DEVELOPMENT AND EVALUATION OF A COMMERCIALLY FEASIBLE HYDROGEN PEROXIDE AND ULTRAVIOLET LIGHT EGG SANITIZATION SYSTEM

A Thesis

by

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ABSTRACT

Egg sanitization is important for hatchery sanitation, hatchability and hatchling health. A method of egg sanitization that applies hydrogen peroxide (H_2O_2) to the eggshell followed by ultraviolet light (UV) exposure has been previously studied (H_2O_2/UV method). This method of sanitization utilizes the photolytic reaction that occurs between H_2O_2 and UV to produce hydroxyl radicals and kill eggshell bacteria. Previous research has applied this method in a rudimentary, lab-scale manner not suitable for commercial implementation. Therefore, the purpose of this study is to further develop the H_2O_2/UV method for commercial implementation. An apparatus was built to mechanize the process and apply to eggs at commercially feasible speeds. A series of experiments was conducted to optimize the parameters of H_2O_2/UV apparatus application. Results from these experiments showed a significant eggshell bacterial reduction ($P < 0.001$) when eggs were treated with the H_2O_2/UV apparatus. To make the implementation of the apparatus in a commercial setting more economically feasible, experiments were also conducted to optimize the treatment of eggs on commercial incubator flats. Samples analyzed in laboratory tests and field trials showed that eggshell bacterial counts were reduced to levels less than the limit of detection (< 20 cfu/egg) for nearly all eggs when treating eggs on commercial incubator flats. Incubation experiments showed that the H_2O_2/UV apparatus treatment did not negatively impact hatchability or chick quality. This research demonstrated that the H_2O_2/UV apparatus treatment can effectively reduce eggshell bacteria of hatching eggs in a

manner that is commercially feasible and does not negatively impact hatchability or chick quality.

DEDICATION

I would like to dedicate this thesis to my wife, Ashley Fuchs, and parents, Susan and Gary Fuchs. Your patience, love, guidance, and support made this possible.

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NOMENCLATURE

d	Day
DI	De-ionized water
H_2O_2	Hydrogen peroxide
UV	Ultraviolet light
cfu	Colony forming unit
Log	Logarithmic
OSHA	Occupational Safety and Health Administration
mL	Milliliter
PBS	Phosphate buffered saline
LOD	Level of detection
APC	Aerobic plate count
min	Minute(s)
s	Second(s)
QAC	Quaternary Ammonium Compounds
HOF	Hatchability of Fertile Eggs
TEM	Total Embryonic Mortality
wk	Week

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CHAPTER I

INTRODUCTION

The implementation of an effective egg sanitization method is an important step in hatchery sanitation. High levels of bacterial contamination can still occur on visibly clean eggs. The microorganisms present on the eggshell have the potential to invade the egg contents, contaminate hatchery surfaces and equipment and infect newly hatched chicks. High levels of microorganisms on the eggshell can potentially negatively impact chick hatchability, infection, and performance (Scott and Swetnam, 1993b). Contaminated farm surfaces, nests, equipment, and vehicles can transmit bacteria to eggshells. Pathogens, such as *Salmonella*, can infect newly hatched chicks and survive with the bird through growth and potentially to the processing plant. Human cases of salmonellosis can be linked back to broilers that obtained *Salmonella* infection from contaminated eggs (Bains and MacKenzie, 1974). An effective egg sanitization program should reduce the eggshell bacterial levels without impacting the viability of the developing embryo. Furthermore, an effective egg sanitization method should be applied in a timely and cost efficient manner in order to be implemented into a commercial breeder farm or hatchery.

Methods of egg sanitization using formaldehyde, chlorine dioxide, hydrogen peroxide (H_2O_2), quaternary ammonium compounds (QAC), and ultraviolet light (UV) have been studied. Formaldehyde fumigation can effectively reduce the eggshell bacterial levels without impacting the embryo, but hazards to human health associated with the use of formaldehyde have caused the Occupational Safety and Health

Administration (OSHA) to regulate its use (Williams, 1970; OSHA, 1991). Due to these health concerns and OSHA regulations, formaldehyde fumigation is no longer used to treat eggs prior to incubation in the United States. It is unclear whether or not chlorine dioxide is effective in reducing eggshell bacterial contamination. Scott and Swetnam (1993b) hypothesized the chlorine dioxide is neutralized by the protein complex of the egg's cuticle before having the opportunity to kill any microorganisms. Quaternary ammonium compounds effectively reduce eggshell bacterial contamination, but also impact eggshell permeability, thus potentially impacting hatchability or chick weight (Brake and Sheldon, 1990). Ultraviolet light (UV) at intensities of 4 to 14 mW/cm^2 reduced eggshell aerobic bacterial levels 1 to 2 $\log_{10}$ cfu/egg in a study performed by Coufal et. al (2003). An effective eggshell sanitization method reduces eggshell bacteria in a safe and time efficient manner without negatively impacting hatchability or chick quality. None of the previously described egg sanitization methods meet this description. Therefore, the development of an effective and commercially feasible egg sanitization method is necessary.

Wells et. al. (2010) studied an egg sanitization method using H_2O_2 and UV in combination (H_2O_2/UV method). The experiments in that study determined that misting eggs with 1.5% H_2O_2 followed by exposure to UV reduced eggshell bacteria to levels lower than H_2O_2 or UV independently. This method utilizes the photolytic reaction between H_2O_2 and UV to create hydroxyl radicals and kill microorganisms. The UV exposure time in that study was 8 min, which would not be feasible for implementation in a commercial breeder farm. However, Gottselig (2011) found that 5 s of UV exposure

was enough time for the photolytic reaction to occur and inactivate bacteria. That study determined the optimal parameters for the H_2O_2/UV method was 3% H_2O_2 mist followed by 5 s of UV exposure, with the process applied twice.

Based on the studies performed by Wells et. al. (2010) and Gottselig (2011), it has been demonstrated that the H_2O_2/UV method reduces the number of aerobic bacteria present on the eggshell to very low levels without negatively impacting the developing embryo. If an apparatus could be built that applies the H_2O_2/UV method in a more time efficient manner, it could likely be implemented at a commercial breeder farm.

The overall goal of this research was to construct an apparatus that could effectively apply the H_2O_2/UV method at commercially feasible speeds (H_2O_2/UV apparatus) and assess the impact of hatching egg treatment on embryo mortality, hatchability and chick quality. To accomplish this goal, the specific objectives of this research were: 1) construct an apparatus that applies the H_2O_2/UV method at commercially feasible speeds; 2) determine the design and operational parameters necessary for maximum eggshell bacterial reduction; 3) determine if eggs could be successfully treated on commercial incubator egg flats; 4) determine the impact of the H_2O_2/UV method on embryo mortality, hatchability and chick quality for eggs stored for various amounts of time.

CHAPTER II

LITERATURE REVIEW

Hatching Egg Contamination

Eggs can become infected through vertical transmission of microorganisms from the hen's reproductive tissue to the egg contents or through horizontal transmission of microorganisms from the environment to the eggshell (De Reu, et al., 2006). Horizontal transmission can also occur from nest boxes, storage rooms, farm equipment, and trucks. Once inside the incubator, microorganisms from hatching eggs can become airborne and travel throughout an incubator, thus risking infection of all chicks in the machine. If the microorganisms are pathogenic they can cause chick mortality or morbidity (Avens, et al., 1975). Previous research also indicates that the microorganisms present on the surface of hatching eggs can penetrate the shell and contaminate the contents of the egg (Cox, et al., 2000).

In a study performed by De Reu et. al. (2006), eggshell factors impacting eggshell bacterial penetration were evaluated. The eggshell characteristics observed included shell surface area, shell thickness, number of pores, weight loss at the pores and cuticle deposition. To determine bacterial penetration, egg contents were drained and the shell was filled with a molten agar that was allowed to harden. Eggs were then candled, and areas where the agar had changed color were identified as areas of bacterial penetration. Results from that study showed that eggshell area, shell thickness, and number of pores had no significant impact on bacterial penetration. The cuticle deposition of penetrated eggs was significantly lower than non-penetrated eggs. Quarles

et. al. (1970) studied bacterial contamination in poultry and its relationship to egg hatchability using 3 houses with wire floor and 3 with litter floors. Air samples were collected to determine total bacteria and fungi per cubic foot of air, and egg samples were collected to determine eggshell contamination. The average bacterial counts per cubic foot of air were approximately 9 times higher in litter floored houses compared to wire floor houses. The average bacterial counts were 4.01, 4.02, and 3.87 $\log_{10}$ cfu/egg in the 3 litter floor houses while average bacterial counts per cubic foot of air from the 3 wire floor houses were 3.05, 2.97, and 3.09 $\log_{10}$ cfu/egg. The average bacterial counts on eggshell surfaces from the three litter floor houses were 4.96, 5.12 and 4.69 $\log_{10}$ cfu/egg compared to 3.43, 3.39 and 3.36 $\log_{10}$ cfu/egg in wire floor houses. In the same study, eggs from the wire and litter floor houses were incubated to measure hatchability. Pipped eggs and late dead embryos from the litter floor houses were 94% positive for coliforms while the same eggs from the wire floor houses were 33% positive for coliforms. These results led to the conclusion that the high percentage of coliform positive embryos and chicks from litter floor houses was related to the higher airborne bacteria concentrations.

Salmonella is one of the microorganisms of concern for eggshell and egg content contamination. *Salmonella* contamination can occur via horizontal and vertical transmission. In the vertical route, the yolk membrane or the albumen of the egg becomes infected because of *Salmonella* infection in the reproductive organs of the hen during egg formation (Messens, et al., 2005). *Salmonella* organisms can be shed through the feces of chickens, leading to contamination of litter and nests, and thus exposing

freshly laid eggs to potential contamination with *Salmonella* (Williams and Dillard, 1973; Williams, et al., 1968). Williams et. al. (1968) determined that *S. typhimurium* could penetrate the eggshell within 6 min after exposure. While the study noted that the incidence of such rapid penetration was low, it was also noted that a single egg contaminated with *S. typhimurium* could contaminate large amounts of adjacent eggs and chicks when broken or when hatching occurs. Bains and MacKenzie (1974) collected samples of eggs, autopsied breeder stock, litter, nest material, day-old broilers, grains and feed from a commercial poultry operation for a 9-month period to investigate the transmission of *Salmonella* within the operation. From these samples, they concluded that *Salmonella* had the potential to travel from breeder feed, to parent stock, to day-old chick and eventually to the processing plant. This conclusion implies that a single source of *Salmonella* has the potential to contaminate an entire integrated poultry complex. If *Salmonella* were to survive with the chick through growth and to the processing plant, human infection becomes a concern (Cox, et al., 2000).

Egg Structure and Natural Protection

The cuticle, shell, shell membranes, and inhibitory proteins in the albumen serve as natural defenses for the egg against bacterial invasion (Brown, et al., 1965). The cuticle of the egg is a thin film-like layer, primarily made up of proteins, that covers the shell of the egg and serves as the first line of defense for prevention of microorganism penetration (Board and Fuller, 1994). Wellman-Labadie et. al. (2008) studied the lysozyme activity of the cuticle. The inhibition of *B. subtilis*, a lysozyme sensitive Gram-positive bacteria, led to the determination that the cuticle can work chemically

through antimicrobial properties to protect the eggs from bacterial invasion. The cuticle does not always exist or cover the entire shell. Board and Halls (1973) studied the eggshell cuticle as a barrier for liquid penetration and reported 8% of the eggs in their study did not have complete cuticle coverage of the eggshell. In the same study, the cuticle was artificially removed using solutions of ethylene diamine tetraacetic acid, sodium hydroxide and sodium sulfide. When the cuticle was removed, an increase in particle and liquid penetration was observed (Board and Halls, 1973). Alls et. al. (1964) observed a microbial contamination increase from 20 to 60% when the cuticle was removed.

The eggshell is covered with between 7,000 and 17,000 pores that can act as gateways to the inside of the egg for bacteria (Solomon, 2010). When the cuticle and egg membranes are not present, the eggshell allows unrestricted passage of bacteria through the pores because the pores are too large to filter bacteria (Garibaldi and Stokes, 1958). Lifshitz et. al. (1964b) measured the resistance time of the eggshell and egg membranes to the penetration of *Pseudomonas fluorescens.* The resistance time of the eggshell, outer membrane and inner membrane combined was similar to the resistance time of the inner membrane independently. This led to the determination that the eggshell and outer membrane had less of an impact on the prevention of bacterial penetration compared to the inner membrane.

The outer shell membrane is made up of felted fibers that lie parallel to the shell. The inner shell membrane is a mesh of small, compact keratin fibers with a mucin-like coating that makes the structure non-porous (Garibaldi and Stokes, 1958;Walden, et al.,

1956). Lifshitz and Baker (1964a) weighed and measured the inner and outer egg membranes and found that the outer membrane was on average 6 times heavier and 3 times thicker than the inner membrane. Though the outer membrane is significantly heavier and wider, the inner membrane is found to be more resistant to bacterial penetration because of the compact nature of its structure (Walden, et al., 1956). In an experiment performed by Garibaldi and Stokes (1958), the eggshell and outer membrane combined were able to hold back 98 to 99% of the bacteria; however, it was noted that hundreds of thousands of bacteria were still able to penetrate these barriers. In the same study, no bacteria were able to penetrate the shell membrane system when the inner membrane was present. Stokes and Osborne (1956) cultured bacteria on an egg membrane and saline solution and observed rapid growth after 5 h of incubation, whereas a decrease of bacteria was observed on a saline only solution. This led to the determination that the eggshell membranes do not contain any chemical antimicrobial properties and that the protection they provide from bacterial penetration is solely mechanical. In fact, Stokes and Osborne (1956) concluded that the eggshell membranes provided the bacteria with the nutrients and protection needed for growth and survival.

Impact of Hatching Egg Storage

Lapão et. al. (1999) studied the impact of storage time and broiler breeder hen age on albumen height, pH, hatchability and embryo mortality. In that study, eggs were collected from two broiler breeder flocks at 32 and 54 wks of age and 42 and 59 wks of age respectively. Some of the eggs collected began incubation the day of collection, while others were stored at interval groups of 1, 4 and 8 d. Albumen pH and height were

measured to determine the impact of hen age and storage. All age groups showed an increase in albumen pH as storage time increased. Eggs collected from older flocks had a higher d 0 albumen pH, but as storage time elapsed, albumen pH between age groups became more comparable. The change in albumen pH increased from 8.20 to 9.15 in eggs stored for 8 d, but the majority of the increase was observed between d 0 and 4 of storage. The number of viable eggs decreased as the storage time increased for both age groups, but more so in the older group. The same phenomenon was observed for hatchability. Because viability decreased at a greater rate in older birds, and pH was similar across age groups for eggs stored longer than 4 d, there was no correlation found between albumen pH and hatchability. However, the height of the albumen, a measure of albumen quality, for the older birds was significantly lower. Therefore, Lapão et. al. (1999) hypothesized that the decrease in albumen quality as the flock age and time of storage increased was the main cause for the decrease in viability and hatchability.

Methods of Hatching Egg Sanitization

Formaldehyde has bactericidal and antifungal properties that make it useful as a disinfectant for many industries (OSHA, 1991), and formaldehyde fumigation is a method of egg sanitization that is highly effective at reducing the bacterial levels present on the eggshell. Williams (1970) studied the bacterial reduction on brown and white eggs when fumigated with varying concentrations of formaldehyde. The standard concentration of formaldehyde fumigation is created by mixing 1.2 mL of formalin with 0.6 g of potassium permanganate per cubic foot of space. In that study, the eggs were exposed to the standard level of formaldehyde fumigation as well as concentrations of 3

and 5 times the standard level. A decrease in bacterial levels was observed when the formaldehyde fumigation concentration increased. In brown eggs, a bacterial kill of 99.82% was observed at standard fumigation levels and 99.85% observed at 5 times the standard fumigation level. Furuta and Maruyama (1981) studied the bacterial reduction of egg washing and formaldehyde fumigation. The bacterial counts on dirty floor eggs decreased from 4.5 to 2.7 $\log_{10}$ cfu/egg after washing with clean water at 40°C. Visibly clean cage and floor eggs had bacterial counts of 3.1 and 3.0 $\log_{10}$ cfu/egg respectively prior to wash, and after wash the average bacterial counts were 2.0 and 2.3 $\log_{10}$ cfu/egg, respectively. The eggs were treated with formaldehyde fumigation after washing. The washed-dirty eggs average bacterial counts were reduced to an average of 0.3 $\log_{10}$ cfu/egg and none of the washed-clean eggs were positive for bacteria after formaldehyde fumigation. Proudfoot and Stewart (1970) studied the impact of post formaldehyde fumigation egg ventilation on hatchability. Regardless of storage time, if eggs were not given time to ventilate after formaldehyde fumigation, a reduction in hatchability was observed. The lack of ventilation time allowed the formaldehyde to diffuse through the eggshell or polymerize and attach to the eggshell. After conducting experiments comparing 24 h of ventilation to 72 h of ventilation, it was determined that 24 h of ventilation was sufficient enough to allow the formaldehyde to naturally dissipate in the atmosphere and avoid hatchability reduction. Williams and Siegel (1969) studied the persistence of formaldehyde on the eggshell surface, within the sub-shell membranes, and in the albumen after formaldehyde fumigation. After the formaldehyde fumigation and a 15 min exhaust period, eggs were held at room temperature from 0 to 120 min.

Formaldehyde levels dropped significantly 30 min after formaldehyde fumigation and exhaust. The levels of formaldehyde in the shell membranes and albumen were low and led to the conclusion that formaldehyde fumigation does not penetrate the egg at any significant level. Williams and Gordon (1970) applied formaldehyde fumigation at 3 and 5 times the usual application of 1.2 mL of formalin and 0.6 g of potassium permanganate to assess the impact of high concentrations of formaldehyde fumigation on chick hatchability. In 2 experiments, the hatchability of eggs treated with standard levels of formaldehyde fumigation was similar to the non-treated control eggs. The hatchability of eggs subjected to high levels of formaldehyde fumigation was slightly lower than the standard fumigation and non-treated control eggs. In both trials, as the concentration of formaldehyde fumigation increased, hatchability decreased. Williams and Dillard (1973) artificially contaminated eggs with *S. typhimurium* after formaldehyde fumigation to determine if the fumigation treatment provided any residual antimicrobial protection. No residual protection from *Salmonellae* penetration was observed, even at twice the standard level of formaldehyde fumigation.

The Occupational Health and Safety Administration (OSHA) regulate formaldehyde use in the workplace. These regulations are due to the health hazards that accompany the use of formaldehyde such as cancer and eye, skin, and respiratory irritation (OSHA, 1991). When considering user friendliness, the strong odor of formaldehyde and the necessity of protective clothing are negative attributes associated with the use of the disinfectant. It was also noted that fumigation gasses are harder to keep from escaping into the workplace than liquid or solid disinfectants (Scott and

Swetnam, 1993). When compared with other eggshell sanitizers, Scott and Swetnam (1993) determined formaldehyde was a severe hazard, citing hazards upon inhalation, eye or skin contact.

Due to the risks associated with the use of formaldehyde, Patterson et al. (1988) studied the use of chlorine dioxide (ClO_2) as a possible egg sanitizer. Temperature differential dipping treatments using ClO_2 were studied on the basis that the room temperature (23°C) eggs dipped into cold (5°C) ClO_2 solution would allow the bactericidal agents to enter shell pores and kill invading microorganisms. It was determined that eggs dipped into 10 ppm ClO_2 solution for 5 min had a slight increase in hatchability compared to non-treated eggs, from 83.7% to 86.4%. However, eggs dipped into ClO_2 solutions of 100 ppm and 1000 ppm reduced hatch by 60% and 80%, respectively. It was concluded that the ability of the temperature differential dipping technique to draw the ClO_2 through the pores of the egg caused embryo death when highly concentrated solutions or lengthy dipping times were used. Patterson et. al. (1988) also studied the use of chlorine dioxide foams compared to formaldehyde fumigation. Eggs were covered with 30 ppm ClO_2 foam for 15 min in the experiments conducted. There was no significant difference in hatchability when comparing ClO_2 foaming to formaldehyde fumigation. In a study using fertile duck eggs, there was no significant improvement in hatchability or number of rotten eggs when comparing clean, non-treated duck eggs to ClO_2 foam-treated eggs. In another experiment conducted by Patterson et. al. (1988), it was concluded that the number of bacteria present on the eggshell was greatly reduced by the use of ClO_2 foam because of its ability to react with

the proteins in the cell wall of microorganisms and kill them. However, Scott and Swetnam (1993), when studying the effectiveness of various egg sanitizers against eggshell microorganisms, determined that when ClO_2 is used as an egg sanitizer, it is neutralized by the protein complex of the cuticle before it can kill any microorganisms.

Quaternary ammonium compounds (QAC) effectively reduce the level of microorganism contamination on the eggshell (Scott and Swetnam, 1993b) in a manner that is both cost effective and user friendly (Scott and Swetnam, 1993). Brake and Sheldon (1990) studied the use of a 1.5 to 3.0% QAC spray. Eggs that were not sprayed with the sanitizer had aerobic plate counts (APC) of 5.17 $\log_{10}$ cfu/egg while the treated eggs had APC of 2.22 $\log_{10}$ cfu/egg. Cox et. al. (1994) studied the use of an automatic spray machine application of a per-oxygenic compound prewash with a QAC sanitization spray after wash. Clean eggs treated with this method of sanitization had a 3.6 $\log_{10}$ cfu/egg reduction in total aerobic bacteria and a 1.4 $\log_{10}$ cfu/egg reduction in coliforms. Dirty eggs treated with the same treatment showed a 3.1 $\log_{10}$ cfu/egg and 0.4 $\log_{10}$ cfu/egg reduction in total aerobic bacteria and coliforms, respectively. Bierer et. al. (1961) determined that QAC were not dependable for killing *S. typhimurium* . While the application of QAC reduces bacterial contamination, the application also affects egg permeability. Brake and Sheldon (1990) observed a significant increase in eggshell permeability when using a 3% QAC spray. The alteration of the eggs natural gaseous exchange and water loss properties complicates the use of QAC on hatching eggs (Brake and Sheldon, 1990). Furthermore, a treatment that alters the cuticle of the egg increases the potential of invading microorganisms (Williams and Dillard, 1973).

Ultraviolet light (UV) radiation is known to kill various types of microorganisms through a photochemical reaction within the nucleic acid of the microorganism (Wells, et al., 2010). Scott (1993) determined UV radiation was an effective means of reducing eggshell bacterial loads. Berrang et. al. (1995) studied the affect of UV exposure on *Salmonella* by exposing eggs inoculated with *S. typhimurium* to UV. The number of *Salmonella*-positive eggs was reduced by 63% and 71% when exposed to 5 and 10 min of UV, respectively. In the same study, there was not a significant reduction of *Salmonella*-positive eggs for eggs that had stains and fecal contamination; therefore, it was determined that UV was not able to penetrate fecal contamination. There was no significant impact on the hatchability of eggs exposed to UV throughout incubation when compared to non-treated control eggs. Chavez et. al. (2002) observed a reduction in aerobic bacteria from 3.96 $\log_{10}$ cfu/egg to 1.98 $\log_{10}$ cfu/egg on eggs exposed to UV at an intensity of 7.35 mW/cm^2. In a subsequent study, Coufal et. al. (2003) observed a significant reduction in aerobic bacteria (1 to 2 $\log_{10}$ cfu/egg), *S. typhimurium* (3 to 4 $\log_{10}$ cfu/egg) and *Escherichia coli* (4 to 5 $\log_{10}$ cfu/egg) on eggs exposed to UV at an intensity of 4 to 14 mW/cm^2 for 4 min. Wells et. al. (2010) exposed eggs to UV for varying amounts of time from 0 to 32 min. Eggs exposed for 16 min experienced the greatest reduction of aerobic bacteria; however, it was found that the extended exposure to UV in the prototype UV cabinet raised the internal temperature of the egg to 37°C. An internal egg temperature that high would risk inducing embryonic development before incubation. Exposing eggs for 8 min to UV resulted in a bacterial reduction of 2.07 $\log_{10}$ cfu/egg without exceeding the 29°C embryonic development threshold.

Hydrogen peroxide (H_2O_2) is a strong oxidizer and effective surface disinfectant. At low concentrations (3 to 6%) used for egg sanitization, toxicity of the solution is low and any waste spray degrades to merely water and oxygen (Sander and Wilson, 1999). Padron (1995) dipped eggs into a H_2O_2 solution using a pressure differential dipping technique to evaluate bacterial reduction. The purpose of dipping is to allow the H_2O_2 to access the shell membranes and kill invading bacteria. Dipping eggs into a 6% H_2O_2 solution decreased the number of *S. typhimurium*-positive eggs 55% and the number of *S. typhimurium* colonies present in the eggshell membranes by 95%. Hatching eggs dipped into 6% H_2O_2 did not significantly affect hatchability compared to non-treated control eggs. Furthermore, there was no significant difference in embryo and chick mortality or chick quality. Sander and Wilson (1999) treated eggs with a H_2O_2 mist during incubation. At d 19 of incubation, the bacterial levels in the treated incubators were significantly lower than in the incubators that were misted with distilled water. An increase in water loss was observed in eggs treated with H_2O_2, but this change caused no significant impact to hatchability of fertile eggs or chick weights. The use of H_2O_2 in that study also had no impact on broiler livability, feed conversion, or body weight.

Bierer et al. (1961) studied egg washing solutions and their ability to kill *S typhimurium* present on eggshells. Their study determined that the chemical ingredients that had a germicidal effect against *S. typhimurium* were: calcium hypochlorite, sodium phosphate tribasic, sodium o-phenylphenate, sodium hypochlorite, formaldehyde, potassium permanganate, pyridine and zinc sulphate. They observed that washing eggs with a 1% zinc sulphate solution for 3 min at 110°F was optimal because of its low

toxicity and effective eggshell germicidal effect. No negative impact on turkey egg hatchability was observed in that study using the zinc sulphate egg washing method.

Hydrogen Peroxide and Ultraviolet Light Egg Sanitization Method

Wells et. al. (2010) studied an egg sanitization method that combined H_2O_2 and UV (H_2O_2/UV method). Their study initiated research into the parameters of UV exposure and H_2O_2 concentration necessary for maximum bacterial reduction on eggshells. Experiments were conducted to determine the most suitable H_2O_2 concentration by pairing 8 min of UV exposure with H_2O_2 concentrations varying from 0.5 to 3%. In all experiments, eggs that were treated with the H_2O_2/UV method had lower bacterial counts than UV exposure or H_2O_2 independently. A H_2O_2 concentration of 1.5% and UV exposure of 8 min was found to reduce bacterial counts by 3.3 $\log_{10}$ cfu/egg compared to non-treated control eggs. They concluded that 1.5% H_2O_2 spray combined with 8 min of UV exposure were the optimal treatment parameters for H_2O_2/UV method application.

The bacterial reduction observed using the H_2O_2/UV method is due to the production of hydroxyl radicals that are created by the irradiation of H_2O_2. Hydroxyl radicals are used in the immune system to kill invading bacteria. It has been proposed that these same bactericidal effects is the mechanism responsible for killing bacteria with this method of egg sanitization (Ikai, et al., 2010) (Gottselig, 2011).

Gottselig (2011) used a 3% H_2O_2 mist and 8 min of UV exposure and observed a 2.62 $\log_{10}$ cfu/egg reduction on treated eggs; however, 8 min of UV exposure was found to be impractical for commercial implementation, prompting further research into

optimal application parameters. Application of 3.0% H_2O_2 followed by 1 min of UV exposure yielded a 2.72 $\log_{10}$ reduction. Experiments using concentrations of H_2O_2 greater than 3.0% and UV exposure longer than 1 min showed no added benefit. It was eventually determined that as little as 5 s of UV exposure after H_2O_2 application was effective at creating the hydroxyl radicals necessary for bacterial reduction. Repeated application of the H_2O_2/UV method showed further bacterial reduction. One application resulted in a 2.57 $\log_{10}$ cfu/egg reduction, while two applications resulted in a 3.05 $\log_{10}$ cfu/egg reduction. Experiments testing more than two applications of the H_2O_2/UV method showed no added benefit. Based on this information, Gottselig (2011) concluded that a 3% H_2O_2 mist, followed by 5 s of UV exposure, repeated twice, with an egg rotation between applications, was the most effective application process for eggshell bacterial reduction.

Gottselig (2011) also evaluated the impact of the H_2O_2/UV method on hatchability and chick parameters. Breeder eggs were collected and treated at a commercial farm with the H_2O_2/UV method and found eggshell bacterial reduction of 3.36 $\log_{10}$ cfu/egg. At the hatchery, an eggshell bacterial reduction of 3.17 $\log_{10}$ cfu/egg was found. There was no significant difference observed in egg moisture loss during incubation. Furthermore, hatchability and chick weight were not impacted by the application of the H_2O_2/UV method to the eggs. Gottselig (2011) concluded that the H_2O_2/UV method was an effective and inexpensive eggshell sanitization method that has the potential to be implemented into a commercial breeder farm setting.

CHAPTER III

DEVELOPMENT AND EVALUATION OF AN APPARATUS TO APPLY THE H_2O_2/UV METHOD AT COMMERCIALLY FEASIBLE SPEEDS

Introduction

Egg sanitization is important for hatchery sanitation, hatchability, chick health and chick quality. Previous research into egg sanitization methods has failed to identify a safe and effective egg sanitization as an alternative to formaldehyde fumigation that does not impact hatchability. Formaldehyde fumigation effectively reduces the number of eggshell bacteria (Williams, 1970), however the use of formaldehyde can be harmful and its use is highly regulated (OSHA, 1991). Chlorine dioxide is presumed to be inactivated by the protein complex of the egg's cuticle before having the opportunity to kill any microorganisms (Scott and Swetnam, 1993b). Quaternary ammonium compounds impact eggshell permeability, possibly impacting hatchability or chick weight (Brake and Sheldon, 1990). Therefore, an egg sanitization method that safely and effectively reduces eggshell bacteria without negatively impacting embryo mortality, hatchability or chick quality needs to be developed.

Wells et. al. (2010) began studying an egg sanitization method that applied H_2O_2 and UV to the eggshell surface to reduce bacterial counts. In that study, the initial parameters of H_2O_2/UV method application were defined as 1.5% H_2O_2 spray followed by 8 min of UV exposure. The application of the H_2O_2/UV method following these parameters resulted in a bacterial reduction of 3.3 $\log_{10}$ cfu/egg. While these application parameters were successful is reducing eggshell bacteria, the application was time

consuming, making the implementation of the H_2O_2/UV method at a commercial breeder farm infeasible. Gottselig (2011) further studied the application parameters of the H_2O_2/UV method in order to determine if this egg sanitization method could be effectively applied in a manner suitable for commercial implementation. From the experiments conducted, it was determined that a 3% H_2O_2 mist, followed by 5 s of UV exposure, repeated twice, with an egg rotation between applications, was the most effective application process for eggshell bacterial reduction. The H_2O_2/UV method applied based on these parameters resulted in a bacterial reduction of 3.36 $\log_{10}$ cfu/egg. While these parameters were more time efficient, the application methodology was all done manually with lab-scale equipment, which still made the process too laborious to be implemented into a commercial setting.

The purpose of this study was to build an apparatus that applies the H_2O_2/UV method at commercially feasible speeds and to determine the optimal parameters of H_2O_2/UV method application. Experiments were conducted to test the parameters of H_2O_2 concentration and source, conveyor speed and the number of applications required. In order to make the H_2O_2/UV method more commercially feasible, the ability to treat eggs arranged on plastic incubator flats was also studied.

Materials and Methods

Design of the prototype egg sanitization apparatus

A prototype apparatus was constructed (H_2O_2/UV apparatus) to apply the H_2O_2/UV method based on the optimal application parameters determined by Gottselig (2011) (Figure 1). The apparatus employs a stainless steel wire conveyor to transport the

eggs through the application chambers. The conveyor was powered by a gear motor and pulley system. Conveyor speed can be altered by moving the belt to different size pulleys on the drive motor or shaft that pulls the conveyor. Along the conveyor, were four application chambers where the H_2O_2/UV method was applied to the eggs. In the initial configuration, 2 H_2O_2 chambers were equipped with4 spray nozzles, 2 located above the conveyor and 2 located below. The H_2O_2 mist was applied using nozzles with a cone-shaped spray pattern in order to achieve maximum H_2O_2 coverage on the eggshell (Figure 2). Following each of the H_2O_2 chambers, a UV chamber was constructed where the eggs could be exposed to UV-C at intensities ranging from 8 to 12 mW/cm^2. Eight UV-C lamps (G20T5, Sankyo Denki, Japan) are located within each UV chamber with 4 above the conveyor and 4 below so that maximum egg exposure is accomplished. Following the parameters described by Gottselig (2011), the H_2O_2/UV apparatus exposes the eggs to 2 applications of H_2O_2 and UV. After the treatment is complete, the eggs travel from the conveyor to an attached collection table where they can dry before being placed onto storage flats, cartons or incubator flats.

Figure 1. Schematic of H_2O_2/UV apparatus.

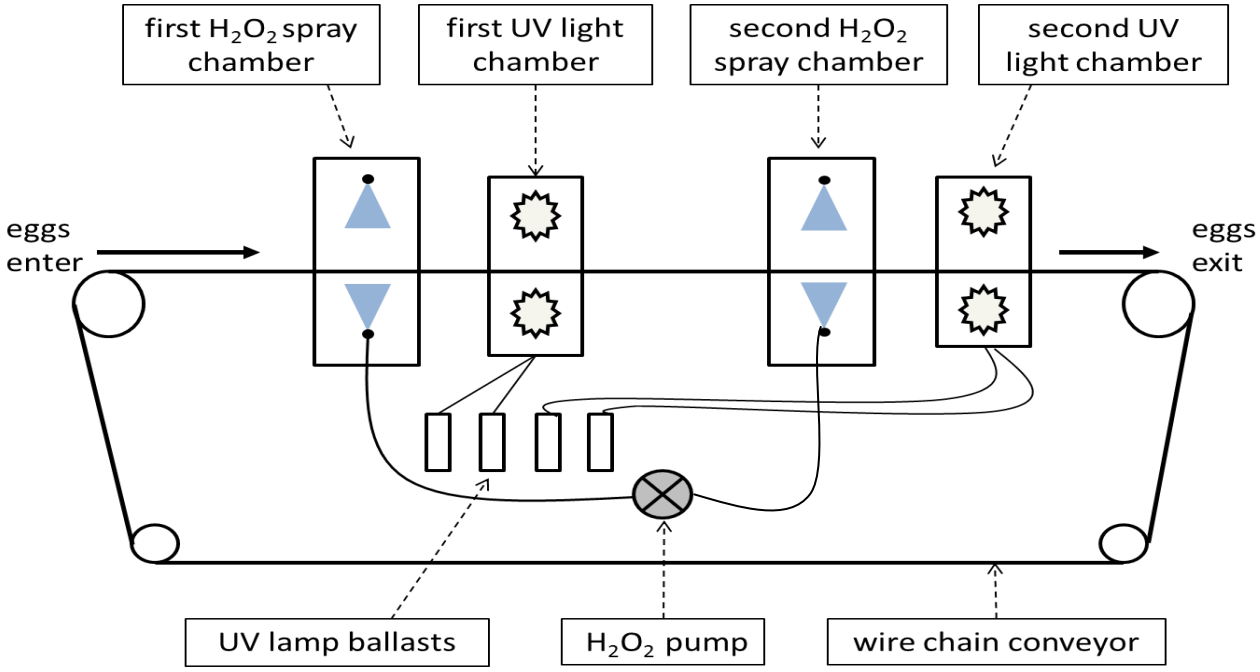
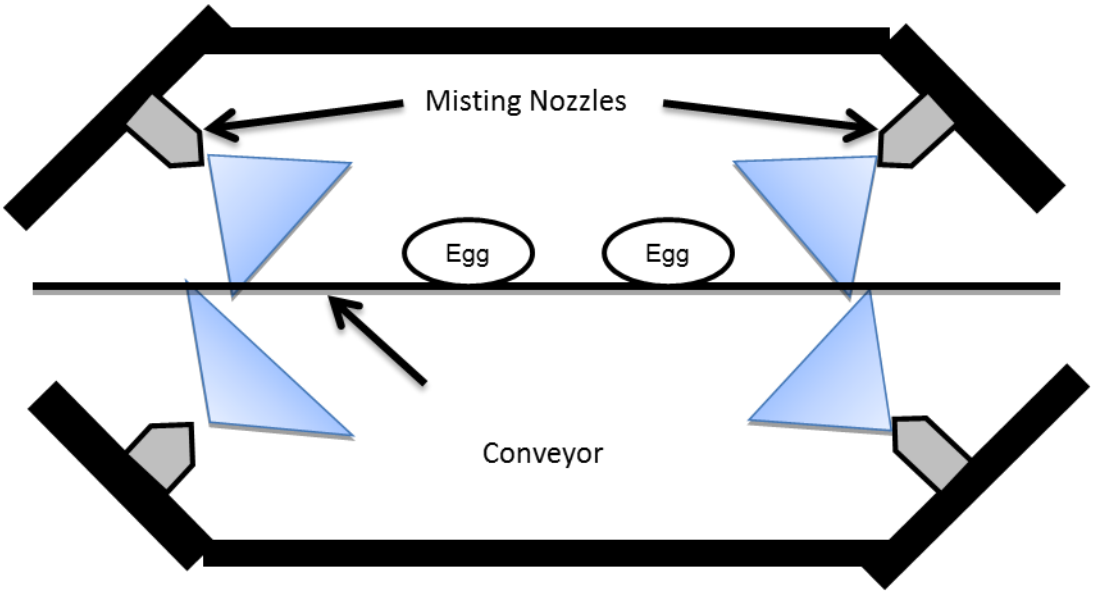


Figure 2. Cross-section of initial H_2O_2 chamber configuration.



Evaluation of the prototype egg sanitization apparatus

A series of experiments were conducted to test the newly constructed H_2O_2/UV apparatus. The purpose of these experiments was to determine the parameters of H_2O_2/UV apparatus operation that would allow for optimal eggshell bacterial reduction. Each experiment was designed according to what was learned in the previous experiment. The H_2O_2/UV apparatus was reconfigured throughout the series of experiments to further optimize bacterial reduction and commercial feasibility.

In Experiment 1, eggs were treated using the H_2O_2/UV apparatus with different sources of H_2O_2 to determine if H_2O_2 diluted on-site from 35% H_2O_2 solution was equally as effective as 3% H_2O_2 solution purchased off the shelf. Gottselig (2011) determined the most effective concentration of H_2O_2 was 3.0%, but was purchased pre-diluted. Six nest eggs were treated using the H_2O_2/UV apparatus with pre-diluted 3.0% H_2O_2, 6 nest eggs were treated with the H_2O_2/UV apparatus using on-site diluted 3.5% H_2O_2, and 5 nest eggs were used as controls. Eggs were exposed to UV intensities of 8 to 12 mW/cm^2 and were treated at a conveyor speed of 7.0 cm/s. At this conveyor speed, the process took 25 s to complete.

Experiment 2 was conducted to determine the efficacy of the H_2O_2/UV apparatus at variable conveyor speeds. Eggs were treated at a 'fast' conveyor speed of 10.2 cm/s and a 'slow' conveyor speed of 5.5 cm/sec. In this experiment, and all following experiments, a 3.5% H_2O_2 concentration diluted on-site with DI water from a 35% solution was used. In total, 6 nest eggs were treated with the H_2O_2/UV apparatus using

the fast conveyor speed, 6 eggs at the slow speed, and 6 nest eggs were used as non-treated controls.

Experiment 3 was conducted to determine the importance of the second application of H_2O_2 and UV. Gottselig (2011) observed a greater eggshell bacterial reduction when repeated applications of H_2O_2 and UV were performed. In this experiment, 6 nest eggs were exposed to both sets of the H_2O_2 and UV chambers while 6 nest eggs were exposed to only 1 H_2O_2 and 1 UV chamber. Six additional nest eggs were used as non-treated controls.

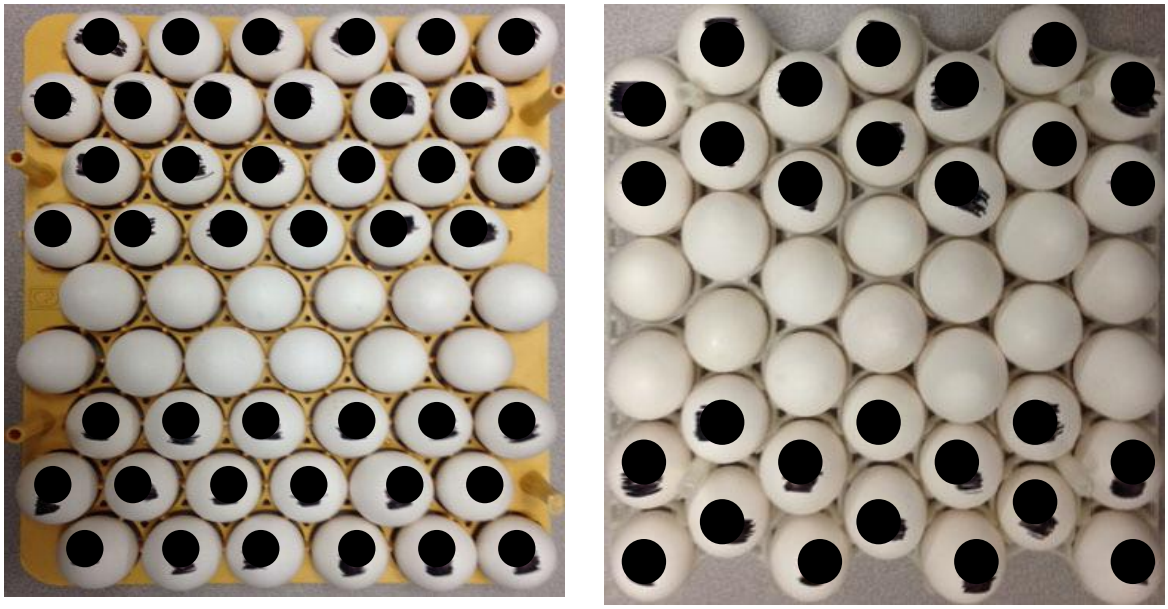
Experiments 1 through 3 were conducted using visibly clean nest eggs. Experiment 4 was conducted to determine if the H_2O_2/UV method could effectively reduce the number of bacteria present on the shell of visibly clean floor eggs. De Reu et. al. (2006) and Quarles et. al. (1970) studied the microbial contamination of eggs in nests, aviaries, and litter houses. These studies found that eggs collected from litter floors had higher bacterial counts than eggs collected from nests or cages. Because floor eggs have higher bacterial counts, the use of floor eggs would provide a greater microbial challenge for the H_2O_2/UV apparatus. In this experiment, 10 visibly clean floor eggs were treated using the H_2O_2/UV apparatus and 5 visibly clean floor eggs were used as controls.

Experiment 5 was conducted to determine the efficacy of the H_2O_2/UV apparatus using 2 different commercial incubator egg flats. A 54-egg plastic flat and a 42-egg plastic flat were used. In this experiment, visibly clean nest eggs were loaded onto the egg flats and treated with the H_2O_2/UV apparatus. Twelve eggs were sampled from the

54-egg flat while 14 eggs were sampled from the 42-egg flat. The position of the eggs sampled from the 54-egg and 42-egg flat are depicted in Figure 3. Eggs without a black dot were sampled. In addition, 6 eggs were treated with the H_2O_2/UV apparatus without a flat and 3 eggs were used as non-treated control eggs.

Figure 3. Position of eggs treated on a 54-egg (a) and 42-egg (b) plastic incubator flat. Eggs without a black dot were the eggs sampled for microbial enumeration (Experiment 5).

(a) (b)



Based on the results from Experiment 5, it was hypothesized that an increased H_2O_2 spray coverage was necessary to effectively treat eggs on commercial incubator flats. Therefore, 2 spray nozzles, 1 nozzle above the conveyor and 1 below it, were added to the original configuration (Figure 4). To further increase the egg exposure to H_2O_2, the conveyor was set to the 'slow speed' used in Experiment 2 (5.5 cm/s).

Experiment 6 was conducted to compare the bacterial reduction of eggs treated with the H_2O_2/UV apparatus on fully loaded flats compared to eggs spaced on flats not filled to capacity. The eggs were spaced in this experiment to determine whether the vertical orientation of the egg on the flat, or the lack of H_2O_2 spray coverage was the cause of low bacterial reduction in Experiment 5. Figure 6a illustrates the position of the eggs sampled from the full 54-egg flat used in this experiment. Figure 5b illustrates how eggs were arranged and sampled on the partially filled flats. Additionally, 6 eggs were treated on the H_2O_2/UV apparatus conveyor and 3 eggs were used as non-treated controls.

Figure 4. Second H_2O_2 nozzle configuration (Experiment 6)

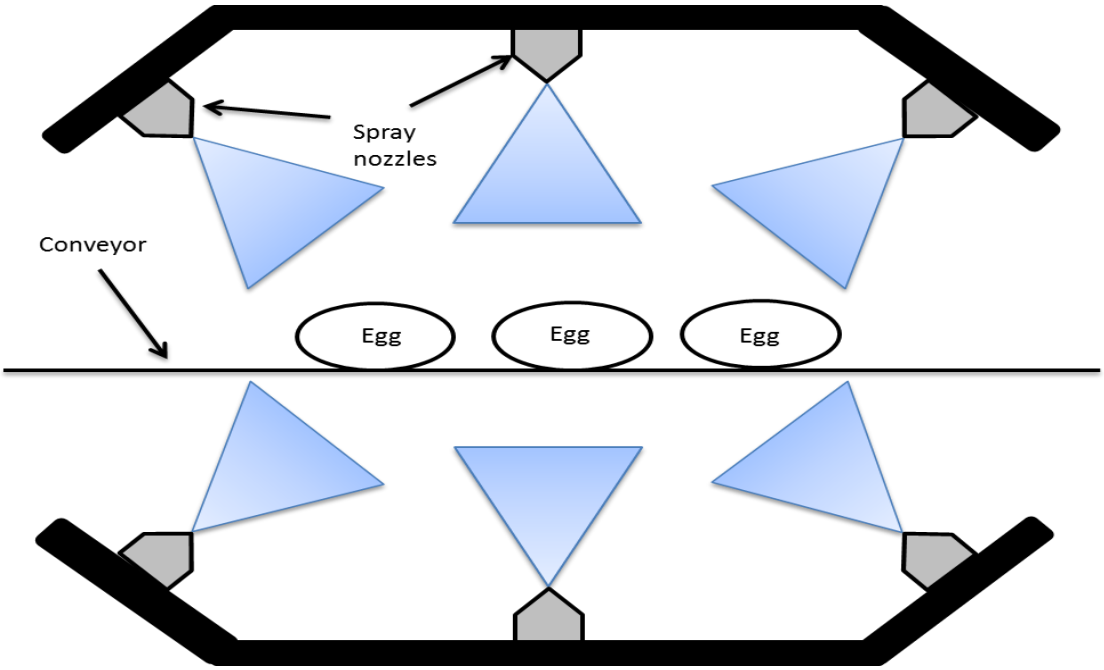
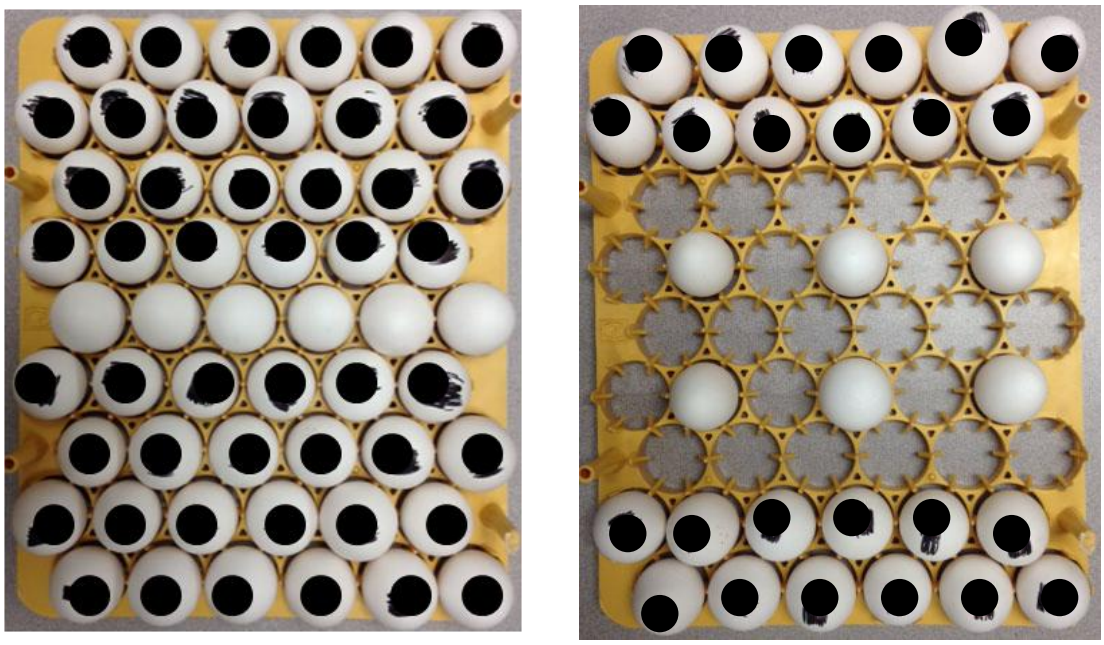


Figure 5. Position of eggs sampled from a full 54-egg flat (a) and a partially filled 54-egg flat (b). Eggs without black dots were the eggs sampled (Experiment 6).



(a) (b)

Based on the results from Experiment 6, it was determined that a further increase of H_2O_2 spray was necessary to treat eggs on incubator flats. Therefore, a mist configuration that used 10 nozzles, 5 above of the conveyor and 5 below (Figure 7), was implemented for Experiment 7. In this experiment, eggs were treated on a full 168-egg plastic flat. Figure 8 illustrates the position of the 14 eggs sampled in this experiment. Additionally, 3 eggs were sampled as non-treated controls.

Figure 6. Third H_2O_2 nozzle configuration (Experiment 7).

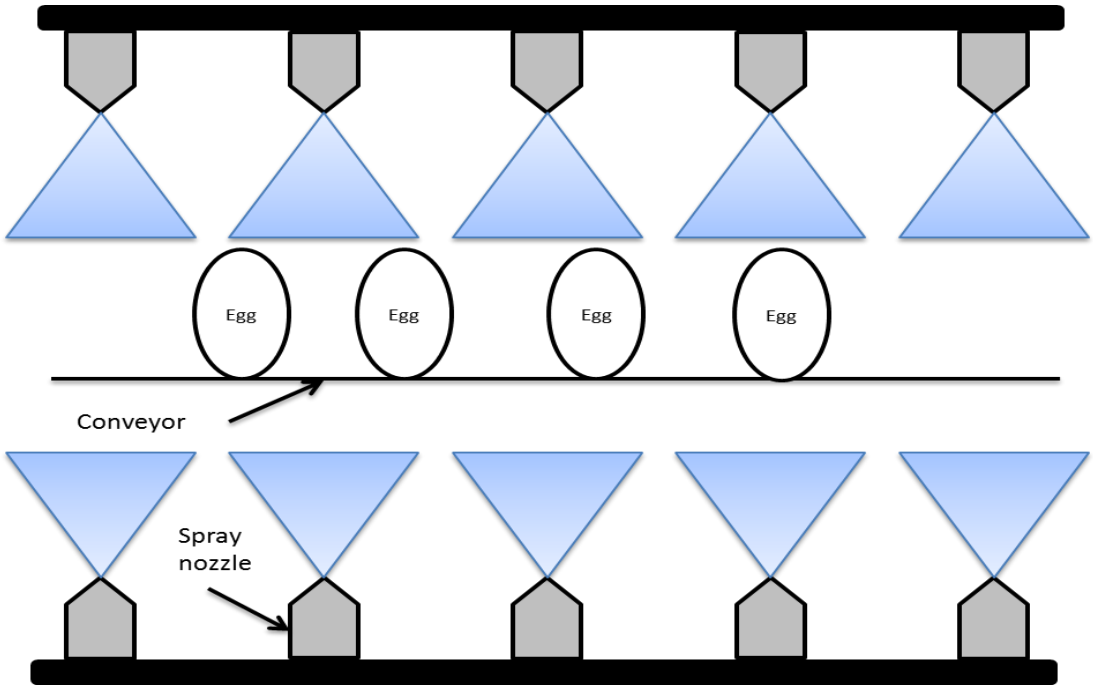


Figure 7. Position of eggs sampled from a full 168-egg flat. Eggs without black dots were the eggs sampled (Experiment 7)

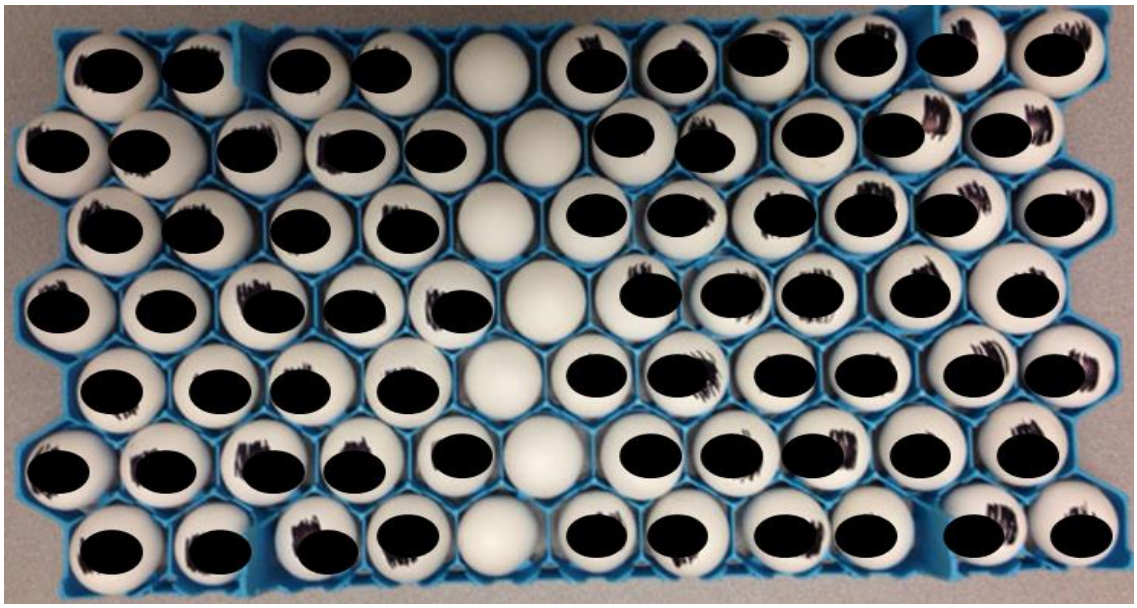
Experiment 8 was conducted at a commercial breeder farm to test the efficacy of the H_2O_2/UV apparatus at reducing the number of eggshell bacteria on eggs treated directly on the conveyor and on flats. This experiment also provided the opportunity to observe a practical implementation of the H_2O_2/UV apparatus in a commercial setting. In this experiment, 5,000 eggs were treated on 168-egg plastic flats and 5,000 eggs were treated directly on the H_2O_2/UV apparatus conveyor. Seven egg samples were taken from each of the treatment groups and 7 non-treated controls were also collected.

Experiment 9 was conducted to evaluate the efficacy of the H_2O_2/UV apparatus with the 10-nozzle H_2O_2 spray configuration at reducing the number of eggshell bacteria of eggs treated on different types of flats other than the 168-egg flat previously tested. In this experiment the use of 3 different plastic incubator flats were examined; a 54-egg flat, a 42-egg flat and an 84-egg flat. After treatment, a 6 egg sample was taken from the 54-egg plastic flat using the sampling procedures depicted in Figure 6a. Figures 9a and 9b illustrate the eggs sampled from the 42-egg and 84-egg flats respectively.

Figure 8. Position of eggs sampled from a 42-egg flat (a) and an 84-egg flat (b). Eggs without black dots were sampled (Experiment 9).

(a)

(b)



Eggshell bacterial enumeration

A rinse and plate method was used to enumerate eggshell bacteria in each experiment. Each egg was placed in a sterile Whirl-pak bag (Nasco, Fort Atkinson, WI) containing 20 mL of sterile phosphate buffered saline (PBS; pH 7.2). Eggs were hand massaged in the PBS solution for 1 min to dislodge bacteria from the eggshell. For treated eggs, 1 mL of the PBS rinsate was placed directly onto an aerobic plate count (APC) petrifilm (3M, St. Paul, Minnesota). For non-treated control eggs, serial dilutions were performed and 1 mL of each dilution plated on petrifilms. Petrifilms were incubated at 37°C for 24 h. After incubation, petrifilms were counted by hand and expressed as $\log_{10}$ colony forming units per egg (cfu/egg). Therefore, the level of detection (LOD) when plating 1 mL of rinsate from a 20 mL rinse was 20 cfu per egg, or 1.30 $\log_{10}$ cfu/egg. Plates that yielded no colonies were assigned a value of half of the LOD (10 cfu/egg or 1.00 $\log_{10}$ cfu/egg).

Egg selection criteria

Visibly clean eggs were used for all of the experiments conducted. Visibly clean eggs were defined as eggs that are void of organic material and visible stains. Eggs were collected from a White Leghorn flock located at the Texas A&M University Poultry Science Research Facility. Eggs were either collected from a nest or from the floor. Nest eggs were collected from a laying nest with sawdust while floor eggs were collected from a litter floor.

Statistical analysis

Means were compared by analysis of variance (ANOVA) using the general linear model (GLM) procedures of SAS (9.2) and means separated by Duncan's Multiple Range Test. Means were considered statistically different at $P \leq 0.05$. The assumptions of the ANOVA were confirmed.

Results and Discussion

The purpose of Experiment 1 was to evaluate the efficacy of the H_2O_2/UV apparatus at reducing the number of eggshell bacteria and to determine if H_2O_2 diluted on-site was equal to store-bought pre-diluted H_2O_2. The 3.0% H_2O_2 used in this experiment was a pre-diluted concentration bought from a retail store. The 3.5% H_2O_2 was created by diluting a 35% H_2O_2 solution with DI water on-site. Aerobic plate counts of non-treated controls were higher than both H_2O_2/UV method treatment groups (Table 1). All of the eggs treated with the pre-diluted 3.0% H_2O_2 and 5 of the 6 eggs treated with 3.5% H_2O_2 had APC lower than the LOD. The results of Experiment 1 established that the H_2O_2/UV method as applied by the H_2O_2/UV apparatus can effectively reduce eggshell bacteria to very low levels, and established that the use of 3.5% solution diluted on-site from a 35% solution and 3.0% pre-diluted store-bought H_2O_2 solution were equally effective. The ability to transport small amounts of highly concentrated H_2O_2 to the treatment site would be easier than transporting the large amounts of pre-diluted 3% H_2O_2 that would be necessary to treat the large number of eggs produced at a commercial breeder farm.

Table 1. Effect of the H_2O_2/UV method application on eggshell APC when using a 3.0% and 3.5% H_2O_2 mist (Experiment 1).

Treatment	N	$\log_{10}$ cfu/egg
Control	5	$4.03^{a} \pm 0.26$
3.0% H_2O_2 + UV	6	$1.00^{b} \pm 0.00$
3.5% H_2O_2 + UV	6	$1.05^{b} \pm 0.05$

[a,b] Values within the same column with different superscripts differ significantly ($P < 0.05$)

The purpose of the H_2O_2/UV apparatus is to apply the H_2O_2/UV method at speeds feasible for implementation in a commercial poultry breeder farm. The goal of Experiment 2 was to determine the efficacy of the H_2O_2/UV apparatus at various speeds. Eggs in this experiment were treated at 2 different conveyor speeds, a fast speed of 10.2 cm/s and a slow speed of 5.5 cm/s. Effective bacterial reduction at faster speeds would make the implementation of the H_2O_2/UV apparatus in a commercial breeder farm more feasible. Eggs treated at slow and fast speeds showed comparable bacterial reduction when compared to non-treated control eggs (Table 2). Furthermore, 5 of the 6 eggs treated at the fast conveyor speed, and 4 of the 6 eggs treated at the slow conveyor speed had APC lower than the LOD. The results from this experiment establish that the H_2O_2/UV method effectively reduces eggshell bacteria at commercially feasible conveyor speeds. Gottselig (2011) determined that UV exposure of 5 s was adequate to achieve maximum eggshell bacterial reduction. However, the eggs in this experiment treated at the fast conveyor speed were only exposed to UV for approximately 3 s. This indicates that the photolytic reaction of the H_2O_2 and UV creates hydroxyl radicals

almost instantaneously and the H_2O_2/UV method can effectively be applied at rates faster than previously believed.

Table 2. Efficacy of the H_2O_2/UV apparatus at various conveyor speeds (Experiment 2).

Treatment	n	$\log_{10}$ cfu/egg
Control	6	$4.54^{a} \pm 0.19$
Fast (10.2 cm/s)	6	$1.22^{b} \pm 0.22$
Slow (5.5 cm/s)	6	$1.15^{b} \pm 0.10$

`[a,b] Values within the same column with different superscripts differ significantly ($P < 0.05$)

The purpose of Experiment 3 was to determine if the second application of H_2O_2 and UV recommended by Gottselig (2011) is necessary to achieve a high eggshell bacterial reduction. If it could be determined that the second application of H_2O_2 and UV was not necessary, the amount of H_2O_2 used in the application would be reduced as well as the cost of the H_2O_2/UV apparatus operation and construction. In this experiment, eggs treated with only 1 application of H_2O_2 and UV resulted in a bacterial reduction of 2.76 $\log_{10}$ cfu/egg while eggs treated with 2 applications of H_2O_2 and UV resulted in a bacterial reduction of 3.89 $\log_{10}$ cfu/egg when compared to non-treated control eggs (Table 3). The bacterial reductions of both treatments were lower than the non-treated controls, but 2 applications yielded a greater reduction than just one application. These results reinforce previous findings that the second application of H_2O_2 and UV is necessary for maximal bacterial reduction.

Table 3. Eggshell APC following 1 and 2 applications of the H_2O_2/UV method using the H_2O_2/UV apparatus (Experiment 3).

Treatment	N	$\log_{10}$ cfu/egg
Control	5	$4.98^{a} \pm 0.14$
Two Applications	10	$1.09^{c} \pm 0.05$
One Application	10	$2.22^{b} \pm 0.29$

[a,b,c] Values within the same column with different superscripts differ significantly ($P < 0.05$)

The aim of Experiment 4 was to determine if the H_2O_2/UV method could effectively reduce the number of eggshell bacteria present on visibly clean floor eggs. If floor eggs could be sanitized, the number of viable hatching eggs available to the breeder farm would increase. The results from this experiment showed a significant bacterial reduction of 3.03 $\log_{10}$ cfu/egg when comparing visibly clean floor eggs treated with the H_2O_2/UV apparatus to non-treated controls (Table 4). Furthermore, of the 10 treated eggs sampled for plating, 8 yielded colony counts of zero

Table 4. Eggshell APC of visibly clean floor eggs treated with the H_2O_2/UV apparatus (Experiment 4).

Treatment	n	$\log_{10}$ cfu/egg
Control	5	$4.38^{a} \pm 0.14$
Treated	10	$1.35^{b} \pm 0.24$

[a,b] Values within the same column with different superscripts differ significantly ($P < 0.05$)

Experiment 5 was conducted to test the efficacy of the H_2O_2/UV apparatus when treating eggs on plastic commercial egg flats. If the H_2O_2/UV apparatus was able to effectively treat eggs on flats, the time taken to load eggs, and the amount of H_2O_2 necessary to operate the apparatus would be reduced. The reduction of these 2 operation parameters would make the implementation of the H_2O_2/UV apparatus into a commercial setting more cost and time efficient. Eggs treated on the 54-egg flat and the 42-egg flat showed a significant bacterial reduction compared to the non-treated controls (Table 5). However, a far greater bacterial reduction was found on eggs treated directly on the conveyor. The substantial difference between bacterial reductions of eggs treated on the conveyor and eggs treated on flats was believed to be due to insufficient H_2O_2 mist coverage of eggs on the flats. The ability to treat eggs on flats is an important step towards commercial implementation of the H_2O_2/UV apparatus. Treating individual eggs on the conveyor increases the amount of time, labor and H_2O_2 necessary to operate the H_2O_2/UV apparatus. The results of this experiment indicated that alterations to the H_2O_2 chambers of the H_2O_2/UV apparatus would be required.

Table 5. Eggshell APC of eggs treated on commercial incubator flats or placed directly on the wire chain conveyor (Experiment 5).

Treatment	N	log_{10} cfu/egg
Control	3	$4.04^{a} \pm 0.06$
Conveyor	6	$1.18^{c} \pm 0.13$
54-Egg Flat Treated	12	$3.26^{b} \pm 0.88$
42-Egg Flat Treated	14	$3.28^{b} \pm 0.17$

[a,b,c] Values within the same column with different superscripts differ significantly ($P < 0.05$)

The purpose of Experiment 6 was to further investigate the ability to treat eggs on incubator egg flats with the H_2O_2/UV apparatus. Based on the results in Experiment 5, it was determined that eggs on full flats did not receive the H_2O_2 mist coverage necessary for maximum bacterial reduction. In this experiment, the H_2O_2 nozzle configuration was altered to include an additional nozzle above and below the conveyor in each spray chamber, eggs were spaced on 54-egg flats and the conveyor was set at a slower speed in order to increase the amount of H_2O_2 mist received by each egg. As in Experiment 5, eggs treated on the conveyor had a substantially greater bacterial reduction compared to eggs treated on full flats (Table 6). Eggs that were spaced out on the flat had a greater bacterial reduction that on the full flat, demonstrating that the vertical orientation of the eggs on the flats was not the cause of the poor bacterial reduction in Experiment 5. This implies that the cause of the poor bacterial reduction of eggs treated on full flats is due to insufficient eggshell H_2O_2 coverage. However, the bacterial reduction of eggs not on flats was still greater than the eggs treated on spaced

flats. Therefore, further alterations to the H_2O_2 nozzle configuration to increase the H_2O_2 spray coverage of eggshells were necessary.

Table 6. Eggshell APC of eggs treated on filled and partially filled egg flats (Experiment 6).

Treatment	n	$\log_{10}$ cfu/egg
Control	3	$4.12^{a} \pm 0.03$
Conveyor	6	$1.05^{c} \pm 0.05$
Full Flat	6	$2.93^{b} \pm 0.27$
Spaced Flat	6	$1.63^{c} \pm 0.25$

[a,b] Values within the same column with different superscripts differ significantly ($P < 0.05$)

Experiment 7 was conducted to test the third H_2O_2 nozzle configuration (Figure 7). The new nozzle configuration was designed to increase the H_2O_2 mist coverage so that maximum bacterial reduction could be accomplished when treating eggs on completely filled flats. The results from this experiment indicated a significant bacterial reduction of 3.66 $\log_{10}$ cfu/egg for eggs treated on flats compared to non-treated control eggs (Table 7). These results are comparable to data for eggs treated directly on the conveyor in previous experiments. Furthermore, of the 14 eggs sampled from the flat, 11 had APC lower than the LOD. Therefore, it can be concluded that the third H_2O_2 mist configuration provided sufficient H_2O_2 coverage, and the H_2O_2/UV apparatus can effectively treat eggs on flats. The ability to treat eggs on flats makes the implementation H_2O_2/apparatus more commercially feasible. In this experiment, instead of having to

load individual eggs onto the H_2O_2/UV apparatus conveyor, eggs could be loaded in groups of 168 eggs at a time. Treating eggs on flats greatly reduces the amount of time, labor, and H_2O_2 necessary to operate the H_2O_2/UV apparatus.

Table 7. Eggshell APC of eggs treated on full egg flats with the third H_2O_2 nozzle configuration (Experiment 7).

Treatment	N	$\log_{10}$ cfu/egg
Control	3	5.04[a]
Treated	14	1.37[b]

[a,b] Values within the same column with different superscripts differ significantly ($P < 0.05$)

The goal of Experiment 8 was to compare, under field trial conditions, eggshell APC for eggs treated on flats using the third H_2O_2 nozzle configuration to eggs treated on the H_2O_2/UV apparatus conveyor. Previous experiments have shown a significant eggshell bacterial reduction when eggs are treated directly on the H_2O_2/UV apparatus conveyor. If the same bacterial reduction can be accomplished when eggs are treated on full flats, the H_2O_2/UV method would become more time efficient and the implementation of the H_2O_2/UV apparatus into a commercial breeder farm would become more feasible. The results from this experiment showed all of the eggs sampled from the conveyor and flat treatment groups had APC lower than the LOD (Table 8). This data confirms that the H_2O_2/UV apparatus can effectively reduce eggshell bacteria when eggs are treated on incubator egg flats at a commercial breeder farm.

Table 8. Eggshell APC for eggs treated on incubator flats and directly on the H_2O_2/UV apparatus conveyor at a commercial breeder farm (Experiment 8).

Treatment	n	$\log_{10}$ cfu/egg
Control	7	4.00[a]
Conveyor	7	1.00[b]
Flat	7	1.00[b]

[a,b] Values within the same column with different superscripts differ significantly ($P < 0.05$)

Experiment 9 was conducted to further evaluate the bacterial reduction of eggs treated on various styles of incubator egg flats. This experiment compared the treatment of eggs on 3 different incubator egg flats to validate that the process worked equally well on different egg flat configurations. The results from this experiment demonstrated a significant bacterial reduction on eggs treated for each style of flat tested (Table 9), validating the findings from Experiments 7 and 8. These results indicate that the H_2O_2/UV apparatus can effectively treat eggs using various types of commercial incubator flats. This is important because various types of incubator flats are used throughout the poultry industry.

Table 9. Eggshell APC of eggs treated on commercial egg flats (Experiment 9).

Treatment	N	log_{10} cfu/egg
Control	4	5.02[a]
54-Egg Flat Treated	6	1.41[b]
42-Egg Flat Treated	7	1.11[b]
84-Egg Flat Treated	7	1.09[b]

[a,b] Values within the same column with different superscripts differ significantly ($P < 0.05$)

Conclusions

The overall goal of these experiments was to test and optimize the newly constructed H_2O_2/UV apparatus. The results from these experiments determined that concentrated H_2O_2 could be diluted on-site and work as effectively as pre-diluted H_2O_2 purchased from a supplier. The transportation of the amount of pre-diluted H_2O_2 necessary to treat the large amount of eggs produced at a commercial breeder farm would be impractical compared to transporting smaller amounts of 35% H_2O_2/UV. This finding has subsequently been incorporated into the design of a new H_2O_2/UV apparatus. On the new apparatus, an on-board DI water filter and H_2O_2 portioning system is built-in. The DI water system filters the incoming tap water and the portioning system dilutes the 35% H_2O_2 with the DI water to the necessary final concentration for spraying eggs. Experiment 2 also demonstrated that the H_2O_2/UV apparatus could effectively treat eggs at conveyor speeds up to 10.2 cm/s. This experiment showed that the photolytic reaction necessary for eggshell bacterial reduction occurs almost instantaneously. Experiment 3 validated the finding of Gottselig (2011) that the second application of H_2O_2 and UV

was necessary for maximum eggshell APC reductions. Experiments 5 through 9 tested and optimized the H_2O_2/UV apparatus for treating eggs on commercial egg flats. Initial experimentation into treating eggs on flats found that maximum eggshell bacterial reduction was not achieved due to insufficient H_2O_2 coverage of the eggshell. The addition of H_2O_2 spray nozzles above and below the wire chain conveyor provided enough H_2O_2 to reach maximum eggshell bacterial reduction. The ability to treat eggs on incubator flats is an important step towards commercial feasibility of the H_2O_2/UV apparatus. Prior to effective treatment of eggs on flats, eggs were loaded onto the wire chain conveyor of the H_2O_2/UV apparatus individually. The process of loading eggs manually, and then putting them back onto flats was very time consuming. Furthermore, more H_2O_2 was being used because eggs could not be loaded by hand onto the conveyor at a speed that utilized the entire conveyor. Loading eggs onto the H_2O_2/UV apparatus on flats is far less labor intensive or time consuming. Eggs on flats can be loaded in groups of 42 to 168, and due to the width of the flat, nearly the entire conveyor is utilized. The results from these experiments demonstrated that the H_2O_2/UV apparatus can effectively reduce eggshell bacteria of eggs on incubator flats at commercially feasible speed. Future studies should determine the minimum H_2O_2 concentration necessary and the impact of egg sanitization on hatchery sanitation and pathogen prevalence. Studies determining the impact of H_2O_2/UV apparatus treatment on hatchability and chick quality are also needed.

CHAPTER IV

EFFECT OF H_2O_2/UV APPARATUS TREATMENT ON HATCHABILITY AND CHICK QUALITY PARAMETERS

Introduction

The application of the H_2O_2/UV method using a prototype apparatus has been shown to effectively reduce eggshell bacterial loads. However, to be implemented into a commercial breeder farm it must be proven that the application of this egg sanitization method does not negatively impact hatchability or chick quality. Gottselig (2011) performed an experiment at a commercial breeder farm to determine the impact of the H_2O_2/UV method on embryo mortality and chick hatchability of hatching eggs. Eggs were treated immediately after collection and a bacterial reduction of 3.36 $\log_{10}$ cfu/egg was observed. Furthermore, the application of the H_2O_2/UV method had no significant impact on embryo mortality or chick hatchability in that experiment. Since the H_2O_2/UV apparatus developed in Chapter IV achieves similar bacterial reduction, hatch results should also be similar. If an improvement in hatch were found after H_2O_2/UV method treatment, the potential economic benefit of hatching egg treatment would improve the economic feasibility of the H_2O_2/UV apparatus.

The purpose of this study was to determine the impact of the H_2O_2/UV method, as applied by the H_2O_2/UV apparatus, on embryo mortality, hatchability and chick quality of hatching eggs. The ability to store hatching eggs for extended periods of time without a detrimental impact on hatch would be of benefit to some breeder farms that transport eggs long distances to hatcheries. It was hypothesized that if eggs with high

amounts of eggshell bacteria were stored for extended periods of time, the storage time would increase the possibility of bacteria penetrating the eggshell and impacting hatchability. Therefore, the effect of eggshell sanitization using the H_2O_2/UV apparatus prior to extended hatching egg storage prior to incubation was investigated.

Materials and Methods

Hatch experiments

In Experiment 1, eggs were collected over a 5-d period from a White Leghorn breeder flock housed at the TAMU poultry Science Research Center. Each day, at least 300 eggs were collected, and half were treated with the H_2O_2/UV apparatus while the other half were used as non-treated controls. Five sample eggs were collected each day from both treated and non-treated control groups for APC testing. After treatment, eggs were stored in an egg cooler at 18.3°C (65°F) and 75% relative humidity (RH) until the 5 d of collection were completed. On the fifth day of collection, eggs were divided into 6 incubators based on treatment (3 incubators per treatment) for incubation.

Experiment 2 was conducted in a similar manner as Experiment 1. In this experiment, 750 total eggs were collected over a 5-d period from the same White Leghorn breeder flock. Ten eggs were sampled from the treated egg group and 5 eggs were sampled from the control egg group. Storage and setting in the incubators were the same as in Experiment 1.

Experiment 3 was conducted to determine the impact of the H_2O_2/UV apparatus on hatchability and chick quality after long-term fertile egg storage. In this experiment, 760 visibly clean nest eggs were collected from a commercial broiler breeder flock and

transported to the laboratory. Half of the eggs were treated with the H_2O_2/UV apparatus upon arrival at the lab and half were used as non-treated controls. After treatment, samples of treated and non-treated eggs were collected for APC testing. Eggs were then placed into an egg cooler and stored for 18 d under the same conditions as in Experiments 1 and 2. After storage, eggs were divided into 6 incubators (3 incubators per treatment) for incubation and hatching.

Incubation

Eggs were incubated in experimental sized GQF model 1500 incubators (GQF, Savannah, Georgia) for 18 d at approximately 37.5°C (99.5°F) and 55 to 60% RH. In Experiment 1, eggs were candled after 10 days and eggs void of embryonic development were removed. After 18 d of incubation, eggs were removed from the incubators and candled so that non-viable eggs could be removed. Viable eggs were then transferred to GQF model 1550 hatching cabinets (GQF, Savannah, Georgia) until hatch on d 21. On d 21, chick weight, mortality and hatchability were recorded. The same individual for all experiments assessed chick quality. Chick quality parameters assessed included unhealed navals/naval tags, chick deformities and chick strength.

Results and Discussion

The APC results of eggs sampled for each of the 5 d of egg collection in Experiments 1 and 2 are presented in Table 10. Eggshell bacterial counts were significantly lower on treated eggs each day of sampling when compared to non-treated control eggs. The average bacterial reduction of treated eggs when compared to non-treated controls was 3.40 $\log_{10}$ cfu/egg in Experiment 1 and 3.26 $\log_{10}$ cfu/egg in

Experiment 2. In order for the H_2O_2/UV apparatus to be implemented in a commercial breeder farm, it must be able to consistently reduce the number of eggshell bacteria day after. This data demonstrated that over the span of a week, the H_2O_2/UV apparatus maintained effectiveness in treating a larger number of eggs than previously tested.

Table 10. Eggshell APC of sampled eggs each day of egg collection (Experiment 1 and 2).

		Experiment 1[1]	**Experiment 2[2]**
Day 1	Control	$4.50^{a} \pm 0.28$	$4.97^{a} \pm 0.13$
	Treated	$1.06^{b} \pm 0.06$	$1.30^{b} \pm 0.26$
Day 2	Control	$4.77^{a} \pm 0.22$	$4.80^{a} \pm 0.07$
	Treated	$1.46^{b} \pm 0.38$	$1.56^{b} \pm 0.26$
Day 3	Control	$4.62^{a} \pm 0.32$	$4.12^{a} \pm 0.09$
	Treated	$1.38^{b} \pm 0.31$	$1.51^{b} \pm 0.17$
Day 4	Control	$4.40^{a} \pm 0.24$	$4.97^{a} \pm 0.30$
	Treated	$1.43^{b} \pm 0.42$	$1.24^{b} \pm 0.17$
Day 5	Control	$5.11^{a} \pm 0.22$	$4.48^{a} \pm 0.12$
	Treated	$1.06^{b} \pm 0.06$	$1.41^{b} \pm 0.17$

[a,b] Mean within a column with different superscript differ significantly ($P \leq 0.05$)
[1]n = 5 eggs/treatment
[2]n = 5 controls, 10 treated

Hatch data for Experiments 1 and 2 is presented in Table 11. In Experiment 1 the hatch of fertile (HOF) of treated eggs was 95.05% compared to 90.66% for the non-treated controls, and in Experiment 2 there was a trend toward a higher HOF for the treated eggs ($p = 0.057$). Total embryonic mortality (TEM) was numerically lower in treated eggs in both experiments and significantly lower in Experiment 2. It is important that the H_2O_2/UV apparatus treatment did not negatively impact hatchability or

embryonic mortality. Other egg sanitization methods, such as QAC, can effectively reduce microorganisms, but can also impact egg permeability and chick hatchability (Brake and Sheldon, 1990). These results indicate that the H_2O_2/UV apparatus can effectively reduce the number of eggshell microorganisms without negatively impacting hatchability. However, combined analysis of the 2 experiments found that H_2O_2/UV apparatus treatment increased hatchability, and therefore could represent an economic benefit to improve the commercial implementation of the apparatus. The difference in hatchability between Experiment 1 and 2 can be attributed to the difference in the age of the hens. The same flock of White Leghorns was used in both experiments, and between experiments the flock aged 23 weeks.

A single individual determined chick quality. Based on that individual's observations, there was a tendency to have fewer chicks with defects in the treated group; however, no significant differences were determined based on the chick quality parameters evaluated (Table 12).

The results from Experiments 1 and 2 indicate that the H_2O_2/UV apparatus does reduce the number of eggshell bacteria to very low levels without negatively impacting embryonic mortality, hatchability or chick quality. Results from these experiments also indicate that H_2O_2/UV apparatus treatment has the potential to decrease embryonic mortality and increase hatchability.

Table 11. Percent embryonic mortality and hatchability of fertile eggs (HOF) of control and H_2O_2/UV treated eggs (Experiments 1 and 2)[1].

	Treatment	Viable Eggs	Early Dead	Mid Dead	Late Dead	Pipped	TEM[2]	HOF
			(%)					
Experiment 1	Control	720	4.47 ± 1.4	0.00 ± 0.0	2.37 ± 0.4	3.42 ± 1.5	10.26 ± 2.1	90.66 ± 2.3
	Treated	728	2.20 ± 0.2	0.14 ± 0.1	2.34 ± 0.8	1.65 ± 0.7	6.32 ± 1.2	95.05 ± 2.1
	p-value	*0.61*	*0.19*	*0.37*	*0.94*	*0.33*	*0.18*	*0.22*
Experiment 2	Control	362	2.76 ± 0.7	0.00 ± 0.0	10.67 ± 0.7	2.40 ± 1.3	16.57^{a} ± 0.5	83.43 ± 0.5
	Treated	355	2.82 ± 1.0	0.00 ± 0.0	6.67 ± 1.7	1.60 ± 0.9	11.55^{b} ± 1.7	88.17 ± 1.7
	p-value	*0.28*	*0.98*	-	*0.09*	*0.64*	*0.046*	*0.057*
Average	Control		3.63 ± 0.8	0.00 ± 0.0	6.71 ± 2.0	2.94 ± 0.9	13.41^{a} ± 1.7	87.06^{b} ± 1.9
	Treated		2.50 ± 0.5	0.07 ± 0.1	4.67 ± 1.4	1.66 ± 0.5	8.90^{b} ± 1.5	91.67^{a} ± 2.0
	p-value		*0.26*	*0.35*	*0.09*	*0.29*	*0.017*	*0.033*

[1] Percentages based on number of viable eggs
[2] TEM = Total embryonic mortality

Table 12. Chick quality parameters assessed for chicks hatched from control and H_2O_2/UV treated eggs (Experiments 1 and 2).[1]

	Treatment	Chicks Hatched	Avg. Chick Wt (g)	Naval Tag	Dirty	Leg Deformities	Good[2] Chicks
				%			
Experiment 1	Control	689	31.34 ± 0.8	15.09 ± 3.6	0.44 ± 0.01	1.89 ± 0.5	82.36 ± 0.03
	Treated	692	32.45 ± 0.7	11.85 ± 1.6	0.82 ± 0.7	1.30 ± 0.3	85.97 ± 0.02
	p-value		*0.36*	*0.46*	*0.55*	*0.38*	*0.43*
Experiment 2	Control	302	42.48 ± 0.5	52.32 ± 5.7	1.66 ± 1.2	5.66 ± 2.1	39.62 ± 0.06
	Treated	313	42.75 ± 0.6	45.69 ± 2.7	1.28 ± 0.3	2.88 ± 1.2	48.75 ± 0.03
	p-value		*0.78*	*0.35*	*0.78*	*0.31*	*0.27*
Average	Control		36.93 ± 2.5	33.72 ± 8.9	1.04 ± 0.6	3.78 ± 1.3	61.00 ± 0.1
	Treated		37.59 ± 2.3	28.83 ± 7.7	1.07 ± 0.3	2.11 ± 0.7	67.36 ± 0.09
	p-value		*0.34*	*0.22*	*0.96*	*0.21*	*0.16*

[1] Percentages based on number of chicks hatched.

[2] Chicks without defects = chicks hatched – chicks with defe

The objective of Experiment 3 was to evaluate the impact of H_2O_2/UV method treatment on hatchability, embryo mortality and chick quality when eggs were stored for 18 d prior to incubation. Eggshell APC were again evaluated to assure the H_2O_2/UV apparatus performed as expected. Samples were taken from treated and control eggs on the day of collection and treatment, and the results are presented in Table 13. As expected, the eggs treated with the H_2O_2/UV apparatus had significantly lower APC when compared to non-treated controls, assuring treatment effectiveness.

Table 13. Eggshell APC of egg samples from control and H_2O_2/UV treated eggs (Experiment 3).

Treatment	**n**	**log_{10} cfu/egg**
Control	10	$4.90^{a} \pm 0.16$
Treated	10	$1.48^{b} \pm 0.23$

[a,b] Values within the same column with different superscripts differ significantly ($P < 0.05$)

Hatchability and embryonic mortality data is presented in Table 14. The low percentage of hatchability and the high percentages of embryo mortality and rotten eggs can be attributed to the prolonged period of egg storage.

The same individual from Experiments 1 and 2 assessed chick quality. Chicks from the treated group had a significantly higher average chick weight ($p = 0.005$), although no other significant differences were determined (Table 15). The results from this experiment suggest that the H_2O_2/UV apparatus has no impact on the hatchability, embryonic mortality or chick quality of long-term stored eggs. The reduction in

hatchability after long term storage probably has more to do with the changes in albumen quality and pH instead of the invasion of microorganisms. The observed increase in the number of rotten eggs in the treated group was unexpected and further research should be conducted to explain this result.

Conclusions

In each experiment conducted, eggshell bacterial reduction for the treated eggs was nearly complete, and only few eggs had recoverable eggshell bacterial counts. Results from Experiment 1, 2 and 3 indicate that the implementation of the H_2O_2/UV method applied to hatching eggs with the H_2O_2/UV apparatus does not negatively impact hatchability, embryonic mortality, or chick quality. Results from Experiments 1 and 2 actually found that after typical lengths of storage, common in the commercial industry, there was a consistent trend toward increases in hatchability and reductions in embryonic mortality observed when hatching eggs are treated with the H_2O_2/UV method. While not statistically different, there was also a consistent trend for improved chick quality. Results from Experiment 3 did not show that the H_2O_2/UV method application significantly impacted hatchability after prolonged storage of hatching eggs. Future studies should attempt to assess the impact of eggshell bacterial reduction on overall hatchery sanitation and pathogen control. Furthermore, a larger scale hatch study should be conducted to validate the trends observed in these experiments. If hatchability and chick quality could be increased by H_2O_2/UV method treatment, the economical benefit of apparatus implementation to the poultry industry could be significant.

Table 14. Percent rotten eggs, embryonic mortality, and HOF of control and H_2O_2/UV treated eggs (Experiment 3).[1]

Treatment	Viable Eggs	Rotten	Early Dead	Mid Dead	Late Dead (%)	Pipped	TEM	HOF
Control	350	11.84[b] ± 0.9	13.43 ± 1.6	2.57 ± 1.4	11.71 ± 0.5	7.14 ± 2.7	35.14 ± 2.1	52.29 ± 1.5
Treated	350	17.89[a] ± 0.6	12.57 ± 1.9	1.43 ± 0.4	6.58 ± 2.4	4.74 ± 2.2	26.29 ± 2.3	54.29 ± 1.7
p-value	*0.79*	*0.005*	*0.85*	*0.46*	*0.17*	*0.65*	*0.07*	*0.61*

[1] Percentages based on number of viable eggs
[a,b] Values within the same column with different superscripts differ significantly ($P < 0.05$)

Table 15. Chick quality parameters assessed from control and H_2O_2/UV treated eggs (Experiment 3).[1]

Treatment	Chicks Hatched	Avg. Chick Weight	Naval Tag	Dirty	Leg Deformities
			(%)		
Control	183	$45.93^{b} \pm 0.08$	17.49 ± 2.0	3.83 ± 2.5	6.56 ± 3.5
Treated	190	$46.42^{a} \pm 0.07$	14.74 ± 4.7	3.16 ± 1.0	7.37 ± 3.2
p-value		*0.01*	*0.72*	*0.74*	*0.92*

[a,b] Values within the same column with different superscripts differ significantly ($P < 0.05$)
[1] Percentages based on number of chicks hatched

CHAPTER V

CONCLUSIONS

The H_2O_2/UV method has been repeatedly demonstrated to effectively reduce eggshell bacteria. When the process was first developed, eggs were sprayed with H_2O_2 by hand and were exposed to 8 min of UV for maximum bacterial reduction. While effective, these application parameters were impractical for implementation in a commercial setting. Further experimentation by Gottselig (2011) showed that only 5 s of UV exposure was necessary for the photolytic reaction between H_2O_2 and UV to occur. This finding suggested that the H_2O_2/UV method had potential to become commercially feasible. In order to maximize commercial potential, a H_2O_2/UV apparatus was constructed in this study. The H_2O_2/UV method can be applied to eggs in less than 30 s with the H_2O_2/UV apparatus and achieve high levels of bacterial reduction. This method of egg sanitization is safe for the user and the developing embryo. The H_2O_2 used in the application is broken down into hydroxyl radicals in the photolytic reaction with UV and consumed in reaction with the organic material on the eggshell surface. Therefore, no chemical residues are left on the eggs. The UV light is contained within the chambers of the apparatus so that damage to the skin or eyes of the operator is avoided.

Experimentation in this study demonstrated that H_2O_2 can be transported to the treatment site in high concentrations and diluted on-site, thus reduding the labor and costs that would be required to transport large quantities of pre-diluted 3% H_2O_2. Currently in the commercial industry, floor eggs are typically not used for hatching. Experiments in this study indicated that H_2O_2/UV apparatus treatment can effectively

reduce eggshell bacteria of floor eggs. If commercial facilities were able to use floor eggs for hatching, the number of viable eggs available would increase.

Important for the commercial feasibility of the H_2O_2/UV apparatus is the ability to treat eggs on commercial incubator flats. If eggs are required to be placed directly on the conveyor for treatment, this means that individual eggs have to be unloaded from eggs flats, placed onto the apparatus conveyor, and then placed back into the incubator flats. This process of treating eggs individually on the apparatus conveyor is time consuming and requires more labor. When treating eggs on flats, moving eggs on and off flats is eliminated, and eggs can be treated in large groups. For instance, in some experiments in this study, egg flats containing as many as 168-eggs were used. This increases the efficiency of egg treatment. When the H_2O_2/UV apparatus was used in a field trial at a commercial breeder farm, eggs were treated on flats and directly on the conveyor to compare treatment effectiveness. All of the samples of eggs that were treated on large 168-egg flats had APC lower than the LOD. The amount of time and H_2O_2 needed to treat 5,000 eggs on flats was far less than what was necessary to treat 5,000 eggs placed directly on the apparatus conveyor. The ease and cost efficiency of treating eggs on flats makes the implementation of H_2O_2/UV apparatus in a commercial breeder farm more feasible.

While the results from incubation Experiments 1 and 2 did not yield statistical differences in hatchability individually due to the small number of incubators used, analysis of combined data across the 2 experiments did yield a statistical difference. It is also important to note that while hatchability was not statistically greater in incubation

Experiment 3, numerically higher hatchability was also observed. This is a trend that should be explored in a larger scale hatch study. If this trend of H_2O_2/UV apparatus treatment improving hatchability were to be validated in future experimentation, the implementation of the apparatus in a commercial setting would have a direct economical benefit.

Future studies should also attempt to assess the impact of H_2O_2/UV apparatus treatment of eggs on hatchery sanitation and pathogen control. Egg sanitization has been shown to have an impact on incubator sanitation and airborne hatchery bacterial contamination. Experiments should be conducted to determine if H_2O_2/UV apparatus treatment at the breeder farm can reduce the occurrence of bacterial contamination at the hatchery. Eggs can be a mode of transportation of microorganisms, including pathogens, from the breeder farm to the hatchery. Experiments should be conducted to determine if the transport of pathogens from the breeder farm to the hatchery can be controlled by egg sanitization through this methodology.

Further study into the necessary parameters and economical impact of H_2O_2/UV apparatus implementation is needed. Experiments to determine the necessary concentration of H_2O_2 and number of UV lamps need to be conducted. The primary cost of the H_2O_2/UV apparatus operation is the H_2O_2, and the higher the H_2O_2 concentration, the higher the cost of operation. If it can be determined that H_2O_2 concentrations lower than 3.5% are effective, then the cost of H_2O_2/UV apparatus application would decrease.

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