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Effects of Pre-chiller Temperature on the Microbial Ecology of Whole Bird Carcass Rinses

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Abstract

Upon entering a poultry processing facility, birds are already contaminated with a variety of microorganisms. It is the responsibility of the processor to reduce these numbers to deliver a wholesome product to customers. This is not an easy task as there are ample opportunities for further microbial contamination. Some of these opportunities are obvious. For example, any areas where contaminants could be washed off one bird and onto another are always an area for concern, i.e. the scalder or the chiller. There are numerous opportunities for contamination that are not so obvious. These incidents of contamination are not necessarily due to the process itself, but could possibly be more related to the management of the process or a lack of understanding of how each part of the process affects the entire system. This includes practices related to water usage, water content, temperature management, and chemical usage. This review will describe common practices from live hang to post chill that could possibly be contributing to elevated microbial counts on whole bird carcasses (WBCR) at post chill. This will give a better understanding of environmental factors, practices, and situations within the processing facility that could be fostering or encouraging increased levels of bacterial contamination. Identifying these areas will enable processors to take a more informed examination of each facility with the intention of reducing microbial populations on broiler carcasses.

List of Published Papers

Literature Review

Blevins, R., S.A. Kim, S.H. Park, R. Rivera, and S.C. Ricke. Chap. 18. Historical, current and future prospects for food safety in poultry product processing systems. In: S.C. Ricke, G.G. Atungulu, S.H. Park, and C.E. Rainwater (Eds.) Food and Feed Safety Systems and Analysis. Elsevier Inc., San Diego, CA pp. 323-345. Published.

Introduction

Microbial quality and contamination is of considerable concern to poultry processors. While much of this might be attributed to the increasing regulatory standards regarding *Campylobacter* and *Salmonella*, shelf-life extension, and the increase in consumer interest in food safety are also major contributing factors. With the recent increase in *Salmonella* and *Campylobacter* regulations on post chill bird carcasses since 2011 and the ever changing economy effecting profit margins, poultry processors are looking for more efficient and more cost effective means of reducing the microbial numbers on poultry products (USDA-FSIS, 2011a; 2014a; 2015a). This means that there is a need for additional methods for reducing bacterial loads without adding more chemicals, extra equipment, or cost to the process. These concerns and corresponding issues have led processors and researchers to take a more in-depth look at the poultry slaughter process to better understand the effects that each processing step has on bacterial contamination. Having a better understanding of how each component of the system affects the final microbial population levels prior to entering second processing will allow processors to fine-tune what a facility is already doing to reduce microbial numbers in a cost effective and efficient manner.

Literature Review

Common Microbial Risks of Poultry Processing-*Salmonella, Campylobacter,* and Spoilage Organisms

Salmonella and *Campylobacter* are two pathogens that should be given consideration when processing raw poultry. Both organisms are responsible for serious gastrointestinal illnesses that have been linked to the consumption or handling of raw or undercooked poultry products; and both organisms are found naturally on raw poultry (Fung, 1987; Barbut, 2002; Horrocks et al., 2009; Owens et al., 2010; Cox et al., 2011; Finstad et al., 2012).

Salmonellosis, the disease caused by *Salmonella* infection, is characterized by diarrhea, fever, and stomach cramps. These symptoms can occur anywhere from 12 to 72 hours after ingestion of the bacterial cells. It is considered self-limiting and symptoms usually diminish after 4 to 7 days. In some cases, usually in very young children, the elderly or in immunocompromised individuals, the disease can be fatal (Cunningham and Cox, 1987; Fung, 1987; Jay, 1996; Foley et al., 2011; Finstad et al., 2012). Campylobacteriosis, the disease caused by *Campylobacter*, has symptoms very similar to that of *Salmonella*. Diarrhea, stomach cramping, and fever are common symptoms. Nausea and vomiting can also accompany *Campylobacter* infection. This normally does not occur with *Salmonella*. *Campylobacter* infection is also considered self-limiting and symptoms usually subside within 2 to 5 days. Again, in very young children, the elderly and in individuals who are considered to be immunocompromised, the disease can be deadly if not diagnosed and treated with antibiotics in a timely manner (Fung, 1987; Horrocks et al., 2009; Forsythe, 2010; Rowe and Madden, 2014; Allos et al., 2015).

Spoilage organisms can diminish the shelf-life of raw poultry products and thus, adversely affect profits (Blackburn, 2006). As raw poultry is stored in a chilled environment, the growth of

normal mesophilic bacteria is slowed. The growth of psychrophiles and psychrotrophs becomes a concern (Fung, 1987; Russell et al., 1995). Psychrophiles prefer refrigerated temperatures, anywhere from 12°C to 15°C (53.6°F to 59°F), while the optimum temperature for mesophiles is 30° to 45°C (86° to 113°F). Psychrotrophs are mesophilic bacteria that are able to grow in refrigerated environments (Rao et al., 1998; Forsythe, 2010). Psychrophiles and psychrotrophs are what cause the off-odors and the break-down of the protein within the product which is essentially spoilage. *Acinetobacter* and *Pseudomonas* spp. are common spoilage organisms that are found in poultry (Barnes, 1972; Fung, 1987; Russell et al., 1995; Cox et al., 1998; Hinton et al., 2004). Once these organisms reach a certain threshold, approximately 10^7 cfu/cm^2, the bacteria deplete the carbohydrates available in the meat. After the carbohydrates are depleted, the bacteria begin to metabolize the amino acids that are present. As the bacteria metabolize the amino acids the off-odors that are associated with spoilage are generated (Russell et al., 1995; Nychas and Drosinos, 2014). As the spoilage bacteria continue to proliferate beyond 10^8 colony forming units (cfu), slime begins to form (Nychas and Drosinos, 2014).

Food Safety Regulations Regarding Pathogen Reduction from Slaughter to Post Chill

In 1996 the United States Department of Agriculture (USDA) Food Safety Inspection Service (FSIS), introduced requirements that meat and poultry facilities must follow to reduce the number and incidence of pathogenic bacteria within poultry facilities. These requirements were established to reduce the number of foodborne illnesses that were associated with the consumption of these products. Thus, the Hazard Analysis and Critical Control Point (HACCP) system was introduced and implemented in the meat industry. Each facility must establish a HACCP system or plan to fit the unique process for each product that is produced. Each critical

control point (CCP) that is established in the HACCP plan will have a corresponding verification check. Checks are simply the documented monitoring of the established critical control points. Checks are used to ensure that the system is working (USDA-FSIS, 1996).

Possible sources of contamination on broilers during production would be from bacteria found on the feathers and skin of the birds (Sofos et al., 2013), as well as the bacteria that are found in the gastrointestinal tract. Ingesta and fecal material are commonly thought to be a source of contamination on broilers within a slaughter facility (Bilgili et al., 2002; Smith and Berrang, 2006; Smith et al., 2007). FSIS Directive 6420.2 states, "In slaughter establishments, fecal contamination of carcasses is the primary avenue for contamination by pathogens. Pathogens may reside in fecal material, both in the gastrointestinal tract and on the exterior surfaces of the animal or bird going to slaughter" (USDA-FSIS, 2004). It is because of this that FSIS enforces a "zero tolerance" policy for any visible fecal material or ingesta on raw poultry. Per the Code of Federal Regulations (CFR), each facility must establish a check within the HACCP system that will prevent contaminated carcasses from entering the chill system. The plant will subsequently perform this check at an established frequency. The USDA inspector at the plant will also inspect for fecal contamination just prior to the chiller by inspecting ten pre-chill carcasses from each evisceration line. This occurs at least two times per shift. Any findings will be evaluated to determine whether or not the finding was an isolated incident or whether or not the root cause can be linked to other findings. This helps the USDA to determine if there is a possible flaw within the process or the HACCP system (USDA-FSIS, 2004).

Temperature can also be considered a CCP and is monitored immediately after the carcasses exit the chiller. The 9 CFR 381.66 (USDA-FSIS, 2014a) regulation states that carcasses

should be adequately chilled after slaughter or upon exiting the chiller to prevent the proliferation of pathogens. Until October 2014, this regulation stated that the internal temperature of the carcasses must be no greater than 40°F (4.4°C). The regulation no longer specifies the temperature and allows each slaughter facility to establish a chilling policy based upon scientific validations of the process being followed. This policy must be documented in either the establishment's HACCP plan, a Sanitation Standard Operating Procedure (SSOP) or another pre-requisite program. The facility can also rely on the previous regulation and use the standard of less than 40°F (4.4°C) as a "safe harbor" (USDA-FSIS, 2014b).

In 1996, along with the introduction of HACCP, the USDA also established the *Salmonella* performance standard. This standard was based upon the baseline data that was collected by USDA and it mandated that all poultry facilities be able to meet the baseline performance for *Salmonella.* At that time, the standard established that the number of positive post chill whole bird carcass rinses in a broiler slaughter facility could not exceed 20% or 12 positives out of 51 broilers carcasses tested. According to the 1996 Final Rule, the purpose of this standard, along with the implementation of HACCP, was to reduce the incidence and number of harmful bacteria, thus drastically reducing the incidence of foodborne illness that had been attributed to these products in the past (USDA-FSIS, 1996). In 2006, in an effort to reduce the incidence of *Salmonella* in young chickens, the USDA initiated a grading system based upon the 1996 performance standard. This grading system divided establishments into 3 categories based upon how successfully each establishment was meeting the standard. Category 1 facilities were any plants that had a positive rate of less than or equal to 10%, which is 50% of the 20% standard implemented in 1996. Category 2 facilities were any facility that were above 10%, but less than

the standard 20%. Category 3 facilities were any facilities that were above the 20% standard established in 1996. In 2008, the FSIS began publishing the names of any facilities that were in either Category 2 or Category 3 (USDA-FSIS, 2008). A new *Salmonella* baseline was conducted in 2007 and 2008. This baseline concluded that the prevalence of *Salmonella* in young chickens was 7.5%. In 2010 the USDA announced that it would be implementing a new performance standard based upon the findings of the 2007 baseline. In 2011, the new standard was implemented. Category 1 now includes plants that are at or below 4% positive (2 of 51). Category 2 is now considered to be above 4%, but at or below 9.8% positive. Category 3 now includes any establishments that exceed 9.8% (5 of 51). Establishments that exceed the new standard (9.8%) are published on the FSIS website in a quarterly report. The new standard of 9.8% was established because it was determined that each facility should be able to meet the 7.5% standard 80% of the time if the facility can stay at or below the 9.8% limit (USDA-FSIS, 2011a).

In addition to the tighter *Salmonella* standard, FSIS also introduced a standard for *Campylobacter* (USDA-FSIS, 2011a). Until 2010, *Campylobacter* testing had not been included. The 2011 performance standard for *Campylobacter* is set up differently than the performance standard for *Salmonella*. It is comprised of two parts. First, 1 mL of a 400 mL rinse is directly plated and then enumerated. Second, if the 1 mL sample is negative, a 30 mL sample is enriched and subsequently direct plated. The 30 mL sample would subsequently be determined to be either positive or negative. The 2006 and 2007 baseline established that the prevalence of *Campylobacter* within the industry was at 10.4%. This estimate was based upon the results of the 1 mL samples. In order to guide the industry to lower the rate of *Campylobacter* within each

facility, FSIS set the standard at 15.7% (8 of 51) for 1 mL samples. Each facility is allowed no more than 52.9% (27 of 51) for a combination of 1 mL and 30 mL samples. FSIS had intended to implement a category system much like the system associated with the *Salmonella* performance standard, but that has not come to fruition at this time (USDA-FSIS, 2011a). In 2014, FSIS discontinued the testing of the 30 mL samples for qualitative *Campylobacter* testing stating that the 1 mL sampling was sufficient to determine process control within an establishment (USDA-FSIS, 2014b).

The effects of these regulations and performance standards have been monitored by the United States Department of Health and Human Services since the implementation in 1996 (CDC, 2012; 2015. All of the regulations and the performance standards discussed previously are in place to mitigate the risk of contamination by pathogens and other bacteria or to slow the rate of proliferation of these organisms. The *Salmonella* and *Campylobacter* performance standards, specifically, were meant to encourage processors to reduce the prevalence of these pathogens in poultry facilities to help reduce the rate of foodborne illness due to the consumption of poultry products (USDA-FSIS, 1996; 2010b; 2011a). The CDC monitors and reports the incidence and the rate of foodborne illness in the Surveillance of Foodborne Disease Outbreaks-United States reports. The CDC states that its foodborne disease surveillance program serves three purposes; disease prevention and control, knowledge of disease causation and administrative guidance. Analysis of these reports can provide evidence as to whether or not these regulations and performance standards are promoting progress in food safety efforts (CDC, 2000; 2014).

According to the CDC, in 1996, the same year that the USDA introduced HACCP and the *Salmonella* performance standards, there were 12,450 confirmed cases of *Salmonellosis*. This

accounted for 55.1% of all foodborne disease for the year. There were also 2 deaths that were attributed to *Salmonella* in 1996. There were 101 cases of *Campylobacter*, which accounted for 0.4% of all foodborne disease in 1996 (CDC, 2000). According to the 2009-2010 report, *Salmonella* accounted for 243 outbreaks or 24% of all foodborne outbreaks. *Salmonella* caused the most hospitalizations that could be attributed to a foodborne outbreak (49%). This is a substantial increase since 1996. *Campylobacter* was the cause of 40 outbreaks or 4% of all foodborne outbreaks for 2009 and 2010 (CDC, 2013a). This report states that the number of foodborne illnesses caused by *Salmonella* did not meet the goals of the Healthy People 2010 program set out by the United States Department of Health and Human Services (CDC, 2013b). "Healthy People" was a program developed by the U.S. Department of Health and Human Services to improve the health of Americans. The program is based upon goals or objectives that are monitored and reassessed every 10 years, upon which new goals are established (CDC, 2015).

In 2014, the CDC released its *Surveillance for Foodborne Disease Outbreaks-United States*: 2012 Annual Report. This was the first annual report that was taken after most of the industry had implemented the 2010 standards and regulations. According to this report, *Salmonella* was responsible for 113 of 831 foodborne outbreaks (20%), and 3,394 of 14,972 foodborne illnesses (28%) (CDC, 2014). As mentioned previously, the *Campylobacter* standards were introduced in 2010 and implemented by most processors by 2011. Per the CDC, in 2012 *Campylobacter* was responsible for 30 out of 425 confirmed foodborne outbreaks (7.1%) as well as 434 of 10,396 confirmed illnesses (4.2%) (CDC, 2014). This report shows that there has been a decline in both *Salmonella* outbreaks and illnesses since the implementation of the most

recent standards. The incidences of *Campylobacter* outbreaks and infections have remained unchanged since 2010 (CDC, 2012; CDC, 2015).

In January 2015, the USDA introduced new measures to further reduce the risk of both *Salmonella* and *Campylobacter*. During 2012, the USDA conducted a baseline study to establish the rate of percent positive for both *Salmonella* and *Campylobacter* in raw chicken parts. This would include chicken breasts, thighs, wings, legs, necks, backs, half- or quarter-carcasses, and internal organs. The rate of *Salmonella* in chicken parts was found to be 26.3% and the rate for *Campylobacter* was 21.4%, with necks having the highest incidence at 54.55% for both microorganisms. *Salmonella* and *Campylobacter* incidence for other parts are as follows: breast 27.06% and 16.11%, legs 24.14% and 20.38%, wings 33.33% and 23.36%, half carcasses 22.15% and 19.46%, quarter carcasses 20.61% and 27.88%, and giblets 40.35% and 43.86%. The results of this baseline were compared to the results from the 2007-2008 young chicken baseline (chickens that are approximately 6 to 10 weeks old). These results were compared as a whole and as individual parts. *Salmonella* and *Campylobacter* percent positive rates in young chickens were 5.9% and 10.6% respectively (USDA-FSIS, 2015a). There is a significant difference between the whole bird baseline and the parts baseline. These findings have resulted in the implementation of both chemical and physical interventions in second processing.

Multiple Hurdle Approach to Microbiological Reduction in First Processing

Because poultry processors cannot control the way consumers prepare or handle products, the industry has been examining multiple ways to reduce the possibility of microbial contamination. In the past two decades, the industry has introduced the use of numerous chemical and physical interventions within the processing environment in hopes of reducing

microbial loading prior to second processing (Ricke et al., 2005). Many of these interventions are used in what is referred to as a "multiple hurdle" approach (Stopforth et al., 2007; Zweifel and Stephan, 2012; Sofos et al., 2013). This means that the processing facility does not rely on one step to reduce or eliminate bacteria, but incorporate many applications or hurdles in place to continually reduce the microbial load. This tactic also helps to reduce the risk of re-contamination, which is a significant concern. Poultry processing consists of several steps and due to the nature of the process, there are multiple areas that can contaminate or re-contaminate the carcasses (Schuler and Badenhop, 1972; Mead, 1974; 2004; Mead et al., 1994; Stopforth et al., 2007; Owens et al., 2010). Each of these processes can expose the poultry product to different microbiological concerns; pathogenic and/or spoilage organisms. Chemical and physical interventions have been studied at multiple locations in the process, including the scalder, the pickers, the inside outside bird wash, the on-line reprocessing system (OLR) and the chiller system. These stages have been studied individually as well as in conjunction with the entirety of the process (Schuler and Badenhop, 1972; Mead, 2004; Stopforth et al., 2007; McKee et al., 2008; Zweifel and Stephan, 2012; Nagel et al., 2013).

Broilers arrive at a poultry processing facility colonized by any number of microorganisms. The amount and the species of microorganisms present on the broilers upon arrival can vary depending upon many variables, i.e. hatchery, geography, grower, transport conditions, as well as many other factors (Mead, 2004). The processing facility has no control over what conditions the birds are exposed to prior to entering the slaughter facility. The birds not only rub against one another during both transport and processing, but carcasses come in contact with common equipment. Shackles, framework, guides, blades, water, and belts are

considered some of the most common points of contact (Mead et al., 1994). This continual exposure can permit the spread of bacteria not only from bird to bird, but from flock to flock as the day progresses within the facility (Mead et al., 1994; Zweifel and Stephan, 2012). Without an effective sanitation program in place, the contamination can continue to persist further from day to day (Schuler and Badenhop, 1972). Because of these conditions, processors must try to accommodate for the worst-case scenario in the most efficient, effective, and economical way.

Broilers are placed on a communal conveyor upon entering the processing facility. Subsequently, the birds are hung upon the overhead shackles. At this point, the birds are still essentially in the same state as at the farm. Nothing has been washed or rinsed in any matter and any fecal material or dirt/soil that was present during transportation is still present on the bird (Mead, 1989; Owens, 2010). The birds are killed, bled, and subsequently scalded. Just prior to the scalder, some facilities have installed what is known as a pre-scald brush (Alter, 2017). Studies have shown that adding a brush prior to the scald tanks may not necessarily provide a practical reduction in microbial populations prior to scalding, as the reduction in populations is often not significant through processing (Berrang and Bailey, 2009). However, these brushes have been proven to be able to reduce the amount of physical debris from the birds prior to entering the scald process. This reduction in physical debris, which can include bedding and feces, can potentially aid in reducing the microbial populations further in the process (Berrang and Bailey, 2009; Pacholewicz et al., 2016). Reducing the amount of organic material on the bird prior to entering the scalder is important, as it is the first of many communal dips, baths, and washes.

The purpose of the scalder is to prepare the carcasses for the removal of the feathers. It also serves to remove the cuticle in some processes depending upon the temperature of the scald (Bowker et al., 2014). Scalding is generally referred to as soft scald or hard scald. The difference between the two types of scalding is determined by time and temperature (Parry, 1995; Barbut, 2002). According to Parry (1995), soft scald consists of 50.0° to 51.5°C for up to 3.5 minutes, which allows for the retention of the cuticle. Hard scald consists of water temperatures of 56.0° to 60.0°C for 2.0 to 2.5 minutes (Barbut, 2002). Scalding is a necessary part of the process, however, it is often perceived as a potential point of cross-contamination since the scalder water is visibly dirty as soon as the first birds of the day pass through the tanks (Mead, 1995; Sams and McKee, 2010). Despite these perceptions, studies have shown that aerobic plate count bacteria, coliform, *Salmonella* and *Campylobacter* populations on broiler carcasses are generally reduced through the scalder (Cason et al., 1999, 2004; Buhr et al., 2005).

Numerous practices have been implemented within the scalder to reduce the possibility of cross-contamination. Some of these practices include the following: a counter-flow water system (i.e. the birds will travel from an area of dirtier water to cleaner water), using a multiple stage counter-flow scalder (i.e. each stage is a separate tank and the water inside is set up as counter-flow, so none of the dirty water from the previous stage is able to intermingle with the water in the tank proceeding it). According to Cason et al (1999), the amount of total solids and APC populations in a three stage scalder were significantly lower in the 3rd stage of a multiple stage scalder as compared to the total solids and the APC populations detected within the end of a single stage scalder, 1.04 grams/Liter (g/L) ± 0.29 vs. 3.46 g/L ± 0.50 and 3.85 $\log_{10}$ cfu/mL vs 4.96 $\log_{10}$ cfu/mL, respectively. This could lead to the conclusion that the carcasses would then

be less contaminated, since the microbial populations of the water that the carcasses are passing through have been significantly reduced. Additionally, the use of scald additives or antimicrobials have also shown promising reductions of *Salmonella* (McKee et al., 2008; Russell, 2008) and *Campylobacter* (Okrend et al., 1986).

Feather removal or plucking is performed in the pickers. Pickers consist of large bays of rubberized fingers. The fingers aggressively brush against the carcasses as the flock is moved through each bay. These fingers remove the feathers that the scalder has loosened (Parry, 1995; Sams and McKee, 2010). Feather removal has been proven to be a prime location for cross-contamination (Berrang and Dickens, 2000; Allen et al., 2003). As the birds pass through the bays of pickers, each carcass rubs against one another as well as the equipment. The pickers are equipped with a rinse to help push the feathers away from the carcasses as the feathers are removed from the skin. This addition of water in conjunction with the sheer force of the picker fingers can aerosolize the bacteria present on the birds (Allen et al., 2003). Studies have proven that microbial numbers can increase significantly as the carcasses move through the pickers (Berrang and Dickens, 2000; Cason et al., 2004). Allen et al (2003), was able to demonstrate how widespread the contamination from the pickers can be. A single "seeder" bird was inoculated on the outside of the carcass with a generic marker organism just prior to entering the pickers. Upon exiting the pickers, birds that had preceded the "seeder" carcass through the pickers were found to be positive for the marker organism, as well as the birds that followed. Positive birds were found as far as ahead of the "seeder" as 30 carcasses and as far behind the "seeder" as 200 carcasses.

According to Berrang and Dickens (2000), *Campylobacter* numbers can increase by as much as 3 logs after passing through the picking bays. The rubber fingers on the picking machines, as stated earlier, rub aggressively over the feathers and skin of all the birds. Arnold (2009), established that the rubber in new picker fingers show resistance to bacterial attachment. However, over time the picker fingers wear down due to the nature of the function as well as the exposure to either sanitation chemicals or chemicals used for microbial intervention (Arnold and Silvers, 2000). This causes the rubber to acquire cracks and crevices that can become harborage areas for spoilage organisms as well as pathogens (Arnold and Silvers, 2000). Bacteria can remain undisturbed once the colonies are allowed to settle into the compromised areas of the picker fingers. This in turn, leads to the formation of biofilms (Arnold and Silvers, 2000; Arnold, 2009). Biofilms are a polysaccharide coating that acts as a protective covering to the bacteria. Biofilms make it much more difficult to completely remove the bacteria from the equipment (Arnold, 2009; Sofos et al., 2013). Because biofilms often accumulate in areas that cannot be physically scrubbed, the bacteria can persist for long periods of time as most chemicals and sanitizers are ineffective at breaking down the structure (Steenackers et al., 2012). Heat is also largely ineffective in the destruction of bacteria that is embedded in a biofilm. In this case, biofilms are usually removed or loosened during production, thus contaminating the carcasses passing over it (Arnold and Silvers, 2000; Arnold, 2009; Forsythe, 2010). This can lead to the spread of psychrophilic and psychrotrophic organisms, which are commonly found on the feathers and skin of the birds (Barnes, 1972; Sofos et al., 2013). This can also lead to the spread of *Salmonella* (Steenackers et al., 2012) and *Campylobacter* (Arnold, 2009).

Another possibility for contamination through the pickers is due to the amount of bacteria that are released from the intestinal contents of the birds as the picker fingers forcefully push on the abdomen of the carcasses. The ceca, crop, and the cloaca all carry significant quantities of bacteria, which includes the pathogens *Campylobacter* and *Salmonella* (Smith and Berrang, 2006). According to Musgrove et al. (2001), 95% to 99% of broiler crops 63% to 100% of the ceca were contaminated with *Campylobacter,* with average counts of 3.6 $\log_{10}$ cfu/gram and 6.8 $\log_{10}$ cfu/gram respectively. Hargis et al. (1995), reported *Salmonella* prevalence in crops and ceca to be 52% and 14.6%, though the samples were not enumerated to determine the loading of *Salmonella* in the crops and ceca. Processors can prolong the amount of time the birds are in the pickers to ensure the extent of feather removal. However, extending the amount of time that the birds are in the picker bays can prolong the extent to which carcasses can be contaminated by fecal material that is released by the bird or by birds that have passed through the pickers previously (Allen et al., 2003; Cason et al., 2004; Burfoot et al., 2007).

Considerable research has been conducted in this area of processing (Musgrove et al., 1997; Cason et al., 1999; 2004; Allen et al., 2003; Buhr et al., 2003; Berrang et al., 2011a). However, there has been very little progress as far as how to mitigate contamination. Some studies have gone so far as to suggest plugging the cloaca before the birds enter the pickers. This method did help reduce the amount of cross-contamination (Musgrove et al., 1997). However, this is not a practical solution as there is not an effective or an efficient way to implement this practice within commercial processing. Other studies have stated the importance of reducing or minimizing the amount of organic loading prior to entering the pickers, thus reducing the contamination potential in the pickers (Allen et al., 2003). While other

studies recommend the application of antimicrobials post pick (Berrang et al., 2011b), and also keeping the entire process of feather removal separate from other processing areas (Mead, 1995; Barbut, 2002). The heads and feet of the birds are also usually removed in the same area as defeathering. In addition, the carcasses are subsequently transferred to the evisceration shackles. This "rehang" process can either happen manually or automatically (Parry, 1995).

Evisceration is the process by which the gastrointestinal tract is removed from the bird carcass, a multi-step process. This area also includes the subsequent washing/rinsing cabinets leading to the chiller area. Evisceration, like the picking area, is an area where extensive research has been conducted in regards to cross-contamination as the potential for cross-contamination is obvious (Russell and Walker, 1997; Berrang and Dickens, 2000; Li et al., 2002; Northcutt et al., 2003; Hinton et al., 2004; Gill et al., 2006; Smith et al., 2007; Reiter et al., 2007; Berrang and Bailey, 2009; Guerin et al., 2010; Berrang et al., 2011a; Liang et al., 2013; Seliwiorstow et al., 2016). Numerous factors in the evisceration process can increase the potential for cross-contamination, but with proper management of equipment operations, facility hygiene and employee hygiene, this risk can be minimized (Barbut, 2002).

In facilities with an automatic process, there are several pieces of equipment in this area, including the oil gland remover, the venter or cutter, the eviscerator, the pack puller, the cropper, and the inside outside bird wash, which all touch the inside of each and every bird within the process (Sams and McKee, 2010). The oil gland remover consists of a rotating blade that excises the gland from the tail (Barbut, 2002). The venter/opener opens the carcass up in preparation for removal of the intestines. The venter probes open the carcass and pulls the lower intestine out of the carcass. If the machine is adjusted properly and managed to

accommodate bird size, the intestine remains intact. If not, the intestines can rip, causing contamination of the machine (Parry, 1995). Chlorinated sprays are often employed on the equipment to reduce contamination (Sams and McKee, 2010). Most automatic eviscerators are comprised of a mechanism that opens the carcass as well as a second mechanism that pulls the viscera from the inside of the carcass. If at any time the intestinal packs are torn, which can occur in both manual and automatic evisceration, the packs can release the contents of the intestinal tract onto the birds and onto the equipment (Barbut, 2002; Smith et al., 2007; Sams and McKee, 2010). Each subsequent carcass that passes through the equipment can then become contaminated. This is a major concern as the intestines are known to be a key reservoir for pathogens such as *Salmonella* and *Campylobacter*, as well as numerous other nonpathogenic organisms (Hargis et al., 1995; Berrang and Dickens, 2000; Musgrove et al., 2001; Hinton et al., 2004; Smith and Berrang, 2006). The giblets may or may not be collected at this point in the process. This depends on the customer market of the processing facility. After the intestines are removed, the crop is removed by the cropper. This consists of a serrated probe that passes through the carcass as it rotates. Once the probe is extended through the neck, the crop is dropped out of the bird (Sams and McKee, 2010).

The crop can also be a concern for contamination. The crop is a part of the digestive tract in broilers, and functions to store food prior to entering the gizzard for digestion (Svihus, 2014). Feed can bypass the crop if birds have food readily available as it would be when fed *ad libitim.* However, when birds are fed at specified times, the crop had significantly higher crop content or feed (Svihus, 2014). As previously stated, Musgrove et al. (2001) found *Campylobacter* in 95% to 99% of the crops sampled. While Hargis et al. (1995), found 52% of the

crops to be contaminated with *Salmonella,* additionally, this study found that crops were more likely to tear than ceca. This data supports the conclusion that proper management of the cropper is important to the hygiene of the carcasses. Croppers are often equipped with an antimicrobial rinse to help mitigate contamination (Sams and McKee, 2010).

After all the inner components of the carcass have been removed, the birds are further transported through the inside/outside bird wash (IOBW). The IOBW is a type of equipment that is designed to wash visual contamination from the inside of the carcass as well as the outside of the carcass. Many IOBWs are set up as cabinets that consist of probes, which may or may not have small rubber fingers that are lowered into the carcasses. As these probes spray the inside of the carcass, spray bars rinse the outside of the carcass with potable water. Since the IOBW is more focused on the removal of organic material and does not necessarily focus specifically on microbial reductions, a potable water rinse is very common. However, IOBWs can be equipped with antimicrobial sprays (Parry, 1995; Barbut, 2002; Sams and McKee, 2010). The probes may or may not touch the insides of the carcass. However, if the probes do touch the inside of the carcass and there is intestinal content remaining within the bird, the sprayers found inside the probes can then propel these contaminants onto each subsequent bird. The IOBW often does not include any type of antimicrobial, thus the rinsing action of the water is the only bacterial deterrent. The water is also frequently set at ambient temperatures and is not cold enough to prevent the proliferation of *Salmonella* (Graziani et al., 2017). It is also not warm enough to inhibit the growth of or reduce the *Campylobacter* population. Li et al., (2002) reported that carcasses that were inoculated with *Campylobacter* showed no reduction in *Campylobacter* populations when exposed to 20°C water in an IOBW. When the temperature of the water was

increased to 55°C, *Campylobacter* populations were reduced by 0.6 $\log_{10}$ cfu/cm^2. Yet, in that same study, when 50 parts per million chlorine were added to the IOBW, *Campylobacter* populations on the carcasses were reduced by 0.5 $\log_{10}$ cfu/cm^2 (Li et al., 2002). This illustrates the importance of considering the use of antimicrobials. Northcutt, et al. (2003), reported that despite the removal of visual contamination through the IOBW, reductions in bacterial populations were not consistent. This was due to the inconsistencies of application of the IOBW in the three facilities that were sampled.

On-line reprocessing (OLR) has become an industry standard in recent years. The use of an OLR relieves a plant from the constraints of off-line reprocessing, which consists of having to pull visibly contaminated birds from the shackle lines and manually wash the carcass (USDA-FSIS, 2014c) . Off-line reprocessing is labor intensive and there is no guarantee that each bird that has visible fecal contamination is removed from the line and washed. An OLR system is generally a cabinet or dip tank that contains an antimicrobial. Every bird on the processing line passes through this system. This ensures to a higher degree of certainty that if a bird is visibly contaminated that it will be properly washed. In order for an establishment to install an OLR system, it must go through a validation to prove to USDA that the system is effective in reducing the microbial counts found on carcasses that are visually contaminated (Bilgili et al., 2002).

In addition to the IOBW and OLRs, many facilities also utilize other wash (spray or dip) and brush cabinets (Kemp et al., 2001). The effectiveness of these applications at reducing bacterial loads is often dependent upon many variables. One of the most important factors is where in the process the application is located, the antimicrobial used, the concentration of the

antimicrobial, the amount of time the antimicrobial is able to stay in contact with the carcass, as well as the upkeep of the brushes/spray bars that are utilized (Cords et al., 2005).

Broilers proceed to the chiller system, after passing through the OLR. The immersion chill system is the most commonly utilized chill system in the United States (Carroll and Alvarado, 2008; Sams and McKee, 2010). Immersion chill systems are set up to immerse the broilers in chilled water to lower the temperatures of the carcasses to less than 40° F (4.4°C). Most modern immersion chillers are set up as multiple tanks with water that flows in a counter flow manner. Clean water is added at the exit end of the chiller and flows back toward the entrance of the chiller. This system ensures that as the birds are exposed to the cleanest water possible upon exiting the chiller (Petrak et al., 1999). The process is often set up as follows: the pre-chiller tank, the main chiller (which usually consists of multiple tanks) and a post chill tank (Sams and McKee, 2010). The pre-chiller has two main functions. It is used to aid in the chilling process by gradually lowering the carcass temperature. Secondly, it is used to wash any remaining organic material from the carcass of the bird prior to it entering the main chiller (Barbut, 2002). The carcasses are generally in the pre-chiller for 10 to 15 minutes and then transferred to the main chiller (Sams and McKee, 2010).

Chilling of carcasses is regulated by the USDA and the process has to be monitored closely by the processing facilities (USDA-FSIS, 2014a). Immersion chillers are used for much more than reducing the temperatures of the birds. The broiler carcasses are in the chiller for a prolonged amount of time-often anywhere from 45 to 110 minutes (Sams and McKee, 2010). This ample dwell time gives processors the opportunity to introduce antimicrobials to the birds and allow the carcasses to have adequate contact time with these antimicrobials to effectively

lower the levels of pathogens and spoilage organisms to a point that these organisms are almost undetectable (Mead and Thomas, 1973; Allen et al., 2000). Numerous studies and reviews have been conducted in regards to which parameters and antimicrobials are the most effective for the chiller application (Blood and Jarvis, 1974; Thomas and McMeekin, 1980; Allen et al., 2000; Buhr et al., 2005; James et al., 2006; Northcutt et al., 2006). Immersion chillers have been criticized because of the potential for cross-contamination (Bailey et al., 1987; Bilgili et al., 2002). If contaminated carcasses enter the chill system, there is a possibility of those contaminants spreading to other carcasses (Petrak et al., 1999; Carroll and Alvarado, 2008; Zheng et al., 2011). Chlorine is commonly used in the main chiller as an effective antimicrobial in the U.S. With the addition of chlorine and the proper parameters for counter flow chilling and make-up water, the immersion chill system can reduce aerobic plate counts by as much as 1.0 log cfu/mL, and can reduce *Salmonella* incidence and *Campylobacter* incidence as well (Bilgili et al., 2002). In recent years, processors have had to look for other antimicrobial options due to fact that the FSIS microbiological standards for post-chill broiler carcasses have become more stringent in recent years (USDA-FSIS, 2011a). This has caused other antimicrobials such as peracetic acid (PAA) to gain popularity (Sukted et al., 2017). PAA has been proven to be an effective antimicrobial in the chiller system. Nagel et al. (2013), reported that poultry chillers treated with PAA showed promising reductions in both *Salmonella* and *Campylobacter*, at approximately 2 $\log_{10}$ cfu/mL for both pathogens. Other studies have demonstrated increased shelf life in poultry products that were treated with PAA during the chiller process (Bauermeister et al., 2008a, b).

Commonly used Antimicrobials in Poultry Processing

There are numerous and varied types of antimicrobials available for use within poultry processing. Processors must choose which chemical is the right choice for each application. There are many factors involved in choosing the correct chemical for the specific applications. These factors generally consist of worker safety, ease of use, antimicrobial efficacy, and cost of use. Furthermore, studies have shown that sometimes the antimicrobials used to eradicate *Salmonella* may not be effective against *Campylobacter* (Arritt et al., 2002)

Chlorine has historically been the most common antimicrobial used in poultry processing in various applications. Chlorine is used for crate washers, belt washers, carcass washes, carcass sprays, chiller interventions and general sanitation (Mead and Thomas, 1973; Kotula et al., 1997; Cords et al., 2005; Bauermeister et al., 2008a; Berrang et al., 2011b; Demirok et al., 2013; Chen and Hung, 2017). Chlorine is an effective antimicrobial as "it is considered a broad-spectrum germicide" (Cords et al., 2005). This means that it is capable of killing a broad range of bacteria, fungi, and algae which is important within a poultry processing facility (Cords et al., 2005). The following equation represents the chemical reaction that takes place when chlorine is added to water: $Cl_2 + H_2O \rightarrow HCl + HOCl$. The chemical reaction that takes place creates hypochlorous acid (HOCl). Hypochlorite ions (OCl^-) can also be present depending upon the chemical makeup of the water solution. The pH of a solution will affect the proportion of HOCl to OCl^- that is present in solution. As the pH rises, the amount of HOCl decreases and the amount of OCl^- increases. HOCl is more efficacious as an antimicrobial, thus making it important to maximize the amount of HOCl present (Dychdala, 1991; Cords et al., 2005). Adjusting the pH of the solution can be a balancing act, as too high a pH can reduce the antimicrobial activity of the chlorine as previously stated, but too low a pH can pose other hazards. As the pH approaches 5,

corrosion to equipment is more likely (Walker and LaGrange, 1991). Additionally, as the pH drops further the chances of off-gassing also increase, creating a serious risk to employees.

The amount of hypochlorite and hypochlorous acid present in the solution is referred to as available chlorine or free chlorine. Free available chlorine is very dependent upon the organic load within the solution (Kotula et al., 1997; Cords et al., 2005; Bauermeister et al., 2008a). Therefore, chlorine is not considered an ideal choice for applications where the carcasses are not visibly clean, which would include such areas as the scalder and the pickers. In these applications, the feathers are still intact and in some instances, contain heavy buildup of fecal matter. The pre-chiller, where much of the residual fat and blood is washed off, is another example of an application where chlorine would not be an ideal antimicrobial choice. Extensive research has been performed on the effectiveness of chlorine within poultry immersion chillers. Chlorine has been proven to be an effective antimicrobial in immersion chillers if the proper parameters are met-water overflow and pH (Mead and Thomas, 1973; Nagel et al., 2013).

PAA has become popular in poultry processing over the last decade (Sukted et al., 2017). PAA, like chlorine, is a broad-spectrum germicide. Additionally, PAA is not significantly affected by pH or organic loading of the solution (Block, 1991; Cords et al., 2005). PAA is not negatively affected by pH in the same manner as chlorine and it is more efficacious at lower pH. Raising the pH above 7 does not inhibit the overall efficacy of PAA, but merely slows the activity. This can be overcome by increasing the concentration of the PAA (Cords et al., 2005).

As PAA is not deactivated by organic loading or pH, it is commonly used in poultry dips, washes and spray applications. Significant amounts of research have been performed showing the effectiveness of PAA as an antimicrobial in poultry applications (Cords et al., 2005). Nagel et

al. (2013), demonstrated that PAA (400 ppm and 1000 ppm) used in a post chill dip application could reduce *Salmonella* and *Campylobacter* populations by approximately 2.0 log cfu/mL, while chlorine (40 ppm total chlorine) reduced the microbial populations by less than 1.0 log cfu/mL. Bauermeister et al. (2008b), demonstrated that PAA is not only an effective antimicrobial for reducing *Salmonella* and *Campylobacter* populations within the immersion chiller, but it can also improve shelf life qualities such as color, flavor, and juiciness.

Sanitation Standard Operating Procedures (SSOPs) and Good Manufacturing Practices (GMPs)

Effective sanitation standard operating procedures and good manufacturing practices are critical to the effectiveness of the microbial intervention strategy and the HACCP plan (Barbut, 2002; Forsythe, 2010; Bilgili, 2010; Davis et al., 2010). Sanitation standard operating procedures are the written details regarding how a plant will start each production run under sanitary conditions, how the plant will maintain sanitary conditions during production and how the plant will be restored to sanitary conditions after the production run has ended. The Code of Federal Regulations Title 9 part 416.1 states, "Each official establishment must be operated and maintained in a manner sufficient to prevent the creation of insanitary conditions and to ensure that product is not adulterated." Part 416 addresses the creation of written SSOPs, the implementation, maintenance, corrective action, proper documentation, and FSIS verification of those SSOPs (USDA-FSIS, 2016). Therefore, it is imperative that a facility not only develops written SSOPs that address sanitation (operational and pre-operational), but that these SSOPs have been established as effective (Davis et al., 2010; USDA-FSIS, 2016). An official SSOP must include the daily procedures that are carried out to prevent the occurrence of insanitary conditions that would contaminate or adulterate product. This includes all procedures that take

place prior to operations, during operations and after operations. The SSOP must include the frequency that the procedure must be carried out, along with the person who is responsible for the execution and upkeep of the procedure (USDA-FSIS, 2011b). The procedures within the SSOP must be monitored in order to verify compliance to the regulation. Monitoring tasks have to be documented and the records must be available to FSIS for review. Corrective actions must be implemented if it is determined that the SSOP was not followed as intended, or if the procedure within the SSOP did not prevent the contamination of product. This is true whether the situation is identified by the facility or by FSIS. Corrective actions must state what the facility intends to do with the affected product, how the facility will restore sanitary conditions, and how any inadequacies within the SSOP will be addressed (USDA-FSIS, 2011b; 2016).

Good manufacturing practices are a critical in the processing of food products. The hygiene of the facility and the employees can have a direct effect on the products produced (Forsythe, 2010; Davis et al., 2010). According to Blackburn (2007), "GMP is concerned with the general (i.e. non-product specific) policies, practices, procedures, processes, and other precautions that are required to consistently yield safe, suitable foods of uniform quality." GMPs generally include the general hygienic practices (GHP) as well. Together, these include the hygienic design of the facility, the hygienic design of the processing equipment, cleaning and disinfection procedures, the microbiological quality of ingredients, sanitary operations of each production process, and the hygiene of production workers (Forsythe, 2010). In order to produce wholesome products, a facility must have a robust HACCP plan in place. Without a proper GMP program, the HACCP plan cannot function as intended (Davis et al., 2010).

Methods of Monitoring Bacterial Contamination within the Poultry Slaughter Facility

Currently, the most common methods for monitoring possible microbiological contamination within poultry processing facilities are pre-operational environmental swabs, operational environmental swabs, and finished product testing. Swabbing of the equipment establishes the sanitary conditions of the equipment either prior to operations or during operations, while finished product testing confirms the effect of the equipment hygiene on the product.

Pre-operational swabs are used to measure and verify the effectiveness of the sanitation procedures (Mead, 1995; Downes and Ito, 2001; Cramer, 2013). Pre-operational swabbing programs often consist of either ATP (adenosine triphosphate swabs) or bacterial plate swabs (Downes and Ito, 2001). Both of these types of swabs are used to focus on non-product contact surfaces, as well as product contact surfaces within the processing area. This data is critical in establishing the effectiveness of sanitation SOPs, essentially ensuring that a facility is starting the production day under sanitary conditions.

In order to measure the presence or the amount of viable bacteria present, a bacterial plate swab is used (Forsythe, 2010). The cotton tipped swabs can either be taken prior to sanitizer application or after the application of a sanitizer, but just prior to the setup of production equipment and tools (Downes and Ito, 2001). If taken after the application of sanitizer, the swab should be moistened in a buffer that is capable of neutralizing the sanitizer applied, which will prevent any residual sanitizer from reducing the bacteria that is present on the swab (Downes and Ito, 2001). These swabs are generally streaked to aerobic plate count agar (APC). Results are typically reported as cfu per square inch or cfu per square centimeter. Because bacterial colonies can be enumerated and further identified if necessary, these swabs

allow food safety teams within the plants to determine potential hygiene risks of the processing environment. The drawback of bacterial swabs is that outcomes of bacterial enumeration are lagging. Results are generally not available for at least 24 to 48 hours after collection (Forsythe, 2010; Cramer, 2013).

ATP swabs, also referred to as bioluminescence swabs, are effective at exposing any residual films or buildup from prior shifts of production before production commences. ATP swabs measure the amount of relative light units emitted by ATP that is found on the surface of the equipment (Aycicek et al., 2006; Cramer, 2013). While this is helpful in determining if there is residue present, ATP swabs do not show whether or not these films are made up of protein residues or of viable bacteria (Forsythe, 2010; Shama and Malik, 2013; Cramer, 2013). Although ATP swabs cannot provide data regarding bacterial counts, these swabs can provide the knowledge that there is a potential for bacterial growth, either due to bacterial populations that are present or due to the presence of a reside that can potentially promote the growth of bacteria (Aycicek et al., 2006; Forsythe, 2010; Shama and Malik, 2013; Cramer, 2013). ATP swabs are convenient as results are provided within minutes as opposed to bacterial plate swabs which usually provide results the following day at the earliest as previously stated. This allows the facility to take immediate action to correct the situation. If a swab result is above the established target, the area or piece of equipment is able to be recleaned before product can be affected (Cramer, 2013). However, care has to be taken when performing ATP swabs, as the presence of residual detergents and sanitizers can adversely affect the results. (Forsythe, 2010; Shama and Malik, 2013). This can potentially cause processors to make decisions based upon erroneous data.

Operational swabs are taken to ensure that sanitary conditions are being maintained throughout the production day. Obviously, the results of these swabs are not able to be directly compared to the results of the pre-operational swabs as the product contact surfaces have been exposed to raw poultry which is not sterile. Because these swabs will have higher counts, it is necessary to make serial dilutions to ensure a countable result. Also, if a facility establishes a baseline of the microbiological counts on the equipment that is swabbed during operations, future results can be compared to the baseline to establish whether or not there is an upward trend that is indicating that a microbiological issue such as spoilage exists.

Finished product testing is the microbiological testing of the product at the end of the production line. Finished product testing can help reduce the amount of contaminated product that reaches consumers (Forsythe, 2010). There are some drawbacks to finished product testing. Davis et al. (2010) states that product testing is normally performed by testing samples that are indicative of a lot or a batch of product as it is not feasible to test 100 percent of the product, which makes sampling plans extremely important. Sampling plans must be vigorous enough to encompass the process to ensure that results are statistically and biologically valid. Microbiological trends must be evaluated on an ongoing basis in order to address issues within the process (Forsythe, 2010; Davis et al., 2010). Microbiological standards or specifications for finished product can be set by different authorities. Some are set up as government regulations, as is the case with broiler carcasses, while other product specifications are set by food companies, either the producer or the end receiver of the product (Forsythe, 2010). Specifications should be established by assessing historical data as well as the risks associated with the microorganism.

Conclusion

Chemical applications have been studied throughout first processing in poultry slaughter. Poultry processors are in need of solutions to reduce microbial populations that are more cost effective and more palatable for customers. As there are multiple opportunities through first processing for cross-contamination or re-contamination, current practices within first processing need to be studied and research gathered in order to better understand how certain common methods or management practices can possibly reduce or increase microbiological risk.

Current Study

Pre-chiller management and the microbiological effect is an area in first processing that has not been studied thoroughly. Most documentation found in regards to pre-chiller management are in reference to moisture loss or moisture pickup. The current study will analyze the relationship between the temperature of the pre-chiller system with APC populations, *Salmonella* spp. populations and incidence and *Campylobacter* spp. populations and incidence on WBCR. This study will analyze WBCR from 2 separate pre-chillers set at different temperatures, one warm (>70°F or 21.1°C) and one cooler (60°F or 15.6°C). The microbiological impact of the differing temperatures will be reviewed to understand if this is an area of processing that should be monitored in order to help reduce microbiological risk.

Effects of Pre-chiller Temperature on the Microbial Ecology of Whole Bird Carcass Rinses

Abstract:

Salmonella and *Campylobacter* are major issues for poultry processors because of increasing regulatory standards in conjunction with public health concerns. In order to assess the impact of pre-chiller temperatures on whole bird carcass rinses, trials were conducted to analyze the effects of pre-chiller temperatures on the incidence of *Campylobacter* spp. and *Salmonella* spp., as well as the aerobic plate counts (APC) on whole bird carcass rinses (WBCR). WBCR samples were tested before and after the pre-chiller to assess the microbiological impact of different temperatures on the microbiological quality of the carcasses. These tests were performed in two separate studies. The results from the first study revealed that APC populations and *Salmonella* rate on WBCR both exhibited significant increases in pre-chillers with warmer temperatures (3.50 $\log_{10}$ cfu/mL to 3.75 $\log_{10}$ cfu/mL and 13% to 73% respectively); greater than 70°F or 21.1°C as compared to 60°F or 15.6°C. *Campylobacter* results demonstrated a significant reduction prior to the pre-chiller to post pre-chiller in both counts and incidence in the two systems (0.36 $\log_{10}$ cfu/mL, 0.41 $\log_{10}$ cfu/mL and 32% to 20%, 30% to 10%). Make-up water within each pre-chiller was not adequately measured at this facility. Thus, the study was repeated at a second facility. These results demonstrated that when pre-chiller water levels were adequate, neither pre-chiller temperatures nor the conditions of the pre-chiller water had a significant adverse impact on the microbial quality of the WBCR. APC populations decreased through both pre-chillers (0.36 $\log_{10}$ cfu/mL and 0.39 $\log_{10}$ cfu/mL). *Salmonella* populations also increased significantly through the cooler pre-chiller, as opposed to 0.27 $\log_{10}$ cfu/mL in the warmer pre-chiller. Although statistically significant, this impact was not

substantial as the incidence of *Salmonella* increased through both systems. These results indicate that there is no significant microbiological benefit to running the pre-chiller at lower temperatures when the make-up water within the system is adequate. This information can allow processors to focus efforts on other parts of the process to reduce the microbiological loads prior to second processing.

Introduction:

Numerous poultry slaughter operations are equipped with multiple stage chill systems. (Barbut, 2002; Sams and McKee, 2010). The function of the chiller system is to cool the carcasses to below 40°F (4 to 5°C). This is to prevent the outgrowth of unwanted bacteria or pathogens (USDA-FSIS, 2014a). This system will often include a pre-chiller. Historically, the pre-chiller has been used to reduce the organic load on carcasses, as well as gradually lower the carcass temperature before the carcasses enter the main chill system (Barbut, 2002; Sams and McKee, 2010). While in the pre-chiller, the carcasses also experience water absorption. Water absorption is dependent upon numerous factors including water to carcass ratio, friction or movement of the carcasses, time, and temperature (Jones and Grey, 1995; Barbut, 2002; Sams and McKee, 2010). Moisture absorption is regulated by United States Department of Agriculture (USDA) due to the economic impact on the final product and is monitored closely by both the processor and USDA officials (USDA-FSIS, 2015b). Some processors may attempt to maximize absorption up to the legal limits by raising pre-chiller temperatures; however, published data that proves or disproves whether this practice has adverse microbiological effects is not available. At elevated temperatures, any microorganisms present may survive and possibly proliferate (Fung, 1987). Proliferation of unwanted bacteria (specifically *Salmonella*,

Campylobacter, spoilage bacteria and aerobic bacteria) may lead to quality and food safety issues, as well as regulatory non-compliances (Thomson et al., 1966; Barnes, 1972; Mead, 1995; Barbut, 2002; Smith et al., 2007; Handley et al., 2010). Understanding the effects of the pre-chill system on the microbial environment during poultry processing could help determine if water temperature exhibits any microbiological impact on the WBCR, and assess whether this impact is carried on throughout the process. There is little information on the parameters or the management of the pre-chiller or the impact that these parameters might have on the microbiological profile of WBCR.

In the current study, the effects of pre-chiller temperatures, greater than 70°F (21.1°C) and approximately 60° to 65°F (15.6° to 18.3°C), on APC, *Salmonella,* and *Campylobacter* populations on WBCR were analyzed. Determining the responses of these microbial populations will aid in understanding the impact of poultry processing conditions as a function of spoilage/shelf-life, and food safety. These results were compared to the conditions of the pre-chiller water-mineral content, chemical oxygen demand, biological oxygen demand, total suspended solids, and total dissolved solids to determine if there was a relationship between the pre-chiller environment to the microbial results of the WBCR. The resulting data could enable processors to make more informed decisions when deciding the processing parameters of the chilling systems, whether it be reducing the pre-chiller temperatures or increasing interventions further in the process.

Methods and Materials:

Trial 1 Sampling:

The first trial was performed in a commercial facility equipped with two evisceration lines, each with a spiral pre-chiller directly before the main chiller. Water temperature in the

body of pre-chiller 1 was targeted at 60°F (15.6°C). The temperature of pre-chiller 2 was set at greater than 70°F (21.1°C). All WBCR samples were collected over the course of a single day throughout two shifts (approximately 15 hours). Each processing line ran the same flock throughout the day. Sampling began 30 minutes after the first carcasses reached the pre-chiller. Thirty birds were collected just prior to each pre-chiller and directly after each pre-chiller, for a total of 60 birds from each line. The WBCR samples were collected at a rate of 3 consecutive birds per line at approximately 1.5 hour intervals. Samples were collected by using sterile gloves to remove the carcass directly from the evisceration line just prior to the pre-chiller. Post pre-chiller samples were collected directly off the pre-chiller exit slide just prior to entering the main chiller. Each carcass was placed inside a sterile shaker bag (Fisher Scientific, 300 Industry Dr., Pittsburgh, PA 15275) and rinsed for 1 minute with a 400 mL solution of sterile, refrigerated Butterfield phosphate buffer (Edge Biologicals, Inc., 598 N. 2nd St, Memphis, TN 38105) with 2.0 mL of 20,000 parts per million (ppm) sodium thiosulfate (Sigma-Aldrich, P.O. Box 14508, St. Louis, MO 63178) as a neutralizing agent. Immediately after rinsing, the carcasses were removed from the shaker bag, and the rinsate was returned to the original Butterfield's container. Samples were held on ice until transported to the on-site plant laboratory. All samples were transported to the onsite laboratory within 30 minutes of collection.

Neither pre-chiller was treated with a chemical intervention such as peracetic acid (PAA) or chlorine, however, the carcasses had previously been exposed to a PAA spray and dip, with both at a concentration of 150-200 ppm. To ensure that the PAA did not affect the pH of the sample, a portion of each sample was poured off and analyzed with a pH meter (HACH Company, P.O. Box 389, Loveland, CO 80539-0389) to confirm that pH adjustment was not necessary.

From each rinsate, a 1.0 mL aliquot was pipetted and plated onto 3M™ APC petrifilm™ (3M, 3M Center Bldg. 275-5W-05, St. Paul, MN 55144-1000) at the onsite plant laboratory. Petrifilm™ was incubated at 35°C (95°F) for 48 hours (AOAC, 2005a). Pathogen analyses of the samples were performed at an off-site laboratory. Prior to transport, aliquots of each sample were prepared for *Salmonella* analysis. A 30 mL aliquot of each sample was added into a specimen cup containing 30 mLs of 2X buffered peptone water (Becton, Dickinson and Company, 1 Becton Drive, Franklin Lakes, NJ 07417-1880). *Campylobacter* analyses were prepared by adding a 30 mL aliquot of each sample to a specimen cup containing 3.0 mL of 10X buffered peptone water (Becton, Dickinson, and Company). These samples were packed on ice and transported to the pathogen testing laboratory to be analyzed within 36 hours of collection. *Salmonella* spp. was tested via Dupont Qualicon™ BAX (Dupont, Experimental Station 400, 200 Powder Mill Road, Wilmington, DE 19803) (USDA-FSIS, 2014d). *Salmonella* enumeration was tested via direct plating on tryptic soy agar plates (Edge Biologicals, 598 N 2nd St, Memphis, TN 38105), with an XLT4 overlay. This method was derived from the following sources: Jay (1996), FSIS (2014a), and Chipley (1987). *Campylobacter sp*. and enumeration were tested via direct plating on Campy Cefex agar plates (Edge Biologicals), and were confirmed via wet mount for typical cell morphology and motility (USDA-FSIS, 2013).

Water samples were taken directly from the body of each pre-chiller at a rate of 1.0 sample per hour. This resulted in a total of 15 water samples per pre-chiller. Each water sample was analyzed for temperature, APC, pH, free chlorine via a HACH pocket colorimeter II (Hach Company P.O. Box 389, Loveland, CO, 80539-0389), and total dissolved solids (TDS) (Standard Methods, 1997a).

Trial 2 Sampling:

Trial 2 was performed in a facility with two processing lines, each with a rocker pre-chiller located directly before the main chiller. The temperature of pre-chiller 1 was set as close as possible to 65°F (18.3°C). The temperature of pre-chiller 2 was targeted be greater than 70°F (21.1°C). Sampling was conducted over two shifts within the same day (approximately 15 hours). Each processing line ran the same flock of birds throughout the day. WBCR samples were collected hourly for two consecutive shifts and analyzed in the same manner as Trial 1.

Water samples were taken directly from the body of each pre-chiller at a frequency of approximately 1 sample per hour, which resulted in a total of 14 water samples per pre-chiller. In addition to the tests that were performed in Trial 1, the water samples in Trial 2 were analyzed for chemical oxygen demand (COD) (Hach Company, 2009) , biological oxygen demand (BOD) (Standard Methods, 2001), total suspended solids (TSS) (Standard Methods, 1997b), percent protein (AOAC, 2006) and mineral analysis-iron, magnesium, copper, phosphorous, potassium, sodium and zinc via inductively coupled plasma optical emission spectroscopy (ICP-OES) (AOAC, 2005b).

Statistical Analysis

SAS (Statistical Analysis System; Version 9.1.3, SAS Institute, Inc., Cary, NC 27513) was utilized for all statistical analyses. Regression analyses were performed on carcass microorganisms over time for APC, *Campylobacter* spp., and *Salmonella* spp. by using the PROC REG function of SAS. An analysis of variance (ANOVA) was performed using the PROC GLM function of SAS. Plate count data were $\log_{10}$ transformed prior to analysis. Counts reported as being less than the detection limit were given a value of 1 log less than the detection limit,

because the $\log_{10}$ value of 0 is undefined. For example, counts reported as less than 1 were given a value of 0.1. For reported chemistry data, values reported below the detection limit were given the value of the detection limit (less than 1 became 1) as any value placed there could potentially influence means and standard deviations and shift these values into a false direction. Chi square analyses were performed on % positive *Campylobacter* spp. and *Salmonella* spp. data using the PROC FREQ function of SAS. M-tests were performed to determine significant differences between the slopes and/or intercepts of two treatments or groups using the PROC REG function of SAS. Results were considered significant at a p-value of less than or equal to 0.05.

Results

Trial 1

The mean temperature for pre-chiller 1 was 59.92°F (15.51°C) and the mean temperature for pre-chiller 2 was 76.89°F (24.94°C). These temperatures were found to be significantly different and remained constant throughout the production day ($p = 0.12$ and $p = 0.28$) (Figure 1). The APC and TDS results were both significantly impacted by pre-chiller temperatures ($p < 0.0001$ and $p < 0.0001$), with the warmer pre-chiller yielding higher results for both. APC and TDS in pre-chiller 2 increased significantly throughout the day ($p < 0.001$ and $p = 0.0369$) (Figure 2 and Figure 3), while APC populations remained steady in the cooler pre-chiller 1.

Mean log10 APC data for WBCR resulted in a significant reduction through pre-chiller 1 (0.49 log10 cfu/mL) ($p < 0.0001$), while pre-chiller 2 WBCR exhibited a significant increase (0.25 log10 cfu/mL ($p < 0.0001$). *Campylobacter* counts were reduced significantly through pre-chiller

1 and pre-chiller 2, 0.36 $\log_{10}$ cfu/mL and 0.39 $\log_{10}$ cfu/mL ($p = 0.013$), respectively. *Salmonella* populations were significantly reduced through pre-chiller 1, 0.45 $\log_{10}$ cfu/mL ($p = 0.0007$). However, pre-chiller 2 *Salmonella* populations did not exhibit any statistical differences between pre and post pre-chiller carcasses ($p = 0.0016$) (Figure 4).

Carcasses sampled prior to pre-chiller 1 yielded no significant increases in APC populations or *Salmonella* throughout the day ($p = 0.36$ and $p = 0.85$). *Campylobacter* counts increased by 0.17 $\log_{10}$ cfu/mL per 1.5 hours throughout the day on samples taken prior to pre-chiller 1. Despite the increase in *Campylobacter* entering pre-chiller 1, there were no significant changes in any microbiological results throughout the day on the post pre-chiller 1 carcasses (APC $p = 0.26$, *Campylobacter* $p = 0.28$, and *Salmonella* $p = 0.07$). Carcasses sampled prior to pre-chiller 2 did not result in significant increases of *Campylobacter* counts or *Salmonella* counts as the production day progressed ($p = 0.2165$ and $p = 0.1300$). APC populations increased significantly by 0.06 $\log_{10}$ cfu/mL per 1.5 hours ($p = 0.0014$) over the course of the day. Post pre-chiller 2 carcasses showed significant changes in all microbiological results over the course of the two shifts (APC $p \leq 0.0001$, *Salmonella* $p = 0.05$ and *Campylobacter* $p = 0.0003$). The APC populations increased by 0.15 $\log_{10}$ cfu/mL per 1.5 hours (Figure 5) and *Campylobacter* increased by 0.06 $\log_{10}$ cfu/mL per 1.5 hours (Figure 6). *Salmonella* populations decreased throughout the day by 0.08 $\log_{10}$ cfu/mL per 1.5 hours (Figure 7).

Salmonella incidence was reduced (42% to 17%) from pre-chill to post chill in pre-chiller 1. Despite the lack of a significant increase in mean $\log_{10}$ *Salmonella* counts in pre-chiller 2, *Salmonella* incidence increased from 13% to 73% ($p < 0.0001$). *Campylobacter* incidence was reflective of the mean $\log_{10}$ data. Although *Campylobacter* incidence was reduced through both

pre-chillers, 32% to 20% through pre-chiller 1% and 29 to 10% in pre-chiller 2, neither of these were found to be significant ($p = 0.16$) (Figure 8).

Trial 2

The mean temperature for pre-chiller 1 was 63.4°F (17.5°C) and pre-chiller 2 was 74.3°F (23.5°C). There were no significant changes in temperature throughout the day in either pre-chiller ($p = 0.94$ and 0.12) (Figure 9). Water data did not result in any significant differences in chemical oxygen demand, biological oxygen demand, chlorine levels, or total dissolved solids based on pre-chiller temperatures ($p = 0.40$, $p = 0.20$, $p = 0.58$, and $p = 0.33$).

Mean TSS values in pre-chiller 1 were significantly higher than the mean TSS values for pre-chiller 2, 1750.6 mg/L and 762.4 mg/L, respectively ($p = 0.03$) (Figure 12). TSS levels increased significantly over the two shifts in both pre-chillers. Increases in pre-chiller 1 were greater than those in pre-chiller 2. Pre-chiller 1 increased by 257.14 mg/L per hour as compared to pre-chiller 2, which only increased by 56.93 mg/L per hour ($p = 0.009$ and $p = 0.002$) (Figure 13). Percent protein values were also significantly higher in pre-chiller 1 than in pre-chiller 2, 0.44 and 0.21 ($p \leq 0.0001$) (Figure 14). Percent protein values were not affected by time in either pre-chiller ($p = 0.49$).

Mineral content of the pre-chiller water was also tested. Copper and zinc values were found to be nearly identical in both pre-chillers throughout the day, therefore these values were not considered in the statistical analyses. Iron, phosphorous, potassium, sodium, and calcium were also determined to have no significant differences between pre-chillers ($p = 0.43$, $p = 0.09$, 0.35, $p = 0.95$ and $p = 0.25$). Magnesium values were significantly lower in pre-chiller 1 (11.61

ppm) than in pre-chiller 2 (14.45 ppm) ($p \leq 0.0001$) (Figure 15). Magnesium levels also increased throughout the course of the day ($p = 0.0157$ and $p = 0.0003$) (Figure 16).

The mean $\log_{10}$ APC populations in the water of each pre-chiller were significantly different ($p = 0.0029$). The APC populations for water from pre-chiller 1 were 3.51 mean $\log_{10}$ cfu/mL and were 3.20 mean $\log_{10}$ cfu/mL in pre-chiller 2 (Figure 10). Both pre-chillers had significant increases in mean $\log_{10}$ APC populations over the course of 2 shifts and both increased at a rate of 0.06 $\log_{10}$ cfu/mL per hour ($p = 0.0079$ and $p = 0.0003$) (Figure 11).

Positive correlations were found between the mean APC populations and COD, TDS, TSS, magnesium, phosphorous, and potassium in pre-chiller 1 ($p = 0.0315$, $p = 0.0196$, $p = 0.022$, $p = 0.0044$, $p = 0.0086$, and $p = 0.0175$). Mean APC populations and chlorine exhibited a negative correlation in pre-chiller 1 ($p = 0.0190$). Pre-chiller 2 showed positive correlations between mean APC populations and BOD, COD, TDS, TSS, magnesium, phosphorous, percent protein, calcium, iron, magnesium, phosphorous, potassium, and sodium ($p = 0.0002$, $p = 0.0020$, $p < 0.0001$, $p = 0.0001$, $p = 0.0011$, $p < 0.0001$, $p = 0.0164$, $p < 0.0001$, $p < 0.0001$, $p < 0.0001$, and $p < 0.0001$ respectively). Pre-chiller 2 mean APC populations exhibited a negative correlation with chlorine ($p = 0.0252$) (Table 1).

Carcasses were collected before and after each pre-chiller at a frequency of every hour, starting one half hour after the first birds of the day entered the pre-chillers. Mean $\log_{10}$ APC results on carcasses exhibited a significant reduction through both pre-chiller 1 and pre-chiller 2, 0.36 $\log_{10}$ cfu/mL and 0.39 $\log_{10}$ cfu/mL, respectively ($p \leq 0.0001$ and $p < 0.0001$). *Campylobacter* mean $\log_{10}$ counts were generally very low. Mean counts on carcasses sampled prior to pre-chiller 1 were below the detection limit (1 cfu/mL). Carcasses sampled post pre-chiller 1, prior to

pre-chiller 2 and post pre-chiller 2 were 0.12 $\log_{10}$ cfu/mL, 0.82 $\log_{10}$ cfu/mL and 1.28 $\log_{10}$ cfu/mL respectively. Despite the very low counts recovered from both pre-chillers, carcasses sampled before and after pre-chiller 2 yielded significantly higher counts than carcasses from pre-chiller 1 (pre pre-chiller $p = 0.0034$ and post pre-chiller $p = 0.0003$). Carcass data showed that mean $\log_{10}$ *Salmonella* cfu/mL counts were also very low. Samples taken prior to pre-chiller 1, post pre-chiller 1, prior to pre-chiller 2 and post pre-chiller 2 resulted in the following counts: -0.61 $\log_{10}$ cfu/mL, -0.06 $\log_{10}$ cfu/mL, -0.32 $\log_{10}$ cfu/mL, and -0.05 $\log_{10}$ cfu/mL. There was a significant increase in mean counts from carcasses pulled before and after pre-chiller 1, 0.55 $\log_{10}$ cfu/mL ($p = 0.0007$). Pre-chiller 2 carcasses showed no significant differences ($p = 0.0987$) (Figure 17).

Throughout the day, carcasses tested before and after pre-chiller 1 did not exhibit increases in APC populations ($p = 0.12$ and $p = 0.19$) or *Salmonella* counts ($p = 0.59$ and $p = 0.33$) (Figure 18 and Figure 20). Carcasses sampled just prior to pre-chiller 1 yielded *Campylobacter* counts that were also constant throughout the day ($p = 0.17$), however, post pre-chiller 1 *Campylobacter* counts increased by 0.14 $\log_{10}$ cfu/mL per hour, which was significant ($p = 0.004$) (Figure 20). Results for carcasses sampled prior to pre-chiller 2 showed no significant changes in APC populations ($p = 0.21$) or *Salmonella* counts ($p = 0.49$). The *Campylobacter* counts increased by 0.16 $\log_{10}$ cfu/mL per hour through the day ($p = 0.004$). Post pre-chiller 2 carcasses showed significant increases in mean $\log_{10}$ APC populations, mean $\log_{10}$ *Campylobacter* counts and mean $\log_{10}$ *Salmonella* counts over time ($p = 0.0002$, $p = 0.0003$ and $p < 0.0001$) (Figure 18, Figure 19, and Figure 20).

Despite the very low *Salmonella* and *Campylobacter* counts*, Campylobacter* incidence and *Salmonella* incidence both increased through each system. Pre-chiller 1 yielded an increase from 40% positive *Campylobacter* samples to 50% and 13.33% positive *Salmonella* samples to 46.67%. Pre-chiller 2 samples increased from 70% positive *Campylobacter* samples to 100% and 3.33% positive *Salmonella* samples to 23.33% (Figure 21).

The water data from the pre-chillers was subsequently compared to the post chill WBCR data from each pre-chiller system to determine if there were any relationships. The APC mean $\log_{10}$ counts showed significant correlations with phosphorous, potassium and sodium ($p = 0.02$, $p = 0.03$ and $p = 0.03$). Other correlations were present, but were not consistent through both pre-chillers. Correlations based on *Salmonella* $\log_{10}$ counts and *Campylobacter* $\log_{10}$ counts were not consistent across both pre-chiller systems for any of the variables tested (Table 1).

Discussion

Trial 1 results were inconclusive due to processing parameters that were not considered by the original study. In order to reach the predetermined temperature of pre-chiller 1, the facility used recycled water from the main chiller. This chiller system was not equipped with a water meter, thus the gallons per minute were indeterminable. The difference in water usage between the two systems was evident as the water levels in pre-chiller 1 were much higher than in pre-chiller 2. The visual clarity of the water in pre-chiller 1 was also drastically different than that of pre-chiller 2. Pre-chiller 1 water was almost clear with little to no fat or grease buildup. While the water in pre-chiller 2 was visually more typical of a pre-chiller, i.e. pinkish water with a greasy residue. All of this makes it impossible to determine if the reductions in *Salmonella* and APC populations through pre-chiller 1 were due to the dilution of the pre-chiller or the

temperature. There is evidence from previous studies supporting the role of both temperature and dilution, as well as incoming bacterial loads in conjunction with one another (Thomas and McMeekin, 1980; 1981; 1982; 1984; Stopforth et al., 2007; Northcutt et al., 2008a). Neither the temperature or the rate of dilution appeared to impact the incidence or the mean $\log_{10}$ count of *Campylobacter* in this trial, as incidence and counts were reduced through both pre-chillers. The WBCR *Salmonella* results in pre-chiller 2 should be noted. Despite the remarkable increase in *Salmonella* incidence through the system (13% to 73%), the *Salmonella* mean $\log_{10}$ counts for pre and post pre-chiller carcasses remained statistically the same (0.16 $\log_{10}$ cfu/mL and 0.26 $\log_{10}$ cfu/mL, $p \leq 0.0001$). This infers that regardless of how low the incoming *Salmonella* counts are, the washing effect of the pre-chiller can aid in the removal of the bacteria from the carcass. Since processors do not usually add antimicrobial interventions to the pre-chiller due to the high organic load, there is nothing in the water to eliminate the bacteria and it is subsequently allowed to re-attach to carcasses that were previously uninfected. Without sufficient water in the pre-chiller, bacteria can subsequently accumulate throughout the day and lead to contamination of subsequent carcasses (Mead and Thomas, 1973; Blood and Jarvis, 1974) .

Trial 2 was carried out in a facility that allowed for the necessary modifications to the evaluation. This facility was not only capable of setting the pre-chiller temperature, but could also ensure that rate of water added back to the pre-chillers throughout the day, 0.18 gallons (0.68 liters) per bird or 25 gallons (94.64 liters) per minute. More analyses were performed in trial 2 than in trial 1 to better understand the environmental variables of the pre-chiller water and the impact that those variables might have on the microbiological quality of the WBCR.

The results of the environmental data showed that despite the correlation found between the pre-chiller environment and the pre-chiller APC populations, there were no statistical relationships between the pre-chiller APC populations and the WBCR microbial counts that were significant through both pre-chiller systems. This correlates with the results of previous studies performed in chiller environments. According to Northcutt et al., (2006; 2008a), bacteria in a chiller without an added chemical intervention, will reach a point of equilibrium. When this equilibrium is reached, the bacteria will no longer be washed from the carcasses within the chiller.

Based on results from trial 2, it appeared that when the water added to the pre-chillers is consistent, the temperature of the water had very little impact on microbial counts. Although statistically the mean APC of the pre-chiller set at 65°F (18.33°C) was significantly higher than the pre-chiller set at >70°F (>21.1°C) (3.51 $\log_{10}$ cfu/mL and 3.2 $\log_{10}$ cfu/mL, $p = 0.0253$), this could be attributed to the higher number of aerobic counts on the carcasses entering the pre-chiller even though the difference in the populations before the pre-chiller was not found to be significant. APC populations on WBCR sampled before and after the pre-chiller set at 65°F (18.33°C) did not decrease as much as the APC populations did through the pre-chiller set at greater than 70° (>21.11°C), 0.36 $\log_{10}$ cfu/mL and 0.39 $\log_{10}$ cfu/mL respectively. The *Salmonella* counts on the WBCR increased slightly through both pre-chiller systems, 0.55 $\log_{10}$ cfu/mL and 0.27 $\log_{10}$ cfu/mL. This could be the result of other variables that were not monitored during the study. Statistical correlations between the mean APC in the pre-chiller water and the microbial populations of the WBCR were not consistent through either pre-chiller. It should also be noted that despite the increase in *Salmonella* counts through the pre-chiller,

the average counts on WBCR from both pre-chillers were extremely low (-0.06 $\log_{10}$ cfu/mL and -0.05 $\log_{10}$ cfu/mL). *Campylobacter* counts on the WBCR were not affected by either pre-chiller. Regardless of the low *Campylobacter* and *Salmonella* counts, the incidence of both pathogens increased significantly through both pre-chillers, further proving that low bacterial counts are not indicative of incidence (Mead and Thomas, 1973; Blood and Jarvis, 1974) . As stated before, the fact that the microbiology of the WBCR was not affected by the conditions of the pre-chiller could most likely have more to do with other processing variables. This is supported by other studies that have shown that immersion of the carcasses will not have a significant impact on the microbiology of the carcasses unless the temperature of the water is such that it can change the topography of the skin in a manner that it can increase the ability of the bacteria to attach to the skin. This change in the structure of the skin generally happens at much higher temperatures than would be found in chilling; scalding temperatures would be more likely to cause this phenomenon (Northcutt et al., 2006; 2008).

Conclusion

In this study, pre-chiller temperature did not have a direct effect on APC, *Salmonella* incidence, *Salmonella* counts, *Campylobacter* incidence or *Campylobacter* counts when water levels within the pre-chiller systems were consistent. There were no consistent significant correlations found between the mineral content of the pre-chiller water and the microbiological data of the WBCR. This infers that facilities that operate pre-chillers without any intervention chemicals should not be concerned with the temperature of the pre-chiller when adequate amounts of make-up water are available. Instead, processors should focus on reducing the microbial contamination prior to the chiller system as well as post-chill recontamination.

More research is needed in regards to first processing parameters and management and the overall microbiological effects that could subsequently occur at post chill or in second processing.

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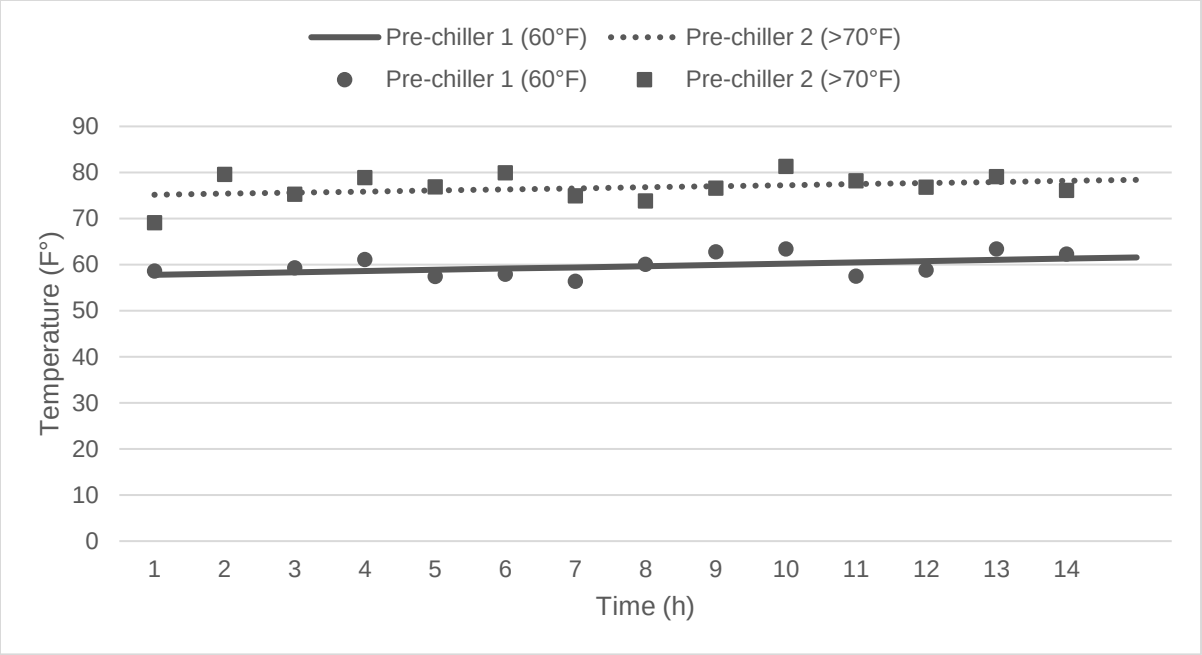

Figure 1: Temperature of Pre-chillers over Time, Trial 1. Temperature data was collected from the body of each pre-chiller every hour over the course of 2 shifts.

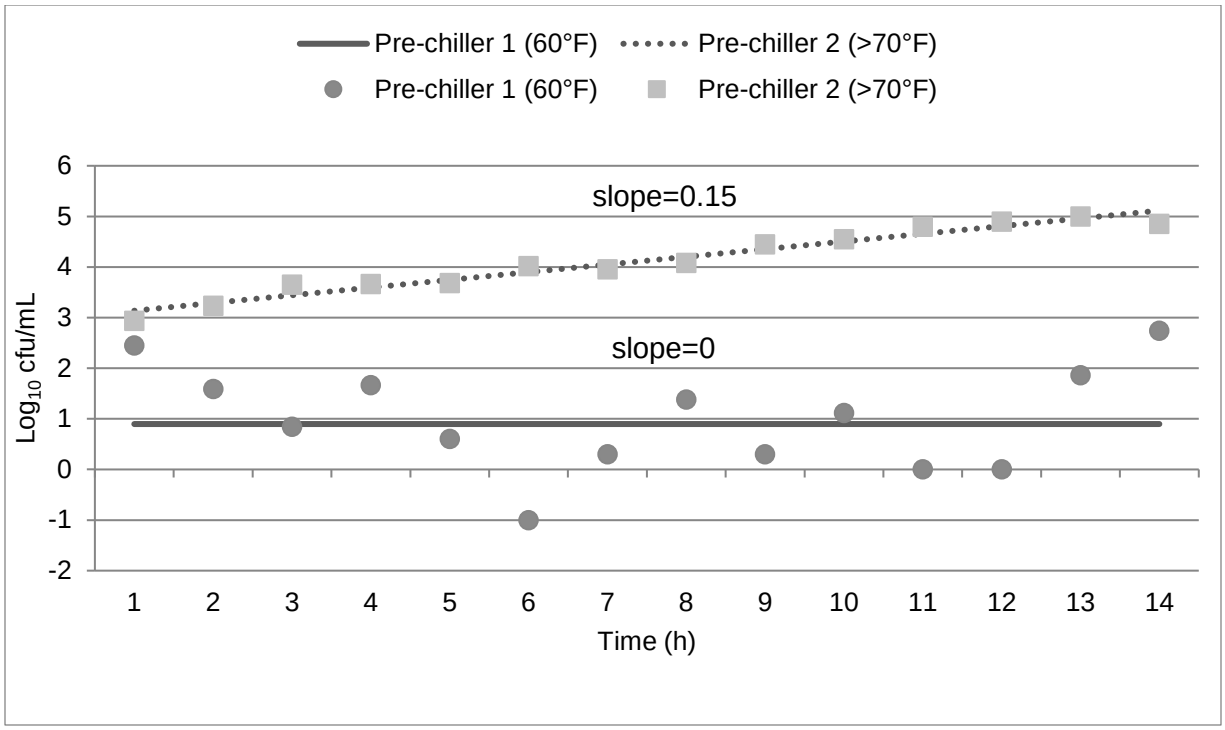

Figure 2: Mean Log_{10} APC Populations in Water over Time by Pre-chiller, Trial 1. Water samples were collected from each pre-chiller at the frequency of 1 per hour over the course of 2 shifts.

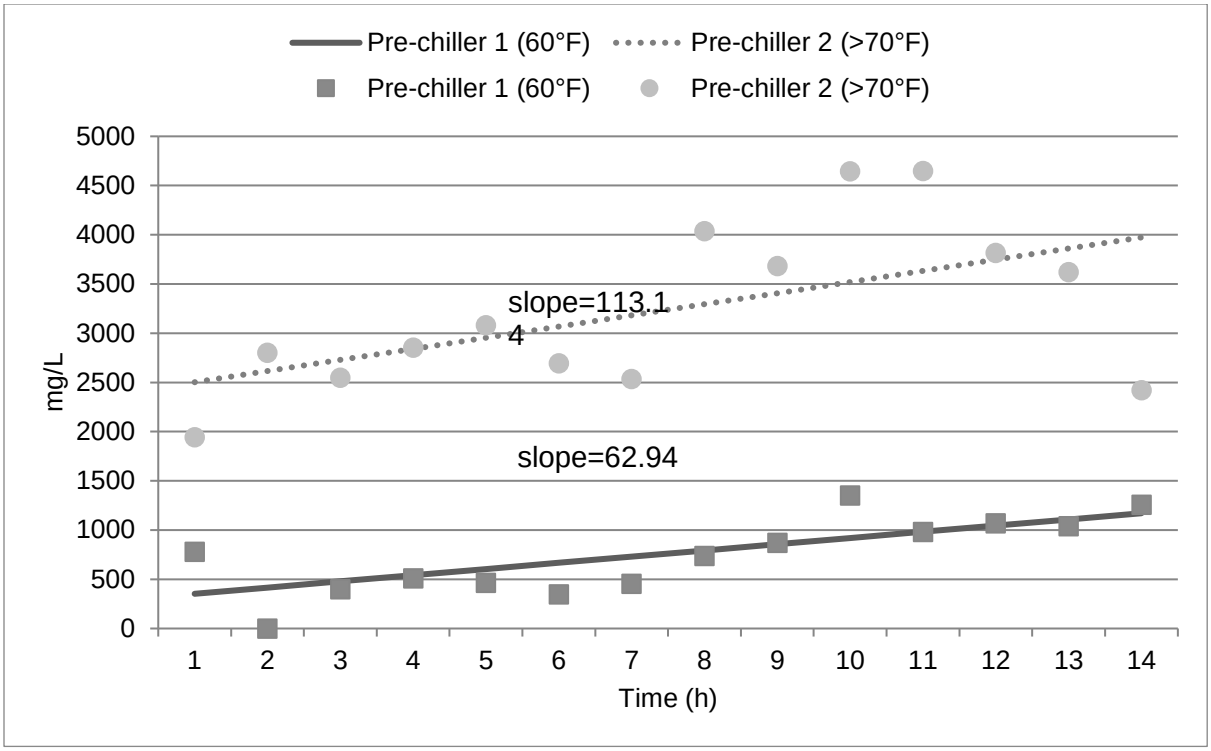

Figure 3: Mean Total Solids in Water over Time by Pre-chiller, Trial 1. Water samples were collected from each pre-chiller at the frequency of 1 per hour over the course of 2 shifts.

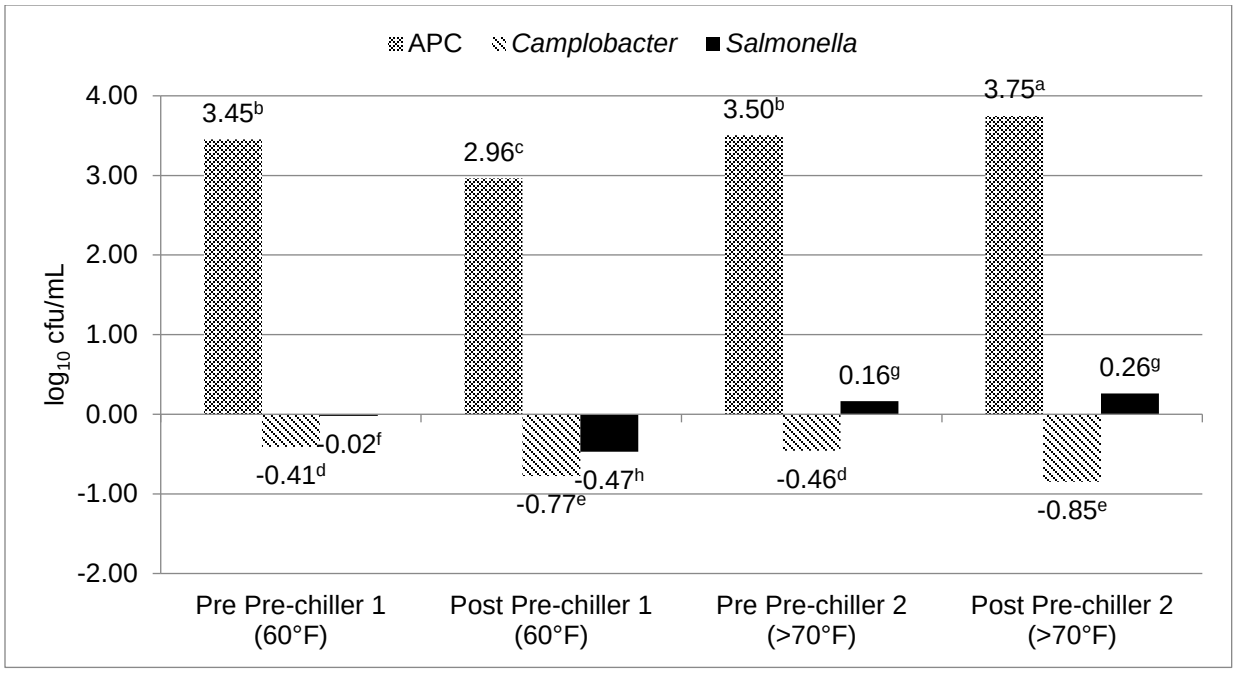

Figure 4: Mean Log_{10} Counts by Pre-chiller and Location, Trial 1. Thirty whole bird carcass rinses (WBCR) were sampled throughout the day from just prior to each pre-chiller as well as just after each pre-chiller for a total of 120 WBCR. These samples were analyzed for *Salmonella* enumeration, *Campylobacter* enumeration and aerobic plate counts. Columns with different superscripts were found to be significantly different.

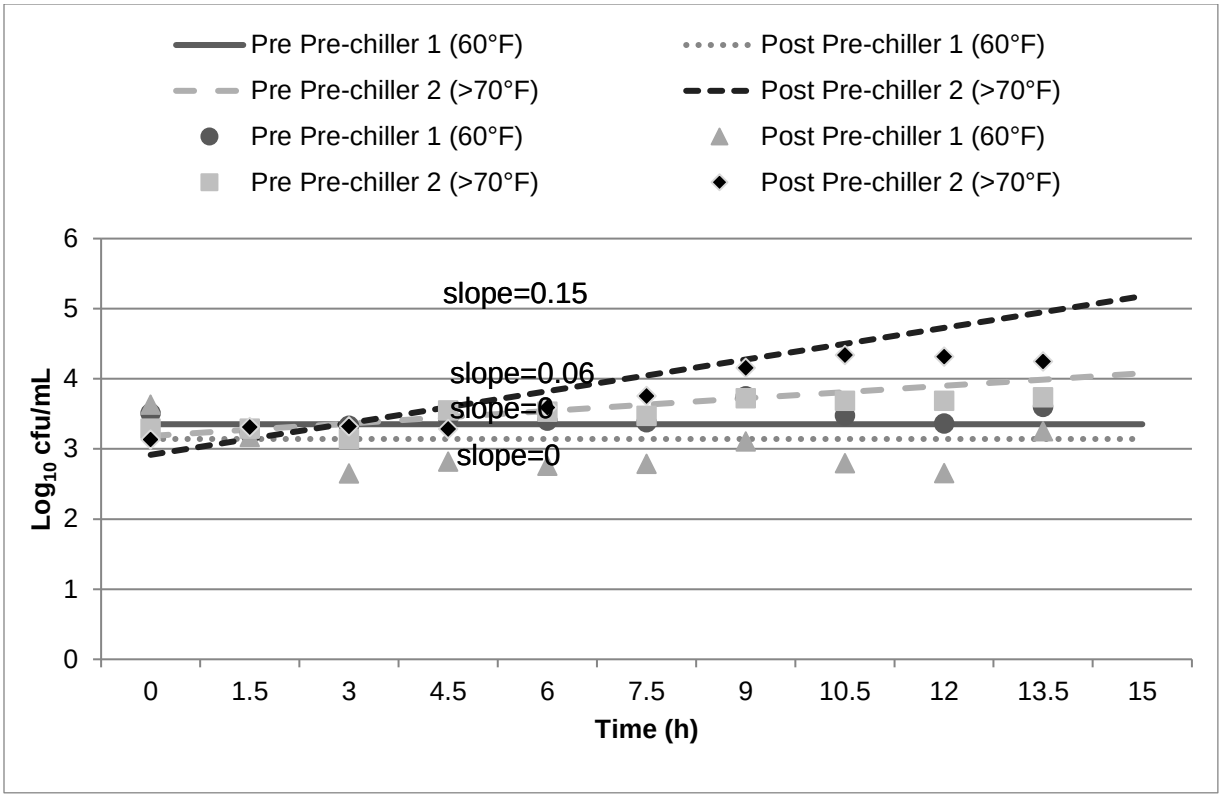

Figure 5. WBCR Mean Log_{10} APC populations by Sampling Location and Pre-chiller over Time, Trial 1. Two whole bird carcass rinses (WBCR) were sampled prior to each pre-chiller as well as directly after each pre-chiller at a frequency of 1.5 hours through the course of 2 shifts.

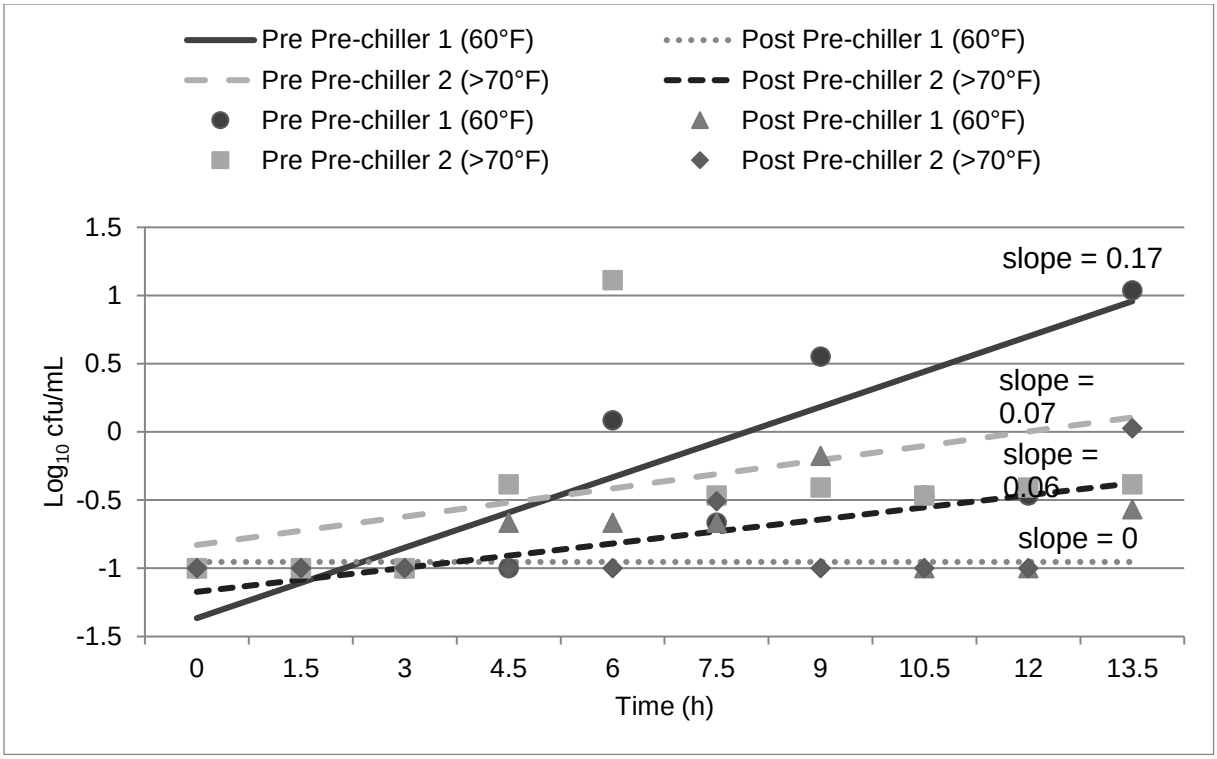

Figure 6. WBCR Mean Log_{10} *Campylobacter* Counts by Sampling Location and Pre-chiller over Time, Trial 1. Two whole bird carcass rinses (WBCR) were sampled prior to each pre-chiller as well as directly after each pre-chiller at a frequency of 1.5 hours through the course of 2 shifts.

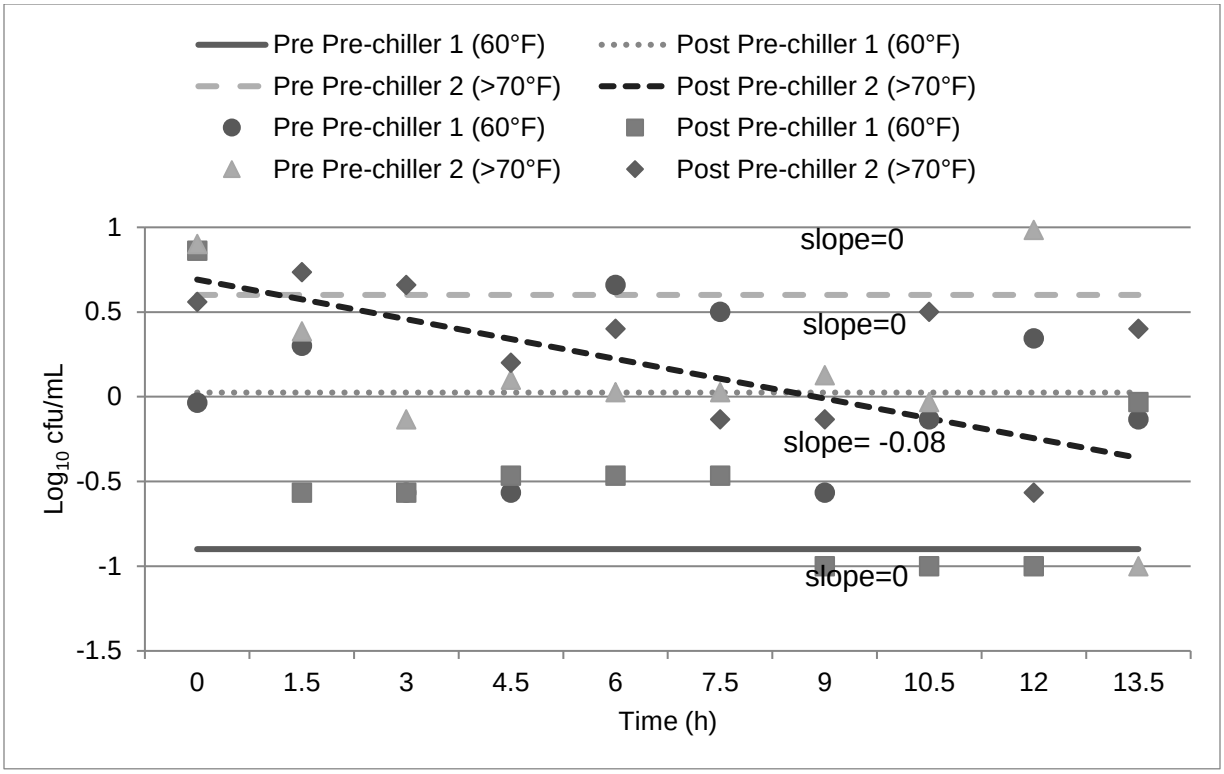

Figure 7. WBCR Mean Log_{10} *Salmonella* Counts by Sampling Location and Pre-chiller over Time, Trial 1. Two whole bird carcass rinses (WBCR) were sampled prior to each pre-chiller as well as directly after each pre-chiller at a frequency of 1.5 hours through the course of 2 shifts.

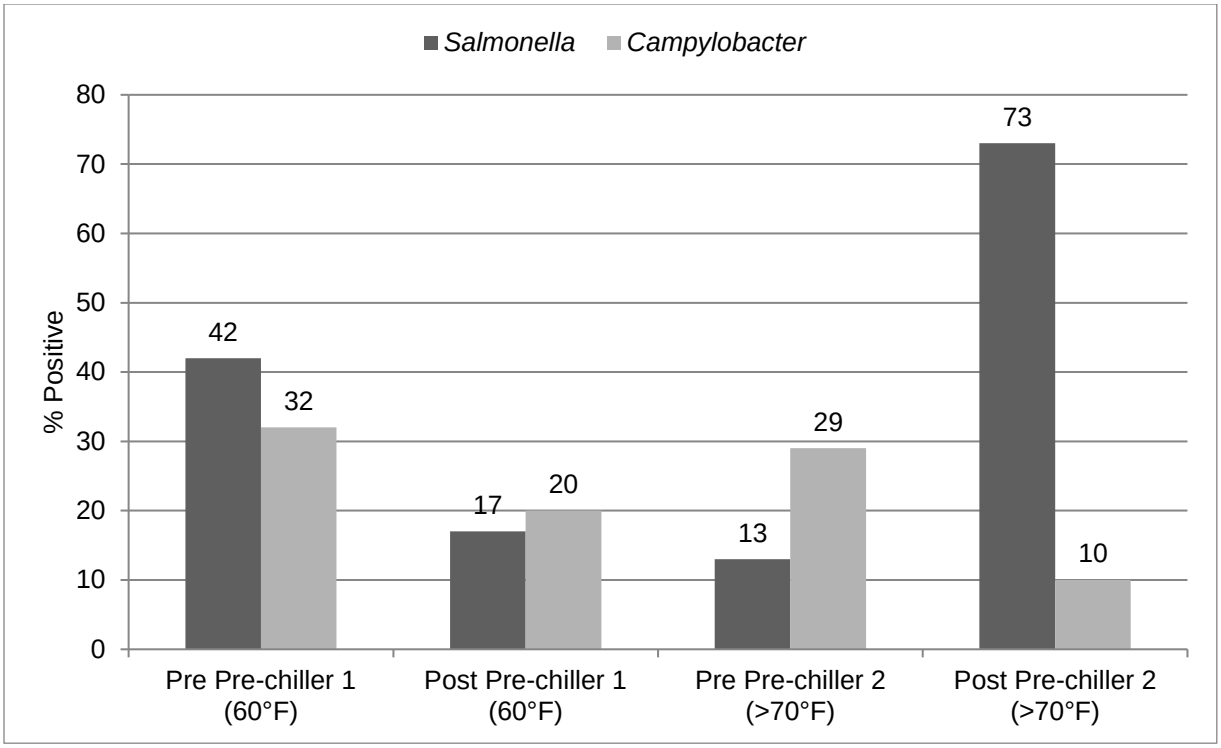

Figure 8. WBCR *Salmonella* and *Campylobacter* Incidence by Pre-chiller and Location, Trial 1. Two whole bird carcass rinses (WBCR) were sampled prior to each pre-chiller as well as directly after each pre-chiller at a frequency of 1.5 hours through the course of 2 shifts.

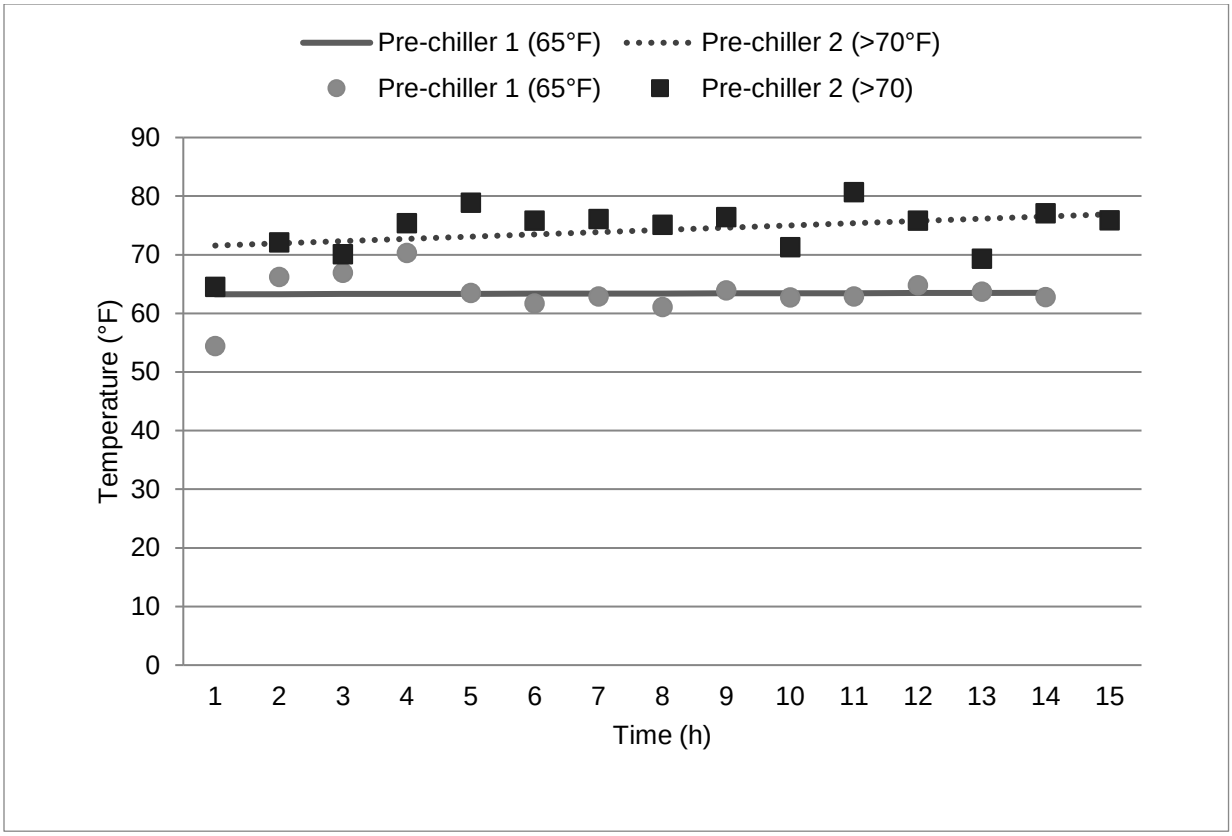

Figure 9: Temperature of Pre-chillers over Time, Trial 2. Temperature data was collected from each pre-chiller at a frequency of every 1 hour over the course of 2 shifts.

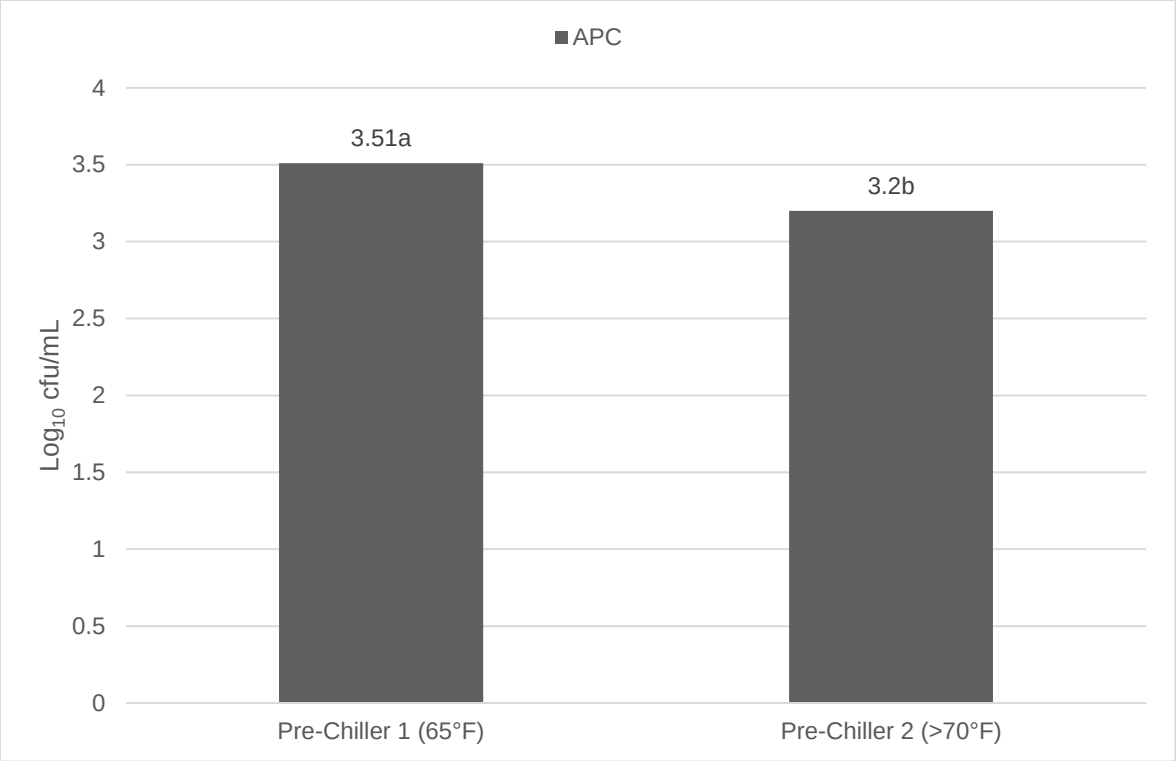

Figure 10. Mean Log_{10} APC populations by Pre-chiller, Trial 2. Water samples were collected from each pre-chiller at the frequency of 1 per hour.

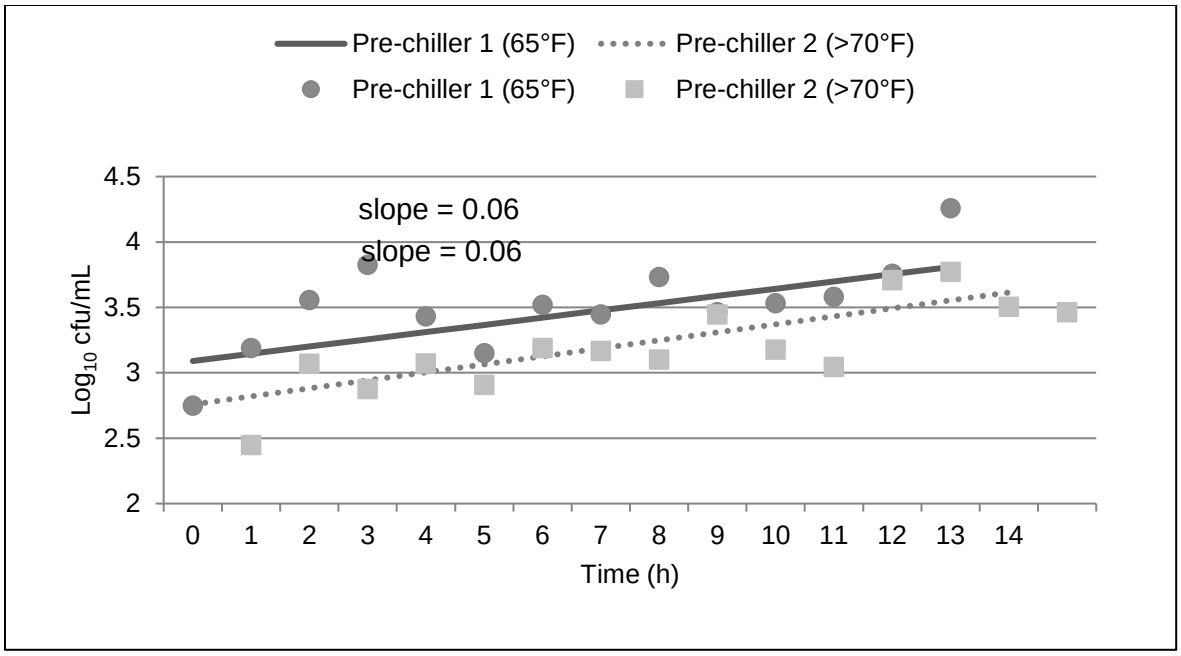

Figure 11. Mean Log_{10} APC populations in Water over Time by Pre-chiller, Trial 2. Water samples were collected from each pre-chiller at the frequency of 1 per hour.

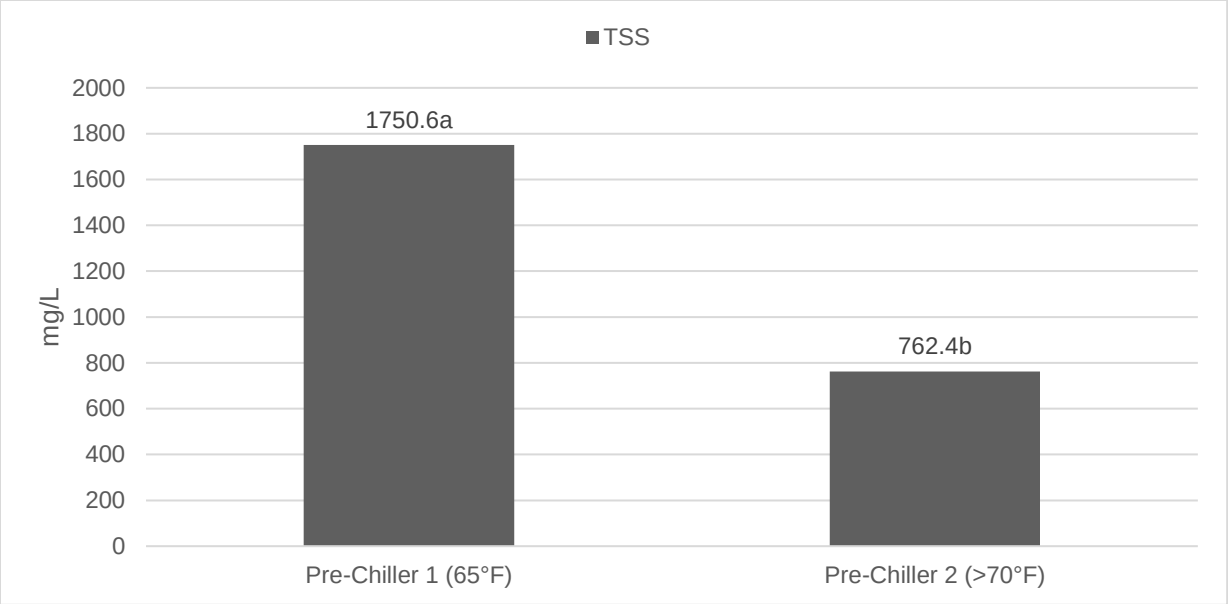

Figure 12. TSS Values by Pre-chiller, Trial 2. Water samples were collected from each pre-chiller at the frequency of 1 per hour over the course of 2 shifts.

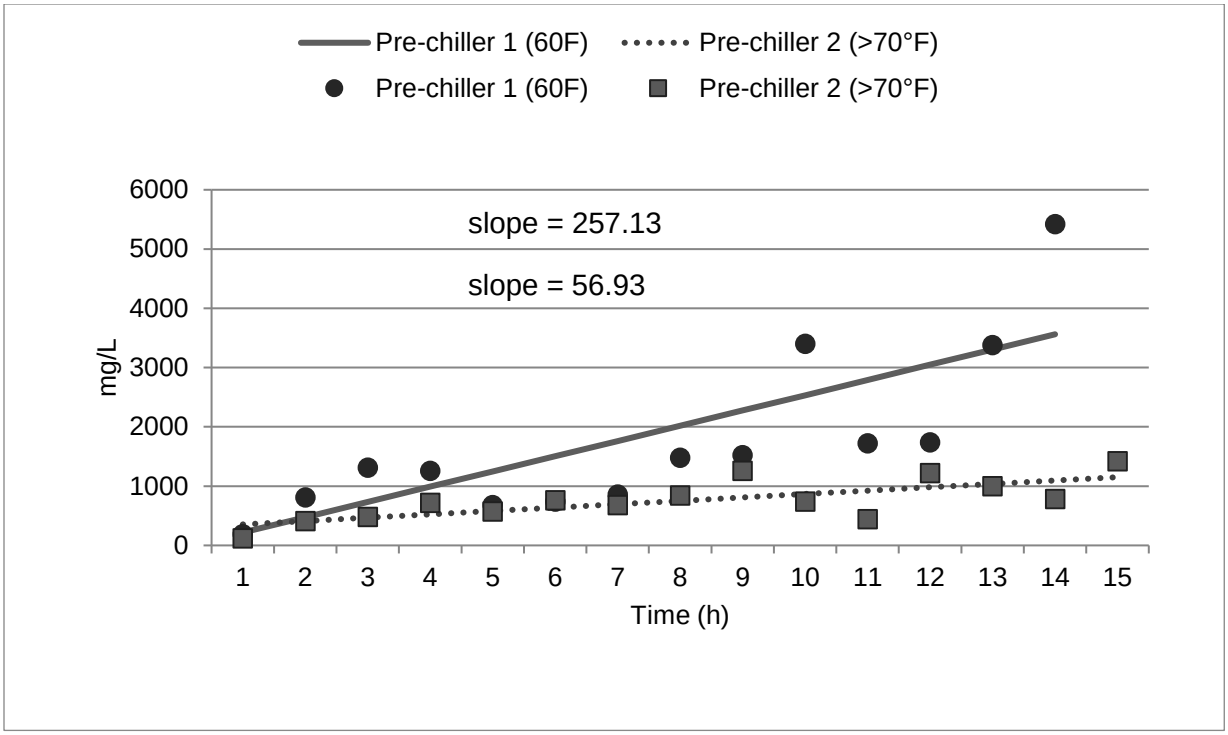

Figure 13. TSS Values over Time by Pre-chiller, Trial 2. Water samples were collected from each pre-chiller at the frequency of 1 per hour over the course of 2 shifts.

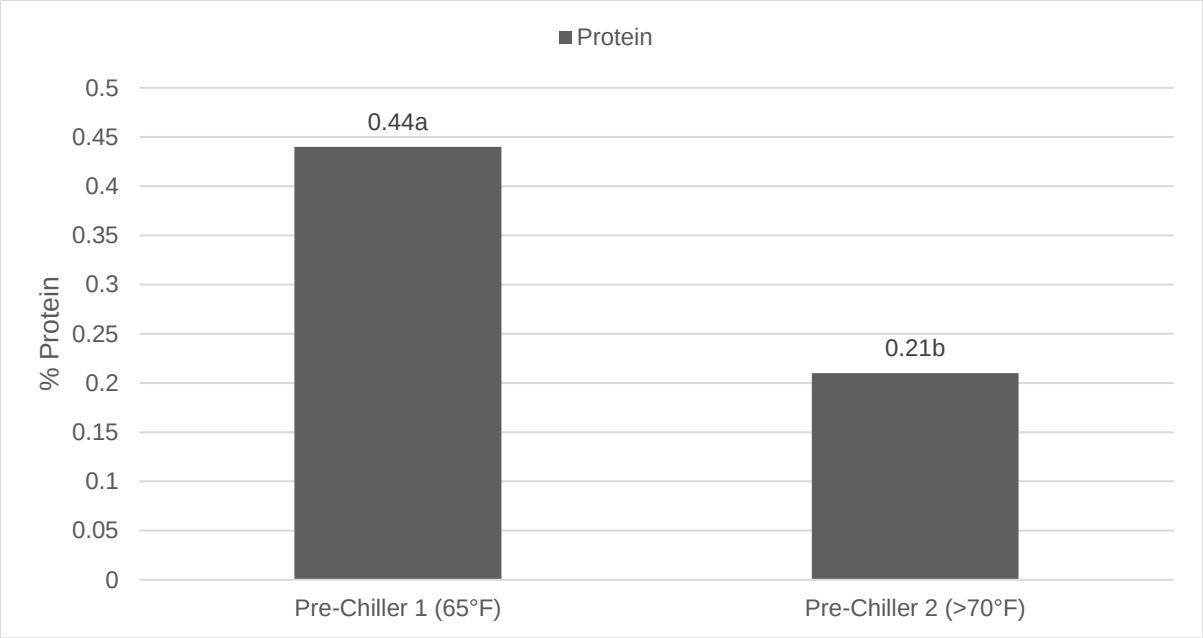

Figure 14. Protein Values by Pre-chiller, Trial 2. Water samples were collected from each pre-chiller at the frequency of 1 per hour over the course of 2 shifts.

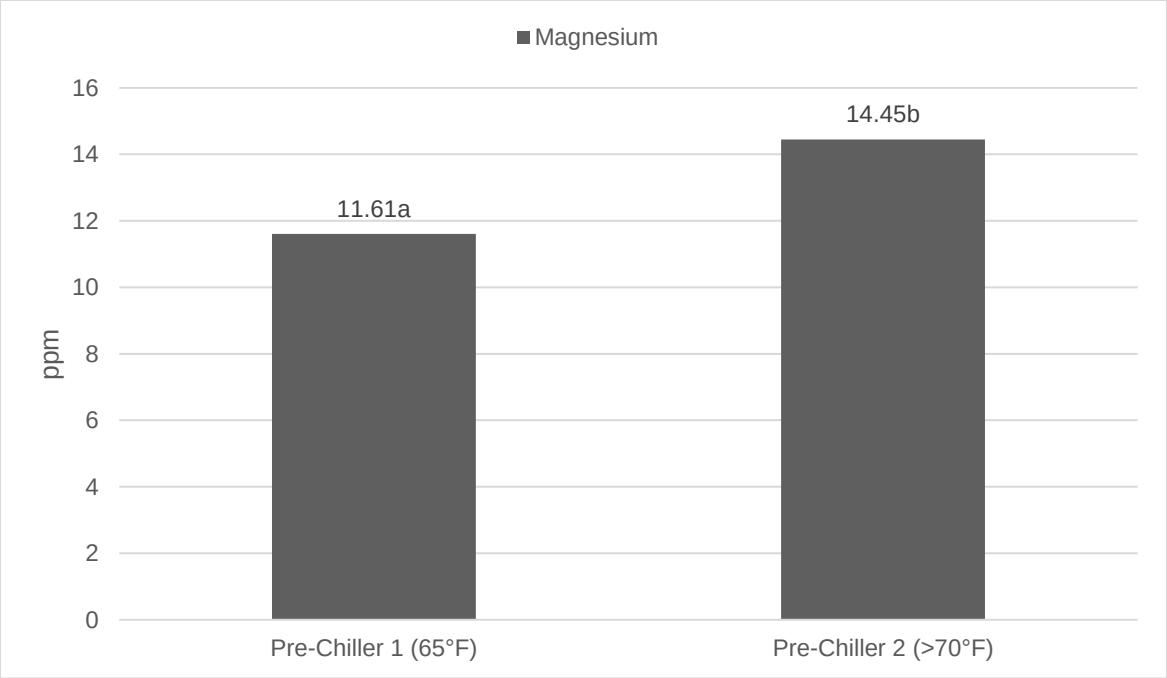

Figure 15. Magnesium by Pre-chiller, Trial 2. Water samples were collected from each pre-chiller at the frequency of 1 per hour over the course of 2 shifts.

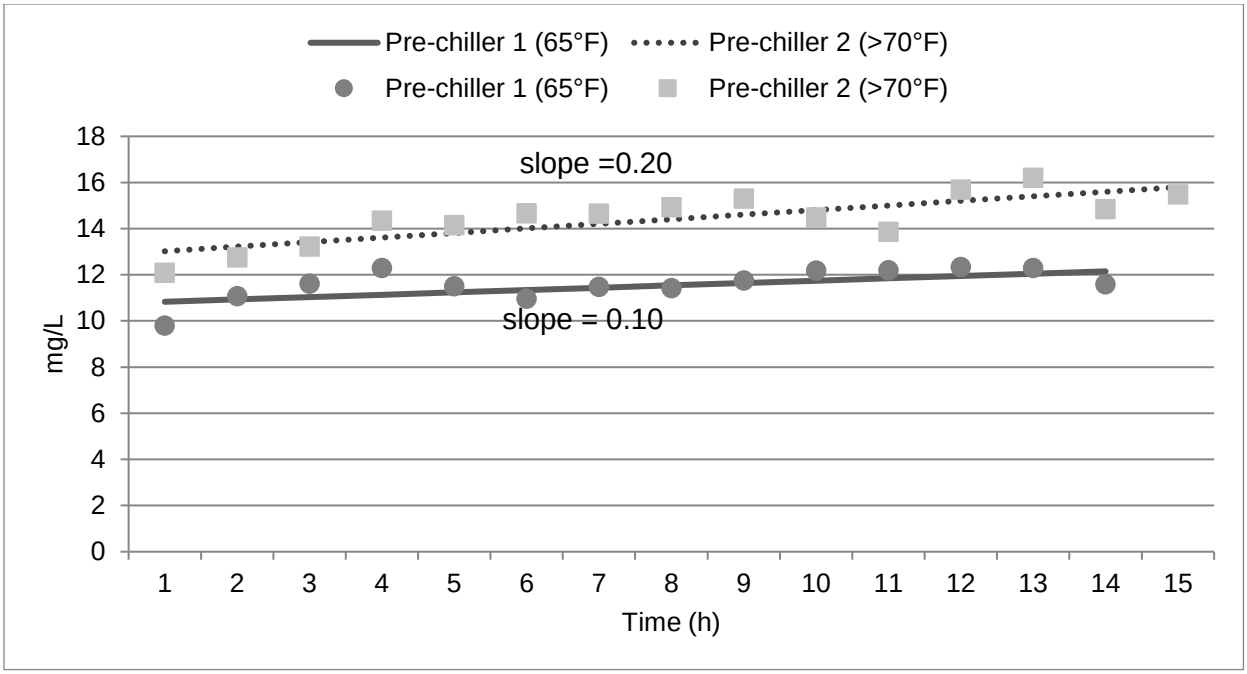

Figure 16. Magnesium Values over Time by Pre-chiller, Trial 2. Water samples were collected from each pre-chiller at the frequency of 1 per hour over the course of 2 shifts. Each sample was analyzed for magnesium.

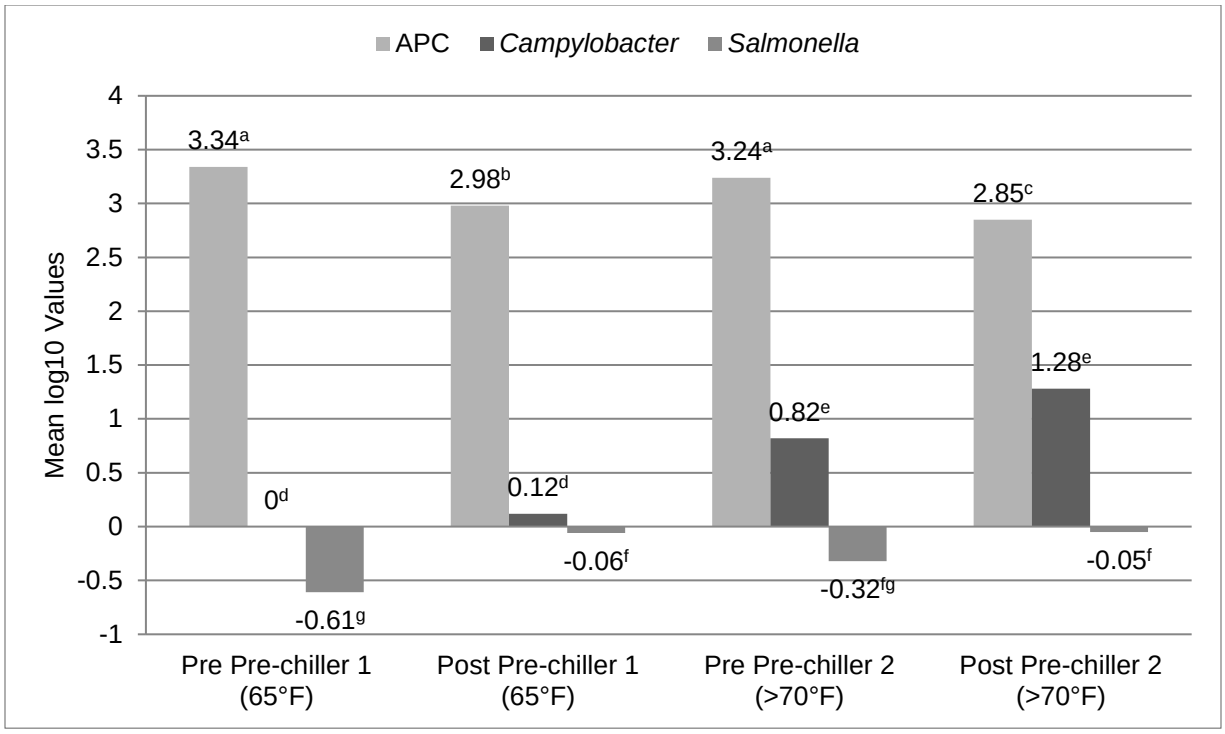

Figure 17. Mean Log_{10} Counts by Pre-chiller and Location, Trial 2. Two whole bird carcass rinses (WBCR) were sampled prior to each pre-chiller as well as directly after each pre-chiller at a frequency of every hour through the course of 2 shifts.

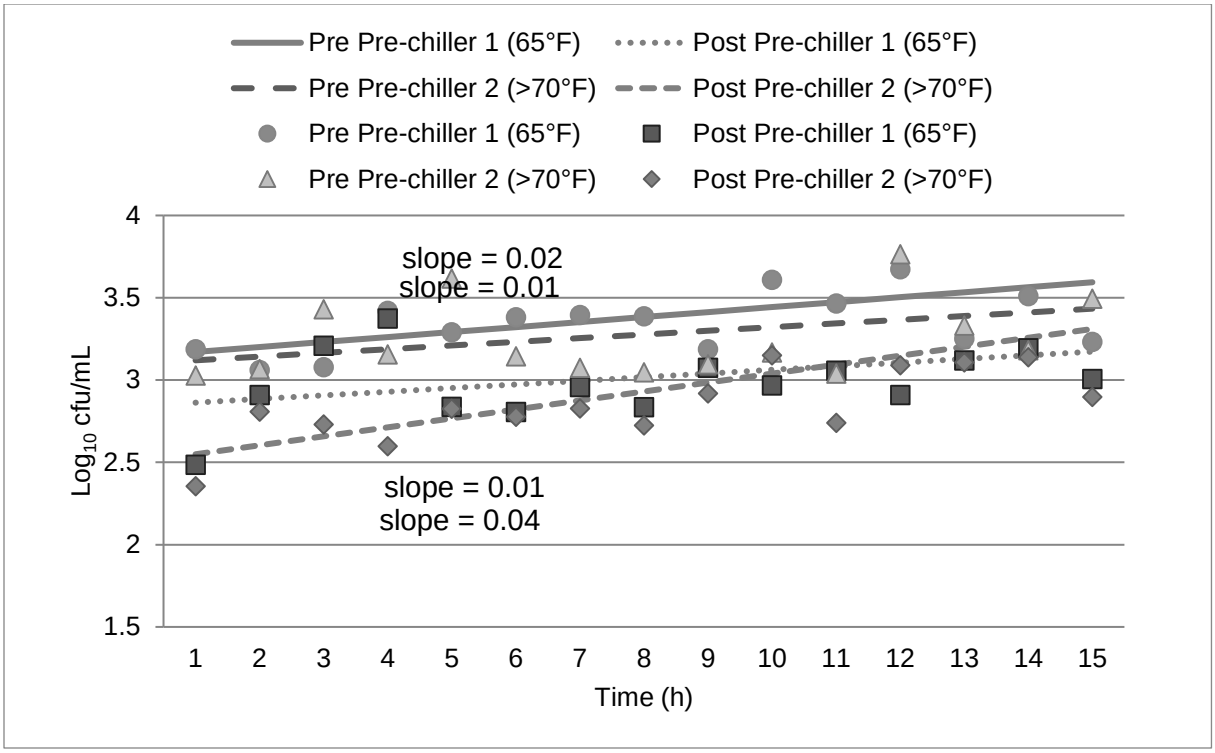

Figure 18. WBCR Mean Log_{10} APC populations by Sampling Location and Pre-chiller over Time, Trial 2. Two whole bird carcass rinses (WBCR) were sampled prior to each pre-chiller as well as directly after each pre-chiller at a frequency of every hour through the course of 2 shifts.

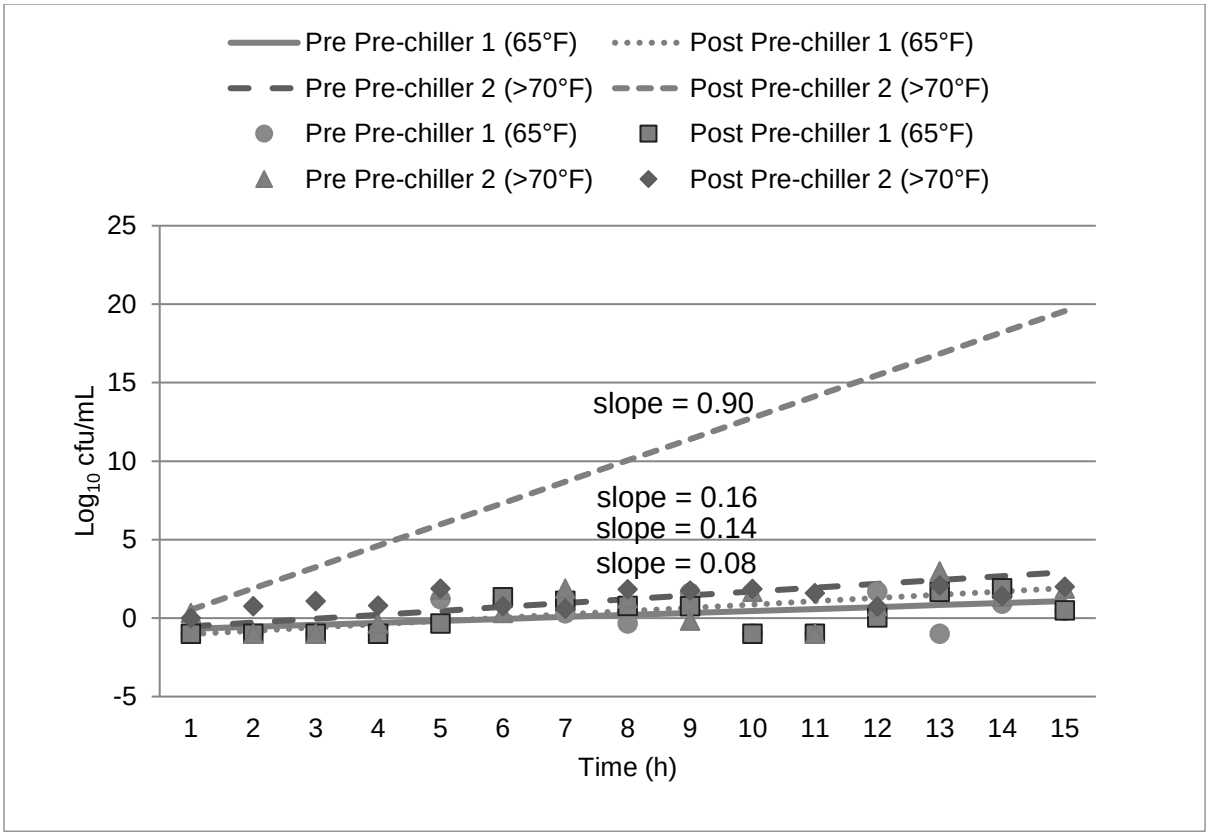

Figure 19. WBCR Mean Log_{10} *Campylobacter* populations by Sampling Location and Pre-chiller over Time, Trial 2. Two whole bird carcass rinses (WBCR) were sampled prior to each pre-chiller as well as directly after each pre-chiller at a frequency of every hour through the course of 2 shifts.

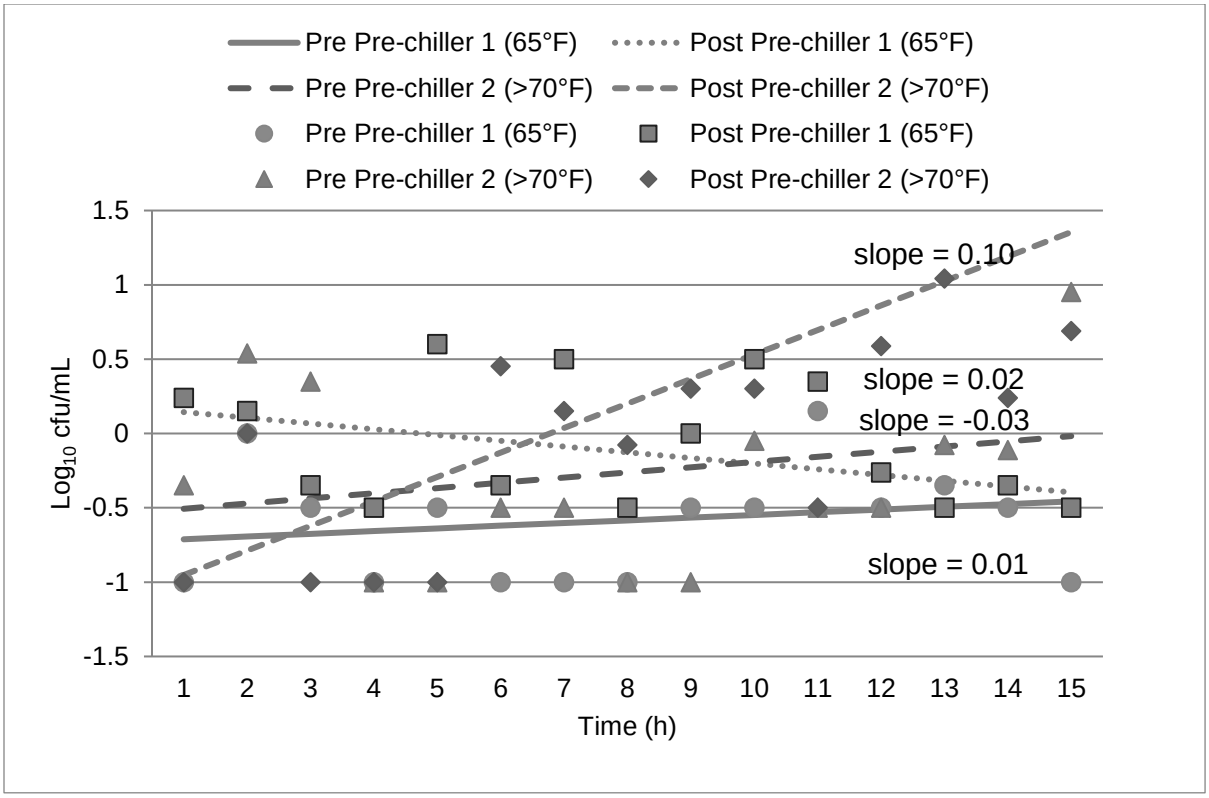

Figure 20. WBCR Mean Log_{10} *Salmonella* populations by Sampling Location and Pre-chiller over Time, Trial 2. Two whole bird carcass rinses (WBCR) were sampled prior to each pre-chiller as well as directly after each pre-chiller at a frequency of every hour through the course of 2 shifts.

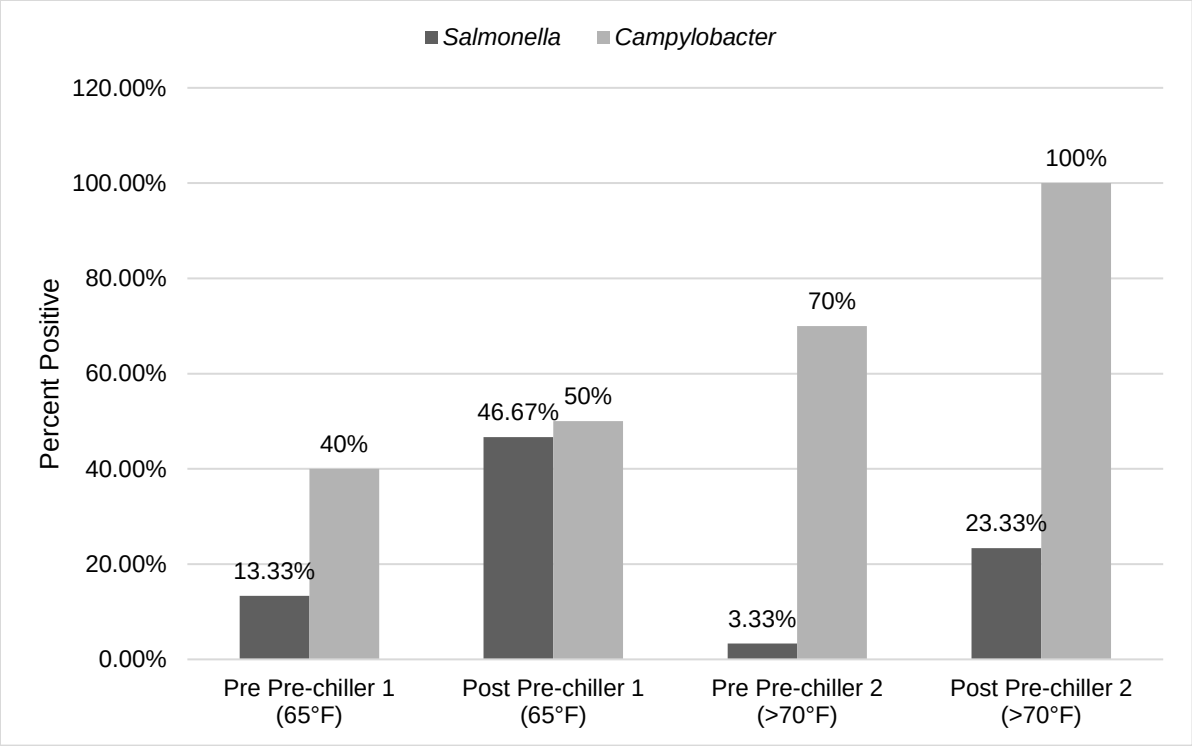

Figure 21. WBCR *Salmonella* and *Campylobacter* Incidence by Pre-chiller and Location, Trial 2.
Two whole bird carcass rinses (WBCR) were sampled prior to each pre-chiller as well as directly after each pre-chiller at a frequency of every hour through the course of 2 shifts.

		Pre Chiller 1 (65°F) Mean Log_{10} APC Counts	Post Chill WBCR Pre Chiller 1 (65°F) Mean Log_{10} *Salmonella* Counts	Post Chill WBCR Pre Chiller 1 (65°F) Mean Log_{10} APC Counts	Post Chill WBCR Pre Chiller 1 (65°F) Mean Log_{10} *Campylobacter* Counts	Pre Chiller 2 (>70°F) Mean Log_{10} APC Counts	Post Chill WBCR Pre Chiller 2 (70°F) Mean Log_{10} *Salmonella* Counts	Post Chill WBCR Pre Chiller 2 (70°F) Mean Log_{10} APC Counts	Post Chill WBCR Pre Chiller 2 (70°F) Mean Log_{10} *Campylobacter* Counts
Salmonella	r=	-0.29091		-0.21405	-0.0997	0.70434		0.18841	0.33993
	p=	0.1189		0.256	0.6002	<0001		0.3187	0.0661
WBCR APC	r=	0.24619	-0.21405		0.041	0.39648	0.18841		0.2715
	p=	0.1897	0.256		0.8297	0.0301	0.3187		0.1467
Campy	r=	0.52682	-0.0997	0.041		0.32116	0.33993	0.2715	
	p=	0.0028	0.6002	0.8297		0.0835	0.0661	0.1467	
Pre Chiller APC	r=		-0.29091	0.24619	0.52682		0.70434	0.39648	0.32116
	p=		0.1189	0.1897	0.0028		<0001	0.0301	0.0835
BOD	r=	0.00058	0.00306	0.23039	-0.05522	0.68193	0.54646	0.33589	0.26512
	p=	0.9976	0.9872	0.2206	0.7719	<0001	0.0018	0.0696	0.1568
COD	r=	0.02453	-0.12817	0.30162	-0.07257	0.62566	0.5229	0.24697	0.26209
	p=	0.8976	0.4997	0.1053	0.7031	0.0002	0.003	0.1883	0.1618
TDS	r=	0.09944	-0.04741	0.34282	-0.01557	0.85524	0.63776	0.38137	0.31141
	p=	0.6011	0.8035	0.0637	0.9349	<0001	0.0002	0.0376	0.0939
TSS	r=	0.88089	-0.21251	0.13633	0.51839	0.70884	0.52311	0.28541	0.45836
	p=	<0001	0.2596	0.4725	0.0033	<0001	0.003	0.1263	0.0109
Protein	r=	0.05108	0.06906	0.31826	-0.04412	0.62477	0.47305	0.2279	0.25065
	p=	0.7887	0.7169	0.0865	0.8169	0.0002	0.0083	0.2258	0.1815
Calcium	r=	0.17917	0.00582	-0.27243	0.26262	-0.7763	-0.56976	-0.37478	-0.29976
	p=	0.3435	0.9757	0.1453	0.1609	<0001	0.001	0.0413	0.1075
Iron	r=	0.06954	-0.19716	0.58236	-0.14707	0.82462	0.71178	0.25631	0.28225
	p=	0.715	0.2963	0.0007	0.438	<0001	<0001	0.1716	0.1307
Magnesium	r=	0.22235	-0.0147	0.34121	-0.01457	0.79595	0.64352	0.35548	0.45405
	p=	0.2376	0.9386	0.065	0.9391	<0001	0.0001	0.0539	0.0117
Phosphorous	r=	0.10527	-0.03631	0.40731	-0.10031	0.85577	0.69612	0.41884	0.42473
	p=	0.5798	0.8489	0.0255	0.5979	<0001	<0001	0.0212	0.0193
Potassium	r=	0.00112	-0.05621	0.39233	-0.1575	0.83729	0.68169	0.39131	0.36566
	p=	0.9953	0.768	0.032	0.4059	<0001	<0001	0.0325	0.0469
Sodium	r=	-0.1497	-0.00849	0.36754	-0.25868	0.8369	0.68508	0.38228	0.33138
	p=	0.4298	0.9645	0.0457	0.1675	<0001	<0001	0.0371	0.0736

Table 1: Correlations between Pre-chiller Water Mean Log_{10} Counts and the Pre-chiller Environment, Trial 2. APC results from water data were compared to the other data that was obtained from the pre-chiller water.