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Evaluation of the Effects of Formaldehyde on Growth Parameters of Broiler Chicks

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Evaluation of the Effects of Formaldehyde on Growth Parameters of Broiler Chicks

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Abstract

Formaldehyde has been used as a disinfectant in poultry hatching cabinets to aid in controlling key pathogenic organisms, such as *Salmonella* and *Escherichia coli* (E.coli). There is some evidence that prolonged exposure of chicks to formaldehyde can reduce tracheal ciliary function and thus reasons to believe that exposure to formaldehyde, in the absence of pathogen challenge, may reduce chick vitality. It has been found that elevated temperatures during incubation may adversely affect body weights of broiler chicks, as well as post-hatch environmental heat stress on performance in poultry. Post-hatch environmental heat stress has been shown to impact performance in poultry. The objective of these experiments was to analyze the effect of formaldehyde treatment or heat stress in the hatch cabinet on body weights (BWs) and body weight gain (BWG). In Exp. 1, 18 day embryos were randomly assigned to either a control, non-treated hatcher or a formaldehyde treated hatcher, where formaldehyde was applied to achieve 1-2 ppm during the hatch period. Chicks from each group were weighed and neck tagged with discrete numbers, and were then co-mingled post-hatch for determination of BW and BWG days 0, 7, and 10. At day 7 and day 10, we measured significantly (p<0.05) lower BW and BWG for chicks in the formaldehyde group as compared to control, non-treated chicks suggesting that this level of formaldehyde exposure in the hatching environment (1-2 ppm) may negatively impact early performance. In Exp. 2, 18 day embryos were randomly assigned to a control, non-treated hatcher, formaldehyde- treated hatcher (1-2 ppm), or heat stress (37.8˚C) treated hatcher. At day 10, BWG was significantly (p<0.05) lower for the formaldehyde and heat stressed treated groups than the control, non-treated group. Based on these results, formaldehyde treatment (1-2 ppm) or heat stress (37.8˚C) in the hatching environment may negatively

influence early broiler performance. While hatching cabinet treatment with formaldehyde under commercial conditions has known beneficial effects on controlling microbial blooms during late hatch, and has been associated with improved livability, formaldehyde treatment, or heat stress, may also be limiting performance potential of broiler chicks.

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Special thanks to my family. The love, support and laughs have helped me get this far and I am forever grateful for all of the phone calls and time sacrificed to help me through this journey. Thank you so much for everything that you do and all that you are.

Dedication

This thesis is dedicated to my mother Mrs. Lyna Johnson and my family. You've been by my side and helped me in ways unimaginable. Thank you for all of the love and support given to help me achieve this endeavor in my life.

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Chapter 1

Introduction

Formaldehyde has been known as a colorless, noxious gas which is soluble in water and used primarily to make building materials and household products (National Cancer Institute 2011; National Toxicology Program 2011). Though its uses vary between construction, automotive, healthcare and clothing, formaldehyde is also used in agriculture. Formaldehyde has been commonly used within the poultry industry. The ability to control microbial blooms, including opportunistic pathogens, during the hatching period is essential for production of quality chicks (Graham, 2015). Low level formaldehyde environmental treatment during hatching is often used as an aid in the control of *Salmonella, E.coli and Pseudomonas* (Sander et al., 1995; Zulkifli et al., 1999, Hayretadağ and Kolankaya, 2006; Cadirci, 2009). Nevertheless, formaldehyde exposure of chicks has been shown to potentially reduce tracheal ciliary function which may predispose to respiratory problems post-hatch (Sander et al., 1995; Zulkifli et al.1999, Hayretadağ and Kolankaya, 2006). Exposure to formaldehyde has also resulted in swelling of the mitochondria, vacuolization, and a significant increase of mucus production (Sander et al.,1995; Zulkifli el al., 1999; Hayretadağ and Kolankaya, 2006). Moreover, formaldehyde exposure causes irritation of the eyes and throat of humans (National Cancer Institute, 2011) and is considered a potential carcinogen (National Toxicology Program, 2011; Agency for Toxic Substances and Disease Registry, 2011). It is an effective disinfectant used in hatcheries, chick transport and sometimes farms to reduce the load of pathogens present, including viruses, bacteria and mold spores (Zulkifli et al., 1999; Hayretdağ and Kolankaya, 2006). Formaldehyde was first reported in 1859 by Alexander Mikhailovich Butlerov when he attempted to synthesize methylene glycol (Formacare, 2014). However, formaldehyde wasn't actually identified until 1868 by, a professor of Chemistry and director of the laboratory of the

University of Berlin, August Wilhelm von Hofmann (Formacare, 2014). His goal was to establish the identity and structure of what we know today as formaldehyde. Cadirci (2008) recognized the first reported use of formaldehyde as a disinfectant was in 1891. Pernot (1908) was the first investigator to demonstrate the use of formaldehyde fumigation of eggs and incubators as a means of controlling poultry diseases.

Although formaldehyde has been known to be useful and very effective, it is a toxic gas, and has been recognized as a human carcinogen by the EPA ((Zulkifli et al., 1999; Hayretdağ and Kolankaya, 2006; Agency for Toxic Substances and Disease Registry, 2011). Nevertheless, this compound is very effective as a disinfectant within the hatching cabinet and is commonly used in commercial hatcheries in some countries at present. Several potential alternatives for formaldehyde have been evaluated and studied by several researchers. One potential alternative to the use of formaldehyde may be the application of beneficial bacteria that can compete with detrimental microflora blooms during hatch or within the gastrointestinal tract of neonatal chicks.

Recently, Graham and co-workers (2018) have demonstrated that selected spores of the genus *Bacillus*, known to produce antimicrobial peptides, could be applied to commercial hatching cabinets and reduce the Gram negative bacterial bloom associated with late hatching. Moreover, Graham (2015) demonstrated that combining these Bacillus spores with selected lyophilized probiotic lactic acid bacteria and demonstrated enhanced commercial performance in comparison with conventional formaldehyde treatment during hatching. Beneficial bacteria such as probiotics may be very important as pioneer colonizers, and replacing the non-commensal organisms in commercial hatching cabinets with beneficial bacteria could have important

benefits (Pedroso et al., 2016). Other potential alternatives to formaldehyde which have been evaluated are discussed below.

Literature Review

Formaldehyde

Formaldehyde is produced in small quantities naturally within vertebrate animals, including humans and chickens (Agency for Toxic Substances & Disease Registry, 2011; American Cancer Society, 2014). Formaldehyde is used in the production of fertilizer, paper, plywood, and urea- formaldehyde resins. It is also used as a preservative in some food and in many products used around the house, such as antiseptics, medicines, cosmetics (Agency for Toxic Substances and Disease Registry, 2011; National Cancer Institute,2011) laboratories, mortuaries and hair smoothing and straightening products (National Toxicology Program,2011;National Cancer Institute,2011)

Formaldehyde is readily soluble in water (Cadirci, 2008) and is commonly distributed as a 37% solution in water. This colorless liquid, with a pungent and irritating odor, is known as formalin. By definition, formalin contains 37-50% formaldehyde by mass, (PubChem, 2004) 1015% methanol and 53% water (Pediaa, 2016). Diluted formalin is also used as a disinfectant and to preserve biological specimens and controlling microbial loads within hatch cabinets (PubChem, 2004; Graham and co-workers, 2015). While formaldehyde is clearly a valuable chemical with a number of uses, formaldehyde is a toxic chemical (Cardirci, 2008; National Toxicology Program 2011) that could have mild to fatal consequences based on the level of exposure. Although the smell of this substance is greatly irritating, hazardous levels of the

substance may be reached without any odor. Concentrations of formaldehyde at as little as 0.1 ppm may be enough to cause irritation to the eyes and throat (Agency for Toxic Substances and Disease Registry, 2011; National Cancer Institute, 2011). National Cancer Institute (2011) reports that the U.S. Environmental Protection Agency (EPA) classified formaldehyde as a human carcinogen (Merk and Speit, 1998; Yildirim, 2003) in 1987. Formaldehyde was reportedly found to be linked to nasal cancer in rats that become exposed to this gas (National Cancer Institute, 2011).

Formaldehyde is not only used as a disinfectant or in clothing and building supplies, but also in agriculture. Formaldehyde is sometimes used on poultry farms to disinfect things such as vehicles, and buildings to reduce the load of pathogens present including viruses, bacteria and mold spores (Zulkifli et al., 1999; Hayretdağ and Kolankaya, 2006; Aulisa, n.d.). Formaldehyde fumigation is frequently used within the hatching cabinet of poultry hatcheries as a disinfectant (Zulkifli et al., 1999; Hayretdağ and Kolankaya, 2006). It is used to reduce the load of pathogenic organisms within the hatcher that can negatively affect the embryos such as *Salmonella, Escherichia coli* and, *Pseudomonas* (Hayretdağ and Kolankaya, 2006; Cadirci, 2009, Sander et al., 1995). Although it is an effective disinfectant, there are concerns about it use and the adverse effects that it can have on embryos and chicks (The National Toxicology Program, 2011; National Cancer Institute, 2011; Cadirci, 2009; Zulkifli et al., 1999; Hayretdağ and Kolankaya, 2006; Banwell, 2013).

Hatchery Sanitation

As indicated above, formaldehyde has been used as a fumigant in commercial poultry hatcheries to reduce the number of pathogens in the hatchery environment (Hayretdağ and Kolankaya, 2006), during the onset of incubation, and immediately after transfer of embryos to the hatcher (Zulkifli et al., 1999). The practice of formaldehyde fumigation has been shown to reduce the level of opportunistic pathogens such as *Salmonella* (Sander et al., 1995a, 1995b; Samberg and Meroz, 1995; Zulkifli et al., 1999; Hayretdağ and Kolankaya, 2006). Infection with *Salmonella* can sometimes cause mild to subclinical disease of poultry that can be vertically passed from the hen to the embryo if the hen is infected. Therefore, control is important due to its ability to be a zoonotic disease. A single chick hatching with *Salmonella* infection can cause hundreds of non-infected chicks to become colonized within the hatching cabinet (Cason et al., 1993; Sander and Wilson, 1998). Cason et al., (1993) evaluated horizontal spread of *Salmonella* by placing fertile eggs inoculated with an acid resistant strain of *Salmonella typhimurium* in the hatching cabinets with control eggs at the same stage of incubation in the same tray. Control eggs were also added to trays above and beneath the trays containing the inoculated eggs. They observed a hatch rate of 86% percent of the fertile inoculated eggs despite the high level of *Salmonella* contamination, indicating embryos contaminated with salmonellae possess the ability to hatch and potentially contaminate other chicks within the same hatcher.

Control of *Salmonella* infection is important for poultry. While usually causing subclinical disease in poultry, poultry products are a source of human infection and salmonellosis (Wilkins et al., 2002). *Salmonella* is an important foodborne disease and accounts for approximately one million foodborne illnesses in the United States, with 19,000 hospitalizations and 380 deaths

(Center for Disease Control,. 2012) . Eggshell penetration by spoilage bacteria such as *Pseudomonas* is correlated to decrease hatchability and contamination of the chick through passage via the blunt air cell end of the embryo (Berrang et al., 1999; Hayretdağ and Kolankaya, 2006). *Salmonella* was found to penetrate the eggshell and membrane of embryos placed in a *Salmonella* contaminated nest box, where 59% of the eggs were penetrated with *Salmonella*. Embryos that possess gram negative bacteria within the hatching cabinet can cause cross contamination, potentially resulting in an increase of gram negative bacteria within the hatching cabinet (Berrang et al., 1999). Although formaldehyde is very affective in sanitation, there are concerns about the potential of the residue migrating into the embryo causing embryonic death or reduced health. Nevertheless, the importance of fumigation in hatchery practice has been clearly demonstrated (Zulkifli et al., 1999; Hayretdağ and Kolankaya, 2006, Cason et al., 1993). However, the impact of hatchery exposure of chicks to formaldehyde on early post-hatch performance of broilers has not been demonstrated (Cardirci, 2008) and evaluation of the effect of fumigation on post-hatch performance is needed.

Effects on Hatchability

Disease organisms adversely affect developing embryos, hatchability, and chick quality (Parkhurst and Mountney, 1988). Clearly, hatchability is important for both small flock and commercial poultry breeder flock owners. Maintaining hatching egg shell quality is important because of its connection with hatchability (Moyle et al., 2008). In modern incubation practices, the levels of ventilation during the early stage of incubation are vastly reduced. This significantly improves hatchability, chick quality, uniformity and post-hatch performance. However, this also causes some of the formaldehyde to remain on the egg shell and enter into the egg, which may

adversely affect hatchability (Proudfoot and Stewart, 1970; Sacco et al., 1989; Yildirim et al., 2003; Banwell, 2013).

Although it has been found that the most effective way of disinfection of hatching eggs is fumigation with formaldehyde (Cadirci, 2008), there are concerns about its use. During fumigation, formaldehyde comes into contact not only with the surface microorganisms but also with the egg shell itself and, if absorbed, with the embryo (Hayretdağ and Kolankaya, 2006; Berrang et al., 1999; Cadirci, 2008). The blastoderm, the layer of cells from which the embryo is developed, is positioned on the upper surface of the yolk which is held in a central position by a combination of the chalazae and the viscous nature of the albumen (Cadirci, 2009; Banwell, 2013). The diffusion of carbon dioxide through the porous shell allows the pH of the albumen to rise. As the pH increases, the interaction between two of the albumen proteins (lysozyme and ovomucin) breaks down, leading to a decrease in the albumen viscosity. This allows the yolk and the blastoderm to float towards the shell and towards any potentially harmful concentration of formaldehyde (Banwell, 2013).

Similar to human skin and even fruits and vegetables, embryos have the eggshell to protect them from things of the environment. This outer layer, the cuticle, acts as a physical barrier preventing passage of microorganisms (Cadirci, 2008). Although there is limited information of the effects of formaldehyde on the cuticle, formaldehyde absorbed at an early stage of embryonic development will alkylate the nitrogen atoms of pyrimidine and purine bases of DNA and RNA inhibiting their function. This is because formaldehyde acts on proteins and also nucleic acids (Cadirci, 2009). The ability to reduce the microbial load is important in overall hatchery

sanitation, hatchability and also viability of the embryo (Sander et al., 1994; Sander et al., 1994; Zulkifli et al., 1999; Hayretdağ and Kolankaya, 2006)

It's normal to experience embryonic mortality within a group of incubated eggs. Mortality generally has two peaks during the incubation period (Parkhurst and Mountney, 1988; Cadirci, 2008). The mortality peaks occur during early incubation – days 2, 3, and 4- totaling around 1.5% and late in the incubation period – days 19, 20, and 21 – totaling around 3.0% in normal hatches. The early peak is associated with the physiological adjustment of the embryo as the various systems of the embryo are initiated. The second peak is associated with pulmonary respiration and the embryo mortality during the second period (Parkhurst and Mountney, 1988).

Studies suggest that embryonic viability is potentially correlated to the hen flock age and strain in some cases. Bruzual et al. observed significantly higher mortality of embryos coming from a broiler breeder flock of 26 weeks vs. 36 weeks old. They found that the BW at hatch and at pull increased with increased hen age. They observed embryos from younger hens showed obvious characteristics such as low egg weight. This is important since the chick weight is greatly influenced by the weight of the egg from which it hatches (Bruzual et al., 2000). Other studies such as that of Williams and Gordon (1970), observed a loss of embryos exposed to formaldehyde from older hens, 55- weeks old, than from younger hens, 35 weeks old. It was suggested this may be due to potentially low calcium levels within the eggshell resulting in some deformities (Cardirci, 2009). However, other researches such as Sander et al., (1995), found that hatchability was not affected by formaldehyde exposure.

Effects on the Respiratory Epithelium

The respiratory system is one of the major systems in the body. It has a number of very important functions including the provision of oxygen, the removal of carbon dioxide, the removal of excess heat (thermoregulation) and vocal communication (Brown et al., 1997). It is important that the respiratory system is functioning properly. However, proper function can be altered by environmental factors.

Formaldehyde, used in hatcheries as a means of sanitation, produces ciliostasis and causes blunting and surface blebbing of the tracheal cilia of exposed chicks. The accumulation of excessive mucus, matted cilia, and areas of deciliation may result in inadequate mucocilliary action (Zulkifli et al., 1999; Hayretdağ and Kolankaya, 2006). Noxious gases act as irritants to the delicate tissues of the upper respiratory system (Sander et al., 1994; Hayretdağ and Kolankaya, 2006). The effects of excessive formaldehyde fumigation on the respiratory epithelium of poultry can be devastating to the overall health of the bird (Cadirci, 2009). Zulkifli et al. (1999) conducted two experiments to evaluate the effect of formaldehyde vaporization of a hatcher on the tracheal epithelium of chick embryos, and on the production. In the first experiment, the embryos were exposed to 23.5 ppm of formaldehyde vapor during the last three days of incubation. Tracheal samples were collected at 0, 6, 30 and 54 hours post exposure for formaldehyde and examined by scanning electron microscopy for pathological changes. In their second experiment, they exposed sixty chicks to formaldehyde vapor, also at 23.5 ppm, and sixty control chicks were used to investigate the effect of formaldehyde fumigation on production performance and behavior.

They observed lesions including excessive accumulation of mucus, matted cilia, and loss of cilia and sloughing of the epithelium. The lesions were seen to be more severe in chicks exposed for 54 hours as compared to those exposed for 6 or 30 hours. In the second experiment, they noticed that formaldehyde vaporization resulted in higher weekly (days 0-6 and 21-27) and total (days 0-41) feed intake and poorer weekly (days 0-6, 7-13, 21-27 and 28-34) and overall (days 0-41) feed conversion ratios.

Hayretdağ and Kolankaya (2006) evaluated the effects of pre-incubation formaldehyde fumigation on the tracheal epithelium of chicken embryos and chicks. Pre-incubation formaldehyde fumigation was administered to 18-day old embryos and 1-day old chicks once, at only 1 or 2 different concentrations (3x, 42 ml of formalin and 21 g of potassium permanganate per m^3 and 4x, 56 ml of formalin and 28 g of potassium permanganate per m^3) for 1 or 2 different duration times (20 minutes or 40 minutes). They also observed a reduction in the number and size of the cilia, vacuolization, swelling of the mitochondria, and spoiling of the cristae, which varied according to fumigation level and time, using a transmission electron microscope (TEM).

Sanders et al. (1995) conducted a study analyzing the effects of formaldehyde on the tracheal epithelium. The chicken embryos were exposed to formaldehyde vapors in the hatcher during the final 3 days of incubation. The measured formaldehyde levels reached 130 ppm. The tracheas were collected at hatch and 5 days post-hatch and were evaluated for functional and morphologic changes. They too found that tracheal cilia motility was reduced in formaldehydeexposed chicks. Scanning electron microscopy revealed blunted cilia and blebs occurring in the cilia surfaces. At 5 days of age, excessive tracheal mucus was present along with sloughing of the tracheal epithelium visible by light microscopy.

The use of formaldehyde irritates mucous membranes, impairs mucociliary mechanisms, and affects the flow of mucus (Sander et.al., 1995; Zulkifli, 1999; Hayretadğ and Kolankaya, 2006). An important factor in the effect of formaldehyde on the tracheal mucosa is the dissolution of the gas in secretions. Formaldehyde dissolved in mucous secretions causes a pH shift toward acidity and these changes in pH cause damage to the membrane structure and ciliary activity (Sander et.al., 1994; Hayretadğ and Kolankaya, 2006). The disruption in the function of the upper respiratory tract makes animals more susceptible to diseases, especially respiratory diseases, such as *Escherichia coli* due to the cilia lacking the ability to remove foreign particles from the body.

Body Weight

Zulkifli et al. (1999) found, during their investigation on respiratory epithelium, production performance and behavior of formaldehyde-exposed broiler chicks, that the use of formaldehyde did not affect body weight, mortality or behavior. However, Khan et al. (2006) found that the implication of less than 10 mL/kg fed to broiler chicks decreased feed consumption and body weight. Khan et al. (2005) also showed that feed containing 20 mL of formalin/kg fed to Japanese quail showed a decrease in body weights. There was also an observance of a decrease in egg production and weight, erythrocyte and leukocyte counts, hemoglobin concentrations, and hematocrits were reported at both 10 and 20 mL of formalin/kg of feed in Japanese quail.

Animal and Human Exposure

The wellbeing and overall safety of the animal is the main priority. However, humans working to supply this level of safety, whether it's in the poultry industry or in general, also need to be considered. Both human safety and animal welfare are becoming increasingly important.

People are commonly exposed to low levels of formaldehyde in the workplace and in home environments, but the highest levels are found in work settings where formaldehyde is used or produced (National Toxicology Program, 2011). Exposure to formaldehyde has been shown to result in irritation of the throat, eyes and nose; coughing, wheezing, nausea and skin irritation (Whistler and Sheldon, 1989; Yildirim, 2003; Cadirci, 2008, 2009; Nation Cancer Institute, 2011). Laboratory studies showed that exposure to formaldehyde could cause nasal cancer in rats (Morgan et al., 1986). After this was discovered, the question of whether formaldehyde had a similar effect on humans became of concern. In 1987, the U.S. Environmental Protection Agency (EPA) classified formaldehyde as a probable human carcinogen under conditions of unusually high or prolonged exposure (U.S. EPA, 1989; National Cancer Institute, 2011). Scientists then began to conduct studies to analyze whether exposure to formaldehyde correlates with cancer in humans. One type of epidemiologic study is called a cohort study. A cohort study is a group of people who may vary in their exposure to a particular factor, such as formaldehyde, and are followed over time to see whether they develop a disease (MacGill, 2016). Another type of epidemiologic study is called a case-control study. This study is designed to analyze individuals who have been diagnosed with a disease (case) and compare to those without a disease (control) to try and identify the difference in factors, such as exposure to formaldehyde,

that might explain why the cases develop the disease but the controls did not (National Cancer Institute, 2011; Kyoungmi,2016).

A cohort study, performed by the by the National Institute for Occupational Safety and Health (NIOSH), found an association between the duration of exposure to formaldehyde and leukemia deaths amongst 11,039 textile workers. However, the evidence remains mixed because a cohort study of 14,014 British industry workers found no association between formaldehyde exposure and leukemia deaths (Coggon et al., 2003; Pinkerton et al., 2004; National Cancer Institute, 2011). Nevertheless, studies have been completed where it was found that individuals who are exposed to a significant level of formaldehyde, such as anatomists and embalmers, had a higher risk of developing myeloid leukemia (National Cancer Institute, 2011).

The general population is exposed to formaldehyde by breathing contaminated indoor or outdoor air and from tobacco smoke (National Toxicology Program, 2011). Items such as a gas stove releasing formaldehyde into the air and could potentially be detrimental in the absence of proper ventilation. The use of formaldehyde has proven to be detrimental in humans but also other animals used in experimental studies.

Fischer (1905) analyzed the toxic effects of formaldehyde. These studies, published over a century ago, demonstrated that even small amounts of formaldehyde in air can result in bronchitis and pneumonia, and that pneumonia is caused by the inhalation of the gas, rather than by secondary infection. This work also indicated that formaldehyde in solution (formalin) may result in gastritis (inflammation of the gastrointestinal tract) when swallowed, potentially leading to acute death. Other regions of the gastrointestinal tract such as the upper jejunum and duodenum may be involved in the inflammatory process**.** Fischer (1905) also observed that,

fibrino-hemorrhagic peritonitis was caused by injecting formalin intraperitoneally. An important factor in the use of formaldehyde and formalin is the fact that it takes very little to cause a reaction and/or be fatal. If formalin is introduced into the peritoneal cavity, it causes massive destruction to important organs such as the pancreas, Fallopian tubes and liver, and causes great inflammation. For instance, it only takes 2 mL of a 1000-fold dilution of formalin for each 100 grams of body weight to cause serious acute disease.

Alternatives

Formaldehyde is a highly effective and inexpensive disinfectant, killing most viruses, bacteria and fungi on contact (Zulkifli et al., 1999; Yildirim, 2003; Hayretadğ and Kolankaya, 2006). However, due to the reasons mentioned above, alternatives to formaldehyde in hatchery applications have been investigated (Brockotter, 2015). Sheldon and Brake (1991) found that hydrogen peroxide could be a potential alternative. They observed a two percent increase in hatchability of fertile eggs from a 44 week old flock after spraying with five percent hydrogen peroxide in comparison to the untreated control group. The level of contaminated eggs and early embryonic death was also significantly reduced. In comparison to formaldehyde fumigation, no significant difference in hatchability due to hydrogen peroxide treatment was detected. Eggshell permeability was not significantly affected by this method of disinfection or formaldehyde fumigation when compared to that of untreated or water-sprayed control eggs. Hydrogen peroxide has been shown to decrease the amount of contaminated eggs and “early-dead” embryos were significantly reduced in eggs treated with hydrogen peroxide (Sheldon and Brake, 1991). Sheldon and Brake (1991) also noted that in comparison to formaldehyde fumigation, hydrogen peroxide treatment caused no significant difference in hatchability or eggshell

permeability. They also observed that hydrogen peroxide compared favorably to formaldehyde as a hatching egg disinfectant without adversely affecting hatching potential. Under certain conditions hydrogen peroxide was found to improve hatching potential of fertile broiler eggs compared to controlled eggs (Sheldon and Brake, 1991). Padron (1994) found that double dipping *Salmonella* Typhimurium-contaminated eggs twice in a 6% hydrogen peroxide solution reduced the average number of organisms in eggshell membranes by 95% and the number of *S.* Typhimurium –positive eggs by 55% compared with the infected untreated group. They also found that dipping eggs in 6% hydrogen peroxide solution did not adversely affect hatchability. Bailey et al., 1996 also observed no significant difference in hatchability and a significant reduction of *Salmonella* on eggshell fragments using hydrogen peroxide. Sander and Wilson (1998) observed a significant reduction in aerosol bacterial counts within the hatcher when incubators were fogged with 3% hydrogen peroxide when compared with water fogged machines, even in the face of high bacterial challenge of *Staphylococcus aureus* contaminated eggs.

Another potential alternative to formaldehyde is Virocid® a disinfectant composed of a combination of multi-chain quaternary ammonium and glutaraldehyde produced by CID Lines (Brocketter, 2015). Glutaraldehyde is known as a colorless, oily, liquid-chemical with a pungent odor, similar to formaldehyde (Centers for Disease Control and Prevention, 2012) that is rapidly bactericidal and sporicidal killing 99.99% of the spores of *Bacillus anthracis* and *Clostridium tetani* in 15 and 30 minutes, respectively (Rubbo et al., 1967; Gorman et al.,1980). It's used in the health care industry, cosmetics, embalming solutions, animal housing and as a fixative for histology (Centers for Disease Control and Prevention, 2012). It has proven to be a potential alternative for formaldehyde for disinfecting hatching eggs prior to setting. It's said to have the

same disinfectant value as formaldehyde (Thermote, 2006). There is a correlation between the droplet size (fog), the angle of contact (wettability), the type of chemicals used, and the levels of concentrations administered are known to have an large impact on the success of the results of the disinfectant on hatching eggs. Virocid® has the same disinfection value as formaldehyde, even in cold foggers, Virocid® is significantly better then formaldehyde (Moyle, 2011; Brockotter, 2015). Tenk et al. observed a significant reduction in the microbial population of the eggshell surfaces of poultry and turkey eggs by fog application of Virocid®. The Virocid® spray applied at a concertation of 0.2% at 43˚C markedly diminished the microbial contamination of turkey egg surfaces (Tenk et al., 2000). However, as Virocid® contains glutaraldehyde, some of the same hazards for humans and chicks may apply. Indeed, glutaraldehyde has been shown to cause many of the issues associated with formaldehyde in animal model studies (van Birgelen, 2000; Takigawa and Endo, 2006).

Ozone has also been considered as a potential alternative to the use of formaldehyde in hatchery applications. The differences in the effects on microbial load and hatchability were observed using ozone misting versus formaldehyde by Whistler and co-workers (1989). They observed a significant decrease in the microbial counts, of over 2.5 log10 ($P < .05$), for watermisted and ozonized (2.83% by weight) eggs or formaldehyde-fumigated (triple strength) eggs than for their control and water-misted eggs. Although the use of ozone does aid in decreasing the microbial load, adverse effects of ozone use were noticed. The use of ozone at certain concentrations showed a decrease in hatchability when compared to either no treatment, water misting or formaldehyde, and may have adverse effects on the development of the embryo when exposed to this gaseous form (Whistler et al., 1989). Various proteins samples extracted from the cuticle of different bird species possess antimicrobial activity against several bacterial

species (Rodríguez-Navarro et al., 2013). It was observed that at low doses ozone treatment completely destroyed the soluble cuticle proteins (Fuhrmann et al., 2010). Degradation of the cuticle and antimicrobial proteins could increase the permeability of the egg, in turn increasing the probability of damaging the eggs by contamination or embryonic death (Fuhrmann et al, 2010).

Probiotics have been the topic of gut health for over a century (Fijan, 2014). Probiotics are viable microorganisms that confer health benefits to the host once consumed in adequate amounts, primarily by promoting the proliferation of beneficial gastrointestinal indigenous microflora (Shi Lye et al., 2009). The most common are the Lactic Acid Bacteria (LAB) such as *Lactobacillus* sp., *Bifidobacterium* sp. and *Enterococcus* sp. (Ljungh and Wadstöm, 2006). LABs are used in functional foods such as yogurt and pharmaceutical preparations based on the capacity to stimulate the host immune system. Potential mechanisms of probiotics include the ability to bind intestinal mucus, modulation of toxin production and action, production of inhibitory metabolites, immunomodulation and modulation of cytokine patterns (Patterson and Burkholder, 2003; Revolledo et al., 2006). It's known that LABs induce distinct mucosal cytokine profiles showing various adjuvant capacities among them in rats (Perdigón et al., 2002).

Direct Fed Microbials are beneficial bacteria administered directly into the feed. These microbes are mostly comprised of *Bacillus* genus (Huyghebaert et al., 2011; Lei et al., 2015). The ability to form spores and withstand heat treatment and pelleting make them ideal candidates for poultry feed (Lei et al., 2015). Utilization of such may result in a shift in the microbiota; however, its benefits are not persistent over long periods of time. Martin and Nisbet (1992) stated that several researchers observed that direct-fed microbials increased cellulolytic bacterial

numbers in the rumen and stimulated the production of some fermentation end products. Thus, suggesting that direct-fed microbials may be providing growth factors for the ruminal microbes.

The mechanism of competitive exclusion is referred to frequently when discussing probiotics. A process in which an organism is prevented from colonizing a given environment due to prior presence of other organisms that have better established and maintained in that environment (Revolledo et al., 2006). Three potential mechanisms of competitive exclusion includes: competition for attachment sites, competition for nutrients and direct support of host innate and acquired immunity through poorly defined mechanisms (Higgins et al., 2007).

Probiotics have gained great attention from scientists in order to further understand their beneficial health effects. An important aspect of probiotics is its composition. They contain microbes, which are usually bacteria. Microbes used in the production of probiotics are that of an array of microorganisms. These organisms include bacteria, mold and yeast. However, some are more prevalent than others. Bacterial components may include members of the *Lactobacillus* family such as Lactobacillus (*L.*) *L. acidophilus, L. sporogenes,* and *L. planturam,* as well as those of the Bifidobacterium (*B.*) genus including *B. bifidum, B. infantis, B. adolescentis, B. longum, B. thermophilum, B. breve, B. lactis,* and *B. animalis.* Also, examples of probiotic species include *Streptococcus (S.) lactis, S. alivarius, and S. thermophilis.* Other examples include various members of the genera *Propionibacterium, Enterococcus, Enterococus, Pediococcus,* and *Bacillus.* Yeast and molds that are reported to be beneficial in some studies include *Aspergillus oryzue, Candida pintolopesii, Saccharomyces cerevisiae* and *Sacaromyces boulardii* (Amara and Shibi, 2015; Kabir, 2009). Other probiotic strains have been found to

demonstrate some health benefits such as selected strains of *Escherichia coli* (Fijan, 2014; Kabir, 2009).

It is believed that if probiotics can be administered during hatch of broiler chicks, the beneficial microorganisms will have the chance to colonize the gut of the embryos, becoming pioneer colonizers, which may permanently alter the phenotype of the avian gut (Oakley et al., 2014). It is believed that some beneficial organisms, such as dry Generally Recognized as Safe (GRAS) Bacillus spores may greatly reduce the number of pathogenic microorganisms in the hatching cabinet due to competition for nutrients and production of antimicrobial factors (Graham et al., 2018). Moreover, other beneficial probiotic flora may colonize the gastrointestinal tract and directly impact development of the gastrointestinal tract through host receptors and may provide further protection from colonization with opportunistic pathogens through the mechanism of competitive exclusion. In reference to Ecology, competitive exclusion, also known as Gause's Law, states that two species that compete for the exact same resources cannot stably coexist (Hardin, 1960). The goal of application of two sets of beneficial bacteria, one able to compete within the environment of the hatching cabinet (*Bacillus spp.)*, and a second set known to beneficially colonize the gut, may provide near optimal options for replacing formaldehyde and benefiting post-hatch production parameters for broiler chicks (Graham et al., 2018). Selected beneficial probiotic microorganisms applied as pioneer colonizers to neonates are known to create a protective barrier of the intestines limiting the colonization of pathogenic microorganisms and combating the occurrence of intestinal disease and reducing food borne pathogens (Jeffrey, 1999). Probiotics are used in both the medical and agriculture fields to combat bacterial antimicrobial resistance (Tellez et al., 2012). Rapid establishment of an adult type intestinal microflora, in newly hatched chicks, via the oral route

almost immediately produces resistance to colonization by any food poisoning Salmonellae that reach the rearing environment (Mead, 2000). To date, probiotic or competitive exclusion products are conventionally applied via the drinking water, post-hatch spray application, or within feed (called Direct Fed Microbials (DFM)), long after true pioneer colonization with opportunistic bacteria within the hatchery cabinets has occurred (Jeffrey, 1999; Graham et al., 2018).

In potentially ground-breaking work, Graham and co-workers (2018) evaluated the use of a spray probiotic + environmental competitive exclusion formulation as an alternative method to control the bacterial bloom within a broiler hatch cabinet versus formaldehyde fumigation. The control hatch cabinets were treated with formaldehyde, the current disinfection method for the commercial hatchery where this approach was evaluated. The probiotic hatch cabinets received a selected mix of *Bacillus subtilis* and *Pediococcus acidilactici.* They found that the percentage of coverage for total recovered non-selective aerobic bacteria (TAB) in the probiotic treated hatch cabinets was significantly ($P < 0.05$) greater than the percentage of coverage for the formaldehyde treated hatch cabinets at all three sampling times, approximately 20% pip; 30% hatch and 85% hatch. However, at 85% hatch, the levels of total gram-negative bacteria (TGB) in the probiotic group were significantly greater than those in the formaldehyde treated hatch cabinets. The probiotic application increased the number of TAB and lactic acid bacteria (LAB) present in the hatching as well as a reduction of TGB in the gastrointestinal tract compared to the formaldehyde group. They also found the reduction in TGB persisted 24 h post-hatch. Their results suggest that spray application of a probiotic in commercial hatcheries can be as effective as formaldehyde in reducing total gram-negative bacteria. Moreover, subsequent large scale field

trial data has indicated that post-hatch performance, all the way to processing, was improved with the application of the microbial treatments as compared to formaldehyde (Graham 2015).

Conclusion

The use of formaldehyde has proven to be extremely effective for an array of uses including clothing production, lumber manufacture, cosmetic manufacture, general disinfectant use, sanitizing poultry eggs, and even use in hatching cabinets during the hatching process, as described above. This molecule is very effective as a disinfectant in the poultry industry in reducing the load of microorganisms. Its role in the reduction of pathogenic microorganism's aids in decreasing the potential for pathogenic or opportunistic pathogenic microorganisms reaching the embryo resulting in decreased hatchability and embryonic death, or neonatal infections resulting in early mortalities after placement. Although formaldehyde is very effective, its potential adverse effects in the poultry industry have caused great interest in finding alternatives with the beneficial properties of this molecule but without the potential hazards described above.

Alternatives for the use of formaldehyde fumigation have resulted in the evaluation of use of Virocid®, ozone, hydrogen peroxide, and even beneficial bacteria including probiotics at the time of hatching. At the present time, probiotics seem to be and exciting and promising alternative. Blankenship et al. (1993) administered a mucosal competitive exclusion culture (MCE) for testing via spray application in the hatchery first, and the drinking water after. They found significantly ($P < 0.05$) lower *Salmonella* contamination of the litter, skin, and ceca after three weeks of growth. Blankenship et al. (1993) study suggests that treatment of chickens in a

commercial setting with MCE cultures can serve as a useful means to reduce *Salmonella* contamination. Of course, further research needs to be conducted to fully understand the effects of probiotics and the mechanisms in which the affect the embryo and bird after hatch. Virocid® has been shown to be just as effective as formaldehyde, even in cold temperatures. This could also be a potential alternative to formaldehyde fumigation. The use of Ozone versus formaldehyde has also been analyzed. It has been shown to successfully decrease the microbial loads significantly, however, just as formaldehyde, causes a decrease in hatchability and the development of the chick embryos. Hydrogen peroxide also exemplifies its ability to be an effective disinfection of chick embryos and a potential alternative to formaldehyde. It was observed that it does not adversely affect hatchability or eggshell permeability, which could potentially aid in the prevention of some embryonic mortality. Formaldehyde is listed on the Agency for Toxic Substances and Disease Registry as an important substance, however, it should be noted that the list is not a list of the most toxic substances, but rather a prioritization of substances based on a combination of their frequency, toxicity, and potential for human exposure at NPL sites (Agency for Toxic Substances and Disease Registry, 2017). Very recent investigations (Graham, 2015; Graham et al., 2018) suggest that an entirely new alternative may be available, using harmless *Bacillus* isolates to competitively exclude the primarily Gram negative bloom of opportunistic pathogens during early hatching, while simultaneously providing beneficial lactic acid bacteria as early pioneer colonizers of the chick gastrointestinal tract. These early studies suggest that most of the antimicrobial benefits can be achieved with this approach and early field trials suggest that improved production parameters, as compared to formaldehyde-treated controls, may be possible (Graham 2015).

In the absence of alternatives that are cost- and labor-effective, formaldehyde will continue to be an effective disinfectant during commercial poultry hatching. Nevertheless, effective and adoptable competing technologies are needed.

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Evaluation of the effect of 1-2ppm formaldehyde or heat stress (37.8˚C) on growth parameters of broiler chicks

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SUMMARY

Formaldehyde has been used as a disinfectant in poultry hatching cabinets, brooder houses, hatcheries and hatchery vehicles of poultry. The present study was designed to evaluate the effects of 1-2 parts per million (ppm) formaldehyde (37.2˚C), or heat stress (37.8˚C) on performance parameters of broiler chicks such as body weights (BW's) and body weight gain (BWG) when hatched in small hatchers with minimal hatch-associated microbial bloom. Three experimental groups (control non-formaldehyde treated (37.2˚C), formaldehyde treated (1-2 ppm) (37.2˚C), and heat stress (37.8˚C) were evaluated. Significantly ($P < 0.05$) lower BW and BWG were observed in the formaldehyde group as compared to the control, non-treated group at d7 and d10 in Exp.1 Similar results were observed in Exp.2 with the formaldehyde-treated group BW and BWG, and heat stress BWG significantly ($P < 0.05$) lower than the control at day 10 post hatch. In large commercial hatchers with the potential for large bacterial contamination blooms, formaldehyde may offer a benefit. Heat stress of embryos/chicks during the hatching period may have a potential effect when temperatures are elevated to 37.8˚C. Temperature fluctuations may occasionally exceed this temperature in commercial hatcheries.

Keywords: formaldehyde, heat stress, weight, chicks, embryos, hatchery

DESCRIPTION OF PROBLEM

The ability to control microbial blooms, including opportunistic pathogens, during the hatching period is essential for production of quality chicks. Low level formaldehyde environmental treatment during hatching is often used as an aid in the control of *Salmonella*, *Escherichia coli* and *Pseudomonas* [1, 2] Nevertheless, formaldehyde exposure of chicks has been shown to potentially reduce tracheal ciliary function which may predispose to respiratory problems post-hatch [1] as well as causing other intracellular signs of cellular distress and increased mucus production [1, 2, 3] However, human exposure to formaldehyde causes irritation of the eyes and throat [4] and is considered a potential carcinogen [5]**.** Nevertheless, control of microbial blooms during hatch is of such critical importance that formaldehyde usage in hatcheries continues to be common [1, 2, 6, 7]**.** While often assumed to be slightly detrimental to chicks, the actual impact of modest formaldehyde exposure during hatch, on neonatal broiler performance, has not been documented. Similarly, elevated temperatures during incubation may adversely affect body weights [8, 9] of broiler chicks, and post-hatch environmental heat stress has been shown to impact performance in poultry [9]. The objective of these experiments was to evaluate the effect of modest (1-2 ppm) formaldehyde exposure or heat stress in the hatch cabinet on body weights (BWs) and body weight gain (BWG) during the neonatal period.

MATERIALS AND METHODS

In experiment 1, a total of 200 candled and viable commercial-cross broiler embryos per group were randomly assigned to non-formaldehyde treated group (control) and formaldehyde treated (1-2 ppm) group (N=100/group) during the hatch period. Formaldehyde was applied using a Watson- Marlow 120U peristaltic pump [10] set at 19 revolutions per minute (rpm) every four hours from day 18 to 20 and hatcher environmental concentrations were measured using an air quality detector. Twelve hours prior to hatch-pull, the pump was stopped, allowing residual formalin to dissipate prior to placing chicks.

At hatch pull, all (~ 195) chicks from each group were weighed and neck-tagged with discrete numbers**,** only keeping 100 of those weighing within one standard deviation of the mean.

The chicks were placed within a co-mingled pen for the duration of the experiment. Individual weights were recorded for each chick on days 7 and 10.

In experiment 2, 600 candled and viable 18 -day old embryos were randomly designated at the hatchery to control (37.2˚C), formaldehyde (1-2ppm; 37.2˚C) or heat stress without formaldehyde (37.8˚C). Chicks were weighed and neck tagged, selected (N= 100/group) and comingled as described above. Individual weights were recorded for each chick on days 7 and 10. Birds were fed a diet consistent with current Aviagen recommendations for starter diets and provided appropriate environmental temperatures during the neonatal evaluation period.

Statistical analysis

For all experiments, weights were subjected to one- way ANOVA comparing the controls to each treatment group utilizing JMP data analysis software [11]. Significance is reported at $P < 0.05$**.**

RESULTS AND DISSUSION

In Exp. 1, formaldehyde treatment during the hatch period caused significantly ($P < 0.05$) lower BWs and BWG than the control, non-treated group at d7 and d10 (Table 1.). The control non-formaldehyde treated group weighed 8g more than it counterparts and 15g more by d10. Similarly, the non-formaldehyde treated group possessed the greatest body weight gain between d0 and d7, d7 and d10, and d0 and d10 (Table. 2).

In Exp. 2**,** 1-2 ppm formaldehyde similarly decreased ($P < 0.05$) BW (Table 3.) at each time point, and decreased ($P < 0.05$) BWG at each interval measured (Table 4.) with twenty percent mortality within the formaldehyde treated group. Exposure of embryos/chicks to modestly elevated hatcher temperatures (37.8 vs 37.2˚C) caused a numerical decrease in BW at d7, and a significant decrease in BW by d10 (Table 3.). Elevated hatcher temperature decreased BWG during the d7-d10 interval, and overall interval d0-d10 (Table 4.). Cloacal temperatures of chicks at pull were measured, and chicks from the hatcher with elevated temperatures were significantly ($P < 0.05$) higher (41.38˚C) than chicks from control hatchers (40.52˚C).

In these experiments, plate counts of total aerobic bacteria collected on non-selective agar were insignificant during the hatch period regardless of treatment (data not shown), which may be related to the small number and carefully candled and selected embryos for placement. Thus, these experiments do not simulate the frequently-observed microbial blooms that are

common in commercial hatchers, generally beginning as humidity increases shortly after pipping occurs. Control of microbial blooms during hatch is of such critical importance that formaldehyde usage in hatcheries continues to be common [1, 2, 6, 7]. This is mainly due to the effectiveness and low cost of formaldehyde. Here, we provide evidence that relatively modest exposure to formaldehyde, in the absence of microbial blooms in hatchers, retards early neonatal performance. While a number of potential formaldehyde alternatives have been investigated, cost and labor friendly alternatives need further exploration for wide-spread adoption in countries where formaldehyde treatment is legally and commonly used in commercial hatchers.

Conclusions and Applications

1. In the absence of high microbial blooms during the hatch period, application of 1-2 ppm environmental formaldehyde in hatching cabinets reduced body weight and body weight gain during the neonatal period.
2. In experiment 2, modest elevation in hatcher temperature also negatively impacted neonatal performance, supporting previously reported observations.

3. While formaldehyde is effective and may be preferable to exposure to high levels of exposure to opportunistic pathogens during hatching, cost-effective and adoptable alternatives are needed.

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Appendix

UNIVERSITY OF ARKANSAS

Office of Research Compliance

To: Billy Hargis
FR: Craig Coon
Date: February 5th, 2018
Subject: IACUC Approval
Expiration Date: April 6th, 2020

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your Modification to protocol # 17073 *Evaulation of GRAS probiotic candidates applied in the hatching environment to control bacterial blooms and improve performance in broiler chickens* Add heat stress and increase animal number..

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond April 6th, 2020 you must submit a newly drafted protocol prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/tmp

Table 1 Effect of 1-2ppm formaldehyde (37.2˚C) on seven and ten day body weights and body weight gain of broiler chicks (Exp.1). Chicks randomly assigned to control treatment group or 1-2 ppm formaldehyde-treated group. Control and 1-2 ppm formaldehyde treated chicks were placed into a single co-mingle pen for the duration of the study. The difference in body weights by day seven and day ten indicated that control group weighed more than its counterparts. The body weight gain was higher within the control group than in the formaldehyde treated group than in the control group.

Treatment	Day 7	Day 10	Day 0-Day 7	Day 7- Day 10	Day 0- Day 10
Control	194 ± 1.21 [a]	317 ± 2.16 [a]	151 ± 1.18 [a]	124 ± 1.22 [a]	275 ± 2.11 [a]
1-2 ppm formaldehyde	185 ± 1.50 [b]	303 ± 2.45 [b]	142 ± 1.41 [b]	118 ± 1.29 [b]	260 ± 2.35 [b]

[a -b] means in each row with different letters are significantly are significantly different data are expressed as mean ± SE

Table 2 Effect of 1-2ppm formaldehyde (37.2˚C) or heat stress (37.8˚C) on average body weights and body weight gain of broiler chicks (Exp.2). Chicks, (N=300), were placed within a co-mingled pen where they remained for ten days. Average body weight was recorded. The results indicate significant difference between the control and 1-2 ppm formaldehyde treated group on day 7. By day 10, the formaldehyde 1-2 ppm and heat stress were significantly different compare to controls. Between day 0 and day 10, body weight gain was again significantly different amongst the treatment. The results indicate significant difference between the control and 1-2 ppm formaldehyde treated group between day 0 and day 7. Similar results were found between day 7 and day 10 amongst treatment groups. Between day 0 and day 10, body weight gain was again significantly different amongst the treatment groups compared to the control.

Treatment	Day 7	Day 10	Day 0 - Day 7	Day 7 - Day 10	Day 0- Day 10
Control	194 ± 1.21 [a]	317 ± 2.16 [a]	151 ± 1.18 [a]	124 ± 1.22 [a]	275 ± 2.11 [a]
1-2 ppm formaldehyde	185 ± 1.50 [b]	303 ± 2.45 [b]	142 ± 1.41 [b]	118 ± 1.29 [b]	260 ± 2.35 [b]
Heat Stress (37.8˚C)	188 ± 1.67 [a]	278 ± 2.59 [b]	147 ± 1.60 [a]	90 ± 1.58 [b]	237 ± 2.52[b]

[a -b] means in each row with different letters are significantly are significantly different data are expressed as mean ± SE