Disinfection of eggshells using ultraviolet light and hydrogen peroxide independently and in combination[1]

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ABSTRACT Poor hatchability can occur due to eggshell bacterial contamination, which can be decreased by UV light or H_2O_2 alone. However, antimicrobial effects of these 2 treatments combined, as well as optimum length of UV exposure, are not known. Therefore, the objectives of this study were to determine the optimum length of UV exposure for maximum bacterial reduction and to determine if a greater bacterial reduction would occur using a combination of UV and H_2O_2 compared with 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 min. In 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 min. Experiment 4 used 10 treatments including a control and 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. Results indicated that every control eggshell contained bacteria, resulting in an average bacterial count of 4 log cfu/egg. Exposure to UV alone for 8 min yielded significant bacterial reductions without excessive egg heating. When administered independently, H_2O_2 and UV each reduced eggshell bacterial counts by 2 log cfu/egg. The combination of 1.5% H_2O_2 and UV for 8 min reduced bacterial counts by a maximum of 3 log cfu/egg, with only 35% of the eggs positive for bacteria. Because bacterial contamination was further reduced by using a combination of UV and H_2O_2, it is possible that hatchability and chick quality of breeder eggs might be improved by such treatments.

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

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INTRODUCTION

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

Ultraviolet 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 (**UV-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) observed a reduction in aerobic bacteria of 2 to 3 $\log_{10}$ cfu/egg for UV-treated eggs after exposing eggshells to 60 s of UV light at an intensity of 75 mW/cm^2. Furthermore, preliminary studies conducted by Gao et al. (1997) demonstrated that UV light was unable to penetrate the eggshell, indicating that UV should not harm the developing embryo's DNA.

Another method used to sanitize eggshells is H_2O_2. Sander and Wilson (1999) demonstrated that fogging eggs with 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 was found in the literature that investigated if UV light and H_2O_2

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could be used in combination to more effectively reduce the bacterial contamination found on the outer eggshell.

Rodriguez-Romo and Yousef (2005) demonstrated that when UV light was combined 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 produces hydroxyl radicals, which results in a more rapid kill of bacteria. In their experiment, a 99.999% reduction in *Bacillus subtilis* 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 could be, when combined with various concentrations of H_2O_2 to result in the highest reduction of bacteria on the eggshell. Therefore, the objectives of these experiments were to determine the optimum length of UV exposure and concentration of H_2O_2 when used in combination for maximum bacterial reduction on eggshells.

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 (Nasco, Fort Atkinson, WI), the bags were then filled with 50 mL of sterile PBS. Each egg was then hand-massaged in the bag for 1 min 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 serially 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 a nonselective media that indicated total bacterial counts (tryptic soy agar plates). All samples were plated in duplicate. Plates were then incubated for 48 h at 37°C. After the incubation period, plates were removed and colony enumeration was performed. All results are 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.

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 visibly clean eggs were collected from 50-wk-old caged White Leghorn hens. Eggs were then separated into 5 treatment groups that 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 min. The UV-C intensity in the UV treatment chamber at egg level was approximately 11 mW/cm^2. This was measured by placing a UV meter on a wire flat inside the machine. 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 lamps in the 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 that did not yield excessive egg heating. Therefore, experiment 2 was performed to determine the optimum concentration of H_2O_2 in combination with 8 min of UV exposure that would maximize bacterial reduction on eggshells. A total of 162 visibly clean eggs were collected from 52-wk-old 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, and 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 min. However, eggs receiving H_2O_2 and UV light were misted with H_2O_2 and immediately placed into the UV chamber for 8 min. 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. 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 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 combina-

tion with 8 min of UV exposure to maximize bacterial reduction on eggshells. This was accomplished by including treatments that were intermediate to the 1 and 2% H_2O_2 treatment (1.5%) and the 2% and 3% H_2O_2 treatments (2.5%). This experiment had a total of 10 treatment groups, each consisting of 18 visibly clean eggs. Treatments groups consisted of 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 and 3% H_2O_2 and UV. Eggs were collected from 53-wk-old caged Single Comb White Leghorn hens. All procedures for control and treated eggs were as mentioned in experiment 2.

Experiment 4

Experiment 4 was conducted to determine the optimum concentration of H_2O_2 in combination with the shortest possible UV exposure time that would yield the greatest reduction of bacteria on eggshells. 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 visibly clean eggs were collected from 55-wk-old caged Single Comb White Leghorn hens. Treatment groups consisted of a control; 1.5% H_2O_2 in combination with 2, 4, or 8 min of UV exposure time; 2% H_2O_2 in combination with 2, 4, or 8 min of UV; and 2.5% H_2O_2 in combination with 2, 4, or 8 min 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 of the above experiments were analyzed as completely randomized designs. Means were separated using Fisher's protected least significant difference test ($P \geq 0.05$). Ultraviolet light and H_2O_2 were applied to individual eggs so that each egg represented an experimental unit (Steel and Torrie, 1980).

RESULTS

Experiment 1

Bacterial counts for eggs treated with different time intervals of UV light compared with control eggs are given in Figure 1. All treatment groups involving UV light exposure had a significant reduction in bacteria when compared with the control group. Also, eggs treated with 16 min of UV light yielded the greatest reduction in bacteria. However, the internal temperature of these eggs reached 37°C, which could induce embryonic development before incubation. The internal temperature of eggs in the treatment group receiving 8 min of UV light did not exceed 29°C, but the bacterial count on the surface of their shells was reduced by 2.07 $\log_{10}$ cfu/egg.

Experiment 2

In experiment 2, all treatment groups that received 8 min of UV light yielded significantly lower eggshell bacterial counts than the control group and the treatment groups that received 1 or 2% H_2O_2 alone (Figure 2). Also, the 3 treatment groups that received the combination of 8 min of UV light and H_2O_2 were significantly lower in bacterial counts than the treatment group that received UV alone or the 3 treatment groups that received only H_2O_2. The treatment group that received 2% H_2O_2 and 8 min of UV light had the greatest reduction in bacteria when compared with the control group, yielding a bacterial reduction of 3.31 $\log_{10}$ cfu/egg.

Experiment 3

A significant bacterial reduction was observed in all treatment groups when compared with the control group (Figure 3). All treatment groups receiving the combination of 8 min of UV light and the various levels of H_2O_2 between 0.5 and 3% were significantly lower than the treatment group receiving only 3% 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 receiving the combination of UV light and any concentration of H_2O_2.

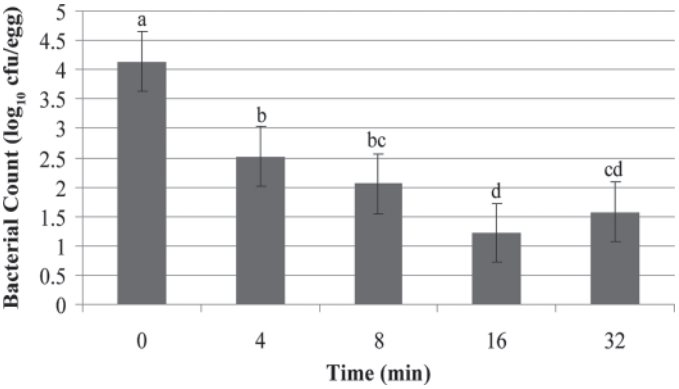

Figure 1. Bacterial counts for eggs treated in experiment 1 with different time intervals of UV light exposure compared with control eggs receiving no treatment. [a–d]Means with different letters are significantly different at $P \leq 0.0001$; n = 18 eggs per treatment.

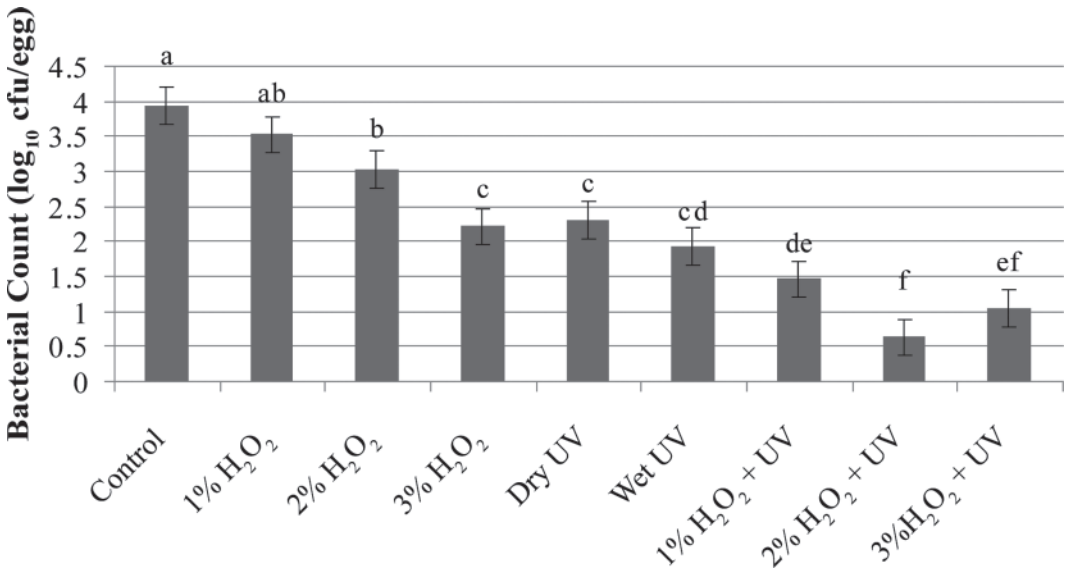

Figure 2. Comparison of different concentrations of H_2O_2 and 8 min of UV light exposure alone and in combination for bacterial eggshell counts in experiment 2. The dry UV received only UV treatment, and the wet UV was misted with sterilized water and treated with UV. [a–f]Means with different letters are significantly different at $P \leq 0.0001$; n = 18 eggs per treatment.

Experiment 4

In the final experiment, a significant reduction in eggshell bacteria was observed when comparing all groups that were treated with the combination of UV light and H_2O_2 to the control group (Figure 4). No significant differences were observed when comparing the treatment groups that received 2 min of UV light in combination with the 3 different concentrations (1.5, 2, and 2.5%) of H_2O_2, nor were there any significant differences among the treatment groups that received 4 min 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 min of UV light and H_2O_2 at the 3 different concentrations, it was found that the eggs treated with 1.5% H_2O_2 were significantly lower in bacterial counts than those eggs treated with 2 or 2.5% H_2O_2. The 1.5% H_2O_2 and 8 min of UV light treatment combination reduced bacterial counts by 3.3 $\log_{10}$ cfu/egg when compared with the control group. Furthermore, that treatment combination caused bacterial counts to be 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 min of UV

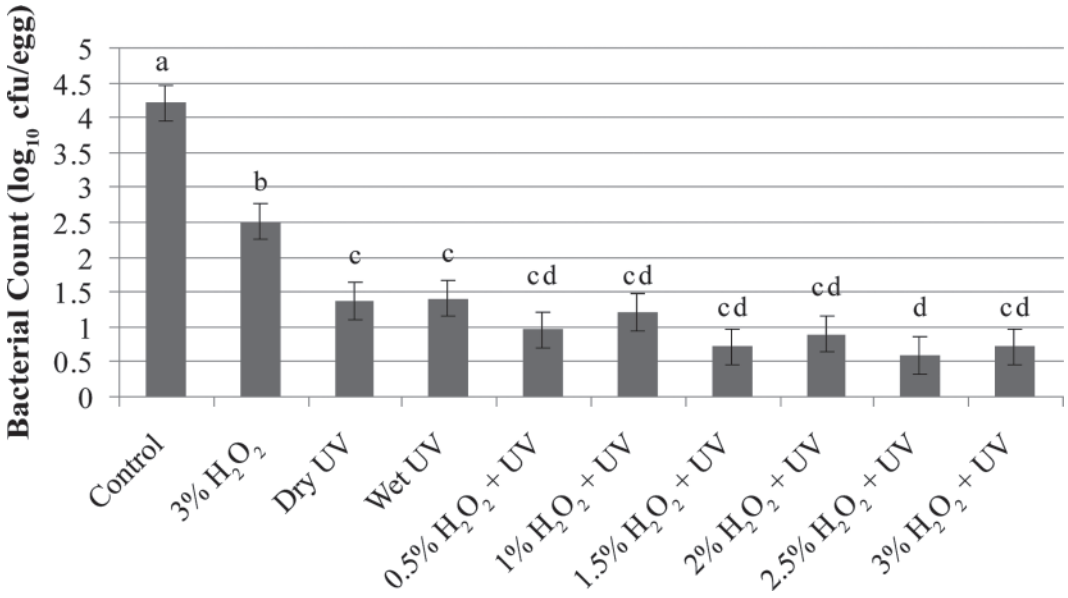

Figure 3. Comparison of bacterial counts in experiment 3 of eggs treated with different concentrations of H_2O_2 and 8 min of UV light. Dry UV was only treated with UV, and wet UV was misted with water and then treated with UV. Treatment groups containing H_2O_2 were misted with H_2O_2, and treatment groups containing H_2O_2 + UV were treated with H_2O_2 and UV. [a–d]Means with different letters are significantly different at $P \leq 0.0001$; n = 18 eggs per treatment.

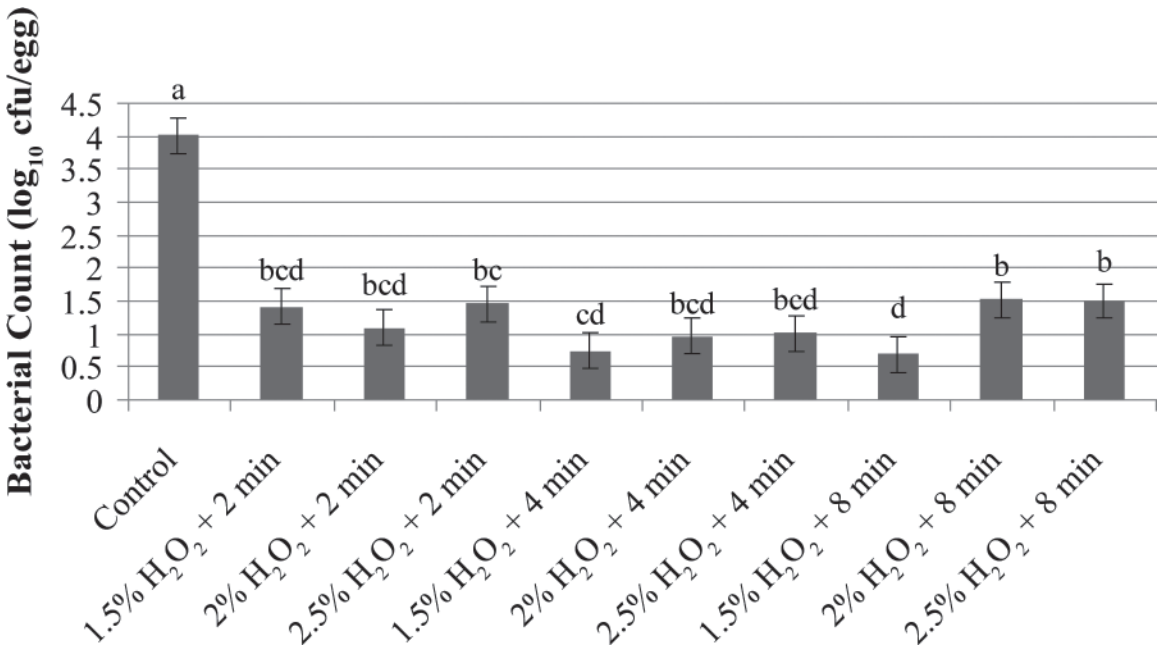

Figure 4. Comparisons of different concentrations of H_2O_2 in combination with different lengths of time of UV light exposure in experiment 4. All treatments labeled with H_2O_2 received treatment with H_2O_2, treatments labeled with 2 min received 2 min of UV light, treatments labeled with 4 min received 4 min of UV light, and treatments labeled 8 min received 8 min of UV light. [a–d]Means with different letters are significantly different at $P \leq 0.0001$; n = 18 eggs per treatment.

light were positive for bacteria. However, 100% of the eggs in the control group were positive for bacteria (Figure 5).

DISCUSSION

In the first experiment, the optimum exposure time to UV light for maximum bacterial reduction on eggshells was found to be 16 min. However, due to the accumulation of excess heat inside the egg, eggs could not be treated more than 8 min 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, which 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 microbial inactivation. (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/

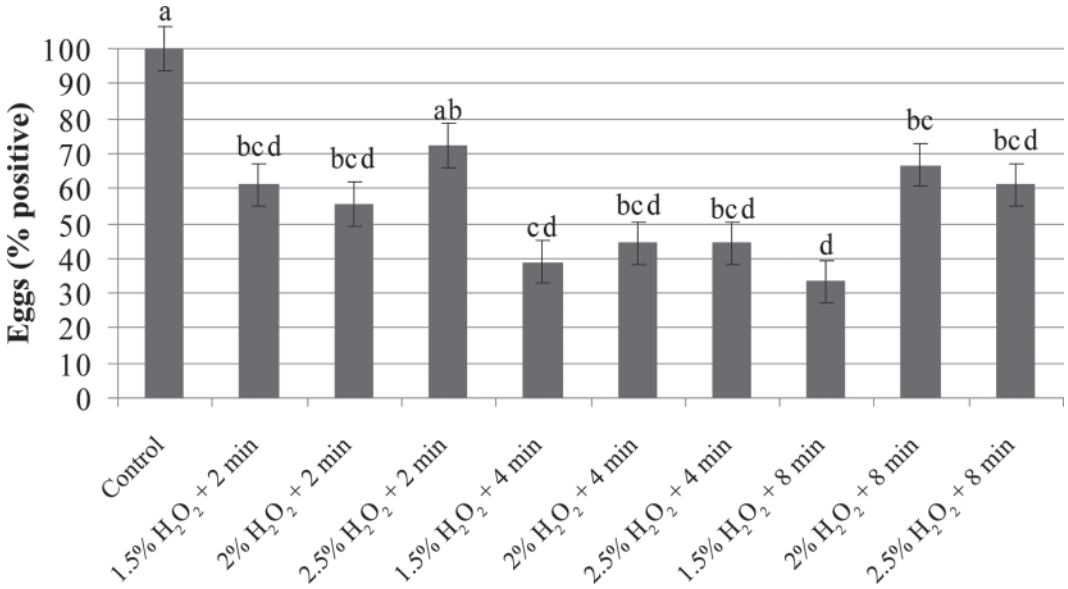

Figure 5. Percentage of eggs positive for bacteria at different concentrations of H_2O_2 in combination with different time lengths of UV light in experiment 4. Treatments labeled with 2, 4, or 8 min received UV for the specified time period of 2, 4, or 8 min, respectively. [a–d]Means with different letters are significantly different at $P \leq 0.0001$; n = 18 eggs per treatment.

egg for 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 min, whereas in this experiment, eggs were treated for 8 min with UV light. Kuo et al. (1997a) also treated eggs with UV light for 1, 3, 5, or 7 min and was able to obtain a 2-log reduction in *Salmonella* 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 experimental results. However, it is possible that some bacteria may have remained in the shell pores and the interior of the egg and may not have been removed with our eggshell rinse method. The shell-and-membrane crush method (Musgrove et al., 2005) could be used to determine bacterial load in the shell pores, and yolk and albumen samples could also be collected to enumerate bacteria from the inside of the egg.

In the second experiment, various concentrations of H_2O_2 were tested in combination with 8 min of UV light. Previous work by Sander and Wilson (1999) demonstrated that when 3% H_2O_2 was administered to broiler breeder eggs over an entire incubation period, a significant reduction in bacteria was obtained. The bacterial counts of eggs in experiment 2 treated only once before incubation with 3% H_2O_2 were significantly lower than control eggs. Padron (1995) dipped eggs twice in 6% H_2O_2 and reduced *Salmonella* Typhimurium-positive eggs by 55%. However, when treating eggs once with only 2% H_2O_2 in conjunction with 8 min of UV light in the present experiment, bacterial counts were reduced from almost 4 log cfu/egg to less than 1 log cfu/egg. Bayliss and Waites (1982) consistently obtained more than a 4-log reduction in *B. subtilis* when administering 2.5% H_2O_2 in combination with UV light in vitro. This was similar to the reduction greater than 3 log in bacteria on eggshells in experiments 2, 3, and 4, in which H_2O_2 was administered at various concentrations less than 3% in combination with 8 min of UV light. This additional reduction in bacteria may be due to the production of hydroxyl radicals when UV light reacts with H_2O_2 (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 bacterial spores. In addition, free radicals produced by the combination of UV light and high concentrations of H_2O_2 are less effective at destroying bacteria. When an excessive concentration of H_2O_2 is administered, inactivation of spores ceases. 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 effects of various intermediate concentrations of H_2O_2 in combination with various lengths of UV light exposure on eggshell bacterial counts were further examined. In experiment 3, H_2O_2 concentrations in excess of 1.5% in combination with UV light were numerically less effective as sanitizing agents. Furthermore, in experiment 4, there were significantly more bacteria present on eggshells when concentrations of H_2O_2 were increased above 1.5% in combination with 8 min of UV light 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 H_2O_2 in combination with UV light.

From the results of experiment 3 and 4, it appears that 8 min of UV exposure and 1.5% H_2O_2 is the optimum combination to consistently reduce numbers of bacteria on eggshells. This data has proven 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 at increasing hatchability. Also, it is possible that the combination of H_2O_2 and UV light could be used to reduce human pathogens on table eggs if the procedure was modified to fit into the modern table egg processing system.

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