EVALUATION OF DRY HYDROGEN PEROXIDE APPLICATION IN A SINGLE STAGE HATCHERY ON EGGSHELL MICROBIAL LOADS AND EFFICACY WHEN COMPARED TO FORMALDEHYDE

by

KYLIE MICHELLE BRUCE

(Under the Direction of Brian J. Jordan)

ABSTRACT

Since the inception of artificial incubation of poultry hatching eggs, sanitation has been a key concern. Optimal hatch requires high levels of cleaning and disinfection, and one of the main chemicals used in poultry hatcheries is formaldehyde. Formaldehyde is a carcinogenic chemical and can be toxic to both hatchery employees and chicks when used improperly. The purpose of the following research was to study Dry Hydrogen Peroxide (DHP) and its potential as a safe, yet effective, alternative to formaldehyde. DHP machines were installed throughout a broiler hatchery equipped with single stage HatchTech incubators, and a 12-month trial was completed. Hatchability among the group of eggs treated with DHP was comparable to the group treated with formaldehyde. Additionally, eggs exposed to DHP during 19 days of incubation had significantly less microbes present on their shell surfaces at transfer when compared to non-treated eggs.

INDEX WORDS: Dry hydrogen peroxide, hatchery, incubator, poultry production

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DEDICATION

I dedicate this work to my dad, mom, sister, and fiancé. Thank you all for teaching me to work hard, love people, and appreciate agriculture.

To my dad, Mike Bruce, it has been an honor to follow in your footsteps and work in the poultry industry. You have led our family with a steadfast faith, and I am thankful that I can call you anytime day or night for sound wisdom and a calm perspective.

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CHAPTER 1

INTRODUCTION

Since the start of the 21st century, poultry consumption has steadily increased throughout the world and remains the most consumed livestock commodity (Miller et al., 2022). Chicken meat, grown and harvested from broiler chickens, made up an average of 67% of all poultry sales from 2013-2022 (USDA ERS, 2023). Poultry is a unique commodity because not only is it the fastest meat producing animal to reach market weight, but most of the poultry industry is vertically integrated, meaning each company owns its own feed mills, hatcheries, trucks, processing plants, and contracts with growers for the use of breeder, pullet, and broiler houses (Miller et al., 2022; Vukina, 2001). Eggs are brought to the hatchery twice per week on egg trucks. They are incubated and hatched, and then chicks are sorted, vaccinated, and taken to broiler farms.

Almost 37 million eggs were set during the first week of September 2023 in Georgia, and yet only 27.9 million chicks were placed on farms for grow out (National Agricultural Statistics Service (NASS), 2023). Hatcheries have always been an interesting part of the vertical integration system because eggs from all breeder farms within a complex are brought together under one roof. With the attempt to hatch millions of eggs each week, there is no way to avoid thousands of eggs entering hatcheries with fecal contamination on the surfaces, cracked and exploding eggs during transportation and incubation, and chicks that do not make it to day 21 of incubation (Quarles et al., 1970). Additionally, microbes from one breeder farm can spread contamination to eggs from other farms since they are all placed together under one roof and in the same machines (Quarles et al., 1970). All of these are examples of variables in the hatchery that can increase contamination

and decrease hatchability. Add to those variables the fact that hatcheries are built differently from a wide range of materials, and operate different machine brands and types (single or multi-stage), making it difficult to create a universal protocol for cleaning and fumigation during hatch that can be successful in all locations (Wright et al., 1959).

The need for sanitation during incubation was recognized as early as 1908, many years before large-scale commercial poultry production in hatcheries began (Pernot, 1908). As chicks pip and hatch, a bloom of microbes fills the hatchers (Magwood, 1964). At this point during hatch, their navels can be slightly open as the yolk sack is drawn into the abdominal cavity (Brandly, 1932). When the level of microbes present in the hatchers is high, that bacteria can enter the navel and cause infection in young chicks, leading to decreased performance and increased 3-day mortality during grow out (Brandly, 1932; Fasenko and O'Dea, 2008). In addition to omphalitis, or infection of the yolk sac, aspergillus is also a common result of contaminated hatchery spaces. Aspergillosis is a respiratory illness caused by the fungus *A. fumigatus.* Throughout the years, a cleaning and disinfecting routine has been developed at each stage of the hatch process that can be adjusted to fit individual hatcheries. Generally, the protocol includes manual removal of organic matter, washing, and disinfecting the space (Ledoux et al., 2006).

In addition to applying a standard soap, scrubbing, and sanitizing spaces, one of the chemicals that has been used for many years throughout poultry hatcheries is formaldehyde (Graham and Michael, 1932; Pernot, 1908). Formaldehyde is applied during the hatch process because it has been proven to decrease the presence of microbes inside the hatcher (J. E. Sander et al., 1995), however it is a toxic chemical and has been known to have a negative impact on both chick and hatchery employee health (Tupper, 2023). Formaldehyde is still being used throughout the industry because of its continued success at reducing bacteria and fungus, including spores

(Cadirci, 2009). An alternative is needed, because the chemical is currently under risk evaluation with the EPA which could lead to its ban in the future, greatly impacting the poultry hatchery industry (US EPA, 2023). If formaldehyde is applied incorrectly, not only can it put employees at risk because of its carcinogenic properties, but it can also damage the tracheal epithelium of the baby chicks (J. Sander et al., 1995). Common alternatives explored in the poultry industry include liquid or vapor hydrogen peroxide, ozone, and UV light to name a few (Al-Shammari et al., 2017; Graham et al., 2022; Sander and Wilson, 1999; Whistler and Sheldon, 1989). Each has benefits and downsides, much like formaldehyde.

An additional potential alternative is Dry Hydrogen Peroxide (DHP) (Lee and Stephens 2018). DHP is a colorless, odorless molecule created by pulling in ambient water (humidity) and oxygen that is naturally present in the surrounding environment, moving the molecules across a photocatalytic membrane, which pulls them apart to then recombine into gaseous H_2O_2 (Lee, 2012). The peroxide molecules are dispersed throughout the space via a fan within the DHP system, the central HVAC, and/or natural air movement within the space. Upon contact with microbes, the DHP attacks essential cell components, including DNA and lipids, thereby killing the pathogen (Centers for Disease Control and Prevention, 2016). One of the downsides to applying aqueous hydrogen peroxide in poultry hatchers is its corrosive properties. Because of the low concentration emitted by DHP (10-25 ppb) and the gaseous state, corrosion is not foreseen as an issue when applied within hatch cabinets.

Previous studies in hatchery environments showed that DHP application in a laboratory egg cooler led to improvements in overall hatch and hatch of fertile eggs (Melo et al., 2020). DHP has also been tested in a commercial broiler hatcher equipped with multistage incubators and hatchers. DHP was supplied to the air spaces within the hatchery and was pulled into machines via

the fresh air intakes (McElreath, 2019). The study resulted in an improvement in overall hatchability and hatch of fertile, decreased early dead, decreased contaminated eggs, decreased prevalence of fungus in lungs of hatched chicks, and decreased 3-day mortality. Additional research is needed that investigates long term exposure effects on hatchery parameters, leading to the present study. If DHP is in contact with eggs beginning in the egg cooler, during incubation in single stage machines, at transfer and during hatch, could it serve as an alternative to the toxic chemical formaldehyde?

For the current trial, DHP machines were installed throughout a commercial broiler hatchery with single stage HatchTech incubators. Hatchability, hatch of fertile, residue breakouts, fluff counts, and microbial level on surfaces throughout the hatchery and eggshells were monitored. Within the overall trial, it was hypothesized that the application of DHP would result in a reduced level of microbes on the eggshell surfaces, which would be the reason DHP may serve as an alternative to formaldehyde.

After 12 months of data collection, and gathering hatchability data from the previous year, results indicated that the eggs with the greatest increase in overall hatchability were eggs that moved through DHP treated incubators and hatchers. The percent increase of hatchability was greater than that of any other group. The evaluation of microbial load on eggshells present in the hatchery using one-way ANOVA indicated that there is a natural decline in the microbial load on eggshells during incubation, but eggs exposed to DHP within single stage incubators shows a significantly lower amount of microbes on day 19 of incubation when compared to the nontreated group. This led to the conclusion that DHP may be a successful alternative to formaldehyde in the poultry industry.

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CHAPTER 2

LITERATURE REVIEW

Poultry Overview

The demand to produce poultry products is increasing daily, as chicken serves as a source of protein, essential nutrients and has a low-fat content (D. Narinç and E. Aydemir, 2021). In the United States in 2021, 59.2 billion pounds of broiler meat was produced with a value of $31.5 billion (USDA, 2021). Commercial broiler production is known to have begun in the early 1920's by Cecille Steele of the Delmarva Peninsula (Williams, 1998). Each year, Cecille would order 50 chicks from Vernon Steen, who operated a local hatchery, to replace the birds she lost throughout the year. In 1923, Mr. Steen sent Cecille 500 chicks, even though she had only ordered 50. Instead of sending them back, she had a shed built to house the chicks. After 18 weeks of growth, the 387 surviving chickens, weighing in at 2.5 pounds each, were sold for 62 cents per pound. This translates to $10 per pound in 2023, making it a highly profitable transaction for the Steele family. The next year, Cecille ordered 1000 chicks, and by 1926, her husband had resigned from his career in the US Coast Guard and built chicken houses that would hold 10,000 birds. The success of the Steele family spread throughout the region, and by 1925, approximately 50,000 broiler chickens were being raised solely to sell for the consumption of their meat in local restaurants and grocery stores (Williams, 1998).

The agricultural patterns that began in Delmarva are thought to have shaped the eating patterns of the nation. As the poultry industry matured, diseases that affected commercial poultry production arose. One of the first poultry related diseases was classified in 1899 as bacillary white

diarrhea, and later was named *Salmonella pullorum* (Rettger, 1900). Pullorum was at first thought to have been due to artificial incubation conditions, and therefore sanitation during incubation became a concern that grew rapidly as commercial poultry production grew in popularity in the early 1900's. Breeder and hatchery industries understood the importance of controlling Pullorum in their facilities and pushed for control programs to be implemented (Hitchner, 2004). In 1934, the National Poultry Improvement Plan (NPIP) was implemented to benefit the poultry industry, and a large portion was dedicated to controlling disease (Georgia Poultry Laboratory Network). The first license to vaccinate poultry was given to University of California, Berkley in 1916 for combating fowl pox (Espeseth and Lasher, 2010), and by 1955, 1937 million doses of poultry vaccines had been created (Espeseth and Lasher, 2010; Hejl, 1968). The control of disease continues to rely on biosecurity and good vaccination practices that begin in the hatchery (Capua and Marangon, 2006). As the industry moves away from the use of antibiotics in the 21st century, hatchery sanitation becomes even more important as bacteria can have a detrimental effect on early chick health, causing diseases such as omphalitis and aspergillosis (Cortes et al., 2005). Production of high-quality poultry begins with providing optimal environmental conditions beginning on the first day on incubation (Narinç and Aydemir, 2021).

Hatchery Operation Overview

Hatcheries are an integral part of the vertical integration system present among poultry companies in the US (Vukina, 2001). They should be designed with one-way flow to reduce cross-contamination and allow for ease of cleaning (Samberg and Meroz, 1995). Fertile eggs from the complex's multiple breeder houses are brought to the hatchery on designated days each week. Eggs are stored in the egg room until they are needed to make a set. Eggs are transferred from farm

egg buggies to incubator racks and placed into incubators. There are two main types of incubators used throughout the poultry industry – single and multi-stage, each having different incubation, temperature, and humidity requirements. Eggs are incubated for 17.5-19.2 days depending on embryo growth rate, which is based on breed (Williams, 2011). Eggs are moved from incubator trays to hatch baskets during a process called transfer. Transfer time is based on positioning of the embryo, with the goal of in-ovo injection of vaccine to be placed in the amniotic fluid within the egg (Ceva Santé Animale–Libourne, 2008). Once injected and transferred from incubator trays to hatch baskets, eggs are placed into hatchers. On day 21, chicks are pulled from the hatchers, separated from eggshell debris, and enter the chick processing room (Bell et al., 2002). Depending on the hatchery, processing within the egg room may include gender identification of the chicks, grading, culling, and additional vaccinations. Chicks are distributed into chick boxes, typically 100 chicks per box, and boxes are stacked throughout the chick holding room until boxes are loaded onto chick trailers and carried to a broiler farm for placement. At the end of each day of processing, hatcheries are thoroughly cleaned (Bell et al., 2002). In addition, hatcheries typically hatch chicks four days per week and one non-hatch day is used to additional intensive cleaning. Organic material such as feces from the breeder farm, yolk from cracked or exploded eggs, and chick fluff produced during hatching are all reservoirs for bacterial growth, and daily sanitation processes are vital to the success of a hatchery and health of chicks. Though the overall hatch process is straight forward, there are many variables present throughout the hatch process that can affect the overall profitability of the hatchery.

Variables Influencing the Success of Hatchery Operations

Industry standards suggest that eggs are brought to the hatchery from each breeder farm twice per week (Bell et al., 2002). Eggs should be collected from the breeder house at least once per day and placed in the on-farm egg cooler for storage (Bell et al., 2002). From the moment eggs are collected from the breeder house and placed on farm buggies, temperature monitoring of embryos becomes a vital aspect of a successful hatchery operation and optimal embryo growth and development. On farm egg coolers are generally kept at 21-25° C. Cooled egg trucks (10-23° C) transport the eggs from the farm to the hatchery to be stored in the hatchery egg room (15-19° C) until eggs are incubated. It is vital to match the temperature and humidity of the on-farm egg cooler, truck, and hatchery egg room to prevent sharp changes in environmental conditions which can cause eggs to condensate or "sweat", which can increase presence of bacteria on the eggshell (Bell et al., 2002). As eggs cool, the water, and therefore bacteria, present on the surface of the egg is drawn into shell, increasing risk of contaminated eggs within the hatchery.

Industry standard is to store eggs for at least 24-48 hours before they are incubated to ensure proper pH within the egg (Bell et al., 2002). At the onset of lay, pH is too high for optimal embryonic growth, but as eggs are stored, CO_2 is released from the egg, increasing the pH of the albumin from 7.6 to between 8.8 to 9.2, which is optimal for proper hatching (Bell et al., 2002). Though storing eggs for a short period of time is ideal, egg storage for an extended period causes egg water loss and increases the size of the air cell (Araújo et al., 2016). Rapid loss of water during egg storage causes albumen deterioration, which leads to early embryo mortality and decreased ability to defend against pathogens (Narinç and Aydemir, 2021). It is therefore recommended that eggs be stored for no more than three days with optimal temperature and humidity conditions being met, to achieve maximum hatchability (Bergoug et al., 2013). To combat albumen degradation

that can lead to a decreased hatchability, some hatcheries that store eggs for 7 days or more implement short periods of incubation during egg storage, or SPIDES (Dymond et al., 2013; Okasha et al., 2023). Heat treating eggs using the SPIDES method can have positive benefits on the embryo that mimic the natural heat that a hen would bring to the nest during each day while she was laying her clutch. The Cobb Vantress hatchery management guide recommends heat treating eggs on day 6, 11, and 16 of egg storage (Bell et al., 2002). Embryonic death can occur if the embryo is above 32° C for more than 13 hours, so optimal heat treatment includes 1 hour of pre-warming at 26.5° C, 6 hours of incubation at 35° C, and 3 hours of cooldown at 23.9° C (Bell et al., 2002). Heat treatment can be completed in specialized equipment or single stage incubators may be programmed for this specific purpose (Bell et al., 2002). Eggs should be immediately placed back in the egg storage room, where the temperature should remain between 13-19° C (Decuypere et al., 2001). This temperature is below physiological zero, or the point in which embryonic cell division is slowed, without causing embryonic mortality (Narinç and Aydemir, 2021). Physiological zero is considered 25-27° C by Brake et. al, and 19-28° C by Decuypere (Brake et al., 1997; Decuypere et al., 2001). Maintaining constant temperature and humidity and limiting the amount of traffic throughout the egg room can mitigate bacterial contamination of hatching eggs while being stored in the egg cooler (Bell et al., 2002).

From the egg cooler, eggs move to the incubation phase of hatching. Broiler chicks require between 461-510 hours of incubation to hatch (Almeida et al., 2008). Temperature of the incubator is one of the most important factors that goes into recreating natural bird incubation (Narinç and Aydemir, 2021), since chicken embryos cannot regulate their own temperature (Hiebert and Noveral, 2007). The optimal temperature of incubation of domestic poultry is 37 to 38° C (Decuypere and Michels, 1992). Not only does temperature affect embryonic development, but it

also affects post hatch chick quality and performance (Șeremet, 2012). Throughout incubation, temperature is adjusted to incorporate natural embryonic heat production in both single and multi-stage incubators (Elibol and Brake, 2004). Humidity present during incubation also effects hatchability. Humidity present in the incubator is directly correlated to egg water loss, a natural requirement for successful hatch (Ar and Rahn, 1980). When incubation humidity is low, the size of the chick upon hatch is smaller, and there is a larger occurrence of unhealed navels (Crespo and Shivaprasad, 2017), which can lead to bacterial infection of young chicks. When humidity is high, the occurrence of early and late embryonic dead's increase (Robertson, 1961). Optimal incubation humidity ranges from 40% to 70%, but 50% is best (Lundy, 1969). Later research completed showed that maximum hatchability is reached with an incubation humidity between 53% to 55% (Kirk et al., 1980). Air circulation and ventilation is also vital for embryo survival during incubation, with gas exchange being the most crucial during the first week of incubation due to the distance between the embryo and the shell (Narinç and Aydemir, 2021). Ventilation includes controlling the level of both oxygen and carbon dioxide within the incubator, and levels within the incubator dictate the levels found within the egg (Bergoug et al., 2013). Embryos receive oxygen via passive diffusion through the pores of the egg, from high to low concentration. Therefore, the rate of diffusion will be influenced based on the concentration of oxygen and CO_2 between the egg compartment and incubator compartment (Visschedijk, 1968).

There are two primary types of incubation: single and multi-stage. Single stage incubators have a slight advantage over multi-stage incubators because the environment within can be matched to the exact needs of the embryo and they are much easier to clean (Fairchild et al., 2007). Incubation begins all at once, allowing the temperature, humidity, and air exchange to be altered based on embryo age (Molenaar et al., 2010). In single stage incubators, the dampers open and

close depending on the age of the embryo (Wineland and Oviedo-Rondon, 2010). Multistage incubators add in three or four different sets of eggs into the same incubator throughout the week, creating one incubator with embryos on different days of incubation (Mesquita et al., 2021). Heat is sourced from the older embryos and air movement allows it to heat younger embryos also present within the machine. This can however create hot spots and excess heat, leading to embryo mortality, which makes it imperative that hatcheries know how each incubator functions when making a incubation (Kolariczyk, 2020). Multistage incubators also keep the damper open to different degrees based upon heating and cooling needs. Both single and multistage incubators incorporate egg turning, as it mimics the natural behavior of poultry (Narinç and Aydemir, 2021). The industry standard is turning the eggs once per hour through at least a 45-degree angle from perpendicular (Funk and Forward, 1960), which moves the egg a total of 90-degrees each hour (Deeming, 2009). By turning eggs, air flow is increased, and the number of hot spots inside incubators is decreased, leading to an increased hatch and post-hatch chick quality (Cutchin et al., 2009). If eggs are not turned during certain periods of embryonic development, abnormal development is likely to occur due to reduced sub-embryonic fluid (Deeming, 1989), reduced chorioallantoic membrane development (Tullett and Deeming, 1987), and embryo malposition (Randles and Romanoff, 1950). When the amount of amniotic fluid present in the egg is reduced, there are less nutrients available to the hatchling, leading to decreased quality chicks. Studies done by Wilson and Wilmering showed that stopping turning on day 10 has a negative effect on hatchability, while stopping turning on day 13 had no effect on the hatchability of the embryo (Wilson and Wilmering, 1988).

For over 50 years, the industry standard has been to transfer eggs from incubator trays that turn to hatch baskets that do not turn on day 18 of incubation (Wilson and Wilmering, 1988).

Because large amounts of fluff and waste are produced during the hatch process, it is more sanitary to control that process independent of incubation, to improve overall hatchery sanitation (Cormick, 2018). During the process of transfer, it is imperative that eggs are not overchilled or overheated at this phase of incubation (Cormick, 2018). Eggs may be either candled by hand or go through an automated candling machine that shines a light through the egg and removes any eggs that allow significant light to pass through, indicating the presence of an infertile egg or early embryo mortality, ideally leaving only viable fertile chicks (Cormick, 2018). Vaccination *in-ovo* on day 18, during the transfer process, was developed in 1992 (de Wit and Montiel, 2022). A small hole is poked into the broad end of the egg, and 0.05-0.1 mL of vaccine is injected into the either the embryo or amniotic fluid (de Wit and Montiel, 2022).

Once vaccination is completed, eggs are placed into hatch cabinets. Industry standards suggest that most hatcheries use temperature step-down profiles in the hatcher to ensure the chick body temperature stays between 39.5-40.5°C upon hatch and as the chick awaits processing (Bell et al., 2002). Research suggests the best way to maintain proper hatchling body temperature is to regularly complete cloacal temperature checks throughout the hatch cabinets and ensure that cloacal temperatures stay within 40-41°C (Bell et al., 2002). Relative humidity throughout the hatcher should be maintained at 52% to 54%. Moisture during hatch is necessary to ensure chicks can successfully pip out of the shell.

The final step of each day of completed hatchery operation is to clean and sanitize the equipment, floors, and spaces impacted by the naturally occurring organic matter in the hatchery (Eckman, 1994). Proper sanitation can have an impact on overall success of the hatchery.

Importance of Hatchery Sanitation

The NPIP of 1959 states that "Section 145.6 (a) Hatcheries, including brooder rooms, shall be kept in a sanitary condition, acceptable to the Official State Agency." A contaminated hatchery can not only cause horizontal transmission of disease but can significantly impact economic loss of poultry (Funk and Irwin, 1955). Florian and Trussell identified 197 different microorganisms from 81 infected eggs, all of which were gram-negative rod-shaped bacteria (Florian and Trussell, 1957). The presence of these microorganisms has three effects on hatcheries throughout the industry: 1) increased numbers of contaminated and unhatched eggs; 2) increased infection in hatched chicks leading to poor chick quality; and 3) decreased performance of surviving birds (Williams, 1970). The microorganisms present on just one or two hatching eggs can be distributed onto surfaces throughout a hatchery, and then workers or natural air flow can distribute those microorganisms into hatchers, contaminating all chicks within the hatcher (Nichols and Leaver, 1967; Sheldon and Brake, 1991). In addition, bacteria present during the final three days of incubation increase drastically because of the bacteria released from within the shell during pipping and hatch, deeming this an important timepoint to mitigate and decrease microbial load (Magwood, 1964; Magwood and Marr, 1964).

Hatchery sanitation is affected by many factors and poor sanitation can lead to many adverse effects on newly hatched chicks. Chicks that hatch within a bacteria filled environment can suffer from health issues, such as omphalitis and aspergillus, that hinder their growth and performance in the field (Chute and Gershman, 1961). Omphalitis occurs as the chick is drawing the remainder of the yolk-sac into the abdominal cavity. Before it completely heals, bacteria, such as E. coli, staphylococci spp., streptococci spp,, and pseudomonas spp., from the environment enters the navel, which is still open at hatch, causing infection that leads to early mortality on the

broiler farm (Stokes et al., 1956). It has been concluded that the major cause of omphalitis is increased bacterial contamination of the air during hatch. Signs of omphalitis in the field include depressed chicks, drooping of heads, and huddling (Khan et al., 2002). *Aspergillus fumigatus* is a fungus that negatively affects the health of birds by impeding the respiratory system (Arné et al., 2011). *A. fumigatus* fungal spores proliferate on organic material present during hatch, filling the space with small airborne particles that are easily inhaled and begin to grow within the respiratory tract of newly hatch chicks (Arné et al., 2011). Infection with *A. fumigatus* in young birds is often referred to as brooder pneumonia and causes high mortality and morbidity (Arné et al., 2011). Signs of infection in the field can include lethargy, ruffled feathers, respiratory signs, such as gasping for air and extended necks, and stunted growth (Arné et al., 2011). The presence of *A. fumigatus* in hatcheries is increased as the amount of disinfectant being used is decreased (Wright et al., 1959).

Historically, antibiotics have been used to improve health of chicks (Nasrin et al., 2012), but as industry standards have gone away from the use of antibiotics, the importance of hatchery sanitation continues to increase. In the last 10 years, the poultry industry has seen a push towards antibiotic free production because of consumer perception and demand (Cervantes, 2015). With the decreased use of antibiotics comes several challenges such as decreased stocking density, longer down-time between flocks, increase requirements of biosecurity measures on farms, and most importantly, an increase in enteric and systemic diseases (Cervantes, 2015). In the past, systemic infection caused by *E. coli* could be treated with antibiotics, but today, hatchery sanitation becomes even more vital as chicks are exposed to *E. coli* without the safety net of antibiotic application in the field (Cervantes, 2015).

Standard operating procedures for sanitation differs throughout different hatchery spaces. In 1959, chemist Herbert Sinner identified four factors that must be present for an effective clean: time, mechanical force, temperature, and chemistry (Sinner, 1960). By decreasing one of the four, the other three will have to be increased to compensate. This model can be applied throughout poultry hatcheries to achieve optimal clean and therefore increase the number of healthy chicks hatched and profit of the hatchery.

The first step when cleaning any area of the hatchery is to remove all organic material present. This is vital because the organic material (i.e, dried yolk, fecal material, chick down) can act as a shield for microbes to escape the action of the cleaning and sanitizing agents applied to the spaces (Samberg and Meroz, 1995). Once organic material has been removed, the area is washed using a soap and scrub brush (Ledoux, 2006). After rinsing, a foaming sanitizer is applied following the direction of the label for proper contact time before rinsing a final time and allowing the space to dry. All egg trays, trolleys, and chick baskets must go through a wash cycle, where water temperature, mechanical force, chemical sanitizer, and time are closely monitored (Wray et al., 1999). Optimal tray wash temperature is 60-66° C (Bell et al., 2002). In addition to equipment sanitation, maintaining clean filters throughout the hatchery is optimal for proper ventilation and microbial control. This is especially important in areas where chick down is present in large quantities. Chemicals used during the sanitation process differs from hatchery to hatchery, but common chemicals seen throughout the industry includes a generic degreasing soap and a sanitizer that can be foamed (Ledoux, 2006).

Use of Formaldehyde in Poultry Hatcheries

Even with the implementation of a sanitation protocol, proper hatchery sanitation begins by limiting the dirt and organic matter that is brought in on the surfaces of the hatching eggs. Eggs coated in organic material, and subsequently in pathogens, have been proven to not only cause increased hatchery contamination, but to also decrease hatchability (Yoho et al., 2008). Pathogens present on the surface of the egg can penetrate both the shell and inner membranes, contaminating both the embryo and other chicks during the hatch process (Berrang et al., 1999). The rate in which bacteria passes through the pores into the eggshell is increased as moisture increases, requiring eggs to be maintained in a properly cooled environment to avoid "sweating" (Lock et al., 1992). This is the precise reason hatching eggshells cannot be sanitized before set, because spraying with water and a sanitizing agent can cause whatever is present on the surface of the shell to be drawn into the shell (Stokes et al., 1956). Additionally, the sanitizing agent can clog the pores and cause a decrease in O_2 and CO_2 diffusion into and out of the shell, causing embryo mortality. Since the goal to achieve maximum hatch limits the ability to sanitize the surface of the egg, it is a key sanitation factor to only bring nest clean eggs into the hatchery, instead of eggs that may have been laid on the floor or slats or in dirty nests at the breeder farm (Mayes and Takeballi, 1983). Since most growers are paid for hatching eggs and not for floor eggs, there can be a strong motivation to include dirty floor eggs in with the egg pack. Visibly dirty eggs may be "cleaned" enough to be included in egg pack, but this activity can be detrimental to the level of contamination seen in the hatchery, as it can impact the integrity of the eggs natural cuticle.

European practices differ from US industry standards on the topic of hatching egg sanitation. Applying formaldehyde directly to the eggshell's surface prior to incubation is the most common method of eggshell sanitation in most European countries (Cadirci, 2009). There are

several different methods of sanitizer application including dipping, wiping, and spraying, but the most popular is fumigation of formaldehyde gas (Cadirci, 2009). Many studies have been completed to analyze the concentration of formaldehyde required to remove microbes from the surface of eggs and for elimination of salmonella species, and nest clean eggs must be fumigated at room temperature and ambient humidity for at least 20 minutes with a minimum formaldehyde concentration of 600 mg of formaldehyde gas per m^3 (Cadirci, 2009). The overarching goal of formaldehyde fumigation is to reduce naturally occurring microbial load on the surface of eggshells while protecting the embryo from the harmful effects of formaldehyde. However, the outermost component of the eggshell, known as the cuticle, is 90% protein (Baker and Balch 1962), and because formaldehyde's mode of action is to react with proteins, it has long been a topic of debate as to whether formaldehyde can degrade the cuticle, allowing microorganisms to easily pass through the pores and come in contact with the embryo (Cadirci, 2009). It has been proven that eggs with a weaker cuticle have an increased water uptake from the environment (Board and Halls, 1973), therefore, by removing the cuticle, any microbes present within the air of incubators or hatchers can be drawn into the egg during water intake (Cadirci, 2009). Eggs are naturally equipped with physical barriers for microbial defense, and by removing the cuticle, that natural defense is also removed (Haines, 1939).

Though the industry in the United States does not use formaldehyde as an eggshell disinfectant in an effort to maintain the cuticle, it has been recognized that the bloom of bacteria that occurs during hatch requires an antimicrobial mitigator, in addition to daily sanitation practices, to decrease bacterial infection of chicks (Chute and Gershman, 1961). Most often, formaldehyde is used as a fog to mitigate the bacteria present during hatch. At room temperature, formaldehyde (CH_2O, formalin) is a gas and is soluble in water (Cadirci, 2009). It is used as a

disinfectant because it is readily available, inexpensive, and kills most bacteria, fungi, and spores and because of its ability to break down protein and nucleic acid bases of microorganisms (Braswell et al., 1970; Fraenkel-Conrat et al., 1945). The first known use of formaldehyde as a disinfectant was noted in 1892; however, the first known use of formaldehyde application to control the spread of poultry disease during incubation and hatching was noted in 1908 (Hugo, 1992; Pernot, 1908). Formaldehyde may be applied in its liquid form, such as on the surfaces of eggs in Europe, but has been deemed most effective when applied as a gas (Harry, 1954). Literature from 1955 suggests that implementation of formaldehyde fumigation may also be effective when applied in incubators at the start of the set, though US industry standard does not currently fumigate formaldehyde during incubation (Funk and Irwin, 1955).

Based on previous research, fumigation of formaldehyde in hatchers is most effective because of the high temperature and humidity. High temperature is necessary to maintain a high humidity, both allowing formaldehyde to remain in the atmosphere at high concentrations for an extended period (Cadirci, 2009). The gas is carried in water droplets, and therefore the efficacy is higher in a warm humid atmosphere (Wright and Truscott, 1954). One of the main factors that can decrease the rate of microbial disinfectant is the amount of organic matter present within the hatch cabinets (Cadirci, 2009). Because the mode of action of formaldehyde is to break down proteins of microbes, formaldehyde efficacy can be decreased because of the chemical reactions caused when formaldehyde encounters and breaks down the protein present in organic matter (Ekelenburg, 1991). Neutralizing the vapors in this manner can leave a lower concentration of the chemical present in the air to focus on microbes that lead to omphalitis and aspergillosis (Cadirci, 2009).

There are a few different methods of vaporizing formaldehyde in hatchers. Some hatcheries place liquid formaldehyde, made up of formalin gas dissolved in water with 5-15% methanol to prevent polymerization, in a small dish within the hatcher which vaporizes and fills the space (Steinlage et al., 2002; Wilson and Mauldin, 1989). Some hatcheries soak a braided wick with dyed formaldehyde, so you can see the liquid in the case of a major leak, and the liquid vaporizes into the hatcher in the same way as the previous method. Other hatcheries may hang an intravenous drip bag filled with liquid formaldehyde, that slowly drips the formaldehyde into the hatcher, again, vaporizing and filling the space (Steinlage et al., 2002). The concentration of formaldehyde must be closely monitored because it is not associated with the amount of liquid formaldehyde applied in the space. Instead, it depends on humidity in the space, the amount of air exchange occurring, and the level of absorption by different surfaces within the hatcher (Cadirci, 2009; Hodgetts, 1987). Research has shown the best method for applying formaldehyde with the intention of microbial reduction is gradual application during the entire hatch duration rather than spacing out formaldehyde application to every 12 hours or no formaldehyde use at all (Cadirci, 2009; Steinlage et al., 2002).

Formaldehyde has long been noted as the most effective antimicrobial to use in poultry hatcheries (Steinlage et al., 2002). However, formaldehyde has been shown to cause cancer and have negative health effects on both chicks and hatchery employee health. For this reason, several states have banned the use of formaldehyde in commercial poultry hatcheries and federal review of the practice is currently ongoing.

Alternatives to Formaldehyde

For years, formaldehyde has been the top choice in hatchery sanitizers because its efficacy as an antimicrobial is undisputed, however, carcinogenic affects have pushed hatcheries and researchers towards the testing of more safe alternatives (Florian and Trussell, 1957; Sander and Wilson, 1999; Scott and Swetnam, 1993). Formaldehyde fumes fill an entire space, and can therefore be absorbed into the shell, causing formaldehyde to impact the growth of the embryo (Cadirci, 2009). The respiratory tract of poultry has a thin layer of aqueous mucous, and because of the mode of action of formaldehyde, the gas can dissolve in the mucous, increasing acidity, which has been known to cause ciliary stasis in trials completed using rats and rabbits (Dalhamn, 1956). Formaldehyde vapor concentration of 80 ppm can damage the tracheal epithelial of chicks, causing lesions that are evident in the field for 5-12 days post formaldehyde exposure (Gerrits and Dijk, 1991).

In addition to impact to chick health, formaldehyde gas negatively impacts human health (Cadirci, 2009), which has since led to regulation by the US Environmental Protection Agency within the Toxic Substances Control Act (Sheldon and Brake, 1991). These regulations have incubation acceptable limits for exposure time and concentration, required records of health of exposed employees and specialized safety equipment and training when dealing with formaldehyde (Wilson and Mauldin, 1989). The acceptable limits are set at 0.75 ppm over an 8-hour period or 2 ppm for short term exposure that is no more than 15 minutes in length; and 200 ppm is considered immediately dangerous to life and health (OSHA). Businesses are required to maintain exposure and health records for 30 years past the end of employment for each person that encounters formaldehyde in the workplace (OSHA). The gas is a strong irritant to the eyes, nose, and mouth, and can cause an immediate immune response upon initial exposure (OSHA). Because

of the carcinogenic affects and amount of record keeping required, research in the last 30 years has shifted to focusing on discovering an alternative to formaldehyde that will be just as good of a biocidal, yet it has proven difficult (Kim and Kim, 2010). A study published in 1993 was completed testing 23 different methods of sanitation, and each was directly compared to eggs sanitized with formaldehyde and eggs that were not sanitized at all (Florian and Trussell, 1957; Scott and Swetnam, 1993).

The most well-known alternative to formaldehyde is hydrogen peroxide (H_2O_2). Hydrogen peroxide is a colorless liquid that has been used as a disinfectant for inanimate objects (Sander and Wilson, 1999). Hydrogen peroxide is an oxidizer and can therefore cause corrosion when applied to many metals (Sheldon and Brake, 1991). Hydrogen peroxide may irritate the skin, eyes, and mucous membranes when used at high concentrations, but because the chemical components are hydrogen and oxygen, when dissipated into the environment, degradation does not leave residues that are harmful to chick or human health (Sander and Wilson, 1999). The mode of action of Hydrogen Peroxide as a sanitizer is the production of a hydroxyl radical which can attack membrane lipids, DNA, and other essential cell components (CDC, 2016). Hydrogen peroxide has been tested for use as a hatchery disinfectant, in attempt to replace formaldehyde, and according to a study completed in 1991 by Sheldon and Brake (Sheldon and Brake, 1991), a concentration of 5% H_2O_2 is required to fully control pathogens present on surfaces. However, a 3% concentration of hydrogen peroxide has also been reported to be successfully bactericidal, while not causing rust on machine metals (Sheldon and Brake, 1991). A study completed in 1999 by Sander and Wilson stated that bacterial populations are naturally reduced on the surfaces of eggs during incubation, and misting hydrogen peroxide during incubation in comparison to no treatment during incubation, yields lower bacteria during hatch (Sander and Wilson, 1999).

Application of the mist did significantly increase the water loss from the eggs, though it did not seem to affect hatchability, livability, body weight, or feed conversion of the broilers (Sander and Wilson, 1999). Recent studies have focused on using hydrogen peroxide gas as an alternative to liquid hydrogen peroxide disinfectants to reduce rust to machines and maintain proper water loss during incubation.

A proprietary Dry Hydrogen Peroxide (DHP) technology has the disinfection properties of aqueous hydrogen peroxide, without the risk of rusting machines or breaking down the cuticle of the egg because it emits a low concentration of hydrogen peroxide molecules that are in a completely gas phase (Lee, 2012). DHP machines pull ambient air containing oxygen (O_2) and humidity (H_2O) over a photocatalytic membrane, and molecules are activated using a non-germicidal light which breaks the bonds of O_2 and H_2O, and forms H_2O_2 (Melo et al., 2020). The DHP molecules are then dispersed throughout the space via the built-in fan or HVAC units. DHP has been used in hospital settings and has been an effective fungicide, bactericide, and virucide (Herman et al., 2015). Few studies have been completed where DHP is applied to a hatchery setting, but the technology may be useful (Lee and Stephens, 2018). The University of Georgia tested the application of DHP to an egg cooler where 1980 eggs were stored. The eggs were hatched, and the eggs treated with DHP had a higher hatchability and hatch of fertile when compared to eggs that were not treated with DHP in the egg cooler (Melo et al., 2020).

Methods of Monitoring Hatchery Sanitation

As standard operating procedures are developed for hatchery sanitation, it is important to use different methods of microbial monitoring to ensure the efficacy of the sanitation protocol as it is put in place. Once hatcheries have implemented a sanitation protocol that is successful at

microbial reduction, routine monitoring is vital to ensure the protocol has continued efficacy. Because the method of microbial mitigation (cleaning and disinfecting procedures, formaldehyde application method, and machine type) differs at each hatchery, it is difficult to interpret when a hatchery is clean (Chute and Gershman, 1961; Wright et al., 1959). The attempt at systematic grading of hatchery sanitation status began in the early 1960's (Magwood, 1964). One method developed to quantify hatchery bacteria and cleaning protocol efficacy is taking samples of the fluff produced while chicks are hatching (Wright et al., 1959).

There are varying methods of fluff sample collection: 1) placing media plates directly into the hatcher and allowing fluff to collect on the agar; 2) collection of fluff into a sterile vial, suspending it into a sterile broth and placing the liquid onto media plates (Wright et al., 1959). Both methods require proper incubation time before colony counts may be performed (Wright et al., 1959). Quantifying the microbes present in fluff has proven to indicate not only cleanliness of a facility, but also bird health throughout the grow out period (Wright et al., 1959).

Fluff samples have long been used to quantify the level of contamination present during hatch, but Magwood studied the microbial presence in the air outside the hatchers and compared findings to fluff samples from the surrounding hatchers (Magwood, 1964). Results indicated that both the air and fluff counts are means of quantifying the level of contamination, but air samples had an advantage because they displayed the wide ranges in microbial load throughout a space (Magwood, 1964). The study concluded that the lowest microbial counts were present early in the hatch period, and increased as the hatch process finished and chicks were processed (Magwood, 1964). A direct correlation has been proven between the number of quantifiable bacteria in relation to the efficacy of the cleaning and sanitation programs (Magwood, 1964). In history, hatcheries that had the lowest microbial counts also had the most intricate cleaning and disinfection procedure

(Magwood, 1964). The study conducted by Magwood in 1964 was continued by comparing the microbes present in air samples to the bacteria gathered from the surfaces inside the hatcher.

Surface swabs can indicate microbial presence on any given surface. Sterile swabs are soaked in a buffered solution before swabbing a predetermined area in four different directions and streaking that onto the media plates at the same angle the surfaces were swabbed (Magwood and Marr, 1964). In addition to swabbing directly onto media, swabs may be saturated in a liquid buffer solution, vortexed, and then that liquid may be plated onto media that will be incubated and quantified. There are limitations to direct surface swabbing. Chemical residues present throughout hatcheries may inhibit microbial growth during incubation or cause contamination (Favero et al., 1968).

In addition to traditional plating of surface swabs, the use of ATP bioluminescence has been increasing in popularity as it gives an instant read (~15 seconds) of the number of ATP molecules present on the swabbed area, which can be correlated to the level of microbes present. Adenosine Triphosphate is present in all living cells and by measuring the level of its presence, the level of microbial contamination may be measured (Bellamy, 2012). As indicated by (Fraga, 2008), ATP bioluminescence in combination with luciferase has been a topic of research since 1885. When using ATP bioluminescence, light is emitted in proportion to the amount of ATP present: the more ATP, the higher the amount of light emitted. The unit of measurement for ATP bioluminescence is reported in Relative Light Units (RLU); therefore, the higher the RLU value, the more contamination present on a surface. Multiple studies have displayed an association between ATP and total aerobic colony counts (Anderson et al., 2011; Lewis et al., 2008; Mulvey et al., 2011), indicating that ATP bioluminescence could serve to give an overview of total cleanliness.

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CHAPTER 3

APPLYING DRY HYDROGEN PEROXIDE IN A HATCHERY EGG ROOM AND IN SINGLE STAGE INCUBATORS REDUCES MICROBIAL LOAD ON SURFACES

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ABSTRACT

In 2022, the United States produced 9.17 billion broiler chickens and each of these was incubated and hatched in a hatchery within the US. With hatching of 9+ billion eggs comes contamination from naturally occurring organic matter present on the surface of freshly laid eggs as well as contamination from the breeder farm environment. Regularly updated cleaning and disinfecting procedures for poultry hatcheries are vital to the success of the broiler industry, and new technology should continually be evaluated to improve hatchery cleanliness and optimize the hatch process. Previous research has shown that application of Dry Hydrogen Peroxide (DHP) in a broiler hatchery resulted in improved performance (total hatch, hatch of fertile), but that hatchery used multi-stage incubators which only allowed for application of DHP to the eggs inside via the fresh air intake plenum. The objective of this study was to determine if DHP, when supplied directly inside single stage incubators, could decrease the prevalence of microbes on eggshells and surfaces. DHP units were installed throughout the egg room and in 1/3 of the incubators of a commercial broiler hatchery equipped with single-stage HatchTech incubators. Pre-treatment data from the egg room and eggs was collected for three months to serve as a baseline for microbial load analysis. DHP systems were installed and turned on, and trial data collection continued for nine months. The microbial load of the eggs in the egg room and the egg room surfaces compared to the baseline were analyzed, as well as the microbial load of treated vs non-treated eggs after incubation. Application of DHP led to a significant reduction of microbes on surfaces in the egg room compared to pre-treatment data, though all values were very low. Interestingly, there was an increase in microbial load on eggshells in the egg storage room after DHP treatment began, but environmental conditions were suboptimal and eggs were sweating. After incubation, a dramatic natural reduction in microbial load was seen on non-treated eggshells, but there was a significant

decline in microbial load on eggshells of eggs incubated with DHP during incubation compared to non-treated eggs. Overall, treatment with DHP during incubation in single stage incubators did result in lower comparative microbial loads on eggshells.

INTRODUCTION

As poultry production has increased over the past century, hatchery size has increased and the number of eggs entering hatcheries on a weekly basis has increased (Samberg and Meroz, 1995). In 2021, 9.88 billion broiler-type chicks were hatched throughout the US (USDA NASS, 2022). To supply the industry with high-quality chicks, poultry hatcheries function to provide proper temperature and ventilation to eggs, and to reduce microbial challenges when sanitation practices are followed. Upon lay at the breeder farm, eggs are placed into an on-farm egg cooler for storage until they are brought to the hatchery. All hatcheries bring eggs into an egg holding room once or twice per week from the breeder farm, and they are stored in this room until incubation (Fasenko et al., 1992). Eggs then move into incubators and are transferred to hatchers between day 17-19, depending on the hatchery protocol. Once chicks are hatched, they are pulled into the chick room for processing, which may include gender identification, culling, and vaccination, before they are placed on the broiler farm for grow-out. Several factors can be controlled on the hatchery level to increase the success of the broiler performance, including proper temperature throughout the hatchery, egg handling, incubator and hatcher management, and sanitation (Bell et al., 2002).

The organic matter present on the surface of eggs coming from various breeder farms is a source of contamination in hatcheries. Indeed, the hatchery is a concentration point for various contaminants as all eggs from all breeder farms within a complex come together and mix, thereby potentially spreading pathogens from eggs from one location within the complex to eggs from

another. It is recommended that breeder farms supply the hatchery with clean eggs that were laid in clean nest boxes, but even visibly clean eggs may carry up to 30,000 microbes, and the temperature and humidity present within hatcheries allow for optimal microbial growth and replication (Samburg and Meroz, 1995). The presence of bacteria in poultry hatcheries causes an increased number of contaminated and unhatched eggs, increased prevalence of infection in hatched chicks leading to poor chick quality, and decreased performance of the chicks that do survive (Williams, 1970). The microorganisms present on just one or two hatching eggs can be distributed onto surfaces throughout a hatchery, and then workers or natural air flow can distribute those microorganisms into hatchers contaminating all chicks within the hatcher (Nichols et al., 1967; Sheldon and Brake, 1991). When microbes spread, they can fill the space, even areas that are difficult to clean, making it more vital to control microbial spread before it occurs.

New hatcheries are built with hard surfaces designed to be easily washed and disinfected, and specific cleaning and disinfecting procedures are carried out daily. Organic matter must be removed before proper disinfection can occur, as the organic matter can serve as a protective layer for harmful microbes, which can ultimately reduce the efficacy of sanitizers. Necessary washing steps include removing dried yolk from the floor, drains, and walls in the egg holding room and removing the remnants of exploding or broken eggs inside incubators and in the transfer space (Bell et al., 2002). Once the spaces throughout the hatchery are washed, they can be sanitized, often using a foaming method to increase contact surface area and time. Most poultry hatcheries operating in the US are not new however, and some are converted buildings originally designed as office spaces, manufacturing plants, or warehouses. Utilizing spaces that were not originally designed for their current role as a hatchery presents challenges for cleaning and disinfection and may require novel technologies and products to achieve success.

One of the potential technologies is Dry Hydrogen Peroxide (DHP). DHP is a colorless, odorless molecule created by deconstructing ambient water (humidity) and oxygen present in the surrounding environment and catalyzing these individual molecules to then recombine into gaseous H_2O_2 as they move across a photocatalytic membrane (Lee, 2012). These peroxide molecules are dispersed throughout the space via a fan within the DHP system, the central HVAC, and/or natural air movement within the space. Previous studies in hatchery environments showed that DHP application in an egg cooler led to improvements in hatch and hatch of fertile (Melo et al., 2020). Additional studies show that when DHP is passively supplied to hatchery spaces using multi-stage incubators via ambient air intake, there was an improvement in overall hatchability and hatch of fertile, decreased early dead, decreased contaminated eggs, decreased prevalence of fungus in lungs of hatched chicks, and decreased 3-day mortality (McElreath, 2019). In these studies, reductions in eggshell specific microbial loads were not assessed and could therefore not be credited as the cause of the improved hatchery metrics, but reductions in contaminated eggs and fungal spores in chicks would indicate that cleaner eggs were hatched. Data from other sources suggests that increased contact time of DHP to surfaces reduces the concentration of aerobic bacteria present (Lee, 2012), so the objective of the present study was to determine if the direct, long-term application of DHP within single stage incubators will reduce the microbial load and contamination on the surface of exposed hatching eggs and potentially explain the improved hatchery performance seen in previous research.

MATERIALS AND METHODS

DHP units were installed throughout a commercial broiler hatchery using single-stage HatchTech incubators. Six DHP Units were installed in the egg room, two DHP units each were installed in incubators 1-12, and nine DHP units were installed in the transfer room. The

comparison portion of the trial ran for 9 months, but samples were taken beginning 3 months prior to the trial start date under normal hatchery operation and conditions.

Each week, microbiological sampling swabs were collected from designated areas within the egg holding room, the incubator hallways (both treated and nontreated), and the transfer room for the total duration of the study. Additionally, ATP-bioluminescent swabs were taken on eggshells from predetermined breeder farms upon entry into the egg cooler at the hatchery once a week. Once a month, selected eggs were swabbed again using the ATP-bioluminescent swabs at transfer from both treated and non-treated incubators.

Traditional Swabbing

Swabs from hatchery spaces were collected as follows: the surfaces were thoroughly swabbed with a Puritan® sterile cotton-tipped applicator with a wood handle that is saturated in sterile PBS. The swab was streaked on a tryptic soy agar (TSA – total aerobic microbial counts) plate while evenly rotating the wood handle. All TSA plates were incubated at 37°C for 24 hours and then bacterial colonies were counted by marking each colony while keeping a numeric tally using a manual cell counting device for accuracy. An additional swab was streaked onto a Sabaroud's dextrose agar (SAB-Dex – fungal spore counts) plate and incubated at 37°C for 48 hours and colony counts were completed using the same methodology as above (McElreath, 2019).

ATP Bioluminescent Swabbing

UltraSnap ATP-based swabs (Hygenia, California, US) were used to swab eggshells according to manufacturer's protocol. In short, eggshells were swabbed in a 2x2cm area on the blunt end surface of the eggshell using the pre-moistened UltraSnap swabs. Manufacturer's directions were followed to bring luciferase enzyme in contact with sample collected. The swabs were immediately placed directly into the EnSURE luminometer (Hygenia, California, US) to

obtain a relative light unit (RLU) value. Twenty-five eggs from different trays of five different breeder farms in the egg holding room were selected each week for each swabbing for a total of 1225 swabs over 49 weeks. Eggs were swabbed again at transfer, but only once a month.

RESULTS AND DISCUSSION

Low amounts of bacteria and fungus were present on surfaces within the hatchery throughout the trial. Most surfaces swabbed were concrete and therefore do not provide optimal conditions for microbial growth even in the presence of humidity and warm temperatures. Additionally, the hatchery does employ a cleaning and disinfection protocol in most hatchery spaces and that protocol was not changed during this study. Based on one-way ANOVA, there was however a significant reduction in bacteria recovered from surface swabs on TSA after DHP treatment in the egg room (Table 3.1). This is likely because the walls of the egg room are one location within this hatchery that is not often washed and/or sanitized, and the DHP filling the space served as a form of microbial reduction. Swabs streaked onto SAB-Dex showed no significant differences in fungal growth using one-way ANOVA. Neither bacterial or fungal loads on surfaces in incubator hallways or the transfer room were affected with the application of DHP and remained low throughout the trial.

Based on previous research where DHP was applied in hatchery settings, it was hypothesized that there would be a statistically significant reduction in the level of microbes detected on the surfaces of eggs in the egg cooler. Overall, the opposite was seen when running an unpaired t-test as there was a significant increase in the average RLU value given by the ATP Bioluminometer while DHP was being applied, even though there was a reduction of microbes in the egg room itself (Table 3.1). The peak in the RLU value of eggs swabbed was in July 2022, four weeks after DHP treatment began, and the RLU value slowly decreased over time. This was most

likely due to “sweating” eggs; the HVAC system was not functioning properly in the egg room and the eggs were visibly wet which is known to increase microbial loads. This is supported by the fact that the peak RLU values were occurring during the warmest months of the year, with a decline in the colder months. Additionally, because of the number of hens in production, eggs were only stored in the hatchery egg storage room for ~24 hours before being put in incubators. Past research shows eggs should be in contact with DHP for at least 48-72 hours in the egg cooler for optimal microbial reduction (McElreath, 2019).

It was also hypothesized that the application of DHP directly into single stage incubators would further reduce the bacterial load on eggshells during incubation. Interestingly, there was a large natural reduction in microbial load on eggshells with no treatment. The average RLU value for eggs swabbed in the holding room was 1356 during the treatment portion of the study (Table 3.1), while the average RLU value of eggs swabbed at transfer that received no treatment was 259 (Figure 3.1). This was somewhat expected as there is no food or energy source for microbes on the surface of eggshells if no organic material is present. Eggs swabbed at transfer after 19 days of incubation and treatment with DHP also had a reduction in microbial load when compared to swabs from the same flock taken in the egg room, with comparative RLU values of 1356 and 192, respectively (Table 3.1 and Figure 3.1). When comparing the RLU values of the treated and non-treated eggs at transfer, eggs treated with DHP during incubation had a significantly lower mean RLU value based on an unpaired t-test, indicating a significant reduction in microbial load (Figure 3.1).

The use of dry hydrogen peroxide in a broiler hatchery equipped with single stage incubators had a significant effect on the overall level of microbes present on the surfaces of the egg room and on eggshells at transfer, even though environmental conditions in the egg storage

room negatively impacted the microbial load on eggshells while in the egg room. The effect of DHP on microbial loads on surfaces seen in this study may help explain why DHP use in previous research has shown to improve hatchery parameters such as hatchability and hatch of fertile. Further studies should be conducted to determine if DHP, when applied in all areas of the hatchery, could serve as an alternative to disinfectants currently being used in poultry hatcheries, such as the toxic chemical formaldehyde.

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FIGURES

Table 3.1. Comparison of Average Bacteria Counts on Surfaces and Eggshells Before and After DHP Application (One-way ANOVA).

Egg Room Surfaces (Log10 CFU/in2)	**N**	**Mean**	**SEM**	**P-Value**
Baseline	52	0.16	±0.04	
DHP Treated	132	0.06	±0.02	0.0287
Eggshells (RLU Value)				
Baseline	275	913.327	±67.84	
DHP Treated	822	1356.14	±47.62	<0.0001
Transfer Room Surfaces (Log10 CFU/in2)				
Baseline	52	0.1	±0.03	
DHP Treated	131	0.08	±0.02	0.64

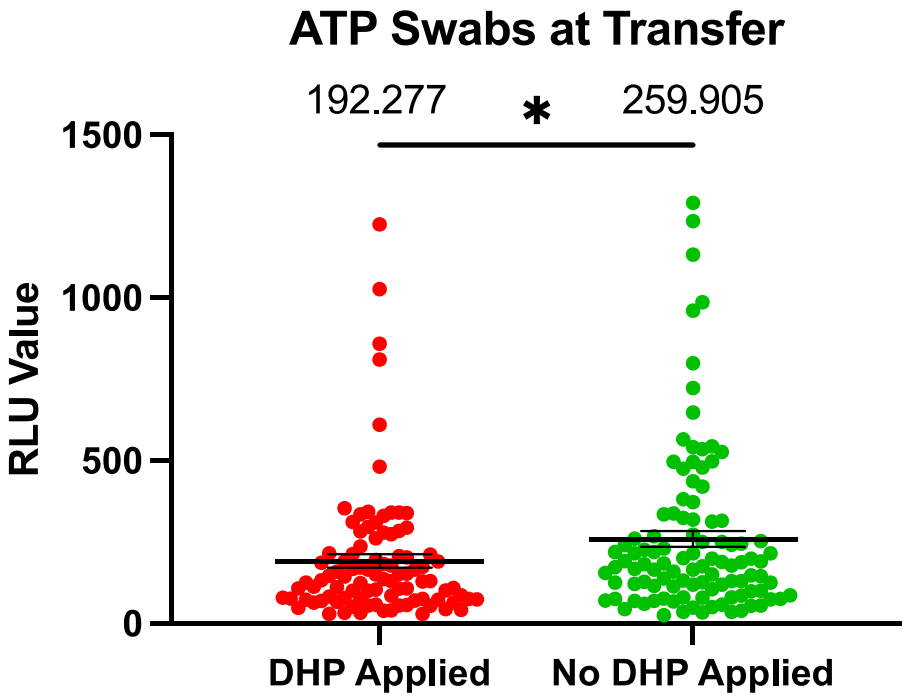

Figure 3.1: Average RLU value of eggs treated with DHP during incubation in comparison to eggs not treated with DHP during incubation (Unpaired t-test).

CHAPTER 4

APPLICATION OF DRY HYDROGEN PEROXIDE IN A SINGLE STAGE POULTRY HATCHERY IMPROVED HATCHABILITY IN TREATED MACHINES AND SHOWED NO DIFFERENCE IN HATCHERY PERFORMANCE COMPARED TO FORMALDEHYDE

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ABSTRACT

Poultry hatcheries serve as the bridge between breeder and broiler farms, and their sanitation is a priority for reducing vertical and horizontal dissemination of microbes and diseases. Daily sanitation practices are necessary for optimal hatchery production, and one of the main chemicals used in poultry hatcheries to mitigate microbial bloom during the pipping and hatching of chicks is formaldehyde. Formaldehyde is a toxic chemical; therefore, an alternative antimicrobial should be researched in the event regulations or consumer opinion lead to its ban. Previous research using Dry Hydrogen Peroxide (DHP) in a multi-stage hatchery led to increased hatchery performance, but in that study DHP was added to the routine sanitation program that also included formaldehyde fogging. The objective of this study was to determine if DHP, when supplied directly into single stage incubators and hatchers, could serve as an alternative to formaldehyde that is safer for both chicks and employees. DHP units were installed throughout the egg room, inside 1/3 of the incubators, transfer room, and in the mezzanine above half the hatchers of a commercial broiler hatchery equipped with single-stage Hatchtech incubators. Baseline data was collected for three months, DHP systems were turned on and used in addition to formaldehyde for 5 months, and then formaldehyde was turned off in the hatchers being treated with DHP for four more months of data collection. A 3% increase in hatchability compared to baseline was observed among the group of eggs and chicks that were treated with DHP throughout incubation and hatch, which was the highest percent increase among all the groups. Additionally, when comparing complete DHP treatment to traditional use of formaldehyde, there was no difference in hatchability. These data show that DHP, in this hatchery, could serve as an alternative to formaldehyde treatment.

INTRODUCTION

Poultry hatcheries play a pivotal role in the production of poultry products. These facilities are responsible for gathering eggs from breeder farms, incubating, vaccinating, hatching the chicks, and taking them to designated broiler farms for grow out. With eggs coming in from multiple locations and chicks going out to multiple other locations, the hatchery is a concentration point and is vital for the control of microbes within the chain of poultry production (Osman et al., 2018). Directly upon lay, eggs are not contaminated. As eggs cool, a vacuum is formed, drawing in any liquid or bacteria that may be present on the surface of the egg, which could have detrimental effects on the cleanliness of the hatchery (Cox et al., 2000). The pathogens the eggs encounter subsequently after lay can be transported via the egg interior or eggshell into the hatchery and, based on previous studies, the higher the level of detectable microbes, the lower the overall hatchability (Mayes and Takeballi, 1983; Quarles et al., 1970). In addition to poor hatchability, chicks that hatch within a bacteria-filled environment are more likely to test positive for aspergillus in the respiratory tract or infection of the yolk sac; both causing increased 3- and/or 7-day mortality (Chute and Gershman, 1961). For optimal hatchery performance, microbial monitoring and sanitation practices should be a daily focus.

There are a variety of methods of sanitation and chemicals used throughout poultry hatcheries. Hatchery surfaces, setters, and hatchers can be washed with a universal cleaner, but first, organic material must be removed (Ledoux, 2006). If not, it can interfere with efficacy of the disinfectant. The universal cleaner used in hatcheries should have the following characteristics: wetting to decrease surface tension, dispersion of dirt particles, emulsifying properties that allows for the suspension of oils and fats, suspension that floats and carries away the dirt particles, and sequestering that dissolves salts (Ledoux, 2006). It is also recommended that, once a month, an

acid soap is introduced to the sanitation procedure to ensure there is no detergent build up. Once soap is applied and rinsed, the space should be allowed time to dry to ensure the disinfectant is not diluted. After dry time, a disinfectant is applied via foam to increase contact time and adhesion to surfaces. In 1959, chemist Herbert Sinner identified four factors critical to achieving an optimal clean: temperature, time, chemistry, and mechanical force (Sinner, 1960). These four factors can be implemented in the hatchery setting for optimized use of employee time and chemical efficacy during regular sanitation.

In addition to sanitation, it is typical to see the application of an antimicrobial during the hatch process to mitigate the bloom of bacteria that is associated with the pipping and hatching of chicks (Pernot, 1908). The warm temperature of hatch cabinets combined with the nutrients within the egg supports replication of bacteria (Selby et al., 2023). One of the more common chemicals applied in hatchers is formaldehyde, which is known for its antimicrobial properties. Formaldehyde works by targeting proteins and alkylating their amino and sulfhydryl groups (CDC, 2016), thereby killing the organism it comes in contact with. Though formaldehyde is a high-level disinfectant, its use has been limited because it has long been thought of as a toxic chemical dangerous to human health. Upon initial exposure, the colorless liquid can cause an immediate immune system response of irritation and itching of the eyes, nose, and throat (OSHA). Previous research shows that, in addition to being hazardous to employee health, formaldehyde can cause damage to the epithelial lining of the respiratory tract of chicks (Sander et al., 1995). Because of the hazardous effects of formaldehyde, and the risk of heightened EPA regulation, a viable alternative is needed for poultry hatchery success in the event formaldehyde is banned.

Common alternatives to formaldehyde in poultry hatcheries include ozone, ultraviolet light, and liquid hydrogen peroxide. They each have bactericidal, virucidal, fungicidal, and

sporicidal properties and have been reported as a successful alternative to formaldehyde in the poultry hatchery setting (Coufal et al., 2003; Sander and Wilson, 1999; Whistler and Sheldon, 1989), though each product also has drawbacks. In addition to these chemistries, a new proprietary technology called Dry Hydrogen Peroxide (DHP) could also potentially serve as a viable alternative to formaldehyde. The DHP units produce peroxide by pulling in ambient oxygen (O_2) and humidity (H_2O) from the environment, separating the molecules into oxygen and hydrogen as they move across a photocatalytic membrane, and then catalyzing a new reaction to form H_2O_2 in a completely gas phase (Lee, 2012). Because the DHP machine emits gas H_2O_2 the potential for corrosion is limited, and extended exposure of DHP to both chicks and employees has not been noted to cause health concerns as it is produced at extremely low concentrations (10-25 ppb), well below OSHA recommended levels. Though the use of DHP in hatchery settings is a relatively new area of research, there have been many studies published where DHP machines are used in hospital, nursing home, and other healthcare settings as an added level of sanitation and for viral/microbial reduction (Huang et al., 2021; Ramirez et al., 2021; Sanguinet and Edmiston, 2021). Many of the studies published in healthcare facilities refer to DHP as a no-touch disinfection system that is an effective addition to manual cleaning. There are similarities between DHP research in poultry facilities when compared to healthcare facilities, such as the fact that DHP has been added in addition to the regular cleaning and disinfecting protocol that the hatchery has previously implemented. One difference between the hatcheries and hospitals, is that healthcare facilities tend to have less organic material present throughout, whereas hatcheries may have more dust, chick fluff, eggshell debris and more.

Despite the level of organic material present, previous research using DHP in poultry hatcheries has shown a successful microbial reduction during egg storage, leading to an increased

hatchability and decreased prevalence of fungus in lungs (Melo et al., 2020). DHP, however, has not been directly compared to formaldehyde in a commercial setting. Therefore, the purpose of this study was to determine if eggs exposed to DHP during storage, incubation in single stage incubators, at transfer, and while in the hatchers will decrease microbial load and increase hatchability when compared to the traditional use of formaldehyde.

MATERIALS AND METHODS

Dry Hydrogen Peroxide (DHP) units were installed throughout a commercial broiler hatchery with single-stage Hatchtech incubators (Figure 4.1). DHP Units were installed throughout the entire egg room, in one of the three incubator hallways and throughout the entire transfer area. For three months, baseline samples were taken prior to the application of DHP. After three months, phase one of the trial began where DHP was turned on and was applied throughout the hatchery in addition to the hatchery's current formaldehyde drop schedule. After five months of data collection, phase two data collection began, where the DHP remained on throughout the hatchery, and the formaldehyde was turned off in hatchers that were being treated with DHP, so direct hatchery treatment comparisons could be made. Phase two data collection continued for four months. In addition to this data, historical hatchery performance for the machines used in this study for 12 months prior to our data collection was provided by the hatchery. The following samples were taken beginning during baseline sampling and continued through the end of phase two.

Each week, traditional microbiological sampling swabs were collected from the hatch hallways (both treated and nontreated) for the total duration of the study.

Once a month, hatch fluff microbial counts and chick quality evaluations (microbial) were performed. Daily hatchery parameters, such as hatchability, were collected, and 3-day mortality

from select farms was assessed. Three-day mortality was also investigated via the Georgia Poultry Laboratory Network to determine the cause, i.e., infection versus hatchery/incubation conditions.

Swabs from hatchery hallway spaces were collected as follows: the surfaces were thoroughly swabbed with a Puritan® sterile cotton-tipped applicator with a wood handle that is saturated in sterile PBS. The swab was streaked on a tryptic soy agar (TSA – total aerobic microbial counts) plate while evenly rotating the wood handle. All TSA plates were incubated at 37°C for 24 hours and then bacterial colonies were counted by marking each colony while keeping a numeric tally using a manual cell counting device for accuracy. Additionally, the swab was streaked onto a Sabaroud's dextrose agar (SAB-Dex – fungal spore counts) plate and incubated at 37°C for 48 hours for fungal colony counts. Raw colony counts were log transformed and are reported as Log10 CFU/in^2.

One day prior to pulling chicks from a hatcher, one plate each of Maconkey agar (Mac), Tryptic Soy Agar (TSA), and Sab-Dex agar were placed into the hatcher for a 5-minute exposure to hatchling fluff. After five minutes, the fluff plates were removed and incubated at 37°C for 24 or 48 hours depending on agar requirement, and bacterial or fungal colonies were counted.

Traditional performance parameters from the hatchery were collected throughout the duration of the trial and analyzed on a daily and weekly basis. The trial consisted of three phases, baseline data collection, phase one and phase two, and during each phase there were four different treatment groups (Figure 4.3):

Baseline:

1. Group 1 Treated/Treated (T/T): Eggs that move throughout DHP treated incubators 1-12 and DHP treated hatchers 1-9. During baseline, these eggs received no treatment in incubators and formaldehyde treatment in hatchers.

2. Group 2 Untreated/Treated (U/T): Eggs that move through non-treated incubators 13-36 and DHP treated hatchers 1-9. During baseline, these eggs received no treatment in incubators and formaldehyde treatment in hatchers.
3. Group 3 Treated/Untreated (T/U): Eggs that move through DHP treated incubators 1-12 and formaldehyde treated hatchers 10-18. During baseline, these eggs received no treatment in incubators and formaldehyde treatment in hatchers.
4. Group 4 Untreated/Untreated (U/U): Eggs that move through non-treated incubators 13-36 and formaldehyde treated hatchers 10-18. During baseline, these eggs received no treatment in incubators and formaldehyde treatment in hatchers.

Phase One:

1. Group 1 Treated/Treated (T/T): Eggs that move throughout DHP treated incubators 1-12 and DHP treated hatchers 1-9. During phase one, these eggs received DHP treatment in incubators and formaldehyde and DHP treatment in hatchers.
2. Group 2 Untreated/Treated (U/T): Eggs that move through non-treated incubators 13-36 and DHP treated hatchers 1-9. During phase one, these eggs received no treatment in incubators and formaldehyde and DHP treatment in hatchers.
3. Group 3 Treated/Untreated (T/U): Eggs that move through DHP treated incubators 1-12 and formaldehyde treated hatchers 10-18. During phase one, these eggs received DHP treatment in incubators and formaldehyde treatment in hatchers.
4. Group 4 Untreated/Untreated (U/U): Eggs that move through non-treated incubators 13-36 and formaldehyde treated hatchers 10-18. During phase one, these eggs received no treatment in incubators and formaldehyde treatment in hatchers.

Phase Two:

1. Group 1 Treated/Treated (T/T): Eggs that move throughout DHP treated incubators 1-12 and DHP treated hatchers 1-9. During phase two, these eggs received DHP treatment in incubators and DHP treatment in hatchers.
2. Group 2 Untreated/Treated (U/T): Eggs that move through non-treated incubators 13-36 and DHP treated hatchers 1-9. During phase two, these eggs received no treatment in incubators and DHP treatment in hatchers.
3. Group 3 Treated/Untreated (T/U): Eggs that move through DHP treated incubators 1-12 and formaldehyde treated hatchers 10-18. During phase two, these eggs received DHP treatment in incubators and formaldehyde treatment in hatchers.
4. Group 4 Untreated/Untreated (U/U): Eggs that move through non-treated incubators 13-36 and formaldehyde treated hatchers 10-18. During phase two, these eggs received no treatment in incubators and formaldehyde treatment in hatchers.

Treatment code: (incubator/hatcher); T (treated with DHP); U (untreated/formaldehyde only).

All eggs were treated with DHP in the egg room and during transfer during phase one and phase two as there was only one egg storage room in the hatchery and every egg had to move through the transfer room for in-ovo vaccination via Embrex. To limit outliers based on natural decline in flock performance in correlation to age, all data presented is from breeder flocks less than 40 weeks of age, and U/T data was not included because it is not the recommended protocol for use of DHP in hatchery settings.

RESULTS

Surface Swabs

Microbial load present on the surfaces of the treated and non-treated hatch hallways remained low throughout the trial with no significant differences, as indicated by surface swabs on both TSA and Sab-Dex agar and one-way ANOVA (Table 4.1).

Fluff Counts

Quantitatively, throughout the duration of the trial, average fluff counts were too numerous to count (TNTC, >250 colonies) on all three types of agars for all treatment combinations. Qualitatively, when comparing the hatchers that were treated with DHP-only (Phase 2) to the same hatchers when they were being treated with formaldehyde-only (Baseline Phase), a visual decrease in the lawn of bacteria present on fluff samples was noticed (Figure 4.2), however the plates were still TNTC.

Hatchability

Hatchability of the eggs from flocks <40 weeks of age that were treated with DHP during egg storage, incubation, at transfer, and during hatch (T/T), had an average hatchability of 85.88%, compared to baseline data hatchability of the same machines at 82.77%, a significant increase of 3.11% in overall hatch based on one-way ANOVA. Hatchability of the eggs from flocks <40 weeks that were treated with DHP during egg storage, during incubation, and at transfer, but not during hatch (T/U), had an average hatchability before treatment of 84.77%. During phase one and two, these eggs had an average hatchability of 82.95% and 83.47%, respectively. Eggs that were not treated with DHP during incubation or during hatch (U/U) had an average baseline hatchability of 86.08%, during phase one average hatchability was 84.6%, and during phase two, 86.1%. (Table 2)

3-day Mortality

Analysis of 3-day mortality using one-way ANOVA indicated a significant decrease from baseline to phase one, but no difference when comparing baseline to phase two data of eggs treated with DHP during incubation and hatch. Eggs not treated with DHP during incubation or hatch had a significantly lower 3-day mortality as the trial progressed. (Table 4.2)

Necropsy of 3-day Mortality

Necropsy of 3-day mortality showed 57.5% chicks positive with yolk sac infection among the T/T group and 64% positive among the U/U group. Infection found in the heart was 22.5% in the T/T group and 7.5% in the U/U group. The same bacteria were found in each group, control and experimental. (Table 4.3)

DISCUSSION

Because surfaces within the hatchery are concrete, it was expected that surface bacteria and fungus counts would be low throughout the duration of the trial, as concrete does not serve as an optimal growth medium for microbes. This was seen in our study, with no differences in these parameters measured between the DHP treated and non-DHP treated hatchery locations. Fluff counts have long been a measurement used for accessing microbial load within hatchers at the time of hatch. Fluff counts throughout the trial were TNTC, further proving the bacterial load present in hatchers as chicks hatch. Though raw counts were TNTC, visual differences could be noted by the plate coverage of the lawn of microbes, with plates sampled from DHP treated locations having a less complete overgrowth compared to other locations. We believe this could be due to the application of DHP rather than formaldehyde, but it must be noted that most plate counts were higher than industry standard regardless of treatment. In hindsight, it may have been better to

collect the fluff in a buffered solution and complete serial dilutions to determine exact microbial load within fluff, but we did not anticipate fluff counts to be TNTC throughout the trial.

The most significant piece of data collected throughout the trial was hatchability. When looking at baseline data collection (February to May), the incubators where DHP was applied once treatment began have an either 2 or 4% lower hatch rate than incubators that were not scheduled to receive DHP treatment. The only physical difference between these machines is egg set capacity. The incubators that received treatment hold 12 incubator racks, which hold 7040 eggs each, for a total of 84,480 eggs set inside. The incubators that were not treated with DHP once phase 1 began hold 18 incubator racks, which also hold 7040 eggs each, for a total of 126,720 eggs set per machine. Both machines hold trolleys that are 16 levels high, with five egg flats per layer. Single stage HatchTech machines are designed to flow air evenly from right to left, no matter the size of the incubator using patented laminar airflow technology (HatchTech). Though air flow is controlled via radiators that are cooled with water, the differing size of incubators could potentially cause hot or cold spots that could affect hatchability. Ideally, all machines would hatch at the same level to reduce variability, but this trial was performed in a commercial hatchery in industry conditions and this type of variability is expected.

As the trial moved into phase one, there was a numerical decrease in hatchability across the hatchery for all machines regardless of treatment. In hatcheries located in the Southeastern US, it is commonly thought that breeder and therefore hatchery performance may decline because of warm weather and humidity, leading to decreased mating/fertility (Bell et al., 2002). In addition, during some of the warmer months of the present trial, there was an increased temperature and humidity recorded during a time when the egg cooler was improperly maintaining the optimal egg room environment. This caused eggs to sweat, which also caused an increase in overall level of

microbes detected on eggshell surfaces using the ATP bioluminometer (Chapter 3). Interestingly, only the T/T group (DHP treatment in incubators and DHP and formaldehyde treatment in hatchers) had a non-significant decline in hatchability during Phase 1, while the T/U group (DHP treatment in incubators with formaldehyde in hatchers) and the U/U group (no treatment in incubators and only formaldehyde in hatchers) had a significant reduction in hatch.

During phase two, hatchability across all treatments increased from the average hatchability during phase one. For the T/U group (DHP treatment in incubators with formaldehyde in hatchers), even though hatchability did increase compared to phase 1, it did not reach the level seen in baseline collection and was therefore down during the overall study. Hatchability in the U/U group (no treatment in incubators and only formaldehyde in hatchers) also increased in phase 2 compared to phase 1, but only back to the same level seen in baseline collection. The only hatchability that significantly increased over baseline in phase 2 was the T/T group (DHP treatment in incubators and hatchers). And though the hatchability of the U/U eggs remained numerically higher than other treatment groups throughout the trial, hatch of T/T during phase two was not significantly different from the hatch of the U/U group, even though baseline hatch data from these machines was significantly different.

The 3-day mortality of all groups decreased throughout phase one, which was an interesting finding. Because hatch decreased, leading to the assumption that normal hatchery success decreased, one might expect to see an increase in 3-day mortality. The opposite could be true, however, in that the decline in hatch was due to poor or unthrifty chicks remaining in eggs unhatched, which would lead to a decrease in mortality as these chicks would ultimately succumb if placed in the field. As the trial moved into phase two, 3-day mortality increased in two groups (T/T and T/U) with the increase in hatchability. Only in the U/U group did 3-day mortality continue

to decline, and it is unclear why this may have been. There was no clear difference in bacterial loads or chicks positive for bacteria in the health assessment. Several factors contribute to the variation in 3-day mortality, such as chick processing and correct entry of mortality on the farm level, so further investigation into this metric is warranted.

Overall, utilizing DHP instead of formaldehyde did not result in a reduction in hatchery performance, and most cleanliness metrics (i.e. surface swabs) were no different between the treated and non-treated groups. This is interesting as previous research in a commercial multi-stage hatchery showed the same positive results in overall hatchability, indicating that we may be missing the most significant mode of action of DHP. The overall purpose of the trial was not to test if DHP was a better antimicrobial when compared to formaldehyde, but to see if it was as successful. According to the visual analyzation of the fluff counts, DHP may have been better at reducing microbial load present in fluff. Based on hatchability, which has proven in past DHP trials to be a good measure of overall hatchery cleanliness, DHP may work as well as formaldehyde. The fact that hatchability among the DHP-treated group (T/T) was similar to formaldehyde-only (U/U) hatchability, paired with the fact that DHP is safe for hatchery employees and chicks, DHP may be better than formaldehyde when used as an antimicrobial in the hatchery.

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FIGURES

Table 4.1. Bacterial and Fungal Surface Swabs Microbial Results using One-way ANOVA

	Bacterial Counts		Fungal Counts	
DHP Treated Hatch Hall 1 (Log10 CFU/in2)	**N**	**Mean±SEM**	**N**	**Mean±SEM**
Baseline	129	0.22±0.04	133	0.07±0.02
Phase 1(DHP+Formaldehyde)	54	0.13±0.02	51	0.04±0.02
Phase 2 (DHP Only)	60	0.21±0.05	55	0.08±0.03
Non-treated Hatch Hall 4 (Log10 CFU/in2)				
Baseline	129	0.28±0.04	119	0.06±0.02
Phase 1 (Formaldehyde Only)	56	0.12±0.04	54	0.09±0.04
Phase 2 (Formaldehyde Only)	59	0.23±0.04	57	0.06±0.02

Table 4.2. Hatchability and 3-Day Mortality Data

	Baseline ± SEM	**Phase 1 ± SEM**	**Phase 2 ± SEM**
Hatchability (%)			
T/T	82.77^{a1} ±0.28	82.51^{a1} ±0.41	85.88^{b1} ±0.30
T/U	84.77^{a2} ±0.22	82.95^{b1} ±0.35	83.47^{bc2} ±0.58
U/U	86.08^{a3} ±0.13	84.6^{b2} ±0.35	86.1^{ac1} ±0.23
3-day Mortality (%)			
T/T	0.94^{a1} ±0.06	0.63^{b1} ±0.03	1.01^{ac1} ±0.11
T/U	0.96^{a1} ±0.09	0.68^{a12} ±0.04	0.99^{ab1} ±0.11
U/U	1.17^{a2} ±0.04	0.84^{b2} ±0.02	0.79^{bc2} ±0.03

Different superscript letters within a row indicates significant differences based on one-way ANOVA. Different superscript numbers within a column indicates significant differences based on one-way ANOVA. All analysis completed at the 5% level of significance.

Table 4.3. Necropsy results of 3-day mortality in groups treated with DHP or formaldehyde.

Treatment Group	**Yolk Sac + (%)**	**Heart + (%)**	**Bacteria Found**
T/T	57.5	22.5	E. coli, Proteus, Pseudamonas, Enterococcus
U/U	64	7.5	E. coli, Proteus, Pseudamonas, Enterococcus

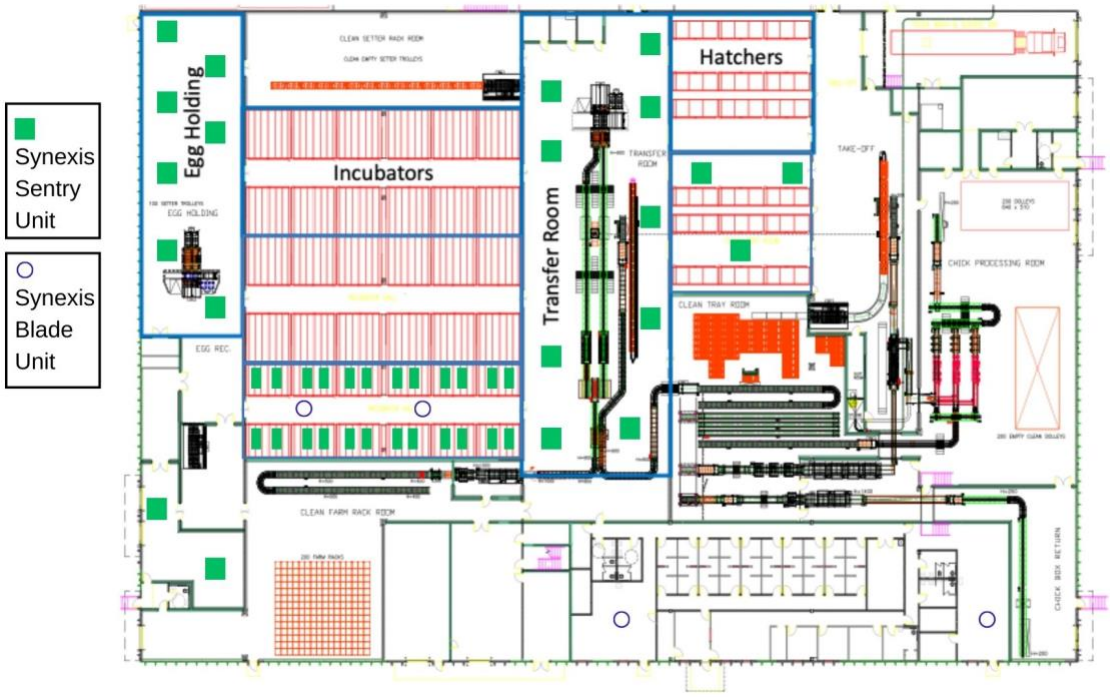

Figure 4.1: Layout of the commercial broiler hatchery used in this study and location of DHP units. Six DHP Sentry (standalone) units were installed in the egg room, two Sentry units per single stage incubator on incubator hall 1, and nine Sentry units in the transfer room. Two DHP Blade units (inline in HVAC ductwork) were installed to supply the incubator hallway. The mezzanine above the hatchers had three Sentry units installed so all fresh air intake would pull DHP-treated air. There is a concrete wall in the mezzanine separating the space above hatch halls one and two from the space above hatch halls three and four.

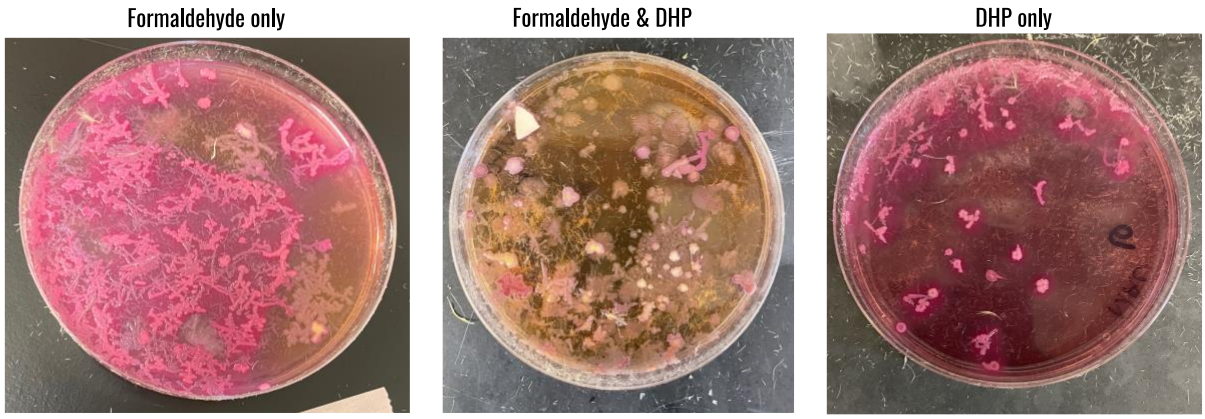

Figure 4.2: Fluff counts from hatcher number six during different antimicrobial treatments, all plated on MacConkey Agar.

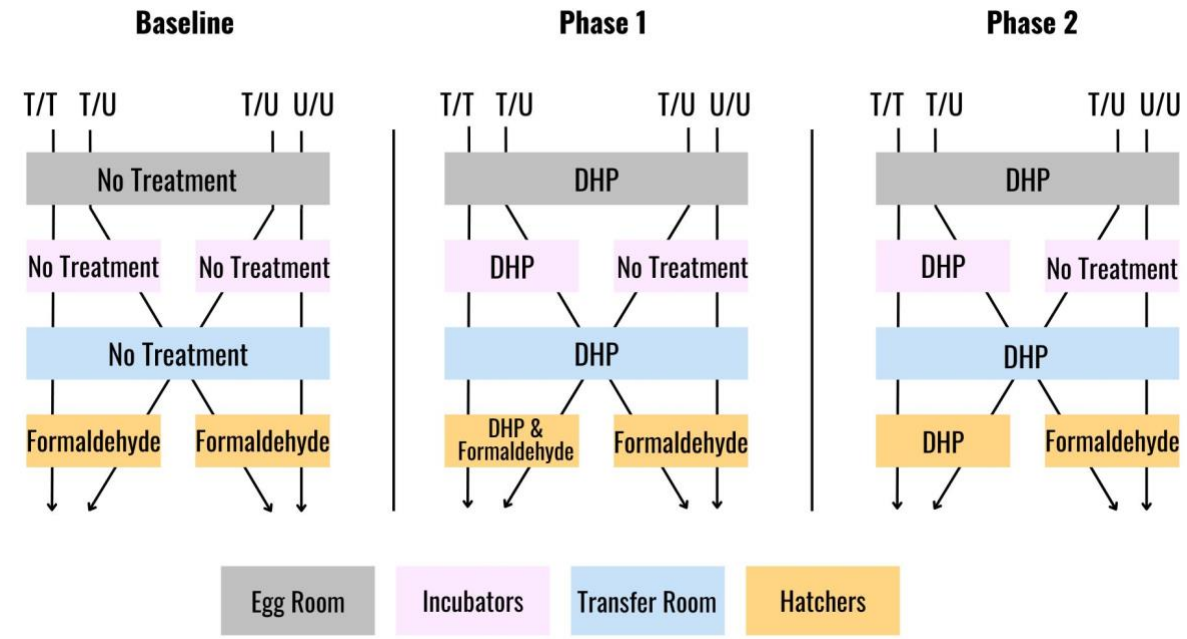

Figure 4.3: The movement of each group of eggs through different machines during each phase of the 12-month trial.

CHAPTER 5

SUMMARY AND CONCLUSIONS

Poultry production in the United States continues to increase, however hatchability has been below 82% since 2018. The integration of the poultry industry is unique, and hatcheries serve as a pivotal point in the process. Eggs from every breeder farm within the complex gather in the hatchery, are set, vaccinated *in-ovo*, and hatched. Upon hatching, the chicks are processed, sorted, and taken to a broiler farm for grow out. Though incubation started out as an art, research has allowed it to become more scientific, showing hatchery employees the parameters necessary for optimal performance. Yes, the number of chicks produced reflects the overall productivity of a hatchery, but so does the health of those chicks in the field. When chicks pip and hatch in hatch cabinets that are full of bacteria and mold, they are likely to develop infection that reflects negatively on the performance of the hatchery. Therefore, proper cleaning and disinfection procedure in the hatchery become a vital step in the process of hatching chicks.

Removal of organic material, scrubbing with a soap, rinsing, and disinfection are popular steps of cleaning and disinfecting in each area of the hatchery. In addition, an antimicrobial such as formaldehyde is incrementally applied in the hatchers throughout the duration of the hatch window, beginning the application before chicks begin to hatch and turning the formaldehyde off 12 hours prior to pull, to prevent a negative health effect on hatchery employees. But because of the toxic nature of formaldehyde, finding an alternative to mitigate microbes that works in poultry hatcheries, while maintaining the integrity of equipment, has been a challenge for many years. Hydrogen peroxide, UV light, ozone, and others have been historically used as alternatives to

formaldehyde, with hydrogen peroxide being the most popular. The downside to hydrogen peroxide is that in its aqueous form, it can be quite corrosive to the metal components of hatch cabinets. The creation of Dry Hydrogen Peroxide (DHP), which is H_2O_2 in a completely gas phase, could be instrumental for the poultry industry if microbial reduction is equal to that of formaldehyde use.

Previous research in our laboratory suggests the use of DHP in poultry hatcheries could mitigate the microbial load present on surfaces and the microbial load that is present during the bloom of chick fluff during pipping. That work also showed that DHP application can reduce the load of microbes on eggshells, which is where the largest number of microbes present in the hatchery enter. Use of DHP in incremental stages has had positive impacts on cleanliness and hatchery performance, but the question still remained about its effectiveness compared to an industry standard such as formaldehyde.

Therefore, this 12 month project had two main aims: 1) to determine if the microbial load on the egg shell surface, when tested via ATP bioluminescence, was reduced when eggs were placed in single stage incubators and exposed to dry hydrogen peroxide during the entire 19 day incubation period; and 2) to determine if DHP treatment through the entire hatchery, including during incubation, could serve as an alternative to the toxic chemical formaldehyde that is currently one of the most popular disinfectants. Because this is the first year-long commercial trial where DHP was supplied directly into single stage incubators and throughout the entire hatchery, the results could have large impacts on the way poultry is hatched throughout the US.

Throughout the duration of this commercial hatchery trial, there were many different hatchery variables that had to be accounted for, such as improperly high temperatures and humidity in the egg cooler during the warmer months, the implementation of an acid soap rotation once a

month to the normal cleaning and disinfecting procedure completed throughout the hatchery, and the effects of breeder flock age on hatchability. Nonetheless, DHP still improved hatchability by 3% in the machines that hatched chicks that were treated with DHP in the egg room, during incubation, at transfer, and in the hatch cabinets compared to the operation of those same machines prior to DHP use. Based on current industry hatchability data, if hatch was increased by 1% in a complex that hatches 5,365,618 chicks per month (industry average), the company would create a monthly dollar savings of $31,296 for the hatchery and add an additional $12,700 profit per pound at the processing plant (personal communication, Mike Donahue, AgriStats). For the hatchery where our study took place, a 3% increase in hatch would result in ~$91,000 in savings and an additional ~$38,000 profit per pound at the processing plant. In addition to hatchability, DHP did have a significant impact on the reduction of microbes present on the eggshell at transfer. This study also confirmed that incubation naturally reduces the level of microbes, but DHP made that microbial reduction even greater. If microbe level on the surface of the egg is reduced as they are placed into hatchers, microbes could also be lower during the hatch process, resulting in an overall healthier chick.

Future research regarding the use of DHP in poultry hatchery settings would be useful to further confirm its efficacy. Because there are many different machine types, it would be interesting to see if DHP improves hatch in other machine types besides single stage HatchTech.