USE OF DISINFECTANTS AND CLEANERS TO REDUCE BACTERIA ON POULTRY TRANSPORTATION COOPS WITH A COMPRESSED AIR FOAM SYSTEM

A Thesis

by

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ABSTRACT

Poultry transport coops are rarely washed and demonstrate to be a major point of broiler carcass contamination. Our laboratory hypothesized that foaming disinfectants and cleaners commonly used within processing plants may be used to clean and disinfect poultry transport coops. The objective of this study was to evaluate treatments consisting of a low-pressure water rinse (LPWR), a foaming additive alone, foaming cleaner or peroxyacetic acid with a foaming additive to reduce bacteria on broiler transport coops. A high-pressure water rinse (HPWR) applied prior to and following treatments was also evaluated. Homogenized feces was evenly applied to the floors of pre-cleaned transport coops and allowed to dry. The first study used fresh layer feces and evaluated the treatments ability to reduce aerobic bacteria from the manure. The second study added a HPWR step to determine whether this technique would reduce bacteria. In the third study, *Salmonella* Typhimurium was added to the homogenized fecal slurry to evaluate how effectively these methods reduce aerobic bacteria and *Salmonella* on coop surfaces. The field study utilized laboratory treatments proven to be most effective on freshly soiled broiler integrator coops.

All foaming treatments were applied using a compressed air foam system (CAFS) using a 1 inch fire hose. Ten minutes post-treatment, all surfaces were rinsed with a LPWR for 30 seconds to remove residual disinfectant. Samples were collected from the transport coops prior to and following treatments utilizing a flame sterilized 5 X 5 cm stainless steel template and a gauze swab pre-applied with buffered peptone

water. All samples were stomached, serially diluted, spread plated onto agar plates, incubated for 24 h at 37° C and enumerated. The foam cleaner and peroxyacetic acid with a foam additive significantly reduced ($P < 0.05$) aerobic bacteria up to 4.84 to 5.17 logs, respectively when compared to the LPWR. The addition of a HPWR following product application significantly reduced bacteria on integrator coops, in the field study, but didn't improve efficacy of our treatments in laboratory trials. These data suggest that a CAFS may be used in combination with disinfectants and cleaners to reduce bacteria on poultry transport coops.

DEDICATION

I would like to dedicate this publication to God and my family. All things are possible through Him. My parents, who are educators, have given me love and support and have always encouraged me to make goals and do my best to accomplish them. They showed my sisters and I that hard work and dedication is a must and that giving up is not an option. I'd also like to dedicate this to my three sisters who are all intelligent and strong. Each of you has been there for me along my journey through my academic career to give me love and support, and I thank you for that.

This is also dedicated to my aunts and uncles who were there with words of encouragement and praise. Your words made me proud of the goals I have accomplished and helped me feel it was possible to achieve anything I set my mind to. My grandma has been a huge role model of mine and is a constant reminder that education can never be taken away from you. You always say a prayer for me to do well in school and are there to listen to me when I need words of encouragement. I knew if you were able to receive a Master's degree during a time when not many women went to get a college degree, then surely I could.

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NOMENCLATURE

ARS	Agriculture Research Service
C	Celsius
CAFS	Compressed air foam system
cm	Centimeter
END	Exotic Newcastle disease
FC	Foam cleaner
FSIS	Food Safety Inspection Service
GBS	Guillain-Barré Syndrome
HPAI	Highly pathogenic avian influenza
HPWR	High-pressure water rinse
gal	Gallon
in	Inch
LPAI	Low pathogenic avian influenza
LPWR	Low-pressure water rinse
oz	Ounce
P	Probability
PAA	Peroxyacetic acid
ST	*Salmonella* Typhimurium
TPC	Total plate count

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CHAPTER I

INTRODUCTION

The poultry industry has continued to grow because the demand for their product has significantly increased in the last several decades. The continued growth could be attributed to the low cost when compared to beef or because of it being a healthier option now that consumers are becoming more conscious of the food they eat (Gotsis, et al., 2013). Per capita consumption of meat in the US is 123 kg, including 50 kg of poultry (Stenhouse, 2008). According to the CDC (2010), *Salmonella* and *Campylobacter* contaminated poultry products were a significant source of foodborne illness from 1998-2010 which makes poultry have the most outbreak cases for a single food commodity. The United States Department of Agriculture - Food Safety Inspection Service published a document entitled "Compliance Guideline to Reduce Levels of *Salmonella* and *Campylobacter* in Poultry," which illustrates the importance of these two pathogens to the industry. This compliance guideline is designed to help reduce foodborne illnesses caused by the consumption of poultry products which would also reduce deaths and costs associated with outbreaks (Food Safety Inspection Service, 2010).

Several hours before broilers are transported to a processing plant, feed is withdrawn to allow for the gut to partially empty its contents, which will minimize potential fecal contamination of the carcass (Papa and Dickens, 1989; Papa, 1991). Transportation of broilers has been shown to be a significant stressor, resulting in increased shedding of foodborne pathogens due to feed withdrawal, coprophagy and

depressed immune function (Stern, et al., 1995; Whyte, et al., 2001). Feed withdrawal increases crop and cecal colonization in market age broilers by *Salmonella* (Ramirez, et al., 1997) and *Campylobacter* (Byrd, et al., 1998) because of a reduction in lactic acid producing bacteria is observed in the crop when food is withdrawn (Corrier, et al., 1999b), followed by an increase in the pH of the crop (Hinton, et al., 2000), creating an environment conducive to pathogen growth. As these changes occur, an increase in coprophagy while searching for feed adds to increased *Salmonella* and *Campylobacter* colonization during feed withdrawal (Corrier, et al., 1999a).

Cross contamination of broilers may occur when flocks are placed into transportation coops that contain feces from previously transported broilers. Dirty transportation coops harbor microorganisms on floor surfaces and become a vector for cross contamination (McCrea and Macklin, 2006). A survey done by Northcutt and Jones (2004) found that only 9% of large poultry facilities clean and disinfect their transportation coops before being reused. Researchers found that catching, loading and transportation are times that cause stress which increases levels of pathogens in broilers (Mulder, 1995). Broilers can spend 3-12 hours in transportation coops which increases levels of *Salmonella* by 20-40% in the gut due to coprophagy and externally on their skin and feathers (Berrang and Northcutt, 2005b; Northcutt and Berrang, 2006; Food Safety Inspection Service, 2010).

Researchers have previously evaluated techniques to reduce the microbial load on transportation coops using a low-pressure water rinse (**LPWR**) followed by an extended drying time of 24 to 48 hours. This method has been recommended by USDA-

FSIS, however, this method may not be practical due to the limited space and availability of coops (Berrang and Northcutt, 2005a; Food Safety Inspection Service, 2010).

Our laboratory evaluated the use of foaming disinfectants and cleaners to reduce bacteria on poultry transportation coops. The addition of a high-pressure water rinse (**HPWR**) prior to or following the treatment was also evaluated to determine if it further reduced the bacterial load. *Salmonella* Typhimurium was added to fresh layer manure in one of the studies to assess how well these methods reduced not only aerobic bacteria but *Salmonella* as well. Once a good evaluation of our disinfectants was seen over several studies, our laboratory took this experimental design to a broiler processing facility for a field study utilizing contaminated coops.

CHAPTER II

LITERATURE REVIEW

Economic Impacts in Poultry

Poultry diseases have a significant impact on production and economics of the industry (Lister, 2008). The U.S. poultry industry and its customers spend millions of dollars every year on medical costs and lost income due to reportable diseases, foodborne pathogens, immunosuppressive viruses and infection costs. United States residents' medical costs in 2009 associated with Campylobacteriosis was $19 million and Salmonellosis was $49 million (Scharff, 2010). Bermudez and Stewart-Brown (2008) mention that humans can be a vehicle in which diseases are spread, "because of their mobility, duties, curiosity, ignorance, indifference, carelessness, or total concentration on current profit margin, humans constitute one of the greatest potential causes of the introduction of disease." People visit poultry farms because they could be a contract worker who is responsible for care of the birds, a neighbor to the area where the farm is located, or a visitor that is interested in seeing what a commercial farm looks like. These visitors provide potential sources for diseases that can be spread and cause outbreaks on farms (Bermudez and Stewart-Brown, 2008). Visitors to a poultry farm should be required to clean and disinfect their shoes and wear freshly cleaned clothing to prevent the spread of disease and those not following these procedures should be prevented from entering the premises. Anyone who discovers a reportable disease must report it within 24 hours of diagnosis to a veterinarian, diagnostic lab, or state

veterinarian so that proper protocol to depopulate birds with this disease may be followed (TAHC, 2012).

Salmonella and *Campylobacter* can cause infections in humans and are responsible for the majority of acute gastroenteritis cases in the world (Mulder, 1995). Finan (2010) estimates that the US spends $152 billion a year on costs associated with foodborne illnesses, this number was calculated by adding total costs associated with all pathogens. The Government Accountability Office reported in 1999 that the federal government spent $1 billion on food safety efforts and state governments spent $300 million, which illustrates the importance of making improvements to reduce foodborne illnesses that occur (General Accounting Office, 2001). Food safety is a major concern due to large economic losses caused by hospitalization and absences from work from bacterial associated enteritis in humans. However, European researchers suggest that between 50% and 80% of reported foodborne illnesses occur in the home (Scott, 1996). This could be during food preparation, due to the cross-contamination of kitchen counter tops with raw food and cooked products (Gough and Dodd, 1998).

Avian Influenza

Avian Influenza is categorized as either highly pathogenic avian influenza (**HPAI**) or low pathogenic avian influenza (**LPAI**), the differences between the classifications depends on the severity of the illness that they cause (Rebel, et al., 2011). According to the Animal and Plant Health Inspection Service (**APHIS**; 2008) a virus strain can be determined by a laboratory-based experiment; eight birds are inoculated with a test virus and if no chickens die due to the virus it is referred to as LPAI but if 6

or more chickens die, the virus is designated as HPAI which gives the virus a 75% or greater mortality rate. Clinical signs that are seen in poultry infected with LPAI include abnormalities in the respiratory, digestive, urinary and reproductive organs. Examples of the respiratory symptoms that are seen include: coughing, sneezing, rales, rattles and excessive lacrimation. Domestic chickens will display clinical signs such as huddling, ruffled feathers, depression, decreased activity, lethargy, decreased feed and water consumption and occasional diarrhea (Swayne and Halvorson, 2008). Clinical signs seen in HPAI include decreased egg production, depression, respiratory issues and a decrease in food and water consumption; however, some infected birds may not display any clinical signs. Outbreaks associated with HPAI have an overall high rate of mortality and morbidity that leads to a need for a depopulation of ill birds (Swayne and Halvorson, 2008). In 2004, estimates were made to determine how much a similar caliber of outbreak would affect the US and it was estimated to cost $100 to $200 billion dollars (McLeod, et al., 2004). A paper written by McLeod and co-workers (2004) describes four main reasons why HPAI is closely watched: 1) highly pathogenic avian influenza may become a zoonotic disease which could negatively affect human health, 2) based on the outbreak of 2003- 2004, farmers experienced severe economic losses due to reduced production, 3) costs associated with keeping the endemic disease in control and 4) migratory birds spreading virus from central Asia to Europe and Africa. Avian influenza can quickly move to other continents with an uncontrolled widespread outbreak, that in turn would be problematic for global trade due to importation restrictions (McLeod, et al., 2004). Keeping avian influenza from spreading globally is a

concern because during the pandemic of 1918 more people died from influenza than from World War I (Billings, 1997).

In 2002, five million birds in Virginia, West Virginia and North Carolina were found to have a strain of low pathogenic avian influenza (LPAI) which cost the industry $149 million (Capua and Alexander, 2004). The prevention and eradication of avian influenza is of significant economic importance to the poultry industry due to production losses and potential human health concerns. Vaillancourt and colleagues (2009) suggest that a better response plan between the poultry industry and government agencies is needed to prevent and eradicate avian influenza outbreaks.

Exotic Newcastle Disease

Exotic Newcastle disease (**END**) is also known as viscerotropic velogenic Newcastle disease (VVND; Miller et al. 2010) . Newcastle disease is caused by a virus which was first reported to have caused an outbreak in 1926 in Java, Indonesia and Newcastle-upon-Tyne, England (Kraneveld, 1926; Alexander and Senne, 2008). Exotic Newcastle disease was first seen in California in 1950 from chukars and pheasants imported from Hong Kong, and any epidemics since are thought to have occurred due to bird smuggling (Kinde, et al., 2005). Exotic Newcastle disease was reported to be in the US again in 1970 in a pet shop bird in New York City and chickens in El Paso County, Texas (Walker, et al., 1972). Towards the end of 2002, an END outbreak was reported in Southern California in small flocks of backyard birds, including some being used for illegal cockfighting which caused a spread of this disease to its neighboring states Nevada and Arizona (Nolen, 2002; Wakamatsu, et al., 2006). This outbreak infected 14

commercial poultry farms that totaled two million birds depopulated (Avian Veterinary Medicine Association, 2003). This disease can cause serious outbreaks that would require quarantine, depopulation, control and cleanup if found to be present in a poultry flock. Clinical signs for non-vaccinated chickens that are known to be infected with END may have depression, anorexia, lethargy, respiratory distress, coughing, gasping, greenish watery diarrhea and fever, but this disease is of great concern due to birds shedding the virus which may never show clinical signs (Kinde, et al., 2005). The economic loss in 1972 in California totaled $56 million and took 3 years to eradicate (Omohundro and Walker, 1973). It was estimated that if an END outbreak occurred that was uncontrolled it would cost the US $200 to $800 million for the first year (Omohundro and Walker, 1973).

Salmonella

The genus *Salmonella* is found in the *Enterobacteriaceae* family (Bennasar, et al., 2000; Grimont, et al., 2000). *Salmonella* is a Gram negative, intracellular, straight rod shaped, non-spore forming, generally motile with peritrichous flagella (Rubin and Weinstein, 1977; Kwang, et al., 1996; Gray and Fedorka-Cray, 2002; Molbak, 2005). Soil, water, food and gastro-intestinal tracts are typical areas were *Salmonella* spp. are found (Anderson and Ziprin, 2001). *Salmonella* spp. are mostly motile with the exception of serotypes of *S. gallinarium* and *S. pullorum* which are found in poultry and can be transmitted both vertically and horizontally (Grimont, et al., 2000). *Salmonella pullorum* can cause an infection known as pullorum disease and *S. gallinarum* causes an infection known as fowl typhoid which can both be controlled through vaccination or at

times positive birds are depopulated (Shivaprasad, 2000). Clinical signs that are seen in chicks and poults include anorexia, diarrhea, dehydration, weakness and high mortality. In mature fowl both pullorum disease and fowl typhoid may cause a decrease in egg production, fertility, hatchability, anorexia and increased mortality (Shivaprasad, 2000). This organism is a facultative anaerobe that can grow with or without the presence of oxygen at a pH growth range between 4.5 to 9.0. However, its most optimal pH is between 6.5 to 7.5 and a temperature of 37^{o} C although it grows from 8 to 45^{o}C (Ziprin, 1994; Garcia-Del Portillo, 2000; Hanes, 2003). *Salmonella* can grow in a high moisture environment but prefers a water activity of (*a*w) 0.93 (Garcia-Del Portillo, 2000; Gray and Fedorka-Cray, 2002) and sodium chloride (NaCl) environment between 3 to 4% and 350 mg/L of sodium nitrite (NaNO2) (Garcia-Del Portillo, 2000).

Human *Salmonella* infections traced to poultry were reported as early as 1899 (Lister and Barrow, 2008). Poultry meat and eggs are considered the primary hosts for salmonellosis (Li and Mustapha, 2002; Capita, et al., 2003; Vadhanasin, et al., 2004). The World Health Organization has released guidelines to monitor and detect *Salmonella* in poultry (Wray and Davies, 1994), which demonstrates the importance of this microorganism to the poultry industry and how it should be set as a critical control point in a company's Hazard Analysis and Critical Control Points (HACCP) because a large amount of this bacteria be an indicator of improper sanitation. Cleaning and disinfection programs for this microorganism are being set by USDA and are being improved upon by researchers (Food Safety Inspection Service, 2010). Poultry houses,

for example, are recommended to be cleaned and disinfected to reduce pathogens that may be present for site decontamination (Lister, 2008).

In 2008, *Salmonella* was the leading foodborne pathogen to be found in poultry and poultry products (Gast, 2008). The CDC (2011a) estimated in 2011 that *Salmonella* caused 1,027,561 illnesses, 19,336 hospitalizations and 378 deaths in the U.S. These data rank *Salmonella* as first in both hospitalizations and deaths and number two in highest number of illnesses for all foodborne pathogens. *Salmonella* is not a new concern for government agencies, such as the USDA- FSIS (Dubbert, 1988). Research to lower *Salmonella* has been a priority for many years and has long been studied by the USDA-Agricultural Research Service (Bailey, 1988). The CDC (2011b) reported that in 2011, 16.47 people per 100,000 population in the U.S. were linked to having a case associated with *Salmonella*.

Campylobacter

Campylobacter is a Gram negative, thermophilic, obligate microaerophilic curved rod bacterium found in the intestinal tract of poultry and other vertebrate animals (Byrd, et al., 1998; Newell and Fearnley, 2003). There are three main species of zoonotic *Campylobacter* that are found in poultry, including *Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter lari* (Evans and Powells, 2008). Campylobacteriosis causes 2.4 million human infections annually (Newell and Fearnley, 2003; Eberle and Kiess, 2012). According to the CDC, *Campylobacter* caused 845,024 illnesses, 8,463 hospitalizations and 76 deaths CDC (2011a). *Campylobacter* is ranked third in hospitalizations, fifth in deaths and fourth in foodborne illnesses. The CDC

(2011b) reported that in 2011, 14.31 people per 100,000 population were treated for Campylobacteriosis; which was a 14% increase from their 2006-2008 evaluation. *Campylobacter jejuni* is found in large numbers in layer feces which can be passed via coprophagy (Ahmed, et al., 2013). It is important to control, prevent and reduce this microorganism because it has the potential for foodborne transmission to humans. It can cause gastroenteritis in humans with symptoms such as self-limiting watery and/or bloody diarrhea, abdominal cramps and fever; however immune compromised patients conditions could be worse which would require the administration of antibiotic treatment (Mead, et al., 1999; Zhang, 2008). This microorganism is linked to poultry and poultry products and is associated with Guillain-Barré Syndrome which is an acute polyneuropathy disorder (Fields and Swerdlow, 1999). Horizontal transmission of this microorganism has been found in old litter, untreated drinking water, farm animals, domestic pets, wildlife, insects, equipment and transportation vehicles (Zhang, 2008). Reducing surface contamination of this pathogen on transportation coops through cleaning and disinfecting may eliminate cross-contamination of carcasses by this pathogen.

Microorganisms in Processing Plants

The demand for poultry products has steadily increased in the past decade with USDA projected demands for poultry as high as 46% for 2011 when compared to 2007 (Mallo, 2010; USDA, 2013). Producing large quantities of meat and eggs safely, for a growing population, becomes a significant challenge for the poultry industry (Stenhouse, 2008). Reducing the bacterial load of microorganisms entering the plant from

transportation coops could result in birds entering the plant with less organic matter on their feathers and lower the possibility for bacterial cross-contamination of carcasses (Ramesh, et al., 2004). One may hypothesize that feed withdrawal and feed changes will increase the amount of foodborne pathogens, however, Northcutt and co-workers (2003b) found that feed changes or the length of feed withdrawal did not affect levels of *Campylobacter* at pre-evisceration or evisceration which may be because birds were only transported a distance of 0.2 km which may have not been a good comparison to actually industry practices.

The picking department at a poultry processing facility tends to be an area of concern due to high numbers of bacteria present on chicken carcasses and is a source of cross contamination (Arnold, 2007). Berrang and Dickens (2000) followed six flocks and found that levels of coliforms, *Escherichia coli*, and *Campylobacter* had all dropped post scalding because birds are exposed to boiling water temperatures that reduces or eliminates pathogens but levels increased post picking due to being in contact with the rubber picker fingers that have removed feathers from all previously processed birds that may have contained feces on their bodies. The chilling department is another area in which cross-contamination may occur. For example, if a *Salmonella* positive carcass enters an immersion chiller it could potentially contaminate carcasses that are free of the pathogen and is why new methods to reduce cross-contamination are still being evaluated by researchers (Smith, et al., 2005). The Food Safety and Inspection Service has recently mandated zero tolerance for visible fecal contamination of carcasses

entering a chiller in 2012; increasing the need for improved methods to reduce carcass adulteration (Food Safety Inspection Service, 2012b).

Experiments done by Northcutt and co-workers (2003a) demonstrated that contamination on the body of the birds entering a processing facility is a critical factor to carcass bacterial counts that are found once processed. Feed withdrawal and transportation caused an increase on carcass counts for *E. coli* and *Campylobacter* recovery in pre-chill but didn't affect post-chill due to the use of chlorine (Northcutt, et al., 2003a). Cleaning of transportation coops could help reduce the bacterial load that enters a plant; which may decrease the levels of harmful microorganisms. Reducing the amount of fecal matter may help with the overall processing of the birds because of less cross-contamination.

Transportation Coop Studies

Researchers have determined that dirty transport coops harbor organisms that may contaminate subsequent flocks (McCrea and Macklin, 2006). Currently only 9% of large poultry facilities are cleaning their coops before being reused (Northcutt and Jones, 2004; Berrang and Northcutt, 2005b). Unwashed coops are a vehicle for cross contamination with approximately 250 birds per coop (Berrang, et al., 2003; Hansson, et al., 2005). Studies show that during the 3-12 hours that broiler chickens spend in transportation coops defecating they tend to have levels of *Salmonella* increase by 20-40% in the gut and externally on the skin and feathers of birds due to being moved and defecating on the cages (Berrang and Northcutt, 2005b; Northcutt and Berrang, 2006;

Food Safety Inspection Service, 2010). Birds become stressed from catching, loading and transportation and this can cause increases of pathogens (Mulder, 1995).

A study performed at four different processing facilities located in different states during all four seasons found transport coops positive with *Salmonella* before being used to pick up a broiler flock and after broilers were removed (Bailey, et al., 2001). Furthermore, *Salmonella* and *Campylobacter* at the receiving department of a processing plant was directly traced from transportation cages (Corry, et al., 2002; Slader, et al., 2002). Cross-contamination of *Campylobacter* was found to be possible when uninfected broilers were placed in transport coops that contained infected broilers; these uninfected broilers were found to be positive after transportation (Newell, et al., 2001; Berrang, et al., 2003).

Berrang and Northcutt (2005a) used recycled fiberglass flooring cut into ten 5 by 5 cm squares and then contaminated with intestinal gut contents from the colon, ceca and small intestines. Forced hot air drying of transport coops for 24 to 48 hours has proven to dry feces and kill bacteria present which may reduce cross-contamination during live haul of broilers (Berrang, et al., 2011a). Another study used gut contents from recently processed birds the addition of a cultured field strain of *Campylobacter* to contaminate their experimental coop flooring. Coop flooring squares were inoculated and allowed to dry for 60 minutes at room temperature, a LPWR and absorbent cornstarch followed by 24 hours of drying. These treated materials had no *Campylobacter* present after 24 hours of drying (Berrang, et al., 2011b). The combination of water spraying and extending drying time (24 or 48h) was also found to lower numbers of *Campylobacter*,

Coliform and *Escherichia coli* bacteria present. This study found that allowing transport coops to dry for 24h and then applying a spray wash was below their limit of detection (less than 50 cfu of *Campylobacter*, 5 cfu of *E. coli* and 5 cfu coliform) for each bacteria evaluated (Berrang and Northcutt, 2005a). Based on the research that has been done on transportation coops; many include extended drying time to lower microorganisms that may be present. As a result of coop costs and space requirements being able to allow extended drying time to all coops being used may not be a practical option for all processing plants (Berrang, et al., 2004).

Additional research on transportation coops that has been done hypothesized whether the type of flooring would affect the amount of bacterial recovery. Fiberglass was compared against wire mesh floors and levels of several microorganisms were found to be marginally higher on birds sampled with their feathers from the fiberglass flooring (Buhr, et al., 2000). Even though birds from the fiberglass floors were noticeably dirtier, their levels of bacterial recovery after being feather removal were not significantly different from the birds that were transported on wire mesh flooring (McCrea and Macklin, 2006).

One study evaluated thirteen chemical disinfectants on the surfaces of transport coops with galvanized steel flooring which suggests that halogens, phenolic, and quaternary ammonium compounds significantly reduced *Salmonella* (Ramesh, et al., 2002). Another similar study used levulinic acid and sodium dodecyl sulfate to lower counts of *Salmonella* present on chicken cages (Zhao, et al., 2011). These experiments proved that *Salmonella* can be lowered by approximately 5 or 6 logs by using levulinic

acid and sodium dodecyl sulfate. They suggested that using foaming disinfectants on transport coops may significantly reduce levels of both *Salmonella* and aerobic bacteria, which could reduce contamination of carcasses.

A recent literature search suggests that there are few procedures on how to clean and sanitize transport coops, this may be because it is currently not a requirement for poultry processing facilities to clean and sanitize their transportation coops before being reused.

Disinfectants and Cleaners

Disinfection of an area or object is needed to reduce, eliminate or prevent microbial populations and the poultry industry uses chemical applications in the live production phases for either sanitation or pest control (Eckman, 1994). Commonly used classes of disinfectants in production agriculture include alcohols, halogens, quaternary ammonium compounds, phenols, aldehydes and oxidizing agents (Smith, 2010). Disinfectants are able to act on microorganisms in two different ways: lethal action and growth inhibition. Lethal action is also known as bactericidal, fungicidal or virucidal effects. Growth inhibition of bacteria or fungi is known as bacteriostasis or fungistasis, respectively. Since killing microorganisms present on transportation coops is preferable, lethal action is the intentional endpoint when using disinfectants (Maris, 1995).The mechanism of action for all disinfectant classes are as follows: alcohols precipitate proteins and denature lipids, halogens such as bleach denature proteins, quaternary ammonium compounds denatures proteins and bind phospholipids of cell membranes, phenols denature proteins and alter cell wall permeability, aldehydes denature proteins

and alkylates nucleic acids and oxidizing agents such as peroxyacetic acid (**PAA**) denature proteins and lipids (Denyer and Stewart, 1998; Dvorak, 2005). The mechanism of action for a soap, also known as a surfactant, which is a type of detergent, can change the tension of water and can disrupt the cell membrane (Desai and Banat, 1997). Understanding the mechanism of action for all types of disinfectants is important to comprehend because different classes are needed depending on the circumstances such as the type pathogen being targeted or whether there will be organic matter present on the surface which is being disinfected.

The disinfectant PAA is commonly used in the poultry industry and is approved for use as an antimicrobial in processing chillers (Bauermeister, et al., 2008) because it has demonstrated the ability to lower numbers of *Salmonella* and *Campylobacter* present in poultry processing chillers.

In an experiment Kassaify and colleagues (2007) reported that PAA was able to kill 99.99% of *Salmonella* present in skim milk using a 0.5% PAA concentration and 100% kill was seen using a 1.0% PAA concentration. In the absence of skimmed milk, PAA at both 0.5% and 1.0% concentrations was able to eliminate all viable *Salmonella* present. This proves the efficacy of PAA based disinfectants against *Salmonella*. Rodgers and co-workers (2001) mention that all disinfectants in his study were effective when tested against strains of *Staphylococcus aureus* at a poultry hatchery but when introduced to hatchery organic matter efficacy suffered. Glutaraldehyde, phenol and quaternary ammonia were evaluated at three commercial chicken hatcheries by Willinghan and colleagues (1996) and found that *Serratia marcescens, Bacillus cereus,*

Bacillus thuringiensis, Bacillus badius, Enterococcus faecalis, Enterococcus faecium, Pseudomonas stutzeri and Enterobacter agglomerans became resistant to the use of one or more of the three disinfectant types that were used at manufacturers recommended concentrations. Resistance can be seen when the concentrations being used are no longer effective in killing viable bacteria present. Correct concentrations and exposure time for the disinfectants are important to its overall performance when the disinfectants are in an environment where organic matter may be present. The mode of action of glutaraldehyde is to alter the RNA, DNA, and protein synthesis of microorganisms (Rutala, et al., 2008). Russell (1982) mentions that pH, temperature, disinfectant concentration, presence of organic matter, presence of particular ions and other factors are important to the efficacy of antiseptics and disinfectants. The researchers in this study were not surprised with the amount of resistance to the glutaraldehyde disinfectant because of it being commonly used in hatcheries for many years. Stringfellow and co-workers (2009) also concluded that when using disinfectants, correct contact time, temperature, and amount of organic matter present plays a factor on product efficacy.

One concern that is periodically brought up in the industry is whether the inclusion disinfectants may cause resistance in microorganisms. A study by Gradel and colleagues (2005) showed that the strains of *Salmonella* and *Escherichia coli* did not become resistant to the five disinfectants between first and last isolate (January 1992-October 2001) that were used in his study when persistently applied to poultry houses . New regulations or guidelines to improve food safety for egg and meat products are implemented by USDA when new research has proven to help the industry (Food Safety

Inspection Service, 2012a). Payne and co-workers (2005) evaluated a phenolic compound, a nascent oxygen compound and a compound that contained potassium peroxymonosulfate/sodium chloride as the active ingredients and applied these disinfectants to the floors of poultry houses and found significant reductions in aerobic and yeast and mold plate counts. These trials were done in a laboratory and at a commercial broiler house for the field trials to prove that similar results could be achieved.

Foam

A typical poultry farm can easily contain 150,000 birds or more and in the case of an outbreak, using firefighting foam to depopulate could be a possible alternative to currently approved methods for the humane euthanasia of poultry (Dawson, et al., 2005; Raj, 2008). The euthanization of a mass production of poultry due to a disease outbreak, natural disaster or structural damage to the facility is needed (Caputo, et al., 2013). The use of water-based foam to depopulate poultry was approved by USDA-APHIS and the American Veterinary Medical Association in 2006 (American Veterinary Medical Association, 2007). Foam was approved because of six reasons which include: being readily available, environmentally friendly, biodegradable, compatible with carcass disposal methods, minimum irritation to poultry and not a significant human health risk (American Veterinary Medical Association, 2007). Comparisons of gas euthanasia of birds versus a water-based depopulation foam suggest that CO_2 and water-based foam were very similar in effectiveness but the water-based foam was more effective than argon-CO_2 gassing (Alphin, et al., 2010). This new method has also been tested to

euthanize floor-reared broilers with a compressed air foam system and found that using these methods would be much less labor intensive, but has currently not been seen to depopulate poultry in the industry (Benson, et al., 2007). Euthanizing a large population of commercial broilers led to the interest in using this method on ducks and researchers found that this was also a practical method to depopulate (Benson, et al., 2009; Caputo, et al., 2013). Caputo and co-workers (2012) found that the combination of foam with atropine used together produced faster rates of unconsciousness in the ducks compared to foam or CO_2 gas used independently. Depopulation using a water-based foam has also been tested on a turkey population and proved to be another valid option for an emergency case to depopulate a flock (Rankin, 2010). Flory and Peer (2010) found that euthanizing turkeys with firefighting foam is a successful way to depopulate.

Currently firefighting foam has only minimally been used to depopulate poultry operations but has not been evaluated to clean and disinfect poultry transportation coops. The addition of a compressed air foam system (**CAFS**) is a new method that has been shown to be a great way to effectively and rapidly depopulate birds (Benson, et al., 2007) but using it to apply disinfectants and cleaners would be a new method that the poultry industry may implement. A CAFS is an apparatus that may be used with the addition of a disinfectant to generate compressed air foam. The additional use of a HPWR step is thought to be effective in reducing microorganisms found on poultry transportation coops due to previous research that demonstrates that a LPWR can significantly reduce bacteria present on transportation coops (Berrang and Northcutt, 2005b). Cleaning and disinfecting poultry transportation coops has been previously

studied by Berrang and co-workers but the addition of using a CAFS to apply disinfectants and cleaners is novel.

CHAPTER III

USE OF FOAMING DISINFECTANTS AND CLEANERS TO REDUCE AEROBIC BACTERIA ON POULTRY TRANSPORT COOPS

Introduction

Transportation coops have been shown to be a vehicle for cross-contamination because of birds defecating and shedding microorganisms on them during transportation (Hansson, et al., 2005). Mulder (1995) described transportation as a stressor for poultry. Broilers may spend from 3 to 12 hours in transport coops before being processed, which increases their levels of *Salmonella* due to these environmental stressors weakening the immune system (Berrang and Northcutt, 2005b; Northcutt and Berrang, 2006; Food Safety Inspection Service, 2010). An animal's immune system causes the lymphatic tissues and organs to regress and as a result they become sensitive toward pathogens (Fillion, et al., 1984; Tufft and Nockles, 1991).

Disinfectants such as peracetic acid (**PAA**) are currently being used in poultry chillers at processing plants because of its ability to reduce microorganisms such as *Campylobacter* and *Salmonella* (Bauermeister, et al., 2008). *Campylobacter* and *Salmonella* are concerns to the poultry industry because they are commonly found in poultry products and poultry is the highest single food commodity that causes foodborne illness (CDC, 2011a). Guidelines are written to prevent and eliminate microorganisms such as *Campylobacter* and *Salmonella* in the poultry industry (Food Safety Inspection Service, 2010). Microorganisms have been extensively investigated in multiple areas of

a poultry processing plant to better understand the areas of processing where the highest microbial levels exist (Berrang and Dickens, 2000). Bacterial levels increase in the picking department because picking fingers come into contact with feathers that at times have organic matter and microorganisms which may cross-contaminate subsequent carcasses (Arnold, 2007). Reducing the amount of organic matter and the associated microbes entering the plant from the transportation coops would lower possible cross-contamination (Ramesh, et al., 2004).

It is currently not a requirement for broiler integrators to clean and disinfect poultry transportation coops before being reused, which is why cross-contamination is a concern. Broilers determined to be negative for *Campylobacter* become positive post-transportation in coops used for transport of *Campylobacter* positive flocks (Berrang, et al., 2003). Other investigators have also suggested that transportation coops may be a source of cross contamination (McCrea and Macklin, 2006). Allowing transportation coops to be washed followed by an extended drying time of 24 to 48h has been evaluated and found to be successful in reducing numbers of microorganisms present. Although these methods may not be a practical for use in the entire industry since this would require more available coops and a large amount of space for drying (Berrang and Northcutt, 2005a).

Firefighting foam is approved for use in the poultry industry to depopulate birds during a reportable disease outbreak (American Veterinary Medical Association, 2007). A common practice to depopulate broilers is to euthanize using CO_2 but this method requires catching all birds and placing in a gas chamber. Benson and co-workers (2007)

concluded that foam is a quick and successful alternative method to depopulate broilers that is less labor intensive. Using a compressed air foam system (**CAFS**) to apply disinfectants or cleaners in foam is a new method that is thought to be a possible approach in the efforts to disinfect but has not yet been evaluated. A CAFS can be an efficient means to disinfect and sanitize poultry transportation coops because it of its great velocity, good contact time and its ability to adhere well to its surface. Minimal previous published research studies have been performed utilizing a CAFS with the exception of it being used to depopulate poultry operations (Benson, et al., 2007).

The objective of these studies was to evaluate the application of disinfectants with a foam additive (**FA)** or a foaming cleaner (**FC**) on bacteria present on poultry transportation coops and to determine if a high-pressure water rinse (**HPWR**) prior to or following foam application improves efficacy. We hypothesized that the application of disinfectants or cleaners with foam using a CAFS will significantly reduce aerobic bacteria on broiler transport coops.

Materials and Methods

Experimental Design

Lab Trial 1 - Peroxyacetic Acid – Aerobes

Lab trial 1 utilized three transportation coops, with each one representing a different treatment. Treatments consisted of: 1) low-pressure water rinse (**LPWR**); 2) FA alone; and 3) peroxyacetic acid with a foaming additive (**PAA+FA**).

Lab Trial 2 - Foam Cleaner - Aerobes

Lab trial 2 utilized three transportation coops, with each one representing a different treatment. Treatments consisted of: 1) LPWR; 2) FA alone; and 3) FC.

Lab Trial 3 - Peroxyacetic Acid with a High-Pressure Water Rinse - Aerobes

Lab trial 3 utilized four transportation coops, with each one representing a different treatment. Treatments consisted of: 1) LPWR; 2) PAA+FA; 3) a HPWR step followed by the PAA+FA; and 4) PAA+FA followed by a HPWR step.

Lab Trial 4 - Foam Cleaner with a High-Pressure Water Rinse - Aerobes

Lab trial 4 utilized four transportation coops, with each one representing a different treatment. Treatments consisted of: 1) LPWR; 2) FC; 3) a HPWR step followed by the FC; and 4) FC followed by a HPWR step.

The control for these studies was a LPWR which involved the use of a standard garden hose to rinse each of the ten compartments of the transportation coop. The standard garden hose was moved from the left side to the right side of each compartment which took less than 30 seconds, in order to perform the LPWR. All treatments were given a 10-minute contact time and were followed by a LPWR of the transportation coops to remove any potential residual chemicals. The concentrations that were used for all trials were the maximum concentrations recommended by the manufacturers. The HPWR was achieved by using a power washer (Briggs & Stratton Elite Series, Milwaukee, WI) for 1 minute at 3000 psi on each transportation coop.

Cleaners and Disinfectants

The FC used in specified lab trials was an alkaline/chlorine based FC (Chlor-A-Foam® XL; DuPont, Wilmington, DE) which was used at 118.29 mL/L (4 oz/gal) concentration. This product contained its own foaming agent so a FA was not added to this product.

The disinfectant (Peraside™; Enviroguard Sanitizer®; Rochester, NY) that was used in specified trials has two main components; peroxyacetic acid and hydrogen peroxide, which was used at a 118.29 mL/L (4 oz/gal) concentration. This product did not contain its own foaming agent so a FA was added to this product when used. The FA (Perafoam®; Enviro Tech Chemical Services INC, Modesto, CA) was added at a 1% concentration.

Compressed Air Foam System (CAFS)

Foam is composed of air, soap and water. We utilized a CAFS that can produce 2,271.25 L **(**600 gallons) of firefighting foam per minute. For each trial, 189.27 L (50 gallons) of tap water was measured into the tank of the CAFS followed by 5.92 L (200 oz) of FC or 5.86 L (198 oz) of PAA with 59.15 mL (2 oz) of the FA (PAA+FA). A 2.54 cm (1 in) fire hose was used to apply the foam from the CAFS to the contaminated coops.

Transportation Coops

Four transportation coops (Bright Coop, Nacogdoches, TX) were obtained from a local broiler integrator for these trials. Each coop had ten holding compartments in a configuration of two columns with five rows. Each coop represented an experimental

unit/treatment. During experiments ten pre-treatment and ten post-treatment samples were taken from each transportation coop.

Fecal Slurry

Feces were collected from single combed white Leghorn chickens (Hy-Line, Bryan, TX) housed at the Texas A&M University Poultry Research Center. Five hundred grams of feces and one liter of tap water was mixed, and filtered to remove large particulates and feathers to make a homogeneous slurry.

Paint Roller Application

Once the homogenous fecal slurry was filtered, it was placed in a paint roller tray and a clean paint roller was used to apply the slurry onto the entrance of each compartment at a width equivalent to the length of one roller (23 cm). The slurry applied onto the transportation coops was given an hour dry time to simulate minimum industry conditions.

Bacterial Recovery/Sampling

Samples were taken from each of the ten compartments of the transportation coops after one-hour of drying time. The samples were taken using a sterile 5 by 5 cm gauze that was pre-applied with buffered peptone water in a 4 oz WHIRL-PAK® bag (Nasco® Fort Atkinson, Wisconsin) using a 5 by 5 cm stainless steel template that was soaked in 100% ethanol and flame sterilized. In order to avoid sampling overlap all pre-treatment samples were taken from the left side of each compartment and all post-treatment samples were taken from the right.

Culture

Samples were kept in the 4 oz WHIRL-PAK® bags and homogenized by stomacher blender (Seward® Bohemia, NY) for 30 seconds at normal speed. The series of 10-fold dilutions were performed into Butterfield's dilution tubes, plated onto tryptic soy agar (Difco Laboratoies, Detroit, MI) and incubated for 24 hours at 37˚ C for a final concentration of 1:4 x 10^6.

Statistical Analysis

Levels of bacterial recovery data were subjected to a one-way ANOVA using the GLM procedure, with means deemed significantly different at $P < 0.05$ and separated using Duncan's multiple range test (SPSS, 2010).

Results and Discussion

The objective of lab trial 1 was to evaluate if PAA+FA could reduce aerobic bacteria on transportation coops (Table 1). Significant reductions (4.17 and 4.77 $\log_{10}$, respectively) of aerobic bacteria were observed from coops treated with PAA+FA in both replications. The FA and the LPWR treatments were statistically similar in both replications, suggesting that the addition of a detergent did not reduce aerobic bacteria as compared to the LPWR control. These data suggest that the use of PPA+FA followed by a LPWR significantly reduced aerobic bacteria present when compared to a LPWR used alone. Berrang and Northcutt (2005a) also evaluated a LPWR as a method to reduce *Campylobacter*, coliforms and *Escherichia coli* on transportation coops. The source of bacterial contamination in this broiler transportation study was obtained from recently slaughtered broiler gut contents that were thoroughly mixed before smearing a

thin layer onto their coop flooring squares and allowed a 60-minute dry time. The source in which to contaminate the surface of our transportation coops was different but both studies applied thin layers of the contaminant to the transportation floorings. However, Berrang and Northcutt (2005a) found that a LPWR significantly reduced bacterial counts in their studies. Further, the addition of a quaternary ammonium chloride compound or sodium hypochlorite didn't cause additional reductions.

Table 1: Lab Trial 1- Peroxyacetic Acid- Aerobes.
All treatments were given a 10-minute contact time and were followed by a LPWR of the transportation coops to remove any residual chemical. Values for reductions in aerobic bacteria recovery were calculated by subtracting post-treatment from pre-treatment samples.

Treatment[1]	Replication 1 Log_{10} reductions aerobic plate count	Replication 2 Log_{10} reductions aerobic plate count
LPWR	*$1.73^{b} \pm 0.52$	$1.76^{b} \pm 0.35$
FA	$2.09^{b} \pm 0.69$	$2.17^{b} \pm 1.18$
PAA+FA	$4.17^{a} \pm 1.47$	$4.77^{a} \pm 0.91$

[1]LPWR= Low-pressure water rinse; FA= Foam additive; and PAA+FA= Peroxyacetic acid with a foam additive
[a-b]Column values with different superscripts differ significantly ($P < 0.05$).
*Data are mean ± standard deviation log_{10} reduction

The limited effect of the disinfectants could be due to the presence of excessive organic matter or limited contact time (5 minutes) on the transportation coops. Stringfellow and co-workers (2009) concluded that when using disinfectants, correct contact time,

temperature, and amount of organic matter present significantly affects the effectiveness of the antimicrobial compounds.

The objective of lab trial 2 was to evaluate if a FC could reduce aerobic bacteria on transportation coops (Table 2). Observations from replication 1 revealed that a similar reduction of aerobic bacteria came from all three treatments and no significant differences were observed. In replication 2, results show that the treatment using the FC and the FA had a similar statistical reduction of aerobic bacteria (1.94 and 2.27 logs, respectively). The LPWR had a 0.79 log reduction of aerobic bacteria, which was statistically lower than the FA and FC.

Table 2: Lab Trial 2- Foam Cleaner- Aerobes.
All treatments were given a 10-minute contact time and were followed by a LPWR of the transportation coops to remove any residual chemical. Values for reductions in aerobic bacteria recovery were calculated by subtracting post-treatment from pre-treatment samples.

Treatment[1]	Replication 1 Log_{10} reductions aerobic plate count	Replication 2 Log_{10} reductions aerobic plate count
LPWR	*0.48 ± 0.42	0.79^{b} ± 0.53
FA	0.72 ± 0.76	1.94^{a} ± 0.71
FC	1.00 ± 0.95	2.27^{a} ± .057

[1]LPWR= Low-pressure water rinse; FA= Foam additive; and FC=Foam Cleaner
[a-b]Column values with different superscripts differ significantly ($P < 0.05$).
*Data are mean ± standard deviation log_{10} reduction

The objective of lab trial 3 was to investigate a HPWR step prior to or following PAA+FA and evaluate whether this additional step was an added benefit. Replication 1 used PAA+FA (Table 3) and results showed all coops treated with PAA+FA alone or with the HPWR prior to or following the treatment were all statistically similar ($P < 0.05$) in achieving reductions in aerobic bacteria, which ranged from 4.37 to 5.17 logs of TPC. The LPWR was consistently associated with the lowest reduction of aerobic bacteria (1.16 logs) when compared to the PAA+FA treatments.

In replication 2 (Table 3) all coops treated with PAA+FA alone or with the HPWR, prior to or following the treatment, were shown to be statistically similar ($P < 0.05$) in the reduction of aerobic bacteria. Significant reductions of aerobic bacteria from the transport coops treated with PAA+FA alone or with the HPWR prior to or following the treatment ranged from 3.96 to 5.06 logs. The LPWR was consistently associated with the lowest reduction of aerobic bacteria (1.33 logs).

The objective of lab trial 4 was to include a HPWR step prior to or following the FC and evaluate whether this additional step improved efficacy. The last lab trial utilized the FC against aerobic bacteria (Table 4) results in replication 1 show that the coop treated with the HPWR followed by the FC (3.60 logs) was statistically different ($P < 0.05$) from the coop treated with the FC following the HPWR and the LPWR, but not different from the FC used alone (3.10 logs). The FC used alone was also statistically similar to the FC followed by the HPWR which had a 2.82 log reduction of TPC. The lowest reduction came from the LPWR at 1.11 logs of aerobic bacteria.

Table 3: Lab Trial 3- Peroxyacetic Acid with a High-Pressure Water Rinse-Aerobes.
All treatments were given a 10-minute contact time and were followed by a LPWR of the transportation coops to remove any residual chemical. Values for reductions in aerobic bacteria recovery were calculated by subtracting post-treatment from pre-treatment samples.

Treatment[1]	Replication 1 Log_{10} reductions aerobic plate count	Replication 2 Log_{10} reductions aerobic plate count
LPWR	*$1.16^{b} \pm 0.35$	$1.33^{b} \pm 0.57$
PAA+FA	$5.17^{a} \pm 1.21$	$3.96^{a} \pm 1.56$
HPWR followed by PAA+FA	$4.37^{a} \pm 1.31$	$4.09^{a} \pm 2.01$
PAA+FA followed by HPWR	$4.95^{a} \pm 1.33$	$5.06^{a} \pm 1.79$

[1]LPWR= Low-pressure water rinse; PAA+FA= Peroxyacetic acid with a foam additive, and HPWR= High-pressure water rinse
[a-b]Column values with different superscripts differ significantly ($P < 0.05$).
*Data are mean ± standard deviation log_{10} reduction

Replication 2 (Table 4) revealed that all treatments using the FC alone or with a HPWR, before or following treatment, had a statistically significant difference ($P < 0.05$) from the LPWR that ranged from (3.46 to 3.71 logs, respectively). The lowest reduction came from the LPWR at 0.71 logs of TPC. Berrang and Northcutt (2005a) evaluated reductions of *Campylobacter*, coliforms and *Escherichia coli* on transportation coop flooring that had been allowed to dry for 15 minutes, 24 hours, or 48 hours followed by a LPWR. They found that a LPWR reduced all bacteria when compared to a control. The use of a disinfectant with an additional HPWR step had not been

evaluated in reducing bacteria on transportation coops until evaluated in the current trials. However, the HPWR did not further improve efficacy in all lab trials.

Table 4: Lab Trial 4- Foam Cleaner with a High-Pressure Water Rinse- Aerobes. All treatments were given a 10-minute contact time and were followed by a LPWR of the transportation coops to remove any residual chemical. Values for reductions in aerobic bacteria recovery were calculated by subtracting post-treatment from pre-treatment samples.

Treatment[1]	Replication 1 Log_{10} reductions aerobic plate count	Replication 2 Log_{10} reductions aerobic plate count
LPWR	*$1.11^{c} \pm 0.79$	$0.71^{b} \pm 0.61$
FC	$3.10^{ab} \pm 0.66$	$3.46^{a} \pm 0.61$
HPWR followed by FC	$3.60^{a} \pm 1.04$	$3.71^{a} \pm 1.25$
FC followed by HPWR	$2.82^{b} \pm 0.70$	$3.46^{a} \pm 0.55$

[1]LPWR= Low-pressure water rinse; FC= Foam cleaner; and HPWR= High-pressure water rinse
[a-c]Column values with different superscripts differ significantly ($P < 0.05$).
*Data are mean ± standard deviation log_{10} reduction

This may be because transportation coops that were used for this study did not have a great deal of organic matter present on them. An actual broiler processing facility would have organic matter in a larger quantity and could be substantially different from the homogeneous fecal slurry used for the laboratory studies. The organic matter used for these studies had water added and was filtered to remove large particulates to avoid

sampling variability. The technique to reduce large particulates from the fecal slurry may be why coops that were cleaned with a disinfectant or with a HPWR step did not alter the results from the different coops evaluated. The amount of organic matter present on a surface may affect the efficacy of the disinfectant used, which is one potential reason why these studies may not have revealed a difference with the disinfectant used alone or with a HPWR step. In these trials the organic matter used may have possibly been present in too thin of a layer. The current study did not prove that the bacteria present were killed on the coops or whether the bacteria were physically washed away. Regardless, bacteria levels were reduced or removed. As such, this observation may be irrelevant since the bacteria is no longer present on the transportation coops that tend to be a vehicle for cross-contamination.

The bacterial load in a field setting is different in quantity when compared to the fecal slurry made for these laboratory studies and a HPWR step may be more beneficial in lowering the amount of bacteria present. According to Dvorak (2005), removal of all organic matter prior to disinfection becomes essential in the efficacy of the disinfectant due to the fact that the organic matter acts as a barrier to the microorganisms present.

CHAPTER IV

USE OF FOAMING DISINFECTANTS AND CLEANERS TO REDUCE AEROBIC BACTERIA AND *SALMONELLA* ON POULTRY TRANSPORT COOPS

Introduction

Transportation coops are shown to be a vector for cross-contamination because of birds defecating and shedding pathogens on them during the 3-12 hours that transportation and holding takes before birds are processed (Hansson, et al., 2005). Transportation coops contain organic matter and microorganisms left by previously transported flocks (McCrea and Macklin, 2006). *Salmonella* levels can increase by 20 to 40% during loading, transportation and holding before being processed (Berrang and Northcutt, 2005b; Northcutt and Berrang, 2006; Food Safety Inspection Service, 2010). Transportation is a known and studied stress factor involved in the poultry industry and is why studies show increasing levels of microorganisms during this event (Mulder, 1995). Poultry transportation coops are not required to be cleaned and disinfected prior to reuse, which may be a possible reason for it being a concern for cross-contamination (Berrang, et al., 2003; McCrea and Macklin, 2006). Researchers have found that broilers determined to be negative for *Campylobacter* become positive post-transportation in coops used for transport of *Campylobacter* positive flocks (Berrang, et al., 2003). Research has been done to evaluate reductions in bacteria present on transportation coops by washing and allowing an extended drying time. This was found

to be successful but may not be a practical method for the industry since this would require more available coops and a large amount of space for drying (Berrang and Northcutt, 2005b).

Campylobacter and *Salmonella* are a concern within the industry because their common presence in poultry products that are high in foodborne illness from consumption of poultry and poultry products (CDC, 2011a). Disinfectants, such as PAA, are currently used in processing plant chillers because of their ability to reduce microorganisms such as *Campylobacter* and *Salmonella* (Bauermeister, et al., 2008). Guidelines to control and prevent these two microorganisms have been written and are in place for the poultry industry (Food Safety Inspection Service, 2010). Poultry processing plant departments have been evaluated to analyze levels and assess where high loads of pathogens are found (Berrang and Dickens, 2000). Mechanical feather removal within the processing plant is one area were the bacterial load increases because of picker fingers contacting feathers with high levels of organic matter containing microorganisms that may possibly cross-contaminate carcasses (Arnold, 2007). Lowering the amount of microorganisms and organic matter entering the plant from the transportation coops should result in less organic matter on feathers and lower possibility for cross-contamination (Ramesh, et al., 2004).

The poultry industry may utilize firefighting foam to depopulate birds during a reportable disease outbreak. This technique has been conditionally approved by the American Veterinary Medical Association and USDA-Animal Plant Health Inspection Service (American Veterinary Medical Association, 2007). Benson and colleagues

(2007) concluded that foam is a quick and successful alternative method to depopulate broilers that is less labor intensive and meets all twelve criteria for an approved euthanasia. Using a compressed air foam system (**CAFS**) may be an efficient way to disinfect and sanitize poultry transportation coops. Researchers observed that this technique washes surfaces due to the velocity of the foam, increases contact time and improves adherence to surfaces. The authors have not found any publications utilizing CAFS for disinfection and sanitization in production agriculture. However, the food industry does utilize foaming disinfectants and cleaners to reduce microbial surface contamination, suggesting that a scalable approach using CAFS has potential.

The objective of the current study was to evaluate the disinfection of poultry transportation coops using a foam cleaner (**FC**), peroxyacetic acid with foam (**PAA+FA**), or PAA+FA with a high-pressure water rinse **(HPWR)** prior to or following the foam application on aerobic bacteria and *Salmonella* recovery. The field study was conducted at a commercial poultry processing facility. This trial evaluated PAA+FA alone and with a HPWR prior to the foam application to evaluate aerobic bacteria and / or *Salmonella* present on poultry transportation coops. We hypothesized that the application of disinfectants or cleaners with foam using the CAFS will significantly reduce *Salmonella* and aerobic bacteria on broiler transport coops.

Materials and Methods

Experimental Design

Lab Trial 1 - Peroxyacetic Acid with a High-Pressure Water Rinse - Aerobes and *Salmonella* Recovery

Lab trial 1 utilized four transportation coops, with each one representing a different treatment. Treatments consisted of a: 1) low-pressure water rinse (**LPWR**); 2) PAA+FA; 3) HPWR step followed by the PAA+FA; and 4) PAA+FA followed by a HPWR step.

Lab Trial 2 - Foam Cleaner with a High-pressure Water Rinse - Aerobes and *Salmonella* Recovery

Lab trial 2 utilized four transportation coops, with each one representing a different treatment. Treatments consisted of: 1) LPWR; 2) FC; 3) HPWR step followed by the FC; and 4) FC followed by a HPWR step.

Field Trial - Peroxyacetic Acid with a High-Pressure Water Rinse – Aerobes

The field trial was conducted at a broiler processing facility and utilized three transportation coops. Treatments consisted of: 1) LPWR; 2) PAA+FA; and 3) a HPWR step followed by PAA+FA.

The control for these studies was the LPWR, which involved the use of a standard garden hose to rinse each of the ten compartments of the transportation coop. The standard garden hose was moved from the left side to the right side of each compartment which took less than 30 seconds to perform the LPWR. All treatments were given a 10-minute contact time and followed by a LPWR of the transportation coops to remove any

chemical residue. The concentrations that were used for all studies were the maximum concentrations recommended by the manufacturers. The HPWR used a (Briggs & Stratton Elite Series, Milwaukee, WI) power washer for 1 minute at 3000 psi on each transportation coop.

Cleaners and Disinfectants

The FC that was used in specified lab trials was an alkaline/chlorine based FC (Chlor-A-Foam® XL; DuPont in Wilmington, DE) and it was used at a 118.29mL/L (4 oz/gal) concentration. This product contained its own foaming agent so a foam additive (**FA**) was not added to this product when used.

The disinfectant (Peraside™; Enviroguard Sanitizer® in Rochester, NY) that was used in specified trials was also used at a 118.29mL/L (4 oz/gal) concentration. This product did not contain its own foaming agent so a FA was added to this product when used. The FA (Phos-chek®; ICL Performance Products LP, St. Louis, MO) was added at a 1% concentration.

Compressed Air Foam System (CAFS)

Foam is composed of air, soap and water. We utilized a CAFS that can produce 2,271.25 L **(**600 gallons) of firefighting foam per minute. For each trial, 189.27 L (50 gallons) of tap water was measured into the tank of the CAFS followed by 5.92 L (200 oz) of FC or 5.92 L (198 oz) of PAA with 59.15 mL (2 oz) of the FA (**PAA+FA**). A 2.54 cm (1 in) fire hose was used to apply the foam from the CAFS to the contaminated coops.

Transportation Coops

Four transportation coops (Bright Coop, Nacogdoches, TX) were obtained from a local commercial broiler integrator for experimental purposes. Each coop represented an experimental unit/treatment and has ten holding compartments in a configuration of two columns with five rows. During experiments ten pre-treatment and ten post-treatment samples were taken from each transportation coop.

The field study utilized three transportation coops containing market-age broilers that had defecated throughout the coops during transport to the processing plant.

Fecal Slurry

Feces were collected from single combed white Leghorn chickens (Hy-Line, College Station, TX) housed at the Texas A&M University Poultry Research Center. Five hundred grams of organic matter, 500 mL *Salmonella* Typhimurium (**ST**) (Corrier, et al., 1990) and 500 mL of tap water was mixed. The ST was cultured in tryptic soy broth (Difco Laboratories, Detroit, MI) for 24 hours at 37° C and passed every eight hours to spike the fecal slurry before being blended and homogenized.

The final study, at the processing facility, did not utilize the homogeneous fecal slurry method since the transport coops were recently contaminated by commercial broiler chickens.

Paint Roller Application

The homogenous fecal slurry was blended and placed in a paint roller tray and a clean paint roller was used to apply the slurry onto the entrance of each compartment at a width equivalent to the length of one roller (23 cm). The slurry applied onto the

transportation coops was given allowed a 30 minute dry time to simulate industry conditions.

Bacterial Recovery/Sampling

Samples were taken from each of the ten compartments of each transportation coop after 30 minutes of drying time. The samples were collected using a sterile 5 by 5 cm gauze padwhich was pre-soaked with XX mL of buffered peptone water and stored in a 4 oz WHIRL-PAK® bag (Nasco® Fort Atkinson, Wisconsin). A 5 by 5 cm stainless steel template was soaked in 100% ethanol and flame sterilized between samples. In order to avoid sampling overlap, all pre-treatment samples were taken from the left side of each compartment and all post-treatment samples were taken from the right.

Culture

Samples were kept in the 4 oz WHIRL-PAK® bags and homogenized by stomacher blender (Seward® Bohemia, NY) for 30 seconds at normal speed. The series of 10-fold dilutions were performed into Butterfield's dilution tubes, plated onto tryptic soy agar (Difco Laboratoies, Detroit, MI) and incubated for 24 hours at 37˚ C for a final concentration of 1:4 x 10^6.

For lab trials 1 and 2 the addition of Xylose-Lysine-Tergitol 4 (XLT4) (Difco Laboratories, Detroit, MI) plates were used to evaluate *Salmonella* bacterial recovery and were plated from the same Butterfield's dilution tubes then incubated for 48 hours at 37˚C for a final concentration of 1:4 x 10^6. Sample WHIRL-PAK® bags were incubated for 24 hours at 37˚C then 100 mL of each sample were transferred into corresponding

Rappaport - Vassiliadis (RV) *Salmonella* enrichment broth (Difco Laboratories, Detroit, MI). The RV tubes were incubated for 24 hours at 37° C and struck onto XLT4 plates and incubated for 24 hours at 37° C to determine how many positive samples were detected through selective enrichment.

Statistical Analysis

Bacterial recovery data were subjected to a one-way ANOVA using the GLM procedure, with means deemed significantly different at $P<0.05$ and separated using Duncan's multiple range test (SPSS, 2010).

Results and Discussion

The objective for lab trial 1 was to spike layer feces with *Salmonella* Typhimurium and evaluate whether a HPWR step prior to or following the PAA+FA treatment would be an added benefit in reducing aerobic bacteria and *Salmonella* (Table 5). Transportation coops treated with PAA+FA alone or with a HPWR step prior to or following the treatment in both replications were statistically similar ($P < 0.05$) in reducing aerobic bacteria (4.10 to 5.17 logs, respectively) and *Salmonella* (3.99 to 4.58 logs, respectively). The LPWR consistently had the lowest reductions in both replications when reducing aerobic bacteria (2.09 and 2.14 logs) and *Salmonella* (2.10 and 2.16 logs).

Table 5: Lab Trial 1- Peroxyacetic Acid with a High-Pressure Water Rinse- Aerobes and *Salmonella* Recovery.
All treatments were given a 10-minute contact time and were followed by a LPWR of the transportation coops to remove any residual chemical. Values for reductions in aerobic bacteria and *Salmonella* recovery were calculated by subtracting post-treatment from pre-treatment samples.

Treatment[1]	Replication 1 Log_{10} reductions aerobic plate count	Replication 1 Log_{10} reductions *Salmonella* plate count	Direct plating incidence	Selective enrichment incidence	Replication 2 Log_{10} reductions aerobic plate count	Replication 2 Log_{10} reductions *Salmonella* plate count	Direct plating incidence	Selective enrichment incidence
LPWR	*$2.14^{b} \pm 0.47$	$2.10^{b} \pm 0.54$	10/10	10/10	$2.09^{b} \pm 0.29$	$2.16^{b} \pm 0.38$	10/10	10/10
PAA+FA	$4.71^{a} \pm 1.33$	$4.12^{a} \pm 0.26$	1/10	10/10	$4.77^{a} \pm 1.17$	$4.22^{a} \pm 0.41$	3/10	10/10
HPWR followed by PAA+FA	$4.10^{a} \pm 0.81$	$3.99^{a} \pm 0.65$	1/10	9/10	$5.17^{a} \pm 0.93$	$4.48^{a} \pm 1.03$	0/10	8/10
PAA+FA followed by HPWR	$4.42^{a} \pm 1.38$	$4.58^{a} \pm 1.21$	1/10	5/10	$4.89^{a} \pm 1.34$	$4.35^{a} \pm 1.35$	2/10	7/10

[1]LPWR= Low-pressure water rinse; PAA+FA= Peroxyacetic acid with a foam additive, and HPWR= High-pressure water rinse

[a-b]Column values with different superscripts differ significantly ($P < 0.05$).

*Data are mean ± standard deviation log_{10} reduction

The objective for lab trial 2 was to spike our feces with *Salmonella* Typhimurium and evaluate whether a HPWR step prior to or following a FC would be an added benefit in reducing aerobic bacteria and *Salmonella* (Table 6). Treatments using a FC varied statistically in both replications. In replication 1, HPWR prior to the FC and also the FC used alone had the greatest reductions and were statistically similar ($P < 0.05$) in reducing aerobic bacteria (4.05 and 4.23 logs, respectively). The FC followed by the HPWR was statistically different ($P < 0.05$) from all other treatments at 3.5 $\log_{10}$ reductions of aerobic bacteria and was greater than the LPWR. The LPWR had the lowest reduction of aerobic bacteria at 1.12 logs.

In the same lab trial *Salmonella* Typhimurium recovery was also evaluated and all three treatments using the FC were statistically similar ($P < 0.05$) to one another (3.17 to 3.65 logs). The LPWR had the lowest reduction at 1.82 logs of *Salmonella* and was statistically different than all other treatments. This demonstrates that the FC is effective in reducing not only aerobes but *Salmonella* as well.

In replication 2 of lab trial 2 (Table 6) aerobic bacteria reductions for all coops were statistically different from one another. The greatest reduction was achieved from the HPWR followed by the FC, which was a 4.84 $\log_{10}$ reduction of TPC. Another significant reduction came from the FC used alone with a reduction at 3.59 logs of TPC. The FC followed by the HPWR with a reduction of 2.78 logs of TPC also had a significant reduction of aerobic bacteria. The lowest reduction was observed with the LPWR treatment at 0.98 log reduction of TPC.

Replication 2 also evaluated the reductions of *Salmonella*. The authors found that the HPWR followed by the FC had the greatest statistically significant reduction of 3.90 logs of TPC. The HPWR used prior to the use of the FC consistently proved to be the most effective way to reduce aerobic bacteria and *Salmonella* in both replications, which could be due to the fact that the organic matter was removed prior to the disinfectant being applied. The organic matter that was used for lab trials had water added and *Salmonella* Typhimurium was blended to allow the slurry to be thicker in consistency and more true to organic matter that is naturally present on broiler transportation coops. According to Dvorak (2005) the removal of organic matter first is essential because it acts as a barrier to the microorganisms present and affects the efficacy of the disinfectant. They concluded that the efficacy of bleach is rapidly reduced when a large amount of organic matter present. Perhaps this is why we saw better results from the coops treated by the HPWR first followed by the disinfectant or cleaner in this lab trial. The FC used alone and followed by the HPWR statistically had similar reductions (2.82 and 3.18 logs). Finally the LPWR statistically showed that it had the lowest reductions of *Salmonella* at 0.65 logs of TPC.

Table 6: Lab Trial 2- Foam Cleaner with a High-pressure Water Rinse - Aerobes and *Salmonella* Recovery.
All treatments were given a 10-minute contact time and were followed by a LPWR of the transportation coops to remove any residual chemical. Values for reductions in aerobic bacteria and *Salmonella* recovery were calculated by subtracting post-treatment from pre-treatment samples.

Treatment[1]	Replication 1 Log_{10} reductions aerobic plate count	Replication 1 Log_{10} reductions *Salmonella* plate count	Direct plating incidence	Selective enrichment incidence	Replication 2 Log_{10} reductions aerobic plate count	Replication 2 Log_{10} reductions *Salmonella* plate count	Direct plating incidence	Selective enrichment incidence
LPWR	*$1.12^{c} \pm 0.39$	$1.82^{b} \pm 0.46$	10/10	10/10	$0.98^{d} \pm 0.51$	$0.65^{c} \pm 0.95$	10/10	10/10
FC	$4.05^{a} \pm 0.71$	$3.71^{a} \pm 0.59$	3/10	10/10	$3.59^{b} \pm 0.81$	$3.18^{b} \pm 0.85$	5/10	10/10
HPWR followed by FC	$4.23^{a} \pm 0.53$	$3.48^{a} \pm 0.54$	2/10	10/10	$4.84^{a} \pm 1.05$	$3.90^{a} \pm 0.33$	1/10	10/10
FC followed by HPWR	$3.50^{b} \pm 0.13$	$3.65^{a} \pm 0.13$	0/10	10/10	$2.78^{c} \pm 0.74$	$2.82^{b} \pm 0.72$	8/10	10/10

[1]LPWR= Low-pressure water rinse; FC= Foam cleaner; and HPWR= High-pressure water rinse
[a-d]Column values with different superscripts differ significantly ($P < 0.05$).
*Data are mean ± standard deviation log_{10} reduction

The objective of the field trial was to evaluate whether PAA+FA alone or after a HPWR step would be effective in reducing aerobic bacteria on freshly contaminated broiler transportation coops from a poultry processing facility (Table 7). Similar results were seen in both replications. Significant reductions (1.72 and 2.32 logs, respectively) of aerobic bacteria were observed from coops treated with HPWR followed by PAA+FA in both replications. The HPWR proved to be effective in a field setting, which may be due to the removal of organic matter present that had not been washed previously.

Table 7: Field Trial- Peroxyacetic Acid with a High-Pressure Water Rinse-Aerobes.
All treatments were given a 10-minute contact time and were followed by a LPWR of the transportation coops to remove any residual chemical. Values for reductions in aerobic bacteria recovery were calculated by subtracting post-treatment from pre-treatment samples.

Treatment[1]	Replication 1 Log_{10} reductions aerobic plate count	Replication 2 Log_{10} reductions aerobic plate count
LPWR	*-0.01[c] ± 0.66	0.42[c] ± 0.37
PAA+FA	0.88[b] ± 0.62	0.80[b] ± 0.34
HPWR followed by PAA+FA	1.72[a] ± 0.57	2.32[a] ± 0.40

[1]LPWR= Low-pressure water rinse; PAA+FA= Peroxyacetic acid with a foam additive, and HPWR= High-pressure water rinse
[a-c]Column values with different superscripts differ significantly ($P < 0.05$).
*Data are mean ± standard deviation log_{10} reduction

Berrang and Northcutt (2005a) suggested that high-pressure rinsing may be more effective to significantly reduce bacterial load than a LPWR. Their hypothesis to apply a

HPWR proved to be effective in a field setting along with removal of organic matter which is what previous research and literature suggests. Stringfellow and co-workers (2009) conclude that when using disinfectants, correct contact time, temperature, and amount of organic matter affects product efficacy. The higher amount of organic matter seen in the present study led to the conclusion that the addition of a HPWR will further reduce bacterial load present on transportation coops. The PAA+FA used alone had a significant reduction of aerobic bacteria (0.88 and 0.80 logs). The LPWR had the lowest reduction concentrations (0.0 and 0.42 logs) for the field trials conducted.

The current study did not demonstrate that the bacteria present were killed on the coops or whether the bacteria were physically washed away but whether the bacteria was reduced or removed. Regardless, bacteria levels were reduced or removed. As such, this observation may be irrelevant since the bacteria are no longer present on the transportation coops that can be a vehicle for cross-contamination. Continued research in a commercial setting may be needed. Furthermore, evaluations of bacterial counts on carcasses that were taken from washed transport coops versus unwashed to determine the bacterial load that is found on the carcasses following cleaned and disinfected coops. These products have already been approved by the Environmental Protection Agency, which means that they may be implemented in being used as a poultry processing facility. These data suggest that a CAFS application of cleaners and disinfectants may be used to significantly reduce *Salmonella* and aerobic bacteria on broiler transport coops. While a direct comparison was not made, coops from a commercial setting were

found to be more difficult to clean and disinfect than coops which were contaminated in the laboratory.

CHAPTER V

CONCLUSION

Food safety and reportable diseases are a significant concern for the poultry industry and is why new guidelines are written and implemented. A previous survey found that only 9% of large poultry processing facilities clean their transportation coops; if a guideline is written to require cleaning and disinfecting of coops these products and methods of application could be considered when implementing a plan of action (Northcutt and Jones, 2004). Berrang and Northcutt have published extensive research on reducing pathogens on contaminated transportation coops. Their studies involve drying or a LPWR to reduce levels of microorganisms present. The effectiveness of allowing a 24 to 48 hour drying time in order to reduce or kill bacteria present on transportation coops gave poultry processing facilities an idea of what could be done, but this method may be difficult to implement with the limited amount of adequate space and equipment available (Berrang, et al., 2004). Facilities will need extra transport coops in order to allow the 24 to 48 hour dry time which may involve purchasing additional transportation coops and thus additional cost. The cost of one transportation coop, depending on the size that is used by the processing facility, can range from $1,400 - $1,500. If cleaning and disinfection of transportation coops did become a common practice in the industry, a standard protocol like a Sanitation Standard Operating Procedures (SSOP) should be developed and followed (Northcutt and Berrang, 2006).

Laboratory trials utilizing a HPWR prior to the FC were found to be most effective in removal of organic matter when compared to the FC used alone or the LPWR which is recommended by the manufactures and written in literature (Dvorak, 2005). The commercial field trial data did identify an added benefit of a HPWR followed by the PAA+FA treatment was more effective in reducing the bacterial load than LPWR. Previous research demonstrated that the use of a LPWR alone can effectively reduce bacterial load and in the present study we observed an additional two log reduction in bacteria when compared to our LPWR control (Berrang and Northcutt, 2005a; Berrang and Northcutt, 2005b). The HPWR prior to PAA+FA treatment being the best in reducing aerobic bacteria wasn't surprising due to the high amount of organic matter seen on the transportation coops that were allowed to accumulate over time.

A compressed air foam system (**CAFS**) can be utilized to apply disinfectants with foam or foaming cleaners to effectively reduce aerobic bacteria and *Salmonella* which can contaminate broiler transportation coops. The use of foam to depopulate commercial poultry operations has already been approved by American Veterinary Medicine Association (AVMA) and USDA-Animal and Plant Health Inspection Service (APHIS) which demonstrates that using this application for cleaning and disinfection of poultry transportation coops may be conceivable (American Veterinary Medical Association, 2007). Disinfectants like PAA that was used for these studies is currently being used by the poultry industry in some of their facilities such as hatcheries or processing plants because of their ability to lower pathogen counts on broiler carcasses (Bauermeister, et al., 2008).

Further research could evaluate that the beneficial effects of using CAFS and the different disinfectants for controlling microorganisms not only on transportation coops but also in hatcheries and processing plant equipment. It would be beneficial for the industry to evaluate whether lower bacterial counts on processed broiler carcasses are seen from broilers transported on disinfected transportation coops when compared to broilers who are transported on unwashed transportation coops.

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