CLEANING AND DISINFECTION OF CAGED LAYER FACILITIES

A Thesis

by

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ABSTRACT

A rapid and effective means to clean and disinfect affected premises is needed by the poultry industry. Commercially available foaming disinfectants and cleaners applied via a compressed air foam system (CAFS) may be used to significantly reduce aerobic bacteria in a commercial caged layer complex. Using a variety of agricultural products against bacterial species may also provide information on which products are most efficacious against specific microorganisms on cage floors. In the first study, six field trials were conducted to evaluate current industry cleaning and disinfection protocols and the proposed CAFS application. A commercially available chlorinated alkaline cleaner (CHL/ALK) in trials 1 & 2 was applied by CAFS to one half of the house, and the other half of the house was not treated. The entire house was then washed with a high pressure water rinse (HPWR). A commercially available peroxyacetic acid (PAA) in trials 3 & 4 or a 14% glutaraldehyde (HI GLUT)/2.5% quaternary ammonia (QAC) blended disinfectant in trials 5 & 6 was applied by CAFS to one half of a washed house. The remainder of each house was treated with 7% (LO GLUT)/26% QAC, which was the spray application applied to cages by the integrator. Environmental swabs of drinker cups and cage floors were collected pre and post treatment to determine if aerobic bacteria levels were reduced. The HPWR and the CHL/ALK treatments did not consistently reduce aerobic bacteria on treated surfaces. Significant differences were observed with each of the CAFS applications of the PAA, HI GLUT/QAC, and LO GLUT/QAC product.

The objective of the second study was to determine the efficacy of commonly used products on soiled layer cages. Trial one was conducted in a small layer barn at the Texas A&M University Poultry Science Research, Teaching, and Extension Center. Trial two was performed at a commercial pullet house. In each trial, treatments were applied by a garden sprayer and six samples per treatment were collected. All products were mixed according to the manufacturer's recommendations. Treatments consisted of a negative control, a low pressure water rinse (LPWR, garden hose), a high pressure water rinse (HPWR, pressure washer), a soap, a chlorinated alkaline cleaner, a QAC, a glutaraldehyde, a peroxyacetic acid, a phenolic, a potassium peroxymonosulfate, a hydrogen peroxide, and a QAC/glutaraldehyde blend product. Swabs of cage floors were collected post treatment to determine if bacterial loads were reduced as compared to the appropriate controls. Aerobic bacteria, coliforms, *Staphylococcus* spp., and *Pseudomonas* ssp. were enumerated to evaluate the efficacy of the treatments. Aerobic bacterial colonization was significantly reduced by the oxidizer, peroxyacetic acid, aldehyde, and QAC disinfectants in trial one and by all seven disinfectants in trial two against the HPWR control. No treatment, in the first trial, significantly decreased coliforms or *Staphylococcus* spp. when compared to controls of nothing and the HPWR. However, reduction ($P < 0.05$) of coliforms and *Staphylococcus* spp. were observed with all disinfectants in trial two. The aldehyde and QAT disinfectant products in trial one, and all disinfectant products except the hydrogen peroxide and QAT/glutaraldehyde compound in trial two significantly reduced the levels of *Pseudomonas* spp. These data suggest that characteristics of cleaning and disinfection regimens can vary significantly.

DEDICATION

I would like to dedicate this publication to my parents. On March 15th, 1996, I moved to America at 6 1/2 years old. Two wonderful individuals, who have sacrificed everything for my brother and I, adopted us from the Ukraine where I lived in an orphanage. All of my accomplishments would not be possible without my parents giving me a second chance at life. I will never be able to repay the both of them for what they have done. Thank you so much mom and dad for everything you continue to do for me, I love you very much.

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This work was supervised by a thesis committee consisting of Dr. Morgan Farnell (chair), Dr. Yuhua Farnell (committee member) of the Department of Poultry Science and Dr. Allen Byrd (committee member) of Diamond V. Thanks to Dr. Luc Berghman (Substitute) for serving as a committee substitute.

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NOMENCLATURE

AI	Avian influenza
C	Celsius
CAFS	Compressed air foam system
C&D	Cleaning and disinfection
cm	Centimeter
HPAI	Highly pathogenic avian influenza
HPWR	High-pressure water rinse
Gal	Gallon
GLUT	Glutaraldehyde
in	Inch
LPWR	Low-pressure water rinse
oz	Ounce
PAA	Peroxyacetic acid
QAC	Quaternary ammonium compound
SE	*Salmonella enteritidis*

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CHAPTER I

LITERATURE REVIEW

Poultry's Impact on the Economy

The poultry industry is a dynamic and vital part of the national economy, which provides 1,814,200 jobs, $100.2 billion in wages, $32.9 billion in government revenue, and $469.6 billion in total economic activity (USDA, 2017). Within the poultry industry, the egg industry provides 128,000 jobs, $7.2 billion in wages, $2.2 billion in government revenue, and $30.7 billion in economic activity (Clyma, 2017). Foodborne and avian diseases cause the poultry industry, and their customers, millions of dollars every year in treatment costs and lost income.

Avian Influenza Outbreak of 2014-2015 in the U.S.

The European Union encountered a Highly Pathogenic Avian Influenza (HPAI) outbreak in 1999 resulting in greater than 50 million bird deaths, which caused severe economic losses to the private and public sectors (Capua and Alexander, 2004).The most recent 2014-2015 Midwest avian influenza (AI) outbreak resulted in 50.4 million bird mortalities and $3.3 billion in economic losses. The virus was discovered from birds of the Asian and American strains, and were introduced in the U.S. during the summer months (USDA-APHIS, 2015). The virus spread down the West Coast by the Pacific Flyway to wintering locations and crossed paths with birds from the Central and Mississippi Flyways (USDA-APHIS, 2015). The HPAI H5N2 was detected in the winter of 2014, which so far has impacted egg laying and turkey industries in the upper

Midwest and has cost the government over $950 million to halt the disease (USDA, 2017).

Billings (1997) stated that keeping AI under control is crucial because of the 1918 pandemic flu outbreak which killed more people than WWI. The 1918 Spanish flu pandemic was among the deadliest public health disasters in human history. The disease killed approximately 675,000 people in the U.S. and an estimated 50-100 million people worldwide (Johnson, et. al., 2002). The Spanish Flu pandemic was caused by an influenza A virus of the H1N1 subtype and the sequence analysis suggests that the ancestral source of this virus was avian (Taubenberger, et. al., 2005). The eradication of AI has been a costly ongoing battle, so prevention of the disease is of significant economic importance to the poultry industry due to production losses and potential human health concerns (Vaillancourt, 2009).

The AI virus requires a host to survive, like most viruses, but flu viruses can survive outside the host if the conditions are amiable (WHO, 2014). Viable viruses in high moisture and low temperature conditions can be recovered from manure for up to 105 days (WHO, 2005). The virus can survive up to four days in 22ºC water, and up to six days in ideal conditions at 37ºC (WHO, 2004). Avian influenza viruses are lipid enveloped, negative sense, segmented, ribonucleic acid viruses belonging to the *Orthomyxoviridae family* (Swayne and Suarez, 2000).Viruses can be divided into A, B, and C categories depending on their resistance to chemical agents, which is according to the lipids on the virus size (Prince and Prince, 2001). Influenza A virus is the only type

reported to cause infections of birds (Capua and Marangon, 2006), and it easy to inactivate by using the major classes of disinfectants (Prince, et. al., 2001).

Reportable Diseases of Poultry

According to the 2017 U.S. National List of Reportable Animal Diseases (NLRAD) and the National Animal Health Reporting System (NAHRS), reportable diseases for poultry are high and low pathogenic avian influenza (H5 or H7 subtypes), exotic Newcastle disease, turkey rhinotracheitis, avian infectious bronchitis, avian infectious laryngotracheitis, duck viral hepatitis, fowl typhoid, infectious bursal disease, avian mycoplasmosis, avian chlamydiosis, and pullorum disease (USDA, 2017). Humans are capable of being vehicles in spreading reportable diseases and constitute potential sources of the introduction of diseases (Bermudez and Brown, 2008).

Diseases of Poultry

Non-reportable diseases including Marek's Disease (MD), *Salmonella enteritidis* (SE) and *Mycoplasma gallisepticum*, are also a concern to the poultry industry. Marek's disease (MD) is associated with neurological and visceral symptoms such as paralysis of legs or wings, and eye lesions along with tumors in the heart, ovary, testis, muscles, and lungs (Nicholls, 1984), which can cause 20.8% mortality in layers (Taylor et al., 1999). The route of infection is commonly respiratory and the highly contagious disease spreads by infective feather follicle dander and fomites. Marek's is more common in birds lacking immunization, and with calcium deficiencies during the laying phase (Farooq, et. al., 2002). Heier and Jarp (2000) also found that there was a higher

incidence of MD in laying hens raised on floors rather than in cages because chickens are coprophagic**.**

On September 8, 2009, the FDA issued a final ruling that required shell egg producers to implement actions to prevent *Salmonella enteritidis* (SE) from contaminating eggs on the farm and from further growth during storage and transportation (FDA-DHHS, 2009). This ruling by the FDA was created because SE is among the leading bacterial cause of foodborne illness in the U.S. and shell eggs are a major source of human SE infections (FDA-DHHS, 2009). Improved biosecurity, implementing pest control, and environmental testing for SE are means to control it in poultry facilities (FDA-DHHS, 2009).

Mycoplasma gallisepticum is responsible for chronic respiratory diseases and infectious bursitis of laying hens (Branton and Deaton, 1985). *Mycoplasma gallisepticum* is considered the leading pathogenic agent for chickens among the 20 species of mycoplasmas isolated from birds (Buim, et. al., 2009). *Mycoplasma gallisepticum* causes economic losses due to decreased in growth rate, and weight loss (Hoerr et al. 1994). Infection can spread through the blood from the hen's respiratory tract to the oviduct, causing reduced egg production and poor egg quality (Patterson, 1994). Infected layer hens have decreased feed consumption, which alters their dietary components to sustain adequate egg formation and egg production. Brown and colleagues (1995) stated that once a bird is infected with MG, it is generally considered chronically infected for life.

The Impact of Biosecurity

A disease outbreak for any small or large livestock farm can lead to animal death, production losses, veterinary expenses, and clean-up cost (Clark, 2002). Without proper sanitation in bird facilities, there is a risk of transmitting a disease to a new flock from a previously infected flock. Diseases have numerous ways of being introduced to birds, but humans do not realize that they are a primary culprit. Humans can transmit diseases to and from the farm through fomites such vehicles, equipment, cloths, and themselves (Clark, 2002). Other wild animals can transmit diseases as vectors such as water fowl, insects, and rodents which are carriers for reportable poultry diseases (Carey, 1999). The utilization of a well-established biosecurity program should prevent pathogens from entering or contaminating animal facilities (Ruano, et. al., 2001). Protocols and procedures within a biosecurity program need to be adhered to by all employees to reduce any chances of possible diseases being introduced on to a farm (Poss, 1998).

Cleaning and Disinfection Programs

Cleaning and disinfection is a disease prevention measure in poultry production in between poultry flocks (Zander, et. al., 1997). Industries can prevent costly diseases by following simple but effective measures in C&D of their poultry houses. The methodology for C&D can be divided into five steps, which are first to dry clean which means to remove any solid contamination, second to wet wash with soap, third to rinse with warm or cool water, fourth to dry, and fifth to disinfect (NAHEMS, 2005). Steps 1-3 are crucial to remove any organic matter before applying disinfectant to ensure nothing can inhibit the disinfectant products. The fourth step prevents the possibility of diluting

out the already mixed solution of product and thereby decreasing its efficacy (NAHEMS, 2005). There are different levels of cleaning depending on the surface and number of organisms that are either removed or eradicated. First is sanitation, which is the physical removal of infectious agents and organic matter on which they thrive. It is an essential step before disinfection and sterilization can occur (Lewis and Mclndoe, 2004). Sanitization is equivalent to a 99.9% or 3 log reduction of microorganism. Out of one million microorganisms, approximately 990,000 of them will be eradicated with sanitation practices (Favero, 2001). Secondly is disinfecting, which is the destruction and reduction in numbers of pathogens. A bio-burden reduction of 99.99% and up to 99.999% or 5 log reduction of microorganisms can occur with proper disinfection procedures. This would be equivalent to destroying 999,990 organisms out of a million (Favero, 2001). Lastly is sterilization, which is the complete destruction and inactivation of all microbes including bacterial spores and viruses (Lewis and Mclndoe, 2004). Sterilization is statistically defined as 99.9999% or a 6 log reduction of microorganisms and their spores. This can be interpreted as zero viable organisms surviving (Favero, 2001). Obtaining sterilization in poultry facilities is nearly impossible, but following a C&D protocol efficiently will decrease pathogens contaminating animal facilities.

Cleaning and Disinfecting Poultry Layer Facilities

Cleaning and disinfection (C&D) in between flocks in broiler and layer houses has been studied before, but with limited effectiveness in many circumstances (Davies and Wray, 1995, 1996). The cleaning of cage layer systems is unsatisfactory because of insufficient time and effort being devoted to the removal of residual organic matter,

which is important before applying disinfectants (Holah, et. al., 1993). Problems arise such as sanitation equipment problems, choice of disinfectants, and influence of wildlife vectors being identified as significant factors (Davies, et. al., 1998). Wales and colleagues (2006) stated that the objective of C&D of poultry houses should be to eliminate contamination of the building and equipment by pathogenic micro-organisms and by organic matter that could possibly shield these organisms.

A study was conducted to compare the efficacy of four commonly used disinfectants in poultry house sanitation procedures utilizing a petri dish designed for direct contact surface sampling (Fate, et. al., 1985). Their findings concluded that the most effective disinfectant for reducing aerobic bacteria was a product that contained glutaraldehyde, while cresylic acid was the most effective disinfectant for reducing mold colony counts. They also looked at a quaternary ammonium product that ranked last out of the four disinfectants in reducing aerobic bacteria but second in reducing molds. Another study done by Carrique-Mas and colleagues (2009) wanted to determine the comparative effectiveness of disinfection programs in Salmonella-positive cage and non-cage houses in the field. The disinfectants used in the research were a formaldehyde disinfectant, a glutaraldehyde, a quaternary ammonium compound, and a standard commercial 10% formalin. The results for the study provided evidence that the use of the 10% formalin dilution was successful in decontaminating infected cage laying flocks. The other products were not as successful in the study because the houses had poor rodent control, and the farmers had a lack of knowledge of how to correctly measure out proper concentrations of each disinfectant (Carrique-Mas, et. al., 2009).

Research was conducted to determine if temperature, humidity, and time treatments were efficacious in eliminating Salmonella in laboratory trials, which were effective against Salmonella in infected layer houses (Gradel, et. al., 2004). Gradel and colleagues concluded that applying a steam treatment and relative humidity with the addition of 30 ppm formaldehyde is recommended for eliminating Salmonella from naturally infected poultry layer houses during a 24-h period. Garber and colleagues (2003) were interested in observing the prevalence of SE in layer houses, determine possible risk factors, and evaluate the occurrence of SE in mice. The manifestation of SE in layer houses was linked with molting, floor reared pullets, and rodents. In conclusion, their study identified multiple management practices such as C&D in between flocks and rodent control can decrease the prevalence of SE in layer houses.

Classes of Disinfectant Products

Choosing an efficacious disinfectant product isn't easy, because over the years there have been numerous products that claim to kill or remove almost all pathogenic infectious diseases (McDonnell and Russell, 1999). Nearly one billion dollars are spent annually on a variety of different types of antimicrobial products with the EPA having over 4,000 products registered, which are considered mixtures of substances used to eradicate or inhibit the growth of dangerous microorganisms such as bacteria, viruses, or fungi on inanimate objects and surfaces (EPA, 2017). Antimicrobial products may contain nearly 300 active ingredients and can be applied as sprays, liquids, powders, gases, and foam (EPA, 2017). Disinfectant products are tested against laboratory bacterial suspensions with success, but this may not always mimic commercial

production conditions and can make it difficult to determine the true effectiveness of these products (Bloomfield, et. al., 1991). Disinfectant efficacy is dependent on the chemical formulation, dilution factor, contact time, organic material load, type of microorganisms present, temperature, pH, water hardness, surface and safety considerations (Zander, et. al., 1997).

The classes of common disinfectants used in production agriculture today to C&D are alcohols, aldehydes, halogens, quaternary ammonium compounds, phenols, and oxidizing agents (Smith, 2010). Alcohols are broad spectrum antimicrobial agents that can damage microorganisms by denaturing proteins, which causes membrane damage and cell lysis (Ewart, 2001). Alcohols are used for both surface disinfection and topical antiseptics, and are fast-acting capable of killing bacteria within five minutes of exposure. Limitations for alcohol include virucidal activity and spores. Alcohols work best in the concentrations between 70-90% with some water required for efficacy to denature proteins because pure alcohol coagulates protein on contact, which would inactivate the cell but not kill it (Quinn, 2001). Alcohols evaporate quickly leaving behind no residue, but their effectiveness is limited in the presence of organic matter.

Aldehydes have a broad spectrum of activity against bacteria, fungi, and viruses (Rubbo, et. al., 1967). Glutaraldehyde's (GLUT) mechanism of action consists of a strong association with the outer layers of bacterial cells by binding to outer layers of organisms such as *E. coli* and *Staphylococcus aureus* (Bruck, 1991; Power, 1995). Gorman and colleagues (1980) revealed that GLUT has high bactericidal and sporicidal activity. Glutaraldehyde is also effective in the inhibition of Gram-negative bacteria

(Gorman and Scott, 1977), inhibition of dehydrogenase activity (Munton and Russell, 1973), inhibition of RNA, DNA, and inhibition of protein synthesis (McGucken and Woodside, 1973).

Halogens need little contact time, are inexpensive, and effective against mycobacteria, fungi, and enveloped/non-enveloped viruses (Block, 2001). Halogen compounds are broad spectrum compounds that lose potency over time and are not active at temperatures above 110ºF (Jeffrey, 1995). In the presence of organic debris, sunlight and some metals, these compounds lose activity quickly and need to be applied to thoroughly cleaned surfaces for disinfection to occur (Kennedy, et. al., 2000). Chlorine compounds denature proteins and are effective against bacteria, enveloped and non-enveloped viruses, and mycobacteria (Maris, 1995). Higher concentrations of chlorine compounds can be sporicidal with sodium hypochlorite being the most widely used chlorine disinfectant (Grooms, 2003).

Quaternary ammonium compounds (QACs) are stable in storage and are used for hard-surface cleaning and deodorization (Tennent, et. al., 1989). The QAC's are attracted to the negatively charged surfaces of microorganisms because of their cationic structure that irreversibly bind phospholipids in the cell membrane and denature proteins (Maris, 1995). There are seven generations of QAC's which depend on their chemical structure and formulations. Generations three to seven of QAC's are more germicidal and more tolerate to organic loads (Kennedy, et. al., 2000). Quaternary ammonium compounds are known for their high efficacy against Gram-positive bacteria, Gram-negative bacteria, fungi and enveloped viruses (Grooms, 2003). Unlike the halogen

disinfectant classes, QAC's are not effective against non-enveloped viruses or mycobacteria and are sporostatic but not sporicidal (Jeffrey, 1995). Quaternary ammonium compounds have little residual effect, and are easily inactivated by organic matter, detergents, soaps and hard water (Tennent, et. al., 1989).

Phenolics have antifungal and antiviral properties causing damage to the plasma membrane, which results in leakage of intracellular constituents (Russell, 1996). Phenolics are also broad spectrum disinfectants that function by denaturing proteins and inactivating membrane bound enzymes to alter the cell wall permeability of microorganisms (McDonnell and Russell, 1999). Phenols have a milky or cloudy appearance when added to water because of their formulations (Shulaw and Markey, 2001). Phenol solutions usually contain soaps to increase their penetration on surfaces, and at 5% concentrations can be considered bactericidal, fungicidal and virucidal for enveloped viruses (Jeffrey, 1995). Phenolics are not effective against non-enveloped viruses and spores, but are effective in hard water and in the presence of organic matter with some residual activity after drying (Kennedy, et. al., 2000).

Peroxides denature proteins, lipids, and eradicate mycobacteria and enveloped/non-enveloped viruses (Block, 1991). Hydrogen peroxide is widely used as a biocide for disinfection, sterilization, and antisepsis. Hydrogen peroxide is a clear, colorless liquid that is commonly applied at a concentration of 3%. Hydrogen peroxide is considered environmentally friendly because of it easily degrades into water and oxygen products (Block, 1991). Higher concentrations of 10-30% of hydrogen peroxide and longer contact times are required for sporicidal activity (Russell, 1991). Hydrogen

peroxide acts as an oxidant, which produces hydroxyl free radicals that attack cell components, including lipids, proteins, and DNA (Block, 1991). Peracetic acid (PAA) is a more potent biocide than hydrogen peroxide, and is considered sporicidal, bactericidal, virucidal, and fungicidal concentrations as low as 0.3% (Block, 1991). Like hydrogen peroxide, PAA also decomposes to safe byproducts of acetic acid and oxygen, but has advantages over hydrogen peroxide by remaining active in the presence of organic matter (Lensing and Oei, 1984).

Microbial Resistance to Disinfectants

Disinfectant products are chemical mixtures that reduce microorganisms on inanimate objects (Rutala and Weber, 2008). Disinfectants are commonly applied to abiotic surfaces such as bathrooms, kitchens, or in production facilities, but may also be added to drinking water or swimming pool water (Wessels and Ingmer, 2013). A risk of a disinfectant can be its toxicity to humans or its susceptibility in developing a resistance to microorganisms (Wessels and Ingmer, 2013). Resistance to disinfectants is a strain of an organism that is insusceptible to a concentration of the disinfectant used in a specific field that normally inhibits the majority of strains of that organism (Russell, 1999). Prions are said to be the most resistant to disinfectants, followed by coccidia (Taylor, 1999). Bacterial spores and mycobacteria are most resistant types of bacteria, with Gram-negative bacteria generally more resistant than Gram-positive cocci such as Staphylococci and Enterococci (McDonald, et. al., 1999).

There are two mechanisms of resistance to disinfectants, which are intrinsic and acquired resistance (Russell, 1999). Intrinsic insusceptibility means that a disinfectant is

unable to reach its target site in sufficiently high concentrations to achieve a lethal effect. Bacterial spores, mycobacteria and Gram-negative bacteria are intrinsically resistant to disinfectants (Brown, et. al., 1993). Acquired resistance results from genetic changes in a bacterial cell that is caused by mutation or the acquisition of plasmids. Acquired resistance has not been associated with spores or mycobacteria, but has with Gram-negative and Gram-positive bacteria (Russell, 1996).

Compressed Air Foam System

Lewis, Morris, and Timpson (1934) invented the first foam forming methods and apparatus for generating a stream of foam consisting of stable bubbles for extinguishing fires. The Royal Navy foamed syntax agents by using compressed air in the 1930's, and the U.S Navy used a compressed air foam systems (CAFS) in the 1940s for flammable liquid fires (Darley, 1995). Mark Cummins (1982) was credited with a patent for inventing the first compressed air foam system (CAFS). First foaming devices used complex designs and expensive nozzles for the inclusion of air into the foaming agent and for agitating the air foaming mixture to produce foam at the nozzle head. The foam generating devices included complex mechanical systems making them difficult to maneuver when fighting a fire. Approximately six years after Mark Cummins's patent of CAFS, Spielholtz (1988) patented the process of using an engine powered portable foam generator to apply disinfectants. A CAFS produces foam by mixing water, compressed air, and foam concentrate. The detergent reduces the surface tension of water, which allows the water to better adhere to and cover the area it is applied to. The components of the compressed air foam system includes a centrifugal water pump, a proportioning

device, an air compressor, a mixing chamber, and a control valve. Water can be carried on a truck in a tank or water can be pumped directly to the system if there is no holding tank from a hydrant, stream, lake, pond, swimming pool, or some similar source. A foam metering device or proportioner is used to accurately inject foam concentrate onto surfaces. Air is utilized from a compressor, which is a mechanical method of introducing a high volume of air by using a vane-type or rotary-screw industrial air compressor (Routley, 1994).

Utilization of Foam

Foam has been used to fight fires for over 100 years and it is still being used today (Rochna, 1991). Foam is used over water for firefighting because it has many purported benefits. Foam has faster knockdown time, rapid heat reduction, lower potential for flare-ups, and reduced water use. The utilization of foam has now expanded to more than just firefighting, which includes depopulation and disinfectant methods in the animal industries. An example is depopulating layer houses containing nearly 200,000 birds in a humane and effective way during a catastrophic disease outbreak. Firefighting foam can be utilized to depopulate poultry facilities as an alternative method (Dawson, et. al., 2005; Raj, 2008). In 2006, the USDA-APHIS and the American Veterinary Medical Association (AVMA) approved the use of water-based foam to depopulate poultry as an alternative (AVMA, 2007). Foam has gained an interest in the field of science and industries because it is environmentally friendly, biodegradable, and not a significant human health risk (AVMA, 2007).

A study in using foam with cleaners and disinfectants to reduce bacterial loads from animal facilities and surfaces could be the next step in improving C&D protocols. Hinojosa and colleagues (2015) did a study on using CAFS to apply cleaners and disinfectants on broiler transportation coops and found up to 5.0 $\log_{10}$ cfu/sample reductions of aerobic bacteria. Previous research has been done with foam to C&D broiler transportation coops, but little research has been evaluated using foam to reduce bacterial loads in poultry facilities such as at layer complexes.

CHAPTER II

COMPRESSED AIR FOAM APPLICATION OF AGRICULTURAL CHEMICALS TO CLEAN AND DISINFECT LAYER CAGES

Description of the Problem

Reportable and non-reportable diseases have a significant economic impact on egg production. The recent outbreak of highly pathogenic avian influenza (**HPAI**) resulted in 50.4 million bird deaths and cost federal taxpayers in excess of $950 million (USDA, 2015). The total economic cost was estimated to be $3.3 billion (Swayne, 2016). Diseases that may cause high mortality in layer flocks include infectious bronchitis, exotic Newcastle disease, and infectious bursal disease (Farooq, et. al., 2002). Exotic Newcastle disease is part of the avian paramyxovirus group, which is an enveloped, single-stranded, negative-sense RNA virus (Hietala, et. al., 2005). From September 2002 to August 2003 in Southern California, the highly contagious virus affected multiple avian species and cost $160 million to eradicate (Hietala, et. al., 2005). *Mycoplasma gallisepticum* has been estimated to reduce egg production by 16 eggs per infected hen resulting in an annual loss of 118 million to the U.S. layer industry (Carpenter, et. al., 1981). Southern California commercial layer producers lost $127 million eggs because of *Mycoplasma gallisepticum* in 1984 (Mohammed, et. al., 1987). In 2009, the FDA issued a final ruling that required shell egg producers to implement actions to prevent *Salmonella enteritidis* from contaminating eggs on the farm and to retard further growth during storage and transportation (FDA-DHHS, 2009).

Salmonellae causes 1.3 million illnesses, 15,000 hospitalizations, and 500 deaths per year in the United States (Zhao, et. al., 2008). *Salmonella enteritidis* is among the leading bacterial causes of foodborne illness in the U.S. (Mead, et. al., 1999).

A rapid and effective means to clean and disinfect infected poultry premises is necessary to recover from outbreaks, such as avian influenza, exotic Newcastle disease, *Mycoplasma gallisepticum*, and *Salmonella enteritidis* (Swayne and Akey, 2003). Establishing a procedure to reduce microbial challenges in between flocks of birds can also be used to avoid cross contamination of pathogens from one flock to another (Beutler, 2007). There are a variety of disinfectant formulations available for use, and they all have different characteristics (McDonnell and Russell, 1999). Selection of a disinfectant will depend on the cost, type of pathogen, organic load, surface material, and worker safety (Rose, et. al., 2000; Gamage, 2003). Common classes of agricultural disinfectants include aldehydes, halogens, peroxides, quaternary ammonium compounds, phenols, and oxidizers. Aldehydes (e.g. glutaraldehyde) are dialdehydes that have a broad spectrum of activity against bacteria, fungi, and viruses (Rubbo, et. al., 1967). Glutaraldehyde possess microbicidal activity which act on the outer layer of bacterial cells, causing an inhibitory action on the transport of ions across the cell wall (Bruck, 1991; Trombetta, et. al., 2002). Halogens (e.g. bleach) require little contact time, are inexpensive, and are effective against mycobacteria, fungi, and enveloped/non-enveloped viruses (Block, 2001). Peroxides (e.g. hydrogen peroxide) denature proteins, lipids, and eliminate similar organisms as the halogens with the exception of fungi (Block, 1991; Russell, 1996). Quaternary ammonium compounds (e.g. QAC,

benzalkonium chloride) are stable in storage and non-irritating to the skin (Heir, et. al., 1995). In addition to having antimicrobial properties, QACs are also used for hard-surface cleaning and deodorization (Tennent, et. al., 1989). Phenolics (e.g. carbolic acid) have antifungal and antiviral properties causing damage to the plasma membrane, which results in leakage of intracellular constituents (McDonnell and Russell, 1999). Oxidizers (e.g. potassium peroxymonosulfate) have a wide spectrum of virucidal, bactericidal, and fungicidal activity. Oxidizers have low toxicity and because of their high detergency and mode of action, can be used for effective cleaning and virucidal disinfection in a single operation (Gasparini, et. al., 1995).

Moustafa and colleagues (2009) evaluated five commercially available disinfectants commonly used in poultry facilities to test against seven selected bacterial, fungal and viral isolates under laboratory conditions. The products were analogous to the ones tested in our study. They concluded that PAA had good antimicrobial activity in the presence of organic matter, but the QAC was not as effective (Moustafa, et. al., 2009). Berrang and Northcutt (2005) examined drying as a means of lowering bacterial numbers on broiler transportation coop flooring. Drying times of 15 min, 24 h, and 48 h were tested on experimentally soiled floor coupons sprayed with water. They determined that after a 24 h drying period, no *Campylobacter*, coliforms, or *E. coli* were detected on the floor surface (Berrang and Northcutt, 2005). Berrang and Northcutt (2006) also conducted a field study to examine the effects of a commercial broiler transportation coop washing system on wastewater characteristics and bacteria recovery from cage flooring. They concluded that a significant bacterial reduction of 1.30 $\log_{10}$

cfu/cm^2 occurred on broiler transportation coop flooring during washing, but only a minimal reduction of 0.80 $\log_{10}$ cfu/cm^2 was observed when sanitizer was applied after washing. Berrang and colleagues (2011) used forced hot air at a temperature of 50°C to dry spray-washed broiler transportation coops as a potential sanitation procedure to control bacterial cross-contamination during live haul of broilers. They found that spray washing followed by 15 min of ambient air-drying time reduced the number of bacteria recovered from broiler transportation coop flooring. Ni and colleagues (2015) sprayed slightly acidic electrolyzed water (SAEW; 60 to 100 mg/L available chlorine) on cages in a layer house, which resulted in a significant reduction of 0.49 to 2.25 $\log_{10}$ cfu/cm^2 for coliforms and 0.53 to 1.13 $\log_{10}$ cfu/cm^2 for Staphylococci. The findings revealed that SAEW could potentially be used as an effective means for lowering microbial contamination on environmental surfaces in layer houses.

Spielholz (1988) originally patented the process of using an engine powered portable foam generator to apply disinfectants, but no research or data has been published evaluating the effectiveness of the system in layer barns. A compressed air foam system (**CAFS**) produces foam by mixing water, compressed air, and foam concentrate. The detergent reduces surface tension of water, which allows the water to better adhere to and cover the area it is applied to. The components of a compressed air foam system includes a centrifugal water pump, a proportioning device, an air compressor, a mixing chamber, and a control valve. Hinojosa and colleagues (2015) incorporated a compressed air foam system, which was used to apply commercially available PAA disinfectant or CHL/ALK foaming cleaner to reduce aerobic bacteria on

experimentally contaminated commercial broiler transportation coops. Significant reductions up to 5.0 $\log_{10}$ cfu were reported in the Hinojosa study. The current study followed similar methodologies to Hinojosa's study, but evaluated layer cages. We hypothesized that a CAFS may be used to quickly and efficiently apply disinfectants and cleaners to layer cages. The objective of this study was to evaluate the application of foaming disinfectants and a cleaner in layer houses, and to observe industry sanitation standard operating procedures. McDonnell and Russell's (1990) review of the literature states that lipid enveloped viruses are the least resistant to disinfectants and antiseptics, but aerobic bacteria are much more difficult to eradicate. In the current study, aerobic bacteria were used as an indicator organism to test disinfectant efficacy.

Material and Methods

Experimental Design

Two treatments were applied to each half of a house (**Table 1**). One half was treated with a disinfectant or cleaner applied via a CAFS, while the remainder of the house was treated with a disinfectant using a custom made sprayer system. A high power water rinse (HPWR) was applied with a power washer by the integrator. Pre and post treatment samples were collected on the cage floors and cup drinkers for both sides of each house per trial.

Pullet and layer houses were used as they became available. Each house had four rows of cages that were three tiers high. One house was 745' (227 m) long by 36' (10 m) wide and could hold 60,293 layers. The other house was 575' (175 m) long by 37' (11

m) wide and could hold 82,000 pullets. The CAFS unit took approximately 2 h per trial to apply 3,407 L (900 gal) of diluted product to treat half of a barn per trial. The custom spray applicator made use of 246 L (65 gallons) of diluted product to treat half of the barn per trial. The spray system took approximately 1 h to treat half of a barn. All treatments were sprayed off before post sampling to prevent residual activity.

Table 1: Field Trial Study 1- Sampling locations

	Pre-Sampling CAFS Method	Pre-Sampling Industry Method	Post Sampling CAFS Method	Post Sampling Industry Method
Cup Drinkers	16	16	16	16
Cage Floor	16	16	16	16

Products

Industry personnel conducted a pre-clean wash with water, via a pressure washer, prior to any disinfectant applications. In trials 1 and 2, a chlorinated alkaline (CHL/ALK) cleaner was applied via CAFS to one half of a house. All cages were then rinsed with a high pressure wash (HPWR) afterwards. In trials 3 and 4, a peracetic acid (PAA) disinfectant with a detergent was applied via CAFS to one half of the house, and a low glutaraldehyde (LO GLUT) disinfectant was applied with a custom made spray system to the other half of the house by the integrator (**Table 2**). In trials 5 and 6, a high glutaraldehyde (HI GLUT) disinfectant was applied via CAFS to one half of the house, and a LO GLUT disinfectant was applied with the spray system on the other half of the house.

Table 2: Field Trial Study 1- List of cleaners and disinfectants

Abbreviation	Ingredients	Diluted Concentration (v/v)	Manufacturers
PAA	-Peroxyacetic acid (5.9%) -Hydrogen peroxide (27.3%)	3.0%	Preserve International, Reno, NV
LO GLUT	-Glutaraldehyde (7%) -Quaternary ammonium compound (26%)	0.4%	EnviroTech Chemical Services Inc., Modesto, CA
HI GLUT	-Glutaraldehyde (14%) -Quaternary ammonium compound (2.5%)	1.6%	Dow Chemical Company, Midland, MI
CHL/ALK	- Potassium hydroxide -Sodium hypochlorite	3.3%	DuPont, Wilmington, DE
Detergent	-Alpha-olefin sulfonate - 2,4-pentanediol, 2-methyl-	1.0%	ICL Performance Products, St. Louis, MO

Application

Foam is composed of air, soap and water. We utilized a CAFS that can produce 1,590 L **(**420 gal) of firefighting foam per minute (Rowe Industries, Hope, AR). For each trial, 1,136 L (300 gal) of water was measured into the tank of the CAFS and mixed with a cleaner or a disinfectant. A total of 137 m (450 ft) of hoses at 3.81 cm (1.5 in), which included a 15.24 m (50 ft) hose at 2.54 cm (1 in) in diameter, were used to apply the foam to cage surfaces via a CAFS smoothbore nozzle (Task Force Tips Inc., Valparaiso, IN). The integrator used a custom made spray system (H&H Farm Machine Co., Monroe, NC) that was connected to a 757 L (200 gal) tank. The sprayer had three levels of nozzles on each side in order to spray each tier of cages in the houses.

Bacterial Recovery/Sampling

Surface swabs were collected from cage floors and cup drinkers. A 5x5 cm (2x2 in) area on the cage floors and the inside ring of the entire cup drinker was swabbed. The samples were taken by a freshly gloved hand using a sterile 5x5 cm (2x2 in) gauze that was pre-wetted with 5 mL of buffered peptone water (Difco Laboratories, Detroit, MI) in

a 118 mL (4 oz) WHIRL-PAK bag (Nasco, Fort Atkinson, WI). In order to avoid sampling overlap, all pre-treatment cages were marked with an ear tag and all post-treatment samples were taken from adjacent cages. All samples were stored in a cooler on ice after each sampling until they were processed approximately 24 h later.

Culture

Samples were homogenized by a paddle blender (Seward, Worthing, England) for 30 sec at normal speed of 230 rpm. One hundred µl was collected directly from the sample bag and spread plated onto a tryptic soy agar plate (Difco Laboratories, Detroit, MI). One mL was then removed from the sample bag and serially diluted into four additional tubes containing 9 mL of phosphate buffered saline (Sigma-Aldrich, St. Louis, MO). One hundred µl of each dilution was spread plated onto individual TSA plates. Plates were incubated at 37°C for 24 h and then counted.

Statistical Analysis

Colony forming units were logarithmically transformed ($\log_{10}$ cfu per mL) prior to analysis. Differences between pre- and post-samples were calculated to determine log reductions of each paired sample. Log reductions were subjected to a one-way ANOVA using the GLM procedure, with means deemed significantly different at $P < 0.05$ (SAS Institute Inc., Cary, NC). Due to differences with treatment application methods and volumes of material used, comparisons were not made between foam and spray applications.

Results and Discussion

A cleaner is designed to wash away organic matter on which microbes may thrive, but does not necessarily kill microorganisms like a disinfectant would (Lewis and Mclndoe, 2004). The CHL/ALK cleaner applied via CAFS did not consistently reduce bacteria on cage surfaces (**Table 3**). The only significant reduction occurred with CHL/ALK during the second repetition on the cup drinkers. The HPWR alone did not reduce bacteria on either surface. In some instances both treatments actually resulted in a statistical increase in bacteria on cage surfaces. Bean (1967) revealed that disinfectants are inhibited in reducing bacteria when applied in the presence of organic matter. The cleaner and HPWR may not have been as effective due to the presence of organic matter on manure boards below the cage floors (Ni, et. al., 2015). The application of the cleaner and water striking the organic matter may also have caused debris to splash onto the cleaned cage surfaces.

Peracetic acid applied at 3% (v/v) significantly reduced bacteria ranging from 1.7 to 2.3 $\log_{10}$ cfu/sample of aerobic bacteria (**Table 4**). Previous researchers reported that the use of CAFS with PAA disinfectant significantly reduced bacteria by 4.45 $\log_{10}$ cfu/sample on broiler transportation coops (Hinojosa, et. al., 2015). However, the coops used in Hinojosa's research were only experimentally soiled with organic matter. The paint roller application of organic matter and associated bacteria may not have adhered as well, resulting in greater reductions than would be observed in the field. Reductions were lower for the current experiment, possibly due to the higher organic load still present on cage surfaces.

The LO GLUT disinfectant applied at a 0.4% (v/v) via a custom spray system did not consistently reduce aerobic bacteria, but did cause a 0.42 to 2.15 $\log_{10}$ cfu/sample reduction for some of the cage surfaces (**Table 4**). Reasons for LO GLUT inconsistency possibly had to do with application methods, time spent applying the product, low dilution rate compared to the other products and amount of organic matter present versus the efficacy of the product.

The HI GLUT disinfectant applied at 1.6% (v/v) via CAFS had significant reductions ranging from 3.11 to 3.78 $\log_{10}$ cfu/sample of aerobic bacteria for cage surfaces (**Table 5**). The LO GLUT disinfectant applied via the custom spray system at one fourth the dilution rate of HI GLUT did not consistently show significant reductions for cage surfaces, but there was a reduction ($P < 0.05$) of aerobic bacteria on the cage floors (Table 5). The LO GLUT disinfectant contained higher concentrations of QAC, which previous research has demonstrated may result in higher bacterial resistance due to the chemical being used in the poultry industry for many years (Tennet, et. al., 1985; Gillespie, et. al., 1986; Russell, 1996; Willinghan, et. al., 1996; Sidhu, et. al., 2002; Moustafa, et. al., 2004 and Gilinsky, 2006). However, the HI GLUT product contains a greater concentration of glutaraldehyde, which has been shown to reduce bacterial loads even in the presence of organic matter (Gelinas and Goulet, 1983).

Diseases, such as avian influenza, exotic Newcastle disease, *Mycoplasmosis gallisepticum*, and *Salmonella enteritidis* are costly to the poultry industry and difficult to control. Using the CAFS to apply a disinfectant product after a disease outbreak may potentially eradicate diseases left from an infected flock and ensure a clean environment

for new birds. The data from this study suggest that the use of a commercially available PAA or HI GLUT applied with a CAFS can significantly reduce aerobic bacteria on cage surfaces.

Table 3: Field Trial Study 1- Reduction of aerobic bacteria on cage surfaces following compressed air foam system application of a cleaner and a HPWR applied via a pressure washer

Treatment	CUPS [1]Pre-Trt	CUPS Post-Trt	CUPS [2]Total Reduction	FLOORS Pre-Trt	FLOORS Post-Trt	FLOORS Total Reduction
[Trial 1] CHL/ALK	[*]7.13[a]±0.11	7.14[a]±0.11	0.00±0.00	6.34[a]±0.10	6.76[a]±0.09	0.00±0.00
HPWR	7.12[a]±0.08	7.31[a]±0.09	0.00±0.00	5.75[a]±0.09	6.50[a]±0.08	0.00±0.00
[Trial 2] CHL/ALK	[*]5.56[a]±0.13	5.22[b]±0.07	0.34±0.13	4.91[a]±0.11	5.75[b]±0.10	0.00±0.00
HPWR	5.84[a]±0.11	5.63[a]±0.10	0.21±0.14	5.41[a]±0.07	6.15[b]±0.08	0.00±0.00

[1]Log_{10} aerobic plate count

[2]Values for total reductions in aerobic bacteria recovery were calculated by subtracting post-treatment from pre-treatment samples.

[a-b]Within a rep, row values with different superscripts differ significantly ($P < 0.05$).

[*]Data are mean ± standard error; n = 16 pooled samples per treatment; log reductions were subjected to a one-way ANOVA using the GLM procedure, with means deemed significantly different at $P < 0.05$.

CHL/ALK = Chlorinated/ alkaline cleaner
HPWR = High pressure water rinse

Table 4: Field Trial Study 1- Reduction of aerobic bacteria on cage surfaces following a compressed air foam system application of peracetic acid or a 7% glutaraldehyde/26% quaternary ammonia compound applied via a custom spray system

Treatment	CUPS [1]Pre-Trt	CUPS Post-Trt	CUPS [2]Total Reduction	FLOORS Pre-Trt	FLOORS Post-Trt	FLOORS Total Reduction
[Trial 3] PAA[3]	[*]7.60[a]±0.04	5.45[b]±0.38	2.15 ±0.40	6.44[a]±0.08	4.61[b]±0.23	1.83 ±0.24
LO GLUT	7.22[a]±0.08	6.80[a]±0.18	0.42 ±0.21	6.44[a]±0.06	5.88[b]±0.13	0.56±0.13
[Trial 4] PAA[1]	[*]6.51[a]±0.17	4.24[b]±0.44	2.27±0.44	5.80[a]±0.14	4.03[b]±0.23	1.77±0.28
LO GLUT	6.32[a]±0.15	5.62[b]±0.15	0.70±0.22	5.84[a]±0.16	3.69[b]±0.41	2.15±0.34

[1]Log_{10} aerobic plate count

[2]Values for total reductions in aerobic bacteria recovery were calculated by subtracting post-treatment from pre-treatment samples.

[3]PAA mixed with a 1% foaming additive

[a-b]Within a rep, row values with different superscripts differ significantly ($P < 0.05$).

[*]Data are mean ± standard error; n = 16 pooled samples per treatment; log reductions were subjected to a one-way ANOVA using the GLM procedure, with means deemed significantly different at $P < 0.05$.

PAA = Peracetic acid
LO GLUT = 7% glutaraldehyde/26% quaternary ammonium compound

Table 5: Field Trial Study 1- Reduction of aerobic bacteria on cage surfaces following a compressed air foam system application of a 14% glutaraldehyde/2.5% quaternary ammonia compound or a 7% glutaraldehyde/26% quaternary ammonia compound applied via a custom spray system at 1.6% and 0.4% (v/v), respectively.

		CUPS		FLOORS		
Treatment	[1]Pre-Trt	Post-Trt	[2]Total Reduction	Pre-Trt	Post-Trt	Total Reduction
[Trial 5] HI GLUT	*5.47[a]±0.12	2.36[b]±0.28	3.11±0.29	5.69[a]±0.11	2.05[b]±0.17	3.64±0.14
LO GLUT	5.43[a]±0.14	5.53[a]±0.21	0.00±0.00	5.61[a]±0.10	4.77[b]±0.14	0.84±0.15
[Trial 6] HI GLUT	*6.48[a]±0.19	2.90[b]±0.31	3.58±0.30	5.66[a]±0.09	1.88[b]±0.15	3.78±0.14
LO GLUT	6.32[a]±0.20	5.98[a]±0.17	0.34±0.29	5.54[a]±0.10	5.46[a]±0.25	0.08±0.26

[1]Log_{10} aerobic plate count

[2]Values for total reductions in aerobic bacteria recovery were calculated by subtracting post-treatment from pre-treatment samples.

[a-b]Within a rep, row values with different superscripts differ significantly ($P < 0.05$).

*Data are mean ± standard error; n = 16 pooled samples per treatment; log reductions were subjected to a one-way ANOVA using the GLM procedure, with means deemed significantly different at $P < 0.05$.

HI GLUT = 14% glutaraldehyde/2.5% quaternary ammonium compound
LO GLUT = 7% glutaraldehyde/26% quaternary ammonium compound

CHAPTER III

EVALUATION OF LAYER CAGE CLEANING AND DISINFECTION REGIMENS

Description of the Problem

The objective of cleaning and disinfection (**C&D**) of poultry facilities should be to reduce bacterial contamination of agricultural surfaces and to remove organic matter that can protect organisms (Whales, et. al., 2006). Biosecurity is an essential focus for the layer industry because of the recent avian influenza (**AI**) disease outbreaks that have caused 50.4 million bird deaths and cost over 3.3 billion dollars in losses (Swayne, et. al., 2016). The layer industry was affected the greatest during the recent outbreak, which resulted in over 200 separate outbreaks across the country (Vilsack, 2015). There are concerns of potentially more disease outbreaks that could affect the layer industry, so it is vital to explore ways to reduce or prevent pathogens from entering poultry layer facilities. Preventing birds from becoming sick at an early age can result in better health and production in the long run. Singh and colleagues (1994) stated that infectious bursal disease, exotic Newcastle disease, *E. coli*, *Salmonella enteritidis* and others cause more problems for pullets between the ages of 6-11 weeks compared to sexually mature layers. *Salmonella enteritidis* (**SE**) outbreaks connected with table egg consumption started increasing in 1985, which resulted in a government inquiry (Davison, et. al., 2003). Sources of SE in commercial layers could consist of contamination from infected breeders, poultry house environment, rodents, feed, and other unknown sources of

infection (Davison, et. al., 2003). In 2009, the FDA issued a final ruling that required shell egg producers to implement actions to prevent SE from contaminating eggs on the farm and during storage and transportation (FDA-DHHS, 2009). Methods to control SE have included improved biosecurity such as C&D of pullet and layer houses in between flocks. Programs have been implemented to control rodents, flies, and other pests. Environmental testing for SE occurs when laying hens are 40 to 45 weeks of age and when a previous environmental test was positive (FDA-DHHS, 2009). Davison and colleagues (2003) evaluated two phenolics, a quaternary ammonia compound **(QAC)**, a QAC/formaldehyde product and one sodium hypochlorite detergent product to determine if SE isolates obtained from environmentally positive layer houses were resistant to commonly used disinfectant products. Laboratory tests concluded that all the disinfectants killed the SE isolates and that the isolates did not vary in their resistance to disinfectants.

There are studies on sanitation practices in poultry facilities (Davies, et. al., 2003) including broiler (Luyckx, et. al., 2015), breeder (Davies, et. al., 1996), and hatchery facilities (Moustafa, 2009), but more C&D studies need to be conducted to improve sanitation practices at caged layer farms. Huneau-Salaun and colleagues (2010) evaluated C&D programs in battery cage and reared layer houses, which included bacteriological monitoring of surfaces with contact plates. The study concluded that bacteriological monitoring with contact plates could be employed by poultry layer farm crews to help inform workers of the importance of consistency and attention to detail when following C&D protocols. A statement by Barrow (1993) indicated that poultry

house design is an important factor in preventing infection of newly housed flocks. Incorporating metal in poultry house structures along with cleanable surfaces such as plastics or other material covering wooden walls can improve C&D (WHO, 1994). Another study by Davies and Breslin (2003) discussed the importance of cleaning, disinfection, and pest control to minimize the chance of an infection being passed on from an infected flock to newly placed birds in commercial layer farms. Davies and Breslin explained that it was problematic to compare the efficacy of different disinfectants used by the farms because insufficient C&D was often due to inadequate application of the products that rendered many of the programs unsuccessful. Commercially available disinfectants used in poultry layer facilities were tested for their efficacy against selected bacteria and viruses in a study by Ruano and colleagues (2001). They found that disinfectants were efficacious against microorganisms at the manufacturer's recommendation within the first 10 minutes of contact time without organic matter. A successful biosecurity program, which regularly includes a C&D program, is one of the best methods used to reduce the level of pathogens in animal facilities (Ruano, et. al., 2001). Wang and colleagues (2010) conducted an international questionnaire filled out by 1,200 commercial layer farms on methods to decrease ectoparasites in caged poultry. They found that only 68.6% of layer facilities cleaned in between flocks and only 54.4% disinfected. Farms that did C&D in between flocks had decreased ectoparasite infestation versus facilities that didn’t. To have a successful C&D program, using the most efficacious product is a must. Not all products work the same

on different species of microbes, therefore, the disinfectant should be tested in the field for the specified application to ensure its effectiveness (Singh, et. al., 2012).

Choosing the most effective disinfectant isn't always easy since there are multiple disinfectant formulations available for use (McDonnell and Russell, 1999). There is no perfect disinfectant, so the use of commercially available products should include a judicious rotational program based on the current efficacy against the microflora present in the facilities being monitored (Doerning, 1998). Disinfectant effectiveness depends on the chemical formulation, dilution factor, contact time, the presence of organic matter, type and microbial load, temperature, pH, water hardness, surface area, and worker safety (Zander, et. al., 1997). Classes of commercially available agricultural disinfectants include aldehydes, halogens, peroxides, quaternary ammonium compounds, phenols, and oxidizers. Aldehydes have a broad spectrum of activity against bacteria, fungi, and viruses (Rubbo, et. al., 1967). Aldehydes possess microbicidal activity which acts on the outer layer of bacterial cells, causing an inhibitory action on the transport of ions across the cell wall (Bruck, 1991). Halogens are inexpensive, require little contact time, and are effective against mycobacteria, fungi, and enveloped/non-enveloped viruses (Block, 2001). Peroxides denature proteins, lipids, and eradicate mycobacteria and enveloped/non-enveloped viruses (Block, 1991). Quaternary ammonium compounds (**QACs**) are stable in storage and are used for hard-surface cleaning and deodorization (Tennent, et. al., 1989). Phenolics have antifungal and antiviral properties, causing damage to the plasma membrane, which results in leakage of intracellular constituents (McDonnell and Russell, 1999). Oxidizers are effective in

reducing viruses, bacteria, and fungi. Oxidizers have low toxicity and because of their high detergency and mode of action they can be used for effective cleaning and virucidal disinfection in a single operation (Gasparini, et. al., 1995).

The current study was conducted to evaluate commercially available agricultural products. We evaluated their efficacy to reduce common microbial species found in poultry facilities by utilizing differential plating to enumerate total aerobes, coliforms, *Staphylococcus* spp. and *Pseudomonas* spp.

Materials and Methods

Experimental Design

The first trial was completed in a small layer barn at the Texas A&M University (TAMU) Poultry Science Research, Teaching, and Extension Center. The second trial was conducted in a commercial pullet house. The first trial tested 11 treatments which were applied to cage floors, while the second study followed the same approach but had an additional treatment (**Table 1**). The house in the first study had two rows of A-frame cages that were two tiers high. The house was 142’ (43 m) long by 30’ (9 m) wide and could hold 1,024 layers. The house in the second study had four rows of H-frame cages that were three tiers high and was 575’ (175 m) long by 37’ (11 m) wide and could hold 82,000 pullets.

Sample locations were chosen by a randomized block design. Treatments for both trials, except the high pressure water rinse (**HPWR**) and the low pressure water rinse (**LPWR**), were applied via a household garden sprayer to the point of saturation

(**Table 6**). The soap or cleaner were applied to dirty cages and allowed a 10-min contact time, prior to the HPWR. Disinfectant treatments were applied after the HPWR. The LPWR was utilized to remove disinfectants from cages and prevent residual activity. Environmental swabs of cage floors were collected after treatment for both studies. Each trial took approximately 2 hours to apply treatments and collect samples.

Application

All products were measured on site and applied via a 7.6 L (2 gal) household garden sprayers to cage floors (Chapin International, Batavia, NY). The LPWR was applied with two 15.24 m (50 ft) by 1.59 cm (5/8 in) diameter garden hoses (Teknor Apex, Pawtucket, RI) attached to a metal pistol nozzle (Yardsmith, Syracuse, NY). The HPWR was applied with a 2,200 PSI / 1.9 GPM gas pressure washer (Briggs and Stratton, Wauwatosa, WI).

Table 6: Field Trial Study 2- List of treatments

Treatments	Ingredients	Dilution (oz/gal)	Manufacturer
Nothing	-NA	NA	NA
Low Pressure Water Rinse	-Water	Saturation	NA
High Pressure Water Rinse	-Water	Saturation	NA
Cleaner	-Potassium hydroxide -Sodium hypochlorite	3.0	Alfa Chem of Georgia, Inc., Ambrose, GA
Soap	-Isopropyl alcohol -Surfactants -Water	2.0	DuPont, Wilmington, DE
Aldehyde	-Glutaraldehyde (20%)	1.5	Dow Chemical Company, Midland, MI
Hydrogen Peroxide	-Hydrogen peroxide (4.3%)	2.0	Virox Animal Health, Oakville, ON
Oxidizer	-Potassium peroxymonosulfate (21.4%) -Sodium chloride (1.5%)	2.7	DuPont, Wilmington, DE
Peracetic Acid	-Peroxyacetic acid (5.9%) -Hydrogen peroxide (27.3%)	2.0	Preserve International, Reno, NV
Phenolic	-Ortho-benzyl-para-chlorophenol (10.1%) -Ortho-phenylphenol (4.9%) -Para-tertiary-amylphenol (2.5%)	0.5	Preserve International, Reno, NV
Quaternary Ammonium Compound	-Dimethyl benzyl ammonium chloride (10%) - Dimethyl ethylbenzyl ammonium chloride (10%)	0.4	Preserve International, Reno, NV
Quaternary/glutaraldehyde blend (2nd Trial Only)	-Alkyl dimethyl benzyl ammonium chloride (26%) -Glutaraldehyde (7%)	0.5	Preserve International, Reno, NV

Bacterial Recovery/Sampling

Surface swabs were collected from cage floors. For the first trial, the entire cage floor of 30x30 cm (1x1 foot) was swabbed because it was designed to house only one bird. For the second trial, a 5x5 cm (2x2 in) area on the cage floors was swabbed. The samples were taken by a freshly gloved hand using a sterile 5x5 cm (2x2 in) gauze that was pre-wetted with 5 ml of buffered peptone water (Difco Laboratories, Detroit, MI) in a 118 mL (4 oz) WHIRL-PAK bag (Nasco, Fort Atkinson, WI). All samples were stored

in a cooler on ice after each sampling until they were processed approximately 24 hours later.

Culture

Samples were homogenized by a paddle blender (Seward, Worthing, England) for 30 seconds at normal speed. One hundred microliters was collected directly from the sample bag and spread plated onto tryptic soy agar (**TSA**), MacConkey agar, Staphylococcus 110 medium, and Pseudomonas isolation agar plates (Difco Laboratories, Detroit, MI). A 0.5 mL aliquot was then removed from the sample bag and serially diluted into four additional tubes containing 4.5 mL of phosphate buffered saline (Sigma-Aldrich, St. Louis, MO). One hundred microliters of each dilution was spread plated onto individual TSA, MacConkey, Staphylococcus 110 medium, and Pseudomonas isolation agar plates. The TSA and MacConkey plates were incubated at 37°C for 24 hours, while the Staphylococcus 110 medium and Pseudomonas isolation agar plates were incubated at 37°C for 48 hours and then counted. A subset of the colonies were confirmed using the Staphaurex coagulation test (Remel Inc., Lenexa, KS), API 20 E test kit (BioMérieux, Marcy-I'Étoile, France), EnteroPluri-Test (Zona Industriale, Roseto degli Abruzzi, Italy), and Gram- staining (Becton, Dickinson and Company, Sparks, MD).

Statistical Analysis

Colony forming units (CFU) were logarithmically transformed ($\log_{10}$ cfu per mL) prior to analysis. Log reductions were subjected to a one-way ANOVA using the GLM

procedure, with means deemed significantly different at $P < 0.05$ (SAS Institute Inc., Cary, NC).

Results and Discussion

Soaps and cleaners are used in meat and poultry industries as a first step process to C&D agricultural surfaces (Salvat, et. al., 1995). Cleaning products are critical in removing organic matter, but they do not necessarily decrease microbial loads (Lewis and Mclndoe, 2004). In our findings, the soaps and cleaners did not reduce bacterial loads on cage flooring in either trials (**Tables 7 & 8**). The HPWR and LPWR treatments did not consistently reduce bacterial load on cage floors, however, both rinses reduced ($P < 0.05$) *Pseudomonas* spp. at the TAMU in trial one and *Staphylococcus* spp. at the commercial pullet house in trial two. Similarly, a study by Hinojosa and colleagues (2015) determined that the LPWR treatment alone did not significantly reduce aerobic bacteria on broiler transportation coops. The same study also evaluated the application of a HPWR prior to or after a treatment, which did not improve product efficacy by decreasing the bacterial load on broiler transportation coops. The LPWR and HPWR can be effective in removing organic materials and debris from cage floors, but not necessarily to disinfect surfaces to significantly reduce bacterial organisms (Berrang and Northcutt, 2005).

Total aerobes for trial one were reduced ($P < 0.05$) by the potassium peroxymonosulfate, peracetic acid, glutaraldehyde, and quaternary ammonium compound treatments, and in trial two aerobes were significantly reduced by all

disinfectant products (**Table 7 & 8**). In the first trial, reductions of aerobic bacteria ranged from 0.89 to 1.11 $\log_{10}$ cfu/sample and 0.67 to 1.35 $\log_{10}$ cfu/sample for the second trial when compared to the HPWR control. No disinfectant treatments in the first trial significantly decreased coliforms or *Staphylococcus* spp. when compared to the HPWR control. In trial two, reductions ($P < 0.05$) of coliforms from 0.62 to 2.54 $\log_{10}$ cfu/sample and *Staphylococcus* spp. with 1.00 to 1.71 $\log_{10}$ cfu/sample occurred for all disinfectant products when compared to the HPWR control. Glutaraldehyde and quaternary ammonium compound treatments were the only products to significantly reduce *Pseudomonas* spp. in the first trial when compared to the control of HPWR, ranging from 0.74 to 0.87 $\log_{10}$ cfu/sample. All disinfectant products in the second trial, except the hydrogen peroxide and quaternary ammonium/ glutaraldehyde blend compound, significantly reduced Pseudomonas spp. from 0.79 to 1.70 $\log_{10}$ cfu/sample when compared to the control of HPWR.

There are numerous commercially available disinfectants, and careful consideration should be taken before choosing the appropriate one. In the current study, seven disinfectant products were used and all of them significantly reduced bacterial species in one way or another. Not all disinfectants are classified as broad spectrum and should be selected for destroying specific problem-causing organisms and other concerns such as contending with organic matter (Stringfellow, et. al., 2009). Attention to detail should be taken into consideration while mixing disinfectants along with concentrations, application rates, contact times, and safety (Payne, et. al., 2005). The current study concluded that soaps, cleaners, LPWR, and HPWR were inconsistent in

reducing bacterial loads but should be included as a cleaning method in a C&D program. All agricultural disinfectants were effective in reducing bacterial organisms, illustrating that there isn't necessarily just one product that works. Implementing C&D protocols correctly and often should be the focus to keep pathogens from contaminating layer facilities.

Table 7: Field Trial Study 2- Texas A&M University Research Farm - Evaluation of cleaning and disinfection protocols on recently soiled layer cages

Treatments	TSA	MacConkey	STAPH	PSEUDO
[2]LPWR	0.00±0.00	0.45±0.31	0.27±0.25	[a]1.07±0.16
[1,2]HPWR	0.00±0.00	0.00±0.00	0.23±0.68	[a]0.70±0.19
Cleaner	0.24±0.13	0.00±0.00	0.08±0.25	0.07±0.13
Soap	0.16±0.17	0.16±0.38	0.00±0.00	0.04±0.20
Aldehyde	[a]1.11±0.18	0.72±0.29	0.45±0.36	[a]0.87±0.22
H202	0.54±0.26	0.21±0.39	0.45±0.36	0.18±0.29
Oxidizer	[a]0.97±0.23	0.47±0.43	0.87±0.21	0.45±0.24
PAA	[a]0.94±0.20	0.81±0.38	0.33±0.35	0.47±0.24
Phenolic	0.50±0.18	0.58±0.37	0.10±0.41	0.06±0.28
QAT	[a]0.89±0.19	0.52±0.30	0.40±0.37	[a]0.74±0.12

Data are mean ± standard error $\log_{10}$ reduction; log reductions were subjected to a one-way ANOVA using the GLM procedure, with means deemed significantly different at $P < 0.05$.

[1]Control for all treatments except LPWR.
[2]Compared to negative control of "Nothing".
[a]Indicates significant difference ($P < 0.05$).
LPWR; Low pressure water rinse
HPWR; High pressure water rinse
H2O2= Hydrogen peroxide
PAA= Peracetic acid
QAT= Quaternary ammonium compound
TSA= Tryptic soy agar
STAPH= *Staphylococcus* spp.
PSEUDO= *Pseudomonas spp.*

Table 8: Field Trial Study 2- Commercial Egg Integrator - Evaluation of cleaning and disinfection protocols on recently soiled pullet cages

Treatments	TSA	MacConkey	STAPH	PSEUDO
[2]LPWR	[a]0.66±0.22	[a]0.93±0.22	[a]0.81±0.01	0.16±0.05
[1,2]HPWR	0.31±0.35	0.23±0.14	[a]0.78±0.14	0.16±0.06
Cleaner	0.03±0.24	0.07±0.08	0.00±0.00	0.24±0.20
Soap	0.00±0.00	0.30±0.15	0.00±0.00	0.04±0.08
Aldehyde	[a]1.35±0.07	[a]2.54±0.00	[a]1.58±0.25	[a]1.70±0.41
H202	[a]0.67±0.07	[a]0.62±0.08	[a]1.00±0.32	0.59±0.12
Oxidizer	[a]0.91±0.31	[a]0.74±0.14	[a]1.41±0.25	[a]0.79±0.17
PAA	[a]1.01±0.08	[a]1.29±0.16	[a]1.10±0.29	[a]0.92±0.09
Phenolic	[a]1.01±0.02	[a]1.50±0.38	[a]1.24±0.35	[a]0.92±0.43
QAT	[a]0.88±0.11	[a]1.46±0.27	[a]1.71±0.25	[a]1.13±0.39
QAT/GLUT	[a]0.70±0.08	[a]0.85±0.16	[a]1.44±0.36	0.39±0.14

Data are mean ± standard error $\log_{10}$ reduction; log reductions were subjected to a one-way ANOVA using the GLM procedure, with means deemed significantly different at $P < 0.05$.

[1]Control for all treatments except LPWR.
[2]Compared to negative control of "Nothing".
[a]Indicates significant difference ($P < 0.05$).
LPWR; Low pressure water rinse
HPWR; High pressure water rinse
H2O2= Hydrogen peroxide
PAA= Peracetic acid
QAT= Quaternary ammonium compound
QAT/GLUT= Quaternary ammonium compound/glutaraldehyde
TSA= Tryptic soy agar
STAPH= *Staphylococcus* spp.
PSEUDO= *Pseudomonas spp.*

CHAPTER IV

CONCLUSION

Poultry diseases cost the poultry industry 10% to 20% in gross value of production in economic losses (USDA, 2017). The ability to identify the causes of disease losses in poultry and to recognize an emerging disease quickly is critical. Avian diseases can wipe out an entire flock of birds worth thousands of dollars to a grower, therefore having forward defenses to exclude diseases through biosecurity programs is crucial for both the growers livelihood and the well-being of the birds.

A CAFS can effectively apply disinfectants to reduce aerobic bacteria in layer cages which was observed in the first study. The cleaner or a HPWR alone were not sufficient to reduce aerobic bacteria on cage surfaces, but can be used to remove organic material before disinfectants are applied. The CAFS maybe utilized after a disease outbreak to apply products in order to eradicate diseases left from an infected flock. The data from the CAFS study suggest that the use of a commercially available PAA or HI GLUT applied with a CAFS can significantly reduce aerobic bacteria on cage surfaces.

In the second study, all of the agricultural disinfectant products were efficacious in reducing bacterial species such as total aerobes, coliforms, *Staphylococcus* spp., and *Pseudomonas* spp. Similar to the first study, the soap/cleaner and LPWR/HPWR treatments were not effective in consistently reducing bacteria on cage floors, but can be beneficial in a C&D program to remove organic material and enhance the efficacy of disinfectant products. Correctly implementing a C&D program in between flocks of

birds or after a disease outbreak can prevent pathogens from contaminating layer facilities.

Research is needed to study the application of more efficacious products with foam for increased microbial reduction on cage surfaces. With the current study of using a CAFS and a study done by Hinojosa and colleagues (2015) on using a CAFS, the application of disinfection products with foam has an increased contact time on surfaces, which increases the time for eradication of bacteria. Foam can reach hard areas for C&D and can be seen when applied to surfaces. The utilization of foam in a C&D protocol within a biosecurity program can potentially be a great asset in poultry facilities, including layer complexes. The current research focused on decreasing bacterial organisms, but further research needs to be done to study the effectiveness of agricultural products against viruses and fungal organisms. Cleaning and disinfectant products work differently depending on chemical formulation, dilution factor, contact time, the presence of organic matter, water hardness, and workers safety. Monitoring and testing of C&D products against all microorganisms can improve the health of the birds by reducing the chances of infection in poultry facilities.

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