle damage did occur. When compared to the conductance of the UV-treated eggs, the observed results reinforce the theory that UV irradiation does not damage the cuticle of the egg.

Effects of UV Irradiation on Embryo Mortality and Hatchability

No significant differences were observed in embryo mortality or hatchability when all three trials were totaled in experiment 5 (Table 2). No significant improvement in hatchability of UV-treated eggs has also been observed by other researchers (Scott, 1993; Berrang et al., 1995; Goerzen and Scott, 1995). Therefore, it can be concluded

### TABLE 1. Effects of ultraviolet (UV) irradiation on aerobic microorganism (APC), *Salmonella typhimurium*, and *Escherichia coli* counts of control and ultraviolet light-treated eggshells

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment</th>
<th>Trial 1 (log₁₀/egg)</th>
<th>Trial 2 (log₁₀/egg)</th>
<th>Trial 3 (log₁₀/egg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC</td>
<td>Control</td>
<td>3.75 a</td>
<td>4.61 a</td>
<td>4.02 a</td>
</tr>
<tr>
<td></td>
<td>UV-treated</td>
<td>1.95 b</td>
<td>2.68 b</td>
<td>2.12 b</td>
</tr>
<tr>
<td></td>
<td>SEM³</td>
<td>0.27</td>
<td>0.16</td>
<td>0.23</td>
</tr>
<tr>
<td><em>S. typhimurium</em></td>
<td>Control</td>
<td>6.33 a</td>
<td>4.67 a</td>
<td>6.26 a</td>
</tr>
<tr>
<td></td>
<td>UV-treated</td>
<td>2.35 b</td>
<td>1.23 b</td>
<td>1.63 b</td>
</tr>
<tr>
<td></td>
<td>SEM³</td>
<td>0.52</td>
<td>0.29</td>
<td>0.47</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Control</td>
<td>7.17 a</td>
<td>7.62 a</td>
<td>7.73 a</td>
</tr>
<tr>
<td></td>
<td>UV-treated</td>
<td>2.26 b</td>
<td>2.81 b</td>
<td>3.15 b</td>
</tr>
<tr>
<td></td>
<td>SEM³</td>
<td>0.30</td>
<td>0.39</td>
<td>0.45</td>
</tr>
</tbody>
</table>

*Values within a column with different superscripts differ significantly (*P* < 0.05).  
¹UV intensity (4 to 14 mW/cm²) for 4 min.  
²Number of observations per trial = 14 eggs (seven control, seven UV-treated).  
³Pooled standard error of the mean.

### TABLE 2. Embryonic mortality and hatchability of control and ultraviolet (UV) light-treated eggs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n¹</th>
<th>1 to 18 d</th>
<th>19 to 21 d</th>
<th>HFE²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>776</td>
<td>8.6⁴</td>
<td>6.8</td>
<td>84.5</td>
</tr>
<tr>
<td>UV-treated</td>
<td>1,561</td>
<td>8.3</td>
<td>6.9</td>
<td>84.9</td>
</tr>
<tr>
<td>SEM³</td>
<td>0.85</td>
<td>0.77</td>
<td>1.10</td>
<td></td>
</tr>
</tbody>
</table>

⁴No significant differences exist between means (*P* > 0.05).  
¹n = total number of fertile eggs.  
²Hatch of fertile eggs.  
³Pooled standard error.
that high-intensity UV irradiation of hatching eggs does not adversely affect embryo viability or development.

Results of swab sampling of the contents of infertile eggs or eggs containing dead embryos resulted in no significant differences for bacterial contamination between control and UV-treated eggs. Over the three trials, 14/73 (19.2%) and 13/71 (18.3%) samples were positive for bacterial contamination on sheep blood agar for control and UV-treated groups, respectively. In control samples, 5 of the 14 positive samples were also positive for microbial growth on MacConkey agar, while 3 of 13 UV-treated positive samples were also positive on MacConkey agar. The lack of a significant reduction in internal contamination could be because the eggs used in these trials were treated with UV light following storage in egg coolers. Fromm (1959) showed that eggs develop a negative internal pressure as the contents of the egg cool. This was evidenced by increased penetration of dye into the shell through the pores. If this principle holds true for penetration of bacteria into the egg, eggs used in these trials may have already been internally contaminated before UV treatment was performed.

In conclusion, the results of these studies indicate that high UV intensities can significantly reduce APC, *S. typhimurium*, and *E. coli* levels on eggshell surfaces in a commercial-style egg-handling system. These investigations suggest that UV irradiation of broiler hatching eggs is not detrimental to the cuticle of the egg or the developing embryo, which agrees with the findings of previous work. More extensive research may reveal that UV treatment improves hatchability and reduces internal contamination of eggs. Improved hatchability may be a direct result of decreased microbial contamination of the egg. Less microbial contamination could also aid in the production of cleaner, healthier chicks. These findings warrant larger field studies in a commercial environment to more accurately determine the effects of ultraviolet radiation on not only the hatchability but also the microbial aspects of broiler hatching eggs and the subsequent day-old chicks.

**REFERENCES**


