MICROBIAL INTERVENTION STRATEGIES FOR *SALMONELLA* AND *CAMPYLOBACTER* REDUCTION IN COMMERCIAL TURKEY PROCESSING

A Thesis

by

SCOTT MICHAEL STEVENS

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

MASTER OF SCIENCE

May 2005

Major Subject: Poultry Science

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Approved as to style and content by:

David J. Caldwell (Chair of Committee)	Audrey P. McElroy (Member)
Allen Byrd (Member)	Gary Acuff (Member)
Alan R. Sams (Head of Department)	

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ABSTRACT

Microbial Intervention Strategies for *Salmonella* and *Campylobacter* Reduction in Commercial Turkey Processing. (May 2005)

Scott Michael Stevens, B.S., Texas A&M University

Chair of Advisory Committee: Dr. David J. Caldwell

One objective of the present investigation was to compare *Salmonella* and *Campylobacter* recovery incidence from commercially processed turkeys immediately prior to and following pre-chill and immersion chiller intervention strategies being used in three distinct turkey processing facilities. In each plant, on a single day of processing, 100 carcass rinse samples prior to and following each post-evisceration, pre-chill intervention and following immersion chilling were obtained for *Salmonella* and *Campylobacter* recovery. Two of three plants demonstrated a trend of decreased *Salmonella* on carcasses following the Inside Outside Bird Wash (IOBW), with reductions of 13%, and 11% being observed for Plants 1 and 2, respectively. Results for reductions of *Campylobacter* contamination were not as straightforward, with only Plant 3 showing decreased levels (11% reduction) following the IOBW. Plant 2 used an additional pre-chill intervention, a low pressure, acetic acid final wash, which was not shown to be effective in causing an additional reduction in either *Salmonella* or *Campylobacter* on carcasses. In all three plants, properly managed immersion chilling systems were the most effective microbial intervention for achieving *Salmonella* and

Campylobacter reduction on processed turkey carcasses. While not as effective, the IOBW present in each plant likely contributed to the effectiveness of immersion chiller interventions. If managed properly these intervention points have demonstrated themselves as a viable means to effectively reduce *Salmonella* and *Campylobacter* on processed turkeys.

Another objective was to modify the scalder environment to an alkaline pH and determine the effects of thermal killing of *Salmonella* and *Campylobacter*. In each plant, on a single day of processing, 50 carcass rinse samples prior to and following scald tank immersion and following feather removal were obtained for *Salmonella* and *Campylobacter* recovery. Modification of the scald water to alkaline conditions (pH 9-10) did not result in increased thermal killing of *Salmonella* or *Campylobacter* on turkey carcasses, as hypothesized before the investigation. Alkaline conditions are known to facilitate a more efficacious pluck and aid in the detachment of bacteria. Due to this, the bacteria that were recovered at these points on the processing line could have had an impact on the observed data.

DEDICATION

I would like to dedicate my work to my mother and father. No matter the circumstance, you have, are, and will always be there for me. Your support is amazing and never ending. I love you. Thank you for being the best two parents a boy could have asked for. You have enabled me to obtain my goals and made me who I am today. Thank you.

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CHAPTER I

INTRODUCTION

Salmonella and *Campylobacter* are the two predominant human food borne pathogens associated with poultry and poultry products. Together they account for 90% of the entire reported bacterial food borne illnesses world wide (Thorns, 2000). With the implementation of the USDA-FSIS mandated pathogen reduction program known as HACCP (Hazard Analysis Critical Control Program) in 1996, commercial poultry producers and processors were, for the first time, required to comply with performance standards for achieving reductions of *Salmonella* on processed broiler carcasses. HACCP was put in place to outline a specific sanitation operating procedure, dictate specific bacteriologic monitoring, establish pathogen performance standards for *Salmonella*, and require a system of preemptive controls to improve the safety of their products. The HACCP system identifies points of control and then requires the individual processing plants to monitor those points with the goal of producing a safer product. One of the overall objectives of the system was to reduce the numbers and incidence of food borne pathogens to reduce human food borne illness (Federal Register, 1996). HACCP, in conjunction with Foodborne Diseases Active Surveillance Network (FoodNet), which joined the efforts of USDA-FSIS, CDC, and FDA, has proven to be successful in the battle against food borne disease in the United States.

This thesis follows the style and format of Poultry Science.

Some factors which have led to the combined success of these pathogen reduction programs (HACCP and FoodNet) has been an increase in public awareness to the dangers of food borne illness, and expanded surveillance network for tracking food borne illness to the source of an outbreak, and an increased accountability on the side of the food producing industry to reduce the incidence of food borne pathogens on products destined for human consumption.

Campylobacter and *Salmonella* commonly represent the most frequently isolated bacteria from human gastroenteritis and both organisms are commonly associated with the consumption of undercooked or improperly handled poultry and poultry products. As such, both organisms, and their relationship with poultry, in both pre-harvest and post-harvest (processing) environments, are reasonable candidates for scientific studies. The economic ramifications of all human food borne illness in the U.S. are staggering with estimated costs ranging between 6.5-34.9 billion dollars annually (Buzby and Roberts, 1997). These costs are a result of nearly 76 million cases of human food borne illness, 325,000 hospitalizations, and 5,000 deaths in the United States alone (Mead et al., 1999). Although these data represent total food borne cases resulting from food animal origin, *Salmonella* and *Campylobacter* are two pathogens that both rank high in infection incidence. The fact that, despite the success of HACCP and the FoodNet systems, these recent estimates continue to be so substantial leads to conclusions that there is still a need for further investigation into successful intervention and reduction strategies for both of these pathogens in commercial poultry operations.

Broiler processing plants have long been used as the model for studies designed to investigate microbial interventions for processed poultry. Although many interventions identified in such investigation have dual application for both broiler and turkey processing, the degree of difference between both broilers and turkeys themselves, and the marked differences in the design and layout of the two processing environments, warrant individual research projects aimed exclusively at turkeys. Historically, the turkey industry has been disadvantaged due to the lack of scientific data related to their processing environment and the evaluation of successful intervention or reduction strategies. To this end, the present investigation focuses upon the evaluation of post-evisceration microbial reduction strategies in three distinct commercial turkey processing facilities in the United States. Research described in this Thesis was aimed at investigating the effectiveness of a post-evisceration, pre-immersion chilling microbial intervention that is very common to both broiler and commercial turkey processing plants, an inside-outside bird wash (IOBW) cabinet. Additionally, to extend previous findings from out laboratory, this investigation will also evaluate the effectiveness of IOBW cabinets as they contribute to the previously described successful microbial reduction strategy of using a properly managed immersion chilling system during commercial turkey processing.

An area of known bacterial reduction during commercial poultry processing is the thermal killing of bacteria on the exterior surface of the bird during scalding prior to feather removal. Several investigations performed with broiler carcasses have demonstrated that scalding at an alkaline pH (pH 9-10) resulted in a higher degree of

thermal killing of bacteria on carcasses. This potential pre-evisceration microbial reduction strategy was also evaluated in the present investigation with the hopes of coupling the potentially synergistic effect of high temperature during scald with unfavorable pH conditions to achieve greater thermal killing of pathogenic bacteria, specifically *Salmonella* and *Campylobacter* on turkeys.

Taking into consideration the lack of data related to microbial intervention and reduction in commercial turkey processing, the primary objective of this investigation was to look at incidence data relative to the combined use of a properly managed chilling system, IOBW spray cabinets, and altered pH scald conditions in three commercial turkey processing facilities to potentially identify effective microbial reduction strategies for reducing *Salmonella* and *Campylobacter* on commercially processed turkeys.

CHAPTER II

REVIEW OF LITERATURE

Introduction

The safety of our food supply in the United States continues to be a matter of paramount importance for federal and state regulatory agencies, researchers, health care professionals, and consumers. Reasons for such importance stem from food associated illnesses accounting for 6.9- 34.9 billion dollars in economic losses each year (Buzby and Roberts, 1997). These losses resulted from an estimated 76 million annual cases of food borne illness which resulted in 325,000 hospitalizations and 5,000 deaths (Mead et al., 1999). Using these estimates for incidence, approximately one-fourth of all Americans will contract a food borne disease during the next year (Tauxe, 2002). Of all food borne pathogens which have been implicated in human outbreaks, a few noteworthy organisms, including *Salmonella*, *Toxoplasma*, and *Campylobacter*, contribute to the bulk of the illnesses or deaths each year. When outbreak data is analyzed on a historical basis, it becomes apparent that throughout time, we have been able to identify, reduce, and eliminate many of these threatening pathogens. This is evident when looking at the pathogens of major concern in the 1900's: typhoid fever, tuberculosis, and brucellosis (Tauxe, 2002). Due to the impacts of food safety, there are currently 21 federally regulated agencies that are bound by approximately fifty regulations or guidelines, which enforce food safety compliance in the U.S. (Forsythe,

1996). These agencies annually spend approximately 200 million dollars in hopes of counter acting some of the burdens to society that food related illnesses cause.

HACCP is a specific governmental program that was established to identify, intervene, and where possible, eliminate food borne pathogens to reduce human food borne disease. HACCP is a preventive system for assuring safe production of food products by aiming to prevent, reduce, or control hazards in foods. The system accomplishes this by outlining specific sanitation procedures, dictating specific bacteriologic monitoring procedures, establishing performance standards for certain organisms including *Salmonella*, and requiring a system of preemptive controls to improve the safety of food products. These preventative systems, coupled with intervention steps at particular points within the processing plant, aim to not only reduce the spread of bacteria but to impede its growth. Within the first year of implementation, the HACCP system was able to reduce *Salmonella* incidence on broiler carcasses from a base line of 20 percent pre-HACCP to 10.9 percent after the first year of implementation (FSIS, 1998). Through the use of numerous microbial intervention strategies which include, but are not limited to, IOBW cabinets, immersion chill tanks, acid baths, and scald tank modification, microbial reduction on commercially processed poultry is indeed possible. Despite the proven success of described interventions to date, the established relationship of pathogenic bacteria to food animals warrants the continued development of new and effective strategies. Developing new strategies for the poultry processing environment is useful and merits the expenditure of resources.

Salmonella and *Campylobacter* are the two main bacterial pathogens associated with raw poultry. Together they account for approximately 90% of all reported bacterial food borne illness world wide (Thorns, 2000). *Campylobacter jejuni* and *C. coli* are thermophilic, gram negative, obligate microaerophilic bacteria that are ubiquitous in moderate environments (Newell and Fearnley, 2003). Colonization of turkeys with these thermophiles takes place in the grow-out environment, where they have been recovered from the air, litter, and drinking water in rearing and finishing houses (Kazwala et al., 1990). Transmission of the *Campylobacter* in poultry is still not very well understood. Although it appears that transmission can be accredited to many diverse horizontal sources, there are some recent studies that suggest that *Campylobacter* may in fact be transmitted vertically (Sahin et al., 2002). *Salmonella* is a gram negative bacillus that requires a facultative anaerobic environment, and is another food borne pathogen very frequently isolated from poultry and poultry products. Of the over 2,800 serovars, only 120 have been isolated as disease causing agents in poultry and humans. Transmission of *Salmonella* varies greatly and includes a multitude of vectors, and can clearly be transmitted vertically from hen to progeny.

The value of turkeys produced in 2003 was 2.72 billion, and despite being slightly lower from last year, the total number of turkeys produced did increase. There were 7.55 billion pounds produced in 2003 as compared to 7.49 billion pounds in 2002 (FSIS, 2004), indicating that despite a slight production increase, the overall value of turkey and turkey products was down. Despite this slight decrease in value, the turkey industry is a billion dollar industry and in years to come will likely continue to grow as

turkey producers continue to find new avenues and markets in which to include their products.

Salmonella and *Campylobacter* in Humans

As outlined by Buzby and Roberts (1997), there are seven major food borne pathogens of human health concern within the United States. These are: *C. jejuni*, *Clostridium perfringens*, *Escherichia coli* O157H7, *Listeria monocytogenes*, *Salmonella*, *Staphylococcus aureus*, *Toxoplasma gondii*. Together, these pathogens account for an estimated 3.3-12.3 million illnesses each year, and as many as 3,900 deaths (Buzby and Roberts, 1997). Due to their inclusion in this group, and coupled with their frequent connection with poultry and poultry products, *Campylobacter* and *Salmonella* are justifiably noteworthy pathogens which clearly represent a major food safety hazard. Numerous studies and reports identify poultry as the most common vehicle associated with human food borne illness related to these two specific pathogens (Mandrell and Wachtel, 1999; Notermans and Hoogenboom-Verdegaal, 1992; White et al., 1997).

Enteritis is the most common clinical presentation of human salmonellosis with symptoms that include nausea, vomiting, diarrhea, headache, myalgia, fever, and other systemic symptoms (Buck and Werker, 1998). Death may be a result of infection particularly in elderly, young, or immunocompromised patients. Of the over 2,800 *Salmonella* isolates, only about 120 have been isolated from enteric disease in humans, animals, and poultry. While many *Salmonella* serovars are host specific, such as *S. abortusequi*, *S. gallinarium*, and *S. typhi*, some have zoonotic potential and tend to have

a broad host range. Transmission of the salmonellae is many times a result of ingestion of fecally contaminated material. Depending on the degree of virulence, the host can either be asymptomatic or show manifestations of clinical infection. Human infection is linked in most cases to animals or animal products.

Salmonellae are gram negative bacilli serogrouped based on the antigenic formula for differing somatic (O) and flagellar (H) antigens. In serogroups that are host-adapted to humans there remains another component of antigenic makeup, a capsular Vi (virulence) antigen. This particular capsular antigen is not used to serotype *Salmonella*, but is very important as a virulence factor for the organism when it is used as a means to evade phagocytosis and serum complement activity (Robbins and Robbins, 1984). O antigens are denoted using Arabic numbers while the H antigen designation is a bit more complicated using small letters for differing phases. Within the differing serovars, there may be separate biovars that exhibit different biochemical characteristics. For such biochemical characterization, the organism produces acid and gas as an end product of carbohydrate fermentation. One species that remains an exception to this rule the human specific *S. typhi*. Salmonellae are facultative anaerobes that are non-hemolytic in nature and are lactose negative when cultured on MacConkey agar.

Given the extensive historical association of *Salmonella* with man and domestic animals, efforts to prevent exposure to reduce contamination has proven difficult throughout the past decades. *Salmonella* spp. are maintained within an animal population via asymptomatic carriers, symptomatic carriers, or environmentally in the feed or soil (Santamaria and Toranzos, 2003). Secondary contamination often occurs

between contaminated food and food handlers, who in turn then subsequently contaminate other food. Improper preparation and handling of food in homes and food establishments continues to be the primary vehicle associated with human outbreaks of salmonellosis. The only way to eradicate the pathogen would be to eliminate all animal and human reservoirs of *Salmonella.* Due to the fact that there are thousands of serovars with a host range just as baud, achieving this is highly unlikely (Patten, 1981). As a result of this, the importance of controlling this preventable disease should be stressed to the public. It seems plausible and more likely with organisms such as *S. typhi, S. gallinarium,* and *S. abortusequi,* in which there is only one reservoir species. In such species the infection has to come from contaminated food by that particular animal or from fecal material of another animal of the same origin.

The genus *Campylobacter* is comprised of 15 species, 12 of which are associated with human campylobacteriosis (Lastovica and le Roux, 2000). *Campylobacter* is the most common cause of bacterial enteritis in humans (Sahin et al., 2002), with an estimated 2.4 million cases occurring annually (Stern et al., 2001). *Campylobacter* has been isolated from the reproductive organs, intestinal tracts, and oral cavities of domestic animals and man alike. Symptoms of infection are similar to salmonellosis, but in many cases are less severe. Enteritis manifests itself in 3-5 days and usually lasts no longer than a week. The most common symptoms include fever, abdominal pain, and bloody diarrhea. The small intestine is the main site of infection, and infections range from mild enteritis to enterocolitis (Chowdhury, 1984). Transmission of the organism has been well documented to be through a fecal to oral route. The organism does not

grow well on foods and survival outside the host is limited. Despite the self-limiting nature of enteritis associated with infection, the infection dose per individual has been shown to vary due to differing levels of immunocompetency and health. Uniformly, the overall estimated dose effective for infection ranges between as low as 500 to 800 colony forming units (cfu)(Black et al., 1988; Wallis, 1994).

The ubiquity of *Campylobacter's* association with poultry is far reaching. The organism can be isolated from many sources in the poultry grow out environment (Newell and Fearnley, 2003). Further, it is present in the intestine of numerous food animal hosts where it usually lives commensally. The organism is a gram negative bacillus that has a characteristic corkscrew-like motion when viewed under normal phase microscopy and possesses one or more polar flagella. *Campylobacter* species have fastidious growth requirements, being microaerophilic in nature requiring an oxygen concentration of 3-15 percent and a carbon dioxide concentration of 3-5 percent. The organism is routinely cultured at 42 C on blood agar and has been shown to be oxidase-positive (Skirrow and Benjamin, 1980).

Campylobacter has only been recognized as a predominant human foodborne pathogen within the past 20 years (Mead et al., 1999). It was most likely always a foodborne pathogen. It was not recognized as such due to its unusual growth characteristics, requiring reduced oxygen levels, and an optimal growth temperature of 42 C. These conditions are not routine lab protocol, and therefore the pathogen was more than likely overlooked during routine diagnosis of an outbreak. One of the first U.S. outbreaks occurred in Vermont in the late 1970's and was traced to the water

supply (Vogt et al., 1982). At present, human campylobacteriosis is considered to be the leading foodborne illness in the United States (Sahin et al., 2002).

Several factors related to our fast paced society within the U.S. today predispose many individuals in our populations to be more at risk for food borne disease. High on this list is an increase in mass food preparation. This favors the spread of not only *Salmonella* and *Campylobacter*, but other pathogenic bacteria as well, by having more people supplied by a common food source. Additionally, a lapse of management or microbiological control within one processing facility on a single day of slaughter or processing represents the potential to contaminate meat products which are designated to feed thousands of individuals. Another factor that often contributes to food borne illness is the improper storage of food. In many cases temperature abuse is the leading cause of outbreaks, and accumulation of large volumes of improperly stored foods can have catastrophic ramifications. With the increase of globalization comes an increase in international trade. This is another factor contributing to the increases of food borne illness being observed (Buck and Werker, 1998). Loss of control over the HACCP system and critical control points becomes an issue when product is imported. Currently there is no system to assure that food animal production outside of our borders is as microbiologically safe as our own. Along with the improvement of general hygiene and overall standards of living has come a decrease in resistance to infection in the human population that is increasing in the number of elderly people (Notermans and Hoogenboom-Verdegaal, 1992). The less an individual is exposed to a microbe, the less resistant they become. The over use of anti-microbial substances and implementing the

most stringent sanitation procedures causes a lack of exposure to individuals and in tern lowers their overall resistance.

Salmonella and *Campylobacter* in Poultry

Salmonella in poultry can remain localized in the gastrointestional tract proving to be asymptomatic or cause varying degrees of enteritis or become generalized as a septicemia having effects on many different organs (Ekperigin and Nagaraja, 1998). These Salmonellae are commonly classified into three groups. The first group consists of the poultry host-adapted, pathogenic, non-motile salmonellae, *S. pullorum* and *S. gallinarum*. *S. pullorum* causes pullorum disease in chickens and *S. gallinarum* is responsible for fowl typhoid (Kwon et al., 2000). Fowl typhoid can be extremely pathogenic in young chickens with mortality being well over 50 percent (Lowry et al., 1999). With the help of vaccinations and new farm based intervention strategies, the two non-motile salmonellae have been eradicated from commercial poultry flocks within the U.S. for decades. The second group of salmonellae are known as the paratyphoid salmonellae and, although they are not known for causing disease in poultry, they contain the two leading serotypes that are responsible for human infection, *S. typhimurium*, and *S. enteritidis*. The third group of salmonellae is reserved for a pathogenic motile serotype of the subgenus *S. arizonae*. These particular salmonellae are commonly associated with causing infection in young turkey poults, where they are commonly isolated from the cecum. Antibiotics, or an early inoculation with a normal

flora that is *S. arizonae* free, have been shown to prevent disease in turkeys (Snoeyenbos et al., 1982).

Transmission of the thermophilic *Campylobacter*s in poultry remains somewhat less understood. At present, measures to develop control systems have been difficult due to the lack of knowledge concerning the source and mode of transmission (Stern et al., 2001). Colonization of turkeys with these thermophiles takes place in the grow out environment where they have been recovered from the air, litter, and drinking water (Kazwala et al., 1990). Although it appears that transmission can be traced to many diverse horizontal sources, there are some recent studies that suggest that *Campylobacter* may in fact be transmitted vertically (Sahin et al., 2002); (Cox et al., 2002). While many *Campylobacter*s are commensal organisms that do not affect the host, they have been observed to cause clinical symptoms of disease in newly hatched chicks two days post inoculation with *C. jejuni* (Sanyal et al., 1984).

Salmonella **and** ***Campylobacter*** **in the Processing Environment**

In 1996, a baseline study assessing the incidence of *Salmonella* on broilers following immersion chilling found carcass incidence to be approximately 20%. Largely due to the implementation of HACCP, the incidence of *Salmonella* on broilers has since dropped to 6.1% in 2001 (Bailey et al., 2001). *Campylobacter* incidence appears to be somewhat higher, with cecal incidence ranging from 60-100% (Wempe et al., 1983) in broilers. The post chill carcass incidence of *Campylobacter* is similarly higher than *Salmonella,* ranging from 20-40% (Stern et al., 2001). In certain instances

these numbers are a misrepresentation of an individual plant's data. The *Salmonella* and *Campylobacter* incidence is highly variable across the nation, differing due to geographic location, on-farm management, or practices of a specific processing facility. These differences can cause these averages to vary. Facilities that are not pro-actively seeking strategies to achieve bacterial reductions can have a large impact on the national averages.

A primary source of plant contamination with *Salmonella* and *Campylobacter* is through infected or intestinally colonized birds (McCapes et al., 1991). Parts of the process can be attributed to the increase in microbial recovery, such as de-feathering, which has been demonstrated to be a process that allows fecal material to escape the bird by way of the cloaca and contaminate the carcass or surrounding carcasses (Berrang et al., 2001). The immersion chill tank, if not managed properly, can also serve as a means to re-distribute bacteria to previously non-contaminated carcasses, leading to an increase bacterial recovery post-chill. Another source of plant contamination is the exterior surface of the bird. Feathers and skin can become contaminated, many times being due to live haul, and subsequently bring bacteria into the plant (Corry et al., 2002). Compounding these potentially hazardous steps in a slaughter process that deals with an extremely high number of animals that are killed on a daily basis at a very fast pace. Poor oversight of management practices in this case represents a potentially problematic situation. It is for this reason that the processing plant must play a pivotal role in the reduction of pathogenic bacteria on processed poultry and poultry carcasses.

Bacterial Attachment and Microbial Reduction Strategies

Understanding the chemical and physical means in which bacteria attach themselves could prove to be imperative for the derivation of new intervention strategies. Interactions between specific cell surface receptors and ligands on the carcass enable bacteria to attach themselves. These specific interactions are highly dependant on extrinsic and intrinsic factors including the tissue type, the particular bacteria, pH, temperature, contact time, and the type of liquid medium or environment in which attachment is occurring. All these factors must be considered when trying to understand bacterial attachment mechanisms and design of successful reduction strategies.

In the complicated process of a bacteria attaching to a tissue, many times the environment can play a vital role. If the surrounding conditions are not within a given range, the bacterium is unable to connect itself to its host. The extrinsic factors are those that which we have control over, and by altering these factors, bacterial reduction or inhibition can be achieved. Temperature, contact time, pH, and the suspension medium can all play a pivotal role in attachment of bacteria to a particular tissue. As a result of these factors being controllable, numerous scientific resources have been devoted to the expansion of knowledge in this particular field.

It has been demonstrated that bacteria posses the ability to use different attachment mechanisms at differing temperatures (Gorski et al., 2003). This adaptation allows the bacteria to maintain invasiveness in a wider range of values. The metabolic activity is adversely affected at lower temperatures, slowing and or stopping the movement of flagella (Holler et al., 1998). It is through this physical mechanism of the

bacteria that can bring forth means to reduce bacterial attachment to tissue. By lowering the temperature of the environment surrounding the bacteria, it is possible to slow and eventually stop the metabolism of the cell. In a study by Notermans and Kampelmacher it was shown that higher temperatures can enable the microorganisms that are attached to the skin to become more resistant to this harsh environment (Notermans and Kampelmacher, 1974).

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

While there exists areas in commercial poultry processing where the potential for the spread of microorganisms is high, the bird washers and immersion chill tank environments can be associated with pathogen reduction, thus presenting processing facilities with tools to successfully reduce bacterial numbers on finished poultry or poultry products. IOBW cabinets are one of the most prevalent pre-chill interventions and if managed properly, can be associated with microbial reductions. Currently,

individual turkey processing facilities differ in management practices surrounding the IOBW cabinets, using a variety of chemical interventions in addition to water only application. The majority of reports focusing on the effectiveness of individual additives for intervention have been preformed using broilers as a model, making it difficult to draw conclusions for the application of such interventions in the turkey processing environment. Chlorine is likely the most common antimicrobial compound utilized in most turkey and broiler plants. As such, it's very commonly used for potentially reducing *Salmonella* in a variety of bird washes. The factors that effect the successful attachment of a bacterium to carcass tissues such as the pH, water flow, temperature, and organic material present, also greatly affect the efficacy of chlorine as well in these and other applications. Other chemical disinfectants such as chlorine dioxide, sodium chlorite acidified with citric or phosphoric acid, and organic acids have also been shown to be successful in the pre-chill reduction of *Salmonella* and *Campylobacter* (Corry and Atabay, 2001). The use of such chemicals, however, as antimicrobials is not as prevalent when compared to chlorine. Bird washers that utilize antimicrobials have been reported to produce bacterial reductions on broiler carcasses ranging from 0.5 to 1.53 logs (Bashor et al., 2004). Individual companies, as well as individual plants within companies, differ in their management practices based on in-house testing of pathogen contamination on carcasses. To date, no "best management" practices exist for the optimized pathogen reduction at this particular point in the processing line.

The immersion chiller environment has also been identified as a critical point for additional bacterial reductions on poultry carcasses (James et al., 1992; White et al.,

1997; Acuff et al., 1986). Immersion chilling has been associated with reduction of aerobic plate counts by approximately 1.8 logs and *Campylobacter* reductions of 1.5 logs (Cason et al., 1997). The immersion chilling systems of processing facilities consist of large, open, ice cold, common water baths that operate under counter current flow and constant agitation. These common baths are required to effectively drop the carcass temperature to 4 C in 8 h for those birds weighing over 8 pounds (FSIS, 1999). Similar to IOBW cabinets, antimicrobial agents are routinely applied to the chill water to assist in the killing of bacteria. Mead and Thorns (1975) demonstrated a ten-fold reduction of fecal and spoilage bacteria on post-processed carcasses when a 20 ppm solution of chlorine was used in the plant (Mead et al., 1975). Similarly, other microbicides, such as ozone (Kim et al., 1999), and ClO_2 (Baran et al., 1973) have shown to be effective in the reduction of bacteria. While the effectiveness of Gas (Cl_2) and hypochlorite (OCl^-) forms of chlorination is highly dependant on the pH in the chiller, the efficacy of ClO_2 is not. Whatever the chosen microbicide used in the chiller, the proper management of the environment is pivotal for achieving reductions on finished carcasses.

The scald tank has been suggested to be a site of cross contamination of *Campylobacter* (Wempe et al., 1983), and *Salmonella* (Mulder et al., 1977). However, proper operation of scald tanks promotes a 2-3 log reduction in the number of *Campylobacter* found on carcasses (Izat et al., 1988). Following scalding, the picking and de-feathering area have also been established as areas of potential cross contamination. Rigby and Pettit determined that the occurrence of *Salmonella* on feather pickers corresponds to an increased contamination of carcasses as the end of the

processing cycle. This could be due to the rubber fingers possibly driving the microorganisms into the skin tissue and feather follicles or by the bacteria becoming aerosolized and contaminating the surrounding equipment in the de-feathering area (Bryan et al., 1968). This further emphasizes the need for optimization of scald tank management for reduction of pathogens prior to feather removal. Research by a group in the U.K. has identified the scald pH modification to alkaline conditions to be effective in decreasing the thermal death time for *Salmonella* on processed chicken carcasses. Even in scald conditions consisting of high levels of organic matter, elevating scald water pH to approximately 9 with sodium carbonate greatly increased *Salmonella* killing in both scald water and on the skin of processed broiler carcasses (Humphrey et al., 1981).

Speciation of *Campylobacter* Isolates Recovered From Poultry

Both *C. jejuni* and *C. coil* attach to cellular membranes and are incorporated into cytoplasmic vacuoles. The level at which the bacterium is able to attach and invade, coupled with the enterotoxin production, has been associated with pathogenicity. In support of this hypothesis, approximately 32% of *Campylobacter* strains isolated from humans with acute enteritis or from the gut of healthy laying hens were determined to be enterotoxigenic. Differences in enterotoxin production between *C. coil* and *C. jejuni* have been observed, with approximately 22% of *C. coli* and approximately 34% of *C. jejuni* being historically shown to be capable of enterotoxin production. These findings suggest that *C. jejuni* is often times more enterotoxigenic and possibly more virulent (Lindblom et al., 1989; Lindblom et al., 1990).

Kapperud and co-workers serotyped thermophillic *Campylobacters* on the basis of heat stable antigens identified by way of passive hemagglutination and found that of these thermophillic *Campylobacters* isolated from human cases of enteritis. In these cases, 86.6% were identified as *C. jejuni* and 13.4% were identified as *C coli* (Kapperud et al., 1984). Other studies have revealed similar findings, with *C.* jejuni being shown to be responsible for causing 89.3% of the cases of human enteritis while *C coil* was only shown to be related to the remaining 10.7% of clinical cases in humans (Kramer et al., 2000). When a similar characterization of recovered *Campylobacters* was preformed on commercial broiler farms, the trend of increased *C. jejuni* when compared to *C. coil* remained. In this investigation, *C jejuni* was isolated in 20.0% of the flocks and *C coil* was isolated in 4.7% of the flocks (Chuma et al., 1997). To date, there is limited literature available describing the in-plant incidence levels of *C coil* and *C. jejuni.* This contributes to the difficulty in tracing the epidemiology of the thermophillic *Campylobacter*s from the grow-out environment through processing and eventually to human infection.

Conclusions

Human food borne illness is the cause of between 6.9- 34.9 billion dollars in economic losses each year in the U.S. alone (Buzby and Roberts, 1997). These losses were calculated based on an estimated 76 million annual cases of food borne illness which resulted in 325,000 hospitalizations and 5,000 deaths (Mead et al., 1999). Using these estimates for incidence, approximately one-fourth of all Americans will contract a

food borne disease during the next year (Tauxe, 2002). The two major bacterial pathogens associated with bacterial food borne illness in this country each year are *Salmonella* and *Campylobacter*. Numerous studies and reports identify poultry as the most common vehicle associated with human food borne illness related to these two specific pathogens (Mandrell and Wachtel, 1999; Notermans and Hoogenboom-Verdegaal, 1992; White et al., 1997).

While some of the responsibility for controlling both *Salmonella* and *Campylobacter* on processed poultry and poultry products falls within the realm of grow-out of live production of both chickens and turkeys, the processing plant has borne the majority of responsibility when evaluated on a historical basis. While there exists areas in commercial poultry processing where the potential for the spread of microorganisms is high, the bird washers and immersion chill tank environments can be associated with pathogen reduction, thus presenting processing facilities with tools to successfully reduce bacterial numbers on finished poultry or poultry products. IOBW cabinets are one of the most prevalent pre-chill interventions and if managed properly, can be associated with microbial reductions. The immersion chiller environment has also been identified as a critical point for additional bacterial reductions on poultry carcasses (James et al., 1992; White et al., 1997).

Broiler processing plants have long been used as the model for studies designed to investigate microbial interventions for processed poultry. Although many interventions identified in such investigation have dual application for both broiler and turkey processing, the degree of difference between both broilers and turkeys

themselves, and the marked differences in the design and layout of the two processing environments, warrant individual research projects aimed exclusively at turkeys. Historically, the turkey industry has been disadvantaged due to the lack of scientific data related to their processing environment and the evaluation of successful intervention or reduction strategies. To this end, the present investigation focuses upon the evaluation of post-evisceration microbial reduction strategies in three distinct commercial turkey processing facilities in the United States. Research described in this thesis was aimed at investigating the effectiveness of a post-evisceration, pre-immersion chilling microbial intervention that is very common to both broiler and commercial turkey processing plants, an inside-outside bird wash (IOBW) cabinet. Additionally, to extend previous findings from out laboratory, this investigation will also evaluate the effectiveness of IOBW cabinets as they contribute to the previously described successful microbial reduction strategy of using a properly managed immersion chilling system during commercial turkey processing.

CHAPTER III

POST-EVISCERATION AND IMMERSION CHILLING MICROBIAL REDUCTION STRATEGIES FOR COMMERCIAL TURKEY PROCESSING

Introduction

Largely due to the ubiquity of many pathogens associated with poultry and the increasing awareness of food safety by regulatory agencies and the general public, there has been a larger responsibility placed on processing facilities within integrated poultry production for achieving pathogen reductions on retail poultry or poultry products. *Salmonella* and *Campylobacter* are the two main bacterial pathogens associated with raw poultry. Together they account for approximately 90% of all reported bacterial food borne illness world wide (Thorns, 2000). Based upon the frequency by which these two organisms are isolated from poultry and poultry products, *Campylobacter* and *Salmonella* are pathogens which clearly represent a major food safety hazard for the commercial poultry industry. To date, the majority of investigations into control measures or bacterial reduction strategies for *Salmonella* and *Campylobacter* in commercial poultry have been performed within the broiler processing environment.

In 1996, a baseline study assessing the incidence of *Salmonella* on broilers following immersion chilling found carcass incidence to be approximately 20% (FSIS, 1998). Largely due to the implementation of HACCP, the incidence of *Salmonella* on broilers has since dropped to 6.1% in 2001 (Bailey et al., 2001). *Campylobacter*

incidence appears to be somewhat higher, with cecal incidence ranging from 60-100% (Wempe et al., 1983) in broilers. The post chill carcass incidence of *Campylobacter* is similarly higher than *Salmonella* ranging from 20-40% (Stern et al., 2001). Without sound management of bacterial reduction strategies through the implementation of a HAACP program, the potential for the spread or amplification of microorganisms on carcasses in commercial poultry processing clearly exists. Through the use of sound management and a proven HACCP plan however, the use of bird washers and immersion chill tanks have been proven to result in pathogen reduction on carcasses. As such, processing facilities routinely use these tools to successfully reduce bacterial numbers on finished poultry or poultry products. Inside outside bird wash (IOBW) cabinets are one of the most prevalent pre-chill interventions found in commercial processing plants, and if managed properly, their use can be associated with microbial reduction. Bashor and co-workers showed that IOBW cabinets are successful in generating 1.5 logs reductions of *Campylobacter* on broiler carcasses (Bashor et al., 2004).

While used extensively in commercial turkey processing, marked differences in management practices surround IOBW cabinet use. Chlorine is the most common antimicrobial compound utilized in most turkey and broiler plants. As such, it's very commonly used for potentially reducing *Salmonella* in a variety of bird washes, including IOBW cabinets. The factors that affect the successful attachment of a bacterium to carcass tissues include the pH, water flow, temperature, and organic material present. These factors also greatly affect the efficacy of chlorine in these and

other applications. Other chemical disinfectants such as chlorine dioxide, sodium chlorite acidified with citric or phosphoric acid, and organic acids have also been shown to be successful in the pre-chill reduction of *Salmonella* and *Campylobacter* in broilers (Corry and Atabay, 2001). The use of such chemicals, however, as antimicrobials is not as prevalent as compared to chlorine. Given the different approach to using IOBW cabinets in turkey processing, "best management practices" currently do not exist for the optimized pathogen reduction at this particular point in the processing line for commercial turkey processors.

The immersion chiller environment has also been identified as a critical point for bacterial reductions on poultry carcasses (James et al., 1992; White et al., 1997). The immersion chilling systems of processing facilities consist of large, open, ice cold, common water baths that operate under counter current flow and constant agitation. These common baths are required to effectively drop the carcass temperature to 4 C in 8 h for birds weighing over 8 pounds (FSIS, 1999). Similar to IOBW cabinets, antimicrobial agents are routinely applied to the chill water to assist in the killing of bacteria. Mead co-workers (1975) demonstrated a ten-fold reduction of fecal and spoilage bacteria on post-processed carcasses when a 20 ppm solution of chlorine was used in the plant (Mead et al., 1975). Similarly, other microbicides, such as ozone (Kim et al., 1999), and ClO_2 (Baran et al., 1973) have been shown to be effective in the reduction of bacteria on carcasses during chilling. While the effectiveness of gas (Cl_2) and hypochlorite (OCl^-) forms of chlorination is highly dependant on the pH of chiller water, the efficacy of ClO_2 is not. Whatever the chosen microbicide used in the chiller,

the proper management of the environment is pivotal for achieving reductions on finished carcasses.

Previous studies conducted by our laboratories demonstrated that properly managed immersion chilling systems in commercial turkey processing are effective intervention tools for *Salmonella* and *Campylobacter* reductions on processed turkey carcasses. Additionally, broiler plant studies have demonstrated that if managed properly, the chiller can be a means to create an inhibitory environment for microorganisms, effectively reducing bacterial load on the finished carcasses. Many have assumed that these findings and observations in broiler processing plants can be directly extrapolated or extended to turkey plants. For this reason, little attention has been given to microbial intervention strategies within commercial turkey processing. Due to the major differences between the processing environments in broiler and turkey processing plants and the major differences in the live production or rearing environments between turkeys and broilers, we contend this argument is flawed. The objective of the present investigation was to evaluate the effectiveness of IOBW cabinets as they contribute to the previously described successful microbial reduction strategy of using a properly managed immersion chilling system during commercial turkey processing.

Materials and Methods

Overview

In this investigation we performed a microbial survey of three commercial turkey processing facilities with the objective of evaluating the effectiveness of intervention strategies for reducing *Salmonella* and *Campylobacter* on commercially processed turkeys. Sites of evaluation in this survey included prior to and following pre-chill and immersion chilling microbial reduction strategies. The plants participating in this investigation were selected since all were using both an immersion chilling system previously proven to be effective and an IOBW cabinet immediately prior to immersion chill. To determine the effectiveness of different interventions currently being used within commercial turkey processing, carcasses were rinsed for bacterial recovery at selected sites post-evisceration. The three individual processing facilities involved in this study were located in three geographically distinct areas of the United States. Table 1 provides an overview of the pre-chill and chiller interventions used in each facility.

TABLE 1. Pre-chill Interventions, Chiller Characteristics, and Management Practices.

Chiller Characteristic	Plant Identification		
	Plant 1	**Plant 2**	**Plant 3**
Chiller Type	Morris Drag Chillers	Morris Drag Chillers	Morris Drag Chillers
Type of Flow	Counter-Current	Counter- Current	Counter- Current
Number of Chillers	4: 2/line	3	3
Chiller Capacity*	15, 45	50	50, 6, 60
Dwell Time**	2:40	6:00	2:15
Volume/Bird Ratio***	1	1.25	1
Overflow Rate***	2	1.25	1
Intervention Strategy	ClO_2	ClO_2 in #3 Ozone Macron Loop	Cl_2 & NaOCl
pH Control System	N/A	N/A	N/A
Pre-Chill Intervention	Chlorinated IOBW	Chlorinated IOBW Chlorinated Transfer Table Acetic Acid Final Wash	Non-Chlorinated IOBW

*1,000 gallons, **hours, ***gallons/bird
ClO_2=chlorine dioxide, Cl_2=chlorine gas injection, NaOCl=sodium hypochlorite, IOBW-inside-outside bird wash

Carcass Rinse Sample Collection

Sample collection and culture of the recovered rinse fluid for the specific isolation of *Salmonella* throughout this study conformed to the FSIS proposed "Mega-Reg" guidelines for sampling and culture as described in the Federal Register (Federal Register, 1996), with slight modification. Sample collection and recovery of *Campylobacter* followed widely accepted protocols as described below. One hundred samples from both pre- and post-IOBW carcass rinses for determination of *Salmonella* and *Campylobacter* incidence and direct recovery from carcass rinse fluid were removed from the processing line in an alternating fashion between the pre- and post-sampling sites. Likewise, 100 post-chill carcass rinses for determination of *Salmonella* and *Campylobacter* incidence and direct recovery were removed in the same fashion. Plant 2 used an additional pre-chill intervention, a low pressure acetic acid wash cabinet immediately following the IOBW cabinet prior to immersion chilling. One hundred pre- and post- carcass rinse samples were also collected from this particular intervention in Plant 2.

Carcasses were removed from the above described locations with an individual pair of sterilized latex gloves and placed into a sterilized polypropylene bag for carcass rinsing. To rinse the individual carcasses, 200 mL of sterile buffered peptone water (BPW; pH 7.2) was added to each individual bag, and the carcasses were rinsed using inverted rotation 30 times each. Approximately one hundred mL of the carcass rinse fluid was then aseptically recovered by allowing the rinse fluid to accumulate in a corner of the rinse bag. The corner and a pair of scissors were sprayed with 70% alcohol,

wiped down, and the corner was then cut. The rinse fluid was then allowed to drain into a sterilized polypropylene collection bottle. All sample collection bottles were placed on wet ice and transported back to our laboratories for the initiation of bacteriologic culture, which was not initiated until 24 h had elapsed from the time of sample collection. The samples were treated equally and recovery incidence between the three sampling locations was compared. Transport-related effects on sensitivity were not a critical concern in the present investigation.

Collection of IOBW and Chill Tank Water

Chill water was sampled for the estimation of bacterial load in the chiller. Five chill water samples were aseptically collected from both the entrance and exit of the chiller prior to the initiation of immersion chilling. Likewise, five water samples were aseptically collected from the entrance and exit of the chiller following the completion of sampling. Approximately 100 mL of chill water was aseptically collected in a sterilized polypropylene collection bottle from the above sites for the specific isolation of *Salmonella*, C*ampylobacter*, and total aerobic plate count bacteria. Certain parameters were also measured to determine the effectiveness of management of the chiller environment. Chill water pH, total chlorine, free chlorine, or chlorine dioxide levels, as applicable, were measured at the entrance and exit of chillers during sampling. These measurements are presented with microbial recovery data below.

Collection of IOBW Water

IOBW water measurements were obtained to determine the effectiveness of management of the IOBW cabinets. The make-up water pH, chlorine level, and temperature were determined during sampling. Along with these measurements, pressure at which water was applied to carcasses within IOBW cabinets was also recorded.

Bacteriologic Culture

For the culture of *Campylobacter*, 30 mL of the recovered rinse fluid from each individual sample was combined and enriched in 30 mL of Bolton's complete enrichment medium for 24 h at 42 C. Following enrichment, each sample was streaked onto Campy-Cefex agar plating medium, and all plates were incubated for 48 h at 42 C, according to the protocol of Stern and co-workers (Stern et al., 1995). All *Campylobacter* culture procedures were conducted under a modified atmosphere containing 5% O_2, 10% CO_2, and 85% N_2 at 42 C. Suspect colonies were confirmed as *Campylobacter* by evaluating and observing both motility and morphology using normal phase contrast microscopy on a wet mount slide. Further speciation to either *C. coil* and *C. jejuni* isolated from collected samples was preformed using a PCR based test identifying divergence in the *ceu*E gene which encodes putative virulence determinates as described by Gonzalez and co-workers (Gonzalez et al., 1997) and specifically modified for such use by Hume and co-workers (Hume et al., 2002). Specific *ceu*E PCR primers (Integrated DNA technologies, Inc.) were used to confirm the specificities of *C.*

coil and *C. jejuni*. Data pertaining to speciation of recovered *Campylobacter* will be reported and discussed in Chapter V of this Thesis.

For the specific culture of *Salmonella*, 30 mL of the recovered rinse fluid was combined and pre-enriched in an additional 30 mL of BPW for 24 h at 37 C. Following pre-enrichment, 100 µl of each sample was sterilely transferred to a sterile culture tube containing 10 mL of Rappaport-Vassiliadis (RV) enrichment broth and incubated for 24 h at 42 C. Following this enrichment procedure, each sample was then streaked onto modified lysine iron agar (MLIA) plating medium for the specific recovery of *Salmonella*. Plates were incubated for 24 h at 37 C. Suspect colonies were confirmed biochemically (triple sugar iron and lysine iron slants) serologically using *Salmonella* o antisera (poly o; a-i)

To enumerate both *Salmonella* and *Campylobacter* recovered from the carcass rinse fluid, an additional 1mL of the rinse fluid from 20% of the carcass rinse samples was sterilely transferred to a polypropylene culture tube containing 9 mL of sterile Butterfield's solution. The suspension was then serially diluted (10X) by transferring 250 µl to an additional 3 tubes containing 2.25 mL of Butterfield's solution. From each dilution 100 µl was spread plated on to Tryptic soy agar, MLIA, or Campy-Cefex plates to allow for the specific determination of colony forming units (cfu) present in each sample. MILA plates and tryptic soy agar plates were incubated at 37 C for 24 h and Campy-Cefex plates were incubated under a modified atmosphere at 42 C for 48 h as described above. The number of cfu TPC bacteria, *Salmonella* or *Campylobacter*, recovered from each rinse sample was determined by manual counting of colonies

identified on respective plates at each dilution. All suspect colonies were confirmed serologically, biochemically, and by visual inspection as described above. Rinse fluid cfu from each rinse sample were converted to Log_{10} cfu/mL of recovered rinse fluid. Samples that were culture negative at the 1:10 dilution, but were positive following selective enrichment, were assigned a value of 1.5 Log_{10} cfu/mL. Samples that were culture negative at the 1:10, dilution and remained negative following selective enrichment, were assigned a Log_{10} cfu/mL value of zero.

Statistical Analysis

Differences in bacterial incidence (+/-) for *Salmonella* or *Campylobacter* recovered by carcass rinsing at specific sampling sites were compared using the Chi-square test of independence and significant ($P<0.05$) and highly significant ($P<0.001$) differences are reported.

Results

Plant 1

On the day of sampling during both respective sampling sets, IOBW cabinet chlorine levels were substantially below the plant's established target range. Total chlorine levels in IOBW make up water were measured at 1.065 or 1.045 for sampling sets 1 and 2, respectively.

These measurements were well below the plant established target range of 20 ppm. Free chlorine values of 0.85 ppm or 0.42 ppm, for sampling sets 1 and 2, respectively were also similarly low. Application pressure of make-up water applied in this IOBW cabinet was set to 110 psi and did not vary during the course of our sampling day.

Measurements obtained from water in the immersion chilling system revealed pH values were 7.97 and 7.40 for sampling sets 1 and 2, respectively. These slightly alkaline values however were not of concern to the management of this facility as pH does not affect ClO_2, the chosen chiller microbicide in this facility, efficacy. During the 1st sampling set of this day of processing, toward the beginning of the processing day, ClO_2 levels were substantially lower (0.196 ppm) than the established plant target range of 1-2 ppm. However a mid-shift change in the management of the application of the chiller ClO_2, yielded markedly higher levels (2.32 ppm) during the 2nd sampling set, bringing the ClO_2 level to slightly above the plant's established range. This mid-shift change in the management of the application of the ClO_2 was associated with substantial post-chill bacterial reductions during the 2nd sampling set (Table 2).

TABLE 2. Plant 1: Post-evisceration Incidence Recovery of *Salmonella* and *Campylobacter*. Recovery following selective enrichment of carcass-rinse samples taken before and after pre-chill and chiller microbial interventions.

Time of Sampling	***Salmonella* Recovery**			***Campylobacter* Recovery**		
	Pre-IOBW Incidence	**Post-IOBW Incidence**	**Post Chill**	**Pre-IOBW Incidence**	**Post-IOBW Incidence**	**Post Chill**
Sampling 1st Lot	29/50 (58.0%)	22/50 (44.0%)	11/50* (22.0%)	49/50 (98.0%)	50/50 (100%)	35/50** (70.0%)
Sampling 2nd Lot	19/50 (38.0%)	13/50 (26.0%)	2/50* (4.0%)	49/50 (98.0%)	49/50 (98.0%)	24/50** (48.0%)

*Represents a significantly ($P<0.05$) different recovery incidence when compared to pre-IOBW carcass incidence.
**Represents a highly significantly ($P<0.001$) different recovery incidence when compared to pre-IOBW carcass incidence.

Despite a suggestion of reduced recovery, *Salmonella* recovery following selective enrichment of rinse fluid was not significantly different during both sampling sets when comparing pre- and post- IOBW sites of sampling. Significant reductions were observed however following immersion chilling ($P<0.05$) during both sampling sets. Likewise, *Campylobacter* incidence after selective enrichment followed very similar trends to *Salmonella* recovery, in that differences were not significant between pre-and post- IOBW sampling sites, but significantly lower ($P<0.001$) following immersion chilling for both sampling sets (Table 2).

Direct recovery data for *Salmonella*, *Campylobacter*, and TPC bacteria are presented in Table 3. Direct recovery of *Salmonella* from recovered rinse fluid was reduced between pre-IOBW, post-IOBW, and post-chill sampling sites as the Log_{10} cfu/mL of recovered carcass rinse fluid decreased throughout each consecutive site. These trends of *Salmonella* reduction highlight the effectiveness of this plant's applied microbial intervention and management strategies. Similar to *Salmonella*, numbers of *Campylobacter* were also reduced throughout the consecutive sampling sites (Table 3). Enumeration of recovered total aerobic plate count (TPC) bacteria, while considerably higher when compared to direct recovery of both *Salmonella* and *Campylobacter*, was also shown to decrease between pre-IOBW, post-IOBW, and post-chill sampling sites (Table 3).

TABLE 3. Plant 1: Post-evisceration Bacterial Enumeration. Enumeration of recovered bacteria following dilution and direct plating of carcass-rinse samples taken before and after pre-chill and chiller microbial interventions.

Day/Time of Sampling	*Salmonella* CFU [1]			*Campylobacter* CFU [1]			Total Plate Count CFU [1]		
	Pre-IOBW	**Post-IOBW**	**Post Chill**	**Pre-IOBW**	**Post-IOBW**	**Post Chill**	**Pre-IOBW**	**Post-IOBW**	**Post Chill**
Sampling 1st Lot	0.9 / (0/10) 0%	0.6 / (0/10) 0%	0.3 / (0/10) 0%	1.3 / (6/10) 60.0%	1.5 / (5/10) 50.0%	1.2 / (0/10) 0%	4.2 / (10/10) 100%	4.1 / (10/10) 100%	3.4 / (10/10) 100%
Sampling 2nd Lot	0.6 / (0/10) 0%	0.4 / (0/10) 0%	0.0 / (0/10) 0%	1.6 / (5/10) 50.0%	1.1 / (6/10) 60.0%	0.6 / (0/10) 0%	4.3 / (10/10) 100%	3.7 / (10/10) 100%	2.9 / (10/10) 100%

[1]Log_{10} cfu/ml rinse fluid/carcass // incidence of cfu positives via direct plating

Plant 2

Similar to Plant 1, on the day of sampling in this facility, IOBW cabinet chlorine levels were substantially below the plant's target range during both sampling sets. Total chlorine levels in IOBW make up water were measured at 0.55 ppm or 0.68 ppm for sampling sets 1 and 2, respectively. Both values were clearly well below the plant established target range of 20 ppm. Free chlorine values of 0.07 ppm or 0.08 ppm, for sampling sets 1 and 2, respectively were also quite low. Application pressure of make-up water applied in this IOBW cabinet was set to 120 psi and did not vary during the course of our sampling day. Measurements obtained from water in the immersion chilling system revealed pH values were 8.76 and 8.4 for sampling sets 1 and 2, respectively, during our sampling day. Similar to Plant 1, this facility was using ClO_2 as its primary chiller microbial intervention strategy, and as such this slightly alkaline pH did not interfere with ClO_2 efficacy. In both sampling sets during this day of processing, ClO_2 levels were substantially lower than the established plant target range of 1-2 ppm. These values were measured at 0.106 ppm in the 1st sampling set and 0.04 ppm during the 2nd sampling set. This facility also used an additional post-evisceration / pre-chill microbial intervention: a low pressure, acetic acid wash cabinet, where a 1% acetic acid water solution was sprayed on carcasses prior to immersion chilling. Measurement of pH from the make up water in this cabinet was invariable and consistently low (pH of 2.4) suggesting that the cabinet was working and being managed properly at the time of sampling.

Salmonella recovery incidence following selective enrichment of recovered rinse fluid was apparently reduced throughout the consecutive sampling sites between pre-IOBW and post-chill sampling sites during the 1st sampling set (Table 4). When compared to pre-IOBW incidence during the 1st sampling set, post-chill *Salmonella* incidence was significantly lower ($P<0.05$). A much overall lower burden of *Salmonella* on carcasses at this stage of processing led to much lower and relatively unchanged recovery incidence between pre-IOBW and post-acid / pre-chill sampling sites (Table 4). When comparing post-chill *Salmonella* incidence to pre-IOBW incidence in the 1st sampling set, it was significantly higher ($P<0.05$), but remained below a 20% total carcass incidence (Table 4). Surprisingly *Campylobacter* recovery incidence was extremely low during both sampling sets, and the only significant difference observed in recovery was a significant increase ($P<0.05$) in post-chill *Campylobacter* incidence during the 1st sampling set (Table 4). This suggests that this particular plant, on this day of processing, did not deal with a high burden of *Campylobacter* on carcasses or within the plant.

Direct recovery of *Salmonella* from the collected rinse fluid was consistently low and essentially equivalent throughout the consecutive sampling sites. Following a similar pattern, *Campylobacter* direct recovery remained consistently low and largely invariable among all sampling sites (Table 5). TPC bacterial recovery from carcass rinse fluid among sampling sites were slightly variable and mostly unchanged during both sampling sets (Table 5).

TABLE 4. Plant 2: Post-evisceration Incidence Recovery of *Salmonella* and *Campylobacter*. Recovery following selective enrichment of carcass-rinse samples taken before and after pre-chill and chiller microbial interventions.

Day/Time of Sampling	***Salmonella* Recovery**				***Campylobacter* Recovery**			
	Pre-IOBW	**Post-IOBW**	**Post Acid**	**Post Chill**	**Pre-IOBW**	**Post-IOBW**	**Post Acid**	**Post Chill**
Sampling 1st Lot	13/50 (26.0%)	2/25 (8.0%)	2/25 (8.0%)	3/50* (6.0%)	0/50 (0%)	0/25 (0%)	0/25 (0%)	11/50* (22.0%)
Sampling 2nd Lot	2/50 (4.0%)	2/75 (2.7%)	7/75 (9.3%)	8/50* (16.0%)	0/50 (0%)	3/75 (4.0%)	2/75 (2.7%)	1/50 (2.0%)

*Represents a significantly (P<.05) different recovery incidence when compared to pre-IOBW carcass incidence.

TABLE 5. Plant 2: Post-evisceration Bacterial Enumeration. Enumeration of recovered bacteria following dilution and direct plating of carcass-rinse samples taken before and after pre-chill and chiller microbial interventions.

Time of Sampling	Salmonella cfu [1]				*Campylobacter* cfu [1]				Total Plate Count cfu [1]			
	Pre-IOBW	Post-IOBW	Post Acid	Post Chill	Pre-IOBW	Post-IOBW	Post Acid	Post Chill	Pre-IOBW	Post-IOBW	Post Acid	Post Chill
1st Sampling	0.4 / (0/10) 0%	0.0 / (0/5) 0%	0.3 / (0/5) 0%	0.1 / (0/10) 0%	0.0 / (0/10) 0%	0.0 / (0/5) 0%	0.0 / (0/5) 0%	0.3 / (0/10) 0%	4.1 (10/10) 100%	5.0 (10/10) 100%	4.1 (10/10) 100%	3.9 (10/10) 100%
2nd Sampling	0.0 / (0/10) 0%	0.0 / (0/15) 0%	0.1 / (0/15) 0%	0.1 / (0/10) 0%	0.0 / (0/10) 0%	0.1 / (1/15) 6.7%	0.1 / (1/15) 6.7%	0.1 / (0/10) 0%	4.5 (10/10) 100%	4.9 (10/10) 100%	4.0 (10/10) 100%	5.5 (10/10) 100%

[1] Log10 cfu/ml rinse fluid/carcass // incidence of cfu positives via direct plating

Plant 3

This facility did not apply chlorine to the IOBW cabinet make-up water and operated the cabinet at a slightly higher application pressure of 125 psi. With measurements of 15.6 ppm and 12.07 ppm for the 1st and 2nd sampling sets, respectively, total chlorine levels in the third and only chlorinated chiller of this plant's immersion chilling system were slightly below the plant established range (20-30 ppm). Despite this lower total chlorine level and elevated pH measurements (8.16 and 7.9 for sampling sets 1 and 2, respectively), free chlorine levels were measured at relatively high levels (6.86 ppm) during the 1st sampling set. In the 2nd sampling set, presumably due to a build up of organic material in chiller water later in the processing day, this value fell considerably (0.133 ppm). Not surprisingly, 1st and 2nd sampling set free chlorine values can be directly linked to effective reductions in post-chill carcass *Salmonella* incidence, as substantial bacterial reductions on processed carcasses were only observed during the 1st sampling set on this day of processing (Table 6).

With the elevated free chlorine levels measured in immersion chilling water during the 1st sampling set, post-chill incidence of *Salmonella* positive carcasses was significantly ($P<0.001$) reduced when compared to pre-IOBW incidence level. With the fall of free chlorine levels in the final chiller later in the processing day, *Salmonella* carcass incidence following selective enrichment did not differ between pre-IOBW, post-IOBW, and post-chill sites during the 2nd sampling set. However, *Campylobacter* recovery following selective enrichment of collected rinse fluid appeared to decrease

during the consecutive sampling sites and was significantly lower ($P<0.001$; $P<0.05$) when comparing pre-IOBW to post-chill incidence during both sampling sets (Table 6).

Corresponding to selective enrichment, direct recovery of *Salmonella* from collected rinse fluid remained low, but was observed to demonstrate consecutive reductions throughout the consecutive sampling sites from pre-IOBW, post-IOBW, to post-chill in the 1st sampling set. However, the 2nd sampling set did not demonstrate such reductions (Table 7), following the trend of incidence seen for *Salmonella* following selective enrichment. Direct recovery of *Campylobacter* followed similar trends to that of *Salmonella*, showing consecutive reductions throughout the sampling sites (Table 7). Recovery of TPC bacteria did not differ between pre- and post-IOBW sampling sites but was reduced by chilling in the 1st sampling set. During the 2nd sampling set, bacterial numbers were mostly invariable and unchanged among all sampling stages of processing (Table 7).

TABLE 6. Plant 3: Post-evisceration Incidence Recovery of *Salmonella* and *Campylobacter*. Recovery following selective enrichment of carcass-rinse samples taken before and after pre-chill and chiller microbial interventions.

Time of Sampling	***Salmonella* Recovery**			***Campylobacter* Recovery**		
	Pre-IOBW Incidence	**Post-IOBW Incidence**	**Post Chill**	**Pre-IOBW Incidence**	**Post-IOBW Incidence**	**Post Chill**
Sampling 1st Lot	17/50 (34.0%)	15/50 (30.0%)	3/50** (6.0%)	23/50 (46.0%)	19/50 (38.0%)	5/50** (10.0%)
Sampling 2nd Lot	20/50 (40.0%)	24/50 (48.0%)	23/50 (46.0%)	12/50 (24.0%)	5/50 (10.0%)	1/50* (2.0%)

*Represents a significantly (P<.05) lower recovery incidence when compared to the pre-IOBW incidence level.
**Represents a highly significantly (P<.001) lower recovery incidence when compared to the pre-IOBW incidence level.

TABLE 7. Plant 3: Post-evisceration Bacterial Enumeration. Enumeration of recovered bacteria following dilution and direct plating of carcass-rinse samples taken before and after pre-chill and chiller microbial interventions.

Time of Sampling	*Salmonella* CFU [1]			*Campylobacter* CFU [1]			Total Plate Count CFU [1]		
	Pre-IOBW	**Post-IOBW**	**Post Chill**	**Pre-IOBW**	**Post-IOBW**	**Post Chill**	**Pre-IOBW**	**Post-IOBW**	**Post Chill**
Sampling 1st Lot	0.6 / (1/10) 10.0%	0.4 / (0/10) 0%	0.1 / (0/10) 0%	2.3 / (10/10) 100%	1.5 / (10/10) 100%	0.1 / (0/10) 0%	4.4 / (10/10) 100%	4.7 / (10/10) 100%	3.4 / (10/10) 100%
Sampling 2nd Lot	0.4 / (4/10) 40.0%	0.5 / (1/10) 10.0%	0.9 / (1/10) 0%	0.5 / (5/10) 50.0%	0.1 / (3/10) 30.0%	0.1 / (1/10) 10.0%	4.7 / (10/10) 100%	4.3 / (10/10) 100%	4.2 / (10/10) 100%

[1] Log_{10} cfu/ml rinse fluid/carcass // incidence of cfu positives via direct plating

Discussion

While evaluating post-evisceration, pre chill microbial interventions in place in the three commercial facilities participating in the current investigation, the most common post-evisceration microbial intervention used was an IOBW cabinet. Despite poor management of the chlorine levels in IOBW cabinets in Plants 1 and 2, all three plants were associated with a trend of decrease for *Salmonella* recovery on carcasses following the IOBW. However the results for *Campylobacter* were not as straightforward, with only Plant 3 demonstrating reductions following the IOBW. As described above, Plant 2 used an additional post-evisceration / pre-chill intervention: a low pressure acetic acid final wash cabinet that we also evaluated in this study. Despite the apparent appropriate application of the acetic acid in this cabinet, data collected before and after this particular intervention did demonstrate an additional benefit, in terms of bacterial reduction for either *Salmonella* or *Campylobacter* by the use of this cabinet. These findings, which represent potentially the first recorded within commercial turkey processing facilities, are mostly in agreement with the findings of Bashor and co-workers (2004) when they showed that IOBW cabinets are successful in generating 1.5 log reductions of *Campylobacter* on broiler carcasses. The fact that our observations suggest greater effectiveness at achieving *Salmonella* reductions on processed turkey carcasses, when Bashor and co-workers (2004) report reductions for *Campylobacter* on broilers, potentially points to the flaw in extrapolating conclusions drawn from research in broiler processing to turkey processing.

Within all three facilities participating in this investigation, despite an apparent contribution by IOBW cabinets, properly managed immersion chilling systems were observed to be the best microbial reduction strategy for achieving reductions in *Salmonella* and *Campylobacter* on processed carcasses. These findings are similar to other investigations conducted in broiler processing facilities where the immersion chilling environment has been identified as a critical focus for controlling bacterial contamination on poultry carcasses (James et al., 1992; White et al., 1997). Measured values of applied microbicides within immersion chiller water in this investigation were clearly associated with the effectiveness of bacterial remediation on processed turkey carcasses, similar to the findings of Mead and co-workers (1975) in their investigations during broiler processing (Mead et al., 1975). While plants participating in this study evaluated different microbicides in chiller and IOBW make-up water, recorded observations underscore the need for proper management for achieving reductions on finished carcasses, regardless of the chosen microbicide applied. Taken together, the present investigation identified effective microbial interventions through the combined use of an IOBW cabinet and a properly managed immersion chilling system in three commercial turkey processing plants.

CHAPTER IV

EFFECT OF SCALD TANK ALKINATION ON *SALMONELLA* AND *CAMPYLOBACTER* REDUCTION IN COMMERCIAL TURKEY PROCESSING

Introduction

Despite a considerable amount of interest within the broiler processing environment, there has been little research performed to date focusing on bacterial contamination or reduction during scalding and feather picking during turkey processing. The scalding environment, due to elevated temperature for thermal killing of bacteria, represents another potential site of microbial intervention or reduction in commercial poultry processing. One focus of research within this area of processing has involved the investigation into different microbicides or additives which might have additive or synergistic effects on thermal killing of bacteria during scalding. Several investigators have demonstrated that exposing bacteria to multiple microbicidal environments of differing types often results in greater bacterial killing when compared to exposure to any single microbicidal environment alone (Miller, 1969; Ueckert et al., 1998; van Asbeck et al., 1983).

Published investigations to date on the effects of the scald environment on *Salmonella* and *Campylobacter* reductions vary in terms of the process being beneficial or detrimental to bacterial contamination of carcasses. Some investigations have shown the scald tank environment to be a site of cross contamination of *Campylobacter*

(Wempe et al., 1983) and *Salmonella* (Mulder et al., 1977) in broilers. However proper operation of scald tanks has also been shown to achieve a 2-3 log reduction in the number of *Campylobacter* found on broiler carcasses (Izat et al., 1988). Similarly, Oosterom and co-workers (1983) reported that *Campylobacter jejuni* levels decreased on turkey carcasses after scalding at a temperature of 50 C or greater. Following scalding, the picking and de-feathering area have also been established as areas of potential carcass cross contamination. Rigby and Pettit (1982) determined that the occurrence of *Salmonella* on feather pickers corresponds to an increased contamination of carcasses at the end of the processing cycle (Rigby et al., 1982). This could be due to the rubber fingers on mechanized feather pickers driving the microorganisms into the skin tissue and feather follicles or by the bacteria becoming aerosolized and contaminating the surrounding equipment in the de-feathering area (Bryan et al., 1968). De-feathering has also been shown to allow fecal material to escape the bird by way of the cloaca and contaminate the carcass or surrounding carcasses (Berrang et al., 2001). These findings emphasize the need for optimization of scald tank management for reduction of these pathogens prior to feather removal.

Turkeys are commonly scalded at a temperature of approximately 60 C, which is considered somewhat to be a mild to high heat treatment for certain bacteria (Ueckert et al., 1998). *Campylobacter coli* and *C. jejuni* are both moderately thermophilic bacteria, capable of survival at temperatures ranging between 50 and 55 C. *Salmonella* on the other hand is believed to be much more sensitive to temperatures within this range. As described above, scald tank additives or microbicides that may additively or

synergistically increase the susceptibility of *Salmonella* or *Campylobacter* to thermal killing during scalding could translate into improved bacterial reductions on carcasses. Research by a group in the U.K. identified scald pH modification to alkaline conditions to be effective in decreasing the thermal death time for *Salmonella* on processed chicken carcasses. Even in scald conditions consisting of high levels of organic matter, elevating scald water pH to approximately 9 with sodium carbonate greatly increased *Salmonella* killing in both scald water and on the skin of processed broiler carcasses (Humphrey et al., 1981). To date, this potential increase in thermal killing of bacteria during alkaline scald conditions has not been researched in turkeys. As such, the present investigation was designed with the experimental objective of evaluating the effects of scalding commercial turkeys under normal or alkaline pH scald conditions on *Salmonella*, *Campylobacter*, or total aerobic plate count (TPC) bacterial recovery.

Materials and Methods

Carcass Sample Collection

Sampling of carcasses before and after scalding was performed by whole carcass rinsing. Rinsing, collection, and culture of recovered rinse fluid for the specific isolation of *Salmonella* during this study conformed to the FSIS proposed "Mega-Reg" guidelines for sampling and culture (Federal Register, 1996), with slight modification. The sample collection and recovery of *Campylobacter* followed widely accepted protocols as described below. Fifty pre-scald and 100 post-feather pick carcass (50 from normal scald and 50 from alkaline scald) rinse samples were obtained for the specific

determination of *Salmonella* and *Campylobacter* incidence and direct recovery from the recovered rinse fluid on an independent day of sampling in three distinct commercial turkey processing facilities.

Carcasses were removed from the processing line in an alternating fashion between the pre-scald and post-feather pick sampling sites. Carcasses were removed from the above described locations with an individual pair of sterilized latex gloves and placed into a sterilized polypropylene bag for carcass rinsing. To rinse individual carcasses, 600 mL of sterile buffered peptone water (BPW; pH 7.2) was added to each individual bag and the carcasses were rinsed using inverted rotation motion 30 times each. Approximately 100 ml of the carcass rinse fluid was then aseptically recovered by allowing the rinse fluid to accumulate in a corner of the rinse bag. The corner and a pair of scissors were sprayed with a 70% alcohol solution, wiped down, and the corner cut. The rinse fluid was then allowed to drain into a sterilized polypropylene collection bottle. All sample collection bottles were placed on wet ice and transported back to our laboratories for the initiation of bacteriologic culture, which did not commence until 24 h had elapsed from the time of sample collection. The samples were treated equally and recovery incidence between the three sampling locations compared. Transport-related effects on sensitivity were not a critical concern in the present investigation.

Collection of Scald Tank Water

Twenty scald water samples (10 from normal pH scald and 10 from alkaline scald ~pH 9) were aseptically collected for the specific bacterial recovery incidence of

Salmonella and *Campylobacter*. Scald temperature and pH measurements were also taken at the entrance and the exit of the scalder at the time of sampling.

Scald Water pH Modifications

For Plant 1 and 2, pH was altered to alkaline conditions (~pH9-10) by addition of sodium carbonate to scald water at an inclusion level of approximately 0.15-0.2 grams per gallon of scald water. Given the results of post-scald bacterial incidence related to pH adjustment to the scald water in Plants 1 and 2 using sodium carbonate (see below), a decision was made to change to sodium hydroxide addition in Plant 3. In this facility, concentrated liquid sodium hydroxide was added to the scald water at a similar inclusion level to achieve and maintain alkaline conditions (~pH9-10). Regardless of the approach, despite our initial attempt to elevate and maintain pH in Plant 1 being slightly below our target, no problems were experienced in the use of either sodium hydroxide or sodium carbonate to meet and maintain the elevated pH in Plants 2 and 3. As such, observed data was not a result of a failure to meet and maintain a minimum pH to test the hypothesis.

Bacteriologic Culture

For the culture of *Campylobacter,* 30 mL of the recovered rinse fluid from each individual sample was combined and pre-enriched in 30 mL Bolton's complete enrichment medium for 24 h at 42 C. Flowing this selective pre-enrichment, each sample was streaked onto Campy-Cefex agar plating medium and all plates were incubated for 48 h at 42 C, based upon the methods of Stern and co-workers (Stern et al., 1995). All *Campylobacter* culture procedures were conducted under a modified

atmosphere containing 5% O_2, 10% CO_2, and 85% N_2 at 42 C. Suspect colonies were confirmed as *Campylobacter* spp. by observing both motility and morphology using normal phase contrast microscopy on a wet mount slide. Further speciation, specifically the difference between *C. coil* and *C. jejuni* of all recovered isolates was performed by a PCR based test (please see Chapter V).

For the specific culture of *Salmonella*, 30 mL of the recovered rinse fluid was combined and pre-enriched in an additional 30 mL of BPW for 24 h at 37 C. Following this pre-enrichment, 100 µl of each sample was sterilely transferred to a sterile culture tube containing 10 mL of Rappaport-Vassiliadis (RV) enrichment broth and incubated for 24 h at 42 C. Following this enrichment procedure, each sample was then streaked onto modified lysine iron agar (MLIA) plating medium for the specific recovery of *Salmonella*. Suspect colonies were confirmed biochemically (triple sugar iron and lysine iron slants) and serologically using *Salmonella* o antisera (poly o; a-i)

To enumerate *Salmonella, Campylobacter*, and TPC bacteria recovered from carcass rinse fluid, an additional 1mL of the rinse fluid from 20% of the carcass rinse samples was sterilely transferred to a polypropylene culture tube containing 9 mL of sterile Butterfield's solution. The suspension was then serially diluted (10X) by transferring 250 µl to an additional 3 tubes containing 2.25 mL of Butterfield's solution. From each dilution 100 µl was spread plated on to Tryptic soy agar, MLIA, or Campy-Cefex plates to allow for the specific determination of colony forming units (cfu) present in each sample. MILA plates and tryptic soy agar plates were incubated at 37 C for 24 h and Campy-Cefex plates were incubated under a modified atmosphere as described

above. The number of *Salmonella*, *Campylobacter*, or TPC bacteria recovered from each carcass rinse sample was determined by way of manual counting of colonies identified on respective plates at each dilution. All suspect colonies were confirmed serologically, biochemically, and by visual inspection as described above. Rinse fluid cfu from each rinse sample were converted to Log_{10} cfu/mL of recovered rinse fluid. Samples that were culture negative at the 1:10 dilution, but were positive following selective enrichment, were assigned a value of 1.5 Log_{10} cfu/mL. Likewise, samples that were culture negative at the 1:10 dilution, and remained negative following selective enrichment, were assigned a Log_{10} cfu/mL value of zero.

Statistical Analysis

Differences in bacterial incidence (+/-) for *Salmonella* or *Campylobacter* recovered by carcass rinsing at specific sampling sites were compared using the Chi-square test of independence and significant ($P<0.05$) and highly significant ($P<0.001$) differences are reported.

Results and Discussion

Plant 1

Recovery of *Salmonella* and *Campylobacter* following selective enrichment and direct recovery are shown in Tables 8 and 9, respectively.

TABLE 8. Plant 1: Pre-evisceration Incidence Recovery of *Salmonella* and *Campylobacter*. Recovery following selective enrichment of carcass-rinse samples taken before and after scald under different pH conditions.

Time of Sampling	***Salmonella* Recovery**		***Campylobacter* Recovery**	
	Normal pH Scald	**Alkaline pH Scald**	**Normal pH Scald**	**Alkaline pH Scald**
Average pH recorded at time of Sampling	7.32	8.72	7.32	8.72
Incidence from recovered rinse fluid	17/50 (34.0%)	28/36 (77.8%)	48/50 (96.0%)	35/36 (97.2%)

TABLE 9. Plant 1: Pre-evisceration Bacterial Enumeration. Enumeration following dilution and direct plating of carcass-rinse samples taken before and after scald under different pH conditions.

Time of Sampling	***Salmonella*** **Recovery** [1]		***Campylobacter*** **Recovery** [1]		**Total Plate Count** [1]	
	Normal pH Scald	**Alkaline pH Scald**	**Normal pH Scald**	**Alkaline pH Scald**	**Normal pH Scald**	**Alkaline pH Scald**
Average pH recorded at time of Sampling	7.32	8.72	7.32	8.72	7.32	8.72
cfu from recovered rinse fluid	1.1 / (10/20) 50.0%	0.9 / (2/20) 10.0%	1.5 / (14/20) 70.0%	1.1 / (15/20) 75.0%	4.1 / (10/10) 100%	4.7 / (10/10) 100%

[1] Log_{10} cfu/ml rinse fluid/carcass

Average pH of the scald water under normal conditions for this facility throughout the sampling set was determined to be 7.32. Our ability to raise the pH to a level of 9-10 for the alkaline scald was not optimized during our first attempt in this facility, and we were only to achieve an average pH of 8.72. Selective recovery incidence for *Salmonella* following carcass rinsing of post-scald carcasses increased during alkaline scald, contradicting our hypothesis of increased thermal killing of bacteria during an alkaline pH scald (Table 8). *Campylobacter* incidence post-scald was near 100%, demonstrating no differences when comparing normal scald conditions to alkaline (Table1 8).

Direct recovery of *Salmonella* from collected rinse fluid demonstrated slight reductions during alkaline pH scald conditions. Similar to that of *Salmonella*, *Campylobacter* Log_{10} cfu/mL of recovered rinse fluid showed slight reductions following alkaline scald. Due to an unforeseen scheduling conflict with the management of this facility, a lot change was experienced while obtaining samples which eliminated our ability to obtaining pre-scald samples within this facility on this day of sampling, thus explaining why these data are not present in Table 9. A comparison of direct recovery of TPC bacteria under normal and alkaline pH scald conditions showed an essentially equivalent level of recovery. However, a slight increase of recovery under alkaline pH scald was observed (Table 9).

Plant 2

Recovery of *Salmonella* and *Campylobacter* following selective enrichment data is shown in Table 10. Direct recovery data are shown in Table 11.

TABLE 10. Plant 2: Pre-evisceration Incidence Recovery of *Salmonella* and *Campylobacter*. Recovery following selective enrichment of carcass-rinse samples taken before and after scald under different pH conditions.

Time of Sampling	***Salmonella* Recovery**			***Campylobacter* Recovery**		
	Pre-Scald	**Normal pH Scald**	**Alkaline pH Scald**	**Pre-Scald**	**Normal pH Scald**	**Alkaline pH Scald**
Average pH recorded at time of Sampling	N/A	7.70	9.75	N/A	7.70	9.75
Incidence from recovered rinse fluid	43/50 (86.0%)	13/50** (26.0%)	27/41* (65.9%)	44/50 (88.0%)	48/50 (96.0%)	42/42 (100%)

*Represents a significantly (P<.05) lower recovery incidence when compared to the prior intervention incidence within lots of each independent day of sampling.
**Represents a highly significantly (P<.001) lower recovery incidence when compared to the prior intervention incidence within lots of each independent day of sampling.

TABLE 11. Plant 2: Pre-evisceration Bacterial Enumeration. Enumeration following dilution and direct plating of carcass-rinse samples taken before and after scald under different pH conditions.

Time of Sampling	*Salmonella* Recovery [1]			*Campylobacter* Recovery [1]			Total Plate Count [1]		
	Pre-Scald	Normal pH Scald	Alkaline pH Scald	Pre-Scald	Normal pH Scald	Alkaline pH Scald	Pre-Scald	Normal pH Scald	Alkaline pH Scald
Average pH recorded at time of Sampling	N/A	7.70	9.75	N/A	7.70	9.75	N/A	7.70	9.75
cfu from recovered rinse fluid	1.6 / (3/10) 30.0%	0.5 / (1/10) 10.0%	1.0 / (3/8) 37.5%	3.1 / (9/10) 90.0%	1.5 / (6/10) 60.0%	1.6 / (7/8) 87.5%	5.6 (10/10) 100%	4.1 (10/10) 100%	4.2 (10/10) 100%

[1] Log_{10} cfu/ml rinse fluid/carcass // incidence of cfu positives via direct plating

Unlike the difficulties we experienced in Plant 1 during this investigation, we were successful in our attempt to raise and maintain an elevated pH during alkaline scald with a pH averaging 9.75. Normal pH scald yielded an average pH of 7.70.

Salmonella incidence from recovered rinse fluid following enrichment was significantly reduced ($P<0.05$) following an alkaline scald when compared to pre-scald incidence levels. However, this reduction was not as significant as the degree of reduction ($P<0.001$) that was achieved under normal scald conditions (Table 10). Regardless of pH, *Campylobacter* incidence levels were essentially unchanged when comparing pre-scald and post-scald levels.

Direct recovery of *Salmonella* revealed reductions following alkaline scald (Table 11). Log_{10} cfu/mL reductions under normal scald were shown to be greater than those observed under an alkaline scald. Direct recovery of *Campylobacter* from the collected rinse fluid showed equivalent reductions during scald regardless of pH (Table 11). Direct recovery of TPC bacteria was shown to be reduced by slightly over a log when the pre-scald sampling site was compared to post scald, with normal and alkaline pH scalds being essentially equivalent (Table 11).

Plant 3

Recovery of *Salmonella* and *Campylobacter* following selective enrichment data is shown in Table 12. Direct recovery data are shown in Table 13.

TABLE 12. Plant 3: Pre-evisceration Incidence Recovery of *Salmonella* and *Campylobacter*. Recovery following selective enrichment of carcass-rinse samples taken before and after scald under different pH conditions.

Time of Sampling	***Salmonella* Recovery**			***Campylobacter* Recovery**		
	Pre-Scald	**Normal pH Scald**	**Alkaline pH Scald**	**Pre-Scald**	**Normal pH Scald**	**Alkaline pH Scald**
Average pH recorded at time of Sampling	N/A	7.06	9.73	N/A	7.06	9.73
Sampling 1st Lot	18/50 (36.0%)	27/50 (54.0%)	7/13 (53.8%)	20/50 (40.0%)	11/50 (22.0%)	1/13 (7.7%)
Sampling 2nd Lot	13/20 (65.0%)	NOT SAMPLED	13/17 (76.5%)	2/20 (10.0%)	NOT SAMPLED	1/17 (5.9%)

*Represents a significantly (P<.05) lower recovery incidence when compared to the prior intervention incidence within lots of each independent day of sampling.
**Represents a highly significantly (P<.001) lower recovery incidence when compared to the prior intervention incidence within lots of each independent day of sampling.

Similar to Plant 2, with the exception of using sodium hydroxide to elevate scald tank pH, we were successful in maintaining the pH of scald water to our target range for sampling with an average pH of 9.73 being observed throughout the sampling set. The average pH under normal scald conditions was measured at 7.07. Similar to the flock scheduling difficulties experienced in Plant 1, it became necessary to sample two independent flocks or lots of turkeys during this sampling day. This inadvertently diminished the sample numbers for the alkaline scald sampling set.

Salmonella incidence recovery following selective enrichment of the collected rinse fluid did not differ respective to scald water pH in either the 1st or 2nd sampling sets. A significant decrease ($P<0.05$) occurred in *Campylobacter* recovery incidence following selective enrichment of carcass rinse fluid from carcasses scalded under alkaline pH conditions during the 1st sampling set, but such reductions were not observed during the 2nd sampling set. Normal scald conditions resulted in equivalent levels of recovery when compared to pre-scald incidence levels (Table 12).

TABLE 13. Plant 3: Pre-evisceration Bacterial Enumeration. Enumeration following dilution and direct plating of carcass-rinse samples taken before and after scald under different pH conditions.

Time of Sampling	*Salmonella* Recovery [1]			*Campylobacter* Recovery [1]			Total Plate Count [1]		
	Pre-Scald	Normal pH Scald	Alkaline pH Scald	Pre-Scald	Normal pH Scald	Alkaline pH Scald	Pre-Scald	Normal pH Scald	Alkaline pH Scald
Average pH recorded at time of Sampling	N/A	7.06	9.73	N/A	7.06	9.73	N/A	7.06	9.73
Sampling 1st Lot	0.7 / (12/20) 60.0%	0.9 / (4/20) 20%	0.6 / (0/1) 0%	1.9 / (20/20) 100%	0.6 / (14/20) 70.0%	1.0 / (2/4) 50.0%	6.2 / (10/10) 100%	4.5 / (10/10) 100%	5.5 / (10/10) 100%
Sampling 2nd Lot	1.1 / (3/8) 37.5%	NOT SAMPLED	1.1 / (2/10) 20.0%	3.0 / (5/7) 71.4%	NOT SAMPLED	1.9 / (5/8) 62.5%	6.2 / (10/10) 100%	NOT SAMPLED	5.9 / (10/10) 100%

[1] Log_{10} cfu/ml rinse fluid/carcass // incidence of cfu positives via direct plating

Direct recovery of *Salmonella* did not differ when the different scald conditions were compared. *Campylobacter* direct recovery between pre- and post-scald sampling sites was approximately one log lower in recovered rinse fluid following scalding, regardless of pH during both sampling sets (Table 13). Direct recovery of TPC bacteria was reduced by approximately 1.5 logs under normal scald conditions and approximately 0.8 logs by alkaline scald conditions during the 1st sampling set. Similar reductions were not observed during the 2nd sampling set scalded under alkaline conditions (Table 13).

Taken together, data collected by rinsing carcasses scalded under different pH conditions in three commercial turkey processing facilities revealed that modification of scald water to alkaline conditions (pH 9-10) did not result in an increased thermal killing of either *Salmonella* or *Campylobacter* on turkey carcasses. As such, these observations did not support the experimental hypothesis of this investigation. These findings are in direct contradiction to the findings of Humphrey and co-workers (Humphrey et al., 1981) which reported significant reductions in the thermal death time for *Salmonella* on processed chicken carcasses scalded under alkaline pH conditions. Since such conditions are known to facilitate more efficient feather picking, greater bacterial detachment from carcasses at this point on the processing line could have impacted observed data. Our laboratory is planning an additional experiment at the present time to test this hypothesis. If proven correct, additional high and low pressure washes down the processing line could actually prove to be more effective following modification of the scald tank environment in such a way.

CHAPTER V

SPECIATION OF *CAMPYLOBACTER* ISOLATES RECOVERED DURING COMMERCIAL TURKEY PROCESSING

Introduction

The two most prevalent species of *Campylobacter* isolated from poultry, domestic animals, and man are *C. jejuni* and *C. coli.* Intestinal colonization and resulting pathogenicity by both species is similar. Both *C. jejuni* and *C. coil* attach to cellular membranes of intestinal epithelial cells and are incorporated into cytoplasmic vacuoles. The level at which the bacterium is able to attach, invade, and produce enterotoxin has been proposed as a determinant of pathogenicity. In support of this, approximately 32% of *Campylobacter* strains isolated from humans with acute enteritis or from the gut of healthy laying hens were determined to be enterotoxigenic (Lindblom et al., 1989). A common hypothesis among health care professionals and researchers alike is that *C. jejuni* is the more pathogenic or virulent of these two species. In support of this hypothesis, differences in enterotoxin production between *C. coil* and *C. jejuni* have been observed. A subsequent investigation conducted by Lindblom and co-workers (Lindblom et al., 1990), revealed that approximately 22% of *C. coli* and approximately 34% of *C. jejuni* evaluated have been shown to be capable of enterotoxin production. These findings suggest that *C. jejuni* is often times more enterotoxigenic and as such potentially more virulent than *C. coli*.

Kapperud and co-workers (Kapperud et al., 1984) serotyped thermophillic campylobacters isolated from human cases of enteritis on the basis of expression of heat stable antigens using passive hemagglutination. In these cases, 86.6% of all isolates were identified as *C. jejuni* and 13.4% were identified as *C coli*. Other studies have revealed similar findings, where *C. jejuni* was shown to be responsible for causing 89.3% of the cases of human enteritis, while *C. coli* was implicated in the remaining 10.7% of clinical cases in humans (Kramer et al., 2000). When a similar characterization of recovered campylobacters was preformed on commercial broiler farms, the trend of increased *C. jejuni* when compared to *C. coil* remained. In this investigation by Chuma and co-workers (Chuma et al., 1997), *C. jejuni* was isolated in 20.0% of the flocks evaluated whereas *C. coli* was only isolated in 4.7% of the flocks. To date, there is limited literature available describing the in-plant incidence levels of *C. coil* and *C. jejuni* isolation during commercial poultry processing. This contributes to the difficulty in tracing the epidemiology of the thermophillic campylobacters from the grow-out environment through processing and eventually to human infection. Given this gap in the knowledge base with respect to species specific isolation of *Campylobacter* during commercial poultry processing, particularly turkey processing, coupled with our ability to screen potentially hundreds of individual isolates recovered in the research described in Chapters III and IV of this Thesis, the objective of this specific investigation was to specifically differentiate recovered *Campylobacter* isolates into *C. coli* or *C. jejuni* using a PCR-based method of speciation as initially described by

(Gonzalez et al., 1997) and subsequently modified by Hume and co-workers (Hume et al., 2002).

Materials and Methods

Overview

Campylobacter speciation, specifically the differentiation between *C. coli* and *C. jejuni* isolated from collected samples (as described in Chapter III and IV of this Thesis) was preformed by a PCR based test identifying a divergence in the *cueE* gene. This gene encodes putative virulence determinates and the procedure was initially described by Gonzalez and co-workers (Gonzalez et al., 1997) and specifically modified for our intended use by Hume and co-workers (Hume et al., 2002).

Bacteriologic Culture

Following the cryopreservation of each of positively identified *Campylobacter* isolate recovered from each of three individual turkey processing facilities, each isolate was streaked onto Campy-Cefex agar plating medium and all plates were incubated for 48 h at 42 C, according to the protocol of Stern and co-workers (Stern et al., 1995). All *Campylobacter* culture procedures were conducted under a modified atmosphere containing 5% O_2, 10% CO_2, and 85% N_2 at 42 C.

DNA Purification

The extraction of DNA from each pure *Campylobacter* culture was preformed using a commercially available tissue kit from QIAgen[1]. The protocol for the isolation of DNA from the isolates followed the manufactures guidelines. Individual

[1] QIAmp tissue kit; QIAgen Ltd., Dorking, United Kingdom

Campylobacter colonies were removed and aseptically placed in a microcentrifuge tube containing a lysis buffer provided by QIAgen. The tubes were centrifuged and exposed to a verity of buffers and centrifugation columns to yield isolated bacterial DNA, which was then stored at -80 C until separated on agarose gels.

Polymerase Chain Reaction and Electrophoretic Gel Migration

This protocol followed the procedures described by the manufacturer. A comparison of the *cueE* gene sequences from both *C. coli* (894 bp; Richardson and Park, 1995) and *C. jejuni* (793 bp; Park and Richardson, 1995) yielded a 86.9% conserved nucleic acid sequence that allowed R&D Systems Europe to determine the species specific sequences in the gene[2]. Both the forward and reverse *C. coli* and *C. jejuni* specific oligonucleotides used in the reaction were obtained from Integrated DNA Technologies. The primers (1 µL each) were added to 12.5 µL of JumpStart REDTaq ReadyMix PCR Reaction Mix and combined with 2.5 µL of the isolated DNA template[3]. Since the optimal reaction volume was 25 µL, the remaining 6µL was made up of PCR grade water. After the amplification of the specific divergences in the genome, the amplicons were frozen until separated and differentiated by gel electrophoresis. For the migration of the amplicons, a bufferless 1.2% agarose commercially available gel was used[4]. Invitrogen's E-gel protocol specified the migration of the protein for 25 min at 60 V. No tracer dye was required due to the inclusion of a red tracer dye in the PCR reaction mixture.

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[2] R&D Systems Europe Ltd., Abingdon, United Kingdom
[3] JumpStart REDTaq ReadyMix PCR Reaction Mix; Sigma, Saint Louis, Missouri
[4] E-Gel; Invitrogen Corp., Carlsbad, California

Results and Discussion

Plant 1

Speciation of recovered *Campylobacter* isolates from Plant 1 was either predominately *C. coli* or *C. jejuni* depending upon location within the plant. Overall recovery of all *Campylobacter* isolates decreased throughout all post-evisceration sampling sites. In total, *C. coli* isolates outnumbered recovery of *C. jejuni* isolates. Despite being more numerous, the proportion of *C. coli* isolates decreased throughout the consecutive stages of post-evisceration sampling between pre-IOBW, post-IOBW, and post- chill sampling sites. *C. jejuni* incidence, however, followed a reversed trend, with recovery incidence actually increasing as a percent of total *Campylobacter* within each sampling site between pre-IOBW and post-chill sampling sites (Table 14). Speciation of *Campylobacter* following scald under normal and alkaline pH conditions revealed an overwhelming recovery of *C. coli* at this stage of processing. No apparent difference was evident between alkaline and normal scald conditions. Recovery of *C. jejuni* following scald was at an overall lower incidence and slightly decreased following an alkaline scald pH (Table 14).

Plant 2

Speciation of recovered *Campylobacter* revealed an exclusive recovery of *C. coli* from post-evisceration sampling sites within this facility during this particular day of sampling. These data were likely influenced by an overall lower incidence of total *Campylobacter* recovery at all sampling sites. Nonetheless, *C. jejuni* was not identified during this day of sample collection at post-evisceration sites (Table 15). *Campylobacter* speciation of recovered isolates at pre-and post scald sampling sites yielded on overwhelming proportion of total isolates being *C. jejuni* as compared to *C. coli* during this sampling day, differing from observations in Plant 1. The incidence of *C. jejuni* isolates were proportionally higher pre-scald as compared to post-scald isolation following alkaline pH scald conditions, but were essentially equivalent to levels observed following post-normal pH scald conditions. *C. coli* incidence while lowest in total recovery at the pre-scald sampling site, increased in frequency following scalding under both normal and alkaline scald conditions (Table 15).

TABLE 14. Plant 1: Speciation of *Campylobacter*.

Isolates Speciated by PCR	***Campylobacter* Isolates**				
	Normal pH Scald	**Alkaline pH Scald**	**Pre-IOBW**	**Post-IOBW**	**Post Chill**
C. coli	17/21 (81%)	9/10 (90%)	14/23 (60.8%)	7/19 (36.8%)	7/18 (38.8%)
C. jejuni	3/21 (14.3%)	1/10 (10%)	8/23 (34.8%)	9/19 (47.3%)	10/18 (55.6%)
Mixed Isolate (*C. coli* & *C. jejuni*)	0/21 (0%)	0/10 (0%)	0/23 (0%)	3/19 (15.8%)	0/18 (0%)
Other	1/21 (4.8%)	0/10 (0%)	1/23 (4.3%)	0/19 (0%)	1/18 (5.6%)

TABLE 15. Plant 2: Speciation of *Campylobacter*.

Isolates Speciated by PCR	***Campylobacter* Isolates**						
	Pre-Scald	**Normal pH Scald**	**Alkaline pH Scald**	**Pre-IOBW**	**Post-IOBW**	**Post-Acid**	**Post Chill**
C. coli	1/14 (7%)	4/21 (19%)	5/15 (33.3%)	0/0 (0%)	1/1 (100%)	1/1 (100%)	7/8 (87.5%)
C. jejuni	11/14 (78.6%)	16/21 (76.2%)	9/15 (60%)	0/0 (0%)	0/0 (0%)	0/0 (0%)	0/0 (0%)
Mixed Isolate (*C. coli* & *C. jejuni*)	0/14 (0%)	1/21 (4.8%)	1/15 (6.7%)	0/0 (0%)	0/0 (0%)	0/0 (0%)	0/0 (0%)
Other	2/14 (14.3%)	0/21 (0%)	0/15 (0%)	0/0 (0%)	0/0 (0%)	0/0 (0%)	1/8 (12.5%)

TABLE 16. Plant 3: Speciation of *Campylobacter*.

Isolates Speciated By PCR	***Campylobacter* Isolates**					
	Pre-Scald	**Normal pH Scald**	**Alkaline pH Scald**	**Pre-IOBW**	**Post-IOBW**	**Post Chill**
C. coli	6/13 (46.2%)	2/5 (40.0%)	2/6 (33.3%)	11/17 (64.7%)	10/15 (66.7%)	0/1 (0%)
C. jejuni	6/13 (46.2%)	3/5 (60.0%)	4/6 (66.6%)	5/17 (29.4%)	4/15 (26.7%)	1/1 (100%)
Mixed Isolate (*C. coli* & *C. Jejuni*)	0/13 (0%)	0/5 (0%)	0/6 (0%)	0/17 (0%)	0/15 (0%)	0/1 (0%)
Other	1/13 (7.7%)	0/5 (0%)	0/6 (0%)	1/17 (5.9%)	1/15 (6.7%)	0/1 (0%)

TABLE 17. Total Speciation of *Campylobacter*.

Isolates Speciated By PCR	**Campylobacter Isolates**		
	Plant 1	**Plant 2**	**Plant 3**
***C.* coli**	54/91 (59.3%)	19/60 (31.7%)	31/57 (54.3%)
C. jejuni	31/91 (34.1%)	36/60 (60.0%)	23/57 (40.4%)
Mixed Isolate (*C. coli* & *C. jejuni*)	3/91 (3.3%)	3/60 (5.0%)	0/57 (0%)
Other	3/91 (3.3%)	2/60 (3.3%)	3/57 (5.3%)

Plant 3

Total *Campylobacter* recovery was markedly reduced by post-evisceration immersion chilling in this facility on this day of sampling. Overall, when evaluating all post-evisceration sampling sites, *C. coli* incidence was greater than *C. jejuni* incidence. *C. coli* and *C. jejuni* incidence levels were largely unaffected by the IOBW cabinet, as both total isolations and the percent of each species per total *Campylobacter* isolation was unchanged between both sampling sites (Table 16). Speciation of *Campylobacter* at both the pre-and post scald sampling sited revealed a similar and balanced recovery of both *C. coli* and *C. jejuni* with each species being reduced during the scalding regardless of pH (Table 16).

Taken together, when evaluating trends of individual species isolation among all three facilities participating in this investigation, speciation of recovered *Campylobacter* was variable depending on the location of sampling within each plant and also between the facilities sampled on an overall basis (Table 17). When all speciated samples were pooled for each facility, *C. coli* predominated in Plants 1 and 3, while *C. jejuni* was isolated at a higher frequency in Plant 2.

CHAPTER VI

CONCLUSIONS

Broiler processing plants have long been used as the model for studies designed to investigate microbial interventions for processed poultry. Although many interventions identified in such investigation have dual application for both broiler and turkey processing, the degree of difference between both broilers and turkeys themselves, and the marked differences in the design and layout of the two processing environments, warrant individual research projects aimed exclusively at turkeys. Historically, the turkey industry has been disadvantaged due to the lack of scientific data related to their processing environment and the evaluation of successful intervention or reduction strategies. To this end, the present investigation focuses upon the evaluation of post-evisceration microbial reduction strategies in three distinct commercial turkey processing facilities in the U.S. Research described in this thesis was aimed at investigating the effectiveness of a post-evisceration, pre-immersion chilling microbial intervention that is very common to both broiler and commercial turkey processing plants, an inside-outside bird wash (IOBW) cabinet. Additionally, to extend previous findings from out laboratory, this investigation will also evaluate the effectiveness of IOBW cabinets as they contribute to the previously described successful microbial reduction strategy of using a properly managed immersion chilling system during commercial turkey processing.

An area of known bacterial reduction during commercial poultry processing is the thermal killing of bacteria on the exterior surface of the bird during scalding prior to feather removal. Several investigations performed with broiler carcasses have demonstrated that scalding at an alkaline pH (pH 9-10) resulted in a higher degree of thermal killing of bacteria on carcasses. This potential pre-evisceration microbial reduction strategy was also evaluated in the present investigation with the hopes of coupling the potentially synergistic effect of high temperature during scald with unfavorable pH conditions to achieve greater thermal killing of pathogenic bacteria, specifically *Salmonella* and *Campylobacter* on turkeys. Taking into consideration the lack of data related to microbial intervention and reduction in commercial turkey processing, the primary objective of this investigation was to look at incidence data relative to the combined use of a properly managed chilling system, IOBW spray cabinets, and altered pH scald conditions in three commercial turkey processing facilities to potentially identify effective microbial reduction strategies for reducing *Salmonella* and *Campylobacter* on commercially processed turkeys.

The experimental objective of the research described in Chapter III of this Thesis was to evaluate the effectiveness of IOBW cabinets as they contribute to the previously described successful microbial reduction strategy of using a properly managed immersion chilling system during commercial turkey processing. While evaluating post-evisceration, pre chill microbial interventions in place in the three commercial facilities participating in the current investigation, the most common post-evisceration microbial intervention used was an IOBW cabinet. Despite poor management of the chlorine

levels in IOBW cabinets in Plants 1 and 2, all three plants were associated with a trend of decrease for *Salmonella* recovery on carcasses following the IOBW. However the results for *Campylobacter* were not as straightforward, with only Plant 3 demonstrating reductions following the IOBW. Plant 2 used an additional post-evisceration / pre-chill intervention, a low pressure acetic acid final wash cabinet that we also evaluated in this study. Despite the apparent appropriate application of the acetic acid in this cabinet, data collected before and after this particular intervention did demonstrate an additional benefit, in terms of bacterial reduction for either *Salmonella* or *Campylobacter* by the use of this cabinet. These findings, which represent potentially the first recorded within commercial turkey processing facilities, are mostly in agreement with the findings of Bashor and co-workers (2004) when they showed that IOBW cabinets are successful in generating 1.5 log reductions of *Campylobacter* on broiler carcasses. The fact that our observations suggest greater effectiveness at achieving *Salmonella* reductions on processed turkey carcasses, when Bashor and co-workers (2004) report reductions for *Campylobacter* on broilers, potentially points to the flaw in extrapolating conclusions drawn from research in broiler processing to turkey processing.

Within all three facilities participating in this investigation, despite an apparent contribution by IOBW cabinets, properly managed immersion chilling systems were observed to be the best microbial reduction strategy for achieving reductions in *Salmonella* and *Campylobacter* on processed carcasses. These findings are similar to other investigations conducted in broiler processing facilities where the immersion chilling environment has been identified as a critical focus for controlling bacterial

contamination on poultry carcasses (James et al., 1992; White et al., 1997). Measured values of applied microbicides within immersion chiller water in this investigation were clearly associated with the effectiveness of bacterial remediation on processed turkey carcasses, similar to the findings of Mead and co-workers (1975) in their investigations during broiler processing (Mead et al., 1975). While plants participating in this study evaluated different microbicides in chiller and IOBW make-up water, recorded observations underscore the need for proper management for achieving reductions on finished carcasses, regardless of the chosen microbicide applied. Taken together, the present investigation identified effective microbial interventions through the combined use of an IOBW cabinet and a properly managed immersion chilling system in three commercial turkey processing plants.

The research conducted in Chapter IV of this Thesis was designed with the experimental objective of evaluating the effects of scalding commercial turkeys under normal or alkaline pH scald conditions achieving reductions in *Salmonella*, *Campylobacter*, or total aerobic plate count (TPC) bacterial recovery. Taken together, data collected by rinsing carcasses scalded under different pH conditions in three commercial turkey processing facilities revealed that modification of scald water to alkaline conditions (pH 9-10) did not result in an increased thermal killing of either *Salmonella* or *Campylobacter* on turkey carcasses. As such, these observations did not support the experimental hypothesis of this investigation. These findings are in direct contradiction to the findings of Humphrey and co-workers (Humphrey et al., 1981) which reported significant reductions in the thermal death time for *Salmonella* on

processed chicken carcasses scalded under alkaline pH conditions. Since such conditions are known to facilitate more efficient feather picking, greater bacterial detachment from carcasses at this point on the processing line could have impacted observed data. Our laboratory is planning an additional experiment at the present time to test this hypothesis. If proven correct, additional high and low pressure washes down the processing line could actually prove to be more effective following modification of the scald tank environment in such a way.

Campylobacter isolated from human cases of enteritis have revealed that over 80% of all isolates are typically identified as *C. jejuni* and the remaining species implicated in human infection has been identified as *C. coli* (Kapperud et al., 1984). When a similar characterization of recovered *Campylobacter* was preformed on commercial broiler farms, the trend of increased *C. jejuni* when compared to *C. coil* remained. In this investigation by Chuma and co-workers (Chuma et al., 1997), *C jejuni* was isolated in 20.0% of the flocks evaluated whereas *C. coli* was only isolated in 4.7% of the flocks. To date, there is limited literature available describing the in-plant incidence levels of *C. coil* and *C. jejuni* isolation during commercial poultry processing. This contributes to the difficulty in tracing the epidemiology of thermophillic *Campylobacter* from the grow-out environment through processing and eventually to human infection. Given this gap in the knowledge base with respect to species specific isolation of *Campylobacter* during commercial poultry processing, particularly turkey processing, coupled with our ability to screen potentially hundreds of individual isolates recovered in the research described in Chapters III and IV of this Thesis, the objective of

this research described in Chapter V of this thesis was to specifically differentiate recovered *Campylobacter* isolates into *C. coli* or *C. jejuni* using a PCR-based method of speciation.

Taken together, when evaluating trends of individual species isolation among all three facilities participating in this investigation, speciation of recovered *Campylobacter* was variable depending on the location of sampling within each plant and also between the facilities sampled on an overall basis. When all speciated samples were pooled for each facility, *C. coli* predominated in Plants 1 and 3, while *C. jejuni* was isolated at a higher frequency in Plant 2.

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VITA

Scott Michael Stevens

Born:

September 5, 1981
New Braunfels, TX

Education:

B.S. Poultry Science, Texas A&M University, 2004

Permanent Address:

2933 Barton Hill Dr.
Bulverde, TX 78163