USE OF A CHILL ADDITIVE TO REDUCE LEVELS OF *SALMONELLA* AND *CAMPYLOBACTER* DURING POULTRY PROCESSING

by

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(Under the Direction of Mark Berrang)

ABSTRACT

Risk of cross-contamination with food-borne pathogens such as *Salmonella* and *Campylobacter* during poultry processing remains of great concern to the poultry industry. One potential cross-contamination step in processing is chilling. The purpose of this research was to evaluate the efficacy of a chill additive, T-128, at reducing levels of *Salmonella* and *Campylobacter* transfer during chilling. Experiments were conducted to evaluate the effectiveness of T-128 alone as well as T-128 plus the addition of 50 ppm chlorine against *Salmonella* and *Campylobacter*. Based on the results of microbial reduction and chlorine interaction, pilot scale experiments were done to evaluate if bench-scale trends withheld through scale up. Reduction trends throughout all experiments suggests that immersion chilling using T-128 in conjunction with chlorine provides an effective and practical intervention strategy for controlling *Salmonella* and *Campylobacter* in poultry processing plants.

INDEX WORDS: Chilling, *Salmonella*, *Campylobacter*, Chlorine

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DEDICATION

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CHAPTER 1

INTRODUCTION

Salmonella and *Campylobacter* are two of the most commonly reported food-borne pathogens in the United States, (24, 26) and are constant safety concerns to the poultry industry and the general population. When infected with *Salmonella*, patients develop diarrhea, fever, and abdominal cramps between 12 and 72 hours after infection. This infection may last from 4 to 7 days (24). The majority of infected people will recover without any treatment; however, some severe cases may require hospitalization. In some cases, the *Salmonella* infection may spread from the intestines and into the blood stream, which can lead to death without proper antibiotic treatment (66). Both pathogens can be transmitted to humans through consumption of food products contaminated with infected animal feces. Symptoms of *Campylobacter* infection include fever, abdominal cramps, and bloody diarrhea. Symptoms appear between 2 and 10 days after infection and can last up to a week (33, 73). Cases of campylobacteriosis may lead to development of into Guillain-Barré syndrome. This condition can appear between 2 and 4 weeks after the infection occurs, generally after all diarrheal symptoms have subsided. Those that develop Guillain-Barré syndrome have symptoms of increasing paralysis in the limbs, and in severe cases, paralysis of the respiratory system requiring extended hospitalization (43).

According to the Foodborne Disease Active Surveillance Network (FoodNet) estimates, as of 2011, the infection rate for *Salmonella* in the United States at 16.5 cases per 100,000 people (1, 24, 50). Estimates of the infection rate for *Campylobacter*, as of 2011, are 14.3 cases per 100,000 people (4, 26). This is only 20 percent of all food-borne illnesses reported yearly

combined (11 percent salmonellosis and 9 percent campylobacteriosis). In addition to the morbidity and mortality there is a major economic burden caused yearly by these pathogens (50). The Center for Food Safety and Applied Nutrition (CFSAN) using figures of $5 million per life lost and roughly 500 deaths per year, taking into account patient suffering, estimated the economic value lost each year to salmonellosis at $2.5 billion (31). The annual economic burden from campylobacteriosis has been estimated between $1-$5.5 billion dollars including both costs from the sickness itself as well as the added cost from cases of Guillain-Barré syndrome associated with a campylobacteriosis infection (23).

As of July 2011, FSIS implemented new performance standards for the poultry industry for *Salmonella* and *Campylobacter* in a continued effort to reduce the number of cases (32). While deaths due to salmonellosis and campylobacteriosis are rare they do occur, mainly in infants, the elderly, or those with pre-existing medical conditions (4). Since the poultry processing industry is highly automated, potential for cross-contamination of carcasses exists at several points, which may aid in the spread of both *Salmonella* and *Campylobacter*. To better understand how to effectively reduce the number of cases of each pathogen, researchers must first strive to understand the microbial ecology of each organism and how it may change throughout the different processing areas, in order to find an exploitable weakness of each pathogen (10).

Figure 1, taken from Jekayinfa (40) describes in general, the trip of a broiler from live bird drop-off at the plant to packaging and shipping of fully processed product. Several points throughout this process are known areas of possible pathogen transfer; these are common "hot spots" in poultry processing plants. Controlling these points along the processing line is crucial to product safety. The point of interest in this study is the carcass chill tank step. In the United

States for the most part, poultry processors utilize ice water chilling instead of cold air chilling which is common in most European countries. Both methods are proven effective for controlling bacterial counts, although cold water chilling may be slightly more effective (12). In the past, chlorine has been the traditional antimicrobial agent used to control contamination in chill tanks, allowable at levels up to 0.005% by volume (76). Many different combinations involving manipulation of water levels and chlorine levels in the chill tank have been evaluated for their antimicrobial qualities (55). Even though chlorine remains the antimicrobial of choice, it does have limitations. For example, simply increasing the amount of chlorine added to the system does not appear to enhance the bacterial reduction capabilities of the chill tank (57). Also, high organic loads and/or pH conditions above 7.0 have both been shown to greatly reduce the effectiveness of chlorine (44). To control build up of organic loads the USDA requires water levels in a chill tank to be maintained at between 2.3-2.6 liters of fresh chill water per carcass (56). As well, pH conditions are carefully monitored within commercial chill tanks.

One alternative to chlorination in a chill tank is the use of organic acids. A range of different organic acids have been evaluated, with various levels of success (18, 29). One major issue with the use of organic acids at high levels in chill tanks is possible product discoloration. Propionic acid for example, has been shown to increase lightness values (L^*) and decrease yellowness values (b*), measured using a reflectance colorimeter, of broiler skin during immersion chilling ($P < 0.01$) (15). To help counteract the possible discoloration problem, several organic acids may be used together or in conjunction with other antimicrobial agents, which lowers the amount of each component necessary to reach the desired microbial reduction. Another option for use in chill tanks is chlorine stabilizers, which interact with the chlorine in the system helping to slow the effects of high organic loads to reduce the amount of active chlorine

in the tank. One formula for the stabilization of chlorine in solution was evaluated and found to be effective against *Escherichia coli* O157:H7 and *Salmonella* spp. This chemical formulation also showed low to moderate ability to stabilize chlorine in the presence of high organic loads (58). However, this chemical blend has not yet been approved for use in poultry chillers. The objectives of this study were to determine the effectiveness of this chemical blend at reducing cross-contamination of broiler carcasses with *Salmonella* and *Campylobacter* spp. and to evaluate the chlorine stabilization capabilities of the chemical blend for possible use in conjunction with chlorine in poultry chillers.

CHAPTER 2

LITERATURE REVIEW

Study Relevance

In the most recently published Unites States Department of Agriculture (USDA) reports, for the third quarter of 2010, an average of 7.4% of chicken carcasses at processing plants nationwide tested positive for detectable levels of *Salmonella* (32). The new USDA performance standard is 7.5%; which is significantly lower than the previous *Salmonella* performance standard, of 20% which was based on the national baseline study conducted by the USDA Food Safety and Inspection Service (FSIS) in 1994 and 1995. The current performance standard also sets a *Campylobacter* standard for the first time. Now, no more than 10.4% of raw broiler carcasses sampled should test positive for *Campylobacter jejuni*, *C. lari* and/or *C. coli* (35). If a plant is found to be operating outside of these performance standards, the plant must re-evaluate their Hazard Analysis and Critical Control Points (HACCP) plan in an effort to reduce *Salmonella* and/or *Campylobacter* prevalence. Hazard Analysis and Critical Control Points or HACCP is a systematic and preventative approach to controlling food safety and allergenic, chemical, biological, and physical hazards in a production process that could potentially cause a finished product to be unsafe for consumption. A second non-compliance event calls for the plant to further modify their HACCP plan. Upon the third non-compliance report, the plant must undergo a food safety audit as verification of their HACCP plan. If a plant continues to operate outside of compliance standards then USDA/FSIS inspection is withdrawn, and the plant is unable to operate legally (75). This increased emphasis on the public safety coupled with stricter

food safety regulations has left the poultry industry searching for new, more effective, and economical intervention strategies.

Salmonella spp.

Broilers and broiler meat are well documented reservoirs for multiple species of *Salmonella* and *Campylobacter* (26). Chickens come into contact with these pathogens while being raised on the farm or in transit from the farm to the processing facility; then, the pathogens set up residence in the digestive tract of the animal. It has been shown that a relatively small dose of these pathogenic cells can effectively colonize the intestines of young broilers (17). Experimentally, as few as 40 viable CFU of *C. jejuni,* for example, are enough to colonize a broiler chick depending on the relative strengths of both the bacterial strain and the chicken strain (52). Once the intestinal tract has been colonized, positive birds become a source of cross-contamination. Other sources of human pathogens on the farm include, but are not limited to, the following: feed, water, litter, insects, rodents, and personnel (59). Although *Salmonella* is pathogenic to humans, these species do not cause any harm to the broiler; however, when not handled properly, there is an obvious risk of an outbreak of food-borne illness (64).

Salmonella is a member of the family Enterobacteriaceae. They are rod-shaped, Gram-negative, non-spore-forming, facultatively aerobic microorganisms that can originate from both food and non-food sources (38). Non-food sources usually include fecal material in some form, human fecal material, wild or domestic animal fecal material, or water sources contaminated by the presence of fecal material. Food sources with a history of *Salmonella* contamination include: mainly raw meats, poultry, eggs, dairy products, and milk (47). *Salmonella* are mesophilic organisms, meaning the growth range for most species is between 7-47°C, with the optimum

growth temperature being 37°C. The optimum growth pH for *Salmonella* species is between 6.5-7.5, but several serotypes have shown growth between pH 4.0-9.0 given all other growth conditions kept ideal (72).

Gastrointestinal symptoms are caused by an infection of *Salmonella enterica* serotypes, which can be found in both human and animal fecal material. Symptoms of this pathogen include fever, headache, nausea, vomiting, cramping, and diarrhea lasting between 24-48 hours. These symptoms will present themselves between 6-48 hours after ingestion of an infective dose of the pathogen (39). Once *Salmonella* is ingested, the organism passes through the stomach and works to invade the epithelial cells of the lining of the small intestines. The bacteria achieve this by binding to receptor sites along the cellular membranes of the small intestines causing these membranes to "fold" around the *Salmonella* cells and absorb them through endocytosis. This process is possible through production of specialized proteins specific to each different serotype of *Salmonella.* Areas where this process occurs in the small intestine become severely inflamed. Some particularly virulent strains of *Salmonella* can penetrate as far as the surrounding lymphatic system after working through the walls of the small intestine leading to widespread infection of many of the major organs (24, 34). Each year there are an estimated 2-4 million cases of gastrointestinal salmonellosis in the United States (30).

***Campylobacter* spp.**

Like *Salmonella*, several species of *Campylobacter*, such as *C. jejuni* and *C. coli*, are harmful human pathogens that cause no physical harm to the chicken reservoir. *Campylobacter* originates in the birds' digestive tract and upon entering the processing facility, can spread from carcass to carcass via cross-contamination along the processing line. As of July 2011, new

Campylobacter performance standards and sampling criteria were put into effect with less than 10.4% positives as the new contamination limits for broilers (61).

Campylobacter are spiral-shaped Gram-negative bacteria. *Campylobacter* is micro-aerophilic in nature, growing best at conditions with oxygen levels under 5%. *Campylobacter* grows well under the conditions found in the small intestines of mammalian and avian species; as the bacteria grow and spread they excrete toxins that destroy the natural gut mucosa. *Campylobacter* organisms are culturally fragile compared to other human pathogens and respond poorly to reduced temperatures and desiccation (26). *Campylobacter* species are also sensitive to changes in salinity and a pH below 5.0 (41). Even though *Campylobacter* may not be the hardiest of pathogens, it is one of the most common causes of diarrheal illness in the United States. The Centers for Disease Control and Prevention has identified campylobacteriosis as one of the most common foodborne bacterial diseases in recent years since 2005 (19). In general, there are more cases in the summer months than the winter months, highest prevalence in infants and young adults, and more cases confirmed in male subjects than in females (26).

Symptoms of *Campylobacter* infection (campylobacteriosis) include diarrhea, abdominal cramping, abdominal pain, and fever. Humans are most often exposed to *Campylobacter* through contaminated milk or water and undercooked meat, specifically poultry. Once exposed, the pathogen will invade the lining of the intestinal tract and begin excreting toxins that break down the mucosal lining of the intestines and causing diarrhea (26). The diarrhea experienced can be bloody in nature; and can be accompanied by vomiting and nausea in some cases. These symptoms will appear between two and five days after adequate exposure occurs. These symptoms can last up to a week and usually are not life threatening unless the patient is elderly or immune-compromised (23).

Most of the time once a person has overcome the initial symptoms of campylobacteriosis, there are no long term consequences. However, in some rare instances, a *Campylobacter* infection may result in long term effects of arthritis and Guillain-Barré syndrome (GBS). GBS is a condition that causes the affected person's own immune system to attack the peripheral nervous system. First signs of this condition include tingling in the extremities or abnormal sensations that spread across the arms and upper body. Eventually, these sensations and paralysis will spread and intensify until certain muscle groups are no longer functioning. This condition can be life threatening if the affected person loses the ability to breathe or begins to experience abnormal heart rhythms and drastic changes in blood pressure (25). Even though this condition is relatively rare (estimated 1 in every 1,000 camplobacteriosis cases develop GBS) cases of GBS caused by a *Campylobacter* infection represent nearly half of all the reported cases in the United States (26).

Cross-contamination Potential

In the face of *Salmonella* and *Campylobacter* performance standards, the poultry industry is examining means to meet the requirements. One approach is to more strenuously exhaust all options to control cross contamination from broiler to broiler before processing or carcass to carcass once both enter a processing facility. When contaminated broilers enter a processing plant, *Salmonella* and *Campylobacter* can then spread throughout the plant during processing (42, 49). While there is potential for cross-contamination of product at any point along the processing line, there have been several "hot" zones identified in which pathogen presence may show noticeable spikes in prevalence if not properly controlled. These points include the scalder, picker, cropper, and chiller (40). Data has shown that processing increases contamination by

Salmonella and *Campylobacter* in studies comparing prevalence on the farm to prevalence on final products (49, 60).

A typical U.S. poultry processing plant consists of many highly automated and specific steps from start to finish. Birds arrive at scheduled times in large coops from which they are unloaded and hung on shackles. From hanging, live birds travel through stunning and throat cutting into the scalder after proper bleed out. Stunning is done to make the slaughter process easier and allow for more efficient processing. Birds are typically electrically stunned for a duration of 2-11 seconds which renders the birds unconscious for 60-90 seconds (65). From stunning the unconscious birds are conveyed directly into slaughter where the jugular vein and carotid arteries are cut and the broilers are bled out for a period of 55 to 120 seconds (9). Following bleed out, broiler carcasses enter an immersion scald tank. Carcasses are exposed to scalding water for various amounts of time and multiple scald tanks depending on the water temperature scheme used. The scalding process serves two major functions, microbial reduction and ease of feather removal from the chicken carcasses. The scald tank begins to denature the proteins of feather follicles, as well as expand the skin pores holding the feathers to allow for easier feather removal (72). Once out of the scald tank, birds enter the picker, which consist of rows of rotating, flexible, rubber picker "fingers" that spin at a high rate of speed rubbing the feathers from the surface of the chicken carcass (65). Feather pickers are often a major source of cross-contamination and physical damage. Levels of *Campylobacter* on the broiler skin have been shown to increase up to 3 logs during picking (13). Carcass bruising, wing breakage, broken hocks, all regularly occur at the picker, especially if the rubber fingers are worn down or positioned incorrectly (21, 51, 53, 54, 79, 82).

After feather removal, the carcasses enter the evisceration process. Carcasses can easily become fecally contaminated if birds entering the evisceration process have not undergone proper feed withdrawal, or the machinery in the evisceration line is not properly lined up (20). One such example of cross-contamination was reported by Lillard (45); Lillard found that the incidence of *Salmonella* spp. was 19% after bleeding, decreased to 11.9% after defeathering, but increased to 14.3% after evisceration.

Past the evisceration step all carcasses will enter a chiller system, either cold air chillers or submerged in a chill tank with chilled water (70). The primary goal of the chilling system is to lower the temperature of the broiler carcass to 4°C or less (65). When a chill tank is properly maintained, the chill tank can be one of the best overall microbiological quality control steps in a poultry processing line. However, if not maintained properly, the chill tank can be a source of cross-contamination. Several studies have shown that improper maintenance of a chill tank can cause increases in incidences of both *Salmonella* and *Campylobacter* from pre-chill to post-chill (80, 81). Poultry processing is a complicated process with many different opportunities for spread of pathogens. Currently, intervention strategies are being implemented at many different stages during processing in order to prevent or reduce cross-contamination spread of pathogens (11).

Current Intervention Strategies

In the late 1990's, when poultry processors were met with the challenges of pathogen reduction set forth by food safety regulations known as HACCP, water usage in plants increased dramatically (55). This dilution factor at the time was believed to be the "quick fix" to fecal contamination issues present in processing plants. Since this time water usage has dropped,

while pathogen levels still met the required safety regulations through the use of several innovative antimicrobials (80). In this way, just as regulations that the poultry industry must follow are continually being revised and changed, so is the poultry industry itself. Innovative intervention strategies and products are constantly being tested and developed. Antimicrobial intervention strategies have been applied at many different stages in the processing environment. These treatments rely on proper control of factors like pH, temperature, levels of all chemicals added, and even factors like proper personnel training and equipment adjustments over time. As of 2009, the three major chemicals used for microbial growth control in an immersion chilling process were chlorine in the form of hypochlorous acid (HClO), peracetic acid, and monochloramine. The three major chemicals used to manage pH within a chill system were carbon dioxide gas, citrus acid, and sulfuric acid (2). These are just a few of the many chemical interventions that have been evaluated, with mixed results. With the poultry industry regulations constantly changing, as well as potential pathogen adaptation to the wide array of antimicrobials used, there is a constant need for exploration into means to more effectively reduce pathogen contamination.

Chlorine Intervention

Hypochlorous acid is the traditional antimicrobial agent used to control contamination in chill tanks, and allowable at levels up to 0.005% by volume (76). Many different combinations involving manipulation of water levels and chlorine levels in the chill tank have been evaluated for their antimicrobial qualities (55). One major benefit of chlorine use is the effectiveness chlorine has at removing residual proteins as well as other visible physical contaminants such as soil or fecal material from the surface of a broiler carcass (56). However, this physical removal

ability is also a key problem with the use of chlorine, as once the active chlorine in a system has removed the organic materials present on the surface of a broiler carcass the active chlorine becomes inactive and bound by these organic materials; the more organic material present the less active/effective chlorine is left in the system. Chlorine by nature is a strong oxidant, binding to organic molecules happens very quickly, and once it has occurred it does not reverse (57). This is not an easy problem to overcome. For example, simply increasing the amount of chlorine added to the system does not appear to enhance the bacterial reduction capabilities of the chill tank (57). Increasing the amount of chlorine input into the chill system simply results in increased occurrence of toxic chlorine by-products and possible generation of toxic chlorine gas-off (58). Also, high organic loads and/or pH conditions above 7.0 have both been shown to greatly reduce the effectiveness of chlorine (44).

One way to maintain active chlorine in a chill system is to control build up of the organic load of a chill system. There are three main factors that determine the organic load of a chill system: water flow rate, the directional flow of the water in the chill system, and the overall cleanliness of the water being circulated. The USDA-FSIS requires water levels in a chill tank to be maintained at between 2.3-2.6 liters of fresh chill water per carcass (56). Most chillers are run in a counter-current fashion, where the carcasses are constantly moving into clean, cold water as they flow through the chiller system. Fresh make-up water is constantly being added to the chiller to ensure that the volume of the chiller does not decrease, and to attempt to maintain a level of water cleanliness. It has been shown that when the amount of fresh water flowing into a chill system drops below 1.9 liters (half a gallon) per carcass, organic matter will build up on the surfaces of the chill tank including the walls and the paddles (3). If all parameters are running properly, chill tanks help to reduce carcass temperature while simultaneously decreasing the

microbial load (32). Water and carcasses are both constantly moved through the chill tank system, in an effort to keep organic build-up to a controlled minimum.

Chlorine in a chill system may be found in several different forms and at various levels of each form based on the chemistry of the chill tank at that specific point in time. The key to having an effective chill process is to keep form and levels of chlorination as steady as possible, minimizing variability in the system. Free or available chlorine refers to the portion of the total chlorine in the system that has not reacted or bound with organic material, and is therefore "free' to react with bacteria and other contaminants. The microbial reduction effect of a chlorinated system is directly proportional to the free chlorine, present as hypochlorous acid (HOCl), concentration within the system. For example, recent fresh produce studies have shown if a wash system contains 10 ppm free chlorine (HOCl), elimination of *Salmonella* species from the produce surface will occur at approximately 120 minutes of exposure (69). If free chlorine within the system is increased to 50 ppm, time necessary for *Salmonella* elimination from the system drops significantly to under 6 minutes (69). This time and free chlorine concentration relationship is important for process validation of the efficiency of any wash or chill system employing chlorine as a cross-contamination control. If proper time and free chlorine concentration is not maintained incoming product will not achieve the proper microbial reduction, leading to the possible spread of harmful pathogens within the system. Bound chlorine is a less frequently used term that refers to the portion of chlorine in a system that has already reacted with some organic material, and is therefore not free to react with new contaminants (69). Total chlorine is a term that refers to all the chlorine within the chill system, a sum of both free chlorine and bound chlorine

The active form of chlorine formed within a chill system is hypochlorous acid, (68) the amount of which is greatly dependent on the pH of the chill system. The best pH range to ensure the greatest amount of active chlorine in the chill system is between 5.0-6.5, nearly all chlorine within the system will be in the active form at a pH of 5.0. As the pH of a chill system rises above 6.5 the amount of active hypochlorous acid present drops significantly with drastically less active chlorine present at even a slightly alkaline pH, for example 8.0 (44). Hypochlorous acid works to inactivate pathogens by penetrating the cell and reacting with cytoplasmic proteins within the cell, destroying cell structure and cellular metabolic functions. Even with all conditions present in a chill system necessary for optimum chlorine efficiency, roughly 50 ppm chlorine has been shown to have very little effectiveness at reducing the bacteria firmly attached to the surface, or entrapment within the skin, of a broiler carcass. One area of pathogen entrapment concern is the feather follicles after feathers are removed from the carcass. There are two steps in the attachment of a pathogen to the surface of broiler tissue; the first step is reversible, but the second stage of attachment is irreversible. Initial attachment of bacteria is dependent on the amount of bacteria present that gets trapped in the liquid film developing around the carcass during chilling. The second stage of attachment involves the formation of polysaccharide fibrils linking the bacteria to the surface of the broiler carcass; this second stage of attachment is a much stronger and more complex process. If caught in the earlier stage of attachment pathogens can be removed from the carcass to the surrounding chill water; once achieved chlorine at proper levels will eliminate virtually all bacteria present (46).

Therefore, one major issue to address with the use of chlorine is how to stabilize chlorine at proper levels, for long enough periods, within a chill system in the presence of an ever increasing amount of organic solids until the bacteria are “washed off” into the chill water.

Another issue that may arise is the continual influx of organic material into a chill tank (69). Introduction of new flocks of birds for processing can lead to a wide range of the amount of organic material that is present in a chill tank at any given time; however, the free chlorine of the system must be held fairly constant to achieve the necessary microbial effectiveness. This kind of situation can create the potential for bacterial pathogens to survive and cross-contaminate.

Organic Acid Intervention

Several antimicrobial alternatives to chlorination have been investigated including a range of organic acids. Organic acids including: acetic, propionic, citric, and formic have all been shown to be effective antimicrobials; however, they may cause negative flavor or color changes in the product (18, 29). Lactic acid has also undergone testing and has the ability to effectively control spoilage in food, specifically fermented foods, at relatively low concentrations without many of the adverse sensory effects experienced by other organic acid compounds at similar levels (48). However, as is the problem with most organic acids, increasing lactic acid concentrations to greater than 1.5%, a concentration in order to achieve greater microbial reduction can cause carcass discoloration (78).

The use of organic acids has been evaluated at multiple points along the processing line. Compared to other points where organic acids have been used in the chill tank seems to impart the least amount of undesirable side effects on the product such as skin discoloration (15). Therefore chilling is the main point of interest in the processing line for the addition of new antimicrobials. To help counteract possible discoloration, several organic acids may be used together or in conjunction with other antimicrobial agents, thus lowering the amount of each component necessary to reach the desired microbial reduction.

Several studies have shown combinations of different antimicrobial agents to be effective for treating broiler carcasses against contamination. Solutions of 1% acetic acid and 3% hydrogen peroxide were shown to be effective at reducing *Escherichia coli*, *Salmonella typhimurium*, and *Listeria innocua* on the surface of beef carcasses (19). Peracetic acid combined with hydrogen peroxide has shown ability to reduce *Salmonella* and *Campylobacter* contamination in poultry chill applications (7). Some of these chemical combinations have been highly effective, reducing *Campylobacter* numbers by as much as 5 logs in some cases (87). Similar strategies have been employed in the produce industry as a way to stabilize free chlorine levels against the presence of heavy organic loads.

Proprietary T-128 Blend

One formula for the stabilization of chlorine in solution was evaluated and found to be effective against *Escherichia coli* O157:H7 and *Salmonella* spp. This chemical formulation (T-128) also showed low to moderate ability to stabilize chlorine in the presence of high organic loads (58). However, this chemical blend has not yet been approved for use in poultry chillers. The main chemical component in T-128 is phosphoric acid, which inactivates pathogens by dropping the pH and affecting the metabolic functions of the pathogen exhausting the cells to death (58). Cells are constantly maintaining an ion homeostasis, when this homeostasis is disrupted the cell will begin to expend stored energy to get back to a homeostatic state (38, 72). The easiest way to produce this effect is pH manipulation; such is the case with T-128 and phosphoric acid additives.

A study was conducted to evaluate the chlorine stabilizing and microbiological reduction claims of T-128 when added to chlorinated produce rinses, specifically in fresh cut lettuce (58).

Results showed that the addition of T-128 to a chlorinated system offered slightly greater free chlorine retention over time in the presence of increased organic loads (58). The same type of study was conducted with specifically organic materials found in soil; the results were promising. Evaluation studies done with high soil organics, opposed to all organic matter, resulted in slightly greater retention of free chlorine. These results are applicable to the poultry industry as broilers coming into a plant for slaughter include varying levels of feces and soil.

The fresh cut produce study (58) also sought to test the bactericidal activities of T-128 alone, and when used in conjunction with proper chlorination. These researchers found that T-128 offers only weak bactericidal activity by itself, unless the concentration of T-128 is increased to a high level (2.5% concentration) or the contact time is extended greatly (58). However, when tests of pathogen inactivation were performed using both chlorine and T-128 together, drastic reduction in pathogen survival was detected, even at extremely high concentrations of organic materials and low measured chlorine levels present. The control treatments for these pathogen inactivation tests contained chlorine alone, and as the concentration of organic material was increased, significantly higher concentrations of bacterial survival were recorded (58).

The exact mechanism by which the addition of T-128 to a properly chlorinated system improves the efficacy of the wash solution against bacterial survival has not yet been determined. However, these results suggest that a synergistic effect exists between T-128 and chlorine in a wash system. Results from the fresh cut produce experiments show that T-128 is effective at pathogen control and cross-contamination reduction for produce. The application of T-128 to other industrial processes to deal with cross-contamination and chlorine stabilization issues could aid the poultry industry in their ongoing efforts to reduce levels of harmful pathogens in the food

chain and continue to meet industry standards as new legislation comes into full effect. Therefore, the objectives of the current study were to determine the effectiveness of T-128 at reducing cross-contamination of broiler carcasses with *Campylobacter* and *Salmonella* spp. as well as to evaluate the chlorine stabilization abilities of the chemical blend for possible use in conjunction with chlorine in poultry chillers. This study will provide the poultry processing industry with information relative to an intervention strategy for prevention and control of carcass cross-contamination with pathogenic bacteria.

CHAPTER 3

MATERIALS AND METHODS

Inoculum Preparation

Selected *Salmonella* and *Campylobacter* poultry isolates originally isolated in 1998-99 from the USDA Bacterial Epidemiology and Antimicrobial Resistance Research Unit (BEARRU) culture collection were used. These cultures have been adapted to be nalidixic acid and gentamicin resistant, respectively, and have been used in previous poultry-related projects reported by Berrang. Nalidixic acid-resistant *Salmonella* Typhimurium inoculums was prepared by streaking the frozen stock culture onto BG-Sulfa agar (Brilliant Green Agar with Sulfapyridine, Acumedia, Lansing, MI.) containing 200 ppm nalidixic acid. The nalidixic acid solution added to the BGS plates was prepared fresh for each batch of plates. One gram of nalidixic acid was dissolved in 50 mL of DI water and filter sterilized. From this 50 mL solution, 10 mL was added per liter of BGS media to attain the 200 ppm nalidixic acid level present in the plates. BGS plates were made fresh for each set of experiments. After incubation for 24 h at 35°C, *Salmonella* was scraped from the agar using a cotton tip swab and added to phosphate buffered saline (PBS) tubes. Gentamicin-resistant *Campylobacter jejuni* inoculum was prepared by streaking the frozen culture onto Campy-Cefex plates with lysed horse blood (50 mL/L) (Acumedia, Lansing, MI) with 33 ppm cefoperazone, 200 ppm cyclohexamide, and 200 ppm gentamicin (Sigma, St. Louis, MO.). The cefoperazone was prepared by dissolving 1 g of powder into 10 mL of DI water. After filter sterilization, 1 mL was added to every 3 L of media made. The cyclohexamide was prepared by dissolving 2 g into 10 mL of a 50/50 v/v

solution of DI water and 100% methanol. Once filter sterilized, 1 mL was added per 1 L of media. For the gentomicin, 1 g was dissolved in 50 mL of DI water. After filter sterilization, 10 mL of this solution was added per 1 L of media. Campy-Cefex plates were made fresh for each set of experiments. After incubation for 48 h at 42°C, the *Campylobacter* was scraped from the agar and transferred to PBS tubes. Stock solutions of 10^6 cells/mL were prepared. Optical density readings (Spectronic 20D+ spectrophotometer, Thermo Fisher Scientific, Waltham, MA) of (.25 and .60) at 540 nm yielded the desired concentrations of *Salmonella* and *Campylobacter*, respectively. Cell density was confirmed by plate counts. Before each of the inoculated experiments were performed, the optical densities of the prepared inoculations were checked and adjusted to closely match these previously established optical density readings. Once achieved, the inoculation vials were stored at refrigerated temperatures (≤ 4°C) until used to inoculate samples.

To inoculate each of the chicken wings used in bench-scale experiments, 10 µL of each of the pathogen solutions prepared was spot inoculated onto each wing using a micropipetor. The inoculums droplets were spread evenly over the surfaces of each inoculated wing using a sterile hockey stick. The wings remained at room temperature (25°C) for 10 min to allow for attachment of the pathogens to the surfaces of each wing before being added to the treatment containers.

For the inoculation of whole carcasses used in the pilot scale experiments, a cocktail of the pathogens was created by combining the *Salmonella* and *Campylobacter* inoculums. Ten µL of the cocktail was added to the feather tract on the breast skin of each carcass and spread over the surface, using a sterile hockey stick. Carcasses were held for 2 min at room temperature

(25°C) to allow for pathogen attachment before the carcasses were placed in the respective treatment containers.

Sampling and Enumeration

Sampling methods for the experiments followed the whole-carcass rinse method as outlined by the USDA Food Safety Inspection Service (FSIS) in the Microbiology Laboratory Guidebook for sampling, detection, and enumeration (77) with the following changes. Depending on the experiment broiler wings or carcasses were removed from the treatment containers and placed into sterile bags and rinsed with 30 mL of PBS for 60 s, or with 100 mL of sterile deionized (DI) water with the addition of 0.6 g sodium thiosulfate (granular anhydrous $Na_2S_2O_3$, J.T. Baker, Phillipsburg, NJ.), respectively. The wings were shaken by hand while the whole carcasses were shaken 12 carcasses at a time on a carcass shaker (1 section of 6 carcasses from each of the two experimental containers). This process was repeated until all carcasses were sampled. Rinsates were transferred to sterile containers and held on ice until further analysis. Thirty mL of the treatment solutions were taken directly from each treatment container and transferred to sterile sampling containers. These were also stored on ice until further analysis. The amount of rinsate used for the wings was less than that described in the USDA method. Previous work reported no effect on the recovery of *Salmonella* (27) from decreased carcass rinse volumes. Decreasing the volume of rinsate has even been shown to increase the recovery of *Campylobacter* (5) in carcass rinse methods.

For both *Salmonella* and *Campylobacter* serial dilutions of the collected samples were spread-plated on BGS (Brilliant Green Agar with Sulfapyridine, Acumedia, Lansing, MI.) with

200 ppm nalidixic acid and Campy-Cefex agar with 5% horse blood, 33 ppm cefoperazone, 200 ppm cyclohexamide, and 200 ppm gentamicin, respectively in duplicate (71).

Plates for *Salmonella* enumeration were incubated aerobically for 24 h at 35°C and those for *Campylobacter* for 48 h at 42°C. *Campylobacter* plates were incubated in a microaerophilic environment of 5% O_2, 10% CO_2, and 75% N_2 in a water-jacketed IR airflow automatic CO_2 incubator (NuAire, Plymouth, MN.). Populations were converted to $\log_{10}$ values and reported as log colony-forming units (CFU) per mL of rinse.

Bench-scale Experiments

Four different treatments were prepared. Treatment containers were filled with the correct ratio of broiler meat, ice, and treatment rinse to represent industry standards. These standards are to ensure that conditions within a chill tank never drop below a water to broiler meat ratio of one half gallons of rinse water for every pound of meat, and to ensure that the whole tank system stays below a temperature of 4°C throughout. Eight containers were prepared, each containing one fresh broiler wing, water, and ice in a weight to weight ratio of 1:2:4 chicken meat to water to ice. Two containers were assigned to each of four treatments. The four treatments were: control [distilled (DI) water no additive], 50 (ppm) chlorine (Clorox germicidal bleach, 6.15% sodium hypochlorite), 0.5% T-128 (by volume) (Smartwash Solutions LLC, Salinas, CA.), and a combination of 50 ppm chlorine and 0.5% T-128. Water temperature, pH, free chlorine, and total chlorine were measured at time 0 (before chilling) and after 45 min of chilling. Water temperatures and pH's were measured each time using the pHTestr 30 (Eutech Instruments, Vernon hills, IL.) pH meter and thermometer. Free and total chlorines were measured each time using a fixed wavelength, silicon detector colorimeter (HACH Pocket

Colorimeter II, Loveland, CO.) which measured mg/L Cl_2 in a test solution at two ranges, low range (0.02-2.00 mg/L Cl_2) and high range (0.10-8.00 mg/L Cl_2). For each experiment, chlorine level was tested and confirmed as approximately50 ppm. Chlorine in the test solutions may exist in two forms, free or total chlorine. Free chlorine is present in the solutions as hypochlorous acid or hypochlorite ions. The total chlorine represents a combination of all the chlorine containing compounds: monochloramine, dichloramine, nitrogen trichloride, and other chloro-derivatives. In the solutions tested, all the total chlorine products present are oxidized to iodine (I_2). Then the iodine and free chlorine are quantified by adding either a free or total chlorine DPD pouch (N,N-diethyl-p-phenylenediamine) (HACH, spectrophotometer DPD powder pillows, lower range or high range) forming a red solution. This red color intensity is proportional to the amount of free or total chlorine present in solution, depending on which DPD pouch is used. The intensity of the red solution was measured using the HACH Pocket Colorimeter, and readings were recorded as amounts of chlorine in parts per million. Also at time 0 and 45 min, one mL of chill water was placed on the surface of plate count agar (PCA, Difco). Plates were incubated for 24 h at 37°C and the resultant colonies were counted.

Ten replications, completed with noninoculated wings, were performed to evaluate the capability of the chemical additive to reduce the natural mcroflora present on processed broiler wings. A second set of ten replications were conducted with inoculated samples to test the effectiveness of the chemical additive (T-128) to lower numbers of *Salmonella* and *Campylobacter* on wings and in chill water. For the second set of experiments, for each replication of eight containers were prepared containing two broiler wings each, water, and ice in a weight to weight ratio of 1:2:4 chicken meat to water to ice. One of the two wings was inoculated on the skin with approximately 10^6 cells each of the antibiotic resistant strains of

Salmonella Typhimurium and *Campylobacter jejuni* prepared following methods previously described; the second wing was left uninoculated. Two containers were assigned to each of the previously described four rinse treatments. After 45 min of agitated chill treatment inside an orbital shaking incubator (Labline Instruments, Rockville, MD.) at 130 rpm, each wing, both uninoculated and inoculated, were removed and sampled as described previously. This was done to measure the transfer of each inocula from one wing to the other in various environments.

Acid Comparison Experiments

To evaluate the possibility that any reduction in pathogen levels could be simply due to lowered pH, another experiment consisting of ten replications was carried out to compare the effectiveness of the chemical additive (T-128) to its main acidic component, *ortho*-phosphoric acid, (Fisher Scientific, Fair Lawn, NJ.). Four containers were used, each containing two broiler wings, ice, and water in the appropriate ratio as described above. One of the two wings was spot inoculated with approximately 10^6 cells each of the antibiotic resistant strains of *Salmonella* and *Campylobacter* in the methods described previously; the second wing was left uninoculated. Treatment containers were assigned to each of two treatments: a combination of 50 ppm free chlorine (hypochlorous acid) and 0.5% T-128 by volume, and a combination of 50 ppm free chlorine (hypochlorous acid) and 0.01% H_3PO_4 by (v/v). All treatment containers were covered and shaken using an orbital shaking incubator at 130 rpm for 45 min. After this period of agitated chill treatment, each wing was sampled as described previously. The pH, free chlorine, and total chlorine, were measured both before and after the 45 min chill treatment, using methods previously described.

Pilot-Scale Experiments

Based on the previous results pilot-scale trials were conducted. Three replications of this scale-up were performed using two pilot-scale prototype four section paddle chillers. These chillers were fabricated and employed in previous poultry chill work performed by the Poultry Microbiological Safety Research Unit (PMSRU) and Bacterial Epidemiology and Antimicrobial Resistance Research Unit (BEARRU) at the USDA-ARS Russell Research Center (RRC) and are not for retail purchase. These paddle chillers were prepared by filling with approximately 75 L of water and 40 kg of ice. To the first paddle chiller, 50 ppm free chlorine was added to the chill water; and to the second paddle chiller both 50 ppm free chlorine and 0.5% by volume T-128 were added to the chill water. All of the ice, water, and chemicals were added once at the beginning of the 45 min chill period. This system was run for a period of 5 min before broiler carcasses were added. Chlorine, pH, and temperature levels were confirmed as previously described.

To each of these two chill systems 24 eviscerated and un-chilled broiler carcasses, obtained from a commercial plant, were added. Six of these 24 carcasses in each chiller were inoculated with approximately 10^6 cells each of both of the antibiotic resistant strains of *Salmonella* and *Campylobacter* following methods described previously. All carcasses (inoculated and un-inoculated) were tagged by attaching a colored zip-tie around the lower leg portion of each broiler carcass for identification. Red zip-ties were used to signify inoculated carcasses, and black zip-ties for un-inoculated carcasses. Carcasses were added to the chillers in groups of six carcasses per quarter section of the chiller; two non-contiguous quarter sections contained only un-inoculated carcasses, and the other two non-contiguous quarter sections contained three un-inoculated carcasses and three inoculated carcasses. The chill tanks were run

for a total of 45 min. Water pH, temperature, ppm free chlorine, and ppm total chlorine were measured at times: 0, 15, 30, and 45 min, using previously described methods. At each time interval, 500 mL of the chill water from each tank were filter concentrated, and plated on BGS agar with the addition of 200 ppm nalidixic acid and Campy-Cefex agar with the addition of gentamicin to detect levels of *Salmonella* and *Campylobacter* present in the chill water. The plates were incubated as described previously. After the 45-min chill treatment, each carcass was sampled as previously described. Figure 2 provides explanation of the experimental set-up utilized in pilot scale experiments.

In the pilot-scale experiments, the possible dilution of pathogens in the paddle chillers was taken into account. Direct plates were re-struck after undergoing enrichment in either buffered peptone water (BP; Acumedia, Lansing, MI.) or Bolton broth (*Campylobacter* enrichment broth (Med-Ox, Lafayette, LA.) that were inoculated at the same time of sampling, if the direct plates came back negative after the proper incubation. This measure was taken to make sure that samples negative for direct plating were truly negative for pathogens, not just the possibility that the amount of pathogen present was below the plating detection limits.

Statistical Analysis

All replications of the four experiments performed were performed with duplicates of each solution tested. Ten replications of the bench-top inoculated work and acid comparison work were done; 3 replications of the pilot-scale work. All data were entered into a spreadsheet program and statistical analysis performed. Results were recorded as least squares means using analysis of variance (ANOVA), student's T-test, or the Tukey's honestly significant difference (HSD) test. Since zero cannot be directly analyzed with these statistical models, a value

equivalent to 1.0 CFU was assigned to all 0 values. Significance was reported using a level of $P \leq 0.05$.

CHAPTER 4

RESULTS

Experiment 1: Affect of T-128 on Aerobic Bacteria

Mean numbers of total aerobic bacteria detected per mL of chill water after 45 min were: 191 colony forming units (CFU) in the control, 72 CFU in the chlorinated samples, 8 CFU and 8 CFU in the T-128 only and T-128 plus chlorine samples, respectively (Table 1). Even with a lower level of active chlorine at time 0 min, samples containing T-128 retained a greater amount of active chlorine over the 45 min chill treatment, and had significantly lower ($p \leq 0.05$) chill water plate counts compared to samples without the additive (Table 2). In fact, samples containing T-128 by itself resulted in lower bacterial numbers comparable to samples containing both T-128 and chlorine, and lower than samples containing only chlorine.

Experiment 2: Affect of T-128 on *Salmonella* and *Campylobacter*

After completion of the general experiments, organism specific reduction capabilities were evaluated. The most effective treatment for the reduction of *Salmonella* in chill water was the combination of 50 ppm chlorine and 0.5% v/v T-128 (Table 3). This treatment significantly reduced ($p \leq 0.05$) the *Salmonella* populations present in chill water by 1.67 logs compared to the control levels. When used separately, neither the 50 ppm chlorine nor the 0.5% T-128 treatments significantly reduced the populations in the chill water. Cross-contamination between the inoculated and uninoculated wings occurred although the population levels on the

uninoculated wings were significantly lower than the corresponding inoculated wings. For the uninoculated wings, the combination of 50 ppm chlorine and 0.5% T-128 was more effective in preventing cross-contamination from the inoculated wings and was able to significantly ($p \leq 0.05$) reduce the *Salmonella* populations (by 1.15 logs) compared to controls. The 0.5% T-128 treatment alone was the least effective at preventing cross-contamination of the wings. None of the treatments significantly reduced *Salmonella* contamination on the inoculated wings compared to the control.

All three treatments significantly ($p \leq 0.05$) reduced *Campylobacter* populations in chill water, with the 0.5% T-128 and 50 ppm chlorine plus 0.5% T-128 treatments being the most effective (Table 4). When used separately, both the 50 ppm chlorine and the 0.5% T-128 treatments significantly reduced the *Campylobacter* populations in the chill water; however the greatest amount of reduction was offered by the treatment solution containing both 50 ppm chlorine and 0.5% T-128. Cross-contamination between the inoculated and uninoculated wings occurred although the population levels on the uninoculated wings were significantly lower than the corresponding inoculated wings. For the uninoculated wings, the combination of 50 ppm chlorine and 0.5% T-128 was most effective in preventing cross-contamination from the inoculated wings, and was able to reduce *Campylobacter* populations present (by 2.05 logs) compared to controls. The 0.5% T-128 treatment alone also effectively prevented cross-contamination between inoculated wings and uninoculated wings, reducing *Campylobacter* populations (by 1.90 logs) compared to controls. The 50 ppm chlorine treatment alone was the least effective at preventing cross-contamination of the wings. None of the three treatments significantly reduced *Campylobacter* contamination on the inoculated wings compared to the control.

The pH was noticeably different for the T-128 and chlorine plus T-128 treatments (2.99-3.32) compared to the chlorine alone treatment (7.09-7.34) (Table 5) at both the initial time of washing and after the 45 min of washing. There was no measurable free or total chlorine in the T-128 alone treatments as expected. However, the chlorine levels in the combined chlorine plus T-128 treatment was similar to those in the chlorine alone treatment. Since the pH of the treatments containing T-128 was considerably lower than the chlorine alone treatment, the question of whether the reduction of *Salmonella* levels was greatly influenced by that factor was raised. This would explain why the more acid susceptible of the two organisms, *Campylobacter*, experienced the largest reduction in populations.

Experiment 3: Determination of pH Effect

There was no statistically significant difference in the number of *Salmonella* detected per mL of chill water between an acidic solution and T-128 solution; however, T-128 treatment resulted in significantly fewer *Campylobacter* per mL of chill water ($p \leq 0.05$). T-128 treatment also resulted in significantly fewer *Salmonella* and *Campylobacter* detected per mL from un-inoculated wing than the acid only treatment ($p \leq 0.05$). These results are summarized in Tables 6 and 7. Maintenance of chlorine activity, shown by the chemical measurements in Table 8, and the statistically significant bacterial results, suggests that T-128 used in conjunction with chlorine may have the ability to control cross contamination of poultry carcasses with human pathogens during immersion chilling by a greater chemical interaction than simply solution pH reduction. From these bench-top results a scaled up trial was undertaken.

Experiment 4: Pilot-Scale Test of T-128 plus Chlorine

Analysis of data generated by the three replications of scaled-up chill work support the earlier trends of T-128 addition to a system. Two tanks were compared, each containing 50 ppm chlorine; to one of the chill tanks the appropriate volume of T-128 treatment was added. Numbers of pathogens on carcasses in the T-128 treated tank show reduced numbers for *Salmonella* and *Campylobacter,* as well as a reduced number of positive plates from re-streaking for both organisms (Table 9). Re-streaking of plates was done from enrichment broths created from the test solution each time all dilutions of a carcass sample came back negative. This measure was taken to increase the chances of accounting for all organisms present in the samples. Earlier trends have shown that the addition of T-128 with chlorine provides roughly a 1 log greater reduction of *Salmonella* cross-contamination in a chill system when compared to the addition of chlorine to the system alone. The addition of T-128 to the chill tank system provides roughly a 2-3 log greater reduction of *Campylobacter* cross-contamination, through either direct physical contact from carcass to carcass or by transfer through the chill water, in a chill system than treating with chlorine alone. Addition of T-128 and chlorine to the chill system resulted in a reduction of *Salmonella* in the chill system, although not statistically significant compared to the addition of chlorine alone to the chill system. However, a statistically significant greater reduction in the number of *Campylobacter* was observed with the addition of T-128 and chlorine to the chill system compared to the addition of chlorine alone to the chill system. In addition to these results, the amount of organism transferred from section to section within the chill tank itself was reduced by the addition of T-128 to the chlorinated system, compared to a system containing only chlorine. Chemical measurements taken for the pilot scale tests are summarized in Table 10.

CHAPTER 5

DISCUSSION

The need for pathogen control in poultry processing, especially at potential cross-contamination points along the line, has created opportunity for new processing innovations to arise. Chemical antimicrobial additives are a key aid in pathogen reduction at points in processing where exposure and contact time are appropriate. In the current study, significant reduction in levels of *Campylobacter* and *Salmonella* present on test carcasses was shown after a 45 minute chill treatment with the addition of T-128 and 50 ppm chlorine. The addition of 50 ppm chlorine to the chill system offered a significant increase in pathogen reduction compared to no treatment as was expected based off previous chill study reports (48, 55, 83). The addition of T-128, at levels of 0.5% by volume, to the chill system in conjunction with 50 ppm chlorine resulted in significantly greater pathogen reduction over the addition of 50 ppm chlorine alone. This result follows previous trends reported on the employment of multiple chemical hurdles for pathogen control (8, 87). Results showing a greater reduction capacity for *Campylobacter* as opposed to *Salmonella* are in keeping with previous studies showing that *Campylobacter* has a greater susceptibility to chemical combinations (87). However, chlorine retention results from the current experiments show an ability to maintain a slightly elevated level of free, or active, chlorine in the presence of high organic loads within the chill tanks compared to a system with chlorine alone. These results could offer some alleviation to the issues inherently present with maintaining proper levels of active chlorine in a chill tank as influx of organic materials into the chill tank varies throughout flock to flock of broilers processed.

While carcasses undergo immersion chilling, bacteria are rinsed off of the carcass, and the number of bacteria found in the chill water seems to equilibrate (42). In the present experiments, chilling water samples averaged 3.2 and 3.8 log cfu/mL for *Salmonella* and *Campylobacter* respectively for the control (no antimicrobials added) samples. These numbers are similar to levels previously reported by Northcutt et al. (42). In the present study, the addition of 50 ppm chlorination caused significant reduction ($p \leq 0.05$) in both *Salmonella* and *Campylobacter* present in the chill water compared to the control solutions. Addition of 50 ppm chlorine also caused significant differences ($p \leq 0.05$) in the amount of these two pathogens recovered from uninoculated wings compared to the wings from the control solutions. These results agree with data presented in previous studies (46). Addition of T-128 alone to the system lead to reduction in the amounts of *Campylobacter* present in both the chill water itself and on the uninoculated wing that were significantly greater ($p \leq 0.05$) than the reduction caused by the addition of 50 ppm chlorine alone. However, the reduction in *Salmonella* was not shown to be significantly greater than reduction caused by the addition of 50 ppm chlorine alone. These reductions support findings of previous studies comparing organic acid antimicrobial solutions to chlorination (7, 19, 87). Addition of both T-128 and 50 ppm chlorine was shown to be the most effective treatment for both organisms tested, showing the greatest amount of reduction for each pathogen when compared to either chlorination alone or T-128 addition alone.

In the present study, a smaller volume of T-128 was required in conjunction with 50 ppm chlorine to achieve reduction results comparative to previously tested antimicrobial combinations (29). Addition of 0.5% by volume T-128 combined with 50 ppm chlorine showed a reduction of 1.25 logs for *Salmonella* and 2.1 logs for *Campylobacter*, which is comparable to the reductions offered by other tested antimicrobial applications. Organic acids such as acetic, propionic, citric,

formic, and lactic acids have all been shown to yield roughly a 1-2 log reduction when used at concentrations of around 1.5% by volume in chill systems (18, 29, 48, 78). Antimicrobial combinations such as 1% acetic acid and 3% hydrogen peroxide, or peracetic acid combined with hydrogen peroxide at similar levels have both been shown as effective interventions against *Escherichia coli*, *Salmonella*, *Listeria innocua,* and *Campylobacter* on meat surfaces (8, 19). The present study results show that a combination of T-128 and 50 ppm chlorine is comparably as effective to reduce *Salmonella* and *Campylobacter* as other previously evaluated antimicrobials at lower volumes within the system.

Most important to note from current results is the amount of reduction offered by combining T-128 with 50 ppm chlorine. Addition of T-128 alone offers reduction comparable to the addition of 50 ppm chlorine alone for *Salmonella*. Addition of T-128 alone results in a greater amount of reduction than addition of 50 ppm chlorination alone for *Campylobacter*. However, in both cases the greatest amount of reduction was obtained by employing both antimicrobials at once. This additive effect is more involved than simply combining an organic acid with chlorine, as was shown in the pH comparison results. The main acidic component of the T-128 blend is *ortho*-phosphoric acid. For both organisms tested, the T-128 plus 50 ppm solution caused a statistically greater reduction in pathogens recovered from both the chill water and the uninoculated wings than the *ortho*-phosphoric acid and 50 ppm chlorine solution. The *ortho*-phosphoric acid and chlorine solution was adjusted to mimic the pH of the T-128 and chlorine solution as closely as possible; therefore the two solutions should have approximately the same antimicrobial effect offered by a lowered pH alone. However, the solution containing T-128 outperformed the *ortho*-phosphoric acid solution for both pathogens tested.

Furthermore, the solution containing T-128 was able to retain an average of approximately 10 ppm free chlorine during the 45 min chill treatment, suggesting that there is stabilization of chlorine by T-128 with the presence of organic material that is not offered by reduction in the pH of the chill solution alone. The amount of reduction from T-128 addition for *Campylobacter* specifically, is not only statistically significant, but also biologically significant. Addition of T-128 with 50 ppm chlorine effectively reduced the amount of *Campylobacter* recovered both from the chill water and the uninoculated wings to below 1 log each from a starting inoculum average of 10^6 cells. This amount of reduction was much greater than the *ortho*-phosphoric acid plus chlorine solution. Increased pathogen reduction and chlorine retention suggest a complementary effect between T-128 and chlorine in the chill tank environment. The exact mechanism of interaction between T-128 and chlorine has not yet been determined, but has also been identified in previous research done on fresh cut produce (58).

An interaction effect between T-128 and chlorine was observed in every stage of the current experiments. Throughout the bench-top work with specific pathogens and organic acid comparison, free and total chlorine retention was observed at higher levels in solutions containing T-128. These observations add merit to the previous experimental results of T-128 evaluation in fresh-cut produce (58). Another notable trend was the amount of pH reduction caused by the addition of T-128 to a chill system. Even when used in conjunction with 50 ppm chlorine in solution T-128 addition to a chill system reduced the overall pH to less than 4. This acidification of the chill system would be beneficial in keeping the highest percentage of active chlorine (hypochlorous acid) in solution; nearly all chlorine in a system can be found in the active form at a pH at or below 5.0 (68). However, if the pH in the chill tank falls too low, product yield may drop from negative effects on the water holding capacity of the product. As

the pH of the broiler meat drops below 5.8-6.0 water holding capacity drops greatly, which could adversely affect further processing of the broiler products (86). Therefore, a balanced chill system with a high percentage of active chlorine, maintained at a pH range of 5.5-6.0, employing multiple antimicrobial hurdles is ideal for pathogen control.

Current results from the pilot scale experiments show microbial reduction trends that are in keeping with earlier bench-scale work. Addition of T-128 in the appropriate volume to the chill system resulted in statistically significant reduction of both *Salmonella* and *Campylobacter* transfer from inoculated carcasses to uninoculated carcasses. As well, the addition of T-128 to the chill system resulted in a significant amount of reduction of *Campylobacter* in the chill water itself; however, not for *Salmonella*. As was shown in earlier bench-scale work, the amount of *Campylobacter* was drastically reduced. However, during the scale-up experiments two results occurred that were unexpectedly contrasting from the trends established by previous results. First, the addition of T-128 to the chill system did not cause as drastic a reduction in the pH of the chill water. The pH of the chill system was reduced only to 6.0-6.2 instead of 3.8-4.0. This result is a positive change. The slightly acidic conditions at a pH around 6.0 are ideal for active chlorine retention in solution, while at the same time less likely to produce negative effects in the water holding properties or sensory qualities of the broiler meat. Secondly, the free chlorine retention trends that were experienced throughout the previous bench-scale work did not hold up through the scale-up experiments. Chemical results show far less than the 8-10 ppm free chlorine retained throughout earlier work. While the cause for this change is unknown, it may have something to do with the act of scaling up each of the test parameters. The relationship between T-128 and chlorine, which is still not fully understood, may have been unknowingly altered in some way when tested on a larger scale. Increased pathogen reduction for both

pathogens tested was still observed in scale up, even with both a pH shift and lack of apparent free chlorine retention occurring. Therefore, while these results may seem unfavorable, further research is needed before the relationship between T-128 and increased chlorine efficacy is fully understood.

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TABLE1. Aerobic plate counts (mean total CFU/mL of chill solution) before and after 45 min simulated chill of broiler wings[1]

Sample	Time (min)	Control	Chlorine only	T-128 only	T-128 + Cl
Chill water	0	604.3	8.4	6.0	2.9
Chill water	45	191.4	71.6	7.9	7.8

[1]Ten replications with duplicate chill vessels (n = 20)

TABLE 2. Chemical parameters of chill water used for 45 minute chill of broiler wings in total aerobic count study[1]

Measurement	Time(min)	Control	Chlorine only	T-128 only	T-128 + Cl
pH	0	7.06	7.36	2.68	3.35
	45	6.94	7.09	3.21	3.8
Free chlorine	0	0.3	41.5	0.0	29.4
	45	0.0	3.8	0.0	4.4
Total chlorine	0	0.3	54.4	0.0	36.8
	45	0.0	10.5	0.0	19.2

[1]Ten replications with duplicate chill vessels (n = 20)

TABLE 3. Populations of *Salmonella* (log CFU/mL) recovered from wing rinses after 45 min chill treatment with chlorine, T-128, or combined solution

Treatment	Chill water	Inoculated wing	Uninoculated wing
Control	3.23 ± 0.49^{A}	2.70 ± 0.38^{Ax}	2.25 ± 0.42^{Ay}
Chlorine	2.47 ± 0.50^{AB}	2.32 ± 0.37^{Ax}	1.68 ± 0.42^{ABy}
T-128	2.62 ± 0.50^{A}	2.43 ± 0.38^{Ax}	2.0 ± 0.42^{Ay}
T-128 + Cl	1.57 ± 0.49^{B}	2.0 ± 0.38^{Ax}	1.1 ± 0.42^{By}

$^{A-C}$Values within the columns with different superscripts are significantly different by Tukey's honestly significant difference (HSD) at $P < 0.05$.
$^{x-y}$Values within the rows and columns with different superscripts represent significantly different mean numbers of each organism by Student's *T* test at $P < 0.05$.

TABLE 4. Populations of *Campylobacter* (log CFU/mL) recovered from wing rinses after 45 min chill treatment with chlorine, T-128, or combined solution

Treatment	Chill water	Inoculated wing	Uninoculated wing
Control	3.75 ± 0.40^{A}	3.03 ± 0.48^{Ax}	2.79 ± 0.40^{Ax}
Chlorine	1.86 ± 0.41^{B}	2.79 ± 0.48^{Ax}	1.32 ± 0.40^{By}
T-128	0.86 ± 0.41^{C}	2.55 ± 0.48^{Ax}	0.89 ± 0.41^{By}
T-128 + Cl	0.59 ± 0.41^{C}	2.65 ± 0.48^{Ax}	0.74 ± 0.41^{By}

[A-C]Values within the columns with different superscripts are significantly different by Tukey's honestly significant difference (HSD) at $P < 0.05$.
[x-y]Values within the rows and columns with different superscripts represent significantly different mean numbers of each organism by Student's *T* test at $P < 0.05$.

TABLE 5. Chemical parameters of chill water used for 45 minute chill of broiler wings in bench-scale inoculated wing rinse study[1]

Measurement	Time (min)	Control	Chlorine only	T-128 only	T-128 + Cl
pH	0	6.97	7.34	2.99	3.59
	45	6.87	7.09	3.32	3.86
Free chlorine	0	0.0	51.9	0.0	50.5
	45	0.0	9.7	0.0	8.2
Total chlorine	0	0.0	53.4	0.0	52.4
	45	0.0	11.4	0.0	10.2

[1]Ten replications with duplicate chill vessels (n = 20)

TABLE 6. Populations of *Salmonella* (log CFU/mL) recovered from wing rinses after 45 min chlorinated chill treatment with T-128 or phosphoric acid

Treatment	T-128 + Chlorine[1]	H_3PO_4 + Chlorine[2]
Chill water	2.58±0.l7[A]	2.84±0.08[A]
Contact wing	1.56±0.28[B]	2.12±0.22[A]
Inoculated wing	2.35±0.17[A]	2.50±0.14[A]

[A-B]Values within the columns with different superscripts are significantly different by Tukey's honestly significant difference (HSD) at $P < 0.05$.
[1]45 min chill treatment with a solution consisting of 0.50% (by volume) T-128 and approximately 50 ppm chlorine.
[2]45 min chill treatment with a solution consisting of 0.01% (by volume) H_3PO_4 and approximately 50 ppm chlorine.
Mean number of bacteria (log cfu $\pm$ 95% confidence interval) recovered from either the chill water itself or 30 mL PBS rinses of the uninoculated wing. (n = 20)
Inoculated wing initially contaminated with approximately 10^6 cells of a marker strain of both *Salmonella* and *Campylobacter*. The broiler wings used in the experiment assumed to be free of any substantial level of either marked strain used before inoculation.

TABLE 7. Populations of *Campylobacter* (log CFU/mL) recovered from wing rinses after 45 min chlorinated chill treatment with T-128 or phosphoric acid

Treatment	T-128 + Chlorine[1]	H_3PO_4 + Chlorine[2]
Chill water	1.0 ± 0.33^{B}	2.72 ± 0.10^{A}
Contact wing	0.75 ± 0.36^{B}	2.07 ± 0.19^{A}
Inoculated wing	2.23 ± 0.22^{A}	2.79 ± 0.18^{A}

[A-B]Values within the columns with different superscripts are significantly different by Tukey's honestly significant difference (HSD) at $P < 0.05$.
[1]45 min chill treatment with a solution consisting of 0.50% (by volume) T-128 and approximately 50 ppm chlorine.
[2]45 min chill treatment with a solution consisting of 0.01% (by volume) H_3PO_4 and approximately 50 ppm chlorine.
Mean number of bacteria (log cfu $\pm$ 95% confidence interval) recovered from either the chill water itself or 30 mL PBS rinses of the uninoculated wing. (n = 20)
Inoculated wing initially contaminated with approximately 10^6 cells of a marker strain of both *Salmonella* and *Campylobacter*. The broiler wings used in the experiment assumed to be free of any substantial level of either marked strain used before inoculation.

TABLE 8. Chemical parameters of chill water used for 45 min chlorinated chill of broiler wings with either T-128 or H_3PO_4 for acid comparison study[1]

Measurement	Time (min)	H_3PO_4 + Chlorine	T-128 + Chlorine
pH	0	3.42	3.55
	45	5.87	6.07
Free chlorine	0	50.5	50.6
	45	0.1	9.1
Total chlorine	0	51.5	51.6
	45	0.3	10.1

[1]Ten replications with duplicate chill vessels (n = 20)

TABLE 9. Pathogen recovery from whole carcass rinses after 45 min chlorinated chill treatment with or without T-128 for pilot-scale study[1]

Section	Treatment	*Salmonella*	*Campylobacter*
1	Chlorine only	6/18	2/18
	T-128 + Chlorine	0/18	0/18
2[2]	Chlorine only	16/18	16/18
	T-128 + Chlorine	14/18	5/18
3	Chlorine only	6/18	6/18
	T-128 + Chlorine	3/18	0/18
4[2]	Chlorine only	17/18	13/18
	T-128 + Chlorine	10/18	6/18

[1]Positive carcass counts could come from either direct physical contact between inoculated and uninoculated carcasses, or from indirect contact between uninoculated carcasses and pathogens within the chill water.
[2]Sections two and four for each of the three runs contained inoculated carcasses.

TABLE 10. Chemical parameters of chill water used for 45 minute chlorinated chill of whole carcass with or without T-128 for pilot-scale study[1]

Measurement	Time (min)	Chlorine only	T-128 + Chlorine
pH	0	7.75	6.31
	45	7.16	6.20
Free chlorine	0	55.6	48.0
	45	2.0	1.0
Total chlorine	0	58.0	57.0
	45	40.0	25.0

[1]Three replications with duplicate chill vessels (n = 6)

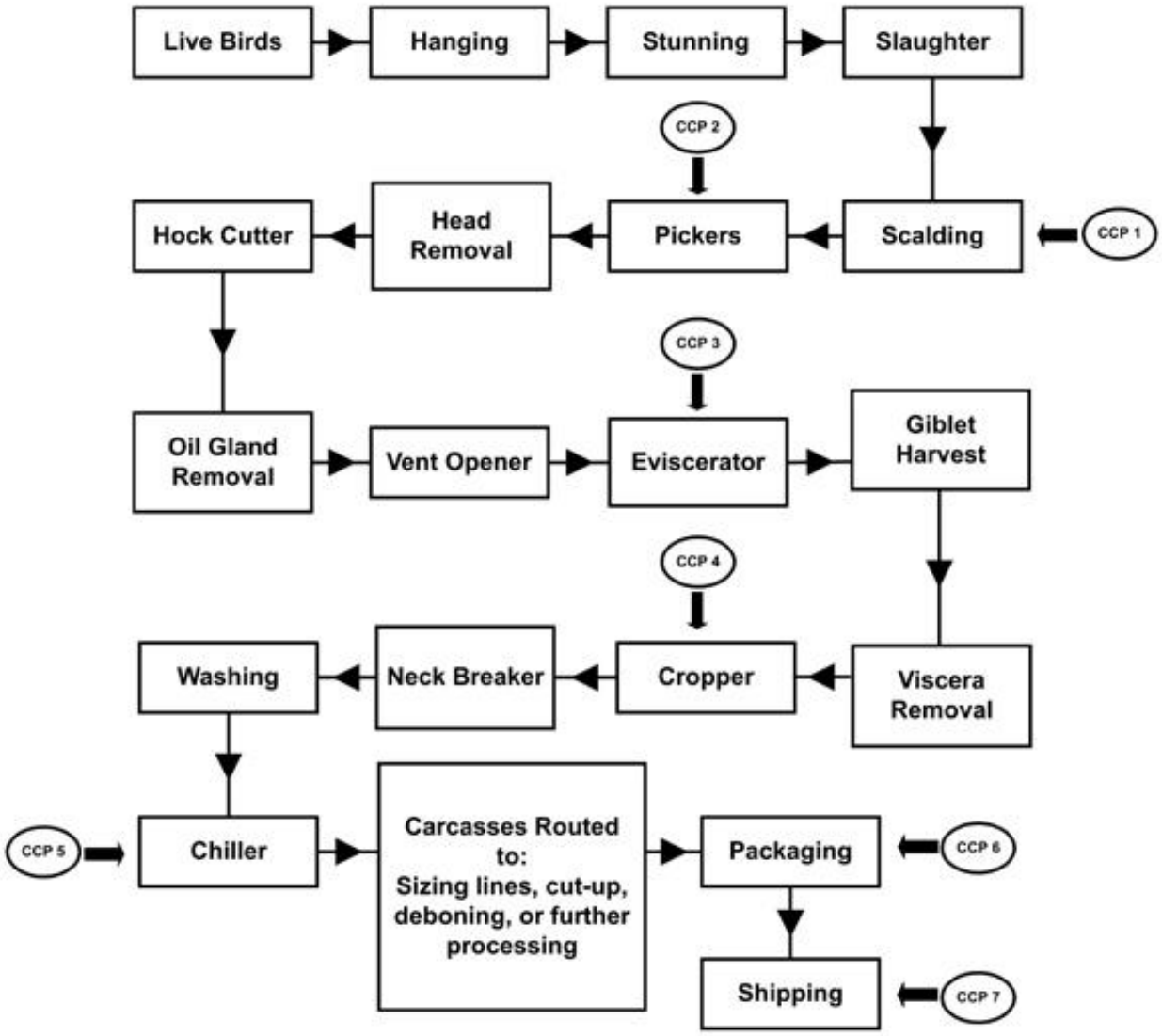

FIGURE 1. Flow diagram of typical commercial poultry slaughter plant (39)

Chiller Section 1	Chiller Section 2
6 uninoculated carcasses (white zip ties)	3 inoculated carcasses (red zip ties) 3 uninculated carcasses (green zip ties)
Chiller Section 4	**Chiller Section 3**
3 inoculated carcasses (red zip ties) 3 uninculated carcasses (green zip ties)	6 uninoculated carcasses (black zip ties)

FIGURE 2. Experimental four-sectioned paddle chiller layout for pilot-scale experiments Above is a diagram of the carcass set-up in each chill tank. Carcasses were banded around the leg with different colored zip ties for easy identification. Each different color specified a section in each tank or whether or not the carcass received inoculation.