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Hatchability of Broiler Breeder Eggs Following Eggshell Sanitization by Repeated Treatment with a Combination of Ultraviolet Light and Hydrogen Peroxide†

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Abstract: Previous research has indicated that a single exposure of eggs to Ultraviolet Light (UV) in combination with 3% hydrogen peroxide (H_2O_2) results in a greater reduction of eggshell microorganisms compared to eggs treated with either UV or H_2O_2 alone. The objective of this study was to determine if hatchability would be affected if eggs were treated by repeated applications of UV and H_2O_2. In the first experiment, eggs receiving H_2O_2 and UV light for 2 min 6 times yielded the greatest reduction in aerobic plate counts (5.3 log_{10}CFU/egg) when compared to other treatment groups that utilized various repetitions of H_2O_2 and UV light. The second experiment determined the effect on hatchability when using this combination tested in Experiment 1. In Experiment 2, a 4 log_{10}CFU/egg reduction in eggshell aerobic plate counts was observed for eggs treated with UV and H_2O_2 when compared to untreated control eggs. There were no differences in hatchability, hatch residue, chick weight, residual yolk weight, or egg weight loss between control and treated groups. In conclusion, multiple applications of UV and H_2O_2 effectively reduced aerobic microorganisms on the eggshell to low levels with no detrimental effects on broiler breeder egg hatchability or chick quality parameters.

Key words: Eggshell, sanitization, bacteria, ultraviolet light, hydrogen peroxide, hatchability

INTRODUCTION

Different methods of sanitizing the eggshell surface have been studied. Methods such as formaldehyde gas, ozone, ethylene oxide, heat treatment and gamma irradiation have been tested in poultry research. However, these methods have characteristics which make their use in commercial production unfavorable. 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). Safer, more effective sanitization methods for eggshells are needed. Scott (1993) stated that ultraviolet light (UV) is safe for the user because it can be contained and is an effective sanitizer. Hydrogen peroxide (H_2O_2) has also been shown 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 administered as a single application created hydroxyl radicals which resulted in a greater kill of bacteria *in vitro* when compared to either UV or H_2O_2 alone. In that research, the combination of H_2O_2 and UV light proved to be effective at killing bacteria on agar slants. More recently, Wells *et al*. (2008) demonstrated that the combination of 1.5% H_2O_2 and 8 min of UV was effective at sanitizing eggshells with a reduction of 2.8 log_{10} CFU/egg. In addition, this method did not result in a significant effect on hatchability. Even though eggshell microbial counts were significantly reduced in that study, refinement of the method could further decrease microbial numbers. If eggshell microorganisms are further reduced by a refined method combining H_2O_2 application and UV exposure, then possibly hatchability would be affected. Therefore, the objective of this study was to determine if hatchability would be affected by increased reduction in eggshell microorganisms through a repetitive application method of H_2O_2 and UV in combination.

MATERIALS AND METHODS

Experiment 1

Egg treatment and microbial enumeration: To determine the optimum number of repetitions for H_2O_2 application and UV exposure, a total of 210 eggs were collected from a single farm of commercial broiler breeders. Eggs were separated into 10 treatment groups, each consisting of 21 eggs. These treatment groups consisted of an untreated control, 2 min of UV (11 mW/cm^2) 6 times with and without egg rotation

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between UV treatments, H_2O_2 spray every 2 min 6 times with rotation, H_2O_2 spray and UV for 30 s 3 and 6 times with rotation, H_2O_2 spray and UV for 1 minute 3 and 6 times with rotation and H_2O_2 spray and UV for 2 min 3 and 6 times with rotation. All H_2O_2 treatments used a 3% H_2O_2 solution applied using a prototype spray chamber. The chamber had 9 nozzles above and 9 nozzles below the wire tray holding the eggs and dispensed 100 mL of H_2O_2 solution in 2 s. This apparatus was constructed to expedite egg spraying and allowed for all eggs to be misted as uniformly as possible with liquid. These eggs were coated by the mist for 2 sec in the prototype spray chamber. Rotation of the egg was examined because Bachmann (1975) determined that eggshell sanitization using UV occurs only in the direct radiation beam, whereas shaded areas are unaffected. Also, Kuo *et al*. (1997) found that when rotating eggs during UV treatment a greater bacterial reduction was found on the eggshell.

After collection, control eggs were placed into Whirl-pak™ (Nasco, Fort Atkinson, WI) bags. All of the remaining eggs were placed onto wire flats according to treatment group. Treatment groups were then treated with H_2O_2 spray and UV as described above. After initial treatment, eggs were placed into Whirl-pak™ bags. Each bag was then filled with 50 mL of peptone (BD Sparks, MD.). The bags were then hand massaged for 1 min to remove any microorganisms. 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, 10- and-100 fold serial dilutions were performed for each control egg. No serial dilutions were performed on the treated eggs. For the control eggs, 0.5 mL of egg rinse and each dilution were spread plated in duplicate onto Tryptic Soy Agar (TSA) plates (BD Sparks, MD.). Egg rinse solution for the treated eggs was directly plated in the same manner. The plates were incubated for 48 h at 37°C before colony enumeration was performed. Colony counts were converted to $\log_{10}$CFU/egg.

Experiment 2

Egg treatment, incubation and hatch: To determine if hatchability was affected by the optimum number of repetitive exposures to UV and H_2O_2 found in Experiment 1, a total of 2,304 eggs from 47 wk-old broiler breeder hens were collected from 3 commercial houses located on a single farm. Eggs were divided equally into 2 treatments with 1,152 serving as untreated control eggs and the remaining 1,152 eggs treated with the combination of H_2O_2 and UV described below. For the treated eggs, 32 eggs were placed horizontally on each of 36 wire trays and misted with 3% H_2O_2. Eggs were immediately placed into the UV chamber after spraying with H_2O_2. The chamber had 20 germicidal lamps (91.4 cm G30T8) mounted above and below the egg trays as close as possible so that all parts of the eggshell were exposed. UV-C intensity was measured at egg level and was approximately 11 mW/cm^2. After 2 min of UV treatment, eggs were rotated 180 degrees and misted again with H_2O_2 and again placed into the UV chamber. This procedure was repeated for a total of 6 times.

After the 6 repetitions of H_2O_2 and UV were complete, eggs were weighed so that incubational egg weight loss could eventually be determined and eggs were then set in 8 separate incubators. To prevent cross contamination, 4 incubators were filled with eggs treated with the H_2O_2 and UV combination and 4 incubators were filled with the untreated eggs serving as controls. After eggs were incubated for 18 d, they were reweighed and transferred into hatching baskets. At 21.5 d of incubation, chicks were removed from the hatchers, weighed and placed into individual floor pens with each pen corresponding to an incubator. All of the remaining eggs that did not hatch were broken out for hatch residue analysis.

Broiler chick rearing and sampling: Chicks from each incubator were placed into 8 corresponding floor pens for grow out. The pens provided 0.084 m^2/bird. Grow out pens had conventional curtain sides with litter floors and a radiant heat brooder. All chicks had *ad libitum* access to a standard broiler diet and nipple drinkers at all times. Each pen was given 23 h of light and 1 h of darkness each day. Birds were grown out for 5 d so that chick weight, residual yolk sac weight and yolk sac bacterial counts could be examined. All birds were treated in accordance with the Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching.

Microbial enumeration: At time of egg treatment, 1 egg from each tray for control and treated groups (72 total eggs) were randomly selected and used for Aerobic Plate Count (APC) enumeration as described for Experiment 1.

At 5 d post hatch, 20 chicks from each pen were randomly selected to obtain body and yolk sac weights to determine relative yolk sac weight. The entire yolk sacs from these chicks were aseptically removed, weighed, masticated and swabbed for the presence of microbial contamination by streaking the contents of the yolk sac directly onto TSA plates.

Statistical analysis: All data were analyzed as a completely randomized design with incubator serving as the experimental unit. Means were separated using Fisher's protected least significant difference test when $p \leq 0.05$ (Steel and Torrie, 1980).

RESULTS

In Experiment 1, all eggs in any of the groups receiving H_2O_2 and/or UV treatment exhibited significantly lower APC than the controls (Fig. 1). Eggs that received both

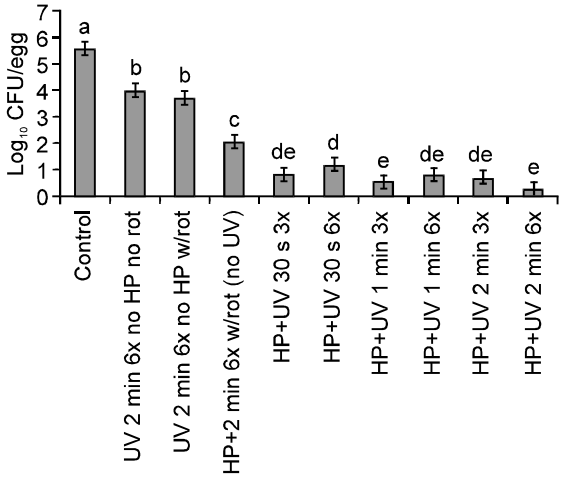

Fig. 1: Aerobic plate counts of eggs treated with 3% hydrogen peroxide (H_2O_2) and Ultraviolet (UV) light for various time lengths and repetitions with or without rotation (rot) in Experiment 1.
[a-e]Means with different letters are significantly different at $p \leq 0.0001$; n = 21 eggs per treatment

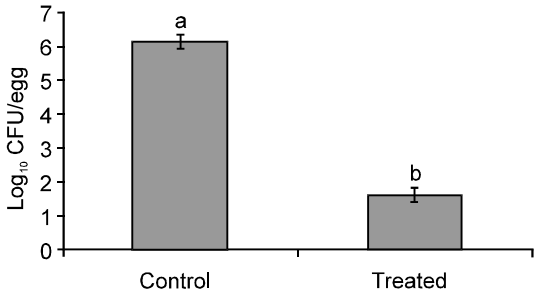

Fig. 2: Effects of hydrogen peroxide (H_2O_2) and Ultraviolet (UV) light sanitization treatment on aerobic plate counts of broiler breeder hatching eggs in Experiment 2.
[a-b]Means with different letters are significantly different at $p \leq 0.0001$; n = 36 trays per treatment (1 egg per tray)

UV and 3% H_2O_2 had significantly lower APC than eggs that received multiple treatment of UV or 3% H_2O_2 only. The treatment groups receiving UV 2 min 6 times with and without rotation were not significantly different. However, numerically, the treatment group receiving 6 repetitions of 3% H_2O_2 and 2 min of UV had the lowest count (0.26 log_{10} CFU/egg, Fig. 1).

In the second experiment, there was a reduction of approximately 4.5 log_{10} CFU/egg in eggshell APC when comparing eggs treated with 6 repetitions of 3% H_2O_2 and 2 min of UV to control eggs (Fig. 2). This resulted in more than 40% of the treated eggs testing negative for aerobic microorganisms by the rinse and plate method

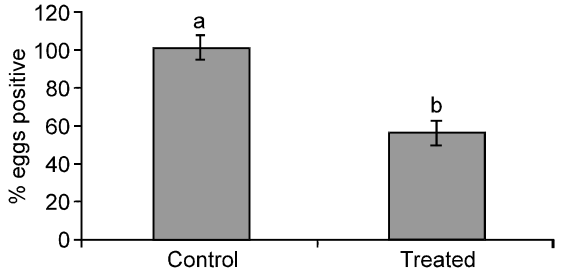

Fig. 3: Percentage of control and treated eggs positive for aerobic plate counts in Experiment 2.
[a-b]Means with different letters are significantly different at $p \leq 0.0001$; n = 36 trays per treatment (1 egg per tray)

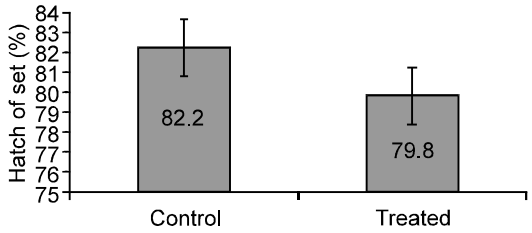

Fig. 4: Percentage hatchability of total eggs set for control and treated groups in Experiment 2. Means are not significantly different at $p \geq 0.27$; n = 4 incubators per treatment with 288 eggs per incubator

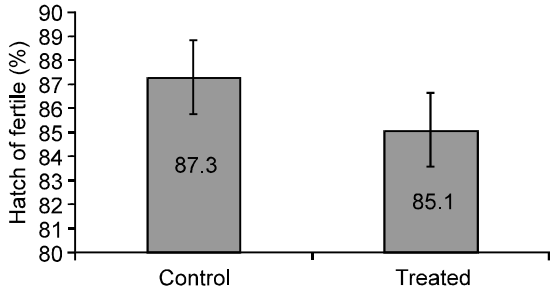

Fig. 5: Percentage hatchability of fertilized eggs between control and treated groups in Experiment 2. Means are not significantly different at $p \geq 0.35$; n = 4 incubators per treatment with 288 eggs per incubator

used (Fig. 3). However, no differences between control and treated groups were observed for hatchability of total eggs set (Fig. 4) and hatchability of fertilized eggs (Fig. 5).

Hatch residue analysis and fertilization level were not different between treatments (Table 1). Chick characteristics shown in Table 2 demonstrate no difference in percentage egg moisture loss, chick

Table 1: Hatch residue analysis and fertility of broiler breeder eggs from experiment 2[1]

Parameter	Fertility (%)	Early dead (%)	Mid dead (%)	Late dead (%)	Pipped (%)	Contaminated (%)
Control	94.20	4.20	0.60	3.30	1.20	0.30
Treated	93.80	3.80	0.60	2.40	2.70	0.30
SEM	0.49	0.91	0.23	0.38	0.54	0.14
P Value	0.61	0.79	0.99	0.14	0.09	0.99

[1]n = 4 incubators per treatment with 288 eggs per incubator

Table 2: Parameters of broiler breeder chicks determined after hatch for experiment 2

Parameter	Egg weight loss (%)[1]	Chick weight (g)[1]	Residual yolks positive for bacteria (%)[2]	Relative residual yolk weight (%)[2]
Control	11.60	46.00	54.00	0.36
Treated	11.20	46.70	59.00	0.41
SEM	0.44	0.59	8.10	0.05
p-value	0.54	0.41	0.05	0.47

[1]n = 4 incubators per treatment with 288 eggs per incubator.
[2]n = 4 pens per treatment with 20 chicks per pen sampled

weight, relative yolk sac weight, or the number of residual yolk sacs that were positive for microorganisms.

DISCUSSION

These experiments demonstrated that repetitive applications for H_2O_2 in combination with UV reduced microbial contamination found on the outer eggshell of broiler breeder hatching eggs by more than 4 log without affecting hatch residue, hatchability and chick characteristics. As shown in previous research conducted by Wells *et al*. (2008), a single treatment of the combination of H_2O_2 and UV administered to the broiler breeder eggshell only resulted in a 2.8 $\log_{10}$ CFU/egg reduction in APC. After obtaining a 4 log reduction in APC, data from this experiment suggest that hydroxyl radical formation from combined H_2O_2 and UV treatment is more effective at killing eggshell surface microorganisms than the photochemical reaction that occurs when UV is administered alone (Bayliss and Waites, 1982). These data also suggest that when the combination of H_2O_2 and UV is administered repetitively it is more effective than just a single application of H_2O_2 and UV. Additionally, although previous research has shown that rotation of eggs is necessary to obtain maximum sanitization using UV, the present study indicates that when UV is used alone rotation is not necessary. This may be due to the high intensity and even distribution of UV to the eggshell used in this experiment.

Even though repetitive treatments of H_2O_2 and UV effectively reduced eggshell microbes prior to incubation, hatch residue characteristics, hatchability and chick characteristics from treated eggs were unaffected. There are two possible explanations for these findings. First, the microbial challenge to the hatching eggs used in this study might not have been sufficient to cause a reduction in hatchability, thus reducing the microbial load on the eggshell would not have been able to improve hatchability. Second, it is possible that microbial contamination responsible for reduced hatchability was already present inside the egg prior to eggshell treatment. It has been shown that UV cannot penetrate the eggshell (Gao *et al*., 1997). Therefore, if bacteria are able to penetrate the eggshell prior to H_2O_2 and UV treatment, these treatments will have no effect on the bacteria located inside the egg. Previous researchers have suggested that microbial contamination found on the outer surface of the eggshell may be able to penetrate the eggshell within the first few minutes following oviposition. Because the cuticle may be ineffective for the first few minutes of lay until it hardens (Sparks, 1987), microbial contamination may occur before egg treatment is performed. Also, immediately after the egg is laid, the negative pressure created within the egg as it cools may pull microorganisms through the eggshell and membranes (Lock *et al*., 1992). Because the egg at the moment of oviposition comes in contact with bacteria found in the nest boxes, 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.

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 deposited in the oviduct. As a result, bacterial contamination present in the oviduct could be detrimental to the chick embryo prior to oviposition and eggshell sanitization.

In conclusion, the repetitive application of the combination of H_2O_2 and UV light to broiler breeder

hatching eggs was effective at reducing eggshell aerobic microorganism contamination to very low levels without significantly impacting hatchability. Also, no differences in egg weight loss during incubation demonstrated that the cuticle on the outer eggshell was not harmed by the sanitization method used in this experiment. Therefore, the combination of H_2O_2 and UV light administered repetitively could be used to reduce pathogenic bacterial contamination in hatcheries by ridding eggshells of bacteria before being transported to the hatchery facility. If this sanitization procedure was developed in a manner that could be used in the commercial industry, it could prove to be a safe method of eggshell sanitization without negatively affecting the cuticle of the egg, hatchability or chick quality.

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