Salmonella and *Campylobacter* **Reduction and Quality Characteristics of Poultry Carcasses Treated with Various Antimicrobials in a Finishing Chiller®**

by

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Abstract

With the implementation of more stringent regulatory guidelines, it is necessary for processors to employ new or additional pathogen intervention strategies for more effective control of *Salmonella* and *Campylobacter* throughout poultry processing. New innovations in poultry include implementation of antimicrobials in a post-chill decontamination tank. Additionally, because antimicrobials can affect the organoleptic properties of a product, it is important to determine quality aspects of carcasses treated with antimicrobials. The objectives of this study were to determine the efficacy of various antimicrobials added to a Finishing Chiller® in reduction of *Salmonella* and *Campylobacter* and to evaluate any associated effects of the antimicrobials on quality characteristics of chicken breast meat. Seven chill water treatments consisting of 0.004% chlorine, 0.04%, 0.1% PAA, 0.003%, 0.01% buffered sulfuric acid, 0.1%, or 0.5% lysozyme were examined using a Finishing Chiller®. A total of 200 broiler carcasses were sampled (10 carcasses X 2 replications X 10 treatments) including postive, negative, and water controls. The skin of carcasses was inoculated with *Salmonella* Typhimurium (10^6 cfu/mL) and *Campylobacter jejuni* (10^6 cfu/mL). Following a 20 min attachment time, carcasses were dipped into the Finishing Chiller® for 20 s. Individual birds were then placed into a sterile rinse bag and rinsed with 200 ml buffered peptone water for 1 min. Serial dilutions were performed and 0.1 ml was spread plated on XLT4 and Campy-Cefex for enumeration of *Salmonella* and *Campylobacter*, respectively. Non-inoculated

chicken breast meat from each treatment was used for sensory analysis. Treatment with 0.04 and 0.1% PAA was found to be most effective ($P<0.05$) in decreasing *Salmonella* and *Campylobacter*. Chlorine treatment at 0.004% in addition to acid treatment at 0.003% and 0.01% and lysozyme applied at 0.1% and 0.5% were found to be less effective ($P<0.05$), resulting in close to a $1\text{-}\log_{10}$ reduction when compared to controls. Treatment with the various antimicrobials was not found to have negative impacts on sensory attributes. Utilizing PAA in a Finishing Chiller® is an effective application for reducing *Salmonella* and *Campylobacter* on carcasses while maintaining product quality.

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"The distance between insanity and genius is measured only by success." –Bruce Feirstein

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CHAPTER I.

INTRODUCTION

The implications due to food-borne illness are enormous, including serious public

health concern as well as significant social and economic burden. Therefore, safe

production and distribution of food across the farm to fork continuum is crucial to ensure

that consumers receive wholesome food products. The Centers for Disease Control and

Prevention (2011) estimated that each year in the United States, 48 million people suffer

from food-borne illness resulting in 128,000 hospitalizations and 3,000 deaths.

Furthermore, the annual basic health-related cost of food-borne illness in the United

States is estimated as $51.0 billion (Scharff, 2011). Contaminated poultry meat represents

the greatest public health impact among foods and is responsible for an estimated $2.4

billion in annual disease burden. *Salmonella* spp. and *Campylobacter* spp. are two food-

borne pathogens most commonly associated with poultry meat. Out of the illnesses

attributed to poultry, *Salmonella* is responsible for 35.1% of illnesses whereas

Campylobacter is responsible for 72% (Batz et al., 2011).

The potential for consumers to acquire food-borne illness typically arises when

food is prepared improperly or cross-contamination occurs. Although advancements in

preventing food-borne illness have been achieved, the ultimate responsibility still relies

with the food industry to produce safe and wholesome products for consumers. The risk of cross-contamination of poultry meat begins with live production and continues through processing to distribution of the final product. There are ongoing challenges and developments toward improving food safety, and any steps that can be taken to prevent cross-contamination and target bacterial reduction should be applied.

With the implementation of more rigorous pathogen reduction standards by the USDA, it is necessary for processors to employ new or additional interventions for effective control of *Salmonella* and *Campylobacter* throughout processing. U.S. Broiler processing facilities are required to comply with criteria set forth by the USDA Food Safety and Inspection Service (FSIS). New Performance standards implemented in response to national baseline studies require routine testing for *Salmonella* and *Campylobacter* in all processing plants, where the percentage of *Salmonella*-positive samples must be below 7.5% (5 positive samples out of 51 samples). Likewise, in the new regulations, *Campylobacter*-positive samples should be less than 10.4% (8 positive samples out of 51 samples).

Current food safety trends focus on achieving best practices in pathogen control. In recent years, methods for reducing pathogens during poultry processing have changed with advances in technology. Intervention strategies such as post-chill decontamination tanks have provided an alternative approach for pathogen reduction during poultry processing. These decontamination tanks are placed directly after the primary immersion chiller. Over the past two years, there has been a considerable increase in U.S. poultry processing facilities that are employing post-chiller antimicrobial interventions (McKee,

2011). These post-chill methods target bacterial reduction and are effective and economical for processors.

A novel post-chill technology developed by Morris & Associates is a Finishing Chiller® that resembles a traditional chiller but has a minimal footprint and volume ranges from 400-600 gallons (Morris & Associates, Garner, NC) while other post-chill dip tanks have a wider volume range depending on size. In addition to this, the contact or dwell time for carcasses in the Finishing Chiller® application is minimal and generally less than 30 s. Since there is a short dwell time in a Finishing Chiller®, a higher concentration of antimicrobials is typically used (McKee, 2011). However, the likelihood of antimicrobial causing negative impacts on carcass quality is diminished because the contact time is so short. As such, applying antimicrobials in a Finishing Chiller® or post-chill dip tank in comparison to applying antimicrobials in primary chillers which hold 20,000 to 50,000 gallons (dwell time of 1.5- 2.0 h) is much more efficient and cost effective (McKee, 2011). Additionally, because organic load may reduce the efficacy of some antimicrobials, and the organic load is low post-chill, there may be an increase in the efficacy of some antimicrobials used at this step (Russell, 2010).

It is thought that targeting a 2-$\log_{10}$ reduction in bacterial counts should eliminate most of the naturally occurring levels of *Salmonella* and/or *Campylobacter* that might remain on carcasses post-chill. When post-chill locations in a processing plant were sampled for *Campylobacter*, a mean $\log_{10}$ CFU/mL of 1.5 was recovered (Berrang and Dickens, 2000), signifying that the normal chilling step is not effective enough in reducing all pathogens. Utilizing antimicrobials with intervention strategies that can yield a 2-$\log_{10}$ reduction should therefore eliminate *Campylobacter* remaining on the carcass

after chilling. Post-chill dip systems have been very successful in reducing *Salmonella* and other pathogens when used in combinations with other interventions throughout the plant (Russell, 2010). This makes them advantageous for processors since post-chiller antimicrobial intervention introduces an additional strategy or "multi-hurdle" approach for pathogen reduction.

While antimicrobials have been validated against *Salmonella* and *Campylobacter* primarily in chillers, this research provides validation results for Finishing Chillers and will be beneficial for the poultry industry as well as consumers. Therefore, the current research was conducted to determine the efficacy of various antimicrobials in reduction of *Salmonella* Typhimurium and *Campylobacter jejuni* on broiler carcasses treated in a Finishing Chiller® to provide industry with recommendations for best practices in pathogen control. Results of this study indicate that peracetic acid (PAA) is the most effective antimicrobial in reducing *Salmonella* and *Campylobacter* on broiler carcasses in a post-chill system when compared to controls and other antimicrobials evaluated. Furthermore, antimicrobial use in this type of application does not exhibit negative impacts on carcass quality attributes. Use of a Finishing Chiller® combined with PAA treatment is both an effective and practical means for pathogen intervention.

CHAPTER II.

LITERATURE REVIEW

Impact of Food Safety on Society

The safety and quality of poultry meat is affected by many steps throughout the flow of production. Consumers have the right and expectation to obtain wholesome food products, so any interventions to reduce the risk of cross-contamination during poultry processing should be practiced. According to the Centers for Disease Control and Prevention (2011), approximately 48 million people become sick, 128,000 are hospitalized, and 3,000 die from food-borne illnesses in the United States each year. The annual health-related cost of food-borne illness in the United States is estimated as $51.0 billion (Scharff, 2011). This figure includes lost wages, hospital bills, and deaths. However, this total does not reflect money that is lost by the meat industry. Two food-borne pathogens that are most often associated with ready-to-cook poultry meat are *Salmonella* spp. and *Campylobacter* spp. In 2011, the CDC estimated that *Salmonella* accounts for 11% of all foodborne illness, 35% of hospitalizations, and 28% of deaths, while *Campylobacter* accounts for 9%, 15%, and 6%, respectively. Moreover, the total basic cost of illness associated with *Salmonella* in the U.S. was reported as $4.43 million and $1.56 million for *Campylobacter*, making infection with these food-borne pathogens a serious economic burden (Scharff, 2011). In addition, a study conducted by Consumer Reports, estimated that 62% of broiler chickens at retail were contaminated with

Campylobacter whereas *Salmonella* was in 14% and both bacteria were found in 9% (Consumer Reports, Anonymous, 2010). Bacterial related food safety hazards are usually a result of poultry meat that has not been cooked thoroughly or due to a cross-contamination event.

An estimated 1.4 million cases of *Salmonella* occur annually in the United States. Of these, approximately 40,000 are culture-confirmed cases reported to the CDC. Approximately 2000 serotypes are known to cause human disease. The top ten most common serotypes associated with human infection in the United States are identified by the CDC. These include Enteritidis, Newport, Typhimurium, Javiana, Heidelberg, Saintpaul, Muenchen, Montevideo, and Infantis. When combined, these particular serotypes account for approximately 75.2% or the majority of human infections in the Foodborne Diseases Active Surveillance Network (FoodNet) sites in 2010 (CDC, 2011). Among 92% *Salmonella* isolates serotyped, the most common serotypes were Enteritidis (22%), Newport (14%), and Typhimurium (13%).

In 2010, FSIS identified the common serotypes among meat and poultry classes (Table 1). Kentucky was recognized as the most frequent serotype isolated in combined chicken classes; Hadar was most common in the ground turkey class; Montevideo was most common in combined cattle classes; and Derby was most common in the market hog class. Upon reviewing CDC data for serotypes isolated from human cases of salmonellosis identified with meat and poultry products and those causing human illness, FSIS indicated that some of the more common serotypes isolated from meat and poultry products are seldom isolated from human illnesses. In contrast, some of the serotypes that are often implicated in human cases of salmonellosis arise in various meat and poultry

products. These serotypes can also occur in other food commodities and non-food sources. Thus, the dominant serotypes identified in 2010 for meat and poultry products (Kentucky, Hadar, and Derby) were not among the top ten serotypes identified in human surveillance data. The CDC reported in 2011 that Enteritidis and Newport incidence was significantly higher, while the incidence of Typhimurium did not change significantly for 2006-2008 (FSIS, 2011).

Serotypes 2010	Number of Isolates	Percent of Total Positive	Percent of Analyzed Samples
Kentucky	208	45.41	3.05
Enteritidis	124	27.07	1.82
Typhimurium 5 -	27	5.90	0.40
Heidelberg	16	3.49	0.23
[a]Typhimurium	14	3.06	0.21
[c]4,5,12:i:-	10	2.18	0.15
Johannesburg	6	1.31	0.09
Schwarzengrund	5	1.09	0.07
Senftenber	5	1.09	0.07
Berta	4	0.87	0.06
Braenderup	4	0.87	0.06
Thompson	4	0.87	0.06
[a]Other serotypes	24	5.24	0.35
[b]Unidentified	5	1.09	0.07
Total serotyped isolates	456		6.68
Not typed	2		0.03
*Total positive	458		6.71
Total number of analyzed samples		6,828	

Table 1. Profile of Serotypes from Analyzed PR/HACCP Verification Samples by Calendar Year. Broilers (1998-2005 'A' Set Samples; 2006-2010 All Samples)

Many people who become infected with *Salmonella* develop diarrhea, fever, and abdominal cramps approximately 12 to 72 hours after infection. Symptoms usually last 4 to 7 days, and most recover without treatment. There are an estimated 400 fatal cases each year, and a few cases which are complicated by chronic arthritis. Resistance to at least five antimicrobial agents is commonly seen in *Salmonella* Typhimurium (CDC, 2010). Floroquinolones are drugs typically used for treatment of salmonellosis. Therefore, the emergence of quinolone resistance in *S.* Typhimurium is cause for concern, especially in animals whose products are known sources of infection for human gastroenteritis (Heurtin-Le Corre, 1999). Furthermore, antibiotic resistance can result in limitation of options for effective treatment of human infections. Continued research and new interventions for quinolone-resistant strains are essential.

Likewise, campylobacteriosis causes mild to severe gastroenteritis. *Campylobacter* is the most common cause of diarrheal illness due to bacteria. According to the CDC, an estimated 2.4 million people are affected each year. Serotypes most commonly implicated in foodborne illness include *C. jejuni*, *C. coli*, and *C. lari*, with most cases resulting from infection with *C. jejuni*. The majority of cases reported have been due to isolated, sporadic occurrences and not associated with large outbreaks (Stern et al., 2001; Jacobs-Reitsma et al., 2008). Furthermore, the minimum infective dose may be as low as 500-800 cells. With a low infective dose and high pathogenicity, poultry products may pose a serious health concern for consumers if mishandled or not cooked thoroughly. Illness typically lasts one week, and symptoms can include diarrhea, fever, abdominal pain and cramps.

Antibiotic treatment for uncomplicated *Campylobacter* infection is rarely necessary. However, antimicrobial resistance to drugs used for treatment, especially floroquinolones and macrolides, has been increasingly reported. In addition, there has been evidence that persons who become infected with antibiotic-resistant strains suffer more complications than those infected with sensitive strains (Helms et al., 2005). There are an estimated 124 fatal cases each year, and seldomly, infection with *Campylobacter* may result in long-term consequences. It is estimated that approximately one in every 1,000 reported *Campylobacter* illnesses leads to Guillain-Barré syndrome, in which the body's immune system attacks part of the peripheral nervous system (CDC, 2010).

In January 2000, the U. S. Department of Health and Human Services (USDHHS) published Healthy People 2010. This 10 year agenda focused on national health promotion and disease prevention with objectives pertaining to the overall reduction of foodborne illness. Each objective had a target to be met by the year 2010. However, the target for foodborne pathogens such as *Salmonella* and *Campylobacter* was not achieved. FoodNet case rate data from 2006-2008 showed the actual number of *Salmonella* illnesses was 15.2 per 100,000; more than double the Healthy People 2010 goal (6.8). Similarly, 12.7 cases on average of laboratory-confirmed *Campylobacter* infections per 100,000 were reported in 2006-2008. Although the target was still not attained, it was closer to the 2010 goal of 12.3. The USDHHS has set a more modest target, however, for *Salmonella* in Healthy People 2020 which was launched in December 2010. The goal is for a 25% reduction with 11.4 cases per 100,000 people. The objective for *Campylobacter* spp. is a target of 33% reduction in illnesses with 8.5 cases per 100,000 people.

Contaminated poultry has the greatest public health impact among all foods. Poultry is the only food product that appears twice in the top 10 ranking of pathogen-food combinations. It is responsible for establishing $2.4 billion in annual disease burden, with the most significant disease burden due to contamination with *Salmonella* and *Campylobacter*. Out of the illnesses attributed to poultry, *Campylobacter* accounts for 72% while *Salmonella* accounts for 35.1%. However, *Salmonella* causes more disease than any other foodborne pathogen. According to FoodNet surveillance data, incidence of this foodborne pathogen has not declined over the last 10 years. What's more, out of the top 5 pathogens reflecting 91% of the costs of illness across all 14 pathogens, *Salmonella* ranks first in cost of illness, contributing 23% of the total cost of illness. *Campylobacter* ties for third with *Listeria monocytogenes*, contributing 12% of the total cost of illness (Batz et al., 2011).

Furthermore, new pathogen reduction data collected during the USDA Food Safety and Inspection Service (FSIS) Nationwide Microbiological Baseline Data Collection Programs: The Young Chicken Baseline Survey (YCBS) of 2007-2008 and the Young Turkey Baseline Survey (YTBS) of 2008-2009, as well as the failure to achieve Healthy People 2010 targets became the driving force for implementing updated performance standards for *Salmonella* and developing standards for *Campylobacter* for the first time in the agency's history. These studies indicate that although there have been improvements, there is still a risk of consumers being exposed to these pathogens through poultry. The new performance standards became effective for sample sets that began on or after July 1, 2011. The number of samples collected for verification sets has not changed; however, each sample collected in a set will be analyzed for both *Salmonella*

and *Campylobacter.* The new USDA performance standard is 7.5% for *Salmonella* and 10.4% for *Campylobacter*.

Federal Register Notice, *New Performance Standards for Salmonella and Campylobacter in Chilled Carcasses at Young Chicken and Turkey Slaughter Establishments* (75 FR 27288), outlines that establishments will pass the updated *Salmonella* standards if FSIS finds no more than five positive samples in the 51-sample set for young chickens and no more than four positive samples in a 56-sample set for turkeys. Likewise, if FSIS finds no more than eight positive samples in a 51-sample set for young chickens and no greater than three positive samples in a 56-sample set for turkeys, establishments will pass the new *Campylobacter* standards. If the acceptable number of samples positive for passing either set of standards at an establishment is exceeded, then the FSIS will collect a follow-up sample set to analyze for both organisms. FSIS estimates that with implementation of the new performance standards, 39,000 illnesses due to *Campylobacter* and 26,000 illnesses due to *Salmonella* will be eliminated. However, these estimates will remain to be seen.

***Salmonella* spp.**

Salmonella are Gram-negative non-spore forming bacilli in the family Enterobacteriaceae. They are facultative anaerobes and are considered mesophiles with an optimal growth temperature between 35 and 37°C or body temperature. Optimal pH for *Salmonella* growth is near neutral, with pH above 9.0 and below 4.0 being detrimental to cells (Jay, 2000). Standardized techniques are performed for human and food testing of *Salmonella*. Samples can be cultured directly onto a selective media. A new medium, xylose lysine tergitol 4 (XLT4) agar, was described by Miller and Tate in 1990 for

isolating *Salmonella*. Today, it is used widely for the isolation of non-typhi *Salmonella* spp. which appear as black or yellow to red colonies with black centers, due to the production of hydrogen sulfide, after 18 to 24 h of incubation.

Chickens are known to be natural reservoirs for pathogenic bacteria such as *Salmonella* and *Campylobacter*. Poultry that carry *Salmonella* can shed the bacteria into eggs and the environment, thereby serving as a source of microbial contamination of poultry meat and eggs (Bishop, 2010). *Salmonella* is spread by two main routes: vertical (trans-ovarian) and horizontal transmission. In vertical transmission, the bacteria are introduced from infected ovaries or oviduct tissue from parents and introduced into eggs prior to shell formation. Horizontal transmission results through fecal contamination on the egg shell or when *Salmonella* is shed into the environment and spreads throughout the flock. Vertical transmission is more invasive and difficult to control (WHO/FAO, 2002).

Transfer of *Salmonella* may begin pre-harvest, specifically in hatcheries and on farms. Because only a few cells of *Salmonella* are required to colonize the intestines of young chicks, it is suggested that contamination in hatcheries is a major concern for introduction of *Salmonella* to chickens (Blankenship et al., 1993). Furthmore, Bailey et al. (2002) found that serotypes isolated from hatchery samples and samples from the previous grow-out were related. However, it was reported that the grow-out environment is considered to be the most solid indicator of *Salmonella* serotypes found in poultry after processing (Lahellec and Colin, 1985). Following oral ingestion of *Salmonella* through contaminated food products, the bacteria colonize the intestines of the host and begin to invade intestinal mucosa. All serotypes of *Salmonella* are capable of invading the host by inducing their own uptake into cells of the intestinal epithelium. Moreover, serotypes

associated with gastroenteritis direct an intestinal inflammatory and secretory response; whereas, serotypes that result in enteric fever establish a systemic infection through their capacity to survive and replicate in mononuclear phagocytes (Ohl and Miller, 2001). Results of a pre-harvest intervention survey for best practices in pathogen control conducted by McKee (2012) indicated that many breeders are using autogenous and commercially killed and live vaccines to reduce the incidence of *Salmonella* at the breeder level. In a study conducted by Berghaus et al. (2011), it was reported that vaccination of broiler breeder pullets increased humoral immunity in breeders and reduced the prevalence and microbial load of *Salmonella* in broiler progeny, but did not show a significant reduction in *Salmonella* at the breeder farm environment.

Campylobacter spp.

Campylobacter is a genus of bacteria belonging to the family Campylobacteriaceae that are small, Gram negative, spiral bacteria, and are unlike other pathogens associated with food-borne illness in that they are microaerophilic. They grow best in an atmosphere containing 10% CO_2 and 5% O_2. The species pathogenic to humans are considered thermophilic campylobacters and have an optimal growth temperature of 42°C. Optimal pH for *Campylobacter* growth and survival is 6.5-7.5, or near neutral. There are 18 species of *Campylobacter* although most infections are caused by *Campylobacter jejuni* and *C. coli*. *Campylobacter* cells have been shown to enter a Viable But Non-Culturable form (VBNC). Reports have indicated that *C. jejuni* can change from a spiral to coccoid morphology causing loss of culturability although the cells are still viable. This evidence suggests that in a protective environment such as chicken skin, *Campylobacter* can be difficult to eliminate (Rollins and Colwell, 1986).

Clinical samples are cultured directly onto selective media. Campy Cefex agar, described by Stern et al., is utilized for the selective isolation of *Campylobacter* spp.

The most important source of *Campylobacter* is thought to be the external environment. The bacteria can be common contaminants in the farm environment and have been linked to those isolated from birds. Vertical transmission, however, remains an area of contention in the epidemiology of *Campylobacter* in chicken production. *C. jejuni* has been recovered from the oviduct, suggesting a possibility of egg contamination. It has also been found in semen samples recovered from breeder cockerels (Humphrey et al., 2007). However, it has yet to be shown explicitly that it can be isolated from newly hatched chicks, which would be the true measure of vertical transmission (Humphrey et al., 2007). It has also been shown that *Campylobacter*-specific maternal antibodies present in chicks delay colonization, and that colonization in birds challenged at 21 days of age occurs much sooner (Sahin et al., 2003). Once ingested by the host, *Campylobacter* initiates infection by penetrating the gastrointestinal mucus through its high motility and spiral shape. The bacteria then adhere to the enterocytes in the host gut and induce diarrhea through toxin release. *C. jejuni* releases several toxins which vary by strain and correlate to the severity of illness. These include mainly enterotoxin and cytotoxins (Wallis, 1994). There are currently no commercial vaccines available for *Campylobacter*. Control is thought to be stringent biosecurity and sanitation practices (McKee, 2012).

Multi-hurdle Approach to Pathogen Control during Processing

There are several stages during broiler processing where cross-contamination of *Salmonella* and *Campylobacter* on carcasses may occur. Sarlin et al. (1998) indicated that

when slaughtered at the beginning of the day, broilers from farms where *Salmonella* was not detectable remained uncontaminated during processing. This suggests a correlation between contamination problems on the farm and during processing. For that reason, bacterial contamination in the plant could be largely precluded through effective pre-harvest intervention strategies.

At the processing plant, broilers undergo stunning, exsanguination, scalding, picking, evisceration, and subsequent carcass chilling. During stunning, broilers are most commonly subjected to electric current resulting in immobilization and rendering them unconscious for approximately 60 to 90 s prior to slaughter. This process gives better positioning for neck-cut efficiency as well as improved bleed-out. The next step following stunning is known as exsanguination. In this process, a rotating circular blade severs the jugular veins and carotid arteries, resulting in a 40-50% blood loss from the broiler carcass after a 1.5-3 min bleed-out time. Feather removal on carcasses is achieved through submersion into a hot water bath, scalder, in order to denature proteins in the feather follicles for easy feather removal during picking. Proteins in feathers start to denature around 51°C. Scalding can be set up in multiple stages or continuous with counter-current flow so birds are moving into increasingly cleaner water (McKee, 2009). When properly controlled, scald tanks typically give a 2-3 $\log_{10}$ reduction in levels of *Campylobacter* on carcasses (Oosterom et al., 1983; Izat et al., 1988; Berrang et al., 2000).

Pickers consist of rows of rapidly rotating flexible, rubber "fingers" that rub the surface of the carcass, removing loosened feathers. The final step of first processing prior to chilling is evisceration in which edible and inedible viscera are removed from the

carcass. During evisceration, the body cavity of the bird is opened, the viscera are scooped out, and the giblets are harvested, trimmed, and washed. Birds undergo an inspection station during evisceration before they pass through the inside/outside bird washer and enter the chiller (Sams, 2001).

Picking and evisceration are two major points during processing of poultry where microbial contamination occurs since *Salmonella* and *Campylobacter* are present in the feces of carrier birds and can be transferred from the intestines to the skin surface (Byrd and McKee, 2005). These steps may require particular attention due to the high rate of cross-contamination (Saleha et al., 1998). During the picking or defeathering stage of processing, cross-contamination of and among carcasses may be increased due to considerable dispersion of microorganisms (Hafez, 1999). In addition, when rubber picking fingers become contaminated with bacteria, the warm, humid environment of the defeathering apparatus provides an atmosphere that may aid in the survival and growth of the pathogens (Mead et al., 1980). Moreover, rubber picking fingers are susceptible to tears which can provide a growth niche for bacteria and are tough to clean and disinfect (McKee, 2009). This may contribute to the transfer of bacteria between processing cycles. In addition, it has been observed that the mechanism of rubber fingers used in defeathering can drive microorganisms into the skin tissue and feather follicles (Bryan et al., 1968). Researchers have reported that when a single carcass becomes contaminated during defeathering it can contaminate more than 200 other carcasses (Van Schothorst et al., 1972; Mead et al., 1975). As a result, it is necessary to maintain optimal conditions in the scalder to reduce the microbial load prior to feather removal.

Furthermore, cutting and tearing of the viscera during carcass evisceration may be a key proponent contributing to cross-contamination of carcasses (Bryan and Doyle, 1995). Other studies have evaluated the leakage of crop contents during processing. It was reported by Hargis et al. (1995) that during processing, crops ruptured 86 times more often than ceca and were very likely to be *Salmonell*a-positive (Hargis et al., 1995; Corrier et al., 1999) as well as contaminated with *Campylobacter* (Byrd et al., 1998). Byrd et al. (2002) reported that when a fluorescent marker was inoculated into the crops of broilers and evaluated through the course of the processing plant, presence of the marker was observed on the carcass surface at incidences of 67% at re-hang, 82.5% post-inspection, 92% pre crop removal, 94% post crop removal, and at 53% after the final wash, before carcasses were chilled.

Consequently, before carcasses can undergo the chilling process, they must pass through an external inspection to evaluate the surface of the bird. If fecal contamination on a carcass exists, the bird may be sent through spray cabinets containing chlorinated water or other antimicrobials for on-line reprocessing (OLR). Additionally, many poultry processing facilities employ an inside-outside bird washer (IOBW) for use after the birds have passed inspection. The IOBW has spray nozzles that rinse the abdominal cavity and the exterior of the bird in order to remove any visible extraneous material before the birds go through chilling. Water in the IOBW also contains some type of antimicrobial compound for disinfection (Sams, 2001). Some bacterial reduction can be attained with these methods, although results may be inconsistent because of contact time, bird coverage, and spray pressure. Xiong et al. (1998) reported a 1.0 $\log_{10}$ reduction in *Salmonella* when the washer was adjusted to 207 kPa for 30 s. Spray pressure should be

adjusted properly since Brashears et al. (2001) found that high pressures may actually be detrimental by forcing bacteria into the skin of the carcasses. Throughout the U.S., various antimicrobials are utilized in spray washes. According to an industry survey conducted by McKee (2011) which included 167 U.S. poultry processing plants, peracetic acid was the intervention used by the majority of processors for OLR and IOBW, followed by chlorine, acids with a pH of 2.0, acidified sodium chlorite, and cetylpyridium chloride, among others that were used less frequently.

Carcass chilling represents one of the most critical steps for controlling microbial growth during processing. Chilling in the poultry industry is a necessary process to reduce carcass temperatures and inhibit microbial growth to meet regulatory requirements as required in 9 CFR 381.66, as well as improve shelf-life of the product. Carcass temperatures must be reduced to 4°C or less within 4 hours of slaughter (USDA, 1995). This is typically achieved within 1 to 2 hours postmortem. Furthermore, USDA carcass testing occurs immediately following chilling. Two approved methods that are utilized today for chilling broiler carcasses include immersion, in which the carcasses are immersed in chilled water, and air chilling, in which the products are misted with water in a room filled with chilled air. Most poultry plants in the United States use the immersion method to chill carcasses since it is efficient and economical for processors.

Immersion chillers typically involve multiple stages of tanks. The prechiller is the first stage that carcasses encounter. Temperatures in the prechiller range from 7 to 12°C, and carcasses remain there for approximately 10 to 15 min. The prechiller generates some washing and chilling effects on the carcass, but the main purpose is to allow water absorption as well as to remove some of the heat load entering the chiller. Carcasses are

about 30 to 35°C when they enter the main chiller tank after prechilling. Water temperature in the chiller is about 4°C at the entrance and about 1°C where birds exit. The low temperatures reduce carcass temperatures rapidly causing lipids in the tissues to solidify and seal in water that was absorbed in the prechiller. Air is injected into the bottom of the chiller in order to create agitation in the water and prevent thermal layering. This mechanism further increases the rate of heat exchange (McKee, 2009).

Most immersion chillers are set up with counter-current flow, in which the carcasses and water flow in opposite directions. This helps to maximize chilling rate and reduce the total bacterial load since product is moving into increasingly cleaner water. However, contact between birds during immersion results in pathogen cross-contamination to other carcasses (Bailey et al., 1987). This cross-contamination can result in a higher incidence of pathogen-positive birds when compared to carcasses that have been air chilled. However, when antimicrobials are utilized in immersion chilling, there is a greater capacity for bacterial reduction on carcasses.

During immersion chilling, carcasses absorb some water in the skin and surrounding fat (Carroll and Alvarado, 2007). It was shown that immersion chilled carcasses absorbed 11.7% moisture during chilling and retained 6.0% of that moisture during cutting and 3.90% during post-cutting storage (Young and Smith, 2004). Due to a rule issued by the USDA Food Safety and Inspection Service in 2001 intended to improve the safety of raw poultry, processors are required to list either the percentage of retained water or the maximum percentage of absorbed water on each product label (USDA, 2001). However, the moisture pickup in carcasses that have been immersion

chilled translates to an increase in yield in product and may be advantageous for processors.

With air chilling, cold air (-7 to 2°C) is used as the method of heat removal. Air is blown over cooling elements and circulated around the room at a moderately high speed (Barbut, 2002) for 1 to 3 hours. The product is often sprayed with water to enhance cooling. Air chilling is increasing in popularity due to the limited availability of water, restrictions on wastewater discharge, and more stringent federal regulations on carcass moisture retention. Additionally, air chilled poultry may be exported to some countries in the European Union that do not favor immersion chilling (Huezo et al., 2007). Air chilling may be more preferred since there is no moisture pickup, and drier products do not show a lot of purge when packaged. The dried skin rehydrates, and typically the appearance returns to normal once packaged. Young and Smith (2004) observed that air-chilled carcasses lost an average of 0.68% of their post-processing weight during storage. Some processors also view the microbial quality of air-chilled product to be better than that of those that were immersion chilled (Barbut, 2002; Sanchez et al., 2002). There is less physical contact between carcasses that are air chilled so the potential for cross-contamination is reduced (Huezo et al., 2007). The increased potential for cross-contamination in water chilling presents an issue in trade restrictions between countries that utilize two different chilling mechanisms. Use of chlorine in product-contact water presents another dynamic limiting trade between countries.

Antimicrobial Intervention Strategies in Poultry Processing

The application of approved chemical treatments during processing is one method to reduce pathogens on poultry carcasses. In order for antimicrobials to be effective and

relevant to the industry, they must be approved and have validated efficacy against microorganisms. In addition, the concentration and contact time needs to be appropriate for a particular processing step. Finally, they must be cost effective and without negative impacts on product quality. Currently approved antimicrobials for use in poultry applications are described in FSIS Directive 7120.1 Revision 9. Some of these include hypochlorous acid (chlorine), chlorine dioxide, acidified sodium chlorite, organic/inorganic acids, peracetic acid, cetyl pyridium chloride, and bromine. Furthermore, when deciding upon an antimicrobial treatment, water quality should be determined for the application. Water with a high mineral content (hard water) can reduce the efficacy of some antimicrobials and render them ineffective. Antimicrobials reduce microbial contamination on carcasses through mechanisms that are inhibitory to the cellular survival and proliferation of bacteria. Because *Salmonella* and *Campylobacter* are typically hindered by a pH above 9.0 and below 4.0, control of these pathogens is often achieved through a low pH range characteristic of some antimicrobials.

In the United States, chlorine has historically been a common antimicrobial utilized for prevention of carcass cross-contamination in immersion chilling systems and throughout the poultry processing plant (McKee, 2011). When chlorine is used, optimal chill water conditions should be maintained. These include a temperature at or below 4°C and a pH between 5.5 and 6. The efficacy of chlorine, however, is also affected by organic load and mineral content in the water. Chlorine reacts with water to form hypochlorous acid (HOCl) and hypochlorite ions, which are both forms of free available chlorine. Hypochlorous acid, however, is the most active form for pathogen reduction. HOCl concentration is dependent upon pH of the solution. A lower pH improves HOCl

formation yet stability decreases. A pH decrease below 4.0 can increase the amount of chlorine gas formation, which is both toxic and corrosive. When water pH is higher than optimal, however, HOCl breaks down forming hypochlorite ions. Chlorine is more stable at a higher pH, but less effective in killing pathogens. Therefore, monitoring pH in the chilling system is very important (McKee, 2009).

Hypochlorous acid destroys microbial cells by hindering carbohydrate metabolism. This is accomplished through inhibiting glucose oxidation by chlorine-oxidizing sulfhydryl groups of enzymes important to the process (Marriott, 2006). Izat et al. (1989) reported effective reduction of *Salmonella* when 100 ppm chlorine was used in chilling systems, although a strong chlorine odor was detectable. It has been determined that a level over 1200 ppm is necessary to achieve a minimum 99% kill. Addition of chlorine to poultry processing water is permitted at levels of 20 to 50 ppm in carcass wash applications and chiller make-up water (USDA-FSIS, 2003).

Chlorine dioxide (ClO_2) is a more stable form of chlorine that is used during poultry processing for pathogen reduction. Chlorine dioxide has been shown to be more effective than chlorine in the presence of organic matter, has greater oxidizing capacity, and is not affected by higher pH (Byrd and McKee, 2005). Additionally, it is somewhat chemically inactive toward individual amino acids and will not result in off-flavors that can be associated with higher concentrations of hypochlorous acid (Sams, 2001). ClO_2 is inhibitory to bacteria by causing loss of cell membrane permeability control with nonspecific oxidative damage to the outer membrane (Berg et al., 1996). Lillard (1979) reported that ClO_2 is four to seven times more effective at reducing the bacterial load in poultry chiller water when compared to the same concentrations of chlorine gas. It is

approved for use in water during poultry processing in levels not to exceed 3 ppm

residual chlorine dioxide (FSIS Directive 7120.1). However, ClO_2 is not widely used

currently because it gives inconsistent results (McKee, 2011).

Organic acids, which are known for their antimicrobial properties, have been used

throughout poultry processing. They typically demonstrate good microbiological efficacy

and are safe for use. Acids destroy bacteria by penetrating and disrupting their cell

membrane. The acid molecule then dissociates and as a result, acidifies the cell interior

(Marriott, 2006). The efficacy of organic acids against microorganisms has been reported

to vary with concentration, contact time, and temperature (Dickson and Anderson, 1992).

Acid treatments are found to be more effective before bacteria are firmly attached to the

surface of the meat. While acids can be effective antimicrobials, studies indicate that they

are associated with negative flavor and color changes (Blankenship et al., 1990). Graying

of carcass wing tips have been reported by some plant personnel when organic acids are

used in higher concentrations. Several organic acids studied for use in poultry

applications include lactic, acetic, formic, and propionic acid (Mulder et al., 1987; Izat et

al., 1990; Dickens et al., 1994). *Salmonella* incidence was reported to be reduced through

addition of 0.5-1.0% lactic acid to chiller water (Izat et al., 1989). Furthermore, Rubin

(1978), found that lactic and acetic acids demonstrated a synergistic inhibitory effect on

S. Typhimurium. Their use in poultry processing is limited, however, since they have

been reported to cause organoleptic effects on raw poultry.

Inorganic acids such as the SteriFx (FreshFx) solution and Aftec 3000 buffered

sulfuric acid have been used as antimicrobials in processing. Additionally, inorganic

acids such as sulfuric, phosphoric, and hydrochloric acid are often used to adjust the pH

in poultry chiller water and processing water in meat and poultry plants. Some companies

have started to utilize strong acids or mixtures of organic/inorganic acids for OLR

purposes (Russell, 2010).

Acids have been combined with other antimicrobials in order to utilize lower

levels of organic acid while still ensuring the efficacy of the compound for bacterial

reduction. An example of this application is peracetic acid (PAA), a mixture of an

organic acid (acetic) and an oxidant (hydrogen peroxide) which in combination, forms an

equilibrium in water (Baldry and Fraser, 1988). Therefore, this antimicrobial kills

bacteria in two separate ways. It was reported that utilizing combinations of 1% acetic

acid and 3% hydrogen peroxide provide the best reduction in *Escherichia coli*,

Salmonella Wentworth, and *Listeria innocua* when sprayed on beef carcasses that had

previously been inoculated (Bell et al., 1997). A greater than 3-log reduction for each

bacterial strain tested was obtained using this combination and was more effective than

each antimicrobial used individually. Bauermeister et al. (2008), found that a peracetic

acid mixture of 15% peracetic acid and 10% hydrogen peroxide (Spectrum™) added

during chilling at 85 ppm caused a 91.8 % reduction in *Salmonella* as compared to a

56.8% reduction with 30 ppm of chlorine. Similarly, treatment with PAA caused a

reduction of 43.4% in *Campylobacter* whereas only a 12.8% reduction was obtained with

chlorine. Based on results from these studies, PAA is an effective antimicrobial at

relatively low levels and provides a synergistic effect due to combined acidic and

oxidizing properties. For antimicrobial applications in poultry, the maximum allowable

concentrations are 220 ppm peracetic acid and 110 ppm hydrogen peroxide in the chiller

and 2000 ppm in a post-chill dip (FSIS Directive 7120.1). The FMC Corporation holds the patent for the higher concentration that can be used in post-chill applications.

Acidified Sodium Chlorite (ASC) is a very common antimicrobial product used for carcass application during processing. ASC, the product of $NaClO_2$ acidification, is a combination of citric acid and sodium chlorite. However, it is not widely used in chiller applications but rather spray applications (Anonymous, 2001). It is approved as a poultry spray or dip in accordance with title 21 of the Code of Federal Regulations at 500 to 1,200 ppm sodium chlorite and pH 2.3 to 2.9 (FSIS Directive 7120.1). In chiller water, ASC is limited to 50 to 150 ppm alone or in combination with other GRAS acids to achieve a pH of 2.8 to 3.2 (Anonymous, 2010). Numerous oxy-chlorous antimicrobial intermediates are formed when ASC comes into contact with organic matter (Gordon et al., 1972; Kross, 1984). The reactive intermediates formed possess germicidal activity over a broad range and act by disrupting oxidative bonds on the cell membrane surface (Kross, 1984). This particular mode of action is thought to minimize the potential for bacterial resistance that may arise following continued exposure to antimicrobial measures.

It has been demonstrated that ASC treatment is an effective method for prechill bacterial decontamination. Kemp et al. (2000) reported the maximum antimicrobial activity of ASC was obtained when carcasses were washed prior to a 5-s dip of phosphoric or citric acid activated ASC used at a level of 1,200 ppm. However, cross-contamination that may occur in the chill tank may limit the efficacy of prechill applications. Therefore, reducing microbial contamination may be better achieved through chemical application during postchill operations opposed to prechill

interventions. Oyarzabal et al. (2004) reported that ASC postchill application done by whole carcass immersion for 15-s significantly reduced *Campylobacter* spp. and *E. coli* in commercial broiler carcasses.

Cetylpyridinium chloride (1-hexadecylpridinium chloride, CPC) is a quarternary ammonium compound with a near neutral pH and antimicrobial properties against a broad range of microorganisms including viruses. It has approved use as an antimicrobial agent in poultry processing for ready-to-cook (RTC) poultry products. CPC destroys bacteria through the interaction of basic cetylpyridinium ions with the acid groups of the bacteria. Weakly ionized compounds are formed through this interaction which subsequently inhibit bacterial metabolism (Sams, 2001). According to title 21 of the Code of Federal Regulations, the concentration of CPC in the solution applied to carcasses shall not exceed 0.8% by weight (FSIS Directive 7120.1). CPC is almost always used in drench cabinets. It gives consistent results, but is expensive and must be recaptured due to lack of EPA approval to be released in waste water (McKee, 2011).

Several studies have verified the effectiveness of CPC as an antimicrobial against *Salmonella*. Yang et al. (1998) reported a 3.62 $\log_{10}$ cfu/mL reduction in *Salmonella* after application of a 0.5% CPC solution to prechill chicken carcasses. Li et al. (1997) applied a 0.1% CPC treatment on prechill chicken carcasses by spraying for 30 or 90 s. They indicated a reduction of 0.59 to 0.85 $\log_{10}$ cfu and 1.20 to 1.63 $\log_{10}$ cfu, respectively. In addition, published findings have demonstrated that between 0.1 and 0.5% CPC (Cecure®) is very effective at controlling *Campylobacter* on poultry carcasses. In a pilot plant study conducted in 1999 at the University of Arkansas in Fayetteville (Waldroup et al., 1999), prechill broilers were subjected to a 10-s dip with 0.5% Cecure® which

completely eliminated *Campylobacter*. The other three treatments which included 0.2 or 0.5% as a mist or 0.2% as a 10-s spray significantly lowered the level of *Campylobacter* on carcasses by 1.7-2.2 logs per mL.

Bromine (AviBrom™) which goes by the chemical name 1,3-dibromo-5,5-dimethylhydantoin (DBDMH), is another broad-spectrum antimicrobial approved for use in poultry. It is a halogen processing aid that is effective over a wide pH range and is less reactive to organic matter than chlorine. McNaughton et al. reported a 2.5 $\log_{10}$ reduction in *Salmonella* and *Campylobacter* on post-chill carcasses using AviBrom™. The compound demonstrates antimicrobial activity as hypobromous acid (HOBr) in water. Hypobromous acid works against bacteria by inhibiting certain essential enzymes through the oxidation of sulfhyrdryl groups and also causes lysis of bacterial cell walls. DBDMH is approved for use in poultry chiller water, IOBW, and for water used in poultry processing of carcasses, parts, and organs at a level not to exceed that required to provide the equivalent of 100 ppm available bromine (FSIS Directive 7120.1).

Many processors are exploring more natural alternatives for bacterial reduction in the plant. Lysozyme, for example, plays an important role in the prevention of bacterial growth in foods of animal origin such as hen eggs and milk. Egg white lysozyme comprises approximately 3.4% of egg white protein. It has an isoelectric point between 10.5 and 11.5 and shows stability in low pH conditions, particularly when thermally treated (Chen et al., 2011).This enzyme exhibits strong antibacterial potential, mainly among Gram-positive bacteria, and because of this it has been used in practical applications in the food and pharmaceutical industries. Gram-negative bacteria, however, are less susceptible to lysozyme activity. Lysozyme has been utilized as a preservative in

foods of which it is not a natural component. However, it currently has only limited applications in the food industry. It is beneficial when used as a food preservative since it is specific in its activity against bacterial cell walls and harmless to humans. It is added to certain hard cheeses in Europe for prevention of gas formation and cracking of the cheese wheels by saccharolytic, butyric-forming *Clostridia*, particularly *Clostridium tyrobutyricum* (Wasserfall and Teuber, 1979).

Lysozyme has natural antibacterial activity because of its ability to lyse certain bacteria. It catalyzes the hydrolysis of the β-1, 4-glycosidic linkages between *N*-acetylglucosamine and *N*-acetylmuramic acid of the peptidoglycan layer in the bacterial cell wall (Lesenierowskie et al., 2004). Hughey and Johnson (1987) indicated that lysozyme effectively lyses and inhibits growth of several food-borne pathogens and spoilage bacteria. They found that certain strains of *Clostridium botulinum* as well as four strains of *Listeria monocytogenes* were lysed by the egg white enzyme lysozyme. In addition, a synergistic relationship has been identified between lysozyme and other preservatives such as organic acids. This association has resulted in significantly improved bacteriostatic activity against a broad range of bacteria. Chen et al. (2011) showed that the enzymatic activity of lysozyme modified with various concentrations of lactic acid maintained consistent antibacterial activity against *Bacillus cereus*, *Escherichia coli*, and *Salmonella* Tyhphimurium. Egg white lysozyme has been approved as a safe and suitable ingredient for use in casings and on cooked RTE meat and poultry products. Its usage level for these applications is limited to 2.5 mg per pound in the finished product when used in casings and 2.0 mg per pound on cooked meat and poultry products (FSIS Directive 7120.1).

There has been a considerable increase in U.S. poultry processing facilities that are currently using post-chiller antimicrobial interventions (McKee, 2011). These methods serve as a last line of defense against pathogens before exiting the chilling system. An example of this is the Morris & Associates FinalKill® Finishing Chiller® (Figure 1). It resembles a traditional chiller, but has a much smaller footprint and volume ranges from 400-600 gallons (Morris & Associates, Garner, NC), while other post-chill dip tanks have a wider volume range depending on size. When comparing antimicrobial treatment in a Finishing Chiller® to the primary chiller which holds approximately 20-50, 000 gallons of water and has a carcass dwell time of 1.5-2.0 hours, it is more economical to treat a much smaller volume of water (McKee, 2011). In addition, the dwell time for carcasses in the Finishing Chiller® application is generally less than 30 s. Because of the short dwell time experienced in Finishing Chillers, higher concentrations of antimicrobials can be utilized with less likelihood of affecting product quality. This makes them very beneficial to poultry processors, since post-chilling introduces an additional strategy or "multi-hurdle" approach for pathogen intervention. Furthermore, employing post-chill systems is advantageous because carcasses are cleanest at this point in the process, since organic loading is very low on carcasses post chilling. Therefore, bacteria can be reduced more effectively. By and large, when used in combination with other interventions throughout the plant, post-chill dip systems have been very successful in lowering *Salmonella* and other pathogens to acceptable levels (Russell, 2010).

Targeting a 2-$\log_{10}$ reduction in bacterial counts is believed to eliminate most naturally occurring levels of *Salmonella* and/or *Campylobacter* that might remain on carcasses post-chill. For instance, different locations in a processing plant were tested for

Campylobacter recovery. *Campylobacter* is a very small organism and therefore is more difficult to eliminate since it can get deep into feather tracks and pores in the skin of carcasses. From the pre-chiller and post-chill locations, a mean $\log_{10}$ CFU/mL of 2.3 and 1.5 were recovered, respectively (Berrang and Dickens, 2000). Therefore, utilizing compounds with intervention strategies that can yield a 2-$\log_{10}$ reduction would likely eliminate *Campylobacter* remaining on the carcass after chilling. Results from an industry survey by McKee (2011) indicated that peracetic acid was the post-chill antimicrobial intervention used by the majority of processors surveyed. It was followed by chlorine, CPC, acids with a pH of 2, and ASC which was used by less than 10% of those surveyed.

Figure 1. Morris & Associates FinalKill® Finishing Chiller®

Importance of Quality Determination

Overall meat quality describes several different attributes of meat such as appearance, juiciness, flavor, and texture. Quality products are those that satisfy all the aspects that affect consumer acceptability while remaining safe and wholesome. Meat quality properties can be measured or determined through both objective and subjective methods which can be used together to make conclusions about quality. Sensory evaluation is recognized by professional organizations such as the Institute of Food Technologists and American Society for Testing and Materials as a scientific method used to evoke, measure, analyze, and interpret product attributes as perceived by the five human senses of sight, smell, touch, taste, and hearing (Stone and Sidel, 2004). During sensory evaluation of a product, panelists assess sensory characteristics of the product and provide a response. Instruments are used for objective measurements and can measure characteristics related to the physical or chemical properties of the product, thereby providing a corollary measurement that supports the sensory evaluation.

There are two main types of sensory testing methods. Laboratory/analytical methods include difference testing and descriptive testing. Difference testing methods focus on determining if there are perceptible differences between products, whereas descriptive testing methods quanitify the perceived intensities of the sensory attributes of a product. These types of panels are composed of panelists who have been selected based upon good acuity of the senses and can be trained to assess products for specific characteristics, not for whether they like or dislike the product. On the other hand, consumer affective test methods measure how consumers feel or respond to the product by attempting to quantify the degree of liking or disliking of the product. These test

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methods require a larger number of panelists, and results can be generalized to the population of interest (Lawless and Heymann, 2010).

Two of the most important quality attributes of poultry meat are appearance and texture. Texture is an important characteristic associated with poultry meat quality since it is most affected by bird age and processing procedures. Meat texture varies because of innate differences within the structure of the meat or muscle tissue relating to contractile protein structures, connective tissue, lipid and carbohydrate components in addition to other factors such as cooking and sample handling (Lee, 2007). Therefore, instrumental methods are used widely to measure tenderness and evaluate the structure of muscle fibers. Tenderness measurements determined from cooked poultry meat are often performed using the Allo-Kramer shear cell. This method is designed to cut through the muscle fibers. Samples are positioned within the instrument so that multiple blades first compress and then shear through the meat sample, cutting perpendicular to the fibers. The total force required to shear through the sample is related to the tenderness or toughness of the cooked sample. Measurements are typically recorded as kg of force/g of sample (Smith et al., 1988). Another method often used for poultry meat products is the Texture Profile Analysis (TPA). The sample is usually prepared by taking a circular core from the cooked meat. Multiple textural aspects can be separated and analyzed from a particular sample such as hardness, springiness, cohesiveness, and chewiness (Lyon and Lyon, 2001).

Appearance or color is typically the first attribute that affects consumer acceptability of poultry meat or products. Instruments used to measure color of a product are based on a light source and a detector. To understand this, numerical values given by

colorimeters used in research and quality are typically associated with a color or appearance term. Color can be measured using the CIELAB color scale (L* a* b*), where the L value represents the degree of whiteness or blackness. Rectangular Cartesian coordinates (a, b) designate the chromatic segment of the color space. Red is represented by +a, green represented by –a, yellow represented by +b, and blue represented by –b (Lawless and Heymann, 2010). Since meat color is important to final product quality, factors that affect color have been researched extensively. These factors include characteristics of the live bird such as age, sex, and strain. In addition, processing methods, exposure to chemicals or antimicrobials, cooking methods, and freezing have been reported to cause variation in poultry meat color. Normal poultry breast meat L* color values on the distal surface of the fillet range from 47 to 49. Lightness values of less than 46 are considered darker than normal whereas values of greater than 50 are considered lighter than normal (Fletcher et al., 2000). These values are based on similar values reported by Allen et al., 1998. Fletcher et al. also reported normal a* and b* values for raw breast fillets as 3.7 and 6.8, respectively.

Because treatment with antimicrobials can affect the organoleptic properties of a product, it is important to determine the quality aspects of carcasses treated with antimicrobials. Ideally, chemical decontamination interventions should reduce the microbial loads without having deleterious impacts on quality characteristics. Some antimicrobial treatments are reported to impact product quality when used in higher concentrations. Therefore, it is important to monitor usage levels as well as contact time to better determine what antimicrobial might be optimal for a particular application. Although the efficacy of chlorine is greater with increasing concentration, discoloration,

off-odor, and off-flavor may be associated with the carcass (Conner et al., 2001). Chlorine dioxide, however, has extremely low residual activity and is relatively unreactive with individual amino acids. Therefore, no off-flavors or odor has been associated with ClO_2 (Sams, 2001). Hecer and Guldas (2011) reported a prolonged shelf-life of 4 days and no off-flavor or negative impacts on sensory properties of broiler wings treated with 0.3 and 0.5% chlorine dioxide.

Higher concentrations of organic acids have been shown to result in discoloration of broiler carcasses. Levels above 3% have been shown to cause carcass discoloration while levels of lactic acid at 1% or greater have been associated with changes in flavor (Blankenship et al., 1990). In addition, Mulder et al. (1987), Izat et al. (1990), and Dickens et al. (1994) reported visual changes including graying of carcasses that were treated with various organic acids. Bilgili et al. (1998) reported consistent results that organic acids as carcass disinfectants can alter the color of processed broiler skin. They observed darkening (L*) and yellowing (b*) with most of the acids in their study. However, treatment with propionic acid resulted in a lighter skin color, similar to the bleaching observed with hydrogen peroxide treatment (Mulder et al., 1987). A number of researchers have found that unacceptable color as well as off-odors may be obtained when organic acids are used in concentrations higher than 1.5% to 2%. Smulders (1995) maintained that unless organic acids are applied in very high concentrations, discoloration tends to return to normal after 24 h.

Acidified sodium chlorite has been shown to have slight effects on poultry skin color. Bolisevac et al. (2004) reported that treatment with ASC at 600 ppm had a significant effect on color and odor when compared to the controls. However, flavor of

samples treated with 300 ppm was given an average or better score than untreated controls. The effects of 1200 ppm ASC treatment on sensory characteristics of poultry carcasses was studied by Scheider et al. (2002). They observed a slight whitening of the skin surface following treatment in both spray and dipped carcasses. Kemp et al. (2000) also observed the whitening effect of ASC on poultry skin. They noticed that the only distinguishable effect of dipping in 1200 ppm for 5 s was a temporary mild whitening of the skin surface. However, these authors indicated that the slight color change disappeared during water chilling and did not result in any organoleptic variations in post-chill or cooked poultry products. Acidified sodium chlorite (Sanova®) had traditionally been used in OLR and IOBW applications (McKee, 2011).

In a study conducted by Dickens et al. (1994), the sensory quality of breast fillets exposed to a prechill treatment of 0.6% acetic acid for 10 min showed no differences when compared to a control. Conversely, studies reveal that when a higher concentration of hydrogen peroxide is used alone, carcasses may have a bloated and bleached appearance (Lillard and Thomson, 1983; Mulder et al., 1987; Izat et al. 1990). Dickens and Whittemore (1997) found that 0.5 to 1.5% solutions of hydrogen peroxide was not effective as antimicrobial and resulted in the bleaching and bloating of the skin of carcasses. It was indicated that when organic acids such as acetic acid is combined with hydrogen peroxide, lower levels can be used to obtain better antimicrobial efficacy while product quality is maintained (Bell et al., 1997). Furthermore, Bauermeister et al. (2008) reported that carcass treatment with peracetic acid did not result in any quality defects, and greater PAA levels may extend product shelf-life. Broilers treated with 0.02% PAA in this study were slightly lighter in color. However, the color disappeared over time and

the slight changes in product color would not be considered a negative impact on product quality. Conversely, plant personnel have reported that higher concentrations of PAA have resulted in graying/darkening of wingtips with increased contact time (Anonymous, 2012).

Other antimicrobials have little to no reported associations with deleterious effects on the organoleptic properties on poultry. Cetylpyridinium chloride has not been associated with negative effects on organoleptic qualities to the birds when applied properly. It is a stable compound with a near neutral pH (Bai et al., 2007). In poultry processing experiments, CPC has not been linked to any associated carcass bloating or skin discoloration and did not corrode equipment (Sams, 2001). Similarly, 1,3-dibromo-5,5-dimethylhydantoin or DMDBH works over a broad pH range thereby reducing or eliminating the need for acidifiers. This minimizes the risk of color or shelf-life issues. In addition, research showed that when various concentrations of lactic acid-induced gelled egg white powder was utilized as a protein additive or natural bacteriostatic agent in sausage and hamburger products, it provided good sensory quality and bacteriostatic activity in ground meat products (Chen et al, 2004; Wang et al., 2005).

Because *Salmonella* and *Campylobacter* continue to be major causes of food-borne illness in the United States, USDA regulations have become more stringent, making it crucial for processors to examine new or additional intervention strategies for effective pathogen control. In recent years, novel intervention strategies such as post-chill decontamination tanks have provided alternative approaches for pathogen reduction during poultry processing (McKee, 2011). This particular study is significant because historically, antimicrobial application was focused in the primary chiller, but new

equipment such as a Finishing Chiller® offers an application point where various antimicrobials can be added using smaller volumes of water. While the efficacy of antimicrobials has been validated against *Salmonella* and *Campylobacter* primarily in chillers, this study provides validation results in Finishing Chillers and will be beneficial for the poultry industry as well as for consumers.

Therefore, the objectives of the current study were to determine the efficacy of various antimicrobials added to the Finishing Chiller® to reduce *Salmonella* and *Campylobacter* on broiler carcasses as well as to evaluate any associated effects of the antimicrobials on quality attributes of chicken breast meat. The efficacy of antimicrobials was evaluated through microbial analyses of carcass rinses while sensory analysis was performed on non-inoculated chicken breast samples. This study provides the poultry industry with a validated practical intervention strategy for pathogen reduction on poultry carcasses.

References

Allen, C. D., D. L. Fletcher, J. K. Northcutt, and S. M. Russell. 1998. The relationship of broiler breast color to meat quality and shelf-life. Poultry Sci. 77:361–366.

Anonymous, 2010. Compliance guideline for controlling *Salmonella* and *Campylobacter* in Poultry, 3rd edition, May 2010. Available at: http://www.fsis.usda.gov/PDF/Compliance_Guide_Controling_Salmonella_Campylobacter_Poultry_0510.pdf. Accessed Nov. 2011.

Anonymous, 2010. How safe is that chicken? Consumer Reports Magazine. January, 2010. Available at: http://www.consumerreports.org/cro/magazine-archive/2010/january/food/chicken-safety/overview/chicken-safety-ov.htm. Accessed Dec. 2011.

Bai, Y., K. R. Coleman, C. W. Coleman, and A. L. Waldroup. 2007. Effect of cetylpyridinium chloride (cecure® cpc antimicrobial) on the refrigerated shelf life of fresh boneless, skinless broiler thigh meat. Inter. J. Poul. Sci. 6 (2):91-94.

Bailey, J. S., N. A. Cox, S. E. Craven, and D. E. Cosby. 2002. Serotype tracking of *Salmonella* through integrated broiler chicken operations. J. Food Prot. 65:742-745.

Baldry, M. G. C., and J. A. L. Fraser. 1988. Disinfection with peroxygens. In Industrial Biocides. Reports on Applied Chemistry. K. R. Payne, ed. John Wiley and Sons, Hoboken, NJ.

Barbut, S. 2002. Poultry Products Processing. CRC Press, BocaRaton, FL.

Batz, M. B., S. Hoffman, and J. G. Morris, Jr. 2011. Ranking the risks: the 10 pathogen-food combinations with the greatest burden on public health. University of Florida, Emerging Pathogens Institute.

Bauermeister, L. J., J. W. J. Bowers, J. C. Townsend, and S. R. McKee. 2008. Validating the efficacy of peracetic acid mixture as an antimicrobial in poultry chillers. J. Food Prot. 71:119-122.

Bauermeister, L. J., J. W. J. Bowers, J. C. Townsend, and S. R. McKee. 2008. The microbial and quality properties of poultry carcasses treated with peracetic acid as an antimicrobial treatment. Poul. Sci. 87:2390-2398.

Bell, K. Y., C. N. Cutter, and S. S. Sumner. 1997. Reduction of foodborne micro-organisms on beef carcass tissue using acetic acid, sodium bicarbonate, and hydrogen peroxide spray washes. Food Microbiol. 14:439-448.

Berg, J. D., P. V. Roberts, and A. Matin. 1986. Effect of chlorine dioxide on selected membrane functions of *Escherichia coli*. J. Appl. Bacteriol. 60: 213-220.

Berghaus, R. D., S. G. Thayer, J. J. Maurer, and C. L. Hofacre. 2011. Effect of vaccinating breeder chickens with a killed *Salmonella* vaccine on *Salmonella* prevalences and loads in breeder and broiler chicken flocks. J. Food Prot. 74:727-734.

Berrang, M. E., and J. A. Dickens. 2000. Presence and level of *Campylobacter* spp. on broiler carcasses throughout the processing plant. J Appl Poult Res 9:43-47.

Bilgili, S. F., D. E. Connor, J. L. Pinion, and K. C. Tamblyn. 1998. Broiler skin as affected by organic acids: influence of concentration and method of application. Poul. Sci. 77:751-757.

Bishop, S. C., R. F. E. Axford, F. W. Nicholas, and J. B. Owen. 2010. Breeding for Disease Resistance in Farm Animals. CABI, Cambridge, MA.

Blankenship, L. C., J. S. Bailey, N. A. Cox, N. J. Stern, R. Brewer, and O. Williams. 1993. Two-step mucosal competitive exclusion flora treatment to diminish salmonellae in commercial broiler chickens. Poult. Sci. 72:1667-1672.

Blankenship, L. C., B. G. Lyon, A. D. Whittemore, and C. E. Lyon. 1990. Efficacy of acid treatment plus freezing to destroy *Salmonella* contaminants of spice-coated chicken fajita meat. Poult. Sci. 69(Suppl. 1):20. (Abstract)

Bolisevac, J. M., S. D. Shackelford, R. Fahle, T. Biela, and M. Koohmaraie. 2004. Decreased dosage of acidified sodium chlorite reduces microbial contamination and maintains organoleptic qualities of ground beef products. J. Food Prot. 67:2248-2254.

Brashears, M. M., D. E. Burson, E. S. Dormedy, L. Vavak, S. R. McKee, W. Fluckey, M. Sanchez. 2001. HACCP implementation and validation in small and very small meat and poultry processing plants. Nebraska Dairy, Food and Environmental Sanitation. 21:20-28.

Bryan, F. L. and M. P. Doyle. 1995. Health risk and consequences of *Salmonella* and *Campylobacter jejuni* in raw poultry. J. Food Prot. 58:326-344.

Bryan, F. L., J. C. Ayres, and A. A. Kraft. 1968. Salmonellae associated with further-processed turkey products. Applied Microbiol. 16:1-9.

Byrd, J. A., B. M. Hargis, D. E. Corrier, R. L. Brewer, D. J. Caldwell, R. H. Bailey, J. L. McReynolds, K. L. Herron, L. H. Stanker. 2002. Flourescent marker for the detection of crop and upper gastrointestinal leakage in poultry processing plants, Poul. Sci. 81:70-74.

Byrd, J. A., D. E. Corrier, M. E. Hume, R. H. Bailey, L. H. Stanker, and B. M. Hargis. 1998. Effect of feed withdrawal on the incidence of *Campylobacter* in crops of preharvest broiler chickens. Avian Diseases 42:802-806.

Byrd, J. A. and S. R. McKee. 2005. Improving slaughter and processing technologies. Pages 310-327 in Food Safety Control in the Poultry Industry. G.C. Mead, ed., CRCPress, LLC, Boca Raton, FL.

Centers for Disease Control and Prevention. 2011. Estimates of Foodborne Illness in the United States. Internet Address: http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html. Accessed Nov. 2011.

Centers for Disease Control and Prevention. 2011. Vital Signs: Incidence and Trends of Infection with Pathogens Transmitted Commonly Through Food – Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 1996-2010. Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6022a5.htm?s_cid=mm6022a5_w. Accessed Jan. 2012.

Centers for Disease Control and Prevention. 2010. National Center for Emerging and Zoonotic Infectious Diseases. Campylobacter. Internet Address: http://www.cdc.gov/nczved/divisions/dfmd/diseases/campylobacter. Accessed Nov. 2011.

Centers for Disease Control and Prevention. 2010. Salmonella. Internet Address: http://www.cdc.gov/salmonella/general/techinical/html. Accessed Nov. 2011.

Chen, S.- L., Y.- M. Weng, J.- J. Huang, and K.- J. Lin. 2011. Physiochemical characteristics and bacteriostatic ability of modified lysozyme from lactic acid-induced gelled egg white powder. J. Food Processing and Preservation. 1745-4549.

Chen, S. L., T. F. Tseng, C. K. Chou, K. J. Lin. 2004. Manufacturing of non-fermented acid Chinese style semi-dry sausage formulated with acid-induced gelled egg white powder. Taiwanese J. Agric. Chem. Food Sci. 42:293–298.

Corrier, D. E., J. A. Byrd, B. M. Hargis, M. E. Hume, R. H. Bailey, L. H. Stanker. 1999a. Presence of *Salmonella* in the crop and ceca of broiler chickens before and after preslaughter feed withdrawal. Poul. Sci. 78:45-49.

Corrier, D. E., J. A. Byrd, B. M. Hargis, M. E. Hume, R. H. Bailey, L. H. Stanker. 1999b. Survival of *Salmonella* in the crop contents of market-age broilers during feed withdrawal. Avian Diseases 43:453-460.

Dickens, J. A., and A. D. Whittemore. 1997. Effects of acetic acid and hydrogen peroxide application during defeathering on the microbioligical quality of broiler carcasses prior to evisceration. Poul. Sci. 76:657-660.

Dickens, J. A., B. G. Lyon, A. D. Whittemore, and C. E. Lyon. 1994. The effect of an acetic acid dip on carcass appearance, microbiological quality, and cooked breast meat texture and flavor. Poultry Sci. 73:576-581.

Dickson, J. S. and M. E. Anderson, 1992. Microbiological decontamination of food animal carcasses by washing and sanitizing systems: A review. J. Food Prot. 55:133-140.

Fletcher, D. L., M. Qiao, and D. P. Smith. 2000. The relationship of raw broiler breast meat color and pH to cooked meat color and pH. Poul. Sci. 79:784-788.

Gordon, G., G. Kieffer, and D. Rosenblatt. 1972. The chemistry of chlorine dioxide, p. 201-286. *In* S. Lippard (ed.), Progress in inorganic chemistry, vol. 15. Wiley Interscience, New York.

Hafez, H. M. 1999. Poultry meat and food safety: pre- and post-harvest approaches to reduce foodborne pathogens. World's Poult. Sci. J. 55:269-280.

Hargis, B. M., D. J. Caldwell, R. L. Brewer, D. E. Corrier, and J. R. DeLoach. 1995. Evaluation of the chicken crop as a source of *Salmonella* contamination for broiler carcasses. Poul. Sci. 74:1548-1552.

Hecer, C. and M. Guldas. 2011. Effects of lactic acid, fumaric acid and chlorine dioxide on shelf-life of broiler wings during storage. African J. Microbiol Research. 5(23):3880-3883.

Helms, M., J. Simonsen, K. E. Olsen, K. Molbak. 2005. Adverse health events associated with antimicrobial drug resistance in *Campylobacter* species: a registry-based cohort study. Journal of Infectious Diseases 191:1050–1055.

Heurtin-Le Corre, C., P. Y. Donnio, M. Perrin, M. F. Travert, and J. L. Avril. 1999. Increasing incidence and comparison of nalidixic acid-resistant *Salmonella* enteric subsp. Enteric serotype Typhimurium isolates from humans and animals. J Clin Microbiol. 37(1):266-269.

Huezo, R., D. P. Smith, J. K. Northcutt, and D. L. Fletcher. 2007. Effect of immersion or dry air chilling on broiler carcass moisture retention and breast fillet functionality. J. Appl. Poult. Res. 16:438-447.

Hughey, V. L. and E. A. Johnson. 1987. Antimicrobial activity of lysozyme against bacteria involved in food spoilage and food-borne disease. Appl. Env. Microbiol. 53, 9: 2165-2170.

Humphrey, T., S. O'Brien, and M. Madsen. 2007. Campylobacters as zoonotic pathogens: a food production perspective. J Food Microbiol. 117:237-257.

Izat, A. L., M. Coldberg, R. A. Thomas, M. H. Adams, and C. D. Driggers. 1990. Effects of lactic acid in processing waters on the incidence of *Salmonella* in broilers. J. Food Qual. 13:295-306.

Izat, A. L., M. Colbert, M. H. Adams, M. A. Reiber, P. W. Waldroup. 1989. Production and processing studies to reduce the incidence of *Salmonella* on commercial broilers. J. Food Prot. 52:670.

Izat, A. L., F. A. Gardner, J. H. Denton, F. A. Golan. 1988. Incidence and level of *Campylobacter jejuni* in broiler processing. Poul. Sci. 67:1568-1572.

Jacobs-Reitsma, W. F., U. Lyhs, J. Wagenaar. *Campylobacter* in the Food Supply. ASM Press, 2008.

Jay, J. M. 2000. Modern Food Microbiology. 6th Ed. Aspen Publishers, Inc. Gaithersburg, MD.

Kemp, G. K., M. L. Aldrich, and A. L. Waldroup. 2000. Acidified sodium chlorite antimicrobial treatment of broiler carcasses. J. Food Prot. 63:1087-1092.

Kross, R. 1984. An innovative demand-release microbiocide. Basis for action and effects of dilution. *In* Second biannual conference on progress in chemical disinfection. SUNY, Binghamton, New York.

Lahellec, C. and P. Colin. 1985. Relationship between serotypes of salmonellae from hatcheries and rearing farms and those from processed poultry carcasses. Br. Poult. Sci. 26:179-186.

Lawless, H. T. and H. Heymann. Sensory evaluation of food: principles and practices. Springer Science+Business Media, LLC 2010.

Lee, Y. S. 2007. Perception, measurement, and prediction of tenderness in poultry breast meat. Thesis (Ph.D.) University of Arkansas, Fayetteville, 2007.

Lesenierowskie, G., C. R. Radziejewska, J. Kijowski. 2004. Thermally and chemical-thermally modified lysozyme and its bacteriostatic activity.World's Poult. Sci. J. 60: 303–309.

Li, Y., M. F. Slavik, J. T. Walker, and H. Xiong. 1997. Prechill spray of chicken carcasses to reduce Salmonella typhimurium. J. Food Sci. 62:605-607.

Lillard, H. S., and J. E. Thomson. 1983. Efficacy of hydrogen peroxide as a bacteriocide in poultry chiller water. J. Food Sci. 48:125-126.

Lillard, H. S. 1979. Levels of chlorine and chlorine dioxide of equivalent bactericidal effect in poultry processing water. J. Food Sci. 44:1594-1597.

Lyon, B. G. and C. E. Lyon. 2001. Meat quality: sensory and instrumental evaluations. Pages 97-120 in Poultry Meat Processing, A. R. Sams, ed., CRC Press LLC, Boca Raton, FL.

Marriott, N. G. and R. B. Gravani. 2006. Principles of Food Sanitation. Springer Science+Business Media, Inc, New York, NY.

McKee, S. R. 2012. Pre-harvest survey for best practices in pathogen control. International Poultry Scientific Forum. Atlanta, Georgia. January 25-26, 2012.

McKee, S. R. 2011. *Salmonella* and *Campylobacter* control during poultry processing. International Poultry Scientific Forum. Atlanta, Georgia. January 24-25, 2011.

McKee, Shelly R. Class lecture. Poultry Further Processing and Products. Auburn University, Auburn, AL. August, 2009.

McNaughton, J. L., M. S. Roberts, R. W. Kuhlmeier, G. M. Ricks & E. W. Liimatta. Efficacy of DBDMH (1,3-Dibromo-5,5-dimethylhydantoin) as a Biocide for Poultry Chill Tank Water & Carcass Bacteria Populations. Data on file. Elanco Reference No. 2318.

Mead, G. C., W. R. Hudson and M. H. Hinton. 1994. Use of a marker organism in poultry processing to identify sites of cross-contamination and evaluate possible measures. Br. Poult. Sci. 35:345-354.

Mead, G. C. 1980. Microbiological control in the processing of chickens and turkeys. Pages 91-104 in Meat Quality in Poultry and Game Birds. G.C Mead and B.M. Freeman, eds., British Poultry Science Ltd, Edinburgh.

Miller, R. G. and C. R. Tate. 1990. XLT4: A highly selective plating medium for the isolation of Salmonella. The Maryland Poultryman, April:2-7.

Mulder, R. W. A. W., M. C. van der Hulst, and N. M. Bolder. 1987. *Salmonella* decontamination of broiler carcasses with lactic acid, l-cysteine, and hydrogen peroxide. Poultry Sci. 66:1555-15557.

"New Performance Standards for *Salmonella* and *Campylobacter* in Chilled Carcasses at Young Chicken and Turkey Slaughter Establishments" 75 FR 27288 (May 14, 2010) p.27288-27294.

Ohl, M. E. and S. I. Miller. 2001. *Salmonella*: A Model for Bacterial Pathogenesis. Annual review of medicine. 52:259-74.

Oosterom J., S. Notermans, H. Karman, G. B. Engels. 1983. Origin and prevalence of *Campylobacter jejuni* in poultry processing. J. Food Prot. 46:339-344.

Oyarzabal, O. A., C. Hawk, S. F. Bilgili, C. C. Warf, and G. K. Kemp. 2004. Effects of postchill application of acidified sodium chlorite to control *Campylobacter* spp. and *Escherichia coli* on commercial broiler carcasses. J. Food Prot. 67:2288-2291.

Rollins, D. M. and R. R. Colwell. 1986. Viable but non-culturable stage of *Campylobacter jejuni* and its role in survival in the natural aquatic environment. Applied and Environmental Microbiol. 52:531-538.

Rubin, H. E., Toxicological model for a two-acid system. 1978. Appl. Environ. Microbiol. 36:623.

Russell, S. M. 2010. *Salmonella* reduction calls for multi-hurdle approach. WATT *PoultryUSA*, June 2010.

Sahin, O., N. Luo, S. Huang and Q. Zhang. 2003. Effect of *Campylobacter*-Specific Maternal Antibodies on *Campylobacter jejuni* Colinization in Young Chickens. Applied and Environmental Microbiology, 69:5372-5379.

Saleha A. A., G. C. Mead, A. L. Ibrahim. 1998. *Campylobacter jejuni* in poultry production and processing in relation to public health. World's Poult. Sci. J. 54:53-58.

Sams, A. R. 2001. Poultry Meat Processing. CRC Press LLC, Boca Raton, FL.

Sanchez, M. X., W. M. Fluckey, M. M. Brashears, and S. R. McKee. 2002. Microbial profile and antibiotic susceptibility of *Campylobacter* spp. and *Salmonella* spp. in broilers processed in air-chilled and immersion-chilled environments. J. Food Prot. 65:948–956.

Sarlin L. L., E. T. Barnhart, D. J. Caldwell, R. W. Moore, J. A. Byrd, D. Y. Caldwell, D. E. Corrier, J. R. DeLoach, B. M. Hargis. 1998. Evaluation of alternative sampling methods for *Salmonella* critical control point determination at broiler processing. Poul. Sci. 77:1253-1257.

Scharff, R. L. 2011. Economic burden from health losses due to foodborne illness in the United States. J. Food Prot. 75(1):123-131.

Schneider, K. R., G. K. Kemp, M. L. Aldrich. 2002. Antimicrobial treatment of air chilled broiler carcasses. Acidified sodium chlorite antimicrobial treatment of air chilled broiler carcasses. Dairy, Food and Environmental Sanitation 22:102-108.

Smith, D. P., C. E. Lyon, and D. L. Fletcher. 1988. Comparison of the allo-kramer shear and texture profile methods of broiler breast meat texture analysis. Poul. Sci. 67: 1549-1556.

Smulders, F. 1995. Preservation by microbial decontamination; the surface of treatment of meats by organic acids. In: Gould, G. W. (Ed.), New Methods of Food Preservation. Blackie Academic and Professional, London.

Stern, N. J. 2001. Campylobacter. Guide to Foodborne Pathogens. 5, 61-67.

Stern, N. J., B. Wojton and K. Kwiatek. 1992. A differential selective medium and dry ice generated atmosphere for recovery of *Campylobacter jejuni*. J. Food Prot. 55:514-517.

Stone, H. and J. L. Sidel. 2004. Sensory Evaluation Practices, Third Edition. Academic, San Deigo.

U.S. Department of Agriculture. 1995. Final rule on pathogen reduction; hazard analysis and critical control points (HACCP) systems. US Dep. Commerce, Washington, DC.

U.S. Department of Agriculture, Food Safety and Inspection Service. 2011. Serotypes profile of *Salmonella* isolates from meat and poultry products January 1998 through December 2010. Available at: http://www.fsis.usda.gov/Science/Serotypes_Profile_Salmonella_Isolates/index.asp. Accessed March, 2011.

U.S. Department of Agriculture, Food Safety and Inspection Service. 2011. New performance standards for salmonella and campylobacter in chilled carcasses at young chicken and turkey slaughter establishments. Available at: http://www.fsis.usda.gov/OPPDE/rdad/FSISNotices/31-11.pdf. Accessed Nov. 2011.

U.S. Department of Agriculture, Food Safety and Inspection Service. 2011. Safe and suitable ingredients used in the production of meat, poultry, and egg products. FSIS Directive 7120.1 Revision 9. Available at: http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7120.1Amend21.pdf. Accessed Nov. 2011.

U.S. Department of Agriculture, Food Safety and Inspection Service. 2003. Use of chlorine to treat poultry chiller water. FSIS Notice 45-03.

Van Schothorst, M., S. Notermans, and E. H. Kampelmacher. 1972. Hygiene in poultry slaughter. Fleischwirttschaft. 52:749-752.

Waldroup, A., C. Coleman and H. Brown. 1999. Pilot plant trials-pre-chill application of cetylpyridinium chloride to broilers. U.S. Food Additive Petition No. 2A4736, Appendix VII. Food and Drug Administration, Department of Health and Human Services. Washington, DC.

Wallis, M. R. 1994. The pathogenesis of *Campylobacter jejuni*. British Journal of Biomedical Science. 51(1):57-64.

Wang, S. C., S. L. Chen, and K. J. Lin. 2005. Manufacturing of ground restructured pork meat patty formulated with acid-induced gelation egg white powder. Taiwan J.Agric. Chem. Food Sci. 43:103–107.

Wasserfall, F., and M. Teuber. 1979. Action of egg white lysozyme on *Clostridium tyrobutyricum*. Appl. Environ. Microbiol. 38:197-199.

World Health Organization, Food and Agriculture Organization of the United Nations. 2002. Risk assessments of *Salmonella* in eggs and broiler chickens. SBN 92 9 156230 7 (WHO) (LC/NLM classification: QW 138.5.S2) ISBN 92 5 104873 8 (FAO) ISSN 1726-5274.

Xiong, H. L., L. Yanbin, M. F. Slavik, and J. T. Walker. 1998. Spraying chicken skin with selected 339 chemicals to reduce *Salmonella* Typhimurium. J. Food Prot. 61:272-275.

Yang, Z., Y. Li., M. Slavik. 1998. Use of antimicrobial spray applied with an inside-outside birdwasher to reduce bacterial contamination on prechilled chicken carcasses. J. Food Prot. 61:829-832.

Young, L. L., and D. P. Smith. 2004. Moisture retention by water and air chilled chicken broilers during processing and cutup operations. Poult. Sci. 83:119–122.

CHAPTER III.

MATERIALS AND METHODS

Pilot Plant Study

This section consists of the materials and methods used to validate antimicrobial application in a Finishing Chiller®. A total of 200 broiler carcasses were sampled (10 carcasses X 2 replications X 10 treatments). During each replication, 100 carcasses were obtained from the Auburn University Poultry Science Research Unit and chilled on ice at 4°C for 24 h before entering the Finishing Chiller®. Ninety carcasses per replication were inoculated in the feather tracks on the skin of the breast portion of the carcass with 1 ml each of *Salmonella enterica* Typhimurium (10^6 cfu/mL) and *Campylobacter jejuni* (10^6 cfu/mL). Once inoculated, the birds were allowed to set for 20 minutes to ensure adequate contact time for bacterial attachment before being dipped into the Finishing Chiller®. Seven finishing chill water treatments consisting of 0.004% chlorine, 0.04% PAA, 0.1% PAA (Spectrum; FMC, Philadelphia, Pa.), 0.003% buffered sulfuric acid, 0.01% buffered sulfuric acid (Aftec 3000; AdvFoodTech, Grand Rapids, Mi.), 0.1% lysozyme, or 0.5% lysozyme (Lysoshield 207; Bioseutica, Zeewolde, Netherlands) were examined using a FinalKill® Finishing Chiller® (model FC-8WHS-S, Morris & Associates, Garner, NC.) at the Auburn University Poultry Science Research Unit.

The Finishing Chiller® is constructed of 10-gauge, 304 stainless steel for durability, and the dimensions are 8'8" x 5' x 8'8". Its unloader mechanism guarantees

that carcasses are fully submerged with consistent dwell time and first in-first out for optimal quality. The Finishing Chiller® held approximately 1,453 liters of water and had a speed of 4 rpm. The water in the chiller was maintained at 4°C, and the carcasses experienced a dwell time of approximately 20 seconds. The finishing chill water treatments included 0.004% chlorine, 0.04%, 0.1% PAA, 0.003%, 0.01% buffered sulfuric acid, 0.1%, 0.5% lysozyme, and water treatment. Positive and negative, or non-inoculated, controls were included. Non-inoculated carcasses were used to determine the prevalence of any background *Salmonella* spp. or *Campylobacter* spp. The positive control treatment consisted of carcasses that were inoculated but not dipped into the Finishing Chiller®. The PAA concentrations were tested and confirmed using a titration drop test kit (LaMotte Co., Chestertown, Md.), while chlorine was measured using Aquachek Water Quality Test Strips (HACH Company, Loveland, Co.). The pH of the treatments was also recorded (HACH Company, Loveland, Co.). The average pH of the PAA treatments was 3.4, and the chlorine and lysozyme treatments were 6.0 and 5.0, respectively. The average pH of the buffered sulfuric acid treatments was 1.6. The chlorine treatment was adjusted to pH 6.0 using 1N HCl.

Furthermore, cleaning and sanitation procedures were conducted in between treatments. After the application of chlorinated foam cleaner (Soft JamCo., Alta Loma, Ca), the chiller was scrubbed and rinsed. BioQuat™ 20 disinfectant sanitizer (HACCO, Inc., Randolph, Wi) was then applied, allowed to set for 20 min, and followed up with a final rinse step. Sanitation procedures were verified using BBL™ RODAC™ plates (Becton, Dickinson, and Co., Franklin Lakes, NJ) containing XLT4 agar and plates containing Campy- Cefex agar at 4 different locations within the Finishing Chiller®.

After exiting the Finishing Chiller®, individual birds were placed into a sterile rinse bag. The USDA whole-carcass rinse method described in the *Microbiological Laboratory Guidebook* was used for bacterial sampling, detection, and enumeration (USDA-FSIS, 2004). However, carcasses were rinsed with 200 mL of buffered peptone water for 1 min instead of rinsing with 400 mL. A reduction in the volume of rinsate has not been shown to impact the recovery of *Salmonella* (Cox et al., 1980) and has been reported to enhance the recovery of *Campylobacter* (Bailey and Berrang, 2007) in carcass rinse methods. The rinsate was transferred into the respective container and stored on ice until subsequent analysis. In order to inactivate residual oxidative activity in the rinsate, 0.1 % sodium thiosulfate was added to the buffered peptone water solution for the peracetic acid and chlorine treatments (Kemp and Schneider, 2000). For lysozyme neutralization, 0.05% sodium polyanethanol sulfonate (SPS) was added to the solution (Traub and Fukushima, 1978).

Campylobacter **Inoculum Preparation**

Tubes containing 10 mL of Brucella-FBP broth were inoculated with one milliliter of *C. jejuni* and incubated for 48 h at 42°C in a Anaero-Jar (Oxoid, Ogdensburg, NY) containing a CampyGen™ sachet (Oxoid) to generate a microaerophilic mixture of 5% O_2, 10% CO_2, and 85% N_2. The culture was streaked for isolation onto Campy-Cefex agar (Stern et al., 1992) and incubated at 42°C for 48 h in microaerophilic conditions as previously described. *Campylobacter jejuni* was removed from the agar and added to buffered peptone water. A stock culture of 10^6cells/mL was prepared. *Campylobacter* cultures were confirmed via phase contrast microscopy.

***Salmonella* Inoculum Preparation**

One milliliter of frozen nalidixic acid-resistant strain of *Salmonella* Typhimurium was added to 10 mL of tryptic soy broth and incubated at 37°C for 24 h. *Salmonella* culture was streaked onto xylose lysine tergitol 4 agar (XLT4, Acumedia Manufactures Inc., Baltimore, MD) containing 35 µL/mL of nalidixic acid. The plates were incubated at 37°C for 24 h. Isolated black colonies were picked from the XLT4 plates and tryptic soy broth tubes were inoculated with 1 colony per tube. The tubes were incubated for 24 h at 37°C and a stock culture of 10^6 cells/mL of *Salmonella* Typhimurium was prepared. Since an antibiotic resistant *Salmonella* marker strain was used, *Salmonella* colonies were confirmed by characteristic growth on XLT4 incorporated with antibiotic. Inoculum levels were verified through direct plating.

Enumeration of *Salmonella* and *Campylobacter*

Enumeration of inoculated samples was done through direct plating. Serial dilutions were prepared using buffered peptone water after carcasses were sampled. The spread plate method was used for enumeration of *Salmonella* onto XLT4 agar (Acumedia) containing nalidixic acid (35 µL; Sigma, St. Louis, MO). The spread plate method involves adding 0.1 mL of sample from the appropriate dilution onto sterile pre-poured XLT4 media and spreading using a sterile plastic disposable spreader. After plates were dried, they were inverted and incubated at 37°C for 24 h. Bacterial populations were converted to log values with the 200 mL of carcass rinsate representing the sample. Results are therefore reported as log colony forming units per sample.

Serial dilutions of *Campylobacter* samples were likewise prepared and plated using the spread plate method onto Campy-Cefex agar (Stern et al., 1992) in duplicate.

Plates were incubated for 42°C for 48 h in AnaeroPack rectangular jars (Mitsubishi Gas Chemical America, Tokyo, Japan) in a microaerophilic environment of 5% O_2, 10% CO_2, and 75% N_2, created by CampyGen sachets (Oxoid). Populations were once more converted to log values with 200 mL of carcass rinsate representing the sample, and results were reported as log colony-forming units per sample.

Quality Determination

A total of 70 broilers were conventionally processed (10 carcasses X 7 treatments) at the Auburn University Poultry Science Research Unit. In particular, the broilers were first hung on shackles and electrically stunned (50 V, 20 mA, 400 Hz) via 1% saline stunner bath (custom built, 1.52 m) with a metal plate along the bottom assembled to an electrical stun control box (model 901-1001IA, Georator Corp., Manassas, VA). After the birds were stunned, they were exsanguinated through a unilateral neck cut followed by a 90-s bleed out. The birds were then scalded in a 2.44-m-long single-pass steam-injected scalder (custom built, Cantrell, Gainesville, GA), defeathered in a 1.22-m-long disk-picker (custom built, Meyn, Oostzaan, the Netherlands), eviscerated (Meyn) and rinsed. Carcasses were chilled at 4°C for 2 h. Broiler carcasses were subsequently divided among the 7 finishing chill water treatments. The following treatments were added to the Finishing Chiller®: 0.004% chlorine, 0.04% PAA, 0.1% PAA, 0.003% buffered sulfuric acid, 0.01% buffered sulfuric acid, 0.1% Lysozyme, and 0.5% Lysozyme. After treatment in the Finishing Chiller®, birds were vacuum packaged according to treatment and stored at 4°C overnight.

An average of 3 color measurements from each carcass was recorded using a Minolta Colorimeter (model DP-301, Minolta Corp., Ramsey, NJ) prior to deboning.

Measurements were taken on the distal side of the breast fillet (skin side). Values were measured using the Hunter L* a* b* color system in which greater L* values designate a sample that is lighter in color. Similarly, larger a* values represent a sample that has more redness, and greater b* values signify a sample that is more yellow in color.

For the sensory analysis, breast fillets were deboned the morning of the panels. Evaluation of sensory attributes was conducted in duplicate (1 panel in the morning and 1 panel in the afternoon) with untrained panelists in the Department of Poultry Science. . Institutional Review Board (IRB) approval was obtained prior to conducting sensory analysis. Thirty panelists participated at each sampling opportunity. In the morning and afternoon of sensory evaluation, fillets were baked to an internal temperature of 76°C in covered aluminum pans with wire racks in a convection oven (Viking Professional Series, VESC Series, Greenwood, MS) set at 177°C. The samples remained in a warmer at 93°C (<1 h) until they were served to panelists. The cooked fillets were prepared as 2 X 2 cm cubes and placed into plastic containers labeled with pre-assigned random 3-digit numbers. One sample at a time was served to panelists, and a modified 8-point hedonic scale was used to rate each sample. The hedonic scale included the aspects of appearance (where 8= like extremely; 1=dislike extremely), texture (where 8= extremely tender; 1= extremely tough), flavor (like to dislike), juiciness (where 8= extremely juicy; 1= extremely dry), and overall acceptability (like to dislike).

Statistical Analysis

Two replicates were conducted for the experiment. Bacterial counts were converted to log colony-forming units per sample with 200 ml of carcass rinse solution representing the sample. Because 0 cannot be directly analyzed with the statistical model, 0.5 $\log_{10}$ CFU was used for statistical analysis (McKee et al., 2008). The data were

analyzed in a 2 x 2 factorial arrangement of antimicrobial treatment and trial. All data were reported as least square means with standard errors and analyzed using the General Linear Model of SAS (SAS Institute, 2003). Significance was reported by P values of $\leq$ 0.05.

References

Bailey, J. S., and M. Berrang. 2007. Effect of rinse volume and sample time on recovery of Salmonella, Campylobacter, Escherichia coli, and Enterobacteriaceae from post-chill chicken carcasses. IAFP Annual Meeting Proceedings. International Association of Food Protection, Des Moines, IA.

Cox, N. A., J. S. Bailey, and J. E. Thomson. 1980. Effect of rinse volume recovery of Salmonella from broiler carcasses. Poult. Sci. Symp. 59:1560. (Abstr.)

Kemp, G. K., and K. R. Schneider. 2000. Validation of thiosulfate for neutralization of acidified sodium chlorite in microbiological testing. Poult. Sci. 79:1857-1860.

McKee, S. R., J. C. Townsend, and S. F. Bilgili. 2008. Use of a scald additive to reduce levels of *Salmonella* Typhimurium during poultry processing. Poult. Sci.87:1672-1677.

SAS Institute. 2003. SAS/STAT® Guide for Personal Computers, Version 9.2 ed. SAS Institute Inc., Cary, NC.

Stern, N. J., B. Wojton, and K. Kwiatek. 1992. A differential-selective medium and dry ice-generated atmosphere for recovery of *Campylobacter jejuni*. J. Food Prot. 55:515-517.

Traub, W. H., and P. I. Fukushima. 1978. Neutralization of human serum lysozyme by sodium polyethol sulfonate but not sodium amylosulfate. J. Clin. Micro. 8(3):306-312.

USDA-FSIS. 2004. Isolation and Identification of Salmonella from Meat, Poultry, and Egg Products. Microbiological Laboratory Guidebook. MLG 4.03. http://www.fsis.usda.gov/science/microbiological_lab_guidebook/index.asp Accessed November 17, 2011.

CHAPTER IV.

RESULTS AND DISCUSSION

In recent years, methods for reducing pathogens during poultry processing have advanced with innovations in technology. Historically, antimicrobial application was predominantly in the primary chiller, but the introduction of new equipment such as a Finishing Chiller® provides a point of application where various antimicrobials can be added using smaller volumes of water (McKee, 2011). This application is more cost effective and advantageous for processors since the primary chiller holds up to 20-50,000 gallons of water, and carcasses remain in the chiller for 1.5-2.0 hours (McKee, 2011). A Finishing Chiller® has volume ranges from 400-600 gallons (Morris & Associates, Garner, NC), while other post-chill dip tanks have a wider volume range depending on size. Utilizing a Finishing Chiller® paired with a short dwell time of generally less than 30 s, a great deal of expense can be saved through the small footprint and cost of antimicrobials. When used in combination with other interventions throughout the plant, utilizing a Finishing Chiller® for antimicrobial application introduces a "multi-hurdle" approach for pathogen reduction. While the efficacy of antimicrobials against *Salmonella* and *Campylobacter* has been validated primarily in chillers, this study provides validation results for a Finishing Chiller®.

When PAA was added to the Finishing Chiller® make-up water, the two concentrations of PAA tested (0.04%, 0.1%) showed better reduction in *Salmonella* Typhimurium on broiler carcasses compared with carcasses treated with 0.004% chlorine, 0.003%, 0.01% buffered sulfuric acid, 0.1% and 0.5% lysozyme, resulting in a 2-$\log_{10}$ reduction (Figure 2). Treatment with chorine, inorganic acid, and lysozyme all resulted in less than a 1-$\log_{10}$ reduction on carcasses. The non-inoculated control was below the detection limit of 5 CFU/mL (0.69 $\log_{10}$) of sample, signifying low levels or no background *Salmonella* spp. on the carcasses before the study was initiated.

PAA was likewise effective in reducing *Campylobacter jejuni* at both 0.04% and 0.1% concentrations. Specifically, both levels of PAA resulted in a 2-$\log_{10}$ reduction against *Campylobacter* when compared to controls (Figure 3). Treatment with chlorine (0.004%) and lysozyme (0.1%, 0.5%) resulted in less than a 1-$\log_{10}$ reduction, while acid treatment (0.003%, 0.01%) showed approximately a 1-$\log_{10}$ reduction on broiler carcasses compared to the positive control. The non-inoculated control for *Campylobacter* was also below the detection limit. Although chlorine, inorganic acid, and lysozyme showed some reduction in *Salmonella* and *Campylobacter*, they were found to be less effective than PAA in decreasing the pathogens in the current study. PAA treatment resulted in a 2-$\log_{10}$ reduction in both *Salmonella* and *Campylobacter* on carcasses (Figures 2 and 3).

The efficacy of acids against microorganisms has been reported to vary with concentration, contact time, and temperature (Dickson and Anderson, 1992). Acids destroy bacteria by penetrating and disrupting their cell membrane. The acid molecule then dissociates and as a result, acidifies the cell interior (Marriott, 2006). *Salmonella*

incidence has been reported to be reduced through addition of 0.5-1.0% lactic acid to chiller water (Izat et al., 1989). Furthermore, Rubin (1978), found that lactic and acetic acids demonstrated a synergistic inhibitory effect on *S*. Typhimurium. Although acids can be effective antimicrobials, their use in poultry processing is limited since they have been reported to cause organoleptic effects on raw poultry such as negative flavor and color changes (Blankenship et al., 1990). The inorganic acid treatments tested in this study might have been less effective in reducing *Salmonella* and *Campylobacter* because the carcasses experienced a short dwell time in the Finishing Chiller®.

Acids have been combined with other antimicrobials in order to utilize lower concentrations of organic acid while still ensuring the efficacy of the compound for bacterial reduction. An example of this application is peracetic acid (PAA), a mixture of an organic acid (acetic) and an oxidant (hydrogen peroxide), which in combination, forms an equilibrium in water (Baldry and Fraser, 1988). Studies have suggested greater antimicrobial efficacy when organic acids are used in combination with hydrogen peroxide (Brinez et al., 2006). Treatment with PAA results in strong oxidation of the cell membrane and other cellular components, thereby resulting in cell death (Oyarzabal, 2005). It was reported that utilizing combinations of 1% acetic acid and 3% hydrogen peroxide provide the best reduction in *Escherichia coli*, *Salmonella Wentworth*, and *Listeria innocua* when sprayed on beef carcasses that had previously been inoculated (Bell et al., 1997). A greater than 3-log reduction for each bacterial strain tested was obtained using this combination and was more effective than each antimicrobial used individually. The combination provided a synergistic effect due to combined acidic and oxidizing properties. Moreover, Bauermeister et al. (2008) reported a 91.8% reduction in

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Salmonella and a 43.4% reduction in *Campylobacter* when 85 ppm PAA was used in a chiller. This was compared to a 56.8% and 12.8% reduction, respectively, when 30 ppm chlorine was applied, indicating that PAA is more effective in reducing *Salmonella* and *Campylobacter* on broiler carcasses than chlorine.

Chlorine reacts with water to form the active antimicrobial hypochlorous acid (HOCl). Chemically, chlorine acts as an oxidizing agent. Hypochlorous acid destroys microbial cells by hindering carbohydrate metabolism. This is accomplished through inhibiting glucose oxidation by chlorine-oxidizing sulfhydryl groups of enzymes important to the process (Marriott, 2006). However, the efficacy of chlorine as an antimicrobial is affected by pH, the presence of organic material, and contact time. The efficacy of chlorine for bacterial reduction decreases with increasing pH and organic load (Byrd and McKee, 2005). Chlorine dioxide and 1,3-dibromo-5,5-dimethylhydantoin (bromine) are also oxidizers and are bactericidal through a similar mechanism to that of chlorine, although they are reported to be less affected by pH and organic load (Berg et al., 1986; McNaughton et al.; Byrd and McKee, 2005). For this study, the Finishing Chiller® make-up water was clean and free of organic matter, and the chlorine was at an optimal pH of 6. Therefore, chlorine may have been less effective in reducing *Salmonella* and *Campylobacter* in the current study due to the short contact time experienced in the Finishing Chiller®. For chlorine to be effective, a contact time of 1 to 1.5 hours is typically required (McKee, 2011). Chlorine was generally regarded as the industry standard for chiller applications in previous years, but has since been surpassed by the use of PAA (McKee, 2011). Because it has been historically used in chiller applications, it served as the point of comparison for antimicrobial efficacy. Although chlorine has

been shown to reduce levels of *Salmonella* and *Campylobacter*, the current study

indicates that it is less effective (P<0.05) than PAA in decreasing *Salmonella* and

Campylobacter on carcasses (Figures 2 and 3) for this application.

Egg white lysozyme has been utilized in practical applications in the food and

pharmaceutical industries for years (Wasserfall and Teuber, 1979), although it is not

indicated by processors as being among the main antimicrobials used in chilling and post-

chill applications in poultry (McKee, 2011). However, many processors are continually

searching for more natural alternatives for bacterial reduction in the plant. Use of egg

white lysozyme provides a more natural alternative to other antimicrobials since it is an

intrinsic component of foods of animal origin such as eggs and milk. Lysozyme has

natural antibacterial activity because of its ability to lyse certain bacteria. It catalyzes the

hydrolysis of the β-1, 4-glycosidic linkages between *N*-acetylglucosamine and *N*-

acetylmuramic acid of the peptidoglycan layer in the bacterial cell wall (Lesenierowskie

et al., 2004). Because lysozyme is specific in its activity against bacterial cell walls, it

exhibits strong antibacterial potential, mainly among Gram-positive bacteria. Gram-

negative bacteria, however, are less susceptible to lysozyme activity (Wasserfall and

Teuber, 1979). A synergistic relationship has been identified between lysozyme and other

preservatives such as organic acids, resulting in improved bacteriostatic activity against a

broad range of bacteria. Chen et al. (2011) reported that the enzymatic activity of

lysozyme modified with various concentrations of lactic acid maintained consistent

antibacterial activity against *Bacillus cereus*, *Escherichia coli*, and *Salmonella*

Typhimurium. In this study, treatment with lysozyme in the Finishing Chiller® resulted

in close to a 1-$\log_{10}$ reduction of *Salmonella* and *Campylobacter* on poultry carcasses

(Figures 2 and 3). It is likely that the short dwell time experienced in the Finishing Chiller® was not long enough for lysozyme to be effective.

The other objective of this study was to evaluate the quality of carcasses treated with the various antimicrobials in order to determine the optimal concentration and antimicrobial application for product safety while preserving product quality. It is important to determine the quality aspects of carcasses treated with antimicrobials since antimicrobial treatment can influence the organoleptic properties of a product. For example, carcasses treated with higher concentrations of chlorine have been associated with discoloration, off-odor, and off-flavor. However, chlorine is typically used at levels of 20-50 ppm and is permitted at levels of up to 50 ppm in carcass wash applications and chiller make-up water (USDA-FSIS, 2003). Furthermore, Bilgili et al. (1998) observed darkening (L*) and yellowing (b*) on broiler skin when organic acids were used as antimicrobials on carcasses. Therefore, acids have been combined with other antimicrobials in order to utilize lower levels of organic acid while still ensuring the efficacy of the compound for bacterial reduction. PAA is an example of this approach. Bauermeister et al. (2008) reported no associated quality defects when carcasses were treated with PAA in the primary chiller, and that levels of 150-200 ppm may extend product shelf-life. However, poultry plant personnel have reported yield loss and/or graying of wing tips observed in carcasses treated with higher concentrations of PAA in the primary chiller (Anonymous, 2012). These described problems have not been observed with PAA used with higher concentrations at the short dwell time experienced in a Finishing Chiller® as determined by this study. In addition, the natural enzymatic activity of lysozyme may be beneficial to sensory properties since good sensory quality

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was reported when various concentrations of lactic-acid induced gelled egg white powder was utilized as a protein additive or natural bacteriostatic agent in sausage and hamburger products (Chen et al, 2004; Wang et al., 2005).

In this particular study, sensory attributes of non-inoculated chicken breast meat were evaluated using a modified 8-point hedonic scale after treatment in the Finishing Chiller® and 24 h of storage at 4° C. The hedonic scale included the aspects of appearance (where 8= like extremely; 1=dislike extremely), texture (where 8= extremely tender; 1= extremely tough), flavor (like to dislike), juiciness (where 8= extremely juicy; 1= extremely dry), and overall acceptability (like to dislike). Since chlorine has historically been a common antimicrobial application used to treat poultry, the 0.004% chlorine treatment served as the control for sensory. For the attribute of appearance, there were no significant (P<0.05) differences between 0.04% PAA, 0.1% PAA, 0.003% acid, 0.01% acid, 0.1% lysozyme, or 0.5% lysozyme when compared to the chlorine control (Table 2). The appearance of all of the treatments were rated as "like slightly." Likewise, panelists were not able to determine differences between the various treatments for the attributes of flavor (Table 3). Flavor of all the treatments were rated by panelists as "like slightly." For sensory texture evaluation, texture of the 0.1% PAA and the 0.01% acid treatment were perceived as more tender by panelists (Table 4). This is possibly due to denaturation of the meat proteins that is commonly seen with breast meat that has been treated with acids (Alvarado and McKee, 2007). Although the higher concentrations of PAA and acid were perceived as more tender, the other treatments were still identified as "slightly tender." Moreover, when panelists evaluated juiciness of the breast fillets, the acid treatments (0.003%, 0.01%) were perceived as being juicier (Table 5), although all

treatments were rated as "slightly moist." In addition, overall acceptability of chicken breast meat from the various treatments was evaluated by panelists and they were unable to determine any significant (P<0.05) differences between the samples (Table 6). All of the treatments were rated as "like slightly."

In addition, color measurements of the breast skin portion of carcasses from each antimicrobial treatment were taken after birds were dipped into the Finishing Chiller®. The chlorine treatment also served as the control for color measurement. There were no differences observed between the various treatments except that the acid treatments (0.003%, 0.01%) had higher yellowness (b*) values (Table 7). This is consistent with results reported by Bilgili et al. (1998) which indicated that acids can have a yellowing (b*) effect on broiler breast skin. However, these color measurements were not part of the experimental design and were not statistically analyzed.

In the current study, none of the antimicrobial treatments exhibited any deleterious impacts on the quality of broiler carcasses. Results from this research indicate that utilizing PAA in a Finishing Chiller® is an effective intervention strategy for reduction of *Salmonella* and *Campylobacter* on poultry carcasses. The optimal concentration of PAA in a Finishing Chiller® is less than 0.1% and around 0.04% as determined by this study. In addition, higher concentrations of PAA at a short contact time do not compromise the organoleptic properties of the product.

References

Alvarado, C. and S. McKee. 2007. Marination to improve functional properties and safety of poultry meat. J. Appl. Poult. Res. 16:113-120.

Anonymous. 2012. Graying of carcasses observed with peracetic acid treatment in the chiller. Alabama poultry processing plant personnel.

Baldry, M. G. C., and J. A. L. Fraser. 1988. Disinfection with peroxygens. In Industrial Biocides. Reports on Applied Chemistry. K. R. Payne, ed. John Wiley and Sons, Hoboken, NJ.

Bauermeister, L. J., J. W. J. Bowers, J. C. Townsend, and S. R. McKee. 2008. Validating the efficacy of peracetic acid mixture as an antimicrobial in poultry chillers. J. Food Prot. 71:119-122.

Bauermeister, L. J., J. W. J. Bowers, J. C. Townsend, and S. R. McKee. 2008. The microbial and quality properties of poultry carcasses treated with peracetic acid as an antimicrobial treatment. Poul. Sci. 87:2390-2398.

Bell, K. Y., C. N. Cutter, and S. S. Sumner. 1997. Reduction of foodborne micro-organisms on beef carcass tissue using acetic acid, sodium bicarbonate, and hydrogen peroxide spray washes. Food Microbiol. 14-439-448.

Berg, J. D., P. V. Roberts, A. Matin. 1986. Effect of chlorine dioxide on selected membrane functions of *Escherichia coli*. J. Appl. Bacteriol. 60: 213-220.

Bilgili, S. F., D. E. Connor, J. L. Pinion, and K. C. Tamblyn. 1998. Broiler skin as affected by organic acids: influence of concentration and method of application. Poul. Sci. 77:751-757.

Blankenship, L. C., B. G. Lyon, A. D. Whittemore, and C. E. Lyon. 1990. Efficacy of acid treatment plus freezing to destroy *Salmonella* contaminants of spice-coated chicken fajita meat. Poult. Sci. 69(Suppl. 1):20. (Abstract)

Brinez, W. J., A. X. Roig-Sagues, M. M. Hernandez Herrero, T. Lopez-Pedemonte, and B. Guamis. 2006. Bactericidal efficacy of peracetic acid in combination with hydrogen peroxide against pathogenic and non pathogenic strains of Staphylococcus spp., Listeria spp. and Escherichia coli. Food Contr. 17:516-521.

Byrd, J. A. and S. R. McKee. 2005. Improving slaughter and processing technologies. Pages 310-327 in Food Safety Control in the Poultry Industry. G.C. Mead, ed., CRCPress, LLC, Boca Raton, FL.

Chen, S.- L., Y.- M. Weng, J.- J. Huang, and K.- J. Lin. 2011. Physiochemical characteristics and bacteriostatic ability of modified lysozyme from lactic acid-

induced gelled egg white powder. J. Food Processing and Preservation. 1745-4549.

Dickson, J. S. and M. E. Anderson, 1992. Microbiological decontamination of food animal carcasses by washing and sanitizing systems: A review. J. Food Prot. 55:133-140.

Izat, A. L., M. Colbert, M. H. Adams, M. A. Reiber, and P. W. Waldroup. 1989. Production and processing studies to reduce the incidence of *Salmonella* on commercial broilers. J. Food Prot. 52:670.

Lesenierowskie, G., C. R. Radziejewska, and J. Kijowski. 2004. Thermally and chemical-thermally modified lysozyme and its bacteriostatic activity.Worlds Poult. Sci. J. 60:303–309.

Marriott, N. G. and R. B. Gravani. 2006. Principles of Food Sanitation. Springer Science+Business Media, Inc, New York, NY.

McKee, S. R. 2011. *Salmonella* and *Campylobacter* control during poultry processing. International Poultry Scientific Forum. Atlanta, Georgia. January 24-25, 2011.

McNaughton, J. L., M. S. Roberts, R. W. Kuhlmeier, G. M. Ricks & E. W. Liimatta. Efficacy of DBDMH (1,3-Dibromo-5,5-dimethylhydantoin) as a Biocide for Poultry Chill Tank Water & Carcass Bacteria Populations. Data on file. Elanco Reference No. 2318.

Oyarzabal, O. A. 2005. Reduction of *campylobacter* spp. by commercial antimicrobials applied during the processing of broiler chickens: a review from the united states perspective. J. Food Prot. 68(8):1752-1760.

Rubin, H. E. Toxicological model for a two-acid system. 1978. Appl. Environ. Microbiol., 36: 623.

Russell, S. M. 2010. *Salmonella* reduction calls for multi-hurdle approach. WATT *PoultryUSA*, June 2010.

U.S. Department of Agriculture, Food Safety and Inspection Service. 2011. Safe and suitable ingredients used in the production of meat, poultry, and egg products. FSIS Directive 7120.1 Revision 9. Available at: http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7120.1Amend21.pdf. Accessed Nov. 2011.

U.S. Department of Agriculture, Food Safety and Inspection Service. 2003. Use of chlorine to treat poultry chiller water. FSIS Notice 45-03.

Wasserfall, F. and M. Teuber. 1979. Action of egg white lysozyme on *Clostridium tyrobutyricum*. Appl. Environ. Microbiol. 38:197-199.

Table 2. Sensory analysis of the appearance of breast fillets from carcasses treated with various antimicrobials in a Finishing Chiller®

Treatment	Appearance[1]
0.004% Chlorine	5.44[a]
0.04% PAA	5.84[a]
0.1% PAA	5.81[a]
0.003% Acid	5.91[a]
0.001% Acid	5.71[a]
0.1% Lysozyme	5.45[a]
0.5% Lysozyme	5.74[a]

[1]Where 8 = like extremely; 1 = dislike extremely.

[a-c]Means with no common superscript differ significantly (P < 0.05).

Table 3. Sensory analysis of the flavor of breast fillets from carcasses treated with various antimicrobials in a Finishing Chiller®

Treatment	Flavor[1]
0.004% Chlorine	5.09[a]
0.04% PAA	5.50[a]
0.1% PAA	5.39[a]
0.003% Acid	5.09[a]
0.001% Acid	5.37[a]
0.1% Lysozyme	5.17[a]
0.5% Lysozyme	5.23[a]

[1]Where 8 = like extremely; 1 = dislike extremely.

[a-c]Means with no common superscript differ significantly (P < 0.05).

Table 4. Sensory analysis of the texture of breast fillets from carcasses treated with various antimicrobials in a Finishing Chiller®

Treatment	Texture[1]
0.004% Chlorine	5.72^{abc}
0.04% PAA	5.86^{ab}
0.1% PAA	6.16^{a}
0.003% Acid	5.70^{abc}
0.001% Acid	6.03^{a}
0.1% Lysozyme	5.51^{bc}
0.5% Lysozyme	5.22^{c}

[1]Where 8 = extremely tender; 1 = extremely tough.

$^{a-c}$Means with no common superscript differ significantly (P < 0.05).

Table 5. Sensory analysis of the juiciness of breast fillets from carcasses treated with various antimicrobials in a Finishing Chiller®

Treatment	Juiciness[1]
0.004% Chlorine	5.40^{ab}
0.04% PAA	5.38^{ab}
0.1% PAA	5.48^{ab}
0.003% Acid	5.66^{a}
0.001% Acid	5.62^{a}
0.1% Lysozyme	5.13^{ab}
0.5% Lysozyme	4.99^{b}

[1]Where 8 = extremely juicy; 1 = extremely dry.

[a-c]Means with no common superscript differ significantly ($P < 0.05$).

Table 6. Sensory analysis of the overall acceptability of breast fillets from carcasses treated with various antimicrobials in a Finishing Chiller®

Treatment	Overall Acceptability[1]
0.004% Chlorine	5.29[a]
0.04% PAA	5.42[a]
0.1% PAA	5.56[a]
0.003% Acid	5.42[a]
0.001% Acid	5.53[a]
0.1% Lysozyme	5.25[a]
0.5% Lysozyme	5.12[a]

[1]Where 8 = like extremely; 1 = dislike extremely.

[a-c]Means with no common superscript differ significantly ($P < 0.05$).

Table 7. Color of chicken skin on carcasses treated with various antimicrobials in a Finishing Chiller®

Treatment	L*	a*	b*
0.004% Chlorine	73.54	3.89	7.13
0.04% PAA	74.08	2.95	7.23
0.01% PAA	73.59	1.00	7.06
0.003% Acid	71.28	1.91	9.44
0.01% Acid	70.97	2.19	12.87
0.1% Lysozyme	69.79	2.89	5.50
0.5% Lysozyme	73.31	3.82	7.73

n=12

Figure 2. *Salmonella* **Typhimurium recovered from inoculated carcasses (n=200) treated with various antimicrobials in a Finishing Chiller®**
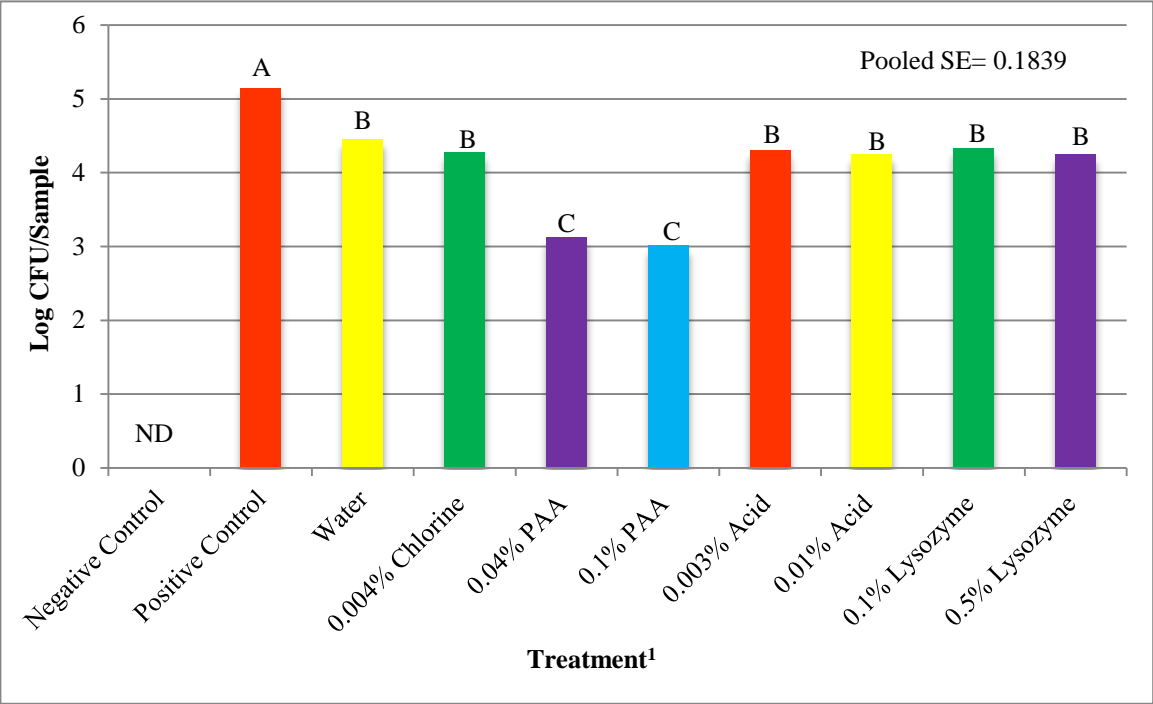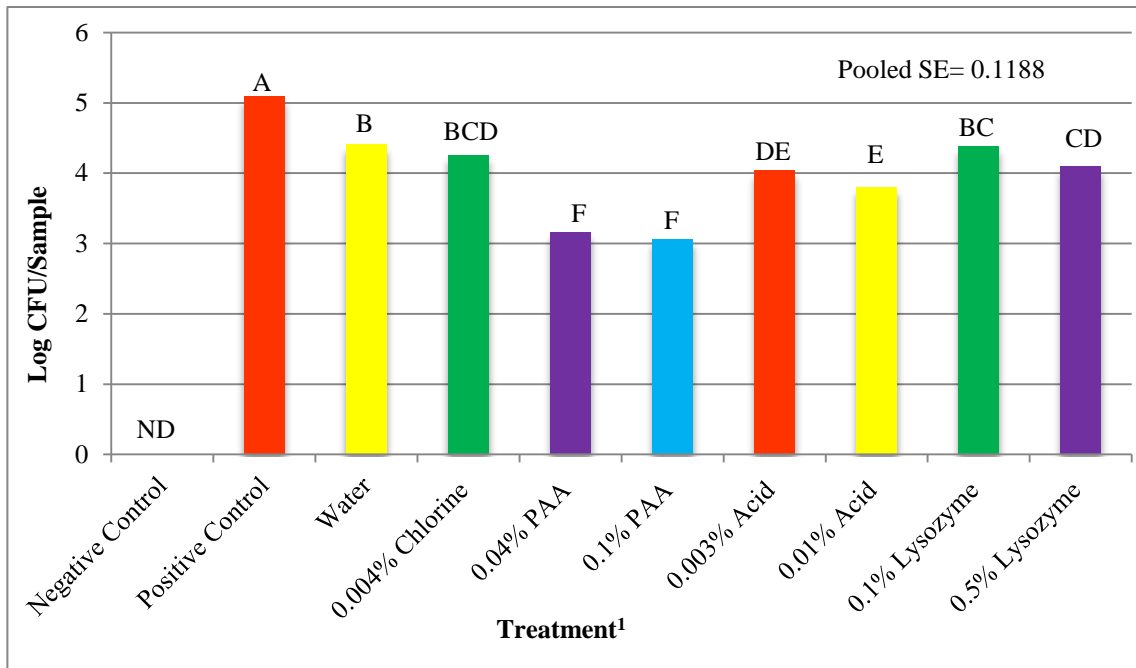

[1]Reported as mean log colony-forming units of *S*. Typhimurium per sample for each treatment group.

ND= Not detectable; $\log_{10}$ CFU< 0.69

[a-c]Means with no common letter differ significantly ($P \leq 0.05$)

Figure 3. *Campylobacter jejuni* **recovered from inoculated carcasses (n=200) treated with various antimicrobials in a Finishing Chiller®**

[1] Reported as mean log colony-forming units of *S*. Typhimurium per sample for each treatment group
ND= Not detectable; $\log_{10}$ CFU< 0.69
[a-c]Means with no common letter differ significantly ($P \leq 0.05$)

REFERENCES

Allen, C. D., D. L. Fletcher, J. K. Northcutt, and S. M. Russell. 1998. The relationship of broiler breast color to meat quality and shelf-life. Poultry Sci. 77:361–366.

Alvarado, C. and S. McKee. 2007. Marination to improve functional properties and safety of poultry meat. J. Appl. Poult. Res. 16:113-120.

Anonymous, 2012. Graying of carcasses observed with peracetic acid treatment in the chiller. Alabama poultry processing plant personnel.

Anonymous, 2010. Compliance guideline for controlling *Salmonella* and *Campylobacter* in Poultry, 3rd edition, May 2010. Available at: http://www.fsis.usda.gov/PDF/Compliance_Guide_Controling_Salmonella_Campylobacter_Poultry_0510.pdf. Accessed Nov. 2011.

Anonymous, 2010. How safe is that chicken? Consumer Reports Magazine. January, 2010. Available at: http://www.consumerreports.org/cro/magazine-archive/2010/january/food/chicken-safety/overview/chicken-safety-ov.htm. Accessed Dec. 2011.

Bai, Y., K. R. Coleman, C. W. Coleman, and A. L. Waldroup. 2007. Effect of cetylpyridinium chloride (cecure® cpc antimicrobial) on the refrigerated shelf life of fresh boneless, skinless broiler thigh meat. Inter. J. Poul. Sci. 6 (2):91-94.

Bailey, J. S., and M. Berrang. 2007. Effect of rinse volume and sample time on recovery of Salmonella, Campylobacter, Escherichia coli, and Enterobacteriaceae from post-chill chicken carcasses. IAFP Annual Meeting Proceedings. International Association of Food Protection, Des Moines, IA.

Bailey, J. S., N. A. Cox, S. E. Craven, and D. E. Cosby. 2002. Serotype tracking of *Salmonella* through integrated broiler chicken operations. J. Food Prot. 65:742-745.

Baldry, M. G. C., and J. A. L. Fraser. 1988. Disinfection with peroxygens. In Industrial Biocides. Reports on Applied Chemistry. K. R. Payne, ed. John Wiley and Sons, Hoboken, NJ.

Barbut, S. 2002. Poultry Products Processing. CRC Press, BocaRaton, FL.

Batz, M. B., S. Hoffman, and J. G. Morris, Jr. 2011. Ranking the risks: the 10 pathogen-

food combinations with the greatest burden on public health. University of Florida, Emerging Pathogens Institute.

Bauermeister, L. J., J. W. J. Bowers, J. C. Townsend, and S. R. McKee. 2008. Validating the efficacy of peracetic acid mixture as an antimicrobial in poultry chillers. J. Food Prot. 71:119-122.

Bauermeister, L. J., J. W. J. Bowers, J. C. Townsend, and S. R. McKee. 2008. The microbial and quality properties of poultry carcasses treated with peracetic acid as an antimicrobial treatment. Poul. Sci. 87:2390-2398.

Bell, K. Y., C. N. Cutter, and S. S. Sumner. 1997. Reduction of foodborne micro-organisms on beef carcass tissue using acetic acid, sodium bicarbonate, and hydrogen peroxide spray washes. Food Microbiol. 14:439-448.

Berg, J. D., P. V. Roberts, A. Matin. 1986. Effect of chlorine dioxide on selected membrane functions of *Escherichia coli*. J. Appl. Bacteriol. 60: 213-220.

Berghaus, R. D., S. G. Thayer, J. J. Maurer, and C. L. Hofacre. 2011. Effect of vaccinating breeder chickens with a killed *Salmonella* vaccine on *Salmonella* prevalences and loads in breeder and broiler chicken flocks. J. Food Prot. 74:727-734.

Berrang, M. E., and J. A. Dickens. 2000. Presence and level of *Campylobacter* spp. on broiler carcasses throughout the processing plant. J Appl Poult Res 9:43-47.

Bilgili, S. F., D. E. Connor, J. L. Pinion, and K. C. Tamblyn. 1998. Broiler skin as affected by organic acids: influence of concentration and method of application. Poul. Sci. 77:751-757.

Bishop, S. C., R. F. E. Axford, F. W. Nicholas, and J. B. Owen. 2010. Breeding for Disease Resistance in Farm Animals. CABI, Cambridge, MA.

Blankenship, L. C., J. S. Bailey, N. A. Cox, N. J. Stern, R. Brewer, and O. Williams. 1993. Two-step mucosal competitive exclusion flora treatment to diminish salmonellae in commercial broiler chickens. Poult. Sci. 72:1667-1672.

Blankenship, L. C., B. G. Lyon, A. D. Whittemore, and C. E. Lyon. 1990. Efficacy of acid treatment plus freezing to destroy *Salmonella* contaminants of spice-coated chicken fajita meat. Poult. Sci. 69(Suppl. 1):20. (Abstract)

Bolisevac, J. M., S. D. Shackelford, R. Fahle, T. Biela, and M. Koohmaraie. 2004. Decreased dosage of acidified sodium chlorite reduces microbial contamination and maintains organoleptic qualities of ground beef products. J. Food Prot. 67:2248-2254.

Brashears, M. M., D. E. Burson, E. S. Dormedy, L. Vavak, S. R. McKee, W. Fluckey, M. Sanchez. 2001. HACCP implementation and validation in small and very small meat and poultry processing plants. Nebraska Dairy, Food and Environmental Sanitation. 21:20-28.

Bryan, F. L. and M. P. Doyle. 1995. Health risk and consequences of *Salmonella* and *Campylobacter jejuni* in raw poultry. J. Food Prot. 58: 326-344.

Bryan, F. L., J. C. Ayres, and A. A. Kraft. 1968. Salmonellae associated with further-processed turkey products. Applied Microbiol. 16:1-9.

Byrd, J. A., B. M. Hargis, D. E. Corrier, R. L. Brewer, D. J. Caldwell, R. H. Bailey, J. L. McReynolds, K. L. Herron, L. H. Stanker. 2002. Flourescent marker for the detection of crop and upper gastrointestinal leakage in poultry processing plants, Poul. Sci. 81:70-74.

Byrd, J. A., D. E. Corrier, M. E. Hume, R. H. Bailey, L. H. Stanker, and B. M. Hargis. 1998. Effect of feed withdrawal on the incidence of *Campylobacter* in crops of preharvest broiler chickens. Avian Diseases 42:802-806.

Byrd, J. A. and S. R. McKee. 2005. Improving slaughter and processing technologies. Pages 310-327 in Food Safety Control in the Poultry Industry. G.C. Mead, ed., CRCPress, LLC, Boca Raton, FL.

Centers for Disease Control and Prevention. 2011. Estimates of Foodborne Illness in the United States. Internet Address: http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html. Accessed Nov. 2011.

Centers for Disease Control and Prevention. 2011. Vital Signs: Incidence and Trends of Infection with Pathogens Transmitted Commonly Through Food – Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 1996-2010. Available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6022a5.htm?s_cid=mm6022a5_w. Accessed Jan. 2012.

Centers for Disease Control and Prevention. 2010. National Center for Emerging and Zoonotic Infectious Diseases. Campylobacter. Internet Address: http://www.cdc.gov/nczved/divisions/dfmd/diseases/campylobacter. Accessed Nov. 2011.

Centers for Disease Control and Prevention. 2010. Salmonella. Internet Address: http://www.cdc.gov/salmonella/general/techinical/html. Accessed Nov. 2011.

Chen, S.- L., Y.- M. Weng, J.- J. Huang, and K.- J. Lin. 2011. Physiochemical characteristics and bacteriostatic ability of modified lysozyme from lactic acid-induced gelled egg white powder. J. Food Processing and Preservation. 1745-4549.

Chen, S. L., T. F. Tseng, C. K. Chou, K. J. Lin. 2004. Manufacturing of non-fermented acid Chinese style semi-dry sausage formulated with acid-induced gelled egg white powder. Taiwanese J. Agric. Chem. Food Sci. 42:293–298.

Corrier, D. E., J. A. Byrd, B. M. Hargis, M. E. Hume, R. H. Bailey, L. H. Stanker. 1999a. Presence of *Salmonella* in the crop and ceca of broiler chickens before and after preslaughter feed withdrawal. Poul. Sci. 78: 45-49.

Corrier, D. E., J. A. Byrd, B. M. Hargis, M. E. Hume, R. H. Bailey, L. H. Stanker. 1999b. Survival of *Salmonella* in the crop contents of market-age broilers during feed withdrawal. Avian Diseases 43:453-460.

Cox, N. A., J. S. Bailey, and J. E. Thomson. 1980. Effect of rinse volume recovery of *Salmonella* from broiler carcasses. Poult. Sci. Symp. 59:1560. (Abstr.)

Dickens, J. A., and A. D. Whittemore. 1997. Effects of acetic acid and hydrogen peroxide application during defeathering on the microbioligical quality of broiler carcasses prior to evisceration. Poul. Sci. 76:657-660.

Dickens, J. A., B. G. Lyon, A. D. Whittemore, and C. E. Lyon. 1994. The effect of an acetic acid dip on carcass appearance, microbiological quality, and cooked breast meat texture and flavor. Poultry Sci. 73:576-581.

Dickson, J. S. and M. E. Anderson, 1992. Microbiological decontamination of food animal carcasses by washing and sanitizing systems: A review. J. Food Prot. 55:133-140.

Fletcher, D. L., M. Qiao, and D. P. Smith. 2000. The relationship of raw broiler breast meat color and pH to cooked meat color and pH. Poul. Sci. 79:784-788.

Gordon, G., G. Kieffer, and D. Rosenblatt. 1972. The chemistry of chlorine dioxide, p. 201-286. *In* S. Lippard (ed.), Progress in inorganic chemistry, vol. 15. Wiley Interscience, New York.

Hafez, H. M. 1999. Poultry meat and food safety: pre- and post-harvest approaches to reduce foodborne pathogens. World's Poult. Sci. J. 55:269-280.

Hargis, B. M., D. J. Caldwell, R. L. Brewer, D. E. Corrier, and J. R. DeLoach. 1995. Evaluation of the chicken crop as a source of *Salmonella* contamination for broiler carcasses. Poul. Sci. 74:1548-1552.

Hecer, C. and M. Guldas. 2011. Effects of lactic acid, fumaric acid and chlorine dioxide on shelf-life of broiler wings during storage. African J. Microbiol Research. 5(23):3880-3883.

Helms, M., J. Simonsen, K. E. Olsen, K. Molbak. 2005. Adverse health events associated with antimicrobial drug resistance in *Campylobacter* species: a

registry-based cohort study. Journal of Infectious Diseases 191:1050–1055.

Heurtin-Le Corre, C., P. Y. Donnio, M. Perrin, M. F. Travert, and J. L. Avril. 1999. Increasing incidence and comparison of nalidixic acid-resistant *Salmonella* enteric subsp. Enteric serotype Typhimurium isolates from humans and animals. J Clin Microbiol. 37(1):266-269.

Huezo, R., D. P. Smith, J. K. Northcutt, and D. L. Fletcher. 2007. Effect of immersion or dry air chilling on broiler carcass moisture retention and breast fillet functionality. J. Appl. Poult. Res. 16:438-447.

Hughey, V. L. and E. A. Johnson. 1987. Antimicrobial activity of lysozyme against bacteria involved in food spoilage and food-borne disease. Appl. Env. Microbiol. 53, 9: 2165-2170.

Humphrey, T., S. O'Brien, and M. Madsen. 2007. Campylobacters as zoonotic pathogens: a food production perspective. J Food Microbiol. 117:237-257.

Izat, A. L., M. Coldberg, R. A. Thomas, M. H. Adams, and C. D. Driggers. 1990. Effects of lactic acid in processing waters on the incidence of *Salmonella* in broilers. J. Food Qual. 13:295-306.

Izat, A. L., M. Colbert, M. H. Adams, M. A. Reiber, P. W. Waldroup. 1989. Production and processing studies to reduce the incidence of *Salmonella* on commercial broilers. J. Food Prot., 52, 670.

Izat A. L., F. A. Gardner, J. H. Denton, F. A. Golan. 1988. Incidence and level of *Campylobacter jejuni* in broiler processing. Poul. Sci. 67:1568-1572.

Jacobs-Reitsma, W. F., U. Lyhs, J. Wagenaar. 2008. *Campylobacter* in the Food Supply. ASM Press, 2008.

Jay, J. M. 2000. Modern Food Microbiology. 6th Ed. Aspen Publishers, Inc. Gaithersburg, MD.

Kemp, G. K., and K. R. Schneider. 2000. Validation of thiosulfate for neutralization of acidified sodium chlorite in microbiological testing. Poul. Sci. 79:1857-1860.

Kemp, G. K., M. L. Aldrich, and A. L. Waldroup. 2000. Acidified sodium chlorite antimicrobial treatment of broiler carcasses. J. Food Prot. 63:1087-1092.

Kross, R. 1984. An innovative demand-release microbiocide. Basis for action and effects of dilution. *In* Second biannual conference on progress in chemical disinfection. SUNY, Binghamton, New York.

Lahellec, C. and P. Colin. 1985. Relationship between serotypes of salmonellae from

hatcheries and rearing farms and those from processed poultry carcasses. Br. Poult. Sci. 26:179-186.

Lawless, H. T. and H. Heymann. Sensory evaluation of food: principles and practices. Springer Science+Business Media, LLC 2010.

Lee, Y. S. 2007. Perception, measurement, and prediction of tenderness in poultry breast meat. Thesis (Ph.D.) University of Arkansas, Fayetteville, 2007.

Lesenierowskie, G., C. R. Radziejewska, J. Kijowski. 2004. Thermally and chemical-thermally modified lysozyme and its bacteriostatic activity.Worlds Poult. Sci. J. 60: 303–309.

Li, Y., M. F. Slavik, J. T. Walker, and H. Xiong. 1997. Prechill spray of chicken carcasses to reduce Salmonella typhimurium. J. Food Sci. 62:605-607.

Lillard, H. S., and J. E. Thomson. 1983. Efficacy of hydrogen peroxide as a bacteriocide in poultry chiller water. J. Food Sci. 48:125-126.

Lillard, H. S. 1979. Levels of chlorine and chlorine dioxide of equivalent bactericidal effect in poultry processing water. J. Food Sci. 44:1594-1597.

Lyon, B. G. and C. E. Lyon. 2001. Meat quality: sensory and instrumental evaluations. Pages 97-120 in Poultry Meat Processing, A. R. Sams, ed., CRC Press LLC, Boca Raton, FL.

Marriott, N. G. and R. B. Gravani. 2006. Principles of Food Sanitation. Springer Science+Business Media, Inc, New York, NY.

McKee, S. R. 2012. Pre-harvest survey for best practices in pathogen control. International Poultry Scientific Forum. Atlanta, Georgia. January 25-26, 2012.

McKee, S. R. 2011. *Salmonella* and *Campylobacter* control during poultry processing. International Poultry Scientific Forum. Atlanta, Georgia. January 24-25, 2011.

McKee, Shelly R. Class lecture. Poultry Further Processing and Products. Auburn University, Auburn, AL. August, 2009.

McKee, S. R., J. C. Townsend, and S. F. Bilgili. 2008. Use of a scald additive to reduce levels of *Salmonella* Typhimurium during poultry processing. Poult. Sci.87:1672-1677.

McNaughton, J. L., M. S. Roberts, R. W. Kuhlmeier, G. M. Ricks & E. W. Liimatta. Efficacy of DBDMH (1,3-Dibromo-5,5-dimethylhydantoin) as a Biocide for Poultry Chill Tank Water & Carcass Bacteria Populations. Data on file. Elanco Reference No. 2318.

Mead, G. C., W. R. Hudson and M. H. Hinton. 1994. Use of a marker organism in poultry processing to identify sites of cross-contamination and evaluate possible measures. Br. Poult. Sci. 35:345-354.

Mead, G. C. 1980. Microbiological control in the processing of chickens and turkeys. Pages 91-104 in Meat Quality in Poultry and Game Birds. G.C Mead and B.M. Freeman, eds., British Poultry Science Ltd, Edinburgh.

Miller, R. G. and C. R. Tate. 1990. XLT4: A highly selective plating medium for the isolation of Salmonella. The Maryland Poultryman, April:2-7.

Mulder, R. W. A. W., M. C. van der Hulst, and N. M. Bolder. 1987. *Salmonella* decontamination of broiler carcasses with lactic acid, l-cysteine, and hydrogen peroxide. Poultry Sci. 66:1555-15557.

"New Performance Standards for *Salmonella* and *Campylobacter* in Chilled Carcasses at Young Chicken and Turkey Slaughter Establishments" 75 FR 27288 (May 14, 2010) p.27288-27294.

Ohl, M. E. and S. I. Miller. 2001. *Salmonella*: A Model for Bacterial Pathogenesis. Annual review of medicine. 52:259-74.

Oosterom J., S. Notermans, H. Karman, G. B. Engels. 1983. Origin and prevalence of *Campylobacter jejuni* in poultry processing. J. Food Prot. 46:339-344.

Oyarzabal, O. A. 2005. Reduction of *Campylobacter* spp. by commercial antimicrobials applied during the processing of broiler chickens: a review from the united states perspective. J. Food Prot. 68(8):1752-1760.

Oyarzabal, O. A., C. Hawk, S. F. Bilgili, C. C. Warf, and G. K. Kemp. 2004. Effects of postchill application of acidified sodium chlorite to control *Campylobacter* spp. and *Escherichia coli* on commercial broiler carcasses. J. Food Prot. 67:2288-2291.

Rollins, D. M. and R. R. Colwell. 1986. Viable but non-culturable stage of *Campylobacter jejuni* and its role in survival in the natural aquatic environment. Applied and Environmental Microbiol. 52:531-538.

Rubin, H. E., Toxicological model for a two-acid system. 1978. Appl. Environ. Microbiol. 36:623.

Russell, S. M. 2010. *Salmonella* reduction calls for multi-hurdle approach. WATT *PoultryUSA*, June 2010.

Sahin, O., N. Luo, S. Huang and Q. Zhang. 2003. Effect of *Campylobacter*-Specific Maternal Antibodies on *Campylobacter* jejuni Colinization in Young Chickens.

Applied and Environmental Microbiology, 69:5372-5379.

Saleha A. A., G. C. Mead, A. L. Ibrahim. 1998. *Campylobacter jejuni* in poultry production and processing in relation to public health. World's Poul. Sci. J. 54:53-58.

Sams, A. R. 2001. Poultry Meat Processing. CRC Press LLC, Boca Raton, FL.

Sanchez, M. X., W. M. Fluckey, M. M. Brashears, and S. R. McKee. 2002. Microbial profile and antibiotic susceptibility of *Campylobacter* spp. and *Salmonella* spp. in broilers processed in air-chilled and immersion-chilled environments. J. Food Prot. 65:948–956.

Sarlin L. L., E. T. Barnhart, D. J. Caldwell, R. W. Moore, J. A. Byrd, D. Y. Caldwell, D. E. Corrier, J. R. DeLoach, B. M. Hargis. 1998. Evaluation of alternative sampling methods for *Salmonella* critical control point determination at broiler processing. Poul. Sci. 77:1253-1257.

SAS Institute. 2003. SAS/STAT® Guide for Personal Computers, Version 9.2 ed. SAS Institute Inc., Cary, NC.

Scharff, R. L. 2011. Economic burden from health losses due to foodborne illness in the United States. J. Food Prot. 75(1):123-131.

Schneider, K. R., G. K. Kemp, M. L. Aldrich. 2002. Antimicrobial treatment of air chilled broiler carcasses. Acidified sodium chlorite antimicrobial treatment of air chilled broiler carcasses. Dairy, Food and Environmental Sanitation. 22:102-108.

Smith, D. P., C. E. Lyon, and D. L. Fletcher. 1988. Comparison of the allo-kramer shear and texture profile methods of broiler breast meat texture analysis. Poul. Sci. 67: 1549-1556.

Smulders, F. 1995. Preservation by microbial decontamination; the surface of treatment of meats by organic acids. In: Gould, G. W. (Ed.), New Methods of Food Preservation. Blackie Academic and Professional, London.

Stern, N. J. 2001. Campylobacter. Guide to Foodborne Pathogens. 5, 61-67.

Stern, N. J., B. Wojton and K. Kwiatek. 1992. A differential selective medium and dry ice generated atmosphere for recovery of *Campylobacter jejuni*. J. Food Prot. 55:514-517.

Stone, H. and J. L. Sidel. 2004. Sensory Evaluation Practices, Third Edition. Academic, San Deigo.

Traub, W. H., and P. I. Fukushima. 1978. Neutralization of human serum lysozyme by

sodium polyethol sulfonate but not sodium amylosulfate. J. Clin. Microbiol. 8(3):306-312.

U.S. Department of Agriculture. 1995. Final rule on pathogen reduction; hazard analysis and critical control points (HACCP) systems. US Dep. Commerce, Washington, DC.

U.S. Department of Agriculture, Food Safety and Inspection Service. 2011. Serotypes profile of *Salmonella* isolates from meat and poultry products January 1998 through December 2010. Available at: http://www.fsis.usda.gov/Science/Serotypes_Profile_Salmonella_Isolates/index.asp. Accessed March, 2011.

U.S. Department of Agriculture, Food Safety and Inspection Service. 2011. New performance standards for salmonella and campylobacter in chilled carcasses at young chicken and turkey slaughter establishments. Available at: http://www.fsis.usda.gov/OPPDE/rdad/FSISNotices/31-11.pdf. Accessed Nov. 2011.

U.S. Department of Agriculture, Food Safety and Inspection Service. 2011. Safe and suitable ingredients used in the production of meat, poultry, and egg products. FSIS Directive 7120.1 Revision 9. Available at: http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7120.1Amend21.pdf. Accessed Nov. 2011.

U.S. Department of Agriculture, Food Safety and Inspection Service. 2003. Use of chlorine to treat poultry chiller water. FSIS Notice 45-03.

Van Schothorst, M., S. Notermans, and E. H. Kampelmacher. 1972. Hygiene in poultry slaughter. Fleischwirttschaft. 52:749-752.

Waldroup, A., C. Coleman and H. Brown. 1999. Pilot plant trials-pre-chill application of cetylpyridinium chloride to broilers. U.S. Food Additive Petition No. 2A4736, Appendix VII. Food and Drug Administration, Department of Health and Human Services. Washington, DC.

Wallis, M. R. 1994. The pathogenesis of *Campylobacter jejuni*. British Journal of Biomedical Science. 51(1):57-64.

Wang, S. C., S. L. Chen, and K. J. Lin. 2005. Manufacturing of ground restructured pork meat patty formulated with acid-induced gelation egg white powder. Taiwan J. Agric. Chem. Food Sci. 43:103–107.

Wasserfall, F., and M. Teuber. 1979. Action of egg white lysozyme on *Clostridium tyrobutyricum*. Appl. Environ. Microbiol. 38:197-199.

World Health Organization, Food and Agriculture Organization of the United Nations. 2002. Risk assessments of *Salmonella* in eggs and broiler chickens. SBN 92 9 156230 7 (WHO) (LC/NLM classification: QW 138.5.S2) ISBN 92 5 104873 8 (FAO) ISSN 1726-5274.

Xiong, H. L., L. Yanbin, M. F. Slavik, and J. T. Walker. 1998. Spraying chicken skin with selected 339 chemicals to reduce *Salmonella* Typhimurium. J. Food Prot. 61:272-275.

Yang, Z., Y. Li., M. Slavik. 1998. Use of antimicrobial spray applied with an inside-outside birdwasher to reduce bacterial contamination on prechilled chicken carcasses. J. Food Prot. 61:829-832.

Young, L. L., and D. P. Smith. 2004. Moisture retention by water and air chilled chicken broilers during processing and cutup operations. Poult. Sci. 83:119–122.