

Evaluation of Poultry Meat Safety and Quality using Peracetic Acid in Poultry Chillers

by

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ABSTRACT

Salmonella spp. and *Campylobacter* spp. continue to be pathogens of concern in poultry processing as they are the leading foodborne illnesses associated with bacteria in foods. In the poultry processing industry, multiple intervention strategies are used in the processing plant to help to reduce and eliminate these pathogens from poultry products. With performance standards becoming more rigid, the control of these organisms has become very important to poultry processors. Peracetic acid (PAA or PHAP) is one of the approved antimicrobials that can be utilized in the immersion chiller used in poultry processing to bring carcass temperatures down to 4 C. Experiments were conducted to validate the efficacy of various levels of PAA (25, 100, 150 and 200 ppm) compared to the more traditional chlorine (30 ppm), in the reduction of *Salmonella* spp. and *Campylobacter* spp. in the poultry processing plant, while also determining the effects of this antimicrobial on product quality and consumer acceptance. After determining which levels of PAA were most effective without changing any organoleptic properties or consumer acceptance, the efficacy of PAA was demonstrated in a commercial poultry processing facility. There were minimal product quality changes in product treated with higher levels of PAA (≥ 150 ppm), however, PAA at higher levels were able to decrease levels of *Salmonella* Typhimurium and *Campylobacter jejuni*, while also demonstrating an extended product shelf-life. When PAA (85 ppm) and chlorine (30 ppm) were used in a commercial processing facility, the prevalence of *Salmonella* spp. between the entrance and exit of the chiller was reduced by 91.8% and 56.8%, respectively. *Campylobacter*

spp. were not reduced as greatly as *Salmonella* spp.; however, PAA still provided a larger reduction in *Campylobacter* spp. (43.4%) when compared to the reduction found when chlorine was used (12.8%). These results indicate that PAA may provide poultry processors with a more effective antimicrobial for use in the immersion chiller, when compared to chlorine, as another hurdle in a multi-hurdle approach to food safety in the poultry processing facility.

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CHAPTER I.

INTRODUCTION AND LITERATURE REVIEW

Salmonella and *Campylobacter* are two major foodborne pathogens in the United States. 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 at \$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 stores were contaminated with *Campylobacter* whereas *Salmonella* was found in 14% and both bacteria were found in 9% of broiler tested (Consumer Reports, Anonymous, 2010). Food safety hazards to consumers are usually a result of poultry meat that has not been cooked thoroughly or via cross-contamination. To help in reducing illness associated with poultry products, updated performance standards issued by USDA FSIS in 2011 require the percentage of *Salmonella* and *Campylobacter* on whole poultry carcasses to be below 7.5% and 10.4% positive, respectively.

Several recalls in recent history have led the United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS) to tighten the performance standards for *Salmonella* spp. and *Campylobacter* spp. in poultry and poultry products. Several of the recent recalls have been highly publicized, including a recall related to ground poultry that occurred in August, 2011, in which 36 million pounds of ground turkey was recalled due to the contamination by a multi-drug resistant strain of *Salmonella* Heidelberg (CDC, 2011b). During this outbreak, 182 people were infected

and 1 case resulted in death. In addition, there have been more recent outbreaks including one which was issued in September 2013 linked to chicken meat, a 13 state outbreak in July of 2013 linked to chicken meat, a 6 state outbreak in November 2011 linked to chicken livers and a 34 state outbreak in August of 2011 linked to ground turkey meat. These outbreaks have resulted in recalls of 23,093 units (approximately 102,635 pounds) of rotisserie chicken, an undetermined amount of chicken livers and a recall of 36 million pounds of ground turkey. As of January 11, 2014, Tyson Foods, Inc. issued a recall of 33,840 pounds of mechanically separated chicken due to an outbreak of *Salmonella* Heidelberg originating at a Tennessee plant. From this recall, 9 persons have contracted Salmonellosis and all 9 were hospitalized. There have been no deaths reported from this outbreak. However, this particular strain of *Salmonella* Heidelberg has been reported to Pulse-Net and has appeared in 12 different states. There is currently an ongoing investigation in order to determine if this recall can be linked to several different recalls involving the same *S. Heidelberg* strain (CDC, 2014). Infection with poultry-borne pathogens such as *Salmonella* and *Campylobacter* presents a serious economic burden in addition to the impacts of illness and loss of life.

LITERATURE REVIEW

Two food-borne pathogens that are most often associated with ready-to-cook poultry meat are *Salmonella* spp. and *Campylobacter* spp. In total, they are responsible for approximately 20% of domestic foodborne illnesses, 50% of foodborne illnesses resulting in hospitalization and 34% of relevant fatal cases, respectively (CDC, 2011a). Contaminated raw or undercooked poultry and poultry products are considered to be the primary food source of transmission for *Salmonella* and *Campylobacter* to humans. 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 Foodborne Diseases Active Surveillance Network (FoodNet) case rate data from 2012 shows that the incidence of infection with *Salmonella* has not changed significantly since 2006-2008 but the incidence of some serotypes of *Salmonella* has increased. Among 92% *Salmonella* isolates serotyped, the most common serotypes implicated in infection were Enteritidis (22%), Newport (14%), and Typhimurium (13%). Furthermore, FoodNet data shows that infection with *Campylobacter* has increased 14% since 2006-2008, reaching 14.3 cases per 100,000 people in 2012. These data emphasize the importance of continuing to identify and address areas where improvements in food safety may be made. Moreover, the Raw Chicken Parts Baseline Survey (RCBS) was conducted by USDA FSIS from January 2012 to August 2012 with the intent to establish microbiological criteria for anticipated industry performance standards. The estimated national prevalence of *Campylobacter* in chicken parts is 21.7% and 24.02% for *Salmonella*. Currently, baseline approaches are under consideration for ground product as well.

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 28. Because *Salmonella*

spp. and *Campylobacter* spp. continue to be major causes of foodborne illness in the U. S., USDA regulations have become more rigid, making it crucial for processors to examine new or additional intervention strategies for effective pathogen control. Historically, antimicrobial application to reduce bacterial cross-contamination on carcasses during poultry processing has been focused in the primary immersion chiller. However, this is an expensive proposition due to the amount of water being treated (20,000-50,000 gallons) and longer dwell times for antimicrobials to be effective (1.5-2 hours). In recent years, novel intervention strategies such as post-chill decontamination tanks have provided alternative approaches for pathogen reduction during poultry processing as part of a multi-hurdle intervention approach.

Based on an industry survey conducted by McKee (2011), the most prevalent chemical used in post-chill applications was peracetic acid (PAA) while cetylpyridinium chloride (CPC) was the predominant post-chill antimicrobial when drench cabinets were used. In previous years, chlorine has been a major antimicrobial used to reduce pathogens on raw poultry but has since been surpassed by the use of PAA for several reasons. The efficacy of chlorine as an antimicrobial is affected by pH, the presence of organic material, and contact time (Byrd and McKee, 2005; Nagel et al., 2013). In addition, Russia, a large export market for domestic poultry meat prohibits the use of chlorine as an antimicrobial treatment in poultry, although PAA is more widely accepted. This is important since Russia still requires poultry imported from the U. S. to meet the demands of its consumers. Tamblyn and Conner (1997) found that chlorine levels must be at least 0.04% (400 ppm) to effectively reduce the attached *Salmonella* on broilers, which is not allowed according to current regulations. Furthermore, Bauermeister et al.

(2008) reported a much better reduction in *Salmonella* and *Campylobacter* prevalence on broiler carcasses when 85 ppm PAA was used over 30 ppm chlorine in a primary chiller. It has also been demonstrated that using PAA at concentrations of 0.4-0.1% produced the best reduction of *Salmonella* and *Campylobacter* levels on broiler carcasses when used in a post-chill decontamination tank for approximately 20 s (Nagel et al., 2013). Validation of the antimicrobials is extremely important because their efficacy is affected by contact time, concentration and coverage. Additionally, 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 microbial loads without having deleterious impacts on quality characteristics (Humphrey et al., 1981). 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.

DEVELOPMENT OF CURRENT REGULATIONS IN POULTRY PROCESSING

The USDA has regulated the production of meat and poultry products since 1906 with the first regulations regarding food safety process controls, sanitation standard operating procedures (SSOPs) being established in 1968 (Ollinger and Taha, 2015). In July of 1996, the US government expanded their regulatory authority by issuance of the Pathogen Reduction; Hazard Analysis and Critical Control Points (PR/HACCP) systems final rule. Not only did this rule require meat and poultry processors to develop and follow a HACCP program that was approved by FSIS inspectors for products produced in their facilities, but it issued the first *Salmonella* performance standards for the poultry

industry. The performance standards issued in these final rules were based on baseline studies completed before the implementation of the HACCP rule and poultry processors were required to have less than a 20% incidence of *Salmonella* on whole carcass products or no more than 11 positive samples in a set of 51 samples (USDA FSIS, 1996).

In January of 2000, the United States Department of Health and Human Services (USDHHS) launched the Healthy People (HP) 2010 campaign. Among the many objectives in this program was to reduce the incidence of Foodborne infections (USDHHS, 2000). While many of the objectives of the HP 2010 campaign were met, the goals for *Campylobacter* were not met. The number of cases per 100,000 people were reduced from 24.6 in 1997 to 12.9 in 2009; however, the target set forth in the HP 2010 initiative was 12.3. Furthermore, the target for *Salmonella* was to reduce the baseline case rate by approximately 50%. The baseline case rate per 100,000 people for *Salmonella* in 1997 was 13.6 and by 2009 the rate had risen to 15.19, so not only was the target not met, but the number of cases increased (

Table 1). Moving forward, in December of 2010, the USDHHS established new objectives for the HP 2020 initiative (Table 2). Again these objectives included the reduction of key pathogens that are transmitted through all food products, including *Campylobacter* and *Salmonella*. The baseline data was taken from averages in reported cases per 100,000 people from 2006-2008 in the FoodNet database. The targets set for *Campylobacter* and *Salmonella* are to reduce their presence from 12.7 to 8.5 cases per 100,000 people and 15.0 to 11.4 cases per 100,000 people, respectively (USDHHS, 2010).

Table 1. Healthy People 2010 progress toward target attainment for foodborne infections (National Center for Health Statistics, 2012).

Objective	Percent of targeted change achieved ²	2010 Target	Baseline (Year)	Final (Year)	Baseline vs. Final			
					Difference ³	Statistically Significant ⁴	Percent Change ⁵	
10-1. Foodborne infections (per 100,000 population)								
a. <i>Campylobacter</i> species	95.1%	12.3	24.6 (1997)	12.9 (2009)	-11.7	Not tested	-47.6%	
b. <i>Escherichia coli</i> O157:H7	100.0%	1.0	2.1 (1997)	1.0 (2009)	-1.1	Not tested	-52.4%	
c. <i>Listeria monocytogenes</i>	73.9%	0.24	0.47 (1997)	0.30 (2009)	-0.17	Not tested	-36.2%	
d. <i>Salmonella</i> species		6.8	13.6 (1997)	15.0 (2009)	1.4	Not tested	10.3%	
f. Cases of postdiarrheal hemolytic uremic syndrome (HUS) (<5 years)		0.90	1.80 (2000)	2.03 (2006)	0.23	Not tested	12.8%	

¹ Movement away from target is not quantified using the percent of targeted change achieved.

² Percent of targeted change achieved = [(Final value – Baseline value) / (Healthy People 2010 target – Baseline value)] × 100.

³ Difference = Final value – Baseline value. Differences between percent's (%) are measured in percentage points.

⁴ When estimates of variability are available, the statistical significance of the difference between the final value and the baseline value is assessed at the 0.05 level.

⁵ Percent change = [(Final value – Baseline value) / Baseline value] × 100.

Table 2. Healthy People 2020 targets and progress for foodborne illnesses from all food sources (USDHHS, 2010; Crim, 2015).

Foodborne Illness	2006-2008 Baseline Case Rate ¹	Healthy People 2010 Target ²	2006-2014 Baseline Case Rate ³	Healthy People 2020 Target ²
<i>Campylobacter</i> species	12.7	12.3	13.5	8.5
<i>Escherichia coli</i> (STEC) O157:H7	1.2	1.0	0.92	0.6
<i>Listeria monocytogenes</i>	0.3	0.24	0.24	0.2
<i>Salmonella</i> species	15.0	6.8	15.45	11.4
Cases of postdiarrheal hemolytic uremic syndrome (HUS) (< 5 years)	2.0	0.90	N/A ⁴	1.0

¹FoodNet Data, cases per 100,000 people

²Cases per 100,000 people

³Estimated FoodNet Data, cases per 100,000 people

⁴No information available

To help reduce the number of foodborne illnesses associated with *Salmonella* and *Campylobacter*, the FSIS published new performance standards for *Salmonella* and *Campylobacter*, in 2010 and 2011, respectively, for young chicken and turkey slaughter facilities. This is the first time that the FSIS has established performance standards for *Campylobacter* and identified it as part of the pathogen reduction initiative. In these new performance standards for broilers, the maximum percent positive allowed was 7.5% (≤ 5 positive out of 51 samples) and 10.4% (≤ 8 positive out of 51 samples) for *Salmonella* and *Campylobacter*, respectively using a moving window (Table 3; FSIS Directive 10,250.1). In August 2014 (FRN Docket No. FSIS-2011-0012), the FSIS issued the final rule for the Modernization of Poultry Slaughter Inspection for a variety of reasons, including the facilitation of pathogen reduction in poultry products. *Salmonella* pathogen reduction performance standards were not included in the Modernization of Poultry Slaughter Inspection; however, in January 2015, FSIS issued new *Salmonella* and *Campylobacter* performance standards for raw chicken parts and not-ready-to-eat (NRTE) comminuted poultry products (FRN Docket No. FSIS-2014-0023). Additionally, they announced that they would use a moving window approach instead of the previous set based approach, to sample for *Salmonella* and *Campylobacter* to determine that an establishment is meeting the performance standards.

Table 3. *Salmonella* and *Campylobacter* performance standards for poultry

Product	Maximum Acceptable % Positive		Performance Standard	
	<i>Salmonella</i>	<i>Campylobacter</i>	<i>Salmonella</i>	<i>Campylobacter</i>
Broiler Carcasses ¹	7.5	10.4	5 of 51	8 of 51
Turkey Carcasses ¹	1.7	0.79	4 of 56	3 of 56
Comminuted Chicken ²	25.0	1.9	13 of 52	1 of 52
Comminuted Turkey ²	13.5	1.9	7 of 52	1 of 52
Chicken Parts ²	15.4	7.7	8 of 52	4 of 52

¹FSIS Directive 10,250.1

²FRN Docket No. FSIS-2014-0023

PATHOGENS OF CONCERN IN POULTRY PROCESSING

Salmonella spp. and *Campylobacter* spp. are the primary pathogens of concern in poultry processing, as they can exist in the intestinal tract of live poultry and can cause cross-contamination externally (e.g., feet, feathers, skin; and internally during live haul and subsequently processing) (Rigby and Pettit, 1980; Stern et al., 1995a). While USDA FSIS has issued best practice guidelines for pre- and post-harvest control of *Salmonella* and *Campylobacter* (USDA FSIS, 2010). It is unlikely that these pathogens could be eliminated from the current broiler production systems in the United States; however, the interventions steps described in the following section can be used to reduce the pathogens in the processing environment.

Salmonella is a facultative anaerobe, Gram-negative rod, which is a non-spore forming microorganism. It belongs to the Enterobacteriaceae family and usually is a motile organism with peritrichous flagella allowing movement (Cosby et al., 2015). There are currently over 2,500 recognized *Salmonella* serotypes, with new serotypes discovered each year. The optimal growth temperature for *Salmonella* is 35 to 40 C; however, dependent on the serotype and growth media used, the bacteria can grow in temperatures ranging between 2 and 54 C (Cosby et al., 2015). The infective dose of *Salmonella* in the most susceptible populations (i.e., infants, children, immunocompromised, elderly) is estimated to be as low as 10^1 to 10^2 cells, while healthy adults usually require an estimated 10^4 to 10^6 cells to cause infection. Ingesting *Salmonella* in foods that are higher in fat can also decrease the infective dose in healthy adults (Bell, 2002; Angulo et al., 2000). The nationwide microbiological baseline data collection program for young chickens (USDA FSIS, 2008) indicated that *Salmonella*

levels from 1,322 or 99.3% confirmed positive samples out of 1,333 total carcass rinse samples were all below 30 MPN/ml of rinsate at re-hang (Table 4).

Table 4. Distribution of quantified *Salmonella* – re-hang samples (USDA FSIS, 2008)

Range (MPN/ml)	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
0.0301 – 0.3	622	41.47	789	52.6
0.301 – 3.0	506	33.73	1,295	86.3
3.01 – 30	194	12.93	1,489	99.3
30.01 – 300.0	7	0.47	1,496	99.7
Undetermined ¹	4	0.27	1,500	100.0
Total	1,333	100.00	-	-

¹Includes 1 value >1,100 and 3 values >11

Campylobacter is usually present in higher numbers than *Salmonella* and is more prevalent in poultry processing than *Salmonella* (Horrocks et al., 2009). *Campylobacter* is a gram-negative fastidious organism, non-spore formers, S-shaped (or spiral rod), that are 0.2 – 0.8 um wide and 0.5 to 5 um long; however, cells that are stressed or old can form a spherical shape (Debruyne et al., 2008; Davis and DiRita, 2008). These organisms are typically motile, moving in a corkscrew type motion by use of unipolar or bipolar flagella (Debruyne et al., 2008; Horrocks et al., 2009). Ideal growth occurs in microaerophilic conditions (85% N₂, 10% CO₂, and 5% O₂) between 37 and 42 C (Davis and DiRita, 2008).

Presently, there are 25 species and 8 subspecies within the *Campylobacter* genus; however, only 2 of these species have an impact on food safety, *Campylobacter jejuni* and *Campylobacter coli* (Perez-Perez and Kienesberger, 2013; Horrocks et al., 2009). When tested in human subject, it was found that the infective dose of *Campylobacter* can be 500 organisms or less (Kothary and Babu, 2001). The nationwide microbiological baseline data collection program for young chickens (USDA FSIS, 2008) indicated that

Campylobacter levels from 438 or 42% confirmed positive samples out of 2,337 total carcass rinse samples were ≤ 100 cfu/ml of rinsate at re-hang (Table 5).

Table 5. Distribution of quantified *Campylobacter* – re-hang samples (USDA FSIS, 2008)

Range (cfu/ml)	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
10 – 100	438	13.37	1,376	42.0
101 – 1,000	841	25.68	2,217	67.7
1,001 – 10,000	719	21.95	2,936	89.6
10,001 – 100,000	310	9.47	3,246	99.1
100,001 – 1,000,000	28	0.85	3,274	100.0
1,000,001 – 10,000,000	1	0.03	3,275	100.0
Total	2,337	100.00	-	-

POULTRY PROCESSING TECHNOLOGIES AND PROCESSING AIDS USED IN MULTI-HURDLE APPROACH TO FOOD SAFETY

Many technologies used in the poultry production and processing continuum help prevent the spread of pathogens and reduce the overall prevalence of the pathogenic and spoilage organisms. Many factors can influence the spread of bacteria between birds in a flock during live haul, such as: crowding, coprophagy, weather, and various stressor to the animal. Additionally, the time period between feed withdrawal and the time until processing can affect the pathogen load that enters the processing facility (Davis et al., 2010). However, precautions can be taken to prevent the spread of pathogens among the flock while transporting them to the processing plant, such as controlling the feed withdrawal period before loading birds and washing and sanitizing of the transport coops. Reducing the level of pathogens during the live haul process can help to reduce the pathogen load entering the processing facility.

During processing, one of the first steps where a physical intervention can be included is just before scalding. This is done by incorporating pre scald brushes to

remove feces and other debris from the carcass before they enter the scalding (Berrang and Bailey, 2009). Scalding is used during processing to denature the proteins in the feather follicle which will loosen the feathers and allow for easier removal of the feathers from the carcass. However, this is the first step in processing where the carcasses are in common water and cross-contamination of carcasses with pathogens can occur at this step of processing (Bailey et al., 1987), with the scald tank even providing a harborage for pathogens (Stern et al., 2001). While there are many different configurations of scalders in poultry processing plants, some commonalities exist to reduce the likelihood of cross-contamination during scalding. Most plants will use multi-stage scalders with countercurrent flow, utilizing high flow rates and agitation so that carcasses are always in contact with the cleanest water to help reduce microbial loads on the carcasses (Sams and McKee, 2010). Carcasses are treated with either a hard or soft scald and this is usually chosen by consumer preferences for color, or for the end use of the product. A soft scald (≤ 55 C) is used to retain the cuticle on the skin, providing a more yellow color to the carcass and a hard scald (> 55 C) will remove the cuticle (National Advisory Committee on Microbiological Criteria for Foods, 1997). The higher water temperatures used with a hard scald system, will provide a greater reduction in bacteria than a soft scald (McKee et al., 2008; Notermans and Kampelmacher, 1975). Additionally, researchers have investigated the use of many different processing aids that affect the pH of the scalding water to reduce the bacterial load in the scalding including; sodium hydroxide (Townsend, 2006; Humphrey et al., 1981), propionic acid (Humphrey et al., 1981), acetic acid (Okrend et al., 1986; Lillard et al., 1987; Tamblyn et al., 1997), glutaraldehyde (Humphrey et al. 1981), chlorine, trisodium phosphate, and sodium metabisulfite

(Tambyln et al., 1997). Helping to control the microbial load at the scalding step will help to minimize the bacteria that continue through the processing facility.

Defeathering is the next major processing step, where the feathers are removed from the carcass by a picker using rubber picker fingers. This step is a known source of pathogen and spoilage organism cross-contamination among carcasses. The physical force used in this process can quickly compromise the integrity of the rubber picker fingers and bacteria can become embedded within the small cracks that form in the rubber picker fingers. When there are no carcasses running on the line, such as during sanitation, these small cracks are closed off and the embedded bacteria may not be reached by sanitizers. This, along with a moist warm environment, that is high in organic material can allow for the growth and transfer of bacteria from carcass to carcass (Wempe et al., 1983; Stern et al., 1995b). Furthermore, the defeathering process can entrap bacteria within the feather follicles. The carcasses are still warm when the feathers have been removed and bacteria can be pushed into the feather follicles during picking. When these carcasses are chilled, the feather follicles close, entrapping the bacteria in the skin. Processing aids, such as antimicrobials, can be applied to the carcass after the feather follicle is closed; however, the antimicrobials will not be able to reach the bacteria within the closed feather follicles (Notermans and Kampelmacher, 1974). Berrang and Dickens (2000) showed an increase in *Campylobacter* counts of 3.7 log after defeathering. Other researchers have also reported higher microbial populations after the defeathering process in broiler and turkey production (Allen et al., 2003; Acuff et al., 1986; Izat et al., 1988). The microflora present on the carcass are impacted by each processing step and it is important to implement a multi-hurdle approach to controlling

food safety at each step to reduce the number of pathogens on carcasses at the end of the processing line.

Evisceration is another processing step that can affect the pathogen load on the carcass in processing. The process of evisceration removes the edible and nonedible viscera from the cavity of the carcass. If the intestines of the bird are compromised when removed from the body cavity, this can lead to fecal contamination of the inside and outside of the carcasses. This fecal contamination can lead to the spread of pathogens to the machinery, line workers, and inspectors, which will lead to cross-contamination of carcasses (NACMCF, 1997). Good manufacturing practices can help to reduce the occurrence of ruptured viscera, including proper maintenance and operation of equipment, as well as attempting to process birds of the same size (Buncic and Sofos, 2012). Minimizing carcass contamination at this step can help to prevent the spread of *Salmonella* through the processing facility (Sarlin et al., 1998).

Wash cabinets (spray or drench) can be used after evisceration and at many other steps throughout the processing line to help remove ingesta and fecal material and offer an additional hurdle in microbial control. There are a variety of different processing aids that can be utilized in these cabinets, such as: peracetic acid, cetylpyridinium chloride, chlorine, acidified sodium chlorite, trisodium phosphate, or other organic acids (FSIS Directive 7120.1, revision 28). Often, multiple wash cabinets are used from the beginning of processing to chilling as the use of multiple cabinets is more effective than a single cabinet used as a final wash (Berrang and Bailey, 2009; Notermans et al., 1980). Berrang and Bailey (2009) did not find a significant reduction in *Campylobacter* and *Escherichia coli* counts or the presence of *Salmonella* during any single step; however,

the use of multiple on-line spray and wash cabinets were able to significantly reduce populations of *Campylobacter* and *E. coli*, while also reducing the presence of *Salmonella*. Xiong et al. (1998) reported a 1.0 log₁₀ reduction in *Salmonella* on inoculated chicken skins when sprayed with water for 30 s at 207 kPa. However, Brashears et al. (2001) found that high pressure sprays may actually force bacteria into the skin of the poultry carcass, so pressure should be validated in the cabinet locations in each individual processing facility.

The primary purpose of the chiller is to chill the carcasses, currently poultry processors are required by regulations in 9 CFR 381.66 to chill carcasses, parts and giblets immediately after processing to prevent the outgrowth of pathogens, with an exception being made for products that will be cooked or frozen within the same facility. Before the Modernization of Poultry Slaughter Inspection was put into practice on October 20, 2014, the USDA FSIS had more prescriptive regulations which required poultry carcasses under 4 lbs to be chilled to 4 C within 4 h of slaughter. Many plants will likely still use this as guidance, however, individual facilities will be required to validate that their process is effective. In the US, the majority of processing facilities use immersion type chillers. There are three types of immersion chillers used in the US, including drag or paddle type chillers, auger type chillers, and rocker type chillers (Figure 1). Most processing facilities use multiple stages in the chillers, the first stage generally includes a prechiller, where birds are immersed for approximately 10-15 minutes in water at about 7 – 12 C in an effort to gradually reduce the carcass temperature to around 30 – 35 C (Sams and McKee, 2010). The next stage usually is the main chiller, where the water temperature in the main chiller is a gradient that ranges from approximately 4 C at

the entrance to about 1 C at the chiller exit and the carcasses are submerged in this chiller anywhere from 45 – 110 min, depending on the carcass size. Some facilities will even employ the use of a 3rd stage chiller which is very useful for antimicrobial treatment. They are generally smaller, with shorter product contact times and have less organic material. Most chillers are designed to have a counter current water flow system, so carcasses are always in contact with the coldest, cleanest water. This counter current water flow system also maximizes the heat exchange rate for rapidly cooling the carcasses and reducing the microbial load on the carcasses. Some chillers are also equipped with air or water jet systems to improve the efficiency of the process by reducing the thermal layering in the chiller, prevent carcass piling in auger chillers, and provide further reduction in microbial load on carcasses (McKee and Sams, 2010).



Figure 1. Immersion chillers: drag chiller (left), auger chiller (middle), rocker chiller (right). Photos courtesy of Morris and Associates, Inc. Garner, NC.

Immersion type chillers allow for the use of processing aids in the chiller as an additional hurdle in a multi-hurdle approach to food safety. Prior to 2010, the most common processing aid used in poultry chillers was chlorine; however, in 2010, chlorine was banned for use on all poultry exported to Russia. Russia will only allow poultry that has been treated with the following processing aids: cetylpyridinium chloride, hydrogen peroxide and peracetic acid (Bottemiller, 2010). Due to this ban, many poultry processors switched to the use of peracetic acid (PAA), as the processing aid used in

immersion chillers, to help reduced pathogens and PAA is currently the most widely used processing aid in the poultry chiller (McKee, 2011). USDA FSIS will schedule *Salmonella* verification testing in a facility to sample carcasses for *Salmonella* and *Campylobacter* when they exit the chiller and broiler processing facilities. Facilities that exceed 5 out of 51 positives or 8 out of 51 positives for *Salmonella* and *Campylobacter*, respectively, would fail under the current regulations (USDA FSIS, 2013).

Post chill dip tanks have recently been incorporated as new technology, developed to apply processing aids after the carcasses have exited the chiller (Nagel et al., 2013) as well as smaller tanks that can be used for poultry parts and giblets (Chen et al., 2014). Dip tanks have a smaller volume of water compared to the immersion chiller and a short dwell time (i.e., < 1 min). Due to this short dwell time, processing aids can be applied in higher concentrations, often reducing or eliminating any organoleptic changes in products. The processing aids used in the dip tank cover more surface area than processing aids applied using a spray or drench cabinet. Using dip technology to apply processing aids provides facilities with an additional tool that can be employed in a multi-hurdle approach to food safety in poultry processing.

Peroxyacetic acid or peracetic acid (PAA) is the most common antimicrobial processing aid used to reduce both spoilage and pathogenic microorganisms in the poultry processing plant (McKee, 2011). PAA is a solution, produced by catalyzing a reaction between acetic acid and hydrogen peroxide and is commercially available in a water solution kept in equilibrium by the use of proprietary ingredients (Block, 1991; Bell et al., 2013). PAA's pKa is 8.2 and it is a strong oxidant with an oxidation potential that is greater than chlorine and many other available disinfectants available for used in

the food industry (Bell et al., 2013). The other advantages of using PAA is that it is effective at a wide range of temperatures (including low temperatures), it is considered to be environmentally friendly (i.e., it decomposes into acetic acid, hydrogen peroxide, water and oxygen), and its efficacy is relatively unaffected by the presence of high organic loads (Kitis, 2004; McDonnell and Russell, 1999).

Some peracetic acid formulations are allowed for use at 2000 ppm in any part of the processing facility and all approved processing aids for meat, poultry and eggs as well as their specific applications are listed in FSIS Directive 7120.1, revision 28. The mode of action for PAA is a little unclear, although it is thought that PAA works very similarly to hydrogen peroxide by disrupting or oxidizing sulfhydryl and sulfur bonds in the proteins and lipids of the microorganism. It begins with bonds in the cellular wall to help increase permeability, and once in the cellular fluid the PAA is able to denature proteins and enzymes that are essential to the reproduction and survival of the microorganism (Baldry and Fraser, 1988; Block, 1991; Marris, 1995). Intracellularly, the disruption of the enzymes and proteins can impair the function of vital biochemical pathways, active transport across membranes and intercellular solute levels (Fraser et al., 1984). Effective inactivation of pathogenic microorganisms can be done by halting the metabolic activity of the organism and disrupting cellular processes to prevent the organism from multiplying, which will in turn prevent the organism from multiplying and causing disease (Randtke, 2010).

Before Russia placed a ban on the use of chlorine on exported poultry products, chlorine was the processing aid most widely used in poultry chillers. Chlorine is allowed in concentrations of up to 50 ppm free available chlorine (FSIS Directive 7120.1, revision

28) in sprays and dips throughout the poultry processing facility. The pKa of hypochlorous acid can vary depending on the water temperature, at 0 C, the pKa is 7.8 (Randtke, 2010), and the final stage chillers are generally around 0 C. When sodium hypochlorite is added to water it readily hydrolyzes to form hypochlorous acid (HOCl), which is the most antimicrobial form of chlorine. The dissociation of the HOCl is highly dependent on the pH of the system, where a decrease in the pH of aqueous chlorine increases the percentage of the HOCl available in the system, thereby increasing the efficacy of the antimicrobial (Randtke, 2010).

There are several factors that affect the inactivation of an organism by chlorine including: the type and concentration of organisms being inactivated, the disinfectant species present (e.g., hypochlorous acid, hypochlorite ions, chloramines, etc.), the concentration of disinfecting species, contact time, temperature, pH and interfering substances (e.g., organic materials and trace minerals; Randtke, 2010; Davis et al., 2010). Hypochlorous acid is more efficient than other chlorine compounds in penetrating cell walls, due to its low molecular weight. Additionally, the oxidation and substitution reactions between hypochlorous acid and organic materials, which include the reactions with critical cellular components, occur quicker than other chlorine compounds (Randtke, 2010). While hypochlorous acid reacts quickly with organic material within the microbial cell, it also quickly reacts with other available organic material within the chiller, leaving it unavailable for its antimicrobial action (Davis et al., 2010). Additionally, lowering the temperature of HOCl, such as the low temperatures found in a chiller, can decrease the rate of reaction with vital cellular components which can also further decrease HOCl's antimicrobial activity (Randtke, 2010). While chlorine has been

an effective processing aid, newer technologies have led to the development of more effective antimicrobials that can be utilized in the immersion chiller.

QUALITY AND SHELF-LIFE OF POULTRY MEAT

The major quality attributes associated with poultry meat include appearance, texture, juiciness, flavor, and functionality. While these are all important aspects of quality, appearance and texture have the greatest impact on consumer selection and overall enjoyment of the eating experience (Fletcher, 2002). Quality of poultry products can be measured in a variety of ways including, objective and subjective measurements. Objective measurements include those measurements taken by instruments which can provide an exact numerical value, while subjective measurements include visual observations or opinions about products (e.g., untrained sensory panel response). The objective measurements generally give researchers a more repeatable measurement that is not as variable as the subjective measurements; however, sensory panels, while more variable, can offer valuable insight into the consumer's perception of the product's organoleptic characteristics related to their sense of taste, smell, sight, feeling, and sound.

Consumers help to define the quality of all food products including poultry meat. They first judge a product by the visual appearance when making a choice to purchase a product in the grocery store, and therefore the overall appearance may be one of the most important sensory attributes (Fletcher, 2002; Chan and Decker, 1994). For the most part, poultry skin color ranges from cream to yellow in color and is primarily influenced by diet (i.e., inclusion levels of carotenoids) which are deposited in the epidermis (McKee, 2007; Totosaus et al., 2007). Scalding temperatures can also impact poultry skin color. For example, a hard scald would remove the cuticle layer of the skin which contains more of

the yellow color components, resulting in a more cream colored appearance. While the coloring of the skin is not indicative of bird health, a more yellow color is viewed as a healthier bird in some regions and is therefore preferred by consumers certain regions of the U.S. (Sams and McKee, 2010; Fletcher, 2002). While color is important an important quality attribute, with more products being used for cut-up or further processed products, the importance placed on this quality attribute has declined in the last 15 years (Fletcher, 2002). The packaging used for meat and poultry products allows the consumer to see the product before purchasing the item. This initial visual appearance can determine if the consumer will purchase the product as they will make assumptions about the products flavor and texture bases on what they see. A lack of consistency in a product can lead a consumer to conclude that the meat product is undesirable or that the product may be spoiled. In previous research, acetic acid used at 6,000 ppm (0.6%) for a contact time of 10 min did not result in any color changes when applied to carcasses; however, when contact time and concentration was increased (5,000 ppm (0.5 %) and 60,000 ppm (6.0 %) at 1 C for 60 min) skin darkening and yellowing were reported when color measurements were compared between pre- and post-treated samples (Dickens et al., 1994; Bilgili et al., 1998).

While the consumer is visually observing the color of the chicken, color is often also measured instrumentally. Typically, there are two types of color measurement that can be done to determine meat color, pigment extraction or color reflectance (Montgomery, 2007; Fletcher, 2002). Color reflectance can be measured using a colorimeter to indicate changes in color or to determine the amount of pigment within the skin or meat; however, these systems have some limitations in measuring opaque

materials, in that, much of the light is either transmitted or absorbed versus reflected to the detector (Fletcher, 2002). The Hunter Lab is one of the common color scales used to measure reflectance. The “L” value is indicative of the lightness of a product, where a value of 100 corresponds to pure white and 0 indicates pure black. The “a” value is indicative of redness and the “b” value is indicative of yellowness, and the more positive a value is, the more red or yellow the product (Montgomery, 2007). Values regarding color are generally highly variable between sensory panelists, so measurements regarding color are generally more accurate and reproducible when taken instrumentally; however, this may not be the most accurate predictor of biochemical meat properties (Fletcher, 2002).

After the consumer selects a product for purchase, the other organoleptic characteristics become important such as odor or aroma, texture, flavor and juiciness, as these characteristics will influence a consumer’s product satisfaction (Fletcher, 2002). Generally, the second sensory attribute a consumer judges is odor or aroma. Consumers will determine if they want to consume the food product based on odor or aroma and will start making assumptions about the products flavor based on the aroma (Coggins, 2007; Montgomery, 2007). Volatile compounds in the meat products are responsible for the odor or aroma in the meat. The primary compounds contributing to the odor and aroma are sulfurous and carbonyl-containing volatiles (Shahidi, 1998). Peracetic acid does have a pungent odor; however, it has been shown that PAA has little or no effect on the odor of the product (Del Rio et al., 2007). Flavor is closely influenced by odor and aroma and is one of the most important factors that determines the eating quality of a meat product. The sensory flavor profile is largely dependent on the activation of the senses of taste and

smell in the oral and nasal passages. Many factors and compounds contribute to flavor including the ratio of lean muscle to fat. Fat carries many of the fat soluble compounds that add to flavor, however, fat can also cause lipid oxidation or a warmed over flavor in meat (Coggins, 2007). Changing the processing conditions could have an impact on the flavor of meat by accelerating the lipid oxidation in a meat product.

Texture (or tenderness) is a very complex characteristic of meat products, but can be simply defined by the amount of force it takes during mastication to cut through the meat fibers. Tenderness is one of the other sensory attributes that influence the consumer's eating quality of a meat product (Coggins, 2007; Fletcher, 2002; Kauffman and Marsh, 1987). While many factors can influence tenderness in poultry processing, deboning time, or rigor mortis development, has the largest impact on the textural properties of the chicken breast meat by influencing the contractile state of the myofibrillar proteins (Fletcher, 2002). Consumers may judge the freshness and quality based on the tenderness of the meat, although these parameters have little impact on the texture of poultry meat (Coggins, 2007). Juiciness is defined as the mouthfeel of a meat product during mastication and is relative to the moisture content or water holding capacity of the meat. As a meat product becomes drier or less juicy, it can be perceived as a tougher product. Moisture content in meat is due to water entrapment in the spaces between the myofibrils and outside the muscle fibers. The spaces between the myofibrils can be increased by certain mechanical actions, such as massaging and tumbling (Cross, 1987). While these processes can impact juiciness in whole muscle products, juiciness is greatly impacted by consumer preparation (e.g., overcooking has a negative impact;

Fletcher, 2002), therefore, it would be unlikely that a surface treatment of an antimicrobial would have an impact on the juiciness of a product.

The use of processing aids can impact a products organoleptic properties; however, there are many conflicting studies when determining the impact of processing aids on the organoleptic properties of broilers. These studies all differ in processing aid application methods, concentration of processing aids and sensory methods; therefore, it is difficult to make any direct comparisons between studies. However, many studies have shown that various processing aids can affect product quality, as both increases and decreases in quality attributes have been reported (Sammant et al, 2015). Product acceptance by consumers is an important factor in determining processing parameters, consumer's acceptance of all the sensory attributes in regard to food products is important to a products success in the market place. While tenderness and juiciness are some of the more important organoleptic properties of a meat product, a consumer must find all attributes acceptable to have a pleasant eating experience and consumers have little tolerance for lack of consistency in the perception of quality. Sensory analysis is an important industry tool used in conjunction with objective measurements in determining how a consumer will perceive product quality.

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CHAPTER II.

THE MICROBIAL AND QUALITY PROPERTIES OF POULTRY CARCASSES
TREATED WITH PERACETIC ACID AS AN ANTIMICROBIAL TREATMENT

ABSTRACT

Salmonella spp. and *Campylobacter* spp. continue to be a prominent food safety concerns for the poultry industry and consumers alike. Peracetic acid (PAA) has been approved as an antimicrobial for use in poultry chillers. To validate its effectiveness, 100 poultry carcasses (per replication x 2) were inoculated with *Salmonella* (10^6 cfu) or *Campylobacter* (10^6 cfu) and were randomly allocated into chill water containing chlorine (30 ppm) or PAA (25, 100, or 200 ppm). Results indicated that PAA concentrations as low as 25 ppm were effective in reducing *Salmonella* spp., while PAA levels of 200 ppm were effective in reducing *Campylobacter* spp. when compared with the chlorine treatment. A sensory study was also conducted with another set of 200 carcasses (not inoculated, per replication x 2). Birds were treated with water, chlorine (30 ppm), or PAA (100, 150, or 200 ppm). Sensory panels and microbial data were collected weekly on randomly sampled carcasses that were stored at 4°C for 21 d. The PAA-treated carcasses at 150 and 200 ppm had an extended shelf-life compared to those treated with water or chlorine. Specifically, on d 15, the only treatments that could be served to sensory panelists were the carcasses treated with 150 or 200 ppm PAA. The carcasses treated with water, chlorine, or 100 ppm PAA had off-colors, off-odors, and high microbial counts. These results suggest that PAA may be an effective antimicrobial when used in poultry chiller applications and higher levels (≥ 150 ppm) may extend product shelf-life.

INTRODUCTION

Salmonella and *Campylobacter* continue to be prominent food safety concerns for the poultry industry and consumers alike. New USDA Food Safety Inspection Service initiatives are to reduce *Salmonella* on poultry to below 10% on sample test sets. Because poultry processing is highly automated, many points exist where cross-contamination may occur.

A critical step during processing where antimicrobials have been used is the poultry chiller. Traditionally, chlorine has been used as an antimicrobial in poultry chiller applications and is allowed up to 50 ppm (Anonymous, 1989). However, chlorine's efficacy as an antimicrobial is greatly reduced by high organic loads and pH levels above 7.0 (Lillard, 1979). Additionally, Tamblyn and Conner (1997) reported that chlorine levels as high as 400 and 800 ppm were needed to kill *Salmonella* attached to broiler skin, but these levels would not be allowable according to current regulations.

Alternative antimicrobial strategies for poultry that have been investigated include organic acids (Izat et al., 1990; Tamblyn and Conner, 1997; Bilgili et al., 1998), hydrogen peroxide (Lillard and Thompson, 1983), trisodium phosphate (Lillard, 1994), chlorine dioxide (Doyle and Waldroup, 1996), acidified sodium chlorite (Kemp et al., 2000; Kemp et al., 2001), and more recently, cetylpyridium chloride (Yang et al., 1998). For antimicrobials to be effective and utilized by the industry, they have to be approved; their efficacy has to be documented; the level and contact time needed has to be appropriate for the particular processing step; they have to be cost effective; and finally, they must not have deleterious effects on product quality. Therefore, some antimicrobial products are more appropriate for rinses, dips, or sprays as opposed to a chiller application where the

volume used may be higher and the contact time would be longer making cost effectiveness and quality factors a consideration.

The most common antimicrobial product other than chlorine used for carcass application during processing is acidified sodium chlorite in spray applications (Anonymous, 2001). However, this compound is not widely used in chiller applications. In chiller applications today, other than chlorine, chlorine dioxide is sometimes selected because it more stable than chlorine; however, its effectiveness is still decreased by organic matter and results have not been consistent in reducing *Salmonella* and *Listeria* at typical usage levels of 1 to 3 ppm (Doyle and Waldroup, 1996).

Several of the organic acids investigated for application in poultry include acetic, formic, citric, lactic and propionic acid (Mulder et al., 1987; Izat et al., 1990; Dickens et al., 1994). While these acids can be effective antimicrobials, it has been reported that levels above 3% can cause carcass discoloration and levels of lactic acid at 1% or above could cause flavor changes (Blankenship et al., 1990). To avoid the quality changes associated with organic acids, the ideal approach is to combine acids with other antimicrobials to be able to use lower levels of organic acid but to maintain the antimicrobial efficacy of the compound. Peracetic acid combined with hydrogen peroxide is an example of this approach. The peracetic acid (PAA) tested in this study was Spectrum™, and it was 15% peracetic acid. In water, PAA forms an equilibrium with acetic acid and hydrogen peroxide (Baldry and Fraser, 1988). As an antimicrobial application in poultry, the maximum allowable concentrations of the chemicals in the mixture are 220 ppm peracetic acid and 120 ppm hydrogen peroxide (Anonymous, 2001).

The combined acidic and oxidizing properties of PAA has been found to be effective against bacteria, spores of bacteria, fungi and yeast (Fatemi and Frank, 1999).

Bell et al. (1997) reported that combinations of 1% acetic acid and 3% hydrogen peroxide provide the greatest reduction in *E. coli*, *S. wentworth* and *L. innocua* when sprayed on beef carcasses that had been previously inoculated. Brinez et al. (2006) also reported that peracetic acid resulted in a greater than 5 log reduction of *Staphylococcus* spp. *Listeria* spp. and *E. coli* regardless of the food matrix tested. These studies suggest there is evidence to support that peracetic acid has demonstrated antimicrobial efficacy at relatively low levels that is likely attributed to PAA's combined acidic properties and oxidizing properties. Although the peracetic acid described above has been approved for use in poultry chillers, there is a lack of published data to indicate its antimicrobial efficacy and its impact on product quality. Therefore, the objectives of the study were to determine the effective levels needed to reduce *Salmonella* and *Campylobacter* when used in poultry chiller applications and to evaluate the quality of carcasses.

MATERIALS AND METHODS

Pilot Plant Study

Poultry chill water was collected in 18.93 L buckets from a commercial poultry processing facility and transported to Auburn University Poultry Processing Unit (<160.93 km). Buckets containing chill water were stored at 4°C overnight. Water samples were collected from each bucket and chlorine levels and organic loads were measured. Chlorine was measured using Aquachek Water Quality Test Strips (HACH Company, Loveland, CO). To determine water quality, chemical oxygen demand (COD) and biological oxygen demand (BOD) were measured (Boyd, 1979). Using sterile

containers, three 500 mL samples of chill water were collected for COD and BOD measurements. Water was collected from the end sections of the chill tank so that the water samples would be representative of the chill water collected for the study. Water samples were placed on ice, and COD and BOD measurements were carried out within 6 h of sampling.

Five treatments consisting of varying levels of PAA (Spectrum™; FMC, Philadelphia, PA) were prepared in the chill water containers. The water was maintained at 4°C and chlorine levels were tested to confirm that they were at minimum concentrations to prevent confounding of results. Five chill water treatments prepared were 25 ppm, 100 ppm, 200 ppm PAA or 30 ppm chlorine and a control where carcasses were not inoculated. Non-inoculated carcasses were used to sample for any background *Salmonella* spp. or *Campylobacter* spp. that may be present. PAA levels were confirmed and tested using LaMotte Peracetic Acid Test Kit (LaMotte Company, Chestertown, MD) and chlorine was measured using Aquachek Water Quality Test Strips (HACH Company). The pH of the treatments was also recorded (Thermo Orion pH Meter, Model 720A, Pittsburg, PA), and the average pH of the peracetic acid treatments was 4.5 and the chlorine treatment was 6.0. The pH of the chlorine treatment was not adjusted because this was actual chill water from a poultry facility and the normal pH of this water was approximately 6.0 and was not adjusted at the plant.

One hundred carcasses were obtained from a commercial processing facility prior to entering the chiller. The carcasses were placed on ice and transported to Auburn University (<160 Km). Carcasses were stored on ice at 4°C prior to being used in the study. In each of 2 replications, 50 carcasses were inoculated on the skin of the carcass

with *Salmonella enterica* Typhimurium (10^6 cfu/mL) and *Campylobacter jejuni* (10^6 cfu/mL). Inoculum preparation is described below. Once the inoculum had dried (10 min), the birds were placed in the assigned treatment for 1 h (5 treatments X 5-8.93 L buckets X 2 carcasses per bucket X 2 replicatons, n=100 birds total). Afterwards, the birds were sampled using the carcass rinse method described below.

Salmonella Inoculum Preparation

Test tubes containing 10 mL of Tryptic Soy Broth (TSB) were inoculated with a frozen culture of a Nalidixic Acid resistant strain of *Salmonella* Typhimurium (isolated from the Auburn University Poultry Research Farm and selected for resistance to Nalidixic Acid). After incubating at 37°C for 24 h, one loop full of the *Salmonella* culture was streaked onto Xylose Lysine Tergitol 4 Agar (XLT4; Acumedia Manufactures, Inc., Baltimore, MD) containing 50 µL/mL of Nalidixic Acid. The plates were incubated at 37°C for 48 h. Black, isolated colonies were picked from the XLT4 plates, and fresh TSB tubes were inoculated with one colony per test tube. The tubes were incubated 20-24 h at 37°C. A stock culture of 10^8 cells per mL *Salmonella* Typhimurium was prepared.

Campylobacter Inoculum Preparation

One mL of the frozen *Campylobacter jejuni* (isolated from the Auburn University Poultry Research Farm) culture was added to 10 mL of Brucella-FBP broth (Acumedia) in a test tube and incubated for 42 h at 42°C in a AnaeroJar™ (Oxoid, Ogdensburg, NY) containing a CampyGen sachet (Oxoid) to generate a microaerophilic mixture of 5% O₂, 10% CO₂ and 75% N₂. The culture was streaked onto Campy-Cefex agar (Stern et al., 1992) and incubated at 42°C for 42 h in microaerophilic conditions as described above to

form a lawn of bacteria. *Campylobacter jejuni* was removed from the agar and added to 0.1% Phosphate Buffered Saline (Fisher BioReagents, Fair Lawn, NJ). A stock culture of 10^6 cells per mL was prepared.

Carcass Sampling

A whole-carcass rinse method described by the USDA in the *Microbiology Laboratory Guidebook* was used for the microbiological sampling, detection and enumeration with the following modifications (USDA FSIS, 2004). Carcasses were placed into sterile stomacher bags and rinsed with 200 mL of Neutralizing Buffer (Difco, Sparks, MD) for 1 min with a rocking motion to assure that all interior and exterior surfaces are rinsed. Reducing the volume of rinsate has no affect the recovery of *Salmonella* (Cox et al., 1980) and has been shown to increase the recovery of *Campylobacter* (Bailey and Berrang, 2007) in carcass rinse methods. The rinsate was transferred to a sterile bottle and placed on ice until analysis.

Direct Plating of Salmonella and Campylobacter

Direct plating was generally used for enumerating inoculated samples. After the carcasses were sampled using the whole-carcass rinse method described previously, serial dilutions were done using BPW and samples were plated in duplicate. For *Salmonella*, the rinsate was direct plated using the pour plate method onto XLT4 agar (Acumedia) containing nalidixic acid (50 $\mu\text{g}/\text{mL}$) (Sigma, St. Louis, MO). Briefly, the pour plate method involved adding 1 mL of sample from the appropriate dilution to a sterile petri dish and then 20 mL of XLT4 Agar that was $<50^\circ\text{C}$ was added to the sample and immediately swirled 8 times counter clockwise and 8 times clockwise. After the plates solidified, they were inverted and incubated at 37°C for 24-48 h. Populations were

converted to log values with the 200 mL of carcass rinsate representing the sample. Therefore, results are reported as log cfu / sample.

For *Campylobacter*, serial dilutions were plated using the spread plate method on Campy-Cefex agar (Stern et al., 1992) in duplicate. Spread plating indicates that 0.1 mL of the appropriate dilution was placed on top of the agar and spread with a spreader. Plates were incubated for 36 h at 42°C in AnaeroPack rectangular jars (Mitsubishi Gas Chemical America, Tokyo, Japan) with a microaerophilic environment of 5% O₂, 10% CO₂, and 75% N₂, generated by CampyGen sachets (Oxoid). Again, populations were converted to log values with the 200 mL of carcass rinsate representing the sample and results were reported as log cfu / sample.

Shelf-life and Quality Determination

Broilers were conventionally processed (n=250 X 2 replications) at the Poultry Science Research Unit. Specifically, the broilers were hung on shackles, electrically stunned (50 V, 20 mA, 400 Hz) via 1% saline stunner bath (custom built, 1.52 m) with a metal plate running along the bottom attached to an electrical stun control box (Georator Corp. model 901-1001IA, Manassas, VA). Following stunning, birds were killed by exsanguination through a unilateral neck cut followed by a 95 s bleed-out. After bleed-out, the birds were scalded in a 2.44 m long single pass, steam injected scald (Cantrell, Gainesville GA, custom built). The birds were defeathered in a 1.22 m long disk-picker (Meyn, The Netherlands, custom built), eviscerated (Meyn, The Netherlands) and rinsed and then broiler carcasses were randomly divided among the 5 chill-water treatments. Chill water (static) treatments were stored at 4°C overnight and the day of processing the following treatments were added to each separate tank 100 ppm PAA, 150 ppm PAA,

200 ppm PAA, 30 ppm chlorine and water (control). Carcasses were allowed to chill at 4°C for 2 h. The birds were packaged in polystyrene foam tray packs with a soaker pad and PVC film over wrap and stored at 4°C. In each replication, five carcasses from each treatment were taken for microbial analysis of total aerobic plate count (APC) and psychrotroph (PSY) enumeration at 1, 7, 10, 15 and 21 d. Briefly, carcasses were rinsed using the carcass rinse method described above using 200 mL of Buffered Peptone Water (BPW) as the rinsate. To enumerate APC and PSY serial dilutions of the rinsate were plated in duplicate on Standard Methods Agar (Acumedia) using the pour plate method. APC were incubated at 37°C for 24 h and PSY were incubated at 4°C for 10 days. Serial dilutions were plated on petrifilm plates (3M Petrifilm, 3M Microbiology Products, St. Paul, MN) in duplicate to enumerate *Escherichia coli* and coliforms and plates were incubated at 37°C for 24 h to determine coliforms and 48 h to determine *E. coli*.

At each storage period (1, 7, 15 d), five carcasses from each group were used for the sensory analysis and color evaluation. An average of three color measurements were recorded from each carcass using a Minolta Colorimeter (Model DP-301, Minolta Corp., 101 Williams Drive, Ramsey, NJ) before they were deboned. Measurements were taken on the breast skin portion of the carcass avoiding feather follicles. Values were recorded using the Hunter L*a*b* color system where higher L* values indicate a lighter colored sample.

For the sensory analysis, breast fillets were deboned the morning of the sensory panels. Sensory evaluation was conducted in duplicate (one panel in the am and one panel in the pm) with untrained panelists in the Department of Poultry Science with 58, 57, and 52 panelists participating on days 1, 7 and 15 respectively. On each day of

sensory testing, fillets were baked on covered aluminum trays in a convection oven (Viking Professional Series, VESC Series, Greenwood, MS) set at 177°C to an internal temperature of 76°C. The samples were held in a warm oven at 93°C (<1 h) until they were served to panelists. The cooked fillets were cut into 2- X 2- cm cubes and placed in plastic containers that were preassigned random three-digit numbers. The panelists were served one sample at a time and asked to rate each sample using a modified eight-point hedonic scale. The hedonic scale included the attributes of appearance (like to dislike), texture (tender to tough), flavor (like to dislike), juiciness (moist to dry), and overall acceptability (like to dislike).

Statistical Analysis

Experiments were all performed in duplicate. All data (bacterial counts were converted to log cfu / sample) was reported as least square means. Data was analyzed using analysis of variance in the general linear model of SAS (SAS Institute, Inc., 2003). Because 0 cannot be directly analyzed with the statistical model, we used a value equivalent to 0.9. Significance was reported using a level of $P \leq 0.05$.

RESULTS AND DISCUSSION

The first objective of this research was to determine the most effective levels of PAA against *Salmonella* spp. and *Campylobacter* spp. *Salmonella* Typhimurium and *Campylobacter jejuni* were selected because they are commonly found species and serotypes on chicken carcasses. When the PAA was added to the poultry chill water, *Salmonella* spp. were reduced by about 1.5 logs at the 25 ppm PAA level compared to the carcasses treated with 30 ppm chlorine; whereas, the 100 and 200 ppm PAA provided a slightly over a 2 log reduction of *Salmonella* Typhimurium (Figure 2). The non-

inoculated control was negative indicating there was no background *Salmonella* spp. on the carcasses prior to initiation of the study.

Although PAA was effective against *Salmonella* spp. at concentrations as low as 25 ppm, higher levels were required to reduce *Campylobacter* spp. Specifically, PAA at 200 ppm resulted in a 1.5 log reduction against *Campylobacter* spp.; however, lower levels of PAA were not found to be different from the chlorine treatment in reducing *Campylobacter* levels (Figure 3). Moreover, *Campylobacter* spp. was found on non-inoculated control indicating birds had background *Campylobacter*. *Campylobacter* is more commonly found on poultry carcasses than *Salmonella* and researchers have reported *Campylobacter* levels of approximately 4,000 cfu per carcass as typical (Oyarzabal, 2005). Another factor that could affect the efficacy of antimicrobials is the level of bacterial attachment to broiler skin. Organic acids at low concentrations have been found to be less effective when bacteria were attached to broiler skin (Tamblyn and Conner, 1997). However, when organic acids and hydrogen peroxide are combined studies have shown greater antimicrobial efficacy (Brinez et al., 2006). In addition, Bell et al. (1997) reported that combinations of 1% acetic acid and 3% hydrogen peroxide gave a greater than 3 log reduction in *E. coli*, *S. wentworth* and *L. innocua* when sprayed on beef carcasses that had been previously inoculated. These researchers found this combination was more effective than each antimicrobial used individually.

While chlorine can be effective against *Salmonella* and *Campylobacter*, it was found to be less effective than PAA in reducing *Salmonella* and *Campylobacter* in the current study (Figure 2 and Figure 3). Generally, chlorine is the industry standard for chiller applications and was used the point of comparison for antimicrobial effectiveness.

For the current study, chlorine may have been less effective due to the pH and presence of organic material in the chill water collected from the processing facility. Since chill water for the treatments in the current study was obtained from a commercial facility, it contained a certain level of organic load (BOD = 144.7 mg/L) and the pH was 6.0 compared to 4.5 when the PAA was added. It is important to note that high pH (>7) and high organic load are known to reduce the efficacy of chlorine as an antimicrobial (Lillard, 1979). Although the pH of the chill water treatment was not alkaline, it is likely that pH and the organic load were factors in the reduced effectiveness of the 30 ppm chlorine treatment compared to the PAA treatments. In addition, the PAA treatments of 200 ppm were effective in reducing both *Salmonella* and *Campylobacter* despite the presence of organic material (Figure 2 and Figure 3).

Brinez et al. (2006) also reported that peracetic acid and hydrogen peroxide were effective antimicrobials in the presence of organic material. Results from that study indicated that as little as a 0.1% concentration of the mixture with a 10 min contact time reduced *Staphylococcus* spp. *Listeria* spp. and *E. coli* greater than 5 logs regardless of the organic matrix tested. Moreover, Bell et al. (1997) used spray washes containing acetic acid, sodium bicarbonate and/or hydrogen peroxide on beef carcasses. They reported that using organic acids, specifically acetic acid, in combination with hydrogen peroxide provided the greatest log reductions in the study. However, King et al. (2005) found that when peracetic acid was applied as a spray to beef carcasses there was not a significant reduction in *E. coli* O157:H7 and *S. Typhimurium*. They suggested that some organisms may be more susceptible to peracetic acid than others and the degree of the organism's attachment to the product's surface could affect the effectiveness of the peracetic acid.

Many studies have demonstrated the effect of initial levels of microorganisms on product treated with organic acids and hydrogen peroxides (Lillard and Thompson, 1983; Dickens et al., 1994; Dickens and Whittemore, 1995; Dickens and Whittemore, 1997; Tambllyn and Conner, 1997), but some studies have noted considerable quality changes in products when organic acids are used (Mulder et al., 1987; Izat et al., 1990; Dickens and Whittemore, 1995). Therefore, the other objective of this study was to validate the quality and shelf-life of carcasses treated with different levels of PAA to determine the optimal level for product safety while maintaining product quality.

On day 1 of the current study, the PAA treatments at 200 ppm had lower of *E. coli* and coliforms and total APC than the carcasses treated with 100 ppm PAA, 30 ppm CL and water (Table 6). On day 1, psychrotrophs were lowest for the 150 ppm PAA while the 200 ppm PAA, 100 ppm PAA and 30 ppm CL treatments were similar (Table 6). Although these treatments were similar, the 200 ppm had lower psychrotrophs compared to the water control. These trends among bacterial populations and treatment effects suggest that as early as day one the higher levels of PAA (150 and 200 ppm) resulted in modest bacteria reductions. By day 7, there were no differences noted in the coliform counts among any of the treatments tested (Table 6). However, differences among *E. coli* counts and psychrotrophs were evident at day 7. Specifically, *E. coli* populations were slightly higher for the 150 ppm PAA and 30 ppm CL treatment when compared to all other treatments; whereas, the 100 ppm PAA, 30 ppm CL, and water control had higher psychrotroph level when compared to the 150 and 200 ppm PAA. By day 10, no differences were detected in *E. coli* and coliforms, while only the water control had a higher level psychrotrophs among the treatments; however, aerobic

populations were lower for the carcasses treated with 150 and 200 ppm PAA. By day 15, *E. coli* were longer detectable; however differences did exist in coliforms, aerobic or psychotrophic bacteria populations. The non-detectable *E. coli* results at day 15 could have been due to the competition of other bacterial populations; however, it is uncertain. It is also important to note that at day 15, the only treatments not exhibiting off-odors and colors indicative of product spoilage were the carcasses treated with 150 and 200 ppm PAA which had the lowest levels of aerobic bacteria and the 200ppm PAA treatment had the lowest level of psychotrophic bacteria. In general, it appeared that PAA at 150 and 200 ppm had positive effects on reduction of bacterial populations early during the storage study; however, these effects were not sustained throughout the duration of the study. Moreover, organoleptic changes indicating product spoilage was not noted in the 150 and 200 ppm PAA which may suggest the microflora on these carcasses were different than microflora from other carcasses and treatments; however, this was not determined in the current study.

PAA may have variable effects on depending on the bacteria type and attachment according as King et al. (2005) found that peracetic acid applied as a spray to beef carcasses did not reduce *E. coli* O157:H7 and *S. Typhimurium*. They suggested that some organisms may be more susceptible to peracetic acid than others and the degree of the organism's attachment to the products service could affect the effectiveness of the peracetic acid. Furthermore, results from the current study also suggest that PAA used in a chiller application was more effective against *Salmonella* than *Campylobacter* (Figures 1 and 2).

Because treatment with antimicrobials can affect the organoleptic properties of a product, it is important to determine quality of carcasses treated with antimicrobials. In the current study, the appearance, flavor, texture, juiciness and overall acceptability of the product was evaluated up to day 15 of storage at 4 C. On day 1 (Table 7), there were no differences between the 100 ppm PAA, 150 ppm PAA, 200 ppm PAA, 30ppm CL and the control in the evaluation of juiciness. The 100 ppm PAA ranked lower than the water control in appearance, flavor and texture evaluation by the panelists, however they were not different from the CL control in appearance and texture. By day 7, panelists were not able to determine any differences in any of the sensory attributes between treatments. At day 15, only two samples were evaluated by panelists and again they were unable to determine any differences between the samples. Similarly, Dickens et al. (1994) found no differences in sensory quality of breast fillets when exposed to a pre-chill treatment of 0.6% acetic acid (10 min) compared to a control. However, results from studies using hydrogen peroxide alone have indicated that carcasses may have a bleached appearance (Lillard and Thompson, 1983; Mulder et al., 1987; Izat et al., 1990). The levels of hydrogen peroxide used in the studies greatly exceeded (6,600-12,000 ppm) the 120 ppm allowed in the PAA mixture and the higher levels of hydrogen peroxide are the likely cause of the negative quality characteristics. Similar to the current study, Bell et al. (1997), found that combining organic acids such as acetic acid with hydrogen peroxide increased the antimicrobial efficacy allowing lower levels (1% acetic/3% H₂O₂) to be utilized in beef carcasses washes while maintaining product quality. In the current study, the PAA treatments did not exhibit any deleterious quality changes and the 150 and 200

ppm PAA were the only samples that could be served to panelist on day 15. These results suggest that the higher levels of PAA may extend product shelf-life.

Throughout the storage study, the 200 ppm PAA treatment was lighter in color than the CL control (Table 8). However, by day 7 there were no differences in the lightness (L^*) values of the lower PAA levels, 100 ppm and 150 ppm, and the CL control. There are slight differences in the redness (a^*) values and the yellowness (b^*) values at day 1 and again at day 15; however, these differences are small and would be difficult for consumers to detect. Lillard and Thomson (1983) reported that when hydrogen peroxide was added to poultry chill water at 1,100 – 12,000 ppm that the birds had a bloated and bleached appearance, but this disappeared after 19 h. Bilgili et al. (1998) reported that found that when breast skin samples were dipped in acetic acid at 0.5, 1.0, 2.0, 4.0 and 6.0% a darkening and yellowing effect was present when comparing measurements taken pre- and post- treatment. The results of the current study suggest while 200 ppm PAA lightened carcasses; there were very few other notable changes in product color and none of these changes would be considered negative in terms of product quality.

Results from this study indicate that PAA could be used in poultry chillers as an effective intervention strategy to reduce the incidence of *Salmonella* Typhimurium and *Campylobacter jejuni*. In addition, PAA may extend the shelf-life of products in the processing facility without compromising the organoleptic properties of the product. In commercial processing plants, PAA is usually added to poultry chill water through controlled pumping equipment. In the current study, it was observed that broilers treated with PAA were slightly lighter in color, but carcass quality was not negatively affected,

and the difference in color disappeared over time. Therefore, PAA may be used as an effective intervention strategy in poultry chiller applications to reduce levels of *Salmonella* and *Campylobacter* while maintaining product quality attributes.

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Figure 2. *Salmonella* Typhimurium recovered from inoculated carcasses (n = 100) treated with varying levels of PAA (25, 100, 200 ppm) and CL (30 ppm) reported as mean log cfu of *S. Typhimurium* per sample for each treatment group. ^{a-d}Means with no common superscript differ significantly ($P \leq 0.05$).

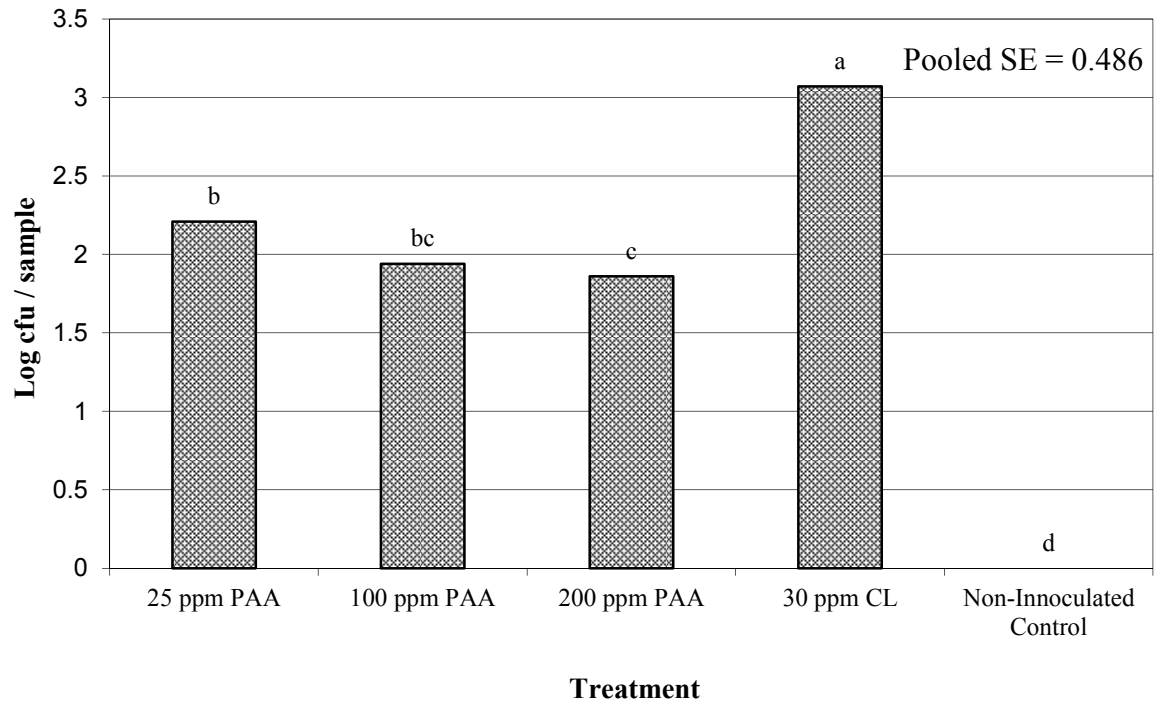


Figure 3. *Campylobacter jejuni* recovered from inoculated carcasses (n = 100) treated with varying levels of PAA (25, 100, 200 ppm) and CL (30 ppm) reported as mean log cfu of *Campylobacter jejuni* per sample for each treatment group. ^{a-d} Means with no common superscript differ significantly ($P \leq 0.05$).

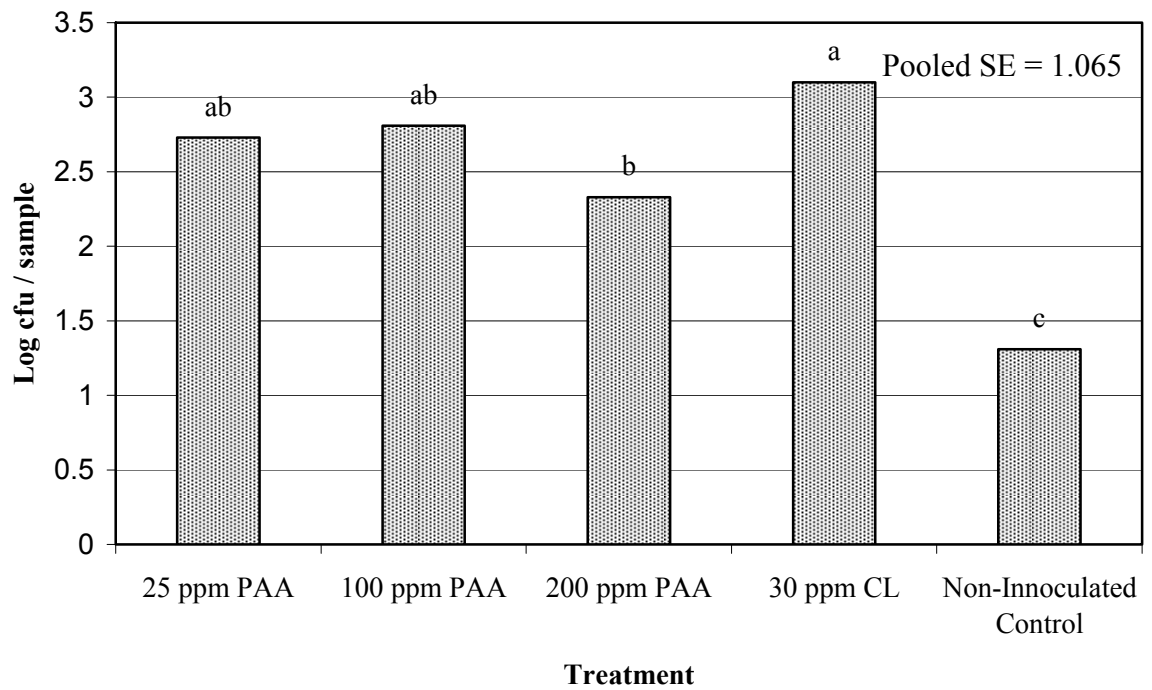


Table 6. Microbial analysis of broiler carcasses treated with various levels of peracetic acid and chlorine during storage (mean \pm SD)¹

Storage Period	Treatment	<i>Escherichia coli</i> log cfu / sample	Coliforms log cfu / sample	Aerobic Plate Count log cfu / sample	Psychrotroph log cfu / sample
Day 1	100ppm PAA	3.17 \pm 0.507 ^c	3.44 \pm 0.408 ^c	4.23 \pm 0.352 ^{ab}	1.57 \pm 0.346 ^{bc}
	150ppm PAA	2.40 \pm 0.815 ^{ab}	2.64 \pm 0.798 ^b	3.37 \pm 0.396 ^{ab}	0.26 \pm 0.253 ^a
	200ppm PAA	1.86 \pm 0.211 ^a	1.96 \pm 0.243 ^a	2.85 \pm 0.489 ^a	1.24 \pm 0.883 ^b
	30ppm CL	3.15 \pm 0.385 ^c	3.54 \pm 0.324 ^c	4.58 \pm 0.389 ^b	2.14 \pm 0.129 ^{bc}
	Control	2.97 \pm 0.332 ^{bc}	3.10 \pm 0.279 ^{bc}	4.24 \pm 0.731 ^{ab}	2.37 \pm 1.159 ^c
Day 7	100ppm PAA	3.14 \pm 0.308 ^a	2.90 \pm 0.328 ^a	4.30 \pm 0.128 ^a	6.05 \pm 0.367 ^b
	150ppm PAA	3.64 \pm 0.958 ^b	3.43 \pm 1.048 ^a	4.25 \pm 0.579 ^a	3.77 \pm 1.129 ^a
	200ppm PAA	3.08 \pm 0.802 ^a	3.03 \pm 0.802 ^a	3.66 \pm 0.727 ^a	4.12 \pm 1.297 ^a
	30ppm CL	3.68 \pm 0.612 ^b	3.33 \pm 0.621 ^a	4.14 \pm 0.543 ^a	6.50 \pm 0.751 ^b
	Control	3.46 \pm 0.664 ^a	3.21 \pm 0.725 ^a	4.24 \pm 0.625 ^a	5.85 \pm 1.034 ^b
Day 10	100ppm PAA	2.31 \pm 0.370 ^a	2.56 \pm 0.513 ^a	6.36 \pm 0.742 ^{bc}	6.24 \pm 2.959 ^a
	150ppm PAA	2.50 \pm 1.262 ^a	2.75 \pm 1.297 ^a	4.73 \pm 0.483 ^a	5.37 \pm 1.163 ^a
	200ppm PAA	2.05 \pm 1.072 ^a	2.15 \pm 1.100 ^a	4.88 \pm 1.504 ^a	5.81 \pm 0.568 ^a
	30ppm CL	2.92 \pm 0.927 ^a	2.87 \pm 0.896 ^a	6.54 \pm 0.534 ^c	6.26 \pm 2.970 ^a
	Control	2.89 \pm 1.214 ^a	2.31 \pm 1.577 ^a	6.26 \pm 0.277 ^{bc}	7.09 \pm 0.336 ^b
Day 15	100ppm PAA	ND ²	1.91 \pm 0.945 ^a	7.03 \pm 0.545 ^c	9.53 \pm 0.622 ^b
	150ppm PAA	ND	2.13 \pm 1.212 ^a	6.29 \pm 1.313 ^b	8.68 \pm 0.970 ^{ab}
	200ppm PAA	ND	2.56 \pm 1.493 ^{ab}	5.89 \pm 0.305 ^a	8.24 \pm 1.041 ^a
	30ppm CL	ND	2.78 \pm 0.374 ^b	6.85 \pm 0.648 ^c	8.83 \pm 0.331 ^{ab}
	Control	ND	2.06 \pm 0.692 ^a	6.88 \pm 0.779 ^c	9.32 \pm 0.299 ^b

^{a-d}Values with the same letter within each storage period and treatment are the same ($P \leq 0.05$).

¹n = 200

²Not detected.

Table 7. Sensory analysis of breast fillets from carcasses treated with varying levels of peracetic acid and chlorine during storage (mean \pm SD)¹

Storage Period	Treatment	Appearance ²	Flavor ²	Texture ³	Juiciness ⁴	Overall ²
Day 1	100ppm PAA	3.00 \pm 0.88 ^a	3.36 \pm 1.07 ^a	3.21 \pm 1.91 ^a	2.91 \pm 1.44 ^a	3.16 \pm 1.02 ^a
	150ppm PAA	2.58 \pm 0.82 ^c	3.12 \pm 1.28 ^{abc}	2.86 \pm 1.27 ^{ab}	3.11 \pm 1.37 ^a	2.98 \pm 1.08 ^{ab}
	200ppm PAA	2.73 \pm 0.88 ^{abc}	3.29 \pm 1.23 ^{ab}	3.00 \pm 1.25 ^{ab}	3.14 \pm 1.37 ^a	3.09 \pm 1.21 ^a
	30ppm CL	2.86 \pm 0.82 ^{ab}	2.95 \pm 1.02 ^{bc}	2.89 \pm 1.18 ^{ab}	2.79 \pm 1.22 ^a	2.89 \pm 0.98 ^{ab}
	Control	2.64 \pm 0.72 ^{bc}	2.77 \pm 1.04 ^c	2.70 \pm 1.09 ^b	2.66 \pm 1.15 ^a	2.63 \pm 0.89 ^b
Day 7	100ppm PAA	2.58 \pm 0.94 ^a	3.00 \pm 1.25 ^a	2.88 \pm 1.13 ^a	2.80 \pm 1.32 ^a	2.89 \pm 1.19 ^a
	150ppm PAA	2.89 \pm 1.18 ^a	3.12 \pm 1.31 ^a	2.82 \pm 1.32 ^a	3.09 \pm 1.42 ^a	3.04 \pm 1.22 ^a
	200ppm PAA	2.96 \pm 1.19 ^a	3.30 \pm 1.34 ^a	3.11 \pm 1.23 ^a	3.01 \pm 1.42 ^a	3.14 \pm 1.22 ^a
	30ppm CL	2.67 \pm 0.89 ^a	3.09 \pm 1.20 ^a	2.93 \pm 1.05 ^a	2.64 \pm 1.26 ^a	2.96 \pm 1.09 ^a
	Control	2.82 \pm 1.21 ^a	3.07 \pm 1.08 ^a	2.86 \pm 1.22 ^a	3.01 \pm 1.34 ^a	2.95 \pm 1.12 ^a
Day 15	100ppm PAA	---	---	---	---	---
	150ppm PAA	2.56 \pm 0.92 ^a	2.83 \pm 1.13 ^a	2.63 \pm 1.16 ^a	3.10 \pm 1.33 ^a	2.90 \pm 1.05 ^a
	200ppm PAA	2.63 \pm 0.99 ^a	3.23 \pm 1.18 ^a	2.94 \pm 1.18 ^a	3.06 \pm 1.24 ^a	2.92 \pm 1.27 ^a
	30ppm CL	---	---	---	---	---
	Control	---	---	---	---	---

^{a-d}Values with the same letter within each storage period are the same ($P \leq 0.05$).

---Data not collected.

¹n = 167 (n = 58 for day 1, n = 57 for day 7, and n = 52 for day 15).

²Where 1 = like extremely; 8 = dislike extremely.

³Where 1 = extremely tender; 8 = extremely tough.

⁴Where 1 = extremely juicy; 8 = extremely dry.

Table 8. Color of chicken skin treated with various levels peracetic acid or chlorine during storage (mean \pm SD)¹

Storage Period	Treatment	L* value ²	a* value ³	b* value ⁴
Day 1	100ppm PAA	73.79 \pm 2.352 ^b	1.94 \pm 0.667 ^{bc}	0.04 \pm 1.820 ^{ab}
	150ppm PAA	73.77 \pm 2.017 ^b	1.38 \pm 0.857 ^{ab}	-0.90 \pm 1.482 ^a
	200ppm PAA	76.17 \pm 1.218 ^b	0.51 \pm 0.389 ^a	-0.14 \pm 1.758 ^{ab}
	30ppm CL	70.95 \pm 2.295 ^a	2.90 \pm 0.772 ^c	3.75 \pm 2.967 ^c
	Control	73.77 \pm 1.470 ^b	2.36 \pm 1.510 ^{bc}	2.43 \pm 2.859 ^{bc}
Day 7	100ppm PAA	73.32 \pm 1.734 ^a	0.01 \pm 0.579 ^a	-0.26 \pm 1.353 ^a
	150ppm PAA	72.94 \pm 2.127 ^a	0.17 \pm 0.651 ^a	-0.10 \pm 2.042 ^a
	200ppm PAA	75.54 \pm 1.925 ^b	-0.13 \pm 0.393 ^a	1.07 \pm 1.904 ^a
	30ppm CL	71.97 \pm 1.490 ^a	0.55 \pm 0.124 ^a	0.17 \pm 1.627 ^a
	Control	71.38 \pm 1.556 ^a	0.42 \pm 0.653 ^a	-0.59 \pm 1.649 ^a
Day 15	100ppm PAA	73.19 \pm 1.348 ^{bc}	-0.29 \pm 0.947 ^{ab}	1.87 \pm 0.978 ^{bc}
	150ppm PAA	72.49 \pm 2.058 ^{ab}	-0.52 \pm 0.350 ^{ab}	-1.57 \pm 3.130 ^a
	200ppm PAA	75.00 \pm 2.143 ^c	-0.59 \pm 0.737 ^a	1.72 \pm 2.379 ^{bc}
	30ppm CL	71.92 \pm 1.307 ^{ab}	-0.14 \pm 0.678 ^{ab}	2.41 \pm 2.050 ^c
	Control	70.44 \pm 1.683 ^a	0.37 \pm 0.455 ^b	-0.35 \pm 1.381 ^{ab}

^{a-d}Values with the same letter within each storage period are the same ($P \leq 0.05$).

¹n = 150.

²Where L* = 0 is black, L* = 100 is white.

³Where +a* is red, -a* is green.

⁴Where +b* is yellow, -b* is blue

CHAPTER III.
VALIDATING THE EFFICACY OF PERACETIC ACID MIXTURE AS AN
ANTIMICROBIAL IN POULTRY CHILLERS

ABSTRACT

Peracetic acid mixture (PAHP) which is a combination of peracetic acid and hydrogen peroxide has been approved as an antimicrobial for use in poultry chillers. To validate its effectiveness, 85 ppm PAHP was compared with the 30 ppm chlorine treatment in a commercial setting. In this trial, 100 carcasses were sampled for *Salmonella* spp. and *Campylobacter* spp. prior to the chiller and 100 were sampled after chilling. In all, 400 carcasses were sampled using 85 ppm PAHP in the chiller and 400 carcasses were sampled using the chlorine treatment. PAHP at 85 ppm reduced *Salmonella* spp. positive carcasses by 92% exiting the chiller; whereas, treatment with 30 ppm chlorine reduced *Salmonella* spp. by 57%. Additionally, PAHP reduced *Campylobacter* spp. positive carcasses by 43% exiting the chiller while chlorine resulted in a 13% reduction. These results suggest that peracetic acid in combination with hydrogen peroxide may be an effective antimicrobial in poultry chiller applications.

INTRODUCTION

Salmonella and *Campylobacter* continue to be the leading causes of foodborne illness in the US (CDC, 2005). Because poultry meat and eggs have been established as sources for *Salmonella*, it is important that effective controls are established when processing poultry (Tauxe, 1991). Currently, USDA performance standards for *Salmonella* on poultry require that levels tested must be below 20% (USDA FSIS, 2004). This performance standard was based upon a nationwide study by the USDA which found the average level of *Salmonella* occurring on processed poultry was 20%. A new FSIS initiative is categorizing plants according to historical levels of *Salmonella* found at a particular location. The best category or category I facilities would have below 10% *Salmonella* and would not be subject to as much testing as category 2 or 3 facilities (USDA FSIS, 2006).

Poultry processing is a highly automated industry where many points exist for cross contamination if *Salmonella*-positive birds enter the processing plant. Therefore, intervention strategies require multiple approaches during processing to prevent *Salmonella* from spreading. A critical step during processing where antimicrobials have been used is the poultry chiller. Traditionally, chlorine has been used as an antimicrobial in poultry chiller applications and is allowed up to 50 ppm (Anonymous, 1989). However, chlorine's efficacy as an antimicrobial is greatly reduced by high organic loads and pH levels above 7.0 (Lillard, 1979). Additionally, researchers (Tamblyn and Conner, 1997) reported that chlorine levels as high as 400 and 800 ppm were needed to kill *Salmonella* attached to broiler skin, but these levels would not be allowable according to current regulations.

Alternative antimicrobial strategies for poultry that have been investigated include organic acids (Bilgili et al., 1998; Tamblyn and Conner, 1997), hydrogen peroxide (Lillard and Thompson, 1983), trisodium phosphate (Lillard, 1994), chlorine dioxide (Doyle and Waldroup, 1996), acidified sodium chlorite (Kemp et al., 2000; Kemp et al., 2001), and more recently cetylpyridium (Yang et al., 1998). For antimicrobials to be effective and utilized by the industry, they have to be approved; their efficacy has to be documented; the level and contact time needed has to be appropriate for the particular processing step; they have to be cost effective; and finally, they must not have deleterious effects on product quality. Therefore, some products are more appropriate for rinses, dips, or sprays as opposed to a chiller application where the volume used would be higher and the contact would be longer making cost effectiveness a consideration.

Several of the organic acids investigated for application in poultry include acetic, formic, citric, lactic and propionic acid (Dickens et al., 1994; Mulder et al., 1987; Izat et al., 1990). While these acids can be effective antimicrobials, it has been reported that levels above 3% can cause carcass discoloration and levels of lactic acid at 1% or above could cause flavor changes (Blankenship et al., 1990). To avoid the quality changes associated with organic acids, the ideal approach is to combine acids with other antimicrobials to be able to use lower levels of organic acid but to maintain the antimicrobial efficacy of the compound. Peracetic acid combined with hydrogen peroxide is an example of this approach. The peracetic acid mixture tested in this study was Spectrum™ and is a mixture of 15% peracetic acid and 10% hydrogen peroxide. As an antimicrobial application in poultry, the maximum allowable concentrations of the chemicals in the mixture are 220 ppm peracetic acid and 120 ppm hydrogen

(Anonymous, 2001). The combination of peracetic acid and hydrogen peroxide has strong oxidizing properties and has been found to be effective against bacteria, spores of bacteria, fungi and yeast (Fatemi and Frank, 1999). Bell et al. (Bell et al., 1997) reported that combinations of 1% acetic acid and 3% hydrogen peroxide provide the greatest reduction in *E coli*, *S. wentworth* and *L. innocua* when sprayed on beef carcasses that had been previously inoculated. These researchers found this combination was more effective than each antimicrobial used individually and gave greater than a 3 log reduction for each bacteria strain tested. Peracetic acid mixture has been approved for use in poultry chillers, but there is a lack of published data to indicate the antimicrobial efficacy. Therefore, the objective of this study was to determine the effectiveness of PAHP used in poultry chiller applications to reduce *Salmonella* spp. and *Campylobacter* spp. on poultry carcasses.

MATERIALS AND METHODS

Sample Collection

For the commercial study, two chill water treatments containing either 30 ppm chlorine or 85 ppm PAHP (Spectrum™; FMC, Philadelphia, PA) were examined using the final chiller as the application point in a commercial facility. Sampling was conducted in the middle of the 1st shift of the operation. PAHP was added only in the final chiller because it was deemed more feasible and more economical to add it to the final chiller. The final chiller was located adjacent to the main chiller and held approximately 15,142 liters of water. The final chiller was 12.19 m long with a residence time between 15-20 min and an overflow rate of 95 liters/min. PAHP was added into the incoming water line for the final chiller using a small pump skid (Sigma ProMinent High

Flow Dual Metering Pump, Model: FMCADMP; ProMinent, Pittsburgh, PA), rated for PAHP. The dual pumping system in the skid is controlled by a universal switch-over box (Model: FMCUSOPL; ProMinent) that is integral to the pump skid design made by Prominent specifically for FMC. A monitoring system with a PAHP ion specific amperometric probe (Model: FMCISAP; ProMinent) monitors the PAHP concentration in the incoming water line and adjusts the pumps accordingly. The carcasses were in contact with the treatment for approximately 20 minutes. Levels of chlorine and PAHP were monitored 30 minutes prior to sampling and every 15 minutes throughout the sampling period using a total chlorine test strip kit (Hach Company, Loveland, CO) and LaMotte Peracetic Acid Test Kit (LaMotte Company, Chestertown, MD).

A total of 800 broiler carcasses were sampled (100 carcasses X 2 replications X 2 sampling sites X 2 treatments). Replications for each treatment were sampled on different days indicating different flocks; however, percent positive carcasses for each replication were similar for each sampling site during the study. During each replication, 100 carcasses were sampled just before entering the chiller (pre-chill sampling site), and 100 carcasses were sampled as they exited the chiller (post-chill sampling site). Birds at the pre-chill sampling site were collected by enclosing them in a sterile bird rinse bag and removing them from the line. As birds were exiting the chiller, they were grabbed with a sterile glove and placed into a sterile bird rinse bag. Carcasses were sampled by the whole-carcass rinse method described by the USDA Food Safety and Inspections service (FSIS) *Microbiological Laboratory Guidebook* (USDA FSIS, 2004), using 200 mL of Buffered Peptone Water (BPW) instead of 400 mL. Reducing the volume of rinsate has no affect on the recovery of *Salmonella* (Cox et al., 1980), but has been shown to

increase the recovery of *Campylobacter* (Bailey and Berrang, 2007) in carcass rinse methods. The rinsate was transferred back to the individual rinse containers and transported to the laboratory on ice, approximately 3 h away.

Through pre-trial investigations we determined that the pH of the rinsate, when using either chlorine or peracetic acid as the chill water additive, was between 6.5 and 7.0, so no pH adjustment was necessary. To address residual oxidative activity in the rinsate, no free chlorine was detected (chlorine test strip kit, Hach Company, Loveland, CO) in the rinsate after birds were rinsed in 200 mL BPW. Additionally, because the PAHP is a mixture of acetic acid and hydrogen peroxide, the residual oxidizing effect of hydrogen peroxide on bacteria would have also been minimal in the rinsate due to the dilution effect. Moreover, other researchers have reported that much higher levels of hydrogen peroxide (5,000 - 15,000ppm) than used in the current study were not shown to be any more effective than water alone in reducing bacteria on carcasses when used in chiller applications (Dickens and Whittemore, 1997).

Campylobacter detection procedure

Campylobacter spp. in carcass rinse samples were analyzed for the presence-absence of *Campylobacter* as described by the USDA (USDA FSIS, 2004) with the following modifications. Sterile tissue culture flasks were used instead of test tubes for the enrichment to increase the surface area to the microaerophilic environment to increase the recovery of *Campylobacter* (Fluckey et al., 2003). Thirty mL of rinsate was used to determine the prevalence of *Campylobacter* in the samples. Media used included Bolton's broth (Oxoid, Hampshire, England) for the enrichment and modified Campy Blood Free Selective Medium (mCCDA) (Acumedia Manufactures, Inc., Baltimore, MD)

for the selective plating. Enrichments and selective plating were incubated for 36 h at 42 C in AnaeroPack rectangular jars (Mitsubishi Gas Chemical America, Tokyo, Japan) with a microaerophilic environment of 5% O₂, 10% CO₂, and 75% N₂, generated by CampyGen sachets (Oxoid). Presumptive colonies were confirmed by agglutination, using the PanBio INDX Campy (jcl) latex agglutination test kit (Integrated Diagnostics, Inc., Baltimore, MD). Results were reported as percent reduction comparing pre-chill samples to post-chill samples.

Salmonella detection procedure

Prevalence of *Salmonella* spp. were determined using methods described by the USDA (USDA FSIS, 2004). Samples were pre-enriched in BPW for 24 h at 37 C. Enrichments were done by transferring 0.1 ml and 0.5 ml of the preenrichment into Rappaport Vassiliadis (Acumedia) broth and Tetrathionate (Acumedia) broth respectively and incubated at 42 C for 24 h. Preenrichments were streaked onto Xylose lysine tergitol 4 (Acumedia) agar and incubated for 24 to 48 h at 37 C. Characteristic colonies were inoculated on slant agar tubes containing triple sugar iron agar (Acumedia) and lysine iron agar (Acumedia) and incubated for 24 h at 37 C. Presumptive positives were confirmed using the polyvalent serum A-Vi serological confirmation test (Difco, Sparks, MD) for salmonellae. Results were reported as percent reduction comparing pre-chill positive carcasses to post-chill positive carcasses.

Statistical analysis

Positive samples were indicated by a 1 and negative samples were indicated by a zero. Data was subjected to the analysis of variance procedure using the general linear model of SAS (SAS Institute, 2003). Significance was indicated by a $P < 0.05$.

RESULTS AND DISCUSSION

In the plant, it was decided to apply PAHP to the final chiller which had a residence or dwell time of approximately 20 min. The PAHP level chosen for the final chiller was 85 ppm, and this level was established as the most effective level for the contact time through pre-trial evaluation. When carcasses were sampled in the plant, pre-chill *Salmonella* positive carcasses were between 25 and 31% positive (Table 9). After using 85 ppm PAHP in the final chiller, only 3% of the carcasses tested positive for *Salmonella* post-chill (Table 9). Compared to the PAHP, higher levels of *Salmonella* (11%) were recovered post-chill when 30 ppm chlorine was used in the chiller. Chiller water pH using PAHP was 4.5; whereas, it was 6.1 for the chlorine. No adjustments were made to equalize pH because the plant normally maintained a pH of 6.0 when using chlorine. Because pH differed among the two treatments, it is likely that the lower pH associated with the PAHP along with the oxidizing properties of the compound attributed to reductions in *Salmonella* observed.

Although there are reports of using hydrogen peroxide in poultry chiller applications, there is no published literature using the combination of peracetic acid and hydrogen peroxide for this application. When hydrogen peroxide alone was used in chiller applications, researchers (Lillard and Thompson, 1983) reported levels of 12,000 ppm were effective in reducing *E. coli*; but, the carcasses treated had a bleached and bloated appearance and would be considered undesirable. The combination of peracetic acid with hydrogen peroxide allows for lower levels which could help alleviate some of the potential quality issues.

Research investigating the antimicrobial properties of similar organic acid and hydrogen peroxide combinations has been documented for various applications. Bell et al. (1997) found bacteria reductions greater than 3 logs when acetic acid and hydrogen peroxide mixture was sprayed onto inoculated beef carcasses. These authors noted that acid combined with the hydrogen peroxide provided a synergistic effect, and it was speculated that the lower pH enhances the oxidizing effects when these two compounds were used together (Bell et al., 1997). In addition, this study reported very minimal changes in product quality. The most noted change in product quality was less redness in beef which was associated with the combined acetic acid and hydrogen peroxide. Therefore, the combinations are not likely to result in major quality changes when applied to poultry; particularly, if the poultry skin is being treated as was the current application in the poultry chiller.

Another study that indicated the positive results of combining an organic acid and hydrogen peroxide was Brinez et al. (2006) who found that the mixture of peracetic acid and hydrogen peroxide was effective in reducing *E. coli* and *Listeria* (>5 logs) in solutions containing organic material from food sources. This study was important because it demonstrated the antimicrobial efficacy of the combined mixture is not greatly diminished by the presence of high organic loads as is often found in commercial poultry chillers. In contrast, the efficacy of chlorine is greatly diminished by organic load (Anonymous, 2006).

In addition to *Salmonella*, the effectiveness of PAHP was also determined against *Campylobacter*. *Campylobacter* is commonly associated with poultry, and 50-70% of food-borne illnesses in humans attributed to *Campylobacter* are related to the

consumption of poultry (Tauxe, 1992). Therefore, reducing *Campylobacter* levels as well as *Salmonella* levels are important food safety goals. In the current study, the percent of carcasses positive for *Campylobacter* spp. before entering the chiller ranged between 78 and 82% (Table 10). After chilling and treatment with 85 ppm PAHP, 47% of carcasses tested positive for *Campylobacter* (Table 10). This level of recovery was lower than that observed with carcasses treated with 30 ppm chlorine. When carcasses were treated with 30 ppm chlorine, 68% of the birds tested positive for *Campylobacter* post-chill. These results suggest that PAHP was more effective than chlorine in reducing *Campylobacter*; however, the overall reduction was not as high as that with *Salmonella*. Specifically, PAHP was effective in reducing *Salmonella* levels over 90% (Table 9), but *Campylobacter* levels were reduced by approximately 40% (Table 10). The difference in bacterial reduction between these two microorganisms may be related to the overall incidence and level of bacteria present on carcasses. In general, *Campylobacter* is more highly prevalent than *Salmonella* on poultry where the prevalence has been reported on retail carcasses as 83% and 25% respectively (Jorgensen et al., 2002).

Although treatment with chlorine was not as effective as PAHP for reduction of *Salmonella* or *Campylobacter*, chlorine did follow a similar trend relative to overall reduction differences between *Salmonella* and *Campylobacter*. Specifically, chlorine had a 55% reduction in *Salmonella* recovery (Table 9) and a 12% reduction in *Campylobacter* (Table 10). Therefore, both PAHP and chlorine were more effective in reducing *Salmonella* compared to *Campylobacter*.

While studies have not reported the combined effect of an organic acid and hydrogen peroxide against *Campylobacter*, studies have been conducted observing the

effects of organic acids and hydrogen peroxide separately. Zhao and Doyle (2006) found that when chicken wings were inoculated with a three strain cocktail of *Campylobacter jejuni* and treated with 0.1 % hydrogen peroxide alone bacterial counts were reduced by 1.0 log CFU/ml and 0.2% hydrogen peroxide had a 3.0 log reduction. Reductions of 2 log CFU/ml in *C. jejuni* were also found when wings were dipped in 0.1% acetic acid with increasing reductions >5 log CFU/ml in 2.0% acetic acid solutions with a 1 min treatment time. However, this study did not combine the effects of acetic acid and hydrogen peroxide as was done in the current study.

Overall, PAHP is approved for use in poultry chill applications up to 220 ppm; yet, lower levels (85 ppm) were found to be an effective antimicrobial treatment in poultry chiller applications to reduce *Salmonella* and *Campylobacter* on carcasses.

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Table 9. Reduction of *Salmonella* positive carcasses treated with PAHP or chlorine during chilling

Chill Water Treatment	Carcass Sampling Point	<i>Salmonella</i> % Positive	% Reduction
85 ppm PAHP	Pre-Chill	30.5 ^a	91.8
	Post-Chill	2.5 ^c	
30 ppm Chlorine	Pre-Chill	25.5 ^a	56.8
	Post-Chill	11.0 ^b	

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

Table 10. Reduction of *Campylobacter* positive carcasses treated with PAHP or chlorine during chilling

Chill Water Treatment	Carcass Sampling Point	<i>Campylobacter</i> % Positive	% Reduction
85 ppm PAHP	Pre-Chill	83.0 ^a	43.4
	Post-Chill	47.0 ^c	
30 ppm Chlorine	Pre-Chill	78.0 ^a	12.8
	Post-Chill	68.0 ^b	

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

CHAPTER IV.

SUMMARY

With an increasing number of illness outbreaks linked to chicken and initiatives by the US government to reduce foodborne illnesses, food safety in poultry products has become an important topic. To help in reducing the number of foodborne illnesses related to meat and poultry, the USDA FSIS has implemented stricter performance standards. As regulations become more stringent, controlling *Salmonella* and *Campylobacter* during poultry processing has become more challenging for poultry processors. While there are new technologies entering the market and new application methods for antimicrobials, these technologies increase production costs. Additionally, many of these technologies or antimicrobials can also have an impact on product yield and product quality. At the same time, the application of these antimicrobials can have some positive impacts in addition to improving food safety. For example, many of these antimicrobials and new application technologies can improve the shelf life of products.

This research compared the use of chlorine and peracetic acid (PAA) in terms of their effectiveness in reducing *Salmonella* and *Campylobacter* in a poultry chiller. When inoculated carcasses were exposed to PAA in a simulated chiller application for 1 hr, *Salmonella* was reduced by 0.8 to 1.2 logs at concentrations of 25 ppm to 200 ppm PAA, compared to 30 ppm chlorine, with higher concentrations of PAA providing the greatest reduction in *Salmonella*. PAA was not as effective in reducing *Campylobacter*; however, PAA still exhibited a larger reduction in *Campylobacter* (0.25 logs to 0.8 logs) with increasing levels of PAA (25 ppm to 200 ppm) compared to the use of 30 ppm chlorine. Since the implementation of the Russian ban on chlorine in poultry products exported to Russia, and stricter performance standards for *Salmonella* and new performance standards for *Campylobacter*, many poultry companies have stopped using chlorine in the

poultry chiller and other processing areas to facilitate the trade of dark meat poultry and meet *Salmonella* and *Campylobacter* performance standards. Since the completion of this research, the regulations regarding the application levels of PAA have changed, currently PAA can be used up to 2000 ppm throughout the processing facility, with the exception of online reprocessing, which is limited at 200 ppm. Additionally, further studies on the application of PAA and other various antimicrobials have looked at reducing the contact time of PAA and increasing the level of PAA, to increase the effectiveness of the application and reduce the negative impacts that PAA has on the organoleptic qualities of poultry products. In addition to the reduction of pathogens in the poultry products, the use of PAA also reduces the spoilage organisms related to poultry products, when compared to the use of chlorine as an antimicrobial, and is able to extend the shelf life of some poultry products.

Peracetic acid can have an impact on some organoleptic properties, such as color and flavor. While the differences in color measured were statistically significant, it would be unlikely that consumer's would be able to detect these differences by visual observation alone. At PAA levels of 100 and 150 ppm, the measured color values were no longer different after 7 days of product storage, so it is likely that by the time this product is shipped to a retail store and a consumer visually inspects the product, visual differences will likely disappear. Additionally, other studies have indicated that by reducing exposure time and increasing the level of PAA you will reduce the negative effects of PAA on the organoleptic properties of the poultry products while still reducing pathogens and improving shelf life.

Peracetic acid offers poultry processors a good option for food safety in that they help reduce the levels and prevalence of *Salmonella* and *Campylobacter* in the processing plant. It would be up to a plant to determine which levels are most appropriate for use in their processing environment. Several items should be considered before determining the usage level of PAA, including levels of pathogens when carcasses enter the chiller and the influence of usage level on product quality, which may be impacted by final product disposition. While baseline studies for *Salmonella* and *Campylobacter* in young chickens indicated a prevalence level of 5.19 and 10.66%, respectively, after the chiller, the prevalence levels of *Salmonella* and *Campylobacter* in raw chicken parts is 26.32 and 21.39%, respectively. This increase in pathogen prevalence from the whole carcasses to the raw parts indicates that there is cross contamination in the processing environment that could be due to comingling of product, extra product handling or passing through production equipment. This demonstrates that there is a need for multiple hurdles in the processing environment to control pathogens in the final products. There are many opportunities to investigate new technologies, new antimicrobials, new application technologies and the combination or effects of serial use of antimicrobials in the poultry processing environment.