

**VALIDATION OF CHEMICAL AND NON-CHEMICAL ANTIMICROBIAL
INTERVENTIONS APPLIED PRE- AND POST-CHILLING TO REDUCE
MICROBIAL POPULATIONS IN BROILER CARCASSES**

A Thesis

by

VERONICA ALEJANDRA MOLINA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2007

Major Subject: Food Science and Technology

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ABSTRACT

Validation of Chemical and Non-Chemical Antimicrobial Interventions Applied Pre- and Post-Chilling to Reduce Microbial Populations in Broiler Carcasses. (August 2007)

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Chair of Advisory Committee: Dr. Marcos X. Sánchez-Plata

Higher risks of food-borne illness associated with increased consumption of poultry products make it necessary to identify potential sources of contamination and apply intervention strategies that will prevent or minimize the risk of contamination during processing. This study investigated the effects of chemical and natural decontamination treatments including sprayed application of acidified calcium sulfate (ACS) in combination with ϵ -polylysine (EPL), dry-rubbing kosher salt coating and molten paraffin wax dipping application on microbial populations of broiler carcasses and parts. Treatments were evaluated for their effectiveness in reducing the numbers of artificially inoculated rifampicin resistant *Salmonella* Typhimurium strain NVSL 95-1776 on the skin surface of bone-in chicken breasts. General model procedures were used to find statistical differences ($P < 0.05$) and separation of means was done with least square means using SAS 9.1.

Chemical interventions (ACS + EPL) caused an overall reduction of ~ 0.65 CFU/ml of rifampicin resistant *Salmonella* Typhimurium populations in inoculated chicken breasts. Similar reductions were observed in validation experiments in whole carcasses when compared to post-eviscerated control samples as well as post chilled

treated samples when compared to post-chill controls. Kosher salt interventions caused ~1.15 CFU/ml log reductions in rifampicin resistant *Salmonella* Typhimurium loads. Significant differences (>2 log reductions) were also observed in validation trials in both pre- and post-chilled samples when compared to non-treated pre- and post-chilled controls. Only for psychrotrophic counts, chilled and post-chill interventions did not have a significant effect ($P>0.05$). The use of molten paraffin wax caused <0.51 CFU/ml log reductions on rifampicin resistant *Salmonella* Typhimurium in chicken breasts. In addition, drip loss on kosher treated samples was 53.8% lower than non-treated counterparts. However, kosher salt application caused a decrease in lightness (*L values) and yellowness (*b values) on treated carcasses when compared to controls, redness (*a values) were not significantly affected. Results indicate that the combined use of ACS and EPL at the stated conditions and the coating application of kosher salt on broiler carcasses significantly reduce pathogen contamination and microbial indicator loads, thus providing an alternative validated antimicrobial intervention for potential use by the poultry industry.

DEDICATION

I would like to dedicate this thesis project to our God, who is my Father and best friend, and helps me, always giving me the strength to continue until the end; to my dear parents: Juan and Lucy, who are my life's motivation and inspiration, my best gift from heaven; to my sister: Natalia Gabriela, my best friend and confidante for being close to me even at a long distance; to my closest friends and relatives in Ecuador, Honduras and College Station, who are my guardian angels, thank you for your support and company in the difficult and good times of my life.

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I would like to express my eternal gratitude and offer my sincere thanks to our Divine Superior Being, who as three holy persons in one, the Father, the Son and the Holy Spirit, helps me always to be who I am and try to do my best, for giving me love and the interior strength to finish every task I begin, including this project. Thanks to our Holy Mother, Mary for helping me feel close to that divinity and for being always beside me. Thank you to my dear family and friends for being close and encouraging me to finish any task no matter how difficult.

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TABLE OF CONTENTS

	Page
ABSTRACT.....	iii
DEDICATION.....	v
ACKNOWLEDGEMENTS.....	vi
TABLE OF CONTENTS.....	vii
LIST OF FIGURES.....	ix
LIST OF TABLES.....	xi
 CHAPTER	
I INTRODUCTION.....	1
II REVIEW OF LITERATURE.....	9
<i>Salmonella</i> spp. in Humans	12
<i>Salmonella</i> spp. in the Poultry Processing Environment	14
Bacterial Attachment and Microbial Reduction Strategies.....	15
Conclusions	20
III VALIDATION OF THE COMBINED USE OF ACIDIFIED CALCIUM SULFATE AND ϵ -POLYLYSINE SOLUTIONS AS MULTIPLE-HURDLE ANTIMICROBIAL INTERVENTIONS IN BROILER PROCESSING.....	22
Materials and Methods	24
Results and Discussion	33
IV VALIDATION OF KOSHER SALT AS AN ANTIMICROBIAL INTERVENTION IN BROILER PROCESSING.....	41
Materials and Methods	44
Results and Discussion	50

CHAPTER	Page
V	EFFECT OF MOLTEN PARAFFIN WAX AS AN ANTIMICROBIAL INTERVENTION IN POULTRY PROCESSING.....62
	Materials and Methods 63
	Results and Discussion 66
VI	CONCLUSIONS.....68
	REFERENCES.....71
	VITA.....76

LIST OF FIGURES

FIGURE	Page
1	Log ₁₀ CFU/ml of <i>Salmonella</i> Typhimurium populations in control chicken breast samples and samples treated with an acidified calcium sulfate (ACS) solution followed with an ε-polylysine solution applied at either 55 or 25°C and sampled at day 0 and 2 of refrigerated storage.....34
2	Log ₁₀ CFU/ml of <i>Salmonella</i> Typhimurium reductions in chicken breast samples treated with ACS and EPL at 55 vs. 25°C and sampled at day 0 and 2 of refrigerated storage34
3	Log ₁₀ CFU/ml of aerobic plate counts in control chicken carcasses and carcasses treated with an acidified calcium sulfate (ACS) solution followed with an ε-polylysine solutions applied at 55°C and sampled at day 0 and 10 of refrigerated storage37
4	Log ₁₀ CFU/ml of coliforms in control chicken carcasses and carcasses treated with an acidified calcium sulfate (ACS) solution followed with an ε-polylysine solutions applied at 55°C and sampled at day 037
5	Log ₁₀ CFU/ml of generic <i>Escherichia coli</i> in control chicken carcasses and carcasses treated with an acidified calcium sulfate (ACS) solution followed with an ε-polylysine solutions applied at 55°C and sampled at day 038
6	Log ₁₀ CFU/ml of psychrotroph counts in control chicken carcasses and carcasses treated with an acidified calcium sulfate (ACS) solution followed with an ε-polylysine solutions applied at 55°C and sampled at day 0 and 10 of refrigerated storage39
7	Log ₁₀ CFU/ml of <i>Salmonella</i> Typhimurium populations in control chicken breast samples and samples treated with kosher salt applied either pre or post-chilling and sampled at day 0 and 5 or refrigerated storage.....51
8	Log ₁₀ CFU/ml of <i>Salmonella</i> Typhimurium reductions in chicken breast samples treated with kosher salt pre or post chill and sampled at day 0 and 5 of refrigerated storage51
9	Log ₁₀ CFU/ml of aerobic plate counts in control chicken carcasses and carcasses treated with kosher salt applied either pre or post chill and sampled at day 0 and 10 of refrigerated storage54

FIGURE	Page
10	Log ₁₀ CFU/ml of coliforms in control chicken carcasses and carcasses treated with kosher salt applied either pre or post chill and sampled at day 0.....55
11	Log ₁₀ CFU/ml of generic <i>Escherichia coli</i> in control chicken carcasses and carcasses treated with kosher salt applied either pre or post chill and sampled at day 056
12	Log ₁₀ CFU/ml of psychrotrophs in control chicken carcasses and carcasses treated with kosher salt applied either pre or post chill and sampled at day 0 and 10 of refrigerated storage.....57
13	Color value *L after kosher salt intervention in chicken carcasses57
14	Color value *a after kosher salt intervention in chicken carcasses58
15	Color value *b after kosher salt intervention in chicken carcasses59
16	Drip Loss (%) in chicken carcasses after kosher salt interventions60
17	Log ₁₀ CFU/ml of <i>Salmonella</i> Typhimurium populations in control chicken breast samples and samples treated with molten paraffin wax67

LIST OF TABLES

TABLE	Page
1	Log ₁₀ CFU/ml of <i>Salmonella</i> Typhimurium populations control chicken breast samples and samples treated with an acidified calcium sulfate (ACS) solution followed with an ε-polylysine solution applied at either 55 or 25°C and sampled at day 0 and 2 of refrigerated storage33
2	Microbial profiles of chicken carcasses treated with a combination of ACS and EPL as an antimicrobial intervention applied either before or after chilling.....35
3	Microbial profiles of chicken carcasses treated with a combination of ACS and EPL as an antimicrobial intervention applied either before or after chilling after 10 days of storage36
4	Log ₁₀ CFU/ml of <i>Salmonella</i> Typhimurium populations in control chicken breast samples and samples treated with kosher salt applied either pre or post-chilling and sampled at day 0 and 5 or refrigerated storage.....50
5	Microbial profiles of chicken carcasses treated with kosher salt as an antimicrobial intervention applied either before or after chilling at day 053
6	Microbial profiles of chicken carcasses treated with kosher salt as an antimicrobial intervention applied either before or after chilling at day 10.....53
7	Mean lightness (L*), redness (a*) and yellowness (b*) of chicken carcasses after kosher salt interventions58
8	Mean drip loss (%) in chicken carcasses after kosher salt interventions59
9	Log ₁₀ CFU/ml of <i>Salmonella</i> Typhimurium populations in control chicken breast samples and samples treated with molten paraffin wax66

CHAPTER I

INTRODUCTION

Salmonella spp. are one of the most frequently reported cause of food-borne outbreaks in the United States. The Center for Disease Control and Prevention (CDC) estimates that *Salmonella* spp. cause approximately 1.5 million cases of foodborne illness each year in the U.S. with more than 15,000 hospitalizations and 500 deaths (Mead et al., 1999). It has been reported that out of the total deaths caused by food-borne pathogens, 95% occurs due to *Salmonella* infection (Santos et al., 2003). However, it should be noted that recent outbreaks and recalls related to *Salmonella* in foods have been traced to non-poultry sources including fruits, vegetables and even peanut butter. Repeated outbreaks associated with this pathogen are of concern to many, and especially the poultry industry, considering that when people get sick, investigators may incorrectly focus on the product with a history of outbreaks, in this case poultry products (Calvin, 2004).

The U.S. Department of Agriculture-Food Safety and Inspection Services (USDA-FSIS) (2006b) reports that a type of *Salmonella* commonly associated with eggs has been frequently isolated from chicken meat in recent cases. In fact, tests conducted in the years 2000 to 2005 indicate a four fold increase in *Salmonella enteritidis* on chicken carcasses. U.S. Department of Agriculture authorities recognize that even with the widespread application of different antimicrobial interventions, *Salmonella* prevalence continues to rise every year. Because of its potential presence in raw poultry,

This thesis follows the style of Poultry Science.

the USDA recommends that poultry should be cooked to 71.1°C (165°F) to kill *Salmonella*. It advises consumers to use food thermometers and follow established guidelines for kitchen safety that involves: washing hands often, separating raw poultry and meat from cooked food, and refrigerating or freezing food right away. All these efforts are in place to minimize the risk of *Salmonella* at the household level, but strategies to minimize its presence when poultry is processed can provide a more significant effect in prevalence reduction while minimize its risks.

The use of organic acids as poultry carcass decontaminants in the meat and poultry industry is well documented. However, there is limited information on the use of acidified calcium sulfate (ACS) and ϵ - (epsilon) polylysine (EPL) in a multiple hurdle fashion to reduce contamination and growth of pathogens. Using organic acids in spray cabinets for carcass decontamination to reduce pathogenic bacteria is now a common industry practice. However, cost effective treatments designed to be used in combination or in sequence as pathogen interventions for small meat and poultry processing plants, have not been validated.

Nowadays, more people are concerned about the safety of the food they consume than at any other time in history. Recent food safety scares have been massively covered by the media and exposure increases the awareness of consumer to potential problems. Despite the fact that a significant share of food-borne diseases has been largely attributed to in home contamination, food processors continue to bear the greatest responsibility for food safety. Food processors are required by the U.S. Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) and the Food and Drug Administration

(FDA) to implement strict measures to reduce or eliminate any potential pathogen or hazard that might be introduced to the food during harvest, processing and handling. Small plants face a particular challenge due to their limited personnel and financial resources. *Salmonella* Typhimurium, *Escherichia coli* O157:H7 and *Listeria monocytogenes* are among the pathogens most commonly associated with contamination of meat and poultry products.

Despite advances in technology and medical sciences, food-borne illnesses continue to be a serious public health problem and have become a significant concern to the food industry. The CDC, one of the 13 operating components of the Department of Health and Human Services, has estimated that there are 76 million cases of food-borne illness annually, resulting in 325,000 hospitalizations and 5,000 deaths. (Mead et al., 1999). Pathogen reduction strategies have been implemented by the regulatory agencies and the food industry to maintain consumer confidence at a time when consumers are increasingly concerned about the safety of what they eat. Agro terrorism also is of concern due to the hazards that could exist if intervention measures are not put in place to detect, reduce or eliminate pathogens introduced intentionally into the food supply.

Meat and poultry are among the top five items implicated in food-borne illness outbreaks on a monthly basis. Frenzen et al. (2000) suggested that pathogen contaminated meat and poultry cause at least 2.5 million illnesses and 1,000 deaths every year. New approaches have been developed to decontaminate meat and poultry products during harvesting processes (Mermelstein 2001; Connor 2001; Huffman 2002; White et al. 2002). These methods include cold and hot water rinses; steam pasteurization or

steam vacuum treatment; carcass trimming; several chemical rinses such as chlorine/chlorine dioxide, ozonated or electrolyzed water, tri-sodium phosphate, acidified calcium sulfate, and organic acid rinses with or without surfactants.

Significant bacterial attachment and accumulation occurs during poultry processing, for this reason, the immediate post harvest application of a combination of more than one antimicrobial intervention treatment to carcasses has often been found to produce a greater antimicrobial effect than any single treatment alone; often working in a synergistic manner. Synergistic effects achieved by different antimicrobial agents have been commonly referred to as hurdle technology. Combined hurdles have been found not only to enhance pathogen reduction, but also serve to improve the quality of meat/poultry resulting in more shelf-stable products.

Acidified Calcium Sulfate (ACS) (Safe₂O®, Mionix Corp., Rocklin, CA) has been found to be very effective as a beef and poultry carcass washing agent (Huffman 2002; Dickens et al., 2004) as well as a rinsing agent for ready-to-eat (RTE) meats. Effects not only include immediate inactivation of some pathogens, but also considerable residual antimicrobial activity (Núñez de González et al., 2004). ACS is a “generally recognized as safe” (GRAS) ingredient to be used on food products. It consists of a complex blend of sulfuric acid, calcium sulfate, calcium hydroxide, and an organic acid, usually lactic acid, adjusted to a final pH of ~1.5. According to the manufacturer, ACS when combined with organic acids disables the proton pumps in bacterial membranes and acts as a metabolic inhibitor, thus attacking bacteria in a

different fashion than organic acids alone (e.g. lactic acid). However, only a few studies have shown its potential residual antimicrobial effects.

On the other hand, ϵ (epsilon) –polylysine (EPL), another commercially available antimicrobial product, has been found to enhance the antimicrobial effects of ACS especially against lactic acid spoilage bacteria (Hiraki, 2002). EPL is also GRAS status and is thought to act by a different mode of action when compared to ACS by causing disruption of the bacterial cell surface (Yoshida et al., 2002). EPL concentrations of 0.02% and 0.04% have been shown to have antimicrobial activity against *E. coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes*. According to recent studies, *Salmonella* Typhimurium is the most sensitive of all three pathogens (Geornaras and Sofos, 2005). As a general finding, when combined with other antimicrobials, EPL has enhanced antimicrobial activity.

Poultry carcasses on a conventional processing line can be contaminated with a variety of pathogenic bacteria, including *Salmonella* Typhimurium, *Campylobacter jejuni*, and *Listeria monocytogenes* (Lillard, 1990; Clouser et al., 1995). It has been reported that poultry processing under kosher guidelines, may introduce even higher risks for microbial contamination when compared to non kosher products. Kosher processing follows strict guidelines based on religious beliefs. The Hebrew word “kosher” means fit or proper (Lipschutz, 1996) and is a religious term used to describe a range of ritualistic objects, activities, or even a state-of-being. Food is kosher when it meets the strict dictates of the laws of kashrus. These laws require that kosher food:

- (1) Should come from a proper source, for example, meat from some animals, such as pork, is inherently non kosher,
- (2) Should be prepared in a specific manner, for example, animals must be slaughtered in a particular manner, and
- (3) Should not be combined improperly with food that might otherwise be considered non-kosher, for example, combining meat and milk.

According to Sigman (1999), kosher poultry is limited to domestic fowl. Scavenging birds, such as eagles or ravens, are not permissible sources of food. In order to make poultry kosher, rabbinic authorities declared that permitted fowl would be limited to a list of birds including chicken, turkey, goose, and domestic duck. Similarly like in the context of beef, the blood from poultry is not considered to be kosher.

Beyond additional rules, the koshering operation is accomplished by soaking the fowl in cold water (8 to 12°C) for 30 minutes, a process called Shriah, to remove coagulated blood. Then, carcasses are salted massively with coarse salt to cover all surfaces for up to 1 hour to allow the blood to drip out. Finally, salted carcasses are rinsed with cold water, a process called Hadacha to remove excess salt (Regenstein and Regenstein, 1988). The two immersion steps in water in the koshering operation are further potential sites for cross-contamination of these birds. Salt, a known antimicrobial may have a deleterious effect on surface bacteria contaminating poultry carcasses, likely by osmotic interactions; however, the effects of poultry koshering on microorganisms has been poorly reported.

Another poultry processing operation that varies from conventional standards has been commercially applied in the slaughtering of ducks. During duck processing, fin feathers cannot be removed by standard picking mechanisms, and an additional step is added during the defeathering operation. Molten paraffin wax is used in the duck industry as an alternative to remove hard-to-pick fin feathers inherent to the duck carcass surface. Duck carcasses are submerged in molten paraffin wax kept at temperatures in the range of 60°C. These hot temperatures may also have a detrimental effect on surface bacterial loads. Subsequent to waxing, carcasses are submerged in cold/ice water to solidify the paraffin product and facilitate wax removal. The molten wax coats most carcass surfaces, including follicles and crevices and when solidified may also grab bacterial organisms on the carcass surface that may not be physically removed by simple washing mechanisms. The effects of hot wax temperature, and potential physical removal of microorganisms caused by the waxing process has not been validated and research is needed to determine its efficacy.

According to the USDA-FSIS (2006a), ducks are plucked soon after killing. Dry plucking combined with wax finishing is recommended by DEFRA (2004). The dry plucking machine is used for strip birds of feathers, being carried out by hand. The tail and large wing feathers are taken out first by hand and kept separately. The machine operation takes one to two minutes. The remaining stubs are removed by hand. The down that clings to the flesh is difficult to remove. For large numbers of carcasses 'wax finishing' is used, where hot paraffin wax is held in tanks at a temperature of 60°C. During duck processing, fin feathers cannot be removed by standard picking

mechanisms, and an additional step is added during the defeathering operation. Molten paraffin wax is used in the duck industry as an alternative to remove hard-to-pick feathers inherent to the duck carcass surface. Duck carcasses are submerged in molten paraffin wax kept at temperatures in the range of 60°C. These hot temperatures may also have a detrimental effect on surface bacterial loads. Subsequent to wax immersion for about 5 seconds, the carcasses are then removed and either sprayed with cold water or immersed in a cold water tank to solidify the paraffin product and facilitate wax removal. The hardened wax is stripped off by hand or by using a rubber-fingered drum plucking machine. During immersion, the molten wax coats most carcass surfaces, including follicles and crevices, thus, when solidified it may also grab bacterial organisms on the carcass surface that may not be physically removed by simple washing mechanisms. The effects of hot wax temperature, and potential physical removal of microorganisms caused by the waxing process has not been validated and research is needed to determine its efficacy.

The purpose of this research project was to validate the use of ACS (1:4 ratio) followed by EPL (200ppm) application as a chemical intervention and to assess the use of kosher salt and molten paraffin wax as natural antimicrobial interventions during poultry processing when applied either before or after the chilling process.

CHAPTER II

REVIEW OF LITERATURE

Meat and poultry are commonly implicated in food-borne illness outbreaks. Pathogens contaminating meat and poultry are thought to cause at least 2.5 million illnesses and 1,000 deaths every year (Frenzen et al., 2000). As a result, several approaches have been developed to decontaminate meat and poultry products during the processing (Mermelstein 2001; Connor 2001; Huffman 2002; White et al. 2002).

The safety of the food supply in the United States continues to be a matter of paramount importance for government agencies, researchers, health care professionals, and consumers. Buzby and Roberts (1997) reported an estimation saying that food-borne illnesses account for 6.9 to 34.9 billion dollars in economic losses each year and these losses resulted from approximately, 76 million annual cases of food related illness with 325,000 hospitalizations and about 5,000 deaths (Mead et al., 1999). Of all food borne pathogens which have been implicated in human outbreaks, *Salmonella*, *Toxoplasma*, and *Campylobacter*, are the most common causes to these illnesses and deaths each year. (Tauxe, 2002). According to Forsythe (1996), almost 200 million dollars is spend each year in 21 federally regulated agencies that enforce approximately fifty regulations or guidelines to find ways to stop the increasing number of foodborne illness cases and enforce food safety compliance in the U.S.

The USDA-FSIS issued a comprehensive final rule establishing pathogen reduction requirements applicable to meat and poultry establishments (USDA-FSIS,

1996). These requirements were designed to reduce the occurrence and numbers of pathogens in or on meat and poultry products, thus reducing the risk of foodborne disease affecting millions of people (Mead et al., 1999). The principal sources of transmission of microbes such as *E. coli* O157:H7, *Salmonella* spp., *Campylobacter*, *Staphylococcus aureus* and others include the hides of animals arriving at processing plants or carcasses that become cross-contaminated with intestinal contents during processing (USDA-FSIS, 2004).

The “Pathogen Reduction: Hazard Analysis and Critical Control Point (HACCP) system” regulation, has four basic components, one is the developing and implementation of a system of preventive controls to improve product safety in every establishment. HACCP is a preventive system established to identify, intervene, and eliminate foodborne pathogens to reduce human foodborne diseases. The system outlines specific sanitation procedures, specific bacteriologic monitoring procedures and performance standards for certain organisms including *Salmonella*. These preventative systems, with intervention steps at particular points within the processing plant, aim to not only reduce the spread of bacteria but to impede its growth. Within the first year of implementation, the HACCP system was credited with reducing *Salmonella* prevalence on broiler carcasses from a baseline of 20% pre-HACCP to 10.9% after the first year of implementation (USDA-FSIS, 1996). Through the use of numerous microbial intervention strategies which include, but are not limited to, inside-outside bird washer (IOBW) cabinets, immersion chill tanks, acid baths, and scalding tank modifications; microbial reductions on commercially processed poultry has been shown to be indeed

possible. Despite the proven success of some of these interventions to date, the established relationship of pathogenic bacteria to food animals and the potential to develop resistance to some of these agents warrants the continued development of novel and effective strategies. Developing new antimicrobial intervention strategies for the poultry processing environment is useful and merits the expenditure of resources.

Salmonella is one of the most prevalent bacterial pathogens and is commonly associated with raw poultry. This pathogen accounts for a significant percentage of all reported bacterial foodborne illness cases worldwide (Thorns, 2000). *Salmonella* is a facultative gram negative bacillus frequently isolated from poultry and poultry products. Tauxe (2002) reported that of the over 2,800 *Salmonella* serovars, 120 have been isolated as disease causing agents in poultry and humans. Transmission of *Salmonella* varies greatly and includes a multitude of vectors, and can clearly be transmitted vertically from hen to progeny.

According to Jensen et al. (1998) the USDA estimates the costs of the pathogen reduction initiative from \$1.1 to 1.3 billion up to 2016; concluding that the regulation provided net positive benefits and the food safety improvement costs increase sharply as control becomes more effective.

***Salmonella* spp. in Humans**

As described by Buzby and Roberts (1997), there are seven major food-borne pathogens of human health concern within the United States. These are: *Campylobacter jejuni*, *Clostridium perfringens*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp, *Staphylococcus aureus* and *Toxoplasma gondii*. Together, these pathogens account for an estimated 3.3 to 12.3 million illnesses each year, and as many as 3,900 deaths (Buzby and Roberts, 1997). *Salmonella* is a justifiably noteworthy pathogen which clearly represents a major food safety hazard. This organism has been identified in poultry as the most common vehicle associated with human foodborne illness (Mandrell and Wachtel, 1999; Notermans and Kampelmacher, 1992; White et al., 2002).

The most common clinical presentation of human salmonellosis is enteritis with symptoms such as: nausea, vomiting, diarrhea, headache, fever, and other systemic symptoms (Buck and Werker, 1998). Death may be a result of infection particularly in elderly, young, or immuno-compromised patients. Buck and Werker, (1998) indicated that only 120 *Salmonella* isolates were recovered from 2,800 *Salmonella* isolates present in enteric disease in humans, animals, and poultry. Many *Salmonella* serovars are host specific, such as *S. abortusequi*, *S. gallinarium*, and *S. typhi*; others, have zoonotic potential and tend to have a broad host range. A common transmission source of *Salmonella* is the result of ingestion of fecally contaminated material. Depending on the degree of virulence, the host can either be asymptomatic or show manifestations of clinical infection.

Salmonellae are gram negative bacilli that can be separated by their somatic groups, O antigens, and flagellar, H antigens (Robbins and Robbins, 1984). O antigens are denoted using arabic numbers while the H antigen designation is more complicated and uses small letters for differing phases. Within the differing serovars, there are biovars with different biochemical characteristics. For biochemical characterization, the organism produces acid and gas as an end product of carbohydrate fermentation. *S. typhi* is an exception to this rule but is not commonly associated with food sources. Salmonellae are non-hemolytic in nature and lactose negative on MacConkey agar. (Robbins and Robbins, 1984).

Due to the extensive historical association of *Salmonella* with humans and domestic animals, efforts to reduce contamination and prevent exposure has proven difficult throughout the past decades. Santamaria and Toranzos, (2003) reported that *Salmonella* spp. are maintained within an animal population via asymptomatic carriers, symptomatic carriers, or environmentally in the feed or soil. Secondary contamination often occurs by cross contamination, usually occurring between contaminated food and food handlers. Improper preparation and handling of food in homes and food establishments is considered one of the primary vehicles associated with human outbreaks of salmonellosis (Patten, 1981). As a result of this, it is important to control strains such as *S. typhi*, *S. gallinarium*, and *S. abortusequi*, in which there is only one reservoir species. In such species the infection has to come from contaminated food by that particular animal or fecal material of another animal of the same origin.

According to the USDA-FSIS (2006b) *Salmonella enteritidis* is considered one of the most common causes of sickness leading to fever, stomach cramps and diarrhea. In some cases, it could spread to the bloodstream in sensitive people and cause systemic syndromes. Increased outbreak cases have been attributed to an increase in mass food preparation, favoring the spread of *Salmonella* and other pathogenic bacteria, by having more people supplied with the same food source. Another factor that often contributes to food-borne illness is the improper storage of food. In many cases, temperature abuse and the accumulation of large volumes of improperly stored foods can have terrible results. With the increase of globalization comes an increase in international trade being another factor contributing to the increases of foodborne illness being observed (Buck and Werker, 1998). Loss of control over the HACCP system becomes an issue when product is imported. Currently there is no infallible system to assure that food animal production is microbiologically safe (Notermans and Hoogenboom-Verdegaal, 1992). Stevens (2005) indicated that over use of antimicrobial substances and implementing stringent sanitation procedures causes a lack of exposure to these agents and its virulent antigens, and this in turn lowers their overall resistance.

***Salmonella* spp. in the Poultry Processing Environment**

In 1996, a baseline study assessing the prevalence of *Salmonella* in broilers following immersion chilling found carcass prevalence in the range of 20% (USDA-FSIS, 1996). Largely, due to the implementation of HACCP; the prevalence of *Salmonella* in broilers dropped to 6.1% in 2001 (Bashor et al., 2001). However,

according to recent reports (2006b) by the USDA-FSIS; there was an increase to 16.3% *Salmonella* prevalence in 2005, with a high degree of variability across the nation; differing due to geographic location, on-farm management, and/ or practices at an specific processing facility.

A primary source of plant contamination with *Salmonella* is through infected or intestinally colonized birds (McCapes et al., 1991). Parts of the process can increase microbial recovery, and individual operations such as defeathering, may allow fecal material to escape the bird by the cloaca and contaminate other carcass (Berrang et al., 2001). The immersion step in a chill tank, can also serve as bacteria distributor to previously non-contaminated carcasses, leading to an increased bacterial recovery post-chill. Another source of plant contamination is the exterior surface of the bird. Feathers and skin can become contaminated and bring bacteria into the plant (Corry and Atabay, 2002). Managing poor oversight practices at the processing facility represents a problematic situation. It is for this reason that the processing plant must play a pivotal role in the reduction of pathogenic bacteria on processed poultry carcasses.

Bacterial Attachment and Microbial Reduction Strategies

Understanding chemical and physical means by which bacteria attach to poultry surfaces could be imperative for the development of novel intervention strategies. Interactions between specific cell surface receptors and ligands on the carcass surface enable bacteria to attach to different microscopic structures. These interactions are highly dependant on extrinsic and intrinsic factors that mediate bacterial attachment. All

these factors must be considered for designing successful inactivation strategies (Stevens, 2005).

According to Gorski et al., (2003), it has been demonstrated that bacteria possess the ability to use different attachment mechanisms at differing temperatures. This adaptation allows bacteria to maintain invasiveness in a wider range of values. The metabolic activity is adversely affected at lower temperatures, slowing and or stopping the movement of flagella (Holler et al., 1998). Gorski et al., (2003) suggested that it is through this physical bacterial mechanism that tissue attachment can be reduced. By lowering the temperature of the environment surrounding the bacteria, it is possible to slow and eventually stop the metabolism of the cell and its attachment. In a study by Notermans and Kampelmacher (1974) it was shown that higher temperatures can enable the microorganisms attached to the skin to become more resistant to this harsh environment.

Notermans and Kampelmacher (1974) demonstrated that bacteria surviving the scalding process and remained attached to the skin were more difficult to remove with further processing steps such as inside outside bird washer (IOBW) cabinets, and chilling. The pH of the environment, in which interaction between the bacteria and the tissues transpires, plays a significant role in the ability to attach to tissue surfaces partially due to the fact that the motility is decreased (Notermans and Kampelmacher, 1974). The variable rate at which bacteria attach to surfaces and themselves may be in part due to the changes that emerge in the structure of the skin after prolonged exposure to an aqueous environment. Contact time and bacterial attachment is directly

proportional to the concentration of the bacteria in the medium that the carcass is being exposed to (Notermans and Kampelmacher, 1974).

While there exists different operations in commercial poultry processing where the potential for the spread of microorganisms is high; it has been shown that the bird washers and immersion chill tank environments provoked significant pathogen reductions, making processing facilities successful in reducing bacterial numbers on finished poultry products. IOBW cabinets are one of the most prevalent pre-chill interventions for achieving microbial reductions. They consist of washing systems used during processing that help reduce the number of bacterial pathogens and microorganisms in general on carcass surfaces. However, the reduction may not be of bacterial significance, nor consistent. In addition, the percentage of *Salmonella* positive samples following the IOBW remains generally the same as pre-IOBW samples (Oyarzabal, 2006). In general, the effectiveness of an IOBW to reduce bacterial pathogens depends on the water volume/pressure and the level of chlorine in the water. These variables may be difficult to control consistently in constantly changing commercial processing environments. The addition of an antimicrobial system to the washes could produce more bacterial reduction in the number of pathogen counts.

According to Oyarzabal (2006) the combined use of IOBWs and chemical sprays have been found effective in removing visible fecal contamination, thus allowing for a continuous online processing of commercial broiler carcasses. Without these efforts, carcasses contaminated with fecal material will need to be removed from the line, and manually washed or trimmed, thus slowing the processing speed. Although

these wash and spray systems reduce *Salmonella* spp.; in some instances, there have been no differences in the numbers of *Salmonella* positives between pre-chill and post-chill samples. Another approach is to apply chemical interventions after chilling with successful reduction of pathogen with the advantage of using cold shock to complement the killing effect of the chemical.

Chlorine is one of the cheapest and most commonly used antimicrobial compounds in broiler processing plants. As such, it is very commonly used for potentially reducing *Salmonella* in a variety of bird washes systems. Other chemical disinfectants such as chlorine dioxide, sodium chlorite acidified with citric or phosphoric acid and organic acids have also been shown to be successful in the pre-chill reduction of *Salmonella* (Corry and Atabay, 2001). The use of such chemicals, however, as antimicrobials is not as consistent when compared to chlorine. Bird washers that utilize antimicrobials have been reported to produce bacterial reductions on broiler carcasses ranging from 0.5 to 1.53 logs (Bashor et al., 2004).

The immersion chiller is considered a critical point for additional bacterial reductions on poultry carcasses (James et al., 1992; White et al., 2002). Cason et al. (1997) reported as approximately 1.8 log₁₀ reductions with immersion chilling systems. Most chillers consist of an open, ice cold, common water bath that operates under counter current flow and constant agitation. These baths drop the carcass temperature to 4°C in 4 to 8 hours for 4 to 8 pound birds. (USDA-FSIS, 1999). Mead (1975) and Thorns (2000) demonstrated a ten-fold reduction of fecal and spoilage bacteria on post-processed carcasses when a 20ppm solution of chlorine was used in the chiller (Mead et

al., 1975). Similarly, microbicides like ozone (Kim et al., 1999) have shown to be effective in the reduction of bacteria. While the effectiveness of gas chlorine (Cl_2) and hypochlorite (HOCl) forms of chlorination is highly dependant on the pH in the chiller, the efficacy of chlorine dioxide (ClO_2) is not. The proper management of the environment is very important for achieving significant reductions on finished carcasses.

The scald tank has been demonstrated to be a site of cross contamination for *Salmonella* (Mulder et al., 1977). Following scalding, the picking and defeathering steps are areas of potential cross contamination (USDA-FSIS, 2006b). Rigby et al., (1982) determined that the occurrence of *Salmonella* on feather pickers corresponds to an increased contamination of carcasses at the end of the processing cycle. This could be due to the rubber fingers possibly driving the microorganisms into the skin tissue and feather follicles or by the bacteria becoming aerosolized and contaminating the surrounding equipment in the defeathering area (Bryan et al., 1968). These events, further emphasize the need for optimization of scald tank management aiming at the reduction of pathogens prior to feather removal. Research by a group of scientists in the U.K. has identified the scald pH modification to alkaline conditions to be more effective in decreasing the thermal death time for *Salmonella* on processed chicken carcasses. Even in scald conditions consisting of high levels of organic matter, elevating scald water pH to approximately 9 with sodium carbonate greatly increased *Salmonella* killing in both scald water and on the skin of processed broiler carcasses (Humphrey et al., 1981). A problem with this approach is that using chlorine in the scalding tank under

alkaline conditions will not be recommended considering that chlorine becomes more effective at pH values below 6.5.

Conclusions

Human food-borne illnesses cost between 6.9 to 34.9 billion dollars in economic losses each year in the U.S. alone (Buzby and Roberts, 1997). Using these estimates for incidence, approximately one-fourth of all Americans will contract a foodborne disease during the next year (Tauxe, 2002). The major bacterial pathogen associated with bacterial foodborne illness in this country each year is *Salmonella*. Numerous studies and reports identify poultry as the most common vehicle associated with human food-borne illness related to this specific pathogen (Mandrell Wachtel, 1999; Notermans and Hoongenboom-Verdegaal, 1992; White et al., 2002).

Despite the enforcement of multiple programs, regulations and guidelines, the USDA-FSIS (2006b) reported increased prevalence of *Salmonella* in 2005 (16.3%) in poultry carcasses processed in the United States, turning to processors as the responsible party to enhance and evaluate pathogen control programs.

IOBW cabinets are one of the most commonly used pre-chill interventions and if managed properly, can be credited with significant microbial reductions. The immersion chiller operation has also been identified as a critical point for additional bacterial reductions on poultry carcasses (James et al., 1992; White et al., 2002), and the combination of pre-chill interventions with the chilling process may provide further reductions not possible if these processes are applied alone.

Broiler processing plants have long been used as the model for studies designed to investigate microbial interventions for processed poultry. To this end, the present investigation focuses on the development and validation of potential chemical and non chemical antimicrobial interventions applied before or after chilling during broiler processing operations.

CHAPTER III

**VALIDATION OF THE COMBINED USE OF ACIDIFIED CALCIUM
SULFATE AND ϵ -POLYLYSINE SOLUTIONS AS MULTIPLE-HURDLE
ANTIMICROBIAL INTERVENTIONS IN BROILER PROCESSING**

Salmonella has been and continues to be one of the leading causes of food-borne illness in the United States. Several studies have shown relative success in using chlorine rinses and its derivatives as antimicrobial interventions to reduce microbial contamination in poultry. Despite increased use of these interventions in commercial settings, *Salmonella* incidence continues to rise (Calvin 2004). The U.S. Department of Agriculture has recently reported that despite initial observed reductions in the late 90's, prevalence data from inspected plants shows a tendency to increase steadily since 2002 from 11.5 % *Salmonella* spp. prevalence in poultry carcasses to 16.3% in 2005. (USDA-FSIS, 2006b).

The use of organic acids as poultry carcass decontaminants in the meat and poultry industry has been extensively reported. However, cost effective treatments designed for use in combination or in sequence as pathogen reduction interventions for small meat and poultry processing plants, have not been validated. Mixed results observed with these antimicrobial agents have prompted the exploration of novel products that may be more promising in reducing pathogenic prevalence. Two novel antimicrobial compounds approved for food applications are acidified calcium sulfate

(ACS; Safe₂O®, Mionix Corp., Rocklin, CA) and ε (epsilon) –polylysine (EPL; Savory® PL–25, Chisso Corp., Yokohama, Japan).

Acidified Calcium Sulfate has been found to be effective as a beef and poultry carcass washing agent (Huffman 2002; Dickens et al., 2004). ACS is a generally recognized as safe (GRAS) antimicrobial ingredient that can be used in certain food product applications. It consists of a complex blend of sulfuric acid, calcium sulfate, calcium hydroxide, and an organic acid, usually lactic acid, adjusted to a final pH of ~1.5. According to its manufacturer, ACS when combined with organic acids disables the proton pumps in bacterial membranes and act as a metabolic inhibitor, thus attacking bacteria in a different fashion than organic acids alone (e.g. lactic acid). However, only a few studies have shown its potential residual antimicrobial effects.

ε-polylysine is a homopolymer of L-lysine, synthesized aerobically by a fermentation process of *Streptomyces albulus*. It is considered as a natural compound because it is produced by a fermentation method rather than a chemical synthesis counterpart. It has been reported that EPL is stable at high temperatures and under acidic and alkaline conditions and has a wide antimicrobial spectrum, including Gram-positive and Gram-negative bacteria, yeast and molds (Shima et al., 1984, Hiraki 2002, Yoshida et al., 2003). EPL is also GRAS and is thought to act in a different manner than ACS by causing disruption of the bacterial cell surface (Yoshida et al., 2002). EPL concentrations of 0.02% and 0.04% have been shown to have antimicrobial activity against *E. coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes*.

Particularly, *Salmonella* Typhimurium was shown to be the most sensitive of all three pathogens (Geornaras and Sofos, 2005).

Considering the potential synergistic effects of these two antimicrobials, the possibility to enhance their overall efficacy by a combined use needs to be explored. Currently, there is limited information on the use of ACS and EPL in a multiple hurdle fashion to reduce contamination and pathogen growth in meat and poultry plants. The purpose of this study was to evaluate the potential combined use of ACS and EPL solutions applied at different temperatures as chemical interventions applied pre- and post-chilling in poultry processing operations. Specific objectives included the determination of the effects of these products on pathogen contamination, chicken parts (skin-on, bone-in chicken breasts) that were artificially challenged with a rifampicin-resistant *Salmonella* Typhimurium strain. Additionally, the use of this chemical treatments was validated in commercially processed carcasses with the enumeration of aerobic plate counts, coliforms, generic *E. coli* counts and psychrotrophic loads immediately after application and after 10 days of storage.

Materials and Methods

Overview

This project was conducted as a collaborative effort between the USDA-ARS, Southern Plains Agricultural Research Center, Food and Feed Safety Research Unit and the Departments of Poultry Science and Animal Science at Texas A&M University, both in College Station, Texas. Bone-in breast samples with the same “sell by” dates and

brand were obtained from a local retail facility and challenged with *Salmonella* Typhimurium to determine the antimicrobial effects of EPL and ACS. Validation experiments were performed with commercially processed chicken carcass samples collected from a local processor after the evisceration process. Samples were immediately transported (20 min) to the Microbial Challenge Pilot Plant at the Poultry Science Center of the Department of Poultry Science at Texas A&M University to be treated with the stated interventions and continue with the remaining processing requirements (chilling operation). Microbial determinations were completed in the Poultry Products Laboratory at the Kleberg Animal and Food Science Center at Texas A&M University.

Media

A selective, differential medium composed of the basic formulation of tryptic soy agar (TSA, Difco, Detroit, MI) supplemented with rifampicin (R8883, Sigma, St. Louis, MO) was used to enumerate rifampicin resistant marker pathogens inoculated onto chicken breast surfaces. TSA was prepared according to manufacturer's recommendations, autoclaved at 121°C for 15 minutes and cooled to 55°C. Rifampicin was dissolved in 5ml methanol, filter-sterilized, and added to the cooled sterile medium prior to pouring into petri plates. The rifampicin resistant *Salmonella* Typhimurium strain produced yellow colonies in this medium.

Bacterial Cultures and Inoculum Preparation

A rifampicin resistant *Salmonella* Typhimurium strain NVSL 95-1776 was provided by the Department of Animal Science, Texas A&M University, College Station, Texas. The rifampicin resistant strains were kept in storage at -80°C before experiments. Before challenge experiments, the cultures were resuscitated by two successive transfers to Tryptic Soy Broth (TSB; Difco) and incubated at 35°C for 18-24 h. The cells were then transferred onto TSA slants and stored at 4.4°C before experiments. The day before the actual challenge experiment, a 12-h culture of *Salmonella* Typhimurium was dispensed into sterile centrifuge tubes (10 ml) and harvested by centrifugation at 1610 g for 15 min at 4.4°C. The cell pellets obtained were re-suspended in buffered peptone water (BPW, Difco) and diluted to a final concentration of c.a. 10^8 cells/ml. This suspension was used as the inoculum for chicken breast samples used to evaluate the interventions.

Collection and Inoculation of Chicken Breasts Samples

Fresh, skin-on, bone-in chicken breast samples were stored at 4.4°C to be processed in less than 24 hours. Inoculation was performed by delivering 1ml of the *Salmonella* Typhimurium rifampicin resistant inoculum into each of two marked piece portions of 6.45 cm² (1x1 in²) on the surface of the longitudinal section of the chicken breast sample. The inoculum was distributed manually by the use of a sterile hockey stick to aid in bacterial attachment. Inoculated breasts were allowed to dry at room

temperature for 10 minutes, before the application of antimicrobial interventions to enhance attachment and distribution of the cultured microorganisms.

Decontamination Procedures

Two decontamination solutions and a control were tested on inoculated samples. Treatment solutions included (i) no chemical intervention (control); (ii) a 1:4 acidified calcium sulfate solution homogenized with distilled water that was applied in sequence with a 200 ppm solution of ϵ (epsilon)-polylysine held at 55°C in a programmable water bath Model 1157P, VWR; and (iii) a 1:4 acidified calcium sulfate solution applied in sequence with a 200 ppm EPL solution kept at room temperature (~25°C). These concentrations were selected based on previous research reported by Keeton J. et al., (2006). Treatment solutions were applied using a pressurized spray system assembled with pressure nozzles especially manufactured for these experiments. The spray cabinet unit delivered a specific volume of 14ml/sec of the treatment solutions which were applied in 20 second intervals. The custom-built isolation spray cabinet was designed by the CHAD Corporation as it was reported by Keeton et al., (2006). The enclosed system was designed to capture and contain all spray residues, pathogen droplets and chicken breasts particles, which were then easily collected in biohazards autoclave bags attachable in the bottom drain. Internal decontamination of the spray cabinet between treatments was performed by using a 20% sodium hypochlorite solution (Clorox®) in a removable reservoir attached to a cleaning/decontamination nozzle through the lid. After each treatment, the cabinet was rinsed with hot water, the residue collected in a

biohazard bag for sterilization and the spray chamber sterilized. Contact between the acidic ACS products with chlorine was avoided to minimize toxic fumes production (Keeton et al, 2006). The cabinet was disinfected with the sodium hypochlorite solution between trials. External cabinet surfaces were swabbed with a 70% alcohol (ethanol) solution for decontamination. All run-off treatment solutions from the cabinet were collected in additional biohazard bags and autoclaved, except for treatments containing ACS, which were treated with additional ACS application for decontamination. Cabinet decontamination was accomplished by adding a 1:2 solution of ACS with water to the soiled cabinet walls and interior parts.

The spray cabinet was fitted with two H 1/8 VVSS 65015 nozzles with a spray angle of 65°, one near the top and bottom of the cylindrical spray chamber, through which treatments were delivered. The spraying pressure applied was 137.9 kPa (20 psi) and the flow rate was maintained at 0.4164 L/min (0.11 gallons per minute (GPM)) per nozzle, resulting in a spray angle of 51° per nozzle. Solution delivery was 0.2776 L/20 sec or 14 ml/sec for a total of 280ml per treatment in the 20 second application. After pathogen inoculation, individual chicken breast samples were sprayed for 20 seconds while continuously rotating at a constant rate of ~5 revolutions/20 sec under the uniform spray stream.

Sample Excision and Microbiological Examination

After the application of the antimicrobial interventions, the two 6.45 cm² (1x1 in²) portions of each of the treated and non-treated chicken breast samples were

aseptically excised from the skin surface using sterile stainless steel forceps and a scalpel. The excised section was cut to a ~2 to 3 mm deep to account for cells deeply attached in the sample. The first excision segment was stored at 7°C in a separate sterile Zip-lock® bag and marked for analysis after two days of refrigeration. The second excision segment was immediately placed in a stomacher bag and diluted with 58 ml of sterile 0.1% buffered peptone water (BPW; Difco) solution. The stomacher bag contents were pummeled in a stomacher for one minute to dislodge pathogens before examination. Serial dilutions in BPW were completed with each sample before plating onto modified TSA plates. Samples plated onto the differential agar were incubated at 37°C for 24 h to determine counts of rifampicin *Salmonella* Typhimurium cells in the excised breast samples.

Commercial Validation of the Intervention

To validate the use of the antimicrobial treatment combination under simulated commercial conditions; chicken carcasses from a local commercial facility were aseptically collected from the processing line post-evisceration, but before the inside-outside bird washer (IOBW) unit at random. Collected carcasses were placed individually in sterile bags and transported (20 minutes) to the laboratory for treatment application and chilling simulation. The antimicrobial treatments were applied either before or after a chilling process simulated in separated batch containers with a potable ice-water mix. Randomly selected carcasses (treated and non-treated) were sampled by

the whole carcass rinse method in a 3,500 stomacher bag and diluted with 400 ml of BPW subsequent to rocking agitation for 1 min.

Three different plant scenarios were simulated during each of three independent plant visits. A control plant scenario consisted in sampling 15 different eviscerated carcasses right after arrival at the laboratory from the processing plant. On each replication, 5 carcasses were sampled after evisceration and arrival, 5 carcasses were sampled after submersion in the ice-water batch chiller for 45 min and the final 5 carcasses were individually bagged after 45 min chilling and stored in a cooler at 4.4°C to be sampled after 10 days for refrigerated shelf life estimations. The antimicrobial interventions strategies proposed in this experiment created 2 additional plant simulation scenarios. The second scenario consisted of pre-chill carcasses sprayed with the acidified calcium sulfate (ACS) 1:4 solution followed by the application of the ϵ -polylysine solution (200ppm) at 55°C. Again 15 separate samples taken after evisceration at the plant were taken to our laboratory and subjected to the intervention application pre-chill. Five of this carcasses were tested immediately after the intervention application, 5 separate carcasses were treated with the chemical sprays and then submerged in separate batch chillers (ice-water bath) and sampled immediately after chilling to determine the combined effects of the intervention and the chilling process. Finally, the 5 remaining carcasses were treated with the chemical interventions, subjected to simulated chilling for 45 minutes in a separate ice-water batch chiller and then individually bagged and stored at 4.4°C for shelf life evaluation after 10 days of storage. The third and final plant scenario created required 10 additional carcass samples collected at the processing plant

to be treated post-chill. Upon arrival the 10 carcasses were submerged in an ice-water bath for 45 minutes to simulate the chilling operation. All 10 carcasses were treated with the ACS (1:4) solution followed with the EPL (200ppm) at 55°C. Five carcasses were sampled immediately after the application of the intervention while the remaining 5 carcasses were individually bagged and stored at 4.4 °C to be sampled after 10 days of storage for shelf-life evaluation.

Microbiological Methods

Standard sampling and plating methods were used to evaluate aerobic plate counts (APC), coliform counts, generic *E. coli* counts and psychrotropic counts as microbial indicators. In addition, samples were tested for the prevalence of *Salmonella* spp. using standardized methods. To determine aerobic plate counts, serial dilutions were prepared using BPW as a diluent and plated in duplicate in plate count agar (PCA; Difco) plates. Plates were incubated at 37°C for 48 h before counting aerobic plate counts in log₁₀CFU/ml of rinse. For psychrotrops enumeration, serial dilutions of each sample were plated in duplicate in tryptic soy agar (TSA; Difco) and incubated at 7 °C for 7 days. Results were recorded as log₁₀ CFU/ml of carcass rinse.

To enumerate coliforms and generic *E. coli* counts, samples were serially diluted and plated in duplicate in 3M™ Petrifilm™ *E. coli*/Coliform Count Plates (3M Inc, St. Paul, MN). Petrifilm plates were incubated at 37°C for 24 h to enumerate total coliform counts (red colonies with gas production) and then re-incubated for 24 additional h to

count generic *E. coli* colonies (blue colonies with gas production). Results were converted to \log_{10} CFU/ ml of carcass rinse.

For *Salmonella* prevalence estimation, whole carcass rinse samples were subjected to a pre-enrichment step, where 25ml of the rinsate were mixed with 25ml of fresh BPW and incubated for 24 hours at 37°C. Samples were then selectively enriched in Rappaport Vassiliadis broth tubes (RV; Difco) incubated for 24 hours at 41°C. Selective plating was done in Xylose Lysine Tergitol 4 plates (XLT4; Difco) with incubation at 37°C for 24 to 48 hours. Suspected colonies (black) were confirmed serologically using the Polyvalent A through I serum for agglutination (VWR).

Statistical Analysis

Count data for each determination were transformed logarithmically before comparison of means by analysis of variance of the General Linear Model system. Each experiment was independently replicated three times in a completely randomized block design. Data were analyzed by one-way analysis of Variance (ANOVA) for each microorganism using SAS statistical analysis software program, version 9.1 (SAS Institute Inc., Cary, NC). When significant differences were observed ($P < 0.05$), separation of means was accomplished by using LSMEANS multiple ranges test using SAS procedures.

Results and Discussion

Salmonella Typhimurium in Chicken Breast Samples

Significant (from control) *Salmonella* Typhimurium population reductions of approximately 0.55 and 0.65 log₁₀ CFU/ml were obtained for samples treated with 14ml/sec for 20 sec intervals at 55°C on day 0 and 2, respectively. A reduction of approximately 0.65 and 0.33 log₁₀ CFU/ml was observed for day 0 and 2, respectively, for samples treated with 14ml/sec for 20 sec intervals at 25°C as shown in table 1 and figures 1 and 2.

Table 1. Log₁₀CFU/ml of *Salmonella* Typhimurium populations in control chicken breast samples and samples treated with an acidified calcium sulfate (ACS) solution followed with an ε-polylysine solution applied at either 55 or 25°C and sampled at day 0 and 2 of refrigerated storage.

<i>Salmonella</i> Typhimurium Populations		
Log ₁₀ CFU/ml		
	Day 0	Day 2
Control	5.89 ^{a*}	5.90 ^a
ACS + EPL @ 55°C	5.34 ^b	5.25 ^b
ACS + EPL @ 25°C	5.24 ^b	5.57 ^b

*Log values followed by different subscript are significantly different ($P < 0.05$) across the table

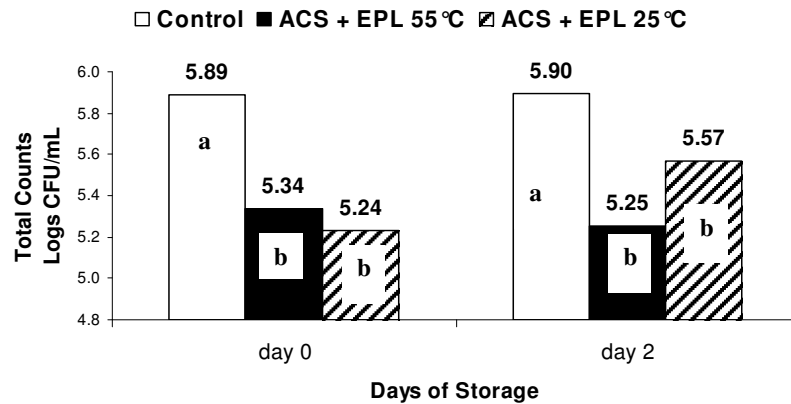


Figure 1. Log₁₀ CFU/ml of *Salmonella* Typhimurium populations in control chicken breast samples and samples treated with an acidified calcium sulfate (ACS) solution followed with an ε-polylysine solution applied at either 55 or 25°C and sampled at day 0 and 2 of refrigerated storage.

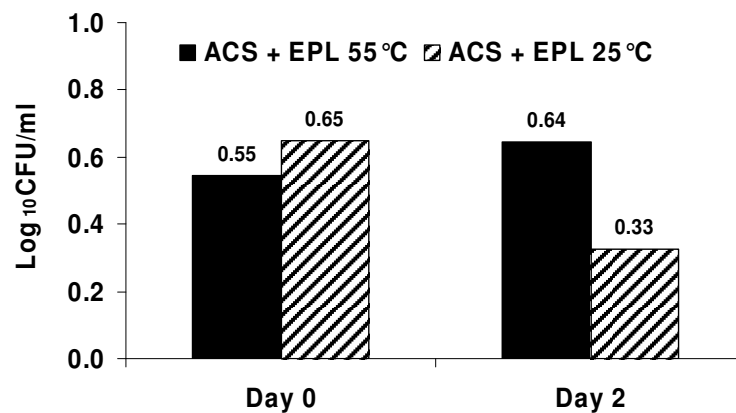


Figure 2. Log₁₀ CFU/ml of *Salmonella* Typhimurium reductions in chicken breast samples treated with ACS and EPL at 55 vs. 25°C and sampled at day 0 and 2 of refrigerated storage.

Commercial Validation of Interventions

Whole chicken carcass samples from a local processing facility were evaluated with the same chemical antimicrobial intervention in order to demonstrated efficacy of

the treatments. Table 2 shows the multiple parameters that were measured with the statistical analysis.

Table 2. Microbial profiles of chicken carcasses treated with a combination of ACS and EPL as an antimicrobial intervention applied either before or after chilling.

Chemical Intervention Treatments					
Log ₁₀ CFU/ml of rinse					
n = 120	Pre-chill Samples		Chilled Samples		Post-chill
	Control	ACS-EPL	Control	ACS-EPL	ACS-EPL
APC	4.66 ^a	3.83 ^{bc}	3.77 ^{bc}	4.00 ^b	3.85 ^{bc}
Coliforms	3.80 ^a	2.02 ^b	2.23 ^b	2.19 ^b	2.13 ^b
Generic <i>E. coli</i>	3.32 ^a	1.75 ^b	1.93 ^b	1.84 ^b	1.73 ^b
Psychrotrophs	2.12 ^a	1.05 ^b	1.11 ^b	1.15 ^b	0.93 ^b

* Means in the same row, followed by different subscript are significantly different ($P < 0.05$)

For aerobic plate counts, pre-chill treatments with the ACS-EPL combination exhibited 0.83 log₁₀ CFU/ml reductions when compared to control eviscerated samples. For coliforms, pre-chill treatments exhibited 1.78 log₁₀ CFU/ml reductions compared with control eviscerated samples, chilled and post-chill chemical interventions exhibited 0.04 and 0.1 log₁₀ CFU/ml reductions, respectively, compared with control chill samples. For generic *E. coli*, pre-chill treatments exhibited 1.57 log₁₀ CFU/ml reductions compared with control eviscerated samples, chilled and post-chill chemical interventions exhibited 0.09 and 0.2 log₁₀ CFU/ml reductions, respectively, compared with control chill samples. For psychrotrophic organisms, pre-chill treatments exhibited 1.07 log₁₀ CFU/ml reductions compared with control eviscerated samples, chilled and post-chill

chemical interventions exhibited -0.04 and 0.18 log₁₀ CFU/ml reductions, respectively, compared with control chill samples as shown in table and figures 3, 4, 5 and 6.

Salmonella organisms were not recovered significantly from control samples (eviscerated and chilled), however after the chemical intervention, there was a 25% of recovery of *Salmonella* from pre and post chill chemical intervened samples. This could be due to the random selection of carcasses for this experiments and the low prevalence of this organism at the facility evaluated.

For APC in stored samples, pre-chill chemical intervened samples tested after chilling, had 0.66 log₁₀ CFU/ml reduction and post-chill interventions exhibited 0.56 log₁₀ CFU/ml reductions at day 10. While for psychrotrophic organisms, pre-chill intervened samples tested after chilling, exhibited 0.01 log₁₀ CFU/ml reductions and post-chill interventions had 0.66 log₁₀ CFU/ml reductions as shown in table 3.

Table 3. Microbial profiles of chicken carcasses treated with a combination of ACS and EPL as an antimicrobial intervention applied either before or after chilling after 10 days of storage.

	Log ₁₀ CFU/ml		
	Chemical interventions		
n = 75	Control Chill	Chilled ACS-EPL	Post-Chill ACS-EPL
APC	6.29 ^b	6.95 ^a	5.73 ^c
Psychrotrophs	6.96 ^a	6.95 ^a	6.30 ^b

* Means in the same row, followed by different subscript are significantly different (P < 0.05)

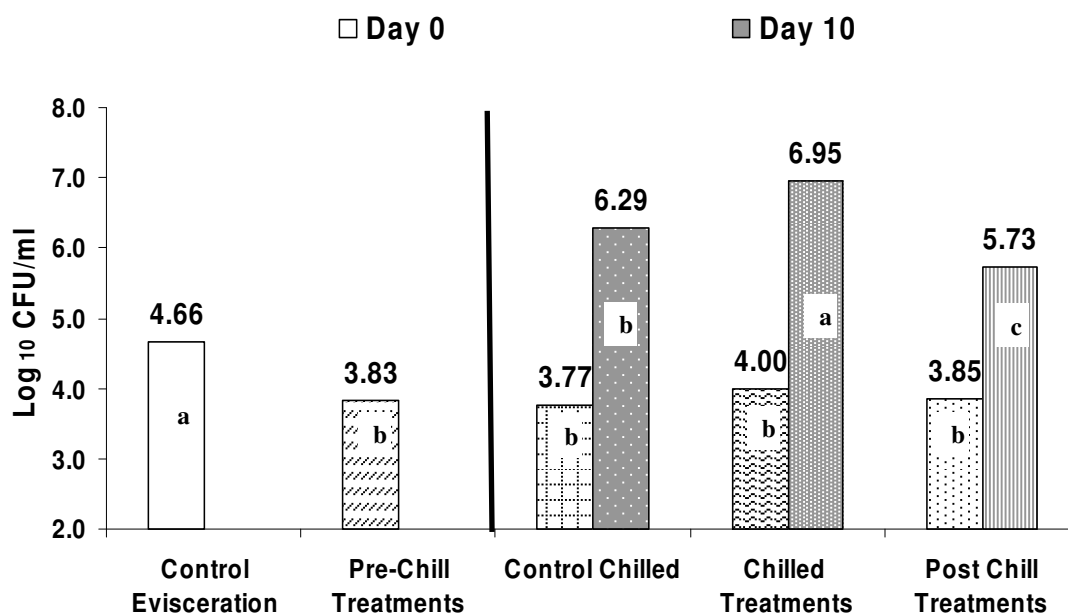


Figure 3. Log₁₀ CFU/ml of aerobic plate counts in control chicken carcasses and carcasses treated with an acidified calcium sulfate (ACS) solution followed with an ϵ -polylysine solution applied at 55°C and sampled at day 0 and 10 of refrigerated storage.

Coliforms

Figure 4 represents the coliform behave before and after chemical intervention.

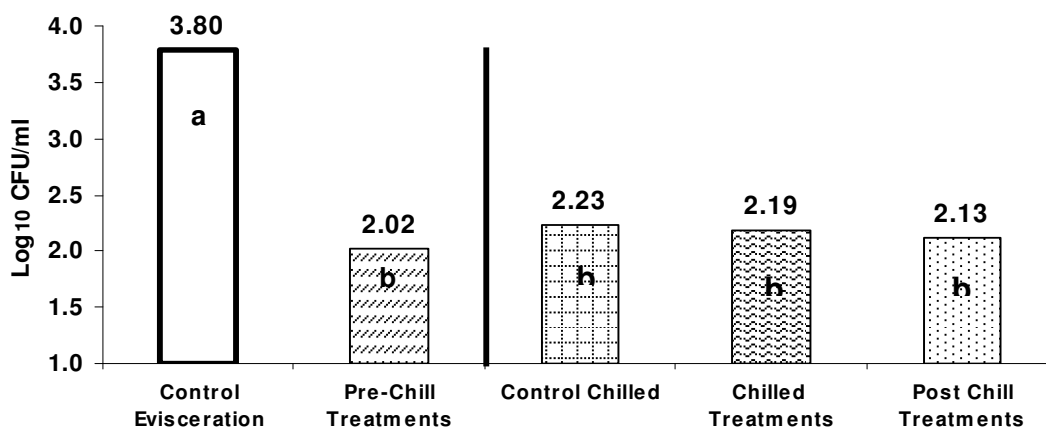


Figure 4. Log₁₀ CFU/ml of coliforms in control chicken carcasses and carcasses treated with an acidified calcium sulfate (ACS) solution followed with an ϵ -polylysine solutions applied at 55°C and sampled at day 0.

Generic *Escherichia coli*

Figure 5 represents the generic *Escherichia coli* behavior before and after chemical antimicrobial intervention in whole chicken carcasses.

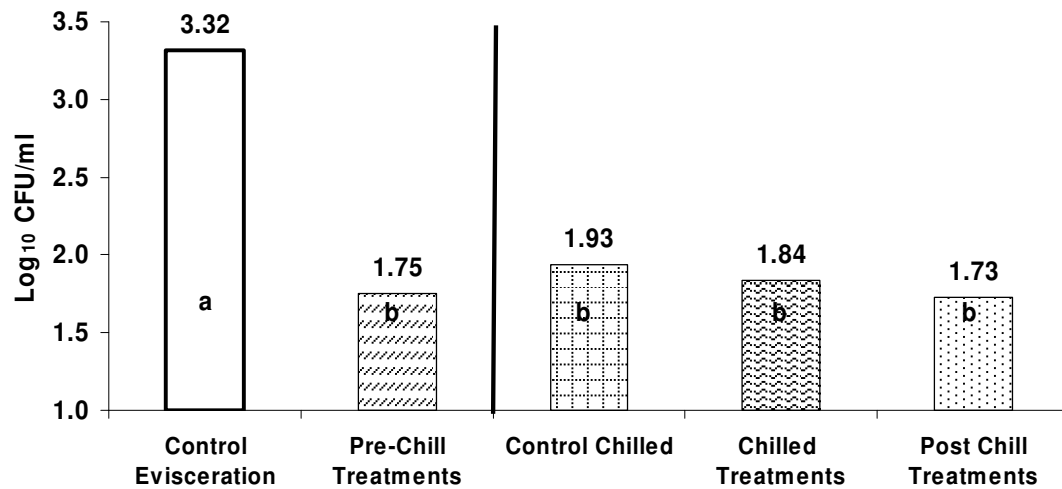


Figure 5. Log₁₀ CFU/ml of generic *Escherichia coli* in control chicken carcasses and carcasses treated with an acidified calcium sulfate (ACS) solution followed with an ε-polylysine solutions applied at 55°C and sampled at day 0.

Psychrotrophs

This graph represents the psychrotroph organisms increasing or reduction after the chemical antimicrobial intervention of the whole chicken carcasses for validate it.

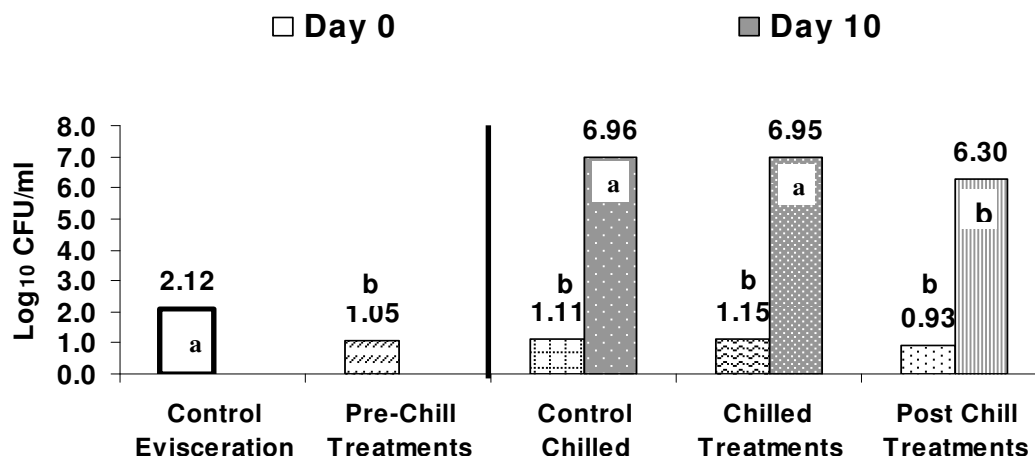


Figure 6. Log₁₀ CFU/ml of psychrotroph counts in control chicken carcasses and carcasses treated with an acidified calcium sulfate (ACS) solution followed with an ε-polylysine solutions applied at 55°C and sampled at day 0 and 10 of refrigerated storage.

In this study, the effectiveness against rifampicin resistant *Salmonella* Typhimurium strain NVSL 95-1776 by the combined treatment with ACS and EPL at either 25°C or 55°C was compared on bone-in chicken breasts samples, as showed in table 1. No significant differences were found between each treatment; however, both strategies produced significant reductions of *Salmonella* when compared to control samples. The application of ACS followed by the EPL solution at 55°C was selected to further validate the combined intervention in a commercial facility due to its apparent residual activity after storage as shown in figures 1 and 2. Similar results were reported

by Keeton et al. (2006), finding ACS + EPL at 55°C more effective than when applied at 25°C when used against *Salmonella* Typhimurium in artificially inoculated beef round samples.

Results observed in the validation trials indicated that pre chill interventions of ACS + EPL at 55°C showed significant different surviving microbial populations when compared to control eviscerated samples. Mean reductions of $>1 \log_{10}$ CFU/ml were observed with all the indicator organisms as it can be seen in figures 3, 4, 5 and 6. With chilled and post chill interventions, the mean \log_{10} reductions were less than 0.5 CFU/ml compared with control chill samples in all the indicator organisms showing not significant differences (Figures 3, 4, 5 & 6), demonstrating that pre chill interventions were more effective than chilled or post chill ones.

At day 10 of storage at 7°C, ACS + EPL at 55°C at post-chill intervention (significant different), produced a mean \log_{10} reduction of 0.56 CFU/ml for APC and 0.66 CFU/ml for psychrotrophs indicating the prolonged effect of the chemicals at 10 days at refrigeration temperatures. Comparing these reductions with those reported by Keeton et al., (2006) and Geornaras and Sofos (2005), EPL has an enhancement effect with acidic antimicrobials (synergistic effect) against microorganisms.

CHAPTER IV
VALIDATION OF KOSHER SALT AS AN ANTIMICROBIAL INTERVENTION
IN BROILER PROCESSING

Several studies have been conducted to evaluate the efficacy of chemicals to reduce *Salmonella* contamination in poultry. Efforts continue to find suitable alternatives that are more efficient and cost-effective compared to existing intervention strategies. Food poisoning is an increasing problem throughout the world, with a high percentage of outbreaks being associated with poultry. Thus, the safety of poultry products is of great concern to the poultry industry, government agencies, and consumers (Yang et al., 1998). Poultry carcasses on a conventional processing line can be contaminated with a variety of pathogenic bacteria, including *Salmonella* Typhimurium, *Campylobacter jejuni*, and *Listeria monocytogenes* (Lillard, 1990; Clouser et al., 1995).

Kosher-slaughtered poultry, passing through the koshering process, may introduce even higher risks for microbial contamination, compared to non kosher products, at several critical points in the koshering line. Two of the more critical operations, which may influence the microbial quality of the end poultry products, are the water temperature in the dressing operation on the non kosher lines and the practice of applying salt in the koshering process (Regenstein and Regenstein, 1988).

Kosher salt is composed mainly by sodium chloride without additives (iodine) and has a much larger grain size than regular table salt with a more open granular structure. The kosher name is attributed because of its use in making meats kosher, by

helping to extract the blood and other pigments from the meat. Because of its larger grain size when compared to regular table salt grains, this product does not dissolve readily when meats are coated in kosher salt. The product tends to remain on the surface of the meat for a longer time, thus allowing fluids to leach out of the meat (Hutchinson, KS. 2007).

The Hebrew word kosher means fit or proper (Lipschutz, 1996) and is a religious term used to describe a range of ritualistic objects, activities, or even a state-of-being. Food is kosher when it meets the strict dictates of the laws of kashrus. The laws of kashrus require that kosher food:

- (1) Come from a proper source, for example, meat from some animals, such as pork, is inherently non kosher.
- (2) Be prepared in a specific manner, for example, animals must be slaughtered in a particular manner, and
- (3) Not be combined improperly with food that might otherwise be considered non-kosher, for example, combining meat and milk.

According to Sigman (1999), kosher poultry is limited to domestic fowl. Scavenging birds, such as eagles or ravens, are not permissible sources of food. Due to historical difficulties in applying the standard of what makes poultry kosher, rabbinic authorities declared that permitted fowl would be limited to a list of birds traditionally known to be kosher (Regenstein et al., 1988). The most well known and consumed from this list are chicken, turkey, goose, and domestic duck. Since there is no definitive

tradition about pheasant, wild ducks and geese, or pigeons, they may not be eaten. Like in the context of beef, the blood from poultry is not considered to be kosher.

The koshering operation is accomplished by soaking the fowl in cold water (8 to 12°C) for 30 minutes, a process called *Shriah*, to remove coagulated blood. Then, the carcasses are salted massively with coarse salt all over their surfaces for 1 hour to allow the blood to drip out. Finally the carcasses are rinsed with cold water, a process called *Hadacha* (Regenstein and Regenstein, 1988). The two immersions in water in the koshering operation are further potential sites for cross-contamination of these birds.

A validation, is defined as the element of verification focused on collecting and evaluating scientific and technical information to determine the effective control of hazards (USDA-FSIS, 2004). Validation activities (9 CFR 417.4) are a critical tool for plants verifying the effectiveness of process control interventions that control pathogenic microorganisms like *Salmonella* spp. In order to determine that any intervention is controlling the pathogen, the validation process must be carried out in the plant, subject to the plant's facilities, processes, and unique conditions. (USDA-FSIS, 2006b).

The purpose of this research is to evaluate and validate the antimicrobial effects of the dry application of kosher salt on the surface of eviscerated chicken parts artificially inoculated with rifampicin-resistant *Salmonella* Typhimurium, and execute its validation in a commercial facility.

Materials and Methods

Overview

In this investigation, the experiments were conducted in the Poultry Processing and Products laboratory, Department of Poultry Science, Texas A&M University. Bone-in breast samples with the same “sell by” dates and brand were obtained from a local retail facility and challenged with *Salmonella* Typhimurium to determine the antimicrobial effects of kosher salt, while the validation of the intervention was accomplished with chicken carcass samples obtained from a local commercial processing facility. Interventions were applied in the Microbial Challenge Pilot Plant of the Poultry Science Center, Texas A&M University; and microbiological analysis was done in the Poultry Processing and Products laboratory.

Media

A selective, differential medium composed of the basic formulation of tryptic soy agar plus rifampicin was developed to simultaneously enumerate rifampicin resistant marker pathogens inoculated onto chicken breast surfaces. The medium consisted of the following ingredients per liter: tryptic soy agar (TSA, Difco, Detroit, MI) and rifampicin (Sigma Chemical code R8883). The medium without rifampicin was autoclaved at 121°C for 15 minutes and cooled to 55°C. 0.1g of Rifampicin was dissolved in 5ml methanol, filter-sterilized, and added to the sterile medium prior to pouring into petri plates. Prepoured plates were dried at 25°C overnight before use. The rifampicin resistant *Salmonella* Typhimurium produced yellow colonies.

Bacterial Cultures and Inoculum Preparation

Rifampicin resistant strains of *Salmonella* Typhimurium strain NVSL 95-1776 were provided by the Department of Animal Science, Texas A&M University, College Station, Texas. The rifampicin resistant strains were kept in storage at -80°C before experiments. Before challenge experiments, the cultures were resuscitated by two successive transfers to Tryptic Soy Broth (TSB; Difco) and incubated at 35°C for 18-24 h. The cells were then transferred onto TSA slants and stored at 4.4°C before experiments. The day before the actual challenge experiment, a 12-h culture of *Salmonella* Typhimurium was dispensed into sterile centrifuge tubes (10 ml) and harvested by centrifugation at 1610 g for 15 min at 4.4°C. The cell pellets obtained were re-suspended in buffered peptone water (BPW, Difco) and diluted to a final concentration of c.a. 10^8 cells/ml. This suspension was used as the inoculum for chicken breast samples used to evaluate the interventions.

Collection and Inoculation of Chicken Breasts Samples

Fresh, skin-on, bone-in chicken breast samples were secured from a local retailer, transported to our laboratories and stored at 4.4°C to be processed in 24 hours. Inoculation was performed by delivering 1ml of the *Salmonella* Typhimurium rifampicin resistant inoculum into each of two marked piece portions of 6.45 cm² (1x1 in²) on the surface of the chicken breasts. The inoculum was distributed manually by the use of a hockey stick to aid in bacterial attachment. Inoculated breasts were allowed to dry at room temperature for 10 minutes, before the application of antimicrobial interventions.

Comparison of Kosher Interventions

For kosher salt intervention, chicken breast samples were inoculated in the same area size (1x1 in²) with *Salmonella* Typhimurium (rifampicin resistant). After 10 minutes to allow bacterial adhesion, the chicken breasts were assigned to be treated immediately by one of the following treatments: (i) not salt application (control), (ii) kosher salt application when chicken breast were at 12°C (pre-chill), and (iii) kosher salt application when chicken breast were at 4.4°C (post-chill). For pre-chill intervention, three chicken breasts were kept at 12°C (30 minutes), 200 grams of kosher salt was applied to the whole breast surface by stocking the breast in a sterile plastic bag, followed by a massage to ensure full coverage of the salt. Samples were left at room temperature for 1 hour. For post-chill intervention, chicken breasts samples were iced for 30 minutes until temperature arrived 4.4°C, then 200 grams of kosher salt was applied to cover the whole breast surface into a sterile plastic bag, followed by a massage, the samples were left at room temperature for one hour. Then, samples were rinsed with 1L of sterile water and the residue was collected in biohazard bags for autoclaving.

Sample Excision and Microbiological Examination

Afterward the application of the antimicrobial interventions, the two 6.45 cm² (1x1 in²) portions of each of the treated and non-treated chicken breast were aseptically excised from the skin surface using sterile stainless steel forceps and a scalpel. The excised section was cut to a ~2 to 3 mm deep to account for cell deeply attached in the sample. The first excision segment was stored at 7°C in a separate sterile Zip-lock® bag

and marked for analysis after two days of refrigeration. The second excision segment was immediately placed in a stomacher bag and diluted with 58 ml of sterile 0.1% buffered peptone water (BPW; Difco) solution. The stomacher bag contents were pummeled in a stomacher for one minute to dislodge pathogens before examination. Serial dilutions in BPW were completed with each sample before plating onto modified TSA plates. Samples plated onto the differential agar were incubated at 37°C for 24 h to determine counts of rifampicin *Salmonella* Typhimurium in the breast samples.

Commercial Validation of the Intervention

Validation was done analyzing the effect of interventions applied before and/ or after chilling in a commercial broiler processing facility. Three different scenarios were simulated during each of three independent plant visits. A control plant scenario consisted in sampling 15 different carcasses after evisceration; 5 were tested after evisceration, 5 simulating chill intervention and 5 were kept on the cooler (4°C) for test shelf life after 10 days. Interventions tested in this experiment will create 2 additional plant scenarios. The second scenario was the pre-chill samples fully covered with kosher salt and sampled 60 minutes after treatment application, and after chilling. The third scenario consisted of 10 samples subjected to the intervention (kosher salt) post-chill keeping 5 of them in the cooler at 4°C for test shelf life. Standard sampling methods were used to evaluate total aerobic counts, coliform, generic *E. coli* and psychrotrophic counts, as well as the prevalence of *Salmonella* spp.

Microbiological Methods

Standard sampling methods were used to evaluate aerobic plate counts, coliform counts, generic *E. coli* counts and psychrotropic counts as microbial indicators. In addition, samples were tested for the prevalence of *Salmonella* spp. using standardized methods. To determine aerobic plate counts serial dilutions were prepared using BPW as a diluent and plated in duplicate in plate count agar (PCA; Difco) plates. Plates were incubated at 37°C for 48 h before counting aerobic plate counts in log₁₀CFU/ ml of rinse. For Psychrotrops enumeration, serial dilutions of each sample were plated in duplicate in tryptic soy agar (TSA; Difco) and incubated at 7 °C for 7 days. Results were recorded as log₁₀ CFU/ ml of carcass rinse.

To enumerate coliforms and generic *E. coli* counts, samples were serially diluted and plated in duplicate in 3M™ Petrifilm™ *E. coli*/Coliform Count Plates (3M Inc, St. Paul, MN). Petrifilm plates were incubated at 37°C for 24 h to enumerate total coliform counts (red colonies with gas production) and then re-incubated for 24 additional h to count generic *E. coli* colonies (blue colonies with gas production). Results were converted to log₁₀CFU/ ml of carcass rinse.

For *Salmonella* prevalence estimation, whole carcass rinse samples were subjected to a pre-enrichment step, where 25ml of the rinsate were mixed with 25ml of fresh BPW and incubated for 24 hours at 37°C. Samples were then selectively enriched in Rappaport Vassiliadis broth tubes (RV; Difco) incubated for 24 hours at 41°C. Selective plating was done in Xylose Lysine Tergitol 4 plates (XLT4; Difco) with

incubation at 37°C for 24 to 48 hours. Suspected colonies (black) were confirmed serologically using the Polyvalent A through I serum for agglutination (VWR).

Color Measurement

A total of 45 samples, 15 from each replicate (5 per treatment) were used for color measurements (L^* = lightness, a^* = redness, b^* = yellowness) that were determined in all samples using a Minolta colorimeter (C.I.E., 1978). Samples were measured immediately after kosher salt interventions and after 24 h. Minolta colorimeter was calibrated each time with a white reference tile ($Y = 88.5$, $x = 0.310$, $y = 0.317$).

Drip Loss Analysis

A total of 45 samples, 15 from each replicate (5 per treatment), were used for drip-loss analysis. Immediately following kosher salt application, chicken carcasses were kept on trays d of an air-tight container as described by Northcutt et al. (1994). Containers were stored for 3 d at 4° C and the individual fillets reweighed to determine drip loss. Drip loss was calculated as (weight of drip loss/initial weight of fillet) X 100.

Statistical Analysis

Count data were transformed logarithmically before comparison of means by Analysis of Variance or General Linear Model procedures. Each experiment was independently replicated three times in a completely randomized block design. Data were analyzed by one-way analysis of Variance (ANOVA) for each microorganism

using SAS statistical analysis software program, version 9.1 (SAS Institute Inc., Cary, N.C. U.S.A.). When significant differences were observed ($P < 0.05$), separation of means was accomplished by using LSMEANS multiple ranges test using SAS procedures (SAS Institute, 1985). Color and drip-loss values were analyzed using the ANOVA procedure, following by a LSMEANS means separation analysis.

Results and Discussion

Salmonella Typhimurium in Chicken Breast Samples

A reduction of approximately 0.88 and 0.94 \log_{10} CFU/ml was obtained on days 0 and 5 respectively, for pre-chill treatment. Post-chill treated samples showed reductions of 1.15 and 1.01 \log_{10} CFU/ml on days 0 and 5 respectively, as shown in table 4 and figures 7 and 8.

Table 4. \log_{10} CFU/ml of *Salmonella* Typhimurium populations in control chicken breast samples and samples treated with kosher salt applied either pre or post-chilling and sampled at day 0 and 5 or refrigerated storage.

<i>Salmonella</i> Typhimurium Populations		
\log_{10} CFU/ml		
	Day 0	Day 5
Control	6.26 ^a	5.90 ^c
Pre-chill Intervention	5.38 ^b	4.96 ^d
Post-Chill Intervention	5.11 ^b	4.89 ^d

*Log values followed by different subscript are significantly different ($P < 0.05$) across the table

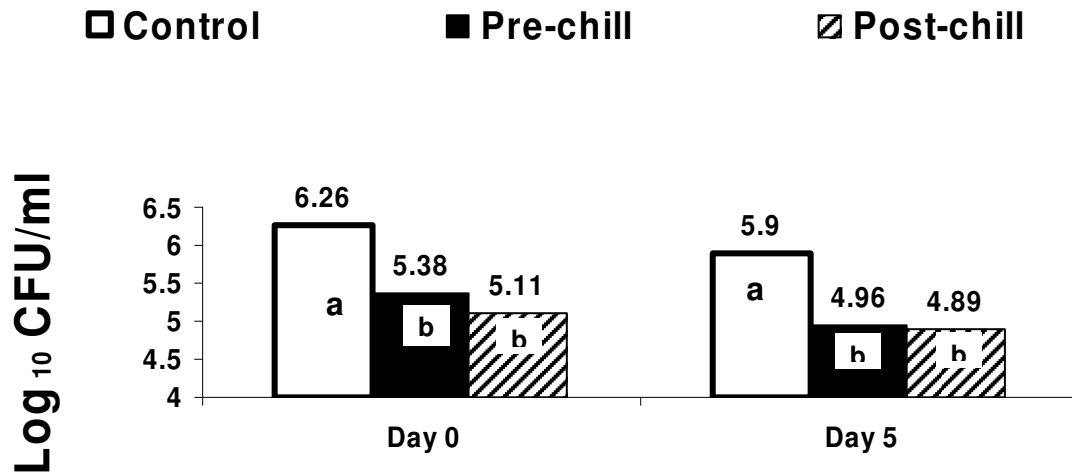


Figure 7. Log_{10} CFU/ml of *Salmonella* Typhimurium populations in control chicken breast samples and samples treated with kosher salt applied either pre or post-chilling and sampled at day 0 and 5 or refrigerated storage.

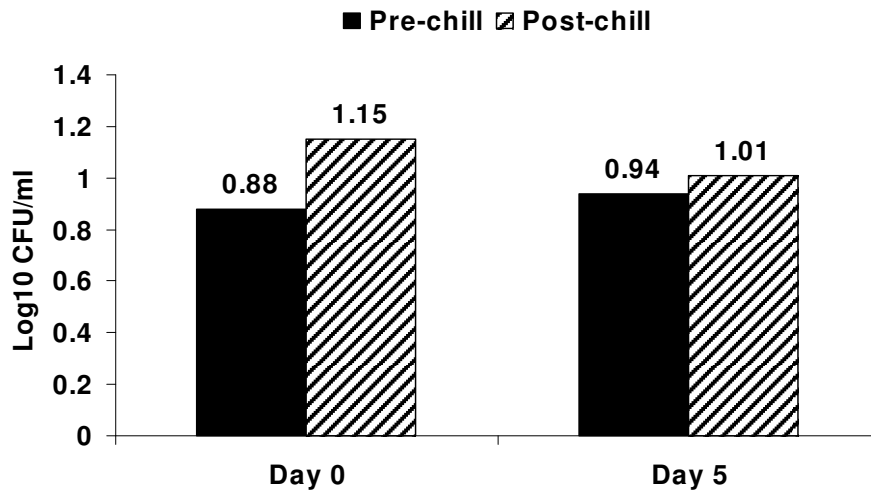


Figure 8. Log_{10} CFU/ml of *Salmonella* Typhimurium reductions in chicken breast samples treated with kosher salt pre or post chill and sampled at day 0 and 5 of refrigerated storage.

Commercial Validation of Interventions

For APC, pre-chill treatment compared with control eviscerated samples exhibited 1.29 Log₁₀ CFU/ml reductions; chilled and post-chill kosher salt interventions exhibited 0.16 and 0.35 Log₁₀ CFU/ml reductions compared with control chill samples, respectively. For coliforms, pre-chill treatment compared with control eviscerated samples exhibited 2.44 Log₁₀ CFU/ml reductions; while chilled and post-chill kosher salt interventions exhibited 0.79 and 1.35 Log₁₀ CFU/ml reductions, respectively, compared with control chill samples. For generic *E. coli*, pre-chill treatment compared with control eviscerated samples exhibited 2.69 Log₁₀ CFU/ml reductions; chilled and post-chill kosher salt interventions exhibited 0.97 and 1.15 Log₁₀ CFU/ml reductions, respectively, compared with control chill samples. For psychotropic organisms, pre-chill treatment compared with control eviscerated samples exhibited 1.33 Log₁₀ CFU/ml reductions; while chilled and post-chill kosher salt interventions exhibited 0.19 and 0.31 Log₁₀ CFU/ml reductions, respectively, compared with control chill samples as shown in table 5 and figures 9, 10, 11 and 12.

Salmonella isolation was not recovered from control samples (eviscerated and chilled), however after the salt intervention, only in one sample, *Salmonella* was recovered from a pre chill kosher salt intervened sample.

Table 5. Microbial profiles of chicken carcasses treated with kosher salt as an antimicrobial intervention applied either before or after chilling at day 0.

Kosher Salt Interventions Treatments					
Log ₁₀ CFU/ml of rinse					
n = 120	Pre-chill Samples		Chilled Samples		Post-chill
	Control	Kosher salt	Control	Kosher salt	Kosher salt
APC	4.66 ^a	3.37 ^c	3.77 ^b	3.61 ^b	3.42 ^b
Coliforms	3.80 ^a	1.36 ^c	2.23 ^b	1.44 ^c	0.88 ^d
Generic <i>E. coli</i>	3.32 ^a	0.63 ^c	1.93 ^b	0.96 ^c	0.78 ^c
Psychrotrophs	2.12 ^a	0.79 ^b	1.11 ^b	0.92 ^b	0.80 ^b

* Means in the same row, followed by different subscript are significantly different (P < 0.05)

For APC, pre-chill kosher salt interventions sampled after chilling samples, got 0.8 Log₁₀ CFU/ml reduction and post-chill interventions exhibited 0.21 Log₁₀ CFU/ml reductions at day 10. While for psychrotrophic organisms, pre-chill intervened samples sampled after chilling exhibited 1.09 Log₁₀ CFU/ml reductions and post-chill 0.16 Log₁₀ CFU/ml reductions as showed in table 6 and figures 9 and 12.

Table 6. Microbial profiles of chicken carcasses treated with kosher salt as an antimicrobial intervention applied either before or after chilling at day 10.

Log ₁₀ CFU/ml			
Kosher Salt Interventions			
n = 75	Control Chill	Chilled	Post-Chill
APC	6.29 ^a	5.49 ^b	6.08 ^a
Psychrotrophs	6.96 ^a	5.87 ^b	6.80 ^a

* Means in the same row, followed by different subscript are significantly different (P < 0.05)

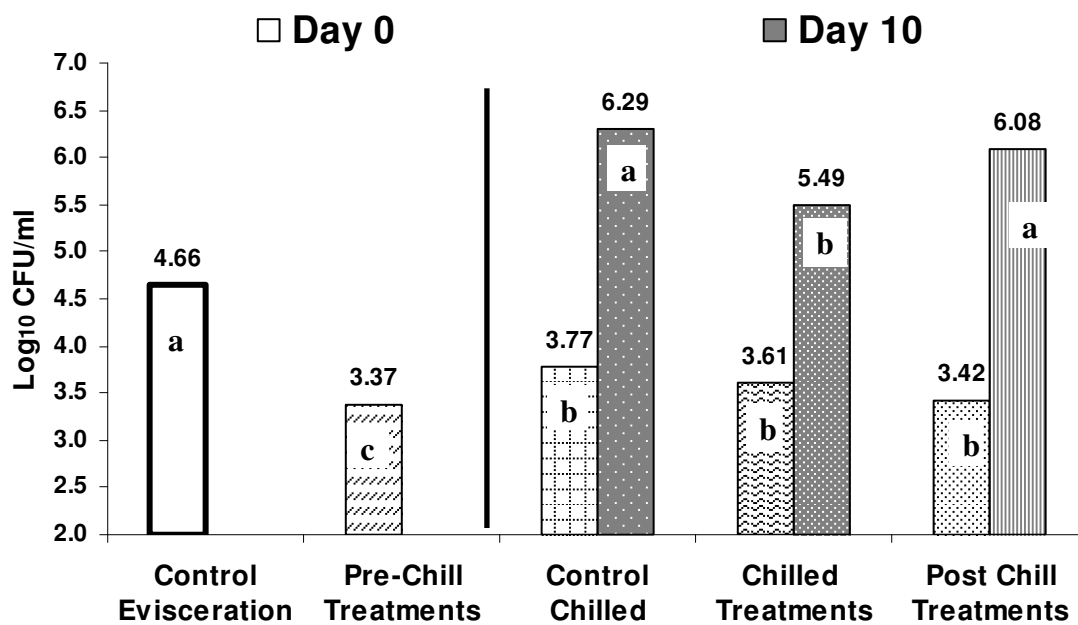


Figure 9. Log₁₀ CFU/ml of aerobic plate counts in control chicken carcasses and carcasses treated with kosher salt applied either pre or post chill and sampled at day 0 and 10 of refrigerated storage.

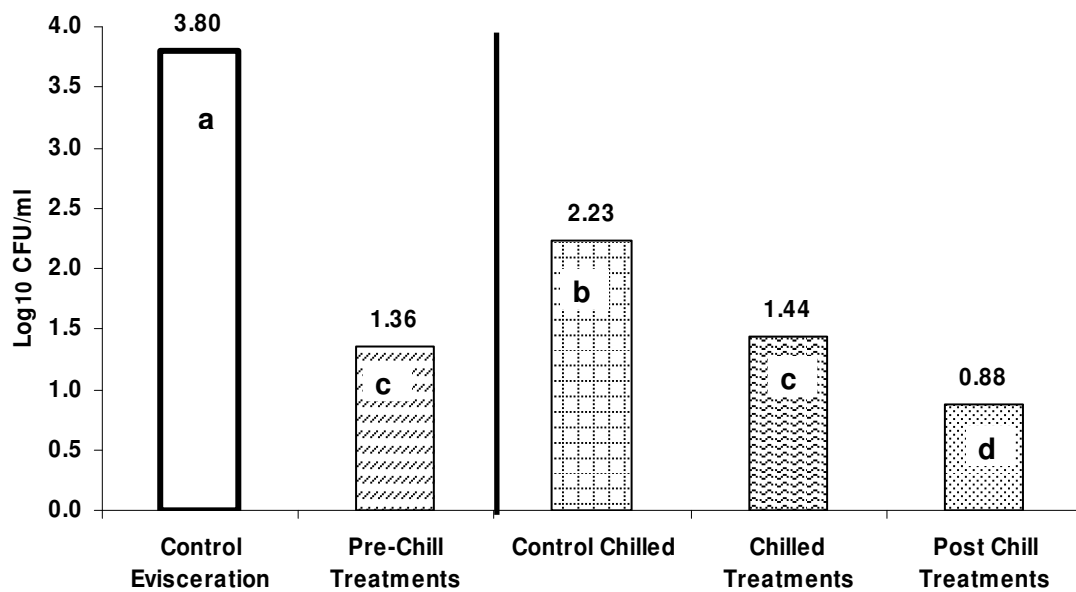


Figure 10. Log₁₀ CFU/ml of coliforms in control chicken carcasses and carcasses treated with kosher salt applied either pre or post chill and sampled at day 0.

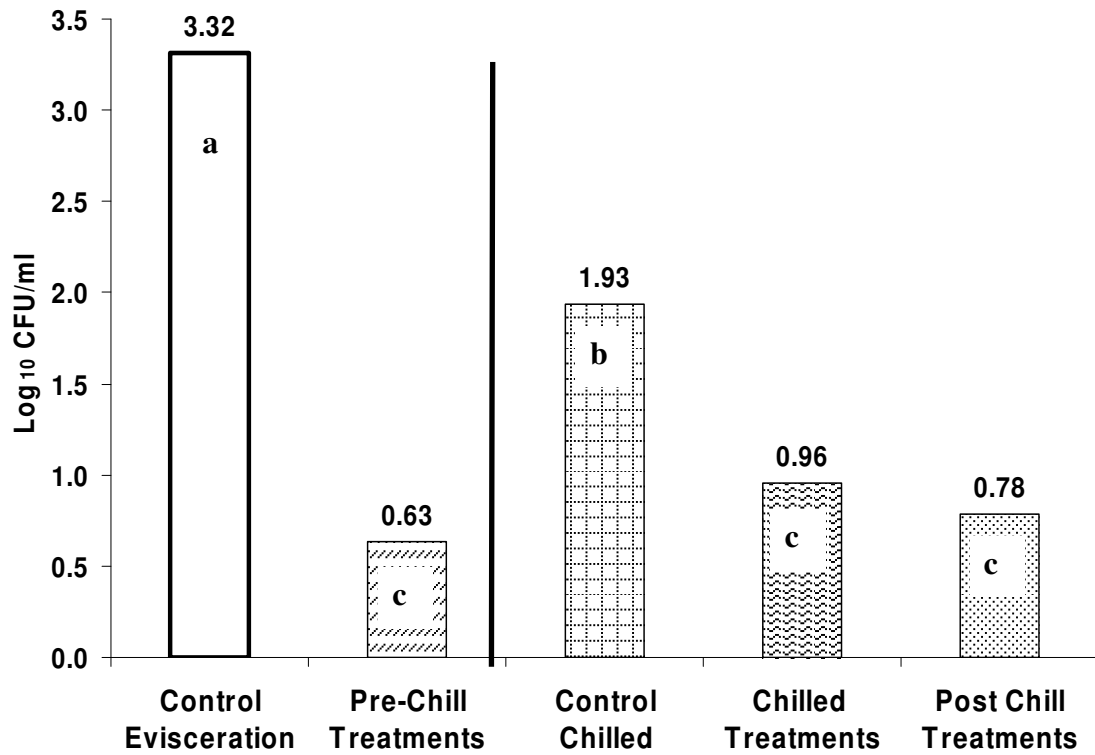


Figure 11. Log₁₀ CFU/ml of generic *Escherichia coli* in control chicken carcasses and carcasses treated with kosher salt applied either pre or post chill and sampled at day 0.

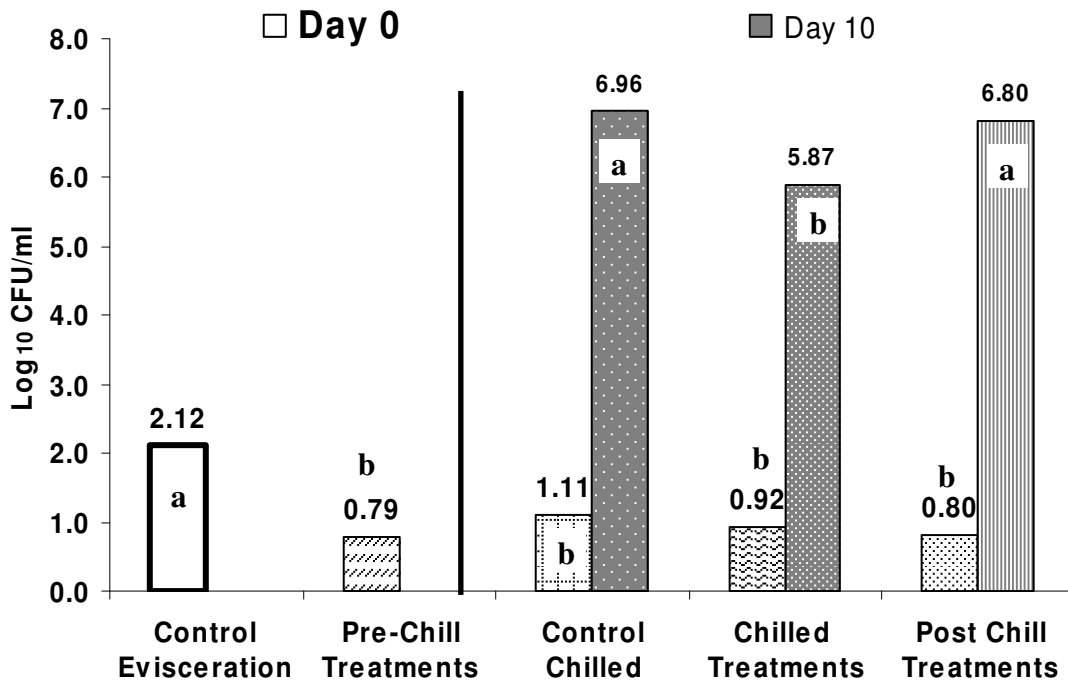


Figure 12. Log₁₀ CFU/ml of psychrotrophs in control chicken carcasses and carcasses treated with kosher salt applied either pre or post chill and sampled at day 0 and 10 of refrigerated storage.

Color

The mean L*, a*, and b* values for chicken carcasses selected are presented in table 7. The control chicken carcasses averaged L* = 74.11, a* = 2.31, and b* = 5.43, the Pre-Chill kosher salt intervened chicken carcasses L* = 70.95, a* = 2.14, and b* = 1.13, and the Post-Chill kosher salt intervened chicken carcasses averaged L* = 70.89, a* = 2.14, and b* = 1.91, all significantly different ($P < 0.05$) from control chill samples with exception of a* value, that has not significant differences. No significant differences were found between each other intervention as shown in figures 13, 14 and 15.

Table 7. Mean lightness (L*), redness (a*) and yellowness (b*) of chicken carcasses after kosher salt interventions

	L*	a*	b*
Control Chilling	74.11 ^a	2.31 ^a	5.43 ^a
Pre-chill Salt Intervention	70.95 ^b	2.14 ^a	1.13 ^b
Post-Chill Salt Intervention	70.89 ^b	2.18 ^a	1.91 ^b

*Log values followed by different subscript are significantly different ($P < 0.05$)

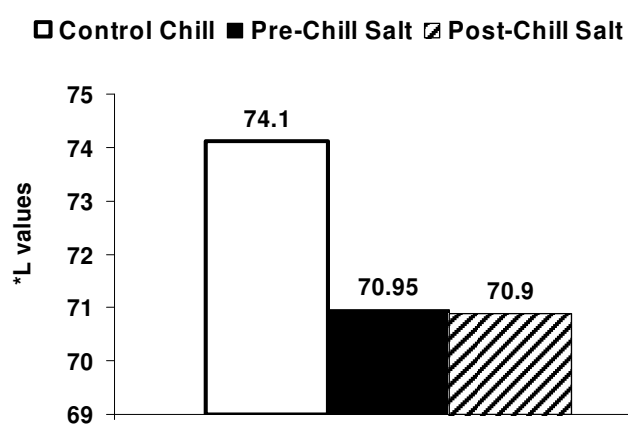


Figure 13. Color value *L after kosher salt intervention in chicken carcasses

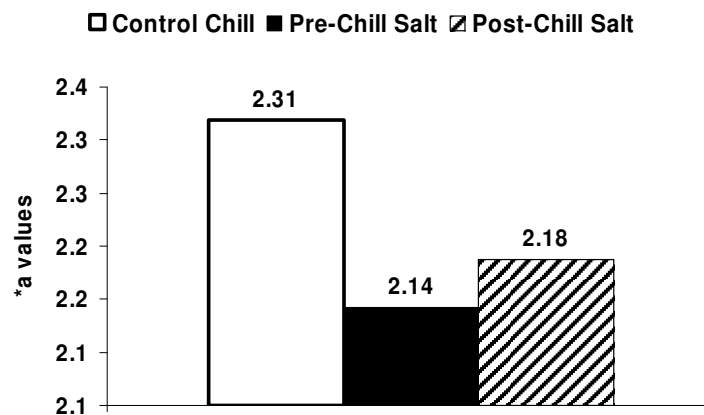


Figure 14. Color value *a after kosher salt intervention in chicken

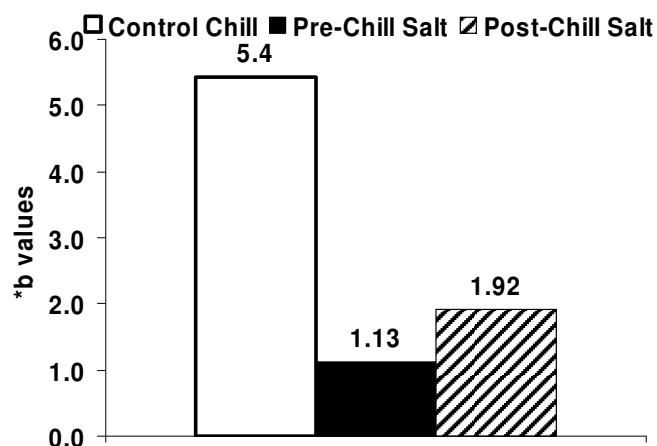


Figure 15. Color value *b after kosher salt intervention in chicken

Drip Loss

Drip loss was measured to obtain an overall assessment of the water binding properties of chicken carcasses after kosher salt applications on their surfaces. Because salt is a natural water binder, the Pre-Chill and Post-Chill kosher salt intervened chicken carcasses exhibited lower percentages of drip loss, 2.42 and 2.43%, respectively, compared with the control chill carcasses 5.26%. (Table 8 and figure 16). The interventions were significant different from the control, but not between each other.

Table 8. Mean drip loss (%) in chicken carcasses after kosher salt interventions

	%
Control Chilling	5.26 ^a
Pre-chill Salt Intervention	4.42 ^b
Post-Chill Salt Intervention	2.43 ^c

*Log values followed by different subscript are significantly different (P < 0.05)

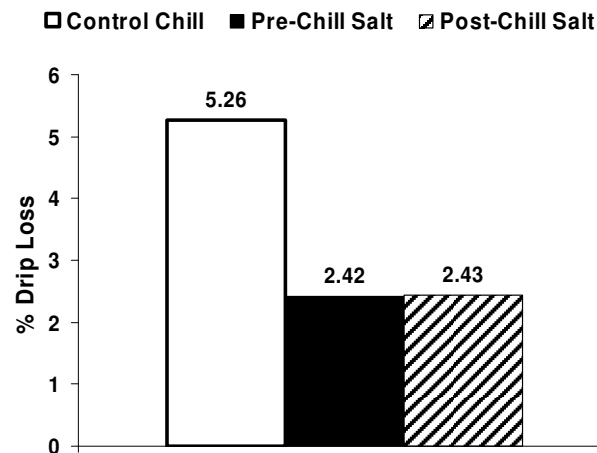


Figure 16. Drip Loss (%) in chicken carcasses after kosher salt interventions

In this study, the effectiveness of dry kosher salt pre and post chill application was compared against rifampicin resistant *Salmonella* Typhimurium strain NVSL 95-1776, on boned chicken breasts samples at day zero and day 5. Significant differences were found between treatment and control, and between days, but not between each treatment; however, post chill application demonstrated a higher mean log reduction (1.15 CFU/ml at day 0) than pre chill (0.88 CFU/ml at day 0), as shown in figures 7 and 8. Both interventions were validated in a commercial facility where kosher salt interventions exhibited mean log reductions of more than 0.5 CFU/ml with all the indicator organisms, except for APC and psychrotrophs. Post chill kosher salt interventions exhibited the highest mean log reduction for coliforms (1.35 CFU/ml) comparing to control chill samples (Figure 10), and pre chill intervention the highest for generic *E. coli* (2.69 CFU/ml), comparing to control eviscerated samples (Figure 11). At

day 10 of storage at 7°C, pre chill kosher salt intervened carcasses sampled after chilling, exhibited bigger mean log reductions than 0.5 CFU/ml for APC, indicating the prolonged effect of the salt application at 10 days at refrigeration temperatures (Figure 12). Pre chill kosher salt intervention could be another alternative for poultry processing lines to decrease microbial counts specially, of pathogens.

Quality effects of kosher salt application were evaluated measuring color and drip loss of chicken carcasses. Statistical analyzes show that kosher salt intervened carcasses exhibited less lightness (pale-white) and yellowness than control, but not dark not blue. Not statistical differences were found in redness between control and intervened carcasses concluding that salt decrease the yellowness of intervened carcasses, as shown figures 13, 14 & 15.

As expected, salt binds water decreasing significantly, drip loss in kosher salt intervened carcasses. Statistical differences were found between control and kosher salt interventions but not between treatments. This could be beneficial for industry who tries to retain more water in the carcass (Figure 16).

CHAPTER V
EFFECT OF MOLTEN PARAFFIN WAX AS AN ANTIMICROBIAL
INTERVENTION IN POULTRY PROCESSING

Several studies have been conducted to evaluate the efficacy of chemicals to reduce *Salmonella* contamination in poultry. Efforts continue to find suitable alternatives that are more efficient and cost-effective compared to existing intervention strategies. Food poisoning is an increasing problem throughout the world, with a high percentage of outbreaks being associated with poultry. Thus, the safety of poultry products is of great concern to the poultry industry, government agencies, and consumers (Yang et al., 1998). Poultry carcasses on a conventional processing line can be contaminated with a variety of pathogenic bacteria, including *Salmonella* Typhimurium, *Campylobacter jejuni*, and *Listeria monocytogenes* (Lillard, 1990; Clouser et al., 1995).

Paraffin wax is used in the duck industry, as another step in the process line, due to the physical characteristics of duck feathers. For its size, it is needed to use wax in order to completely defeathering in ducks.

According to FSIS (2006a), ducks are plucked soon after killing. Dry plucking combined with wax finishing is recommended by DEFRA (2004). The dry plucking machine is used for strip birds of feathers, being carried out by hand. The tail and large wing feathers are taken out first by hand and kept separately. The machine operation takes one to two minutes. The remaining stubs are removed by hand. The down that clings to the flesh is difficult to remove. For large numbers of carcasses 'wax finishing' is

used, where hot paraffin wax is held in tanks at a temperature of 60°C. After immersion in the wax for about five seconds the carcasses are removed and either sprayed with cold water or immersed in a cold water tank. The hardened wax is stripped off by hand or by using a rubber-fingered drum plucking machine.

The purpose of this research is to evaluate and validate the antimicrobial effects of molten paraffin wax on the surface of eviscerated chicken parts artificially inoculated with rifampicin-resistant *Salmonella* Typhimurium.

Materials and Methods

Overview

In this investigation, the experiments were conducted in the Poultry Products laboratory, Department of Poultry Science, University of Texas A&M, USA. Bone-breast samples were obtained from a local commercial facility.

Media

A selective, differential medium composed of the basic formulation of tryptic soy agar (TSA, Difco, Detroit, MI) supplemented with rifampicin (code R8883) Sigma, St. Louis, MO) was used to enumerate rifampicin resistant marker pathogens inoculated onto chicken breast surfaces. TSA was prepared according to manufacturer's recommendations, autoclaved at 121°C for 15 minutes and cooled to 55°C. Rifampicin was dissolved in 5ml methanol, filter-sterilized, and added to the cooled sterile medium

prior to pouring into petri plates. The rifampicin resistant *Salmonella* Typhimurium strain produced yellow colonies in this medium.

Bacterial Cultures and Inoculum Preparation

Rifampicin resistant strains of *Salmonella* Typhimurium strain NVSL 95-1776 were provided by the Department of Animal Science, Texas A&M University, College Station, Texas. The rifampicin resistant strains were kept in storage at -80°C before experiments. Before challenge experiments, the cultures were resuscitated by two successive transfers to Tryptic Soy Broth (TSB; Difco) and incubated at 35°C for 18-24 h. The cells were then transferred onto TSA slants and stored at 4.4°C before experiments. The day before the actual challenge experiment, a 12-h culture of *Salmonella* Typhimurium was dispensed into sterile centrifuge tubes (10 ml) and harvested by centrifugation at 1610 g for 15 min at 4.4°C. The cell pellets obtained were re-suspended in buffered peptone water (BPW, Difco) and diluted to a final concentration of c.a. 10^8 cells/ml. This suspension was used as the inoculum for chicken breast samples and intervention trials.

Collection and Inoculation of Chicken Breasts Samples

Fresh, skin-on, bone-in chicken breast samples were secured from a local retailer, transported to our laboratories and stored at 4.4°C to be processed in 24 hours. Inoculation was performed by delivering 1ml of the *Salmonella* Typhimurium rifampicin resistant inoculum into one marked piece portion of 6.45 cm² (1x1 in²) on the surface of

the chicken breasts. The inoculum was distributed manually by the use of a hockey stick to aid in bacterial attachment. Inoculated breasts were allowed to dry at room temperature for 10 minutes, before the application of antimicrobial interventions.

Comparison of Molten Paraffin Wax Interventions

For molten paraffin wax interventions, chicken breast samples were inoculated in the same area size (1x1 in²) with *Salmonella* Typhimurium (rifampicin resistant). After 10 minutes to allow bacterial adhesion, the chicken breasts were assigned to be treated immediately by one of the following treatments: (i) not wax application (control), (ii) paraffin wax application at 60°C on chicken breast surface and (iii) used paraffin wax. For application of paraffin wax, water baths were used to heat wax until 60°C, in an approximately time of 5 minutes, the samples were kept outside at room temperature until solidification and immediately immersed in ice for separation of wax from chicken breast surface. The wax was collected in stomacher bags as the samples.

Sample Excision and Microbiological Examination

Afterward, one 6.45 cm² (1x1 in²) portion of each chicken breast was excised from the skin side surface using sterile stainless steel forceps and a scalpel (approximately 2 to 3 mm deep). The portion was immediately placed in a stomacher bag into which, 58 ml of sterile 0.1% buffer peptone water (BPW) solution was added and the contents pummel in a stomacher for one minute to dislodge pathogens before examination. Dilutions 4, 5 & 6 were done for each treatment. Counts of rifampicin

Salmonella Typhimurium were determined by plating appropriate dilutions of the samples onto duplicate plates of the selective, differential agar described above and incubating at 37°C for 24 h.

Statistical Analysis

Count data was transformed logarithmically before comparison of means by Analysis of Variance or General Linear Model procedures. Each experiment was replicated three times in a completely randomized design. Data were analyzed using SAS statistical analysis software program, version 9.1 (SAS Institute Inc., Cary, N.C. U.S.A.). When significant differences were observed ($P < 0.05$), separation of means was accomplished by using LSMEANS multiple ranges test using SAS procedures (SAS Institute, 1985).

Results and Discussion

A reduction of approximately 0.51 log₁₀ CFU/ml was obtained after molten paraffin wax treatment, as shown in table 9 and figure 17.

Table 9. Log₁₀ CFU/ml of *Salmonella* Typhimurium populations in control chicken breast samples and samples treated with molten paraffin wax.

	Log₁₀ CFU/ml
Control	6.87 ^a
Waxed chicken	6.36 ^b
Waste wax	5.41 ^c

*Log values followed by different subscript are significantly different ($P < 0.05$)

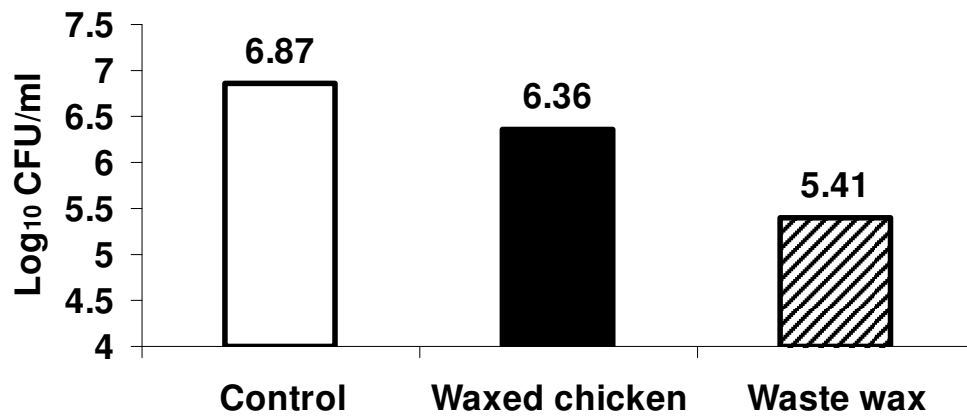


Figure 17. Log₁₀ CFU/ml of *Salmonella* Typhimurium populations in control chicken breast samples and samples treated with molten paraffin wax.

Significant statistical differences were found between control and paraffin wax intervened chicken, however the total mean log₁₀ reduction was less than one (0.51 CFU/ml). Further investigation is needed, comparing these results with other industry paraffin waxes, used in the duck processing, as another step in the process line.

The attachment of rifampicin resistant *Salmonella* Typhimurium in the paraffin wax was approximately 1.46 CFU/ml, however this attachment was not enough to get lower reduction on the chicken breast surface.

After these results, paraffin wax intervention was not considered for validation, in the actual conditions.

CHAPTER VI

CONCLUSIONS

In this study, the effectiveness of ACS + EPL at 25°C and 55°C was compared against rifampicin resistant *Salmonella* Typhimurium strain NVSL 95-1776, on boned chicken breasts samples. Even not significant differences were found between each treatment (was found only compared with control), ACS with EPL at 55°C was selected to further validation in a commercial facility, because of the demonstrated increase in the mean log reduction at day 2 (0.65 CFU/ml) compared with the first treatment (0.33 CFU/ml). Also, considering the increased mean log reductions reported by Keeton J. et al. (2006) of ACS + EPL at 55°C against *Salmonella* Typhimurium in beef round samples.

After validation, pre chill interventions of ACS + EPL at 55°C were significant different than control eviscerated samples, showing \log_{10} mean reductions >1 CFU/ml with all the indicator organisms. With chilled and post chill interventions, the mean \log_{10} reductions were less than 0.5 CFU/ml compared with control chill samples in all the indicator organisms showing not significant differences demonstrating that pre chill interventions were more effective than chilled or post chill ones.

At day 10 of storage at 7°C, ACS + EPL at 55°C at post-chill intervention (significant different), produced a mean \log_{10} reduction of 0.56 CFU/ml for APC and 0.66 CFU/ml for psychrotrophs indicating the prolonged effect of the chemicals at 10 days at refrigeration temperatures. Comparing these reductions with those reported by

Keeton (2006) and Geornaras and Sofos (2005), EPL has an enhancement effect with acidic antimicrobials (synergistic effect) against microorganisms.

In this study, the effectiveness of dry kosher salt pre and post chill application was compared against rifampicin resistant *Salmonella* Typhimurium strain NVSL 95-1776, on boned chicken breasts samples at day zero and day 5. Significant differences were found between treatment and control, and between days, but not between each treatment; however, post chill application demonstrated a higher mean log reduction (1.15 CFU/ml at day 0) than pre chill (0.88 CFU/ml at day 0). Both interventions were validated in a commercial facility where kosher salt interventions exhibited mean log reductions of more than 0.5 CFU/ml with all the indicator organisms, except for APC and psychrotrophs. Post chill kosher salt interventions exhibited the highest mean log₁₀ reduction for coliforms (1.35 CFU/ml) comparing to control chill samples, and pre chill intervention the highest for generic *E. coli* (2.69 CFU/ml), comparing to control eviscerated samples. At day 10 of storage at 7°C, pre chill kosher salt intervened carcasses sampled after chilling, exhibited bigger mean log reductions than 0.5 CFU/ml for APC, indicating the prolonged effect of the salt application at 10 days at refrigeration temperatures. Pre chill kosher salt intervention could be another alternative for poultry processing lines to decrease microbial counts specially, of pathogens.

Quality effects of kosher salt application were evaluated measuring color and drip loss of chicken carcasses. Statistical analyzes show that kosher salt intervened carcasses exhibited less lightness (pale-white) and yellowness than control, but not dark not blue. Not statistical differences were found in redness between control and

intervened carcasses concluding that salt decrease the yellowness of intervened carcasses.

As expected, salt binds water decreasing significantly, drip loss in kosher salt intervened carcasses. Statistical differences were found between control and kosher salt interventions but not between treatments. This could be beneficial for industry who tries to retain more water in the carcass.

Significant statistical differences were found between control and paraffin wax intervened chicken, however the total mean \log_{10} reduction was less than one (0.51 CFU/ml). Further investigation is needed, comparing these results with other industry paraffin waxes, used in the duck processing, as another step in the process line.

The attachment of rifampicin resistant *Salmonella* Typhimurium in the paraffin wax was approximately 1.46 CFU/ml, however this attachment was not enough to get lower reduction on the chicken breast surface.

After these results, paraffin wax intervention was not considered for validation, in the actual conditions.

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