8-1-2009

Sanitization of broiler breeder hatching eggs using ultraviolet light and hydrogen peroxide

Jessica Benoit Wells

SANITIZATION OF BROILER BREEDER HATCHING EGGS USING ULTRAVIOLET LIGHT AND HYDROGEN PEROXIDE

By

Jessica Benoit Wells

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirement
for the Degree of Master of Science
in Agricultural Science
in the Department of Poultry Science

Mississippi State, Mississippi

August 2009

SANITIZATION OF BROILER BREEDER HATCHING EGGS USING

ULTRAVIOLET LIGHT AND HYDROGEN PEROXIDE

By

Jessica Benoit Wells

Approved:

Christopher McDaniel Professor of Poultry Science (Director of Thesis)	David Peebles Professor of Poultry Science (Committee Member)
Craig Coufal Assistant Professor of Poultry Science (Committee Member)	Christopher McDaniel Professor of Poultry Science Director of Graduate Studies in the Department of Poultry Science
Melissa Mixon Interim Dean of the College of Agriculture and Life Sciences	

Name: Jessica Benoit Wells

Date of Degree: August 8, 2009

Institution: Mississippi State University

Major Field: Agricultural Science (Poultry)

Major Professor: Dr. Christopher McDaniel

Title of Study: SANITIZATION OF BROILER BREEDER HATCHING EGGS USING ULTRAVIOLET LIGHT AND HYDROGEN PEROXIDE

Pages in Study: 77

Candidate for Degree of Master of Science

Ultraviolet light (UV) and hydrogen peroxide (H_2O_2) decrease eggshell bacteria. However, when combined, the optimum amount of each and effects on hatchability are unknown. In Experiment 1, when compared to other concentrations of H_2O_2 and lengths of UV, the combination of 1.5% H_2O_2 and 8 minutes of UV yielded optimum results with a 3 $\log_{10}$ CFU/egg reduction in bacteria on the eggshell. In Experiment 2, exposing eggs to this optimum combination yielded a 1000 fold reduction in eggshell bacteria but only a numerical increase in hatch of set and hatch of fertile. In Experiment 3, eggs exposed to repetitive treatments of H_2O_2 and UV yielded a 4 log reduction in eggshell bacteria but no differences in hatchability or chick characteristics. In conclusion, the combination of H_2O_2 and UV proved to be effective for eggshell sanitization, especially when used repetitively, and did not alter hatchability.

Key words: eggshell sanitization, bacteria, ultraviolet light, hydrogen peroxide, hatchability.

DEDICATION

I would like to dedicate this work to my supportive and loving husband Gathian Wells, my wonderful parents, Robert and Sandy Benoit, and my family.

ACKNOWLEDGMENTS

The author expresses her sincere gratitude to all of those who helped with this research and were supportive along the way. First, a special thanks is due to Dr. Chris McDaniel, for being such a wonderful major professor and for all of the much needed guidance and support through my masters study. Thank you to the committee members Dr. Craig Coufal and Dr. David Peebles. Appreciation is also expressed to Dr. Holly Parker for her assistance and great friendship through all of my research and education. To all who assisted in my experiments in the lab and to the farm crew, I thank you for the much needed helping hands. Lastly, to my parents, husband, and family, without your encouragement and support none of this would have been accomplishable.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Hatching eggs are often not sanitized in the commercial broiler breeder industry, allowing for possible bacterial contamination of the egg. Bacterial contamination, contributed by the hens from fecal material, can be found in nest materials (Mine et al., 2003). Eggshells will also come into contact with bacteria in farm egg storage rooms, hatchery trucks, as well as within the hatchery environment (Cox et al., 2000). The bacteria found in the environment surrounding the egg can pose serious problems because it has been suggested they can easily penetrate the eggshell (Berrang et al., 1999). Once bacterial contamination of hatching eggs occurs, it can lead to embryonic mortality, high chick mortality, as well as poor growth (Wilson, 1997).

However, the egg does have some natural defenses against bacterial contamination. The first defense of the egg is the cuticle. The cuticle is a waxy coating on the surface of the outer eggshell that covers the pores on the eggshell to prevent invasion of bacteria. However, the cuticle is not effective immediately after lay, but instead has to dry before it can serve as protection against bacteria (Berrang et al., 1999). The shell membranes are also able to protect the egg against bacteria. Because of the tight meshwork of the inner and outer membranes, it is difficult for bacteria to enter the egg (Garibaldi and Stokes, 1958). Another protective mechanism is the albumen.

Albumen has a high pH which is uninviting for bacteria and makes it difficult for bacteria to grow inside the egg (Sharp and Powell, 1931). Albumen also has an iron-binding agent, conalbumin, which prevents bacterial growth within the egg (Board, 1974). Even though the egg has numerous natural defenses against bacteria, these defenses are not flawless. This is why artificial egg sanitization methods are necessary.

Safe and effective egg sanitization methods have been widely studied for use in the poultry industry. These methods are needed not only to disinfect the eggshell, but in turn they may have a positive effect on hatchability. Ultraviolet (UV) light is one method of sanitization that effectively reduces bacteria on the eggshell and can be safe for the user. Because UV light can be contained, it is harmless to the user when administering it to the eggshells (Scott, 1993). Kuo et al. (1997a) demonstrated that UV radiation was effective at reducing yeast, molds, and aerobes on eggshell surfaces. Chavez et al. (2002) also found that high intensity UV light reduced aerobic bacterial counts. However, in studies by Berrang et al. (1995) and Goerzen and Scott (1995), even though UV light was effective at killing bacteria located on the eggshell, it had no effect on hatchability. Another method that can be safely used to sanitize the eggshell surface is hydrogen peroxide (H_2O_2). Sander and Wilson (1999) demonstrated a significant reduction in bacteria when using 3% H_2O_2 on broiler breeder hatching eggs, but observed no effect on hatchability. Padron (1995) also used H_2O_2 in an experiment and reduced organisms on the eggshell by 95%, but again no effect on hatchability was observed.

Bayliss and Waites (1982) demonstrated that a greater kill of bacteria could be obtained when using H_2O_2 and UV light simultaneously in vitro. This combination had a damaging effect on bacterial spores because it produces hydroxyl radicals. After

administering 2.5% H_2O_2 and UV light to *Bacillus subtilis* cultured on agar slopes, a 2000-fold reduction of this bacteria was obtained (Bayliss and Waite, 1982).

Even though it is known that the combination of H_2O_2 and UV light is more effective at reducing bacteria in vitro than either H_2O_2 or UV light alone, it is still unknown if this combination will be more effective when administered to the eggshell. It is also unknown whether additional sanitization of the outer eggshell by administering H_2O_2 and UV light will have an effect on hatchability. Therefore, the objectives of this thesis research were to: 1) determine which combination of H_2O_2 and UV light is most effective at egg sanitization, and 2) determine if hatchability is affected when eggs are sanitized by this combination.

CHAPTER II

LITERATURE REVIEW

The Egg and Eggshell

The egg is a very complex structure. It consists of several components, such as the crystalline shell, organic matrix, albumen, and ovum (Whittow, 2000). All of these components of the egg help protect it from microorganisms as well as provide nutrients for the embryo (Mine, 2003). The external and internal components of the egg are formed in the oviduct of the hen. This structure is where the development of the egg begins. The oviduct is approximately 600 mm in length and consists of 6 distinct regions. These regions are the infundibulum, magnum, isthmus, tubular shell gland, shell gland pouch, also referred to as the uterus, and the vagina (Solomon, 1997).

The process of producing an egg begins when the ovum or egg yolk is released by the ovary and engulfed by the infundibulum. The infundibulum is funnel shaped and approximately 3.5 inches in length (North and Bell, 1990). The yolk will remain in the infundibulum for approximately 18 minutes, while fertilization as well as production of the first layer of albumen occurs (Whittow, 2000). After the egg leaves the infundibulum it enters the magnum. The magnum is made of mucosal folds which give it a thick diameter (Solomon, 1997) and is approximately 13 inches long. The egg remains in this portion of the oviduct for 3 hours (North and Bell, 1990). In this span of 3 hours, the

magnum will form the albumen, which will surround the outside of the ovum (Whittow, 2000).

The next portion of the oviduct is the isthmus. The isthmus is only 33 centimeters in length (North and Bell, 1990) and the egg remains there for 1-2 hours. During this time period the inner and outer shell membranes are formed (Whittow, 2000). After leaving the isthmus, the egg enters the tubular shell gland. The egg is only present in this region of the oviduct for a short period of time. The tubular shell gland is where calcium salts are added onto the shell membrane fibers (Solomon, 1997). Next, the egg enters the shell gland pouch or uterus. The shell gland pouch is 10 to 12 cm in length and holds the egg for 18 to 20 hours (North and Bell, 1990). While in the uterus, the egg becomes approximately 15 grams heavier because of an infusion of fluid (Solomon, 1997). In addition to the influx of fluid, the calcification of the eggshell also occurs in the shell gland pouch. Here the shell will be placed around the outer shell membrane of the egg, and a waxy cuticle will be the final layer added to the eggshell (Whittow, 2000). Lastly, the finished egg will enter the vagina. The vagina is 12 cm in length and holds the egg for only a few minutes (North and Bell, 1990). The vagina has no role in the formation of the egg, and only serves to expel the egg from the oviduct during oviposition (Whittow, 2000).

As previously mentioned, the outermost surface of the eggshell is covered by a waxy substance known as the cuticle. The cuticle is composed of 85-87% protein, 2.5-3.5% fat, 4.4% carbohydrates, and 3.5% ash (Wedral et al., 1974). The cuticle thickness on the surface of an eggshell varies between 0.5 and 12.8 μm, and the average dry weight

is approximately 12 mg (Simons, 1971). Formation of the cuticle occurs prior to oviposition (Solomon, 1997).

The cuticle serves many functions. For example, prior to oviposition, the water content of the cuticle is high and serves as a natural lubricant as the egg is laid (North and Bell, 1990). After lay, the cuticle dries and covers the pores located on the outer surface of the eggshell, which in turn prevents bacterial penetration and reduces internal moisture loss from the egg (Wedral et al., 1974).

Located directly beneath the cuticle is the calcified portion of the egg commonly known as the eggshell. Furthermore, the calcified portion of the eggshell can be divided into the outer surface crystal layer, palisade layer, and mammillary knob layer, which are composed of approximately 97% inorganic material (Whittow, 2000). The outer surface crystal layer is composed of a thin monolayer of crystals (Mine et al., 2003). The crystalline structure of the outer surface crystal layer is dense and has a thickness of 3-8 μm (Whittow, 2000). The palisade layer is made up of calcite crystals and has a chalky texture (North and Bell, 1990). It is approximately 200 μm thick and represents the major portion of the eggshell (Whittow, 2000). The inner most layer of the calcified portion of the eggshell is the mammillary knob layer (North and Bell, 1990). This layer, like the palisade layer, is also composed mainly of calcite. The formation of the mammillary knob layer occurs in the first 5 hours after entering the uterus. These mammillary knobs are the seeding sites for calcification (Whittow, 2000).

The majority of the calcified portion of the eggshell is produced in the uterus portion of the oviduct (Cregar et al,. 1976). The formation of this calcified portion takes approximately 17 hours and requires 5 to 6 g of calcium carbonate which are created in

the uterus (Lavelin et al., 2000). During the formation of the three layers found in the calcified portion of the eggshell, the crystals do not grow completely together. As a result, the eggshell contains pores which range in diameter from 0.3 to 0.9 μm (Whittow, 2000).

The inner portion of the eggshell is referred to as the shell membrane, which contains two distinct layers known as the inner and outer membrane (Whittlow, 2000). These shell membranes are produced by the isthmus portion of the oviduct (Solomon, 1997). Interestingly, the formation of these two shell membranes determines the eggs final shape (North and Bell, 1990). The inner shell membrane is approximately 50-70 μm thick, whereas the outer shell membrane is only 15-25 μm thick (Whittow, 2000). The combined weight of the inner and outer shell membranes is approximately 143 mg (Solomon, 1997), and they are composed of 10% collagen, 70-75% protein and approximately 15-20% glycoprotein (Candlish, 1972). The inner and outer shell membranes are semi-permeable which allows gas, water and crystalloid passage through the eggshell. Located on the inner side of the inner thin membrane is a thin film which appears to be solid instead of a continuation of the meshwork from the inner membranes (Rahn et al., 1979). However, these membranes do not allow albumen to pass through the eggshell (Whittow, 2000).

Beneath the shell membranes is the albumen. Development of albumen occurs within 3 hours after entering the magnum (North and Bell, 1990). At lay, the pH of albumen is 7.6 and rises to 9.5 after storage (Sharp and Powell, 1931). Albumen can be separated into 4 layers. These layers include the outer thin, outer thick, inner thin, and

the chalaziferous layers (Whittow, 2000). All of these layers are produced in the magnum region of the oviduct and consist of 40 different proteins (Solomon, 1997).

The inner most portion of the egg is the ovum or yolk. The greatest portion of the ovum is composed of water. However, yolk dry matter is composed primarily of lipids and proteins (North and Bell, 1990). Furthermore, the main function of the yolk is to provide nourishment in the form of lipids and proteins to the developing embryo (Whittow, 2000).

The entire structure of the egg is to provide nutrition and to prevent harm from occurring to the embryo. The cuticle prevents excessive moisture loss from occurring which could be detrimental to a chick embryo (Wedral et al., 1974). The calcified portion of the eggshell can provide protection from external invasive factors and predators. This portion of the eggshell along with the shell membranes and albumen can also prevent bacterial invasion from occurring (Berrang et al., 1999).

Bacteria on the Eggshell

Studies have shown that the outer eggshell is exposed to many contaminants through contact with nest materials (Mine et al., 2003). For example, hens bring feces and soil into the nest boxes where eggs are laid, and these materials can contain different types of bacteria such as *Salmonella* (Cox et al., 2000). Cox et al. (2000) also stated that *Salmonella* can be found in farm egg storage rooms, hatchery trucks, as well as within the hatchery environment itself. Research has shown that bacteria located on the outer eggshell of hatching eggs can pose serious problems, as it can easily penetrate the egg shell and contaminate the embryo. As a result, numerous chicks are contaminated during

the hatching process (Berrang et al., 1999). Wilson (1997) reported that contamination of hatching eggs can lead to embryonic mortality, high chick mortality, weak chicks, as well as poor growth.

The first defense an egg has to bacterial contamination is the cuticle. The cuticle acts as a filter allowing vital gas exchange and water loss to occur between the eggshell and embryo, while preventing the invasion of bacteria and the excessive loss of moisture through the eggshell (Mine et al., 2003). However, at lay the cuticle is ineffective until the egg dries (Sparks, 1987). After the egg dries the cuticle hardens and covers the pores located on the outer eggshell (Berrang et al., 1999). The eggshell itself is not an effective defense mechanism to prevent bacterial contamination because of the large pores covering its surface (Sauter and Petersen, 1974). However, the inner and outer shell membranes also offer protection from bacterial contamination over a short period of time (Garibaldi and Stokes, 1958). Because of its tight meshwork, the inner membrane is more effective at protecting the embryo from bacterial contamination than the outer membrane (Lifshitz et al., 1964). However, the meshwork of both the inner and outer shell membranes makes it difficult for bacteria to enter the egg (Garibaldi and Stokes, 1958). Also, the solid film located beneath the inner membrane acts as a barrier against bacterial contamination (Rahn et al., 1979).

Another protective mechanism against bacteria is the albumen. It is uninviting to bacteria because the pH is approximately 7.6 at lay, but over storage the pH rises to 9.6. Most bacteria thrive in a neutral pH of 7.2, therefore, they are unable to grow properly in the high pH found in albumen (Sharp and Powell, 1931). Albumen also has an iron-binding agent, conalbumin, which reduces bacterial growth within the egg (Board, 1974).

Even though the egg has numerous natural defenses against bacteria, the egg is not flawless in its defenses. For example, Lock et al. (1992) revealed that *Pseudomonas putida* was still able to penetrate the albumen and reproduce quickly after coming in contact with the egg yolk.

Egg Sanitization Methods

Once bacteria penetrate the outer eggshell and the inner hatching egg, embryo contamination cannot be prevented. This is why sanitization methods for the outer eggshell are crucial in the commercial industry and are needed to prevent bacteria from penetrating the eggshell (Cox et al., 2000).

Egg sanitization methods have been widely used in the poultry industry to reduce bacterial growth on hatching eggs. For example, formaldehyde gas fumigation is a method that has been used for many years in commercial hatcheries. It is a very effective decontaminant but is also a known carcinogen (Mitchell et al., 2000). Due to the restrictions on formaldehyde use, other methods of eggshell sanitization have been and are currently being studied. Ozone is one such method studied for hatching egg sanitation (Rodriguez-Romo and Yousef, 2005). Rodriguez-Romo and Yousef (2005) treated *Salmonella* infected eggshells with ozone for 0 – 20 minutes and determined that ozone significantly reduced the concentration of *Salmonella* located on the outer eggshell. Although ozone is a good disinfectant and can spontaneously decompose to form a nontoxic product, in its initial state, it is harmful to the respiratory system of the person administering it in hatcheries (Rodriguez-Romo and Yousef, 2005).

Other sanitation methods such as ethylene oxide, heat treatment, and gamma irradiation have also been frequently used but pose problems when used in the egg industry (Kuo et al., 1997b). For example, in a study conducted by Coretti and Inal (1969), ethylene oxide produced toxic residues that were harmful to users. Gamma irradiation is also not widely accepted because of safety concerns (Shama, 1992). However, Gao et al. (1997) demonstrated that ultraviolet (UV) irradiation is safe and effective at eliminating *Salmonella* on whole eggs. The use of UV light is thought to be a safer sanitization alternative than formaldehyde gas, because unlike formaldehyde gas, UV light can easily be administered in an enclosed environment. Because UV light can be contained, this prevents the user from coming in direct contact with it (Scott, 1993).

Ultraviolet Light Sanitization and Effects on Hatchability

There are 3 types of UV light emitted by the sun, UV-A, UV-B, and UV-C. Out of these three, UV-C is commonly used for sanitization because it has the most detrimental effects on bacterial DNA (Tapper and Hicks, 1998). Ultraviolet-C causes a photochemical reaction within the nucleic acids found in microorganisms, resulting in inactivation of these microorganisms. For bacteria, the maximum effect of this inactivation is found when the UV-C wavelength is between 250 and 270 nm (Bachmann, 1975). Ultraviolet radiation causes thymine dimers to form on DNA strands, which is lethal to bacteria. It also has a germicidal property that destroys bacteria by degrading their cell walls (Kuo et al., 1997b). Because UV light has these properties, it has been shown to be an effective method for sanitization of eggshells. For example, Kuo et al. (1997a) demonstrated that UV radiation for 1, 3, 5, and 7 minutes of exposure was

effective at reducing yeast, molds, and aerobes on eggshell surfaces. Chavez et al. (2002) also found that exposing eggshells to high intensity UV light for 30 and 60 seconds significantly reduced aerobic bacterial counts. They reported a reduction of 2 to 3 $\log_{10}$ CFU/egg when using UV light to sanitize eggshells. When exposing eggshells for 1, 3, and 5 min of UV light, *Salmonella* was decreased by 3.4, 3.0, and 4.3 log units, respectively (Rodriguez-Romo and Yousef, 2005). After exposing eggs to UV light for only 4.7 and 18.8 seconds, bacteria were reduced from 4.47 to 3.57 $\log_{10}$ CFU/egg (De Reu et al., 2006). Bachmann (1975) determined that eggshell sanitization using UV light occurs only within direct radiation beams. Therefore, shaded areas, holes, and pores are unaffected by UV radiation. However, when compared to eggs that were not rotated, eggs that were rotated and exposed to high intensity UV light yielded less bacteria on the shell (Kuo et al., 1997b).

It is commonly known that bacterial contamination may affect the hatchability of broiler breeder eggs (Wilson, 1997). Recently, studies have been conducted to determine if a reduction in bacteria, using UV light, can affect hatchability without being detrimental to the embryo. For example, a preliminary study conducted by Gao et al. (1997) showed that UV light was unable to penetrate the eggshell. In another study, Berrang et al. (1995) exposed broiler breeder eggshells to continuous UV light at 254 nm during the entire 21 days of incubation and reported no effect on hatchability. Goerzen and Scott (1995) observed that microbial counts were significantly reduced when eggshells of commercial broiler hatching eggs were exposed to 5 seconds of UV light located above egg collection belts, yet there were no significant differences observed in hatchability or embryonic development. Similarly, Coufal et al. (2003) reduced aerobic

bacteria plate counts by 1.3 log, *S. typhumurium* by 4 log and *E. coli* by 4 to 5 log on broiler breeder eggs, but observed no effects on hatchability or eggshell conductance. However, hatching eggs treated with a commercial sanitizer and then incubated under 1, 3, and 5 minutes of UV light resulted in a significant increase in embryo viability (Scott, 1993). Also, Rodriguez-Romo and Yousef (2005) combined 1 minute of UV light with 1 minute of ozone and were able to reduce bacteria on shell eggs by 4.6 log.

Hydrogen Peroxide Sanitization and Affects on Hatchability

Another method examined for eggshell sanitization is hydrogen peroxide (H_2O_2). For example, H_2O_2, when used at 3%, significantly reduced bacterial counts on broiler breeder hatching eggs. Although H_2O_2 was shown to increase egg moisture loss, it had no subsequent effect on hatchability (Sander and Wilson, 1999). Padron (1995) dipped *Salmonella typhimurium* contaminated eggs in 6% hydrogen peroxide, twice, and reduced the average number of organisms on the eggshell by 95%. They also reduced the amount of eggs positive for *Salmonella typhimurium* by 55%. It was also noted that hatchability was not adversely affected (Padron, 1995). In another study, Sheldon and Brake (1991) found that hand spraying 5% hydrogen peroxide onto broiler breeder hatching eggs yielded a 5 log reduction in bacteria and increased hatchability of fertile eggs by 2 to 3%.

Combination of Ultraviolet Light and Hydrogen Peroxide

When hydrogen peroxide is combined with UV light, it produces hydroxyl radicals, which have a damaging effect on spores of *Bacillus subtilis* (Bayliss and Waites, 1982). Hydroxyl radicals generated by photolysis are extremely reactive with microbial

cells and organic matter. This reaction is detrimental to microbial cells (Rodriguez-Romo and Yousef, 2005).

In a study where 2.5 % hydrogen peroxide and UV light were used simultaneously in vitro, a rapid kill in spores of *Bacillus subtilis* was obtained (Bayliss and Waites, 1979). This reduction was 2000-fold greater for the nutrient agar slopes which were treated with a combination of hydrogen peroxide and UV light as opposed to nutrient agar slopes treated only with UV light (Bayliss and Waites, 1979). In another experiment, Bayliss and Waites (1982) again demonstrated a 2000-fold kill in *Bacillus subtilis* bacteria with 2.5 % H_2O_2 and UV light and found that concentrations of hydrogen peroxide higher than 2.5% protected the *Bacillus subtilis* spores against the effects of irradiation. When combining 2.5% hydrogen peroxide with high intensity UV light, they consistently produced more than a 4 log reduction in *Bacillus subtilis* spores which are normally highly resistant to hydrogen peroxide treatment alone. The effects of irradiation and hydrogen peroxide were only evident if this combination was administered simultaneously (Bayliss, 1982).

Although it was demonstrated by Bayliss and Waites (1982) that the combination of hydrogen peroxide and UV light was detrimental to bacterial growth on nutrient agar slopes in vitro, it is still unknown if it will have the same affects on bacteria found on eggshells. It is possible that the combination of UV and H_2O_2 would be effective at disinfecting the outer eggshell of a chicken egg. Therefore, studies need to be conducted to determine if the combination of UV light and H_2O_2 can reduce bacterial contamination on the eggshell as well as affect hatchability, chick quality, and livability.

REFERENCES

Bachmann, R., 1975. Sterilization by intense ultraviolet radiation. Brown Boveri Rev. 62:206-209.

Bayliss, C., and W.M. Waites, 1979. The combined effect of hydrogen peroxide and ultraviolet irradiation on bacterial spores. J. Appl. Bacteriology. 47:263-269.

Bayliss, C., and W.M. Waites, 1982. Effect of simultaneous high intensity ultraviolet irradiation and hydrogen peroxide on bacterial spores. J. Fd Technol. 17:467-470.

Berrang, M., N.A. Cox, and J.S. Bailey, 1995. Efficacy of ultraviolet light for elimination of *Salmonella* on broiler hatching eggs. J. Appl. Poultry Res. 4:422-429.

Berrang, M.E., N.A. Cox J.F. Frank and R.J.Buhr, 1999. Bacterial penetration of the eggshell and shell membranes of the chicken hatching egg: A review. Appl.Poultry Res. 8:499-504

Board, R.G., 1974. Non-specific antimicrobial defenses of the avian egg, embryo, and neonate. Biol. Rev. 49:15-49.

Candlish, J., 1972. The role of the shell membranes in the functional integrity of the egg. In "Egg Formation and Production" British Poultry Science Ltd.., Edinburgh.

Chavez, C., K.D. Knape, C.D. Coufal, and J.B. Carey, 2002. Reduction of eggshell aerobic plate counts by ultraviolet irradiation. Poult. Sci. 81:1132-1135.

Coretti, K., and T. Inal. 1969. Residue problems in cold sterilization of spices using T-gas (ethylene oxide). Fleischwirtschaft 49:599-604.

Coufal, C., C Chavez, K. Knape, and J. Carey. 2003. Evaluation of a method of ultraviolet light sanitation of broiler hatching eggs. Poult. Sci. 82:754-759.

Cox, N., M.E. Berrang, and J.A. Cason, 2000. *Salmonella* penetration of egg shells and proliferation in broiler hatching eggs: A review. Poult. Sci. 79:1571-1574.

Cregar, C., H. Phillips, and J. Scott, 1976. Formation of an eggshell. Poult. Sci. 55:1717-1723

De Reu, K., K. Grijspeerdt, L. Herman, M. Heyndrickx, M. Uyttendaele, J. Debevere, F.F. Putirulan, and N.M. Bolder. 2006. The effect of a commercial ultraviolet disinfection system on the bacterial load of shell eggs. Society for Appl. Microbiology. 42:144-148.

Garibaldi, J.A. and J.L. Stokes, 1958. Protective role of shell membranes in bacterial spoilage of eggs. Food Res. 23:283-290.

Gao, F., L.E. Stewart, S.W. Joseph, and L.E. Carr, 1997. Effectiveness of ultraviolet irradiation in reducing the numbers of *Salmonella* on eggs and egg belt conveyor materials. Appl. Engineering in Agriculture. 13(3):355-359

Goerzen, P., and T.A. Scott, 1995. Ultraviolet light sanitation for broiler hatching eggs. Poult. Sci. 74:83.

Kuo, F., J.B. Carey, and S.C. Ricke, 1997a. Ultraviolet irradiation of shell eggs: Effect on populations of aerobes, molds, and inoculated *Salmonella* typhimurium. J. Food Protection. 60(6):639-643.

Kuo, F., S.C. Ricke, and J.B. Carey, 1997b. Shell egg sanitation: Ultraviolet radiation and egg rotation to effectively reduce populations of aerobes, yeasts, and molds. J. Food Protection. 60(6):694-697.

Lavelin, I., M. Meiri, and M. Pines, 2000. New insight in eggshell Formation. Poult. Sci. 79:1014-1017.

Lifshitz, A., R.C. Baker, and H.B. Naylor, 1964. The relative importance of chicken egg exterior structures in resisting bacterial penetration. J. Food Sci. 29:94-99.

Lock, J., J. Dolman, and R.G. Board, 1992. Observations on the mode of bacterial infection of hen's eggs. FEMS Microbiol. Letters 100:71-74.

Mine, Y., C. Oberle, and Z. Kassaify, 2003. Eggshell matrix proteins as defense mechanism of avian eggs. J. Agric. Food Chem. 51(1):249-253.

Mitchell, B., G.C. Witcher, and J. Hammond, 2000. Automated fumigation and neutralization systems for formaldehyde gas. J. Appl. Poultry Res. 9:359-363.

North, M. and D. Bell, 1990. Commercial chicken production manual fourth edition. Van Nostrand Reinholk Library of Congress. New York, NY

Padron, M., 1995. Egg dipping in hydrogen peroxide solution to eliminate *Salmonella* typhimurium from eggshell membranes. Avian Dis. 39:627-630.

Rahn, H., A. Ar, and C. Pagenelli, 1979. How bird eggs breathe. Sci. Direct. 240: 46-55.

Rodriguez-Romo, L., and A.E. Yousef, 2005. Inactivation of *Salmonella* enteric serovar enteritidis on shell eggs by ozone and ultraviolet radiation. J. Food Protection. 68(4):711-717.

Sander, J.E., and J.L. Wilson, 1999. Effect of hydrogen peroxide disinfection during incubation of chicken eggs on microbial levels and productivity. Avian Diseases.43:227-233.

Sauter, E. and C.F. Peterson, 1974. The effect of egg shell quality on penetration by various *Salmonella*e. Poultry Sci. 53:2159-2162.

Scott, T., 1993. The effect of ultraviolet light and air filtering system on embryo viability and microorganism load on the egg shell. J. Appl. Poultry Res. 2:19-25.

Shama, G. 1992. Ultraviolet irradiation apparatus for disinfecting liquids of high ultraviolet absorptivities. Lett. Appl. Microbiol. 15:69-72.

Sharp, P. and C.K. Powell, 1931. Increase in the pH of the white and yolk of hens' eggs. Ind. Eng. Chem. 23:196-199.

Sheldon, B. and J. Brake, 1991. Hydrogen peroxide as an alternative hatching egg disinfectant. Poultry Sci. 70(5):1092-1098.

Simons, P., 1971. Ultrastructure of the hen eggshell and its physiological interpretation; Communication No. 175; Central Institute Poultry Research: Beekbergen, The Netherlands.

Solomon, S., 1997. Egg and Eggshell Quality. Manson Publishing Ltd. Ames, IA.

Sparks, N., 1987. The hen's eggshell: A resistance network. Aslib Index Theses 36:294.

Tapper, M. and R. Hicks. 1998. Temperate viruses and lysogeny in lake superior bacterioplankton. Limnol. Oceanogr. 43(1):95-103

Wedral, E., D. Vadehra, and R. Baker. 1974. Chemical composition of the cuticle and inner and outer membranes from eggs of Gallus gallus. Comp. Biochem. Physiol. 47B:231-240.

Whittow, C., 2000. Sturkie's Avian Physiology Fifth Edition. Academic Press. San Diego, CA.

Wilson, H., 1997. Hatching egg sanitation. University of Florida, Institute of Food and Agricultural Sciences (UF/IFAS)

CHAPTER III

DISINFECTION OF EGGSHELLS USING ULTRAVIOLET LIGHT AND HYDROGEN PEROXIDE INDEPENDENTLY AND IN COMBINATION

Abstract

Eggshell bacteria are decreased by ultraviolet light (UV) or hydrogen peroxide (H_2O_2) alone, however, the antimicrobial effects of these two treatments combined as well as optimum length for UV exposure are not known. Therefore, the objectives of this study were to obtain the optimum length of UV exposure for maximum bacterial reduction and to determine if a greater bacterial reduction would occur when using a combination of UV and H_2O_2, as opposed to either treatment alone. The first experiment was conducted to determine the optimum length of UV exposure by exposing eggs to 4, 8, 16 and 32 min of UV. Three experiments were also conducted to determine what concentration of H_2O_2 in combination with UV exposure would yield maximum bacterial reduction. For experiment 2, treatments consisted of a control and UV alone, as well as 0, 1, 2 and 3% H_2O_2 alone and in combination with UV for 8 minutes. For experiment 3, treatments consisted of a control, UV alone, 3% H_2O_2 alone, as well as 0, 0.5, 1, 1.5, 2, 2.5 and 3% H_2O_2 in combination with UV for 8 minutes. Experiment 4 contained 10 treatments including control, 1.5, 2 and 2.5% H_2O_2 at UV exposure times of 2, 4 and 8 min for each H_2O_2 concentration. Every control eggshell contained bacteria with an

average bacterial count of 4 log. Exposure to only UV for 8 min yielded optimum bacterial reduction. When administered independently, H_2O_2 and UV each reduced the bacterial count by 2 log, yielding no bacteria on 16% and 30% of the plates for H_2O_2 and UV, respectively. The combination of 1.5% H_2O_2 and UV for 8 minutes reduced bacterial counts by a maximum of 3 log and the percentage of eggs positive for bacteria by 65%. Because bacterial contamination was further reduced by using a combination of UV and H_2O_2, hatchability and chick quality of broiler breeder eggs may be improved by these treatments.

Introduction

The outer eggshell is exposed to many contaminants (Mine et al., 2003). Hens bring fecal material into nest boxes and these feces can contain many different types of bacteria (Cox et al., 2000). Cox et al. (2000) also suggested that *Salmonella* can not only be found in nest boxes but also in farm egg storage rooms, hatchery trucks, as well as in the hatchery environment. Because of the bacterial contamination found on the outer eggshell, safe and effective methods for sanitization of the eggshell are researched.

Ultraviolet (UV) light is commonly administered as a disinfection method in many different industries to sanitize such products as air, dairy products, water, meat, maple syrup, fresh cider, and packing materials (Huang and Toledo, 1982). Ultraviolet light-C, which is the most effective UV emitted by the sun (Tapper and Hicks, 1998), can cause a photochemical reaction within the nucleic acid of microorganisms resulting in cellular inactivation. Chavez et al. (2002) found that after exposing eggshells to high

intensity UV light, a reduction in bacteria of 2 to 3 $\log_{10}$ CFU/egg was obtained in the treated eggs.

Another method used to sanitize eggshells is hydrogen peroxide (H_2O_2). Sander and Wilson (1999) demonstrated that using 3% H_2O_2 significantly reduced bacterial counts on broiler hatching eggs. Padron (1995) also demonstrated that H_2O_2 could reduce bacteria on eggshells by 95%. Both UV light and H_2O_2 have been shown to be effective disinfectant agents on the outer surface of the eggshell. However, no research has currently been conducted to determine if the combination of UV light and H_2O_2 would more effectively reduce the bacterial contamination found on the outer eggshell.

Rodriguez-Romo and Yousef (2005) demonstrated that when combining UV light with ozone a 4.6 log reduction in bacteria on shell eggs was obtained. Furthermore, Bayliss and Waites (1982) demonstrated that the combination of UV light and H_2O_2 can produce hydroxyl radicals, which results in a more rapid kill of bacteria. In their experiment, a 2000 fold reduction in *Bacillus subtillis* was obtained when simultaneously administering the combination of UV light and H_2O_2 to agar slopes in vitro. However, no research has been conducted to determine if this combination will have the same effect on bacteria located on the eggshell. Also, it is unknown what the optimum length of UV exposure would be when combined with H_2O_2, nor is it known what the optimum concentration of H_2O_2 should be when combined with UV light to produce the highest reduction in bacteria on the eggshell. Therefore, the objectives of these experiments were to determine the optimum length of UV exposure and the optimum concentration of H_2O_2 used in combination with UV light for maximum bacterial reduction.

Materials and Methods

Bacterial Enumeration Procedure

For the following 4 experiments, bacterial enumeration was performed in the same manner. After individual eggs from both treated and control groups were placed into Whirl-pak™ bags, the bags were then filled with 50 mL of sterile phosphate buffered saline (PBS). Each egg was then massaged in the bag for 1 minute to remove bacteria located on the outer eggshell. After massaging was complete, the bags were opened and 10 mL of the rinse solution was aseptically pipetted into an empty sterile culture tube. Rinse solutions collected from all eggs receiving any treatment in the following 4 experiments were not serial diluted. However, 2 serial dilutions were necessary for all control eggs due to high bacterial loads. After dilutions were complete, 0.5 mL of all rinse samples and dilutions were spread-plated on tryptic soy agar (TSA) plates. All samples were plated in duplicate. Plates were then incubated for 48 hours at 37 C. After the incubation period, plates were removed and colony enumeration was performed. All of the following results were reported as $\log_{10}$ CFU/egg. All eggs with no bacteria detected using the aforementioned bacterial enumeration procedure were considered negative for bacteria when calculating percentage of eggs positive for bacteria. However, due to the dilutions that were performed, if an egg only had 1 CFU there would be a 1% chance that it would be detected using the above bacterial enumeration procedure.

Experiment 1

In the first experiment, eggs were exposed to UV light for different amounts of time. This was done to determine the optimum time of UV exposure that would achieve maximum bacterial reduction on eggshells. The length of UV exposure that yielded the greatest reduction in eggshell bacteria would then be used in Experiments 2 and 3. A total of 90 eggs were collected from caged White Leghorn hens. Eggs were then separated into 5 treatment groups which included a control group. Each treatment group consisted of 18 eggs. All 18 eggs from a single treatment group were placed horizontally on a wire flat. The eggs were then placed into a UV chamber for 4, 8, 16, or 32 minutes. The UV-C intensity in the UV treatment chamber was approximately 11 mW/cm^2. Immediately after the 18 eggs from each treatment group were removed from the UV chamber, each individual egg was placed into a sterile Whirl-pak™ bag for the bacterial enumeration procedure. Because the UV chamber generated heat during the sanitization procedures that could be harmful to the embryo, internal egg temperatures were also measured using an internal thermistor probe.

Experiment 2

Eight minutes of UV exposure was determined to be the optimum length of time for bacterial reduction in Experiment 1. Therefore, Experiment 2 was performed to determine the optimum concentration of H_2O_2 in combination with 8 minutes of UV exposure that would maximize bacterial reduction on eggshells. A total of 162 eggs were collected from caged White Leghorn hens. Eggs were then divided into 9 treatment groups, each consisting of 18 eggs. Treatment groups were as follows: untreated control,

1% H_2O_2, 2% H_2O_2, 3% H_2O_2, Dry UV, Wet UV (UV + sterile water), 1% H_2O_2 and UV, 2% H_2O_2 and UV, 3% H_2O_2 and UV. Control eggs were placed directly into sterile Whirl-pak™ bags and bacterial enumeration was performed. All eggs that were treated with H_2O_2 alone were evenly hand misted from many angles until the eggshell was completely coated but not dripping with one of the concentrations of H_2O_2, depending on treatment group. Those eggs were then allowed to dry for 8 minutes. However, eggs receiving H_2O_2 and UV light were misted with H_2O_2 and immediately placed into the UV chamber for 8 minutes. This was done so that all treatment groups receiving H_2O_2, regardless of UV treatment, were given the same amount of time to dry after misting with H_2O_2. After initial treatment, all eggs from every treatment group were placed into sterile Whirl-pak™ bags for bacterial enumeration.

Experiment 3

The third experiment was performed to further refine the optimum concentration of H_2O_2 in combination with 8 minutes of UV exposure to maximize bacterial reduction on eggshells. This experiment had a total of 10 treatment groups, each consisting of 18 eggs. There was a control group, 3% H_2O_2, Dry UV, Wet UV, 0.5% H_2O_2 and UV, 1% H_2O_2 and UV, 1.5% H_2O_2 and UV, 2% H_2O_2 and UV, 2.5% H_2O_2 and UV, and 3% H_2O_2 and UV. Eggs were collected from caged Single Comb White Leghorn hens. The control eggs were immediately processed for bacterial enumeration. Eggs receiving H_2O_2 treatment were hand misted with the designated concentration of H_2O_2 for their treatment group while located on wire flats. Eggs that received and UV treatment were misted with H_2O_2, placed into the UV chamber, and removed after 8 minutes. The eggs from the

single treatment group that only received UV exposure were placed onto wire flats and put into the UV chamber without being sprayed. After treatment, eggs were placed into sterile Whirl-pak™ bags for bacterial enumeration.

Experiment 4

Experiment 4 was conducted to determine the optimum concentration of H_2O_2 in combination with the optimum UV exposure time that would yield the greatest reduction of bacteria on eggshells with the shortest length of exposure to UV. This shorter exposure time to UV would be more appealing to the commercial poultry industry, due to the tremendous number of eggs that would need to be sanitized daily. A total of 10 treatment groups each consisting of 18 eggs were collected from caged Single Comb White Leghorn hens. Treatment groups consisted of a control, 1.5% H_2O_2 in combination with 2, 4, or 8 minutes of UV exposure time, 2% H_2O_2 in combination with 2, 4 or 8 minutes of UV , as well as 2.5% H_2O_2 in combination with 2, 4 or 8 minutes of UV. Control eggs received no treatment and were placed directly into Whirl-pak™ bags for bacterial enumeration. Eggs from each treatment group, excluding controls, were misted with H_2O_2 using a hand sprayer from many angles until they were completely coated but not dripping. The eggs were then placed immediately into the UV chamber for their designated time period. Once eggs were removed from the UV chamber they were placed into Whirl-pak™ bags for bacterial enumeration.

Statistical Analysis

Data collected from all 4 of the above experiments were analyzed as completely randomized designs. Means were separated using Fisher's protected least significant difference test ($P \geq 0.05$).

Results

Experiment 1

Eggs treated with different time intervals of UV light compared to control eggs can be seen in Figure 3.1. All treatment groups treated with UV light had a significant reduction in bacteria when compared to the control group. Also, eggs treated with 16 minutes of UV light yielded the greatest reduction in bacteria. However, the internal temperature of these eggs reached 37 C. The treatment group receiving 8 minutes of UV light did not exceed internal egg temperatures of 29 C and had a reduction in bacteria of 2.07 $\log_{10}$ CFU/egg.

Experiment 2

In Experiment 2, all treatment groups that received 8 minutes of UV light yielded significantly lower bacterial counts than the treatment groups that received 1 % or 2 % H_2O_2 alone (Figure 3.2). Also, the 3 treatment groups that received the combination of sanitization of UV light and H_2O_2 were significantly lower in bacterial counts than the treatment groups that received UV alone or the groups receiving only 1 % and 2 % H_2O_2. Numerically, the treatment group that received 2% H_2O_2 and UV light had the greatest

reduction in bacteria, and when compared to the control group, it yielded a bacterial reduction of 3.31 $\log_{10}$ CFU/egg.

Experiment 3

A significant bacterial reduction was observed in all treatment groups when compared to the control group (Figure 3.3). Also, all treatment groups receiving the combination of UV light and H_2O_2 were significantly lower than the treatment group receiving only H_2O_2. Additionally, the 2.5 % H_2O_2 and UV treatment yielded lower bacterial counts than the H_2O_2 or UV treatment alone. No significant differences were observed among any of the treatment groups treated with the combination of UV light and H_2O_2 at any of the 6 concentrations.

Experiment 4

In the final experiment, a significant reduction in bacteria was observed when comparing all treatment groups that were treated with the combination of UV light and H_2O_2, to the control group (Figure 3.4). No significant differences were observed when comparing the treatment groups that received 2 minutes of UV light in combination with the 3 different concentrations of H_2O_2, nor were there any significant differences among the treatment groups that received 4 minutes of UV light in combination with different concentrations of H_2O_2. However, when comparing the treatment groups that were treated with the combination of 8 minutes of UV light and H_2O_2 at the 3 different concentrations, the eggs treated with 1.5% H_2O_2 were significantly lower in bacterial counts than those eggs treated with 2% H_2O_2 or 2.5% H_2O_2. Also, the 1.5% H_2O_2 and 8

minutes of UV light reduced bacterial counts by 3.3 $\log_{10}$ CFU/egg when compared to the control group and was numerically lower than any other treatment group. Only 33.3% of the eggs in the treatment group that received 1.5 % H_2O_2 and 8 minutes of UV light were positive for bacteria. However, 100 % of the eggs in the control group were positive for bacteria (Figure 3.5).

Discussion

In the first experiment, the optimum exposure time of UV light was found to be 16 minutes. However, due to the accumulation of excess heat inside the egg, eggs could not be treated more than 8 minutes with UV or embryo damage might occur. The significant reduction of bacteria found on the eggshell is the result of the germicidal property of UV light that is able to destroy bacteria by degrading the cell wall (Kuo et al., 1997b). Also, UV-C causes a photochemical reaction within the nucleic acids of microorganisms and results in inactivation of these microorganisms (Bachmann, 1975). The data from Experiment 1 suggest that this inactivation did occur. Coufal et al. (2003) obtained a reduction of 1 to 2 $\log_{10}$ CFU/egg in aerobic bacterial counts when using high intensity UV light of 4 to 14 mW/cm^2. This was similar to the 2.07 $\log_{10}$ CFU/ egg reduction at approximately 11 mW/cm^2 that was obtained in this experiment. However, Coufal et al. (2003) only treated eggs with UV light for 4 minutes, where as in this experiment, eggs were treated for 8 minutes with UV light. Kuo et al. (1997a), also treated eggs with UV light for 1, 3, 5, or 7 minutes and was able to obtain a 2 log reduction in *S. typhimurium.* A 1 to 2 log reduction was also obtained by Chavez et al.,

(1999) when using only a 7.5 mW/cm^2 UV intensity. The 2 log reduction in bacteria that was obtained in this experiment was similar to all of the previous experiment's results.

In the second experiment, H_2O_2 and the optimum length of UV light (8 minutes) were combined to determine the concentration of H_2O_2 that should be administered. Previous work by Sander and Wilson (1999) demonstrated that when 3 % H_2O_2 was administered to broiler breeder eggs during the entire incubation period a significant reduction of bacteria was obtained. Also, the bacterial counts of eggs in Experiment 2 treated only once prior to incubation with 3 % H_2O_2 were significantly lower than the control eggs. Padron (1995) dipped eggs twice in 6 % H_2O_2 and reduced *S. Typhimurium* positive eggs by 55 %. However, when combining only 2 % H_2O_2 with UV light and treating eggs only once in the present experiment, bacterial counts were reduced from almost 4 log to less than 1 log. Bayliss and Waites (1982) consistently obtained more than a 4 log reduction in *Bacillus subtilis* when administering 2.5 % H_2O_2 in combination with UV light in vitro. This was similar to the greater than 3 log reduction in bacteria on eggshells in Experiments 2, 3, and 4 when administering H_2O_2 at various concentrations less than 3% in combination with UV light. This additional reduction in bacteria may be due to the production of hydroxyl radicals when H_2O_2 reacts with UV light (Bayliss and Waites, 1982). However, a numerical increase in bacterial counts was observed as concentrations of H_2O_2 increased above 2%. This could be due to the protection of bacterial spores by H_2O_2. Bayliss and Waites (1982) determined that when higher concentrations of H_2O_2 are used, the H_2O_2 will actually absorb the UV light and in turn provide protection for the bacterial spores. In addition, the free radicals produced by the combination of UV light and H_2O_2 at higher concentrations are also not as effective.

When an excessive concentration of H_2O_2 is administered, inactivation of the spores will no longer occur. Instead, breakdown will occur within the products produced by the H_2O_2 molecules (Bayliss and Waites, 1982).

In the third and fourth experiments, the concentrations of H_2O_2 and length of UV light were further refined. In Experiment 3 the sanitizing ability of H_2O_2 concentrations in excess of 1.5% was numerically reduced. Furthermore in Experiment 4 there were significantly more bacteria present when concentrations of H_2O_2 were increased above 1.5% in combination with 8 minutes of UV exposure. This further supports the results of Bayliss and Waites (1982), suggesting that higher concentrations of H_2O_2 can protect bacterial spores from the sanitizing effects of the combination of H_2O_2 and UV light.

From the results of Experiment 3 and 4, it appears that 8 minutes of UV exposure and 1.5% H_2O_2 is the optimum combination to consistently reduce bacteria on eggshells. This data proved that the combination of H_2O_2 and UV light can effectively kill bacteria located on the outer surface of the eggshell. However, because a greater reduction in bacteria was achieved when UV and H_2O_2 were administered together rather than separately, future research is needed to determine if this combination is effective in increasing hatchability.

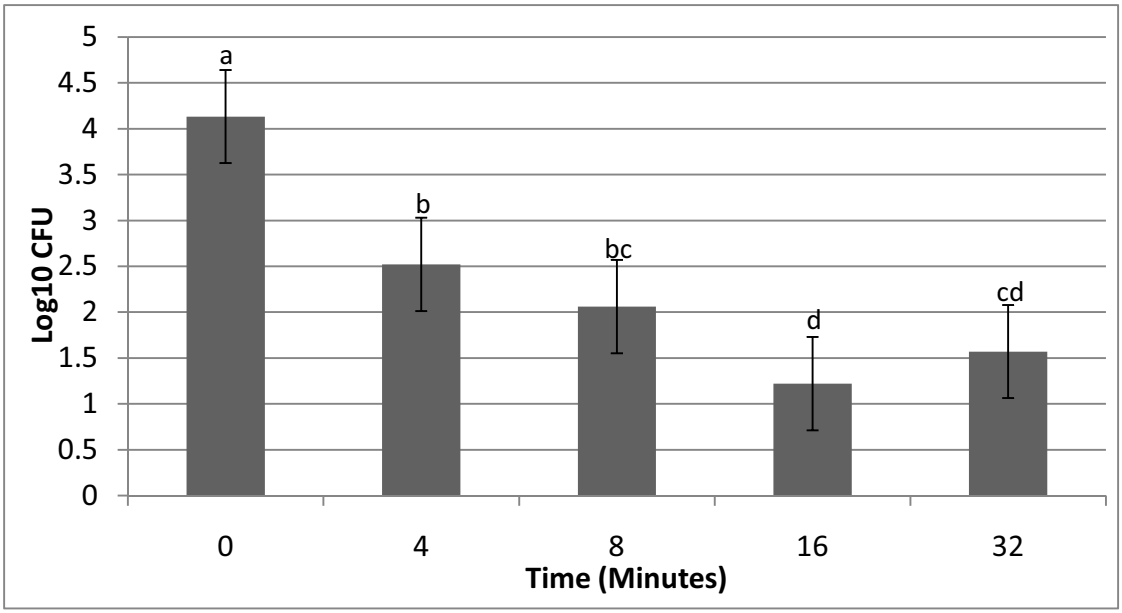

Figure 3.1

Bacterial counts for eggs treated in Experiment 1 with different time intervals of ultraviolet (UV) light compared to control eggs receiving no treatment.

[a-d] Means with different letters are significantly different at $P \leq 0.0001$.

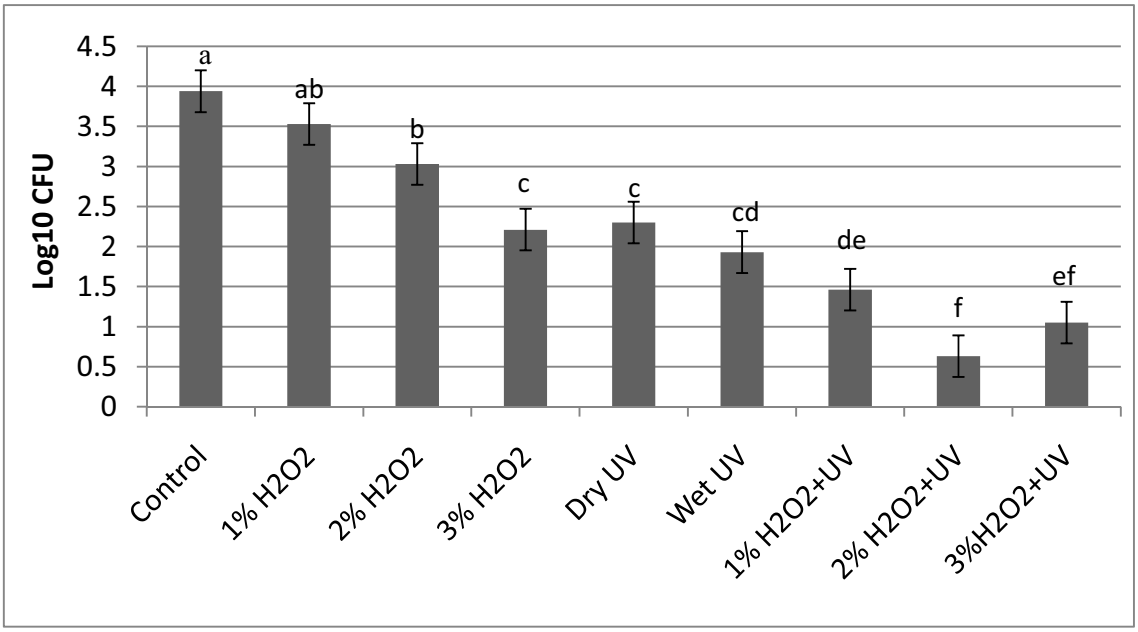

Figure 3.2

Comparison of treatments of different concentrations of hydrogen peroxide (H_2O_2) and ultraviolet light (UV) alone and in combination for bacterial presence on the eggshell in Experiment 2.

The Dry UV received only UV treatment, and the Wet UV was misted with sterilized water and treated with UV. [a-f] Means with different letters are significantly different at $P \leq 0.0001$.

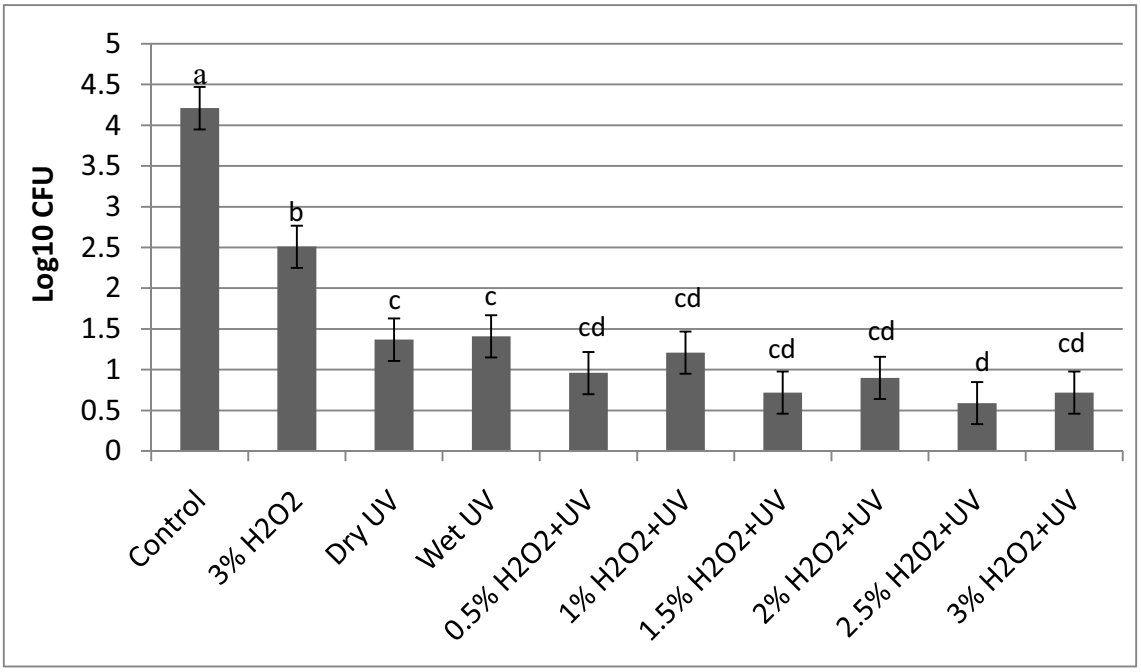

Figure 3.3

Comparison of bacterial counts in Experiment 3 of eggs treated with different concentrations of hydrogen peroxide (H_2O_2) and ultraviolet (UV) light.

Dry UV was only treated with UV, Wet UV was misted with water and then treated with UV, treatment groups containing H2O2 were misted with H_2O_2, and treatment groups containing H2O2+UV were treated with H_2O_2 and UV. [a-d] Means with different letters are significantly different at $P \leq 0.0001$.

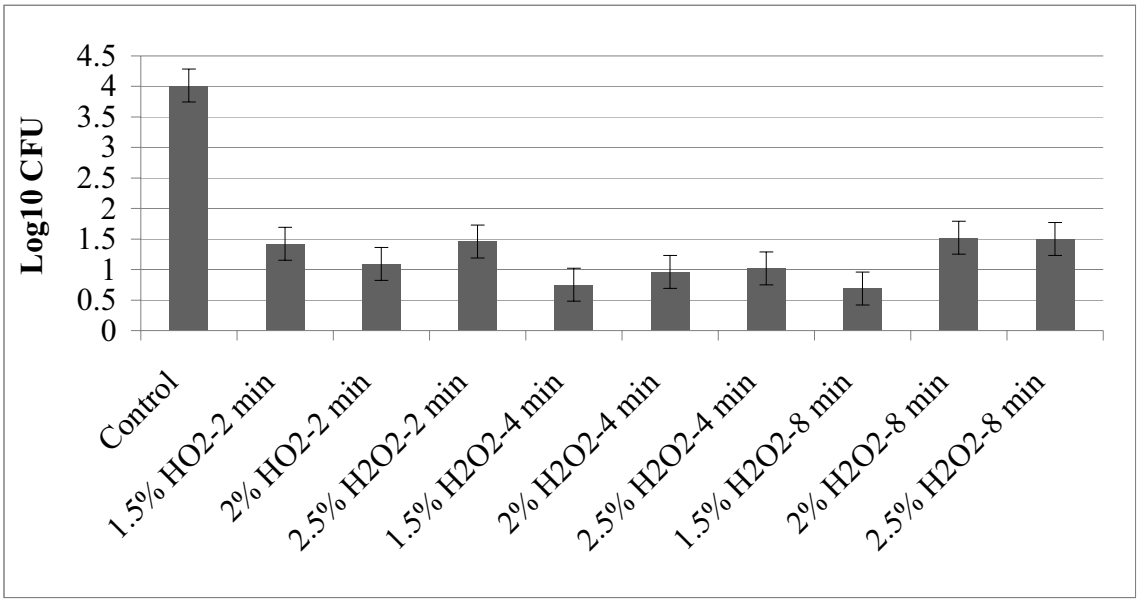

Figure 3.4

Comparisons of different concentrations of hydrogen peroxide (H_2O_2) in combination with different time lengths of ultraviolet (UV) light in Experiment 4.

All treatments labeled with H_2O_2 received treatment with H_2O_2, treatments labeled with 2 min received 2 minutes of UV light, treatments labeled with 4 min received 4 minutes of UV light, and treatments labeled 8 min received 8 minutes of UV light. $^{a\text{-}d}$ Means with different letters are significantly different at $P \leq 0.0001$.

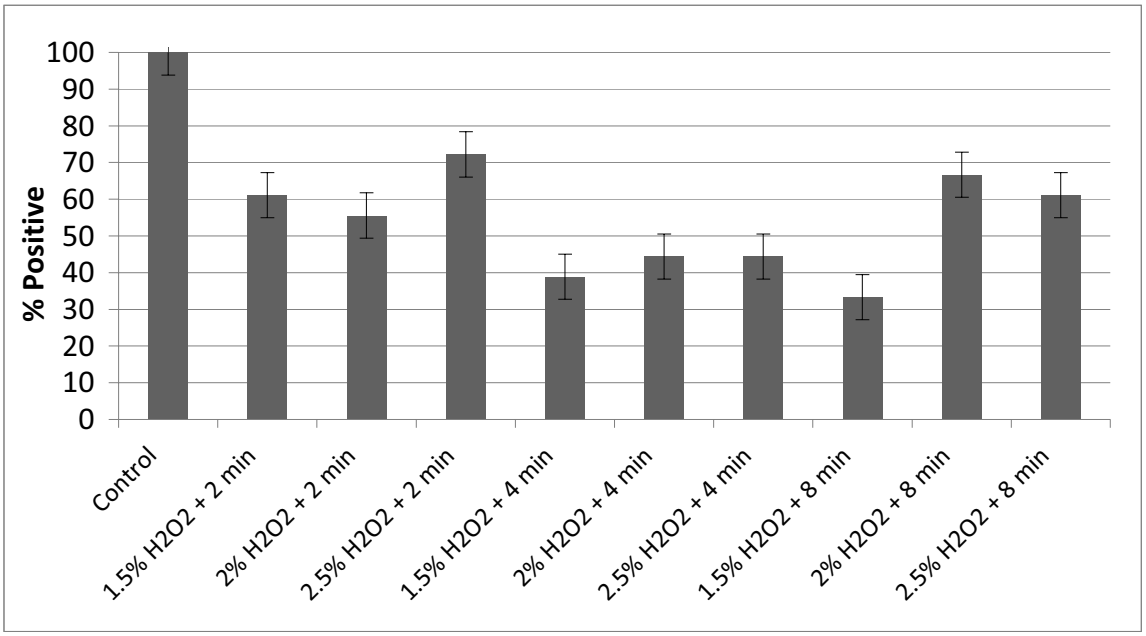

Figure 3.5

Percentage of eggs positive for bacteria at different concentrations of hydrogen peroxide (H_2O_2) in combination with different time lengths of ultraviolet light (UV) light in Experiment 4.

Treatments labeled with 2 min, 4 min, or 8 min received UV for the specified time period of 2, 4, or 8 minutes. [a-d] Means with different letters are significantly different at $P \leq 0.0001$.

REFERENCES

Bachmann, R., 1975. Sterilization by intense ultraviolet radiation. Brown Boveri Rev. 62:206-209.

Bayliss, C., and W.M. Waites, 1982. Effect of simultaneous high intensity ultraviolet irradiation and hydrogen peroxide on bacterial spores. J. Fd Technol. 17:467-470.

Chavez, C., K. Knape, and J. Carey. 1999. Reduction of egg shell aerobic plate counts by ultraviolet light irradiation. Poult. Sci. 78(Suppl. 1):67

Chavez, C., K.D. Knape, C.D. Coufal, and J.B. Carey, 2002. Reduction of eggshell aerobic plate counts by ultraviolet irradiation. Poult. Sci. 81:1132-1135.

Cox, N., M.E. Berrang, and J.A. Cason, 2000. *Salmonella* penetration of egg shells and proliferation in broiler hatching eggs: A review. Poult. Sci. 79:1571-1574.

Coufal, C., C Chavez, K. Knape, and J. Carey. 2003. Evaluation of a method of ultraviolet light sanitation of broiler hatching eggs. Poult. Sci. 82:754-759.

Huang. Y., and R. Toledo. 1982. Effect of high doses of high and low intensity UV irradiation on surface microbiological counts and storage-life of fish. J. Food Sci. 47:1667-1669,1731.

Kuo, F., J.B. Carey, and S.C. Ricke, 1997a. Ultraviolet irradiation of shell eggs: Effect on populations of aerobes, molds, and inoculated *Salmonella* typhimurium. J. Food Protection. 60(6):639-643.

Kuo, F., S.C. Ricke, and J.B. Carey, 1997b. Shell egg sanitation: Ultraviolet radiation and egg rotation to effectively reduce populations of aerobes, yeasts, and molds. J. Food Protection. 60(6):694-697.

Mine, Y., C. Oberle, and Z. Kassaify, 2003. Eggshell matrix proteins as defense mechanism of avian eggs. J. Agric. Food Chem. 51(1):249-253.

Padron, M., 1995. Egg dipping in hydrogen peroxide solution to eliminate *Salmonella* typhimurium from eggshell membranes. Avian Dis. 39:627-630.

Rodriguez-Romo, L., and A.E. Yousef, 2005. Inactivation of *Salmonella* enteric serovar enteritidis on shell eggs by ozone and ultraviolet radiation. J. Food Protection. 68(4):711-717.

Sander, J.E., and J.L. Wilson, 1999. Effect of hydrogen peroxide disinfection during incubation of chicken eggs on microbial levels and productivity. Avian Dis.43:227-233.

Tapper, M. and R. Hicks. 1998. Temperate viruses and lysogeny in lake superior bacterioplankton. Limnol. Oceanogr. 43(1):95-103

CHAPTER IV

EFFECTS OF EGGSHELL SANITIZATION USING ULTRAVIOLET LIGHT AND HYDROGEN PEROXIDE IN COMBINATION ON HATCH PARAMETERS OF BROILER BREEDER EGGS.

Abstract

Previous research in our lab has shown that exterior eggshell bacteria are greatly decreased using the combination of ultraviolet light (UV) and hydrogen peroxide (H_2O_2). However, it is unknown whether this reduction in eggshell bacteria has any effect on egg hatchability. Therefore, the objective of this experiment was to determine if eggshell bacterial reduction using UV irradiation for 8 minutes and 1.5% H_2O_2 in combination could improve hatchability of broiler breeder eggs. Eggs from 3 commercial houses (57 wk-old-broiler breeders) were collected over 2 d. A total of 1944 eggs were transported to the lab, where half were treated with UV and H_2O_2 and the other half served as controls, for a total of 54 replications per treatment with 18 eggs per replication. At time of treatment, 1 egg was randomly selected from each replication for bacterial enumeration on TSA. The remaining eggs were stored at 18.3 C up to 2 d. Prior to set, 1 egg per replication from Day 1 of collection was sampled for bacterial enumeration, and the 16 remaining eggs designated for incubation were weighed prior to set. Eggs were incubated for 21 day using 6 incubators, 3 each for control and treated eggs. At 18 d,

eggs were weighed to determine egg weight loss during incubation. At hatch, chick weights were obtained for each replication, and meconium samples were collected from 1 chick per replication. Meconium samples were plated on TSA agar to determine the presence of intestinal bacteria. Hatch residue analysis was performed on unhatched eggs after 21 days of incubation. A 1000 fold reduction in eggshell bacterial counts was found for treated eggs in comparison to control eggs. At hatch, no statistically significant differences in chick weight, egg moisture loss, positive meconium samples, or hatchability were observed between treatments. However, numerically, hatchability of total eggs set and hatchability of fertilized eggs were 2 and 6 percentage points, respectively, greater in the treated egg group in comparison to the control group off eggs. In conclusion, UV irradiation for 8 minutes in combination with 1.5% H_2O_2 reduced eggshell bacteria on broiler breeder eggs and shows potential for improving hatchability.

Introduction

Bacterial contamination located on the surface of the eggshell may have a negative effect on hatching eggs. It has been shown that this contamination can easily penetrate the eggshell and contaminate the embryo (Berrang et al., 1999). Wilson (1997) also reported that the contamination found on hatching eggs can lead to embryonic mortality, weak chicks, poor growth, and even chick mortality.

Safe and effective sanitization methods have been widely researched for many years. Ultraviolet light (UV) has been shown to be an effective method of sanitization for the eggshell. Unfortunately, in research conducted by Berrang et al. (1995) as well as Goerzen and Scott (1995), it was determined that even though UV light was effective at

reducing bacterial contamination on the eggshell surface of broiler breeder eggs it was not effective at increasing hatchability.

Sanitization using hydrogen peroxide (H_2O_2) has been researched to determine if it can affectively increase hatchability. Research conducted by Sander and Wilson (1999) demonstrated that 3% H_2O_2 was effective at reducing bacteria but it had no effect on hatchability. Padron (1995) also found no increase in hatchability when eggs were dipped in 6% H_2O_2, even though 95% of the bacteria were reduced on the eggshell surface.

Bayliss and Waites (1982) reported that the combination of UV light and H_2O_2 can reduce bacterial counts in nutrient agar slopes by more than 4 logs. In more recent studies conducted by Wells et al. (2008), the combination of UV light and H_2O_2 was administered to the outer surface of eggshells, and like the results of Bayliss and Waites (1982), bacterial contamination on the eggshell was reduced by 4 logs. However, it is still undetermined if this treatment combination can improve the hatchability of broiler breeder eggs. Therefore, the objective of this project was to determine if bacterial reduction on the eggshell using H_2O_2 and UV light in combination can affect hatchability of broiler breeder eggs.

Materials and Methods

Egg Collection

A total of 1944 eggs from 57 wk-old-broiler breeder hens were collected. Egg collection was completed over a 2 day period, with 864 eggs collected on the first day at 10:00 am.

The following day 1080 eggs were collected during the same time frame, beginning at 10:00 am. All eggs were collected directly off of the egg belts of 3 houses located on a single commercial breeder farm. All 1944 eggs from the 2 days of collection were divided equally into a control group and a treated group.

Egg Treatment, Incubation, and Hatch

After all eggs were collected, the treated eggs were then placed horizontally on wire trays and hand misted until completely coated with 1.5% H_2O_2. The eggs received enough H_2O_2 on the surface to be entirely covered without dripping. Immediately after treating each tray with H_2O_2, the eggs were placed into a UV chamber for 8 minutes. The UV chamber used in this experiment had a UV-C intensity of approximately 11 mW/cm^2. Immediately after all of the eggs were treated, they were placed into clean paper flats.

All of the eggs, including the control eggs, were then placed into a cooler at 18.3 C. The eggs collected on the first day were stored for 2 days in the cooler, and the eggs collected on the second day were only stored for 1 day. After being stored in the cooler, the eggs were removed. At this time, initial egg weights were then recorded for calculating incubational egg weight loss. This was performed to determine if treatment altered moisture loss through the eggshell. Out of the eggs that had been stored in the cooler for 2 days, 24 eggs were selected for bacterial enumeration to determine the effect of storage on bacterial growth following sanitization.

On the third day of the experiment, eggs from Days 1 and 2 of collection were set. These eggs were separated between 6 different incubators. There were 3 incubators that contained all control eggs and 3 incubators that only contained treated eggs with

approximately 300 eggs per incubator. Treated and control eggs were placed in different incubators to prevent bacterial cross contamination between the control and treated groups. One incubator of control eggs and 1 incubator of treated eggs represented a single breeder house.

After 19 days of incubation, eggs were removed from each incubator and weighed. Egg weights were obtained to determine weight loss during the incubation period relative to set egg weight. After weighing was complete, all of the eggs were placed into hatching baskets (8 baskets/incubator). After a total incubation period of 21.5 days, chicks hatched at this time were removed from all 6 incubators. Every chick was counted and weighed. A single chick from each hatching basket was randomly selected during weighing, and a meconium sample was obtained by forced fecal expulsion. After chicks were removed from hatching baskets, the remaining eggs that had not hatched at this time were collected, and hatch residue analysis was performed.

Bacterial Enumeration Procedure

From both treated and control eggs, 168 randomly selected eggs were used for bacterial enumeration. These eggs were individually placed into a sterile Whirl-pak™ bag containing 50 mL of sterilized peptone. Eggs were then massaged to remove bacteria located on the outer surface of the eggshell. After the massage, 10 mL of the rinse solution was aseptically pipetted into sterile culture tubes. Preliminary research revealed that control eggs were highly contaminated (Wells et al., 2009). Therefore, 2 serial dilutions were performed for each control egg. However, no serial dilutions were performed on the treated eggs. For the control eggs, 0.5 mL of the egg rinse and diluted

samples were spread plated in duplicate on tryptic soy agar (TSA). Also, 0.5 mL of egg rinse from the treated eggs was spread plated in duplicate onto TSA plates. The plates were incubated for 48 hours at 37 C before colony enumeration was performed.

At hatch, 108 chicks were randomly selected, and meconium samples were obtained. Meconium was expressed from the cloaca of each chick directly into a sterile Whirl-pak™ bag and 5 ml of sterile peptone was added. This solution of peptone and meconium was mixed, and 0.5 ml was spread plated in duplicate on TSA.

Statistical Analysis

Data from the entire experiment were analyzed as a completely randomized block design and means were separated using Fisher's protected least significant difference ($P \leq 0.05$). Each of the 3 breeder houses served as a block. Also, a 2 X 2 factorial arrangement of treatments was used to analyze egg sanitization and length of egg storage.

Results

Effects of H_2O_2 and UV light sanitization on bacterial contamination from broiler breeder hatching eggs can be seen in Figure 4.1. Bacterial counts from eggs treated with the combination of H_2O_2 and UV light were significantly lower than the control eggs. There was a 2.8 $\log_{10}$CFU/egg reduction in bacteria when comparing the treated to control eggs. Also found in the bacterial enumeration data was an interaction of sanitization and egg storage. When analyzing the interaction of egg sanitization and storage, a significant bacterial reduction during egg storage was observed in treated eggs

but not control eggs (Figure 4.2). A 1.25 log reduction was found in treated eggs that were stored when comparing them to the treated eggs that were fresh.

There were no significant differences between treatments for infertile, early dead, mid dead, late dead, or pip embryonic mortalities (Table 4.1). However, the late dead and early dead embryos were numerically lower in the treated group when compared to the control group. Also, there were no significant differences observed in the chick weight, egg weight loss, and meconium samples positive for bacteria (Table 4.2). In addition, when comparing hatchability of total eggs set and hatchability of fertilized eggs, the treated groups had a numerical increase of 2 and 6 percentage points, respectively, (Figure 4.3 and 4.4), but were not statistically different ($p \geq 0.7$).

Discussion

As shown by Wells et al. (2008) for White Leghorn eggs, the combination of UV light and H_2O_2 effectively reduced bacterial contamination found on the surface of broiler breeder eggshells. Wells et al. (2008) demonstrated a 3.3 log reduction in bacteria when treating White Leghorn eggs with 1.5 % H_2O_2 and 8 minutes of UV light. However, in the current study, there was only a 2.8 log reduction in bacteria. This lower reduction in bacteria in the current study was probably due to differences in breeds and in housing of the birds that laid the eggs. For example the White Leghorn eggs were from caged hens and had 1.9 $\log_{10}$ CFU/egg fewer bacteria than the broiler breeder eggs used in this experiment which were from litter floor commercial houses. However, regardless of chicken breed, both aforementioned studies suggest the formation of free radicals that

occur when H_2O_2 is combined with UV light is effective at producing a rapid kill of bacteria, as suggested by Bayliss and Waites (1982).

Eggs that were treated and then stored in the cooler also had significantly lower bacterial counts than those fresh eggs that were treated and sampled for bacterial enumeration. Possibly the combination of UV and H_2O_2 damaged the bacterial cell wall (Kuo et al., 1997), which in turn caused the bacteria to be unable to withstand colder temperatures. Thieringer et al. (1998) suggested that the stress of low temperatures can hinder stabilization of DNA and RNA secondary structures in bacteria. Phadtare et al. (1999) also suggested that colder temperatures can cause DNA replication to be less efficient in bacteria. If the treatment of UV and H_2O_2 had already caused thymine dimers to form on DNA strands (Bachmann, 1975), then it may be possible once eggs were placed into the cooler, that stabilization was impossible for the bacteria, which resulted in their death.

The data from this experiment also demonstrated that treatment did not significantly affect hatchability. In other experiments using only UV light to sanitize eggs, researchers also demonstrated no effects on hatchability. Berrang et al. (1995) exposed broiler breeder eggshells to continuous UV light at 254 nm over the entire 21 days of incubation had no effect on hatchability. However, using hatching eggs that were treated with a commercial sanitizer and then exposed to 1, 3 or 5 minutes of UV light resulted in a significant increase in embryo viability (Scott, 1993). This was similar to the embryonic mortality trend in the current experiment in which eggs were sanitized using H_2O_2 before UV light treatment for 8 minutes. However, in the current study, no significant increase occurred in hatchability. This could be the result of bacterial

contamination found on the outer eggshell penetrating the egg before treatment could occur. Cox et al. (2000) suggests that as soon as eggs are laid in nest boxes they come in contact with bacteria. Williams et al. (1968) demonstrated that *Salmonella* was able to penetrate the cuticle and entire shell almost immediately after exposing the shell to bacteria. However, the *Salmonella* used in that experiment was inoculated onto the egg using a liquid. This liquid could have made it easier for bacteria to enter the pores of the egg. In a commercial setting, eggs are rarely submersed in water. Therefore, bacteria within the broiler breeder house that these eggs came into contact with may not so easily penetrate the cuticle and entire shell immediately.

However, even though hatchability in this study was not significantly increased, a numerical increase in hatchability of total eggs set and hatchability of fertilized eggs was observed. This suggest that even though it may be possible for bacteria to penetrate the egg before sanitization can occur, enough bacteria can be removed during sanitization that have not already entered the eggshell. Also, the fertility in both control and treated eggs from this experiment was extremely low. The percentage of infertile eggs for the control and treated eggs was 25 and 28%, respectively. This excessive infertility may have hindered our ability to detect a significant effect on hatchability. In conclusion, the numerical increase in hatchability observed in this experiment suggests that with further research it may be possible to obtain a statistically significant difference in hatchability of broiler breeder eggs if a greater kill in bacteria can be obtained with UV and H_2O_2.

Table 4.1

Means for Hatch Residue Analysis Data for Broiler Breeder Eggs

Parameter	Control	Treated	SEM	P Value
Infertile (%)	25.1	28.4	1.1	0.18
Early Dead (%)	5.8	4.1	1.0	0.35
Mid Dead (%)	1.3	1.3	0.3	0.99
Late Dead (%)	9.6	5.3	1.7	0.47
Pip (%)	1.9	3.7	2.0	0.50
Contaminated (%)	1.62	0.92	0.7	0.53

Table 4.2

Means of Chick Characteristics of Broiler Breeder Chicks at Hatch

Characteristic	Control	Treated	SEM	P Value
Chick Weight (g)	48.2	48.4	0.68	0.83
Egg Weight Loss (%)	11.9	11.6	0.06	0.09
Meconium Samples Positive for Bacteria (%)	72.2	79.6	3.5	0.27

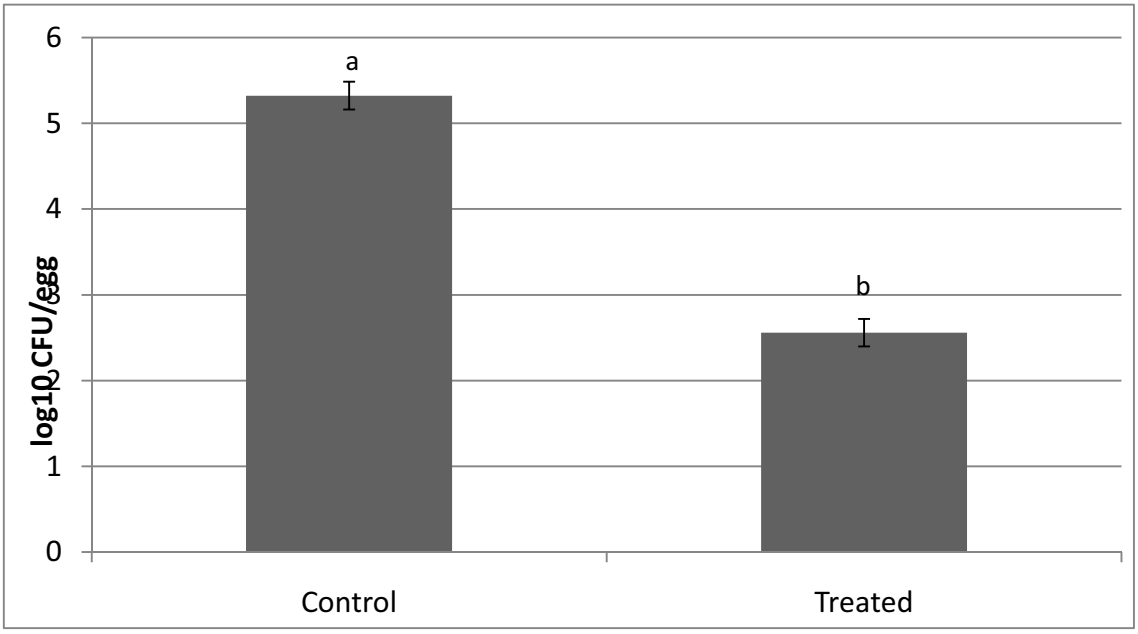

Figure 4.1

Effect of hydrogen peroxide H_2O_2 and ultraviolet UV light sanitization on bacterial contamination of broiler breeder hatching eggs.

[a-b] Means with different letters are significantly different at $P \leq 0.002$.

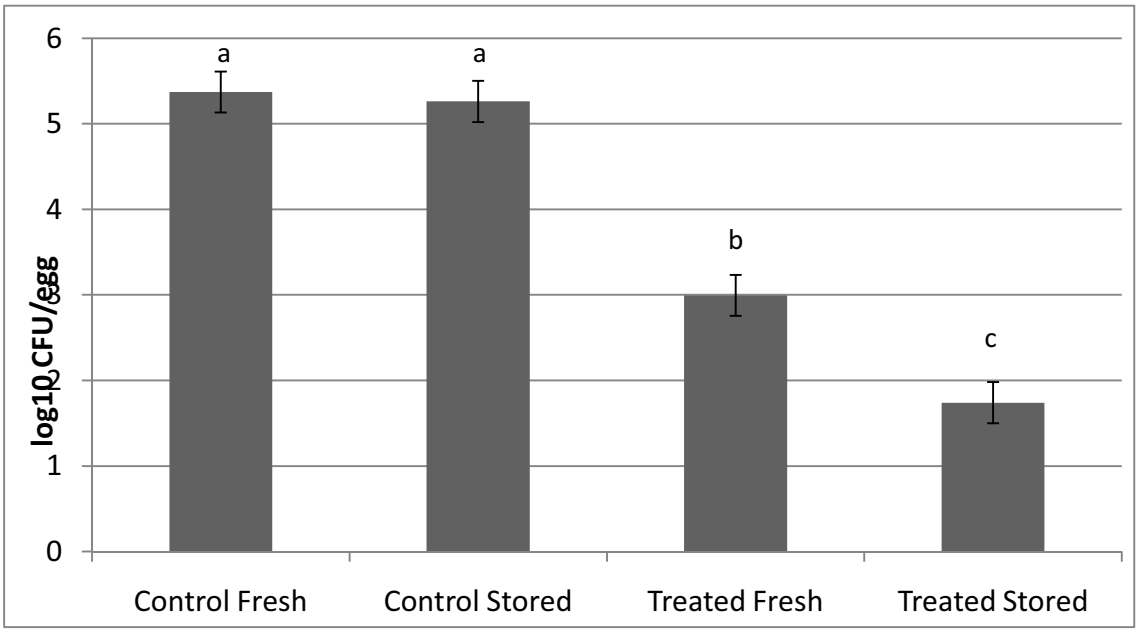

Figure 4.2

Effects of sanitization and egg storage on eggshell bacterial counts.

[a-c] Means with different letters are significantly different at $P \leq 0.05$.

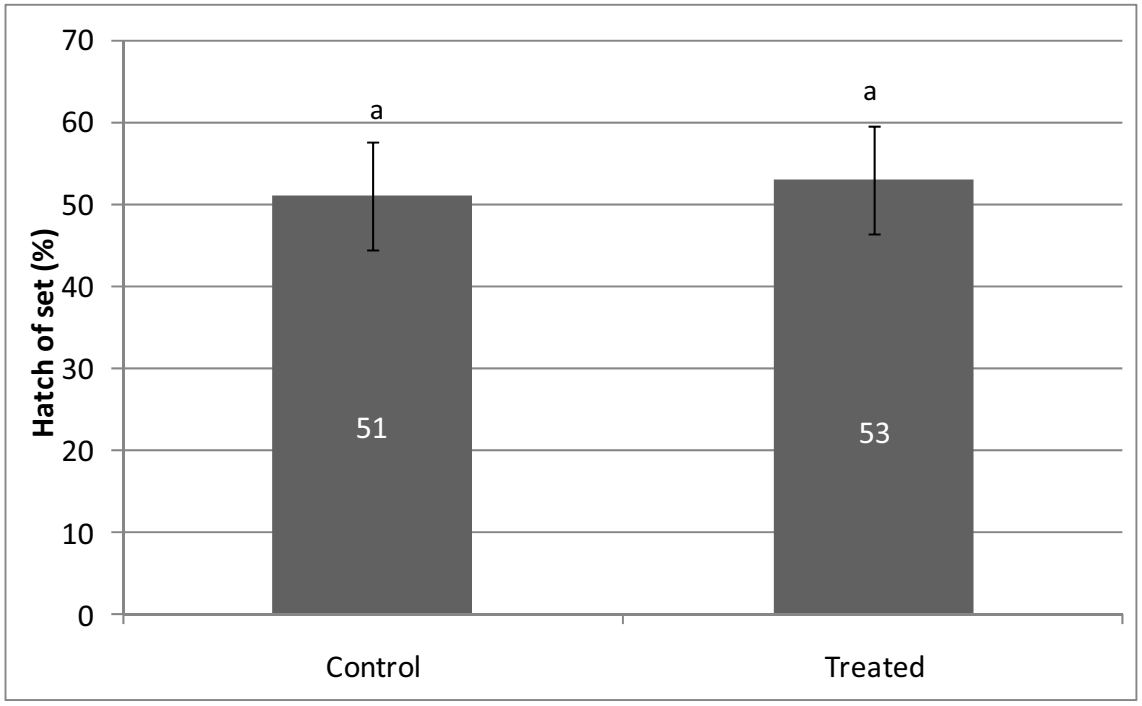

Figure 4.3

Percentage hatchability of total eggs set for control and treated eggs.

Means are not significantly different at P≥0.9.

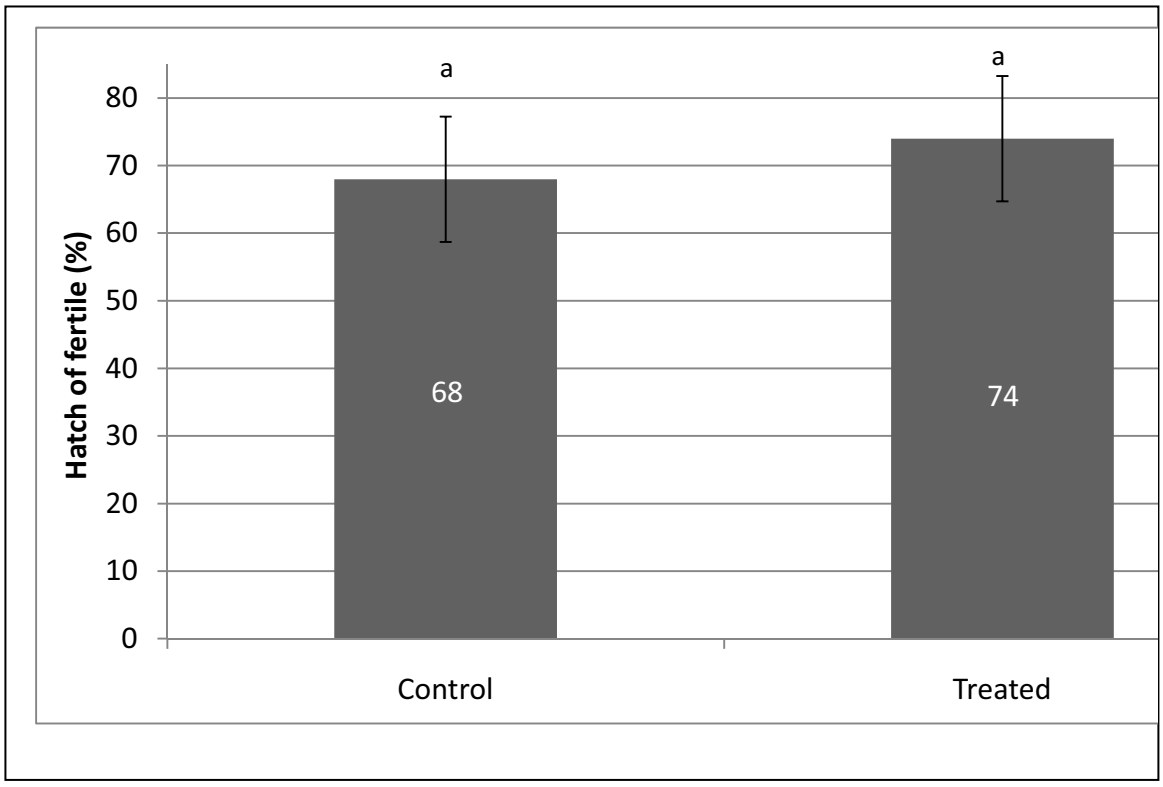

Figure 4.4

Percentage hatchability of total fertilized eggs for control and treated eggs. Means are not significantly different at P≥0.7.

REFERENCES

Bachmann, R., 1975. Sterilization by intense ultraviolet radiation. Brown Boveri Rev. 62:206-209.

Bayliss, C., and W.M. Waites, 1982. Effect of simultaneous high intensity ultraviolet irradiation and hydrogen peroxide on bacterial spores. J. Fd Technol. 17:467-470.

Berrang, M., N.A. Cox, and J.S. Bailey, 1995. Efficacy of ultraviolet light for elimination of *Salmonella* on broiler hatching eggs. J. Appl. Poultry Res. 4:422-429.

Berrang, M.E., N.A. Cox J.F. Frank and R.J.Buhr, 1999. Bacterial penetration of the eggshell and shell membranes of the chicken hatching egg: A review. Appl.Poultry Res. 8:499-504

Cox, N., M.E. Berrang, and J.A. Cason, 2000. *Salmonella* penetration of egg shells and proliferation in broiler hatching eggs: A review. Poult. Sci. 79:1571-1574

Goerzen, P., and T.A. Scott, 1995. Ultraviolet light sanitation for broiler hatching eggs. Poult. Sci. 74:83.

Kuo, F., S.C. Ricke, and J.B. Carey, 1997. Shell egg sanitation: Ultraviolet radiation and egg rotation to effectively reduce populations of aerobes, yeasts, and molds. J. Food Protection. 60(6):694-697.

Padron, M., 1995. Egg dipping in hydrogen peroxide solution to eliminate *Salmonella* typhimurium from eggshell membranes. Avian Dis. 39:627-630.

Phadtare, S., J. Alsina, and M. Inouye, 1999. Cold-shock response and cold-shock protein. Current Opinion in Micro. 2:175-180.

Sander, J.E., and J.L. Wilson, 1999. Effect of hydrogen peroxide disinfection during incubation of chicken eggs on microbial levels and productivity. Avian Diseases.43:227-233.

Scott, T., 1993. The effect of ultraviolet light and air filtering system on embryo viability and microorganism load on the egg shell. J. Appl. Poultry Res. 2:19-25.

Thieringer, H. A., P. G. Jones, and M. Inouye, 1998. Cold shock and adaptation. Bioessays. 20:49-57.

Wells, J., C. Coufal, H. Parker, and C. McDaniel, 2008. Disinfection of Eggshells Using Ultraviolet Light and Hydrogen Peroxide Independently and in Combination. Poult. Sci. 87(Suppl. 1):143.

Williams, J., l. Dillard, and G. Hall, 1968. The penetration patterns of Salmonella typhimurium through the outer structures of chicken eggs. Avian Dis. 12:445-466

Wilson, H., 1997. Hatching egg sanitation. University of Florida, Institute of Food and Agricultural Sciences (UF/IFAS).

CHAPTER V

HATCHABILITY OF BROILER BREEDER EGGS FOLLOWING EGGSHELL SANITIZATION BY REPEATED TREATMENT WITH A COMBINATION OF ULTRAVIOLET LIGHT AND HYDROGEN PEROXIDE.

Abstract

Exposing eggs to ultraviolet light (UV) in combination with 3% hydrogen peroxide (H_2O_2) results in a greater reduction of eggshell bacteria compared to eggs treated with only UV and H_2O_2. However, it is unknown if repetitive treatment of the eggshell with UV and H_2O_2 will result in a greater reduction in bacteria and improve hatchability. Therefore, the objective of this experiment was to determine if hatchability would be affected by reducing eggshell bacteria with repeated uses of UV and H_2O_2. In the first experiment, a total of 10 treatment groups were used. A control, UV for 2 minutes 6 times with and without egg rotation, H_2O_2 for 2 minutes 6 times with rotation, H_2O_2 and UV for 30 seconds 3 and 6 times with rotation, H_2O_2 and UV for 1 minute 3 and 6 times with rotation, and H_2O_2 and UV light for 2 minutes for 3 and 6 times with rotation. A total of 21 eggs were used in each treatment group. The eggs receiving H_2O_2 and UV light for 2 minutes 6 times yielded the greatest reduction in bacteria of 5.3 logs when compared to the treated group. The second experiment determined the effect on hatchability when using this combination found in Experiment 1. A total of 2,208 eggs

from 47 wk-old-broiler breeders were collected from a commercial house and transported to the lab. Half of these eggs were treated and the other half served as controls. The treated eggs were misted with H_2O_2 and then exposed to 2 min of UV. This procedure was repeated a total of 6 times. Thirty-six eggs per treatment were used for bacterial enumeration. The remaining eggs were weighed and placed in 8 separate incubators (4 control and 4 treated). At 18 days of incubation, the eggs were weighed to determine percentage egg moisture loss and placed in hatching baskets. At hatch chick weights were determined. Also, meconium samples were obtained from 10 randomly selected chicks from each incubator to determine the presence of intestinal bacteria. All chicks from each incubator were placed into a corresponding grow out pen, so that each incubator was represented by a single pen. Hatch residue analysis was performed on unhatched eggs. At 5 days post hatch, yolk sacs from 20 chicks per room were removed, weighed, and yolk sac material was plated for presence of bacteria. A 4 log reduction in eggshell bacteria was observed for eggs treated with UV and H_2O_2 compared to control eggs. There were no differences in hatchability, hatch residue, chick weight, residual yolk weight, egg moisture loss, or bacterial presence in meconium, residual yolks, or incubator air samples between control and treated groups. In conclusion, multiple applications of UV and H_2O_2 effectively reduced bacteria on the eggshell with no effects on broiler breeder egg hatchability.

Introduction

Different methods of sanitizing the outer surface of the eggshell have been studied. Methods which include the use of formaldehyde gas, ozone, ethylene oxide, heat

treatment, and gamma irradiation have been used in poultry research. However, formaldehyde is a known carcinogen, and ozone can be harmful to the respiratory system of workers who are applying it to the eggshells (Rodriguez-Romo and Yousef, 2005). Also, ethylene oxide, heat treatment, and gamma irradiation are not widely accepted because they can be detrimental to chick embryos and pose safety concerns for the user as well (Shama, 1992). Therefore, safe and effective sanitization methods used on the outer eggshell surface are needed. Scott (1993) stated that ultraviolet (UV) light is safe for the user because it can be contained and is an effective sanitizer. Also, hydrogen peroxide (H_2O_2) has proven to be an effective sanitization method. Sander and Wilson (1999) were able to significantly reduce bacterial counts when administering 3 % H_2O_2 to eggshells.

Research by Bayliss and Waites (1982) demonstrated that the combination of H_2O_2 and UV light administered as a single application created hydroxyl radicals which resulted in a greater kill of bacteria in vitro when compared to either UV light or H_2O_2 alone. This research, using the combination of H_2O_2 and UV light, proved to be effective at killing bacteria on agar slopes. More recently, Wells et al. (2008) proved that the combination of H_2O_2 and UV light was effective at sanitizing eggshells, with a reduction of 2.8 $\log_{10}$ of CFU/egg, but it did not have a significant effect on hatchability. However, numerical increases (2 %) in hatchability of total eggs set and hatchability of fertilized eggs (6%) were observed. This data demonstrates that if bacterial contamination of the eggshell can be decreased further by a refined method combining H_2O_2 application and UV light exposure, then hatchability may be significantly increased. Therefore, the

objective of this study was to determine if hatchability would be affected by reducing eggshell bacteria with the repetitive use of the combination of H_2O_2 and UV light.

Materials and Methods

Experiment 1

Egg Treatment and Bacterial Enumeration

To determine the optimum number of repetitions of UV application and H_2O_2 exposure, a total of 210 eggs were collected from caged White Leghorn hens. The eggs were then separated into 10 treatment groups, each consisting of 21 eggs. These treatment groups consisted of a control, 2 minutes of UV 6 times with and without egg rotation, H_2O_2 for 2 minutes 6 times with rotation, H_2O_2 and UV for 30 seconds 3 and 6 times with rotation, H_2O_2 and UV for 1 minute 3 and 6 times with rotation, and H_2O_2 and UV for 2 minutes 3 and 6 times with rotation. Rotation of the egg was examined because Bachmann (1975) determined that eggshell sanitization using UV light occurs only in the direct radiation beam, whereas shaded areas are unaffected. Also, Kuo et al. (1997) found that when rotating eggs during UV light treatment less bacteria were found on the eggshell. After collection, control eggs were placed into Whirl-pak™ bags. All of the remaining eggs were placed onto wire flats according to treatment group. Treatment groups were then treated with H_2O_2 and or UV light as described above. After initial treatment, eggs were placed into Whirl-pak™ bags. Each bag was then filled with 50 mL of peptone. The bags were then massaged for 1 minute to remove any excess bacteria.

Rinse solution (10 mL) was aseptically pipetted into sterile culture tubes. Preliminary research revealed that control eggs were highly contaminated (Wells et al. 2008). Therefore, 2 serial dilutions were performed for each control egg. However, no serial dilutions were performed on the treated eggs. For the control eggs, 0.5 mL of the egg rinse and diluted samples and, 0.5 mL of egg rinse from the treated eggs were spread plated in duplicate onto tryptic soy agar (TSA) plates. The plates were incubated for 48 hours at 37ºC before colony enumeration was performed.

Experiment 2

Egg Treatment, Incubation, and Hatch

To determine if hatchability is affected by the optimum number of repetitive exposures to UV and H_2O_2 found in Experiment 1, a total of 2208 eggs from 47 wk-old-broiler breeder hens were collected from 3 commercial houses located on a single farm. Eggs were divided as follows, 1104 served as control eggs, and the remaining eggs were treated with a combination of H_2O_2 and UV light. For the treated eggs, eggs were placed horizontally on wire trays and misted with 3% H_2O_2. These eggs were coated by the mist but not saturated. Immediately after eggs were treated with H_2O_2, they were rotated and placed into an UV light chamber. The chamber had a UV-C intensity of approximately 11mW/cm^2. After 2 min of UV light treatment, eggs were misted again with H_2O_2 and placed into the UV light chamber. This procedure was repeated for a total of six times.

After the six repetitions of H_2O_2 and UV light were complete, eggs were weighed so that incubational egg weight loss could eventually be determined, and eggs were then

set into 8 separate incubators. To prevent cross contamination, 4 incubators were filled with eggs treated with the combination of H_2O_2 and UV light, and 4 incubators were filled with the eggs serving as controls. After eggs were incubated for 18 d, they were weighed to determine egg moisture loss. Eggs were then transferred into hatching baskets. At 21.5 day of incubation, chicks were removed, weighed, and placed into individual rooms, and all of the remaining eggs that did not hatch were broken out for hatch residue analysis.

Chicks from a single incubator were placed into a corresponding room for grow out. The rooms provided 0.084 m^2/bird. Grow out rooms had conventional curtain sides with litter floors and a radiant heat brooder. All chicks were fed a standard ad libitum broiler diet and had access to nipple drinkers at all times. Each room was given 23 hours of light and 1 hour of darkness each day. All birds were treated in accordance with the Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching.

Bacterial Enumeration

From both treated and control groups, 36 randomly selected eggs were used for bacterial enumeration. These eggs were individually placed into a sterile Whirl-pak™ bag containing 50 ml of sterilized peptone. Eggs were then massaged to remove bacteria located on the outer surface of the eggshell. After the massage, 10 mL of the rinse solution was aseptically pipetted into sterile culture tubes. Preliminary research revealed that control eggs were highly contaminated (Wells et al., 2008). Therefore, 2 serial dilutions were performed for each control egg. However, no serial dilutions were performed on the treated eggs. For the control eggs, 0.5 mL of the egg rinse and diluted

samples and, 0.5 mL of egg rinse from the treated eggs were spread plated in duplicate onto TSA plates. The plates were incubated for 48 hours at 37ºC before colony enumeration was performed.

At hatch, 10 chicks from each incubator were randomly selected and meconium samples were obtained. Meconium was expressed from the cloaca of each chick directly into a sterile Whirl-pak™ bag and 5 ml of peptone was added. This solution of peptone and meconium was mixed, and 0.5 mL was spread plated on TSA. At 5 days post hatch, 20 chicks from each room were randomly selected to obtain body and yolk sac weights to determine relative yolk sac weight. The yolk sacs from these chicks were aseptically removed, weighed, and sampled for bacterial enumeration by streaking the contents of the yolk sac directly onto TSA plates.

Statistical Analysis

All data were analyzed as completely random designs with incubator serving as experimental unit. Means were separated using Fishers protected least significant difference test ($P \leq 0.05$).

Results

In the initial experiment all eggs treated multiple times with any treatment yielded significantly fewer bacteria than the controls (Figure 5.1). Those eggs receiving both UV and H_2O_2 had significantly lower bacterial counts than eggs receiving multiple treatment of UV or H_2O_2 only. However, numerically, the treatment group receiving 3 % H_2O_2 and

2 minutes of UV light six times consecutively had the lowest bacterial count (0.26 $\log_{10}$ CFU/egg, Figure 5.1).

In the second experiment, there was a reduction of approximately 4.5 $\log_{10}$ CFU/egg in bacteria when comparing eggs treated with 3 % H_2O_2 and 2 minutes of UV light 6 times to control eggs (Figure 5.2). This resulted in more than 40% of the treated eggs testing negative for bacteria (Figure 5.3). However, for hatchability of total eggs set (Figure 5.4) and hatchability of fertilized eggs (Figure 5.5), there were no significant differences between control and treated groups.

The results for hatch residue analysis are illustrated in Table 5.1. Hatch residue analysis and fertilization level were not significantly different between treatments. However, early and late mortality were numerically lower for the treated group when compared to the control group. Furthermore, chick characteristics shown in Table 5.2 demonstrate no significant difference in percentage egg moisture loss, chick weight, relative yolk sac weight, or for the number of residual yolk sacs that were positive for bacteria.

Discussion

These experiments demonstrated that repetitive applications of UV light in combination with H_2O_2 reduced bacterial contamination found on the outer eggshell of broiler breeder hatching eggs by more than 4 logs. As shown in previous research conducted by Wells et al. (2008), a single treatment of the combination of H_2O_2 and UV light administered to the broiler breeder eggshell only resulted in a 2.8 $\log_{10}$ CFU/egg reduction in bacteria. Bayliss and Waites (1982) stated that the hydroxyl radicals created

by the combination of UV light and H_2O_2 proved to be very effective at producing a rapid kill of bacteria located on agar slopes. After obtaining a 4 log reduction in bacteria, data from this present experiment suggest that hydroxyl radical formation is more effective at killing bacteria than the photochemical reaction that occurs when UV light is administered alone (Bayliss and Waites, 1982). These data also suggest that when the combination of H_2O_2 and UV light is administered repetitively it is more effective than just a single application of H_2O_2 and UV light. For example, the 4 $\log_{10}$ CFU/egg reduction obtained in this experiment compared to previous research by Wells et al. (2008), where only a reduction of 2.8 $\log_{10}$ CFU/egg was obtained, demonstrates that repetitive applications of H_2O_2 and UV light is more effective than single applications. Bacteria that may have only been damaged with a single treatment of UV light and H_2O_2 may have been able to regenerate during the egg incubation period. However, it is apparent that bacteria are vulnerable to multiple applications of H_2O_2 and UV light.

Even though repetitive treatments of H_2O_2 and UV light killed bacteria, hatch residue analysis as well as chick characteristics demonstrated that hatchability of treated eggs was unaffected. The hatchability data suggest that bacterial contamination found on the outer surface of the eggshell may be able to penetrate the eggshell within the first few minutes of oviposition. Because the cuticle may be ineffective for the first few minutes of lay until it hardens (Sparks, 1987), bacterial contamination may occur before treatment of eggs is able to be performed. Also, immediately after the egg is laid, the effect of negative pressure within the egg allows bacteria to be pulled through the eggshell and membranes (Lock et al., 1992). Because the egg immediately comes in contact with bacteria found in the nest boxes when it is laid and these bacteria are able to penetrate the

egg almost immediately, Cox et al. (2000) suggested that some bacterial contamination is already within the egg before treatment can ever occur. It has also been shown that UV light cannot penetrate the eggshell (Gao et al., 1997). Therefore, if bacteria are able to penetrate the eggshell prior to UV light and H_2O_2 treatment, it will have no effect on the bacteria located inside the egg.

Another method of bacterial contamination is vertical transmission. Research conducted by Timoney et al. (1989) suggested that orally inoculating hens with bacteria can result in bacterial infection of the reproductive tract. Miyamoto et al. (1997) also demonstrated that intravenous inoculation causes contamination of eggs forming in the oviduct. This bacterial contamination in the reproductive tract could actually contaminate the ovum before the eggshell is even deposited in the oviduct. As a result, bacterial contamination present in the oviduct could be detrimental to the chick embryo prior to oviposition. Therefore, no sanitization method applied to the eggshell would be beneficial.

In the present study, the combination of H_2O_2 and UV light had no negative impact on hatchability. Also, no significant difference in egg moisture loss demonstrated that the cuticle on the outer eggshell was not harmed by the sanitization method used in this experiment. This suggests that the combination of H_2O_2 and UV light administered repetitively could be used to reduce bacterial contamination in hatcheries by ridding eggshells of bacteria before being transported to the hatchery facility. This experiment has proven that high intensity UV light in combination with H_2O_2 can be used as a safe method of sanitization without negatively effecting hatchability of broiler breeder eggs.

In conclusion, the initial objectives of this experiment were to determine if repetitive applications of H_2O_2 and UV light would yield a greater kill in bacteria and would affect hatchability. It was concluded that there was a greater kill in eggshell bacteria when the combination is used repetitively rather than as a single application. Finally, even though hatchability was not improved in the current study, there were no negative effects from the combination of H_2O_2 and UV light.

Table 5.1

Hatch Residue Analysis and Fertility of Broiler Breeder Eggs

Parameter	Control	Treated	SEM	P Value
Fertility (%)	94.2	93.8	0.49	0.61
Early Dead (%)	4.2	3.8	0.91	0.79
Mid Dead (%)	0.6	0.6	0.23	0.99
Late Dead (%)	3.3	2.4	0.38	0.14
Pip (%)	1.2	2.7	0.54	0.09
Contaminated (%)	0.3	0.3	0.14	0.99

Table 5.2

Parameters of Broiler Breeder Chicks Determined After Hatch

Parameter	Control	Treated	SEM	P Value
Egg Weight Loss (%)	11.6	11.2	0.44	0.54
Chick Weight (g)	46.0	46.7	0.59	0.46
Residual Yolks positive for bacteria (%)	54	59	8.1	0.73
Relative Residual Yolk Weight (%)	0.36	0.41	0.05	0.47

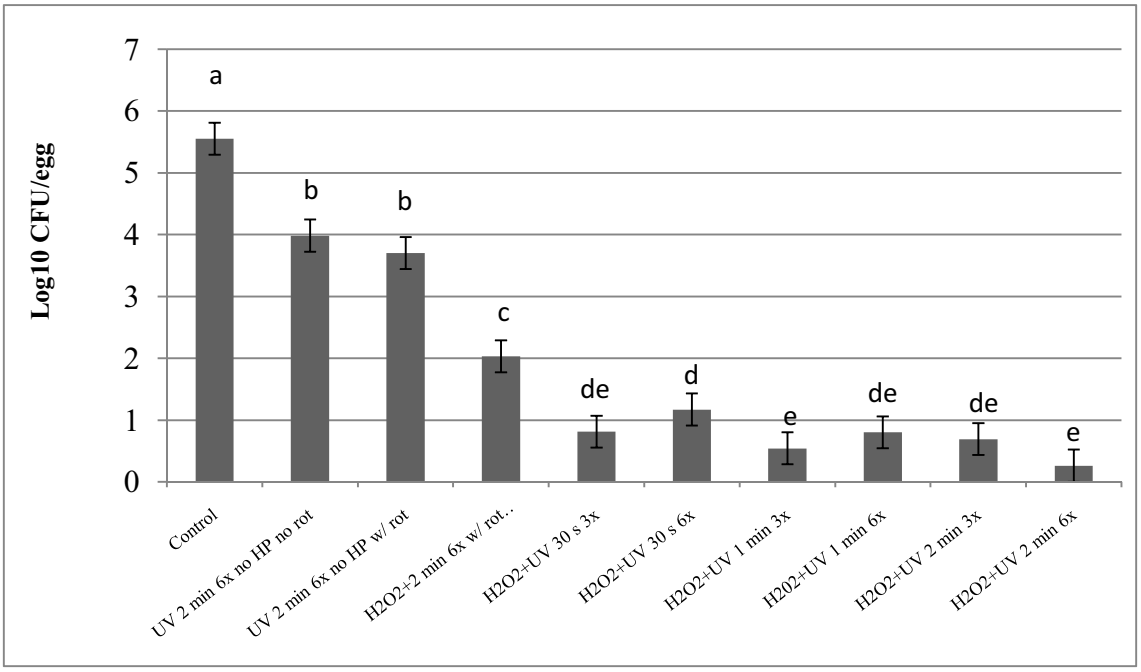

Figure 5.1

Bacterial counts of eggs treated with 3% hydrogen peroxide (H_2O_2) and ultraviolet (UV) light for various time lengths and repetitions with or without rotation.

[a-e] Means with different letters are significantly different at $P \leq 0.0001$.

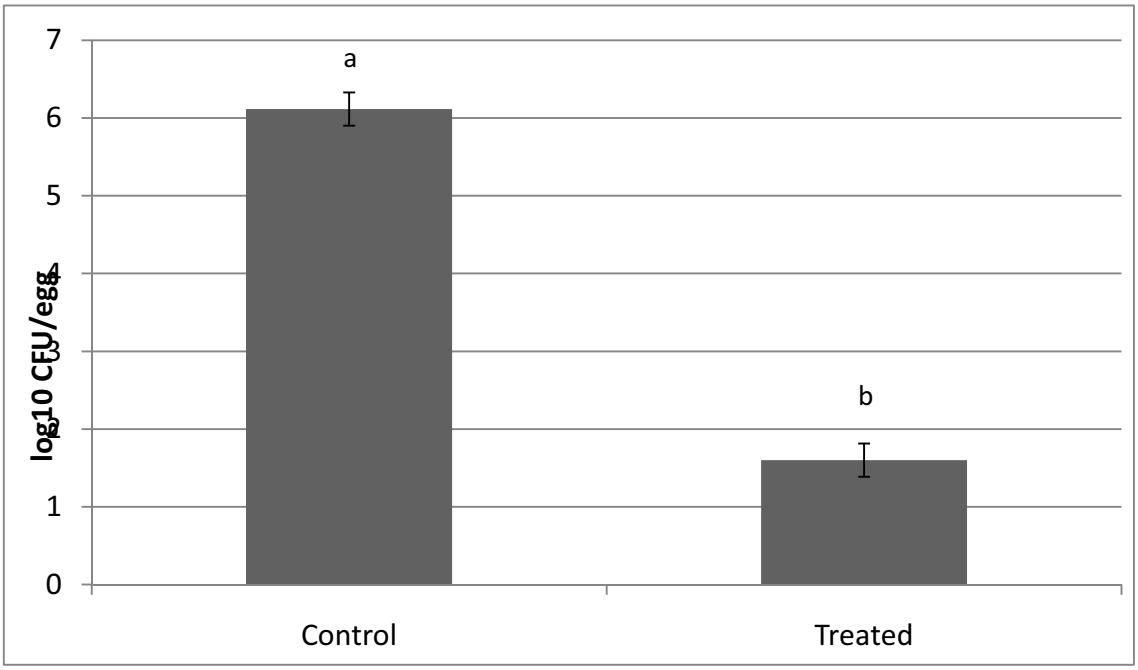

Figure 5.2

Effects of hydrogen peroxide (H_2O_2) and ultraviolet (UV) light sanitization treatment on bacterial counts of broiler breeder hatching eggs.

[a-b] Means with different letters are significantly different at $P \leq 0.0001$.

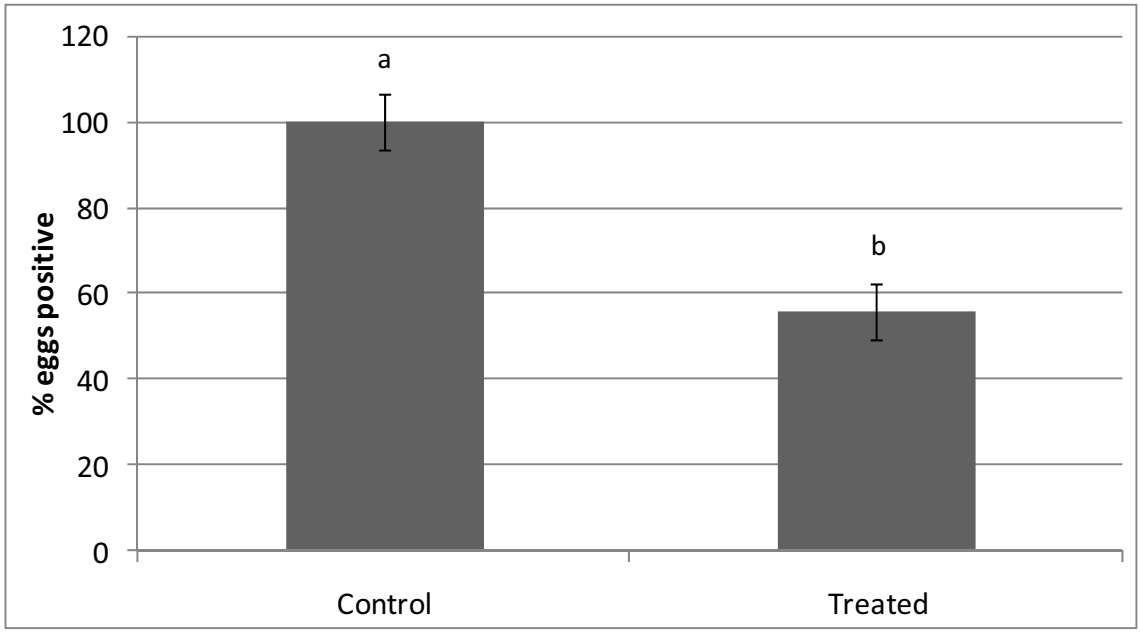

Figure 5.3

Percentage of control and treated eggs positive for bacteria.

[a-b] Means with different letters are significantly different at P≤0.0001.

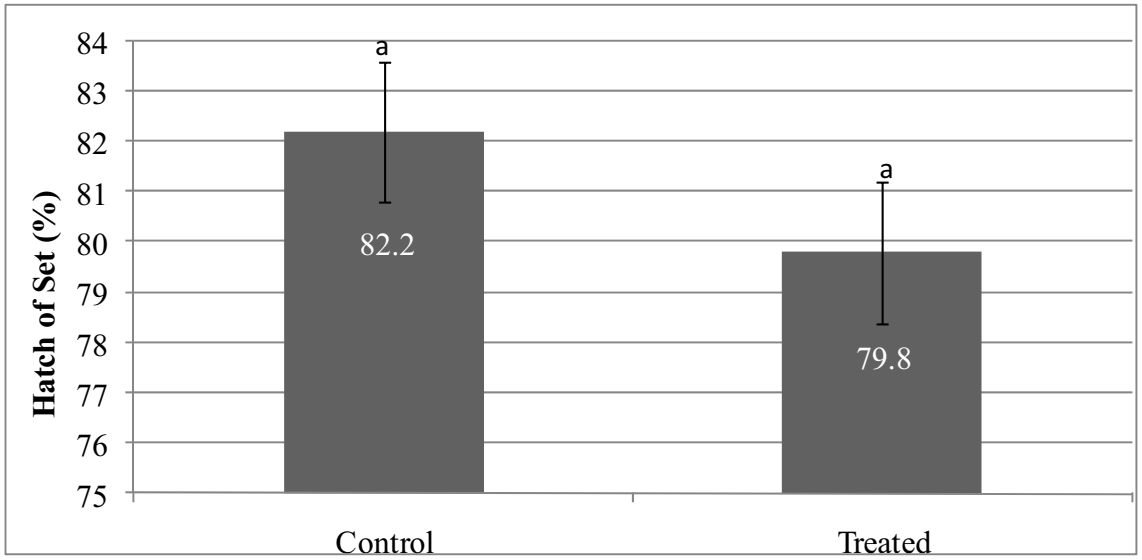

Figure 5.4

Percentage hatchability of total eggs set for control and treated groups.

Means are not significantly different at P≥0.27.

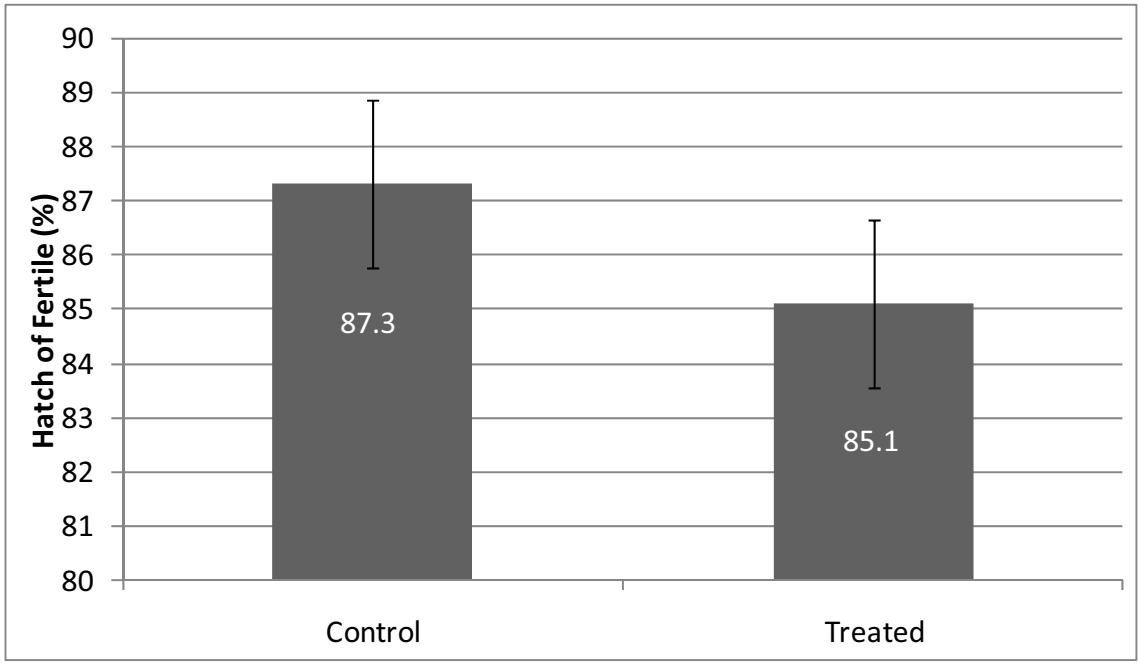

Figure 5.5

Percentage hatchability of fertilized eggs between control and treated groups. Means are not significantly different at P≥0.35.

REFERENCES

Bachmann, R., 1975. Sterilization by intense ultraviolet radiation. Brown Boveri Rev. 62:206-209.

Bayliss, C., and W.M. Waites, 1982. Effect of simultaneous high intensity ultraviolet irradiation and hydrogen peroxide on bacterial spores. J. Fd Technol. 17:467-470.

Cox, N., M.E. Berrang, and J.A. Cason, 2000. *Salmonella* penetration of egg shells and proliferation in broiler hatching eggs: A review. Poult. Sci. 79:1571-1574

Gao, F., L.E. Stewart, S.W. Joseph, and L.E. Carr, 1997. Effectiveness of ultraviolet irradiation in reducing the numbers of *Salmonella* on eggs and egg belt conveyor materials. Appl. Engineering in Agriculture. 13(3):355-359

Kuo, F., S.C. Ricke, and J.B. Carey, 1997. Shell egg sanitation: Ultraviolet radiation and egg rotation to effectively reduce populations of aerobes, yeasts, and molds. J. Food Protection. 60(6):694-697.

Lock, J., J. Dolman, and R.G. Board, 1992. Observations on the mode of bacterial infection of hen's eggs. FEMS Microbiol. Letters 100:71-74.

Miyamoto, T., E. Baba, T. Tanaka, D. Sasai, T. Fukata, and A. Hrakawa, 1997.Salmonella enteritidis contamination of eggs from hens inoculated by vaginal, cloacal and intravenous routes. Avian Dis. 41:296-303

Rodriguez-Romo, L., and A.E. Yousef, 2005. Inactivation of *Salmonella* enteric serovar enteritidis on shell eggs by ozone and ultraviolet radiation. J. Food Protection. 68(4):711-717.

Sander, J.E., and J.L. Wilson, 1999. Effect of hydrogen peroxide disinfection during incubation of chicken eggs on microbial levels and productivity. Avian Diseases.43:227-233.

Scott, T., 1993. The effect of ultraviolet light and air filtering system on embryo viability and microorganism load on the egg shell. J. Appl. Poultry Res. 2:19-25.

Shama, G. 1992. Ultraviolet irradiation apparatus for disinfecting liquids of high ultraviolet absorptivities. Lett. Appl. Microbiol. 15:69-72.

Sparks, N., 1987. The hen's eggshell: A resistance network. Aslib Index Theses 36:294.

Timoney, J., H. Shivaprasad, R. Baker, and B. Rowe, 1989. Egg transmission after infection of hens with Salmonella enteritidis page type 4. Vet. Rec. 125:600-601

Wells, J., C. Coufal, H. Parker, and C. McDaniel, 2008. Effects of eggshell sanitization using ultraviolet light and hydrogen peroxide in combination on hatch parameters of broiler breeder eggs. Poult. Sci. 87(Suppl. 1):47.

CHAPTER VI

CONCLUSION

It is known that the combination of H_2O_2 and UV light is a more effective sanitizing method than either H_2O_2 or UV light administered alone in vitro (Bayliss and Waites, 1982). However, the present research proved that the combination of H_2O_2 and UV light is also more effective at sanitizing the outer eggshell of broiler breeder eggs than H_2O_2 or UV light administered alone, and the combination proved to be more effective when used repetitively. The optimum concentration and exposure time of UV light was also determined in this experiment. Also, it was determined that the combination of H_2O_2 and UV light had no negative effects on hatchability of broiler breeder eggs.

The combination of H_2O_2 and UV light is more effective than UV light or H_2O_2 administered individually, because of hydroxyl radical formation when the combination is used simultaneously. These hydroxyl radicals help produce a more rapid kill of bacteria located on the eggshell. The present study also determined that the concentration of H_2O_2 must be limited in order to be effective. The optimum H_2O_2 concentration for a single exposure was determined to be 1.5% and when higher concentrations were used in combination with UV light, bacterial reduction decreased. This may be the result of H_2O_2 protecting bacterial spores. When a higher concentration is used, the H_2O_2 will

actually absorb the UV light and in turn protect the bacteria on the surface of the eggshell. Also, these higher concentrations of H_2O_2 will cause degradation to occur within the products produced by the H_2O_2 molecules. Since degradation is occurring in the H_2O_2 molecules it will no longer occur within the bacterial spores (Bayliss and Waites, 1982).

It was also determined in the present experiments that eggs treated and stored in the cooler had significantly lower bacterial counts than those fresh eggs that were treated and sampled for bacterial enumeration. This may be the result of the combination of UV and H_2O_2 damaging the bacterial cell wall (Kuo et al., 1997b), which in turn causes the bacteria to be unable to withstand colder temperatures. Thieringer et al. (1998) suggested that the stress of low temperatures can cause the stability of DNA and RNA secondary structures to be reduced in bacteria. Phadtare et al. (1999) also suggested that colder temperatures can cause DNA replication to be less efficient in bacteria. If the treatment of UV and H_2O_2 has already caused thymine dimers to form on DNA strands (Bachmann, 1975), then it may be possible that once eggs are placed into the cooler that stabilization is impossible for the bacteria, which would result in their death.

Even though a greater kill in bacteria was obtained when using the combination of H_2O_2 and UV light, there were still no effects observed on hatchability. It was determined that repetitive applications of H_2O_2 and UV light could achieve a greater kill in bacteria when compared to a single application. When compared to the single application, repetitive applications of H_2O_2 and UV light reduced bacteria on the eggshell 2 fold. This may be because bacteria that were only damaged by a single treatment of UV light and H_2O_2, were able to regenerate during the egg incubation period. However,

it is apparent that bacteria are vulnerable to multiple applications of H_2O_2 and UV light and are not able to survive all of the repetitive treatments.

Although the kill in bacteria was greater with the repetitive applications of H_2O_2 and UV light, hatchability was still unaffected. The hatchability data from these experiments demonstrate that bacteria on the outer surface of the eggshell may be able to penetrate the eggshell within the first few minutes of oviposition. Sparks (1987) stated that the cuticle, which protects the outer eggshell surface from bacterial contamination, is not effective for the first few minutes after ovipostion. Cox (2000) suggests that bacterial contamination is already present in the egg before oviposition because of vertical transmission. If this is true, then it may explain why hatchability was not increased by sanitizing the eggshell with the combination of H_2O_2 and UV light.

Even though the combination of H_2O_2 and UV light was not effective in increasing hatchability, it did not have a negative effect on hatchability. If this combination administered repetitively can reduce bacterial counts on broiler breeder eggshells it could be used to decrease contamination in hatcheries. Contamination found on broiler breeder farms, in nest boxes, and in transportation trucks are carried back to the hatchery on the eggshell surface. If this contamination could be decreased before the eggs reached the hatchery, then contamination within the hatchery could ultimately be decreased. This method would also be safe for users and would have no detrimental effects on the chick embryo.