MANAGEMENT AND PRODUCTION

Effects of a dry hydrogen peroxide disinfection system used in an egg cooler on hatchability and chick quality

E. F. Melo,[*] J. S. McElreath,[†] J. L. Wilson,[†] L. J. C. Lara,[*] N. A. Cox,[#] and B. J. Jordan[‡,†,1]

Department of Animal Science, College of Veterinary Medicine, Universidade Federal de Minas Gerais, Belo Horizonte 31270-901, Brazil; [†]Department of Poultry Science, The University of Georgia, Athens, GA; [‡]Department of Population Health, The University of Georgia, Athens, GA; and [#]USDA, Agricultural Research Service-Russell Research Center, Athens, GA

ABSTRACT A sanitation method that could continually clean and disinfect the air and surfaces in a hatchery could provide a second layer of microbial reduction on top of routine cleaning and disinfection. A gaseous dry hydrogen peroxide (**DHP**) system has been used in other facilities for this purpose and could have potential for use in chicken hatcheries. Because the DHP is a true gas and can permeate through the entire hatchery space, contact with eggs during storage and incubation could potentially interfere with normal hatching processes. Therefore, the aim of this study was to evaluate the effects of the DHP system on hatching parameters and chick quality. A total of 3,960 hatching eggs were collected from an ∼40-week-old Ross 308 broiler breeder flock and distributed in 2 treatments: treated and nontreated. For the treated group, the egg cooler was cleaned, and 1 DHP generator was placed inside. Two other DHP generators were placed in the common area outside as well. Both areas were treated for 7 D before placement of eggs, and then eggs were collected and placed inside the cooler over a 4-day period. Eggs were then stored for an additional 3 D after the last collection. Dry hydrogen peroxide levels were recorded each day during storage. For the nontreated group, all DHP machines were removed from the cooler and external room, and the egg cooler was cleaned. Eggs were collected in the same way for the control group as the treated group. After storage, eggs were placed into a single stage Natureform incubator. The eggs exposed to DHP showed higher ($P < 0.05$) hatchability of fertile eggs and lower ($P < 0.05$) early embryonic dead than eggs from the nontreated group. No other parameters evaluated were different between groups. Based on this work, the DHP treatment of fertile eggs had no detrimental effect on any performance parameter, with potential positive effects seen on hatch of fertile eggs and early embryonic dead embryos.

Key words: hatching eggs, hatch of fertile eggs, embryonic mortality

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INTRODUCTION

In commercial poultry production, hatcheries are a source of continual potential contamination of hatching eggs and hatched chicks. Egg contamination can occur through 3 possible routes: trans-ovarian, trans-oviductal, and trans-shell (Board and Tanter, 1995). Trans-ovarian and trans-oviductal contamination cannot be affected by hatchery practices, but trans-shell contamination can be influenced by several factors. Eggs are more vulnerable to trans-shell contamination immediately after laying, when the eggs are being cooled, and bacteria on the shell are being drawn inside the shell to the membrane. This is why any surface that comes in contact with the egg after laying can be a source of contamination (Berrang et al., 1999).

Egg disinfection processes should aim to reduce microbial contamination on eggshell surfaces while causing little damage to the developing embryo. Several methods to sanitize hatching eggs can be used: spraying, dipping, fumigation, and radiation, with many products available: formaldehyde, peracetic acid, hydrogen peroxide, ozone, and ultraviolet light (Cony et al., 2008; Braun et al., 2011; Gottselig et al., 2016; Keita et al., 2016; Vinayananda et al., 2017). These methods all have potential drawbacks however, including removing the protective cuticle layer from the egg, being harmful to

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[1]Corresponding author: brian89@uga.edu

Table 1. Colony forming units (CFU) per 100 mm blood agar petri dish per day of storage.

Groups	24 h prestorage	At time of initial egg placement	24 h postegg placement	72 h postegg placement	168 h postegg placement
Control	16	22	6	10	9
Treated	36	12	6	3	3

human health, or requiring dedicated additional personnel to use.

Hydrogen peroxide is a strong oxidant with a low molecular weight, able to pass through cell walls/membranes of microorganisms, and reacting with internal components, which leads to cell death (Finnegan et al., 2010). Spraying hydrogen peroxide has been used for years as an efficient product to sanitize hatching eggs (Sheldon and Brake, 1991; Sander and Wilson, 1999; Rehkopf et al., 2017); however, the spraying process requires a specific device or a hand-held manual sprayer which can become vary laborious and can damage the cuticle.

Several systems using hydrogen peroxide–based room disinfection technologies (vapor or aerosol) to decontaminate laboratories cabinets, environmental surfaces, and objects in hospital rooms have been studied (Pottage et al., 2010; Fu et al., 2012). According to Fu et al. (2012), these news systems can reach areas that are inaccessible by regular disinfectants with minimal impact on sensitive medical equipment that sometimes is not compatible with liquid cleaning. A new dry hydrogen peroxide (**DHP**) technology (Synexis Biodefense, Kansas City, MO) that utilizes ambient air to produce hydrogen peroxide as a near-ideal gas was developed for this purpose. The DHP is produced by passing ambient air (containing O_2 and vapor H_2O [humidity]) across a membrane containing a proprietary photocatalyst. The catalyst is activated with a nongermicidal light and breaks the O_2 and H_2O molecules in the air into H_2O_2. The newly formed DHP is then blown into the space by the fan used to move air across the membrane. In hospital environments, DHP has been demonstrated effective against a variety of bacteria, fungi, and viruses (Herman et al., 2015).

The DHP system could also be very useful for commercial poultry production to passively combat microbes in a hatchery environment. However, there are no studies evaluating the use of DHP system in a hatchery space with fertile eggs. Therefore, the objective of this study was to determine if the DHP system could be applied to fertile hatching eggs in an egg cooler without negatively affecting hatchability and chick quality.

MATERIAL AND METHODS

Egg Groups and Disinfection Procedure

A total of 1,980 hatching eggs were collected from an ~40-week-old Ross 308 broiler breeder flock at 2 different times and were used for the 2 groups: treated and nontreated. For the treated group (collected first), the egg cooler at the University of Georgia Poultry Science research farm was cleaned with a chlorine–phenol–based solution (0.9%) and rinsed, and 1 DHP generator (Synexis Biodefense, Kansas City, MO) was placed inside. Two other DHP generators were placed in the room outside the egg cooler as well. Both areas were treated for 7 D before placement of eggs, and then, eggs were collected and placed inside the cooler over a 4-day period and stored for 3 D after the last collection. The eggs were held in cardboard flats, and the temperature in the egg cooler was maintained at 16°C to 18°C, and the relative humidity 65 to 75%. During pretreatment and egg storage, DHP levels were measured daily inside the cooler using an Interscan 4,000 Series Portable Gas Analyzer (Interscan Corporation, Simi Valley, CA), and air samples were taken to evaluate environmental microbial load. For the nontreated group (collected 1 wk later), all DHP machines were removed from the cooler and external room 5 D before placing eggs in the cooler. Before storing eggs in the cooler, the walls and floor of the egg cooler were cleaned as before.

Microbial Load Evaluation

A 100 mm petri dish containing blood agar (**BA**) was placed into the egg cooler and left open for 10 min to passively sample the air in the space. This sampling procedure matches those used to monitor hatcheries in the U.S. poultry industry. A BA plate was placed in the

Table 2. Mean values ± SEM of total hatchability, hatch of fertile, chick weight, grade A, and grade B chicks according to treatment.

Groups	Total hatchability (%)	Hatch of fertile (%)[1]	Chick weight (g)	Grade A chicks (%)	Grade B chicks (%)
Control	90.20 ± 0.55	91.48^{b} ± 0.56	43.69^{b} ± 0.13	97.11 ± 0.35	2.89 ± 0.35
Treated	91.36 ± 0.76	93.93^{a} ± 0.61	44.46^{a} ± 0.12	97.33 ± 0.30	2.67 ± 0.30

[1]Means having different superscripts within a column are different by unpaired t test ($P < 0.05$). Grade A chicks characterized by closed navels, fully dry, absence of red hocks, no buttons, or strings on navels, etc. Grade B chicks characterized by exhibition of 1 or more of the grade A qualifications.

Table 3. Mean values ± SEM of percent early (0–7 D), mid (8–14 D), and late (14–21 D) dead embryos and contaminated eggs according to treatment.

Groups	Early dead (%)[1]	Mid dead (%)	Late dead (%)	Contaminated (%)
Control	4.48[b] ± 0.41	0.15 ± 0.15	2.64 ± 0.40	0.05 ± 0.05
Treated	2.78[a] ± 0.33	0.61 ± 0.16	1.67 ± 0.29	0.00 ± 0.00

[1]Means having different superscripts within a column are different by unpaired t test ($P < 0.05$).

cooler 1 D before egg set and then on days 0, 1, 3, 5, and 7 of storage. After sampling, plates were incubated at 37°C for 24 h, and then, total colonies were counted.

Incubation and Hatching

Before setting for each group, all nonhatching eggs (dirty, double yolk, misshapen, cracked, and small) were removed, and hatching eggs were placed in trays with a capacity of 90 eggs each. Each treatment had 22 trays, totaling 1,980 eggs per treatment. Then, eggs were placed into a NMC-2000 single stage incubator (Natureform, Jacksonville, FL) at a temperature of 37.5°C and relative humidity of 53% during the first 18 D. The eggs were then transferred from setter to a NMC-2000 hatcher (Natureform). The hatcher temperature was 36.9°C, and relative humidity was 65% until hatch. Trays were distributed throughout all positions in the setter and hatcher to account for possible small machine position effects that could be because of differences in air flow. At the 12th day of incubation, all eggs were candled to remove infertile eggs or early embryonic mortality. All eggs removed were opened to assess infertile or embryonic mortality status.

After 21 D of incubation, all chicks were removed from the hatcher and counted and weighed. Chick quality was visually graded, and chicks deemed not saleable (chicks with unhealed navels, red hocks, or obvious abnormalities) were graded as B quality. The number of unhatched eggs were counted, opened, and examined macroscopically to determine the percentage of embryonic mortality (early [0–7 D], middle [8–14 D], and late [15–21 D]), contaminated, and percentage of hatchability of fertile and total eggs. The hatchability of eggs was calculated as the number of chicks hatched per 100 eggs set or per 100 fertile eggs set.

Experimental Design and Data Analysis

The experimental design was completely randomized with 2 treatments (treated and nontreated group). Twenty-two replications per treatment were used, where each tray of 90 eggs constituted a replicate. Data were subjected to paired t test analysis of each data set using Prism 6 software. Statistical significance for all data was considered at $P < 0.05$.

RESULTS AND DISCUSSION

During the treated phase of the trial, a daily increasing level of DHP was measured in the egg cooler with an average level of 12 ppb. A concomitant reduction in microbial load from air samples was also seen, 36 CFU/100 mm plate on day 1 of storage to 3 CFU/100 mm plate on day 7 of storage (Table 1), confirming that the room was treated, and the system was killing microbes as expected. This result is in agreement with Herman et al. (2015), who demonstrated a significant reduction in microbial load in rooms treated with DHP. During the nontreated phase of the trial, microbial load on BA plates fluctuated daily, between 9 and 22 CFU/100 mm plate (Table 1). Total hatchability and chick quality were similar ($P > 0.05$) between the treatments (Table 2), which shows that the DHP system did not negatively impact these parameters. Alternatively, the eggs disinfected with gaseous DHP showed higher ($P \leq 0.0001$) hatch of fertile than the eggs from the nontreated group (Table 2), indicating a potential positive effect of DHP treatment. Chick weights were also significantly increased in the treated group compared with the nontreated group (Table 2). No significant differences were observed ($P > 0.05$) between the treatments for mid embryonic dead, late embryonic dead, or contaminated eggs (Table 3); however, the early embryonic dead was higher ($P = 0.01$) in the nontreated group of eggs compared with the treated group (Table 3), which could account for the difference in hatch of fertile eggs between the groups.

The results of this trial support previous work by Keita et al. (2016) who investigated 4 different disinfection processes as an alternative to formaldehyde. Liquid-based hydrogen peroxide was used in 2 of the treatments: one as a solution of hydrogen peroxide (6%) applied by nebulization and the other as hydrogen peroxide (30%) vapor. The products were not applied directly onto the eggs but by aerial disinfection in a dedicated room at a hatchery, similar to how DHP was applied in this trial. The treatment with hydrogen peroxide vapor showed lower total aerobic bacteria than the nontreated group, and hatching results were significantly higher than the other treatments with no difference in chick quality. Herman et al. (2015) found a complete eradication of *Staphylococcus aureus* and *Pseudomonas putida*, as well as a reduction in *Alcaligenes* (68%), *Pseudomonas aeruginosa* (95%), and *Enterobacter* (50%) over 7 D of treatment with DHP technology in a healthcare environment. *P. putida* and *P. aeruginosa* are associated with rotten eggs, and the other bacteria listed can be found on eggshells (Board and Tranter, 1995), which shows the potential of the system to reduce bacteria present in the poultry environment.

These results demonstrate the DHP system applied in a hatchery environment will have no negative impact on hatchery performance. In fact, this trial demonstrates potentially positive impacts, with increased hatch of fertile and decreased early dead embryos seen. In this trial, the eggs were only treated for 7 D while in storage in an egg cooler. In practice, most hatching eggs in the United States will not be stored for this length of time before setting. The gas form DHP has the potential to be used inside incubators and setters however, so the overall treatment time in a commercial setting could be much longer. In future studies, longer treatments throughout the storage, incubation, and hatch phase should be evaluated to ensure the same benefits are seen. More extensive microbial load reduction data could also be captured to evaluate the system's potential in a commercial poultry setting. The data from this trial are promising, and the DHP system should be considered for sanitation of environments for improved hatchery performance as well as comparing to other common hatchery disinfectants such as formaldehyde.

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