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Hatchablility of Broiler Breeder Eggs Sanitized with a Combination of Ultraviolet Light and Hydrogen Peroxide*

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Abstract: Research has shown that exterior eggshell Aerobic Plate Counts (APC) are greatly decreased using a combination of Ultraviolet Light (UV) and hydrogen peroxide (H_2O_2). However, it is unknown how this treatment process would impact hatchability. Therefore, the objective of this experiment was to determine if spraying eggs with 1.5% H_2O_2 followed by UV irradiation for 8 min to reduce eggshell APC would affect hatchability. Eggs from 3 commercial houses (57 wk-old broiler breeders) were collected over 2 d (n = 1,944 eggs). Half were treated with 1.5% H_2O_2 and UV and the other half served as untreated controls (18 eggs/ tray). At time of treatment, 1 egg was randomly selected from each of 108 trays (n = 54 per treatment) for eggshell APC enumeration on TSA. Remaining eggs were stored at 18.3°C. Prior to set, 1 egg per tray from d 1 of collection was sampled for APC enumeration. The 16 remaining eggs/tray were weighed prior to incubation and at 18 d to determine egg weight loss. At hatch (21.5 d), chick weights were obtained, meconium samples were collected from 18 chicks per incubator and samples were plated on TSA to determine the presence of intestinal microorganisms. A 3 log_{10}CFU/egg reduction in eggshell APC was found for treated eggs when compared to control eggs. At hatch, no differences in chick weight, egg weight loss, positive meconium samples, or hatchability were observed between treatments. In conclusion, UV irradiation for 8 min with 1.5% H_2O_2 reduced eggshell APC on broiler breeder eggs with no affect on hatchability.

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

INTRODUCTION

Microbial 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 (Berrang *et al*., 1999). Wilson (1997) also suggested 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 (Berrang *et al*., 1999; Goerzen and Scott, 1995). 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 alters hatchability. Research conducted by Sander and Wilson (1999) demonstrated that fogging with 3% H_2O_2 was effective at reducing bacteria but it had no effect on hatchability. Padron (1995) also found no affect on 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 on nutrient agar slopes by more than 4 log_{10}CFU In more recent studies conducted by Wells *et al*. (2008), the combination of UV light and H_2O_2 was administered to the surface of eggshells and like the results of Bayliss and Waites (1982) microbial contamination on the eggshell was reduced by 4 log_{10}CFU/egg. However, it is still undetermined if this treatment combination will alter the hatchability of broiler breeder eggs. Therefore, the objective of this project was to determine if the application of H_2O_2 and UV light in combination to reduce eggshell contamination will affect the hatchability of broiler breeder eggs.

MATERIALS AND METHODS

Egg collection: A total of 1,944 eggs from 57 wk-old broiler breeder hens were utilized in this study. Egg collection was completed over a 2 d period, with 864 eggs collected on the first day at 10:00 am. The following day 1,080 eggs were collected during the same time frame, beginning at 10:00 am. All eggs were collected directly from the egg belts of 3 houses located on a single commercial breeder farm. All 1,944 eggs from the 2 d of collection were divided equally into a control group and a treated group.

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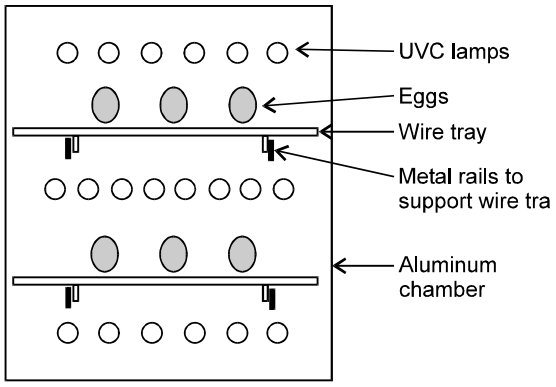

Fig. 1: Experimental UV light chamber configuration for the treatment of hatching eggs on wire trays

Egg treatment and handling: Each day after all eggs were collected, the treated eggs were placed horizontally on wire trays (36.8 cm x 72.4 cm with 18 eggs per tray) and hand misted until completely coated with 1.5% H_2O_2. The eggs were misted with enough H_2O_2 on the surface to be entirely covered without dripping. Immediately after treating each tray of eggs with H_2O_2, the eggs were placed in a UV chamber for 8 min. The UV chamber used in this experiment had a UV-C intensity of approximately 11 mW/cm^2 measured at egg level. The chamber was designed with 2 levels so that 2 trays could be treated simultaneously. A total of 20 UV-C lamps (91.4 cm G30T8) were mounted in the chamber as close to the eggs as possible (see Fig. 1). Immediately after each tray of eggs was treated with UV light, 1 egg from each tray (54 per treatment) was collected from both treated and control groups and used for Aerobic Plate Count (APC) enumeration as described below. Using clean gloves for each repetition, the remaining 17 eggs per tray were then placed into clean paper flats for storage.

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 d in the cooler and the eggs collected on the second day were only stored for 1 d. All eggs were removed from the cooler on d 3 of the experiment. Prior to setting both the treated and control eggs in the incubator, initial egg weights were recorded for calculating incubational egg weight loss. This was performed to determine if the treatment altered moisture loss through the eggshell. From control and treated eggs that had been stored in the cooler for 2 d, 1 egg from each tray (24 total eggs) per treatment were randomly selected for APC enumeration to determine the effect of storage on microbial survival following sanitization. Eggs were then set into 6 different incubators (3 per treatment). All eggs were incubated under standard incubation conditions for chicken eggs. Each incubator contained either all control or all treated eggs from a single breeder house with approximately 300 eggs per incubator. Control and treated eggs were placed in different incubators to prevent microbial cross contamination between the groups.

After 18 d of incubation, eggs were removed from each incubator and reweighed 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 (18 baskets/incubator). At 21.5 d of incubation, all hatched chicks were removed from the incubators, counted and weighed by treatment. 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 the hatching baskets, all remaining eggs were collected and hatch residue analysis was performed.

Bacterial enumeration procedure: Each egg was individually placed into a sterile Whirl-pak™ bag (Nasco, Fort Atkinson, WI) containing 50 mL of 10% sterilized peptone water. Eggs were then massaged for 1 min to remove microorganisms located on the outer surface of the eggshell. After the massage, 10 mL of each rinse solution was aseptically pipetted into a sterile culture tube. Preliminary research revealed that control eggs were highly contaminated (Wells *et al*., 2008). Therefore, 2 serial dilutions were performed for each control egg sample. However, no serial dilutions were performed on the treated egg samples. For control eggs, 0.5 mL of each 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. All plates were incubated for 48 h at 37°C, after which colony enumeration was performed.

At hatch, a total of 108 chicks (1 chick per hatch tray) 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 water was added. This solution of peptone and meconium was mixed and 0.5 mL was spread plated in duplicate on TSA. These plates were incubated for approximately 48 h at 37°C and assessed for positive or negative microbial growth.

Statistical analysis: All data from this study were analyzed as a randomized complete 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 (Steel and Torrie, 1980).

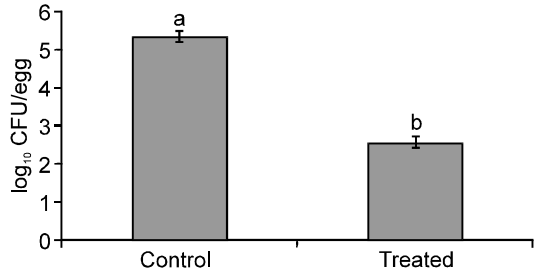

Fig. 2: Effect of hydrogen peroxide (H_2O_2) and Ultraviolet (UV) light sanitization on average eggshell APC counts of broiler breeder hatching eggs collected over a 2 day period. Each treatment contained 54 replications (24 replications for d 1 and 30 replications for d 2) with 1 egg per replication (108 eggs total).
[a-b]Means with different letters are significantly different at $p \leq 0.002$

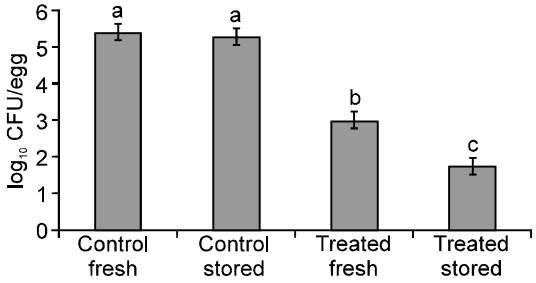

Fig. 3: Effects of egg sanitization and storage on eggshell bacterial counts for eggs stored for 2 days. Fresh eggs were sampled immediately after treatment and collection (n = 54 replications per treatment; 24 replications for d 1 plus 30 replications for d 2, 1 egg per replication). Stored eggs were sampled only from eggs collected on d1 and stored 2 d (n = 24 reps per treatment with 1 egg per replication).
[a-c]Means with different letters are significantly different at $p \leq 0.05$

Table 1: Means for hatch residue analysis data for broiler breeder eggs[1]

Parameter	Infertile	Early dead	Middle dead	Late dead	Pipped	Contam.
	(%)					
Control	25.1	5.8	1.3	9.6	1.9	0.18
Treated	28.4	4.1	1.3	5.3	3.7	0.92
SEM	1.1	1.0	0.3	1.7	2.0	0.70
p-value	0.18	0.35	0.99	0.47	0.50	0.53

[1]n = 3 incubators containing approximately 300 eggs each per treatment. Contam. = Contaminated

Table 2: Means of chick characteristics of broiler breeder chicks at hatch

Characteristic	Chick weight[1] (g)	Egg weight loss[2] (%)	Meconium samples positive for bacteria[3] (%)
Control	48.2	11.9	72.2
Treated	48.4	11.6	79.6
SEM	0.68	0.06	0.09
p-value	0.83	0.09	0.27

[1]n = 3 incubators per treatment containing approximately 150 chicks each per incubator.
[2]n = 3 incubators containing approximately 300 eggs each per treatment.
[3]n = 3 incubators per treatment with 18 chicks sampled per incubator

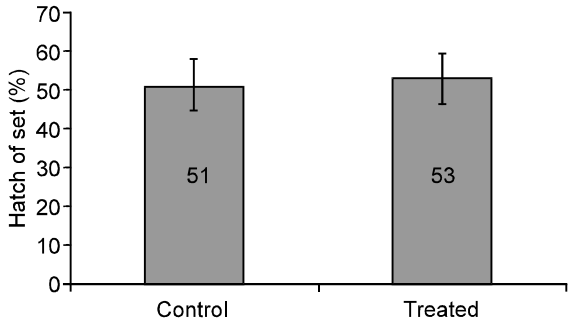

Fig. 4: Percentage hatchability of total eggs set for control and treated eggs. Means are not different at $p \geq 0.9$; n = 3 incubators containing approximately 300 eggs each per treatment

RESULTS

Effects of H_2O_2 and UV light sanitization on average APC counts from broiler breeder hatching eggs are given in Fig. 2. APC 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 APC on the treated eggs when compared to the control eggs. An interaction between the sanitization treatment and egg storage time was observed for the APC enumeration data. A significant microbial reduction during egg storage was observed in treated eggs but not control eggs (Fig. 3). A 1.25 log_{10}CFU/egg reduction was found in treated eggs that were stored for 2 d when compared to treated eggs sampled immediately following treatment.

There were no differences between treatments for infertile eggs, early dead (1-7 d), mid dead (8-14 d), late dead (15-21 d), or pipped embryonic mortalities (Table 1). There were also no treatment effects observed for chick weight, egg weight loss and meconium samples positive for microbial growth (Table 2). In addition, when comparing hatchability of total eggs set and hatchability of fertilized eggs, no significant treatment effects were observed (Fig. 4 and 5).

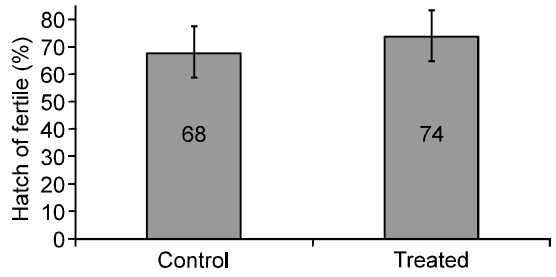

Fig. 5: Percentage hatchability of total fertilized eggs for control and treated eggs. Means are not different at $p \geq 0.7$; n = 3 incubators containing approximately 300 eggs each per treatment

DISCUSSION

As shown by Wells *et al*. (2008) using White Leghorn eggs, the combination of UV light and H_2O_2 effectively reduced microbial contamination found on the surface of broiler breeder eggshells. Wells *et al*. (2008) demonstrated a 3.3 $\log_{10}$ CFU/egg reduction in APC when treating White Leghorn eggs with 1.5% H_2O_2 and 8 min of UV light. However, in the current study, there was only a 2.8 $\log_{10}$ CFU/egg reduction in APC with the use of this treatment. The lower reduction in the current study can likely be attributed to the different sources of eggs between the two studies. In Wells *et al*. (2008), the White Leghorn eggs were from caged hens which had 1.9 $\log_{10}$ CFU/egg fewer APC than the broiler breeder eggs used in this experiment, which were from litter and slat-floored commercial houses equipped with mechanical nest collection systems. Therefore, the broiler breeder eggs contained higher initial microbial counts and likely greater amounts of organic material that would also react with the H_2O_2. However, both aforementioned studies suggest that the formation of hydroxyl radicals, which occurs when H_2O_2 is split by 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 for 2 d in the cooler also had significantly lower bacterial counts than freshly treated eggs. 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 that once eggs were placed into the cooler, stabilization was impossible for the bacteria, which resulted in their death.

The data from this experiment also demonstrated that treatment did not affect hatchability. In other experiments using only UV light to sanitize eggs, researchers also demonstrated no effects on hatchability. Berrang *et al*. (1995) showed that the exposure of broiler breeder eggshells to continuous UV light at 254 nm over the entire 21 d of incubation demonstrated no effect on hatchability. However, when using hatching eggs that were treated with a commercial sanitizer, 1% formalin, or water and then incubated in an incubator equipped with a UV light/air filtering system there was a significant increase in embryo viability (Scott, 1993). However, in the current study, no significant increase in embryo viability or hatchability was observed. Cox *et al*. (2000) suggested 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 enter the shell almost immediately after exposing the shell to bacteria. However, the *Salmonella* used in that experiment was inoculated onto the egg using a liquid. The liquid may have facilitated the entry of the bacteria into the egg through the eggshell pores. In a commercial setting, eggs are rarely submersed in water. Therefore, the bacteria that these eggs came in contact with in the broiler breeder house may not have penetrated the cuticle and shell so easily, resulting in a low challenge of internal contamination in the control group.

Additionally, 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, eggshell surface microbial counts were significantly lowered without negatively affecting hatchability.

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