USE OF A SCALD ADDITIVE TO REDUCE LEVELS OF *SALMONELLA* DURING

POULTRY PROCESSING

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USE OF A SCALD ADDITIVE TO REDUCE LEVELS OF *SALMONELLA* DURING POULTRY PROCESSING

Julie Carol Townsend

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USE OF A SCALD ADDITIVE TO REDUCE LEVELS OF *SALMONELLA*

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Julie Carol Townsend

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Julie Carol Townsend, daughter of Harmon and Janice Townsend, was born September 17, 1982, in Pine Bluff, Arkansas. She graduated magna cum laude from Malvern High School in 2000. She attended the University of Arkansas in Fayetteville, Arkansas, for four years, and she graduated magna cum laude with a Bachelor of Science in Agriculture degree in Poultry Science in May 2004. She then entered Graduate School at Auburn University in August 2004 in the Department of Poultry Science under the direction of Shelly R. McKee, Ph.D.

THESIS ABSTRACT

USE OF A SCALD ADDITIVE TO REDUCE LEVELS OF *SALMONELLA* DURING POULTRY PROCESSING

Julie Carol Townsend

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The risk of cross contamination of food-borne pathogens such as *Salmonella* during poultry processing remains of great concern to the poultry industry. One of the most critical steps in poultry processing where cross contamination may occur is scalding. The purpose of this research was to evaluate the efficacy of the commercial scald additive, RP Scald, in reducing levels of *Salmonella* during the scalding phase of poultry processing.

Preliminary in vitro experiments were conducted to assess the effectiveness of RP Scald as an antimicrobial at hard scald (56.6°C), soft scald (50°C), and room temperatures (~25°C) in tap water and actual scald water containing high organic loads. Water pH and temperature were monitored to gain insight on how RP Scald achieves microbial reductions. In-plant experiments were conducted to determine the capability of

RP Scald in a commercial setting to reduce levels of *Salmonella* in hard scald and soft scald environments. In order to determine the effects of organic matter and microbial loads on the efficacy of RP Scald, water quality measurements were also monitored.

In vitro and in-plant results indicated that RP Scald was effective in reducing levels of *Salmonella* at hard scald, soft scald and room temperatures in tap water as well as in scald water. The effectiveness of RP Scald was attributed to the alkaline environment created by the scald additive. Furthermore, water quality parameters such as high organic loads in scald water did not reduce the antimicrobial effectiveness of RP Scald. Data suggests that RP Scald could provide an effective and practical intervention strategy in poultry processing plants to reduce levels of *Salmonella*.

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CHAPTER I.

INTRODUCTION

Each year in the United States, it is estimated that 76 million people suffer from food-borne illness, resulting in 325,000 hospitalizations and 5,000 deaths (Mead et al., 1999). Food-borne diseases not only pose health risks to humans, but they are also costly to both consumers and the food industry. According to the United States Department of Agriculture's (USDA) Economic Research Service (ERS), costs associated with food-borne illness total $6.9 billion annually (USDA ERS, 2000). Two food-borne pathogens that are commonly associated with poultry meat are *Salmonella* spp. and *Campylobacter* spp. (Izat et al., 1988; Bailey et al., 1987; Bailey, 1993). The CDC reported that out of 16,614 cases of food-borne infections, *Salmonella* caused 6,471 of the cases (CDC, 2006). When 91% of those *Salmonella* isolates were serotyped, *S.* Typhimurium accounted for 19% of the serotypes causing salmonellosis.

Food safety risks to consumers are usually a result of undercooked poultry meat or cross contamination. Furthermore, there are many steps during poultry production in which cross contamination may occur, beginning at the farm and continuing through preparation of the final product. Although many improvements to prevent the risk of harmful pathogens such as *Salmonella* entering the food chain have been made through consumer education and the implementation of HACCP, these pathogens are still being

detected, and consumers are still at risk. Practical and effective intervention strategies should be implemented in order to reduce health risks to humans.

Two of the most common food-borne pathogens, *Salmonella* and *Campylobacter*, originate in the digestive tracts of chickens, and these pathogens can spread by cross contamination. In fact, studies have shown that the level of microorganisms on carcasses leaving a processing plant is a direct indication of the microbial quality of the same flock during the live production phase (Stern et al., 1995). Specifically, major points of cross contamination of *Salmonella* include the parent flocks, hatcheries (Bailey et al., 2001), feed, water sources, farm animals, farm personnel, litter (Opara et al., 1992), wild birds (Davies and Wray, 1994a), insects and rodents. Pre-harvest management steps that can result in the transfer of *Salmonella* to poultry include feed withdrawal time and transportation time. Prior to the birds arriving at the processing plant, there are many opportunities in which cross contamination may occur. Therefore, when broilers enter processing facilities, they may carry food-borne pathogens such as *Salmonella* and *Campylobacter* with them, and there is a risk of cross contamination through various stages of processing (Mead et al., 1994; Kotula and Pandya, 1995).

Within a poultry processing plant, two of the most critical steps in which cross contamination may occur are scalding and picking (Bryan et al., 1968; Woodburn and Stadelman, 1968; Van Schothorst et al., 1972; Nivas et al., 1973; Notermans et al., 1975; Mulder et al., 1978). Scald tanks provide a unique environment in which some bacteria may be able to survive. The warm scalding temperatures (generally ranging from 50°C to 60°C), neutral pH, and high levels of organic matter may provide microorganisms with proper nutrients, enabling them to survive and spread to other carcasses. The scald tank

is also the first communal water bath in processing plants through which all carcasses pass. When broilers enter the scald tank, blood, litter, fecal matter, and bacteria on the carcasses are introduced into the scald water (Notermans et al., 1977). Because substantial cross contamination may occur during the scalding phase of processing, bacteria may continue to spread throughout the plant, specifically during the defeathering phase. During defeathering, carcasses pass through machinery containing rubber picking fingers, and this action may contribute to the cross-contamination between carcasses (Hafez, 1999). By reducing the risk of cross contamination of poultry during the scalding phase, cross contamination during stages further along during processing may be reduced.

During scalding, the scald water undergoes many quality changes. As, blood, litter, fecal matter, and bacteria are introduced into the water, a shift in pH occurs, and the level of organic matter increases. The pH of the scald water is lowered due to the formation of uric acid (Weast and Selby, 1966), which is a product of the dissociation of ammonium urate from chicken feces. It has been reported that the pH of scald water may affect the heat resistance of microorganisms such as *S*. Typhimurium. Humphrey (1981) reported that *S*. Typhimurium was most heat resistant at pH 6.1. The pH for optimum growth of salmonellae is around neutrality, with pH values greater than 9.0 and below 4.0 being bactericidal (Jay, 2000). The amount of organic matter in scald water may also impact the chemical oxygen demand (COD), biological oxygen demand (BOD), and total suspended solids (TSS), all measures of water quality. COD is a measure of the amount of oxygen needed to oxidize organic matter into carbon dioxide and water (Boyd and Tucker, 1992). High levels of organic matter provide a means of oxidation for

microorganisms, and BOD is a way of measuring the amount of oxygen in water that is required by the microorganisms to oxidize the organic matter (EPA, 1997). Generally, high levels of organic matter and microorganisms in water result in increased COD and BOD, as well as TSS, which is a measure of particulate matter in suspension (Boyd and Tucker, 1992. The relationships of water temperature, pH, COD, BOD, and TSS to the survival of harmful pathogens in scald water are very important from a food safety aspect.

Since the risk of cross contamination is likely to occur during the scalding process, intervention strategies should be implemented. Because scalding is the first stage in processing where birds are in communal water, this step represents a unique opportunity to implement an antimicrobial intervention strategy. Many studies have shown the antibacterial effects of various scald additives such as sodium hydroxide (Humphrey et al., 1981), propionic acid (Humphrey et al., 1981), acetic acid (Okrend et al., 1986; Lillard et al., 1987; Tamblyn et al., 1997), glutaraldahyde (Humphrey et al., 1981), chlorine, trisodium phosphate and sodium metabisulfite (Tambyln et al., 1997) on bacterial levels in scald water. Scald water additives not only directly affect the pH, but can also affect the suspension of organic matter in the scald water. Sodium hydroxide has shown to reduce the amount of fecal matter adhering to feathers, as well as reduce total bacterial counts in scald water (Humphrey et al., 1981). The additions of acetic acid, propionic acid, and gluteraldehyde increased death rates of bacteria in scald water (Okrend et al., 1986; Humphrey et al., 1981), but have not shown to reduce levels of bacteria on broiler carcasses. Higher levels of acetic acid have been shown to reduce *S.*

Typhimurium on chicken skin samples, but discoloration of the skin samples was observed (Tamblyn et al., 1997).

RP Scald is an alkaline scald water additive that is currently being used in the commercial poultry industry to reduce the appearance of bruising on broilers, but it has been suggested as a means to reduce microbial loads in the scald tank. The main ingredient is sodium hydroxide, which is a strong alkaline compound that destroys microorganisms, dissolves proteins, and disperses and emulsifies solids (Marriott, 1997). The high pH produced by RP Scald has been shown to cause organic matter to precipitate out of solution. Little, if any, research has been conducted to validate the efficacy of RP Scald in reducing levels of *Salmonella*.

Since *Salmonella* continues to be one of the largest causes of food-borne illness in the United States, research such as this would benefit consumers, as well as the poultry industry. All broiler processing plants in the United States must meet the USDA Food Safety and Inspection Service (FSIS) *Salmonella* performance standards. Implemented after the national baseline study by the USDA, these performance standards require *Salmonella* testing in all broiler processing plants and require the percentage of *Salmonella*-positive samples to be less than 20% (12 positive samples out of 51 samples) (USDA FSIS, 1996).

Currently, one of the most common antimicrobial compounds used in poultry processing plants is chlorine. However, the warm temperature, high organic load, and neutral pH of scald water places limitations on the use of chlorine in scald tanks. Therefore, the current study was conducted in order to assess the effectiveness of RP Scald in reducing levels of *Salmonella* both in vitro and in a processing plant setting.

Four preliminary in vitro experiments and two in-plant experiments were conducted using an antibiotic resistant strain of *S.* Typhimurium in order to determine the antibacterial efficacy of RP Scald using various water temperatures consistent with hard scald and soft scald industry standards. Results of the current study demonstrate that RP Scald has the ability to reduce levels of *Salmonella*, thereby reducing the risk of cross contamination further down the processing scheme. RP Scald provides an effective and practical means of improving the quality of poultry products from a food safety standpoint.

CHAPTER II.

LITERATURE REVIEW

Relevance of Food Safety

Many steps during poultry processing may directly affect meat quality and safety of the final product. Two food-borne pathogens that are often associated with poultry meat are *Salmonella* spp. and *Campylobacter* spp. (Izat et al., 1988; Bailey et al., 1987; Bailey, 1993). The Centers for Disease Control and Prevention (CDC) estimates that 76 million people will suffer from food-borne illness resulting in approximately 325,000 hospitalizations and 5,000 deaths annually (Mead et al., 1999). More specifically, the CDC estimates that *Salmonella* infections alone are responsible for 1.4 million illnesses, 16,000 hospitalizations and nearly 600 deaths annually.

The implications of food-borne illnesses to the food industry as well as consumers can be overwhelming. The United States Department of Agriculture's (USDA) Economic Research Service (ERS) estimates that costs associated with food-borne illnesses total $6.9 billion annually (USDA ERS, 2000). Costs associated solely to food-borne illnesses caused by *Salmonella* are estimated to be $2.9 billion (USDA ERS, 2003). The risk of cross contamination of pathogenic microorganisms such as *Salmonella* poses hazards to consumers, and preventative measures should be taken during processing, from live production through packaging. *Salmonella* most commonly presents human health risks when poultry products are inadequately cooked or cross contamination

occurs. From a production standpoint, poultry production involves a number of steps in which cross contamination may occur, beginning at the farm and continuing through processing. Data has shown that processing increases contamination by *Salmonella* and *Campylobacter* in studies comparing prevalence on the farm to prevalence on final products (Oosterom et al., 1983; Mead et al., 1994). In response to the risk of cross contamination of pathogens during processing, the government has conducted studies and enforced regulations to help prevent the incidence of pathogens in poultry products.

In July 1996, the USDA Food Safety and Inspection Services (FSIS) published "The Final Rule on Pathogen Reduction and Hazard Analysis and Critical Control Point (HACCP) Systems," which requires that all plants develop, adopt, and implement a HACCP plan for each process in their plant (USDA FSIS, 1996). Under the mandated HACCP system, there are microbiological testing procedures including performance standards for *Salmonella* and testing for generic *E. coli* (USDA FSIS, 1996). The *Salmonella* performance standards are based on the national baseline study conducted by the USDA FSIS in 1994 and 1995. In these studies, 20% of broilers were positive for salmonellae after the chilling stage, and the current performance standards are based on this level. For broilers, if 20% or more samples (13 samples out of a moving window of 51 samples) are positive, then the plant does not meet the performance standards of the Final Rule. In the event that a processing plant does not comply with the performance standards of the Final Rule, the plant must re-evaluate their HACCP plan and find ways to reduce *Salmonella* and improve microbiological quality. A second non-compliance calls for the plant to modify their HACCP plan. If a plant fails to comply with the performance standards a third time, the plant must undergo a food safety audit as an in

depth verification of their HACCP plan. If a plant fails to comply, then inspection by the USDA FSIS is withdrawn, and the plant may be forced to close down.

Although consumer education and the introduction of HACCP in meat processing have lead to improvements in reducing the risk of food-borne pathogens, these harmful microorganisms are still present during processing. Food-borne hazards may be derived from physical, chemical, or microbiological origin, but currently microbial food-borne hazards are recognized as the greatest risk to consumers (Hafez, 1999). With stricter food safety regulations and increased emphasis on public health, processors are searching for effective food safety intervention strategies that are practical and economical. Specifically, strategies to reduce the incidence of food-borne *Salmonella* should be sought.

Salmonella spp.

Chickens are known natural reservoirs for many pathogenic microorganisms such as *Salmonella* and *Campylobacter*. While chickens may carry these pathogens into processing facilities, *Salmonella* and *Campylobacter* that are harmful to humans are not necessarily pathogenic to chickens. Specifically, *Salmonella* and *Campylobacter* originate in the birds' digestive tracts and can spread by cross contamination. Cross contamination may occur during pre-harvest steps or during poultry processing. Since the USDA set performance standards for *Salmonella*, poultry companies have implemented numerous interventions to lower the incidence of *Salmonella*. Those implementations lowered the national prevalence of *Salmonella* initially, but more recently, the number of processing plants failing to meet the standards has jumped from 3.6% in 2000 to 11.7% in 2003 (Russell, 2005). Recent data in the United States shows

33.9% of carcasses tested positive for *Salmonella* over a 20-week sampling period (Simmons et al., 2003).

Salmonella spp. are small, rod-shaped, Gram-negative, facultative microorganisms. *Salmonella* spp. are motile, with the exception of *S. gallinarum* and *S. pullorum*, and non-spore-forming microorganisms. *Salmonella* spp. are mesophilic, meaning that cell growth may occur between 6°C and 47°C, with the optimum temperature being 37°C. Specifically, *S.* Typhimurium has been reported to grow at temperatures as low as 6.2°C (Jay, 2000). Generally, pH for optimum growth of *Salmonella* spp. is around neutrality, with pH above 9.0 and below 4.0 being bactericidal to the cells (Jay, 2000).

In the family Enterobacteriaceae, *Salmonella* may originate from both food and non-food sources. Some of the common food sources include raw meats, poultry, eggs, milk and dairy products, fish, shrimp, frog legs, and coconut. Non-food sources may include human and animal fecal matter, domestic and wild animals, and contaminated water. *Salmonella* spp. may cause three types of human illness: typhoid fever, paratyphoid fever, and gastroenteritis. The latter is caused by *Salmonella enterica* serotypes, found in both human and animal intestinal tracts, and with poultry identified as a major reservoir. Acute symptoms of salmonellosis are nausea, vomiting, cramping, fever, headaches, and diarrhea, which usually last for 1-2 days. The onset period of salmonellosis is 6-48 hours, and the infective dose is usually 10,000-1,000,000 cells, depending on host age and health status, as well as strain characteristics. *Salmonella* bacteria pass through the gut lumen and penetrate the epithelial wall of the small

intestine, resulting in inflammation. It is estimated that there are 2 to 4 million cases of salmonellosis in the United States annually (US FDA, 2006).

Although there are more than 2300 serotypes of *Salmonella*, in 2005 the CDC found that five of the most common food-borne serotypes of *Salmonella* in poultry include *S*. Typhimurium, *S*. Enteritidis, *S*. Newport, *S*. Heidelberg, and *S*. Javiana (CDC, 2006). The two serotypes most commonly associated with human illness from salmonellae infections are *S*. Typhimurium and *S*. Enteritidis. The Foodborne Diseases Active Surveillance Network (FoodNet) of the Centers for Disease Control and Prevention (CDC) Emerging Infections Program reported in 2005 that there were a total of 16,614 cases of food-borne infections with 6,471 of those cases being caused by *Salmonella* (CDC, 2006). When 91% of these *Salmonella* isolates were serotyped, *S*. Typhimurium accounted for 19% of the serotypes causing salmonellosis (CDC, 2006). Although there was a 9% decrease in *Salmonella* from 1998 to 2005 (CDC, 2006), there is currently a need to control this pathogen, which is still a leading cause of food-borne illness in the United States.

It has been reported that numerous pre-harvest management practices can affect contamination of carcasses in processing facilities (Stern et al., 1995; Bailey, 1993), and levels of microorganisms on the carcasses leaving the plant are a reflection of the microbial quality of the flock (Stern et al., 1995). The transfer of pathogens such as *Salmonella* may begin during production, specifically in hatcheries and on farms. Because it takes relatively few cells of *Salmonella* to colonize in the intestines of young chicks, researchers suggest that contamination in hatcheries is a major concern for the introduction of *Salmonella* to chickens (Blankenship et al., 1993). Bailey et al. (2002)

found that serotypes isolated from hatchery samples and samples from the previous grow-out house samples were associated. However Lahellec and Colin (1985) reported that the grow-out environment is considered to be the strongest indicator of *Salmonella* serotypes found in poultry after processing. Major points of cross contamination of *Salmonella* have been found to be the parent flocks, hatcheries (Bailey et al., 2001), feed, water sources, farm animals, farm personnel, litter (Opara et al., 1992), wild birds (Davies and Wray, 1994a), insects and rodents. In a large study by Bailey et al. (2001), environmental sources for *Salmonella* included hatchery paper pads, flies, drag swabs, and boot swabs. In addition, *Salmonella* has been detected in wild birds near hatcheries and in poultry processing units (Davies and Wray, 1994a). Specifically, humidity and water activity of the litter are associated with the presence of *Salmonella* on broilers entering the processing plants. Higher water activity values (0.90-0.95) have been associated with *Salmonella*-positive flocks (Hayes et al., 2000), and it is believed that high humidity of the litter increases the chances of microbial survival since the water activity level is closer to the optimal growth values (Opara et al., 1992).

Two other pre-harvest management steps that can ultimately affect the transfer of *Salmonella* to poultry are feed withdrawal time (Bilgili, 1988; Izat et al., 1989) and transportation to processing facilities (McNab et al., 1993). Before processing, feed is typically withdrawn from broilers 8 to 12 hours prior to processing in order to allow an adequate length of time for clearance of intestinal contents. However, variations of feed withdrawal times may result in greater risks of cross contamination during processing. In the case of a shorter feed withdrawal time, the likelihood of the full intestines rupturing during evisceration increases. A longer feed withdrawal time may weaken the intestines,

which may be filled with watery feces, thereby increasing chances of contamination during processing. Specifically, Ramirez et al. (1997) reported that feed withdrawal increased the number of *Salmonella* isolations from the crops of broilers. Lengthy transportation times to processing facilities may result in overall longer feed withdrawal times. Additionally, levels of contamination may be increased during transport due to bird-to-bird contact. Stern et al. (1995) reported that the transport of broilers to the processing plant increased the prevalence of *Campylobacter*, and reasons could be because of fecal contamination of skin and feathers between the birds. Crates that have been in contact with previous infected flocks that have not been sufficiently sanitized may also result in cross contamination. Rigby and Pettit (1980) reported that crates are a source of *Salmonella* contamination. Crates that are not effectively cleaned and sanitized before transportation could become potential vectors of contamination to broilers being transported to processing facilities.

Cross Contamination During Poultry Processing

Cross contamination may occur at many points during the poultry production and processing scheme, beginning at the farm and continuing through the processing plant (Bailey et al., 1987). Even in modern, fully automated processing facilities, it can be impossible to isolate individual contaminated carcasses from others, or from contaminated equipment, employees, and production materials (Hafez, 1999). When broilers enter the processing plants, they may carry food-borne pathogens such as *Salmonella* and *Campylobacter*, which can then spread throughout plants during various stages of processing (Mead et al., 1994; Kotula and Pandya, 1995).

After arrival to the processing plant, broilers are unloaded from coops, hung on shackles, stunned, killed, and then continue though scalding, picking, evisceration, and chilling operations. During stunning, broilers are most commonly subjected to an electric current, rendering the birds unconscious for 60 to 90 sec (Sams, 2001). Stunning immobilizes the birds so that there is improved killing efficiency, complete blood loss, and better feather removal during defeathering (Sams, 2001). All of these variables may impact final product quality. Immediately after stunning, the birds continue on the shackles to the killing machine, in which a rotating circular blade cuts the jugular vein and carotid arteries in the neck of the birds (Sams, 2001). The killing blade has been cited as a potential area where cross contamination of *Salmonella* may occur. After being tested for *Salmonella* contamination, the blade of a neck-cutting knife in a Spanish processing plant was found to be positive in 50% of the samples (Carramiñana et al., 1997). Mead et al. (1994) used a marker organism and found that an automatic killing knife was capable of spreading the microorganism to the 500^{th} bird passing through the machine. Research such as this indicates that the killing blade can be a major source of contamination during poultry processing. For 2 to 3 minutes after killing, the birds are allowed a bleed-out time in which 30 to 50% of blood in the bird is lost (Sams, 2001). In order to remove the feathers from the carcasses, the carcasses are then submerged in a hot water bath, the scald tank, which denatures the proteins of feather follicles to allow for easier feather removal during defeathering. During defeathering, rotating, flexible, rubber “fingers” abrade the surface of the carcass and pull out the loosened feathers (Sams, 2001).

Scalding and picking are two of the most critical areas in processing plants in which cross contamination of bacteria may occur (Bryan et al., 1968; Woodburn and Stadelman, 1968; Van Schothorst et al., 1972; Nivas et al., 1973; Notermans et al., 1975; Mulder et al., 1978), and more specifically, of *Salmonella* alone (Okrend et al., 1986). According to the USDA, scalding is the first step in poultry processing that may impact final product safety and quality. Therefore, intervention strategies to reduce cross contamination among carcasses should be implemented during the scalding step of poultry processing. By relaxing the feather follicles on the carcass, scalding in hot water allows the proteins to denature so that the feathers can be easily removed during defeathering (Thaxton, 2002). However, it is thought that the follicles remain open until the chilling stage of poultry processing, meaning that microorganisms may be retained in the follicles even after chilling (Keener et al., 2004), allowing for cross contamination further down the processing scheme.

Some factors contributing to the buildup of microorganisms in scald tanks include the rate of fresh water added to the scalder, scald water temperature, and the heat resistance of some microorganisms. When broilers enter the scald tank, blood, litter, fecal matter, and bacteria on the exterior of the carcasses are removed due to the agitation of the scald water (Notermans et al., 1977). It has been suggested that carcasses excrete fecal matter into the scald water as a result of the scalding process (Crabb and Walker, 1971), thereby introducing more organic matter and enterobacteria such as *Salmonella*. Another factor affecting the degree of contamination is the actual construction of the scald tanks. Some scalders are constructed with counter-current water flow, meaning that the water moves in the opposite direction of the birds, from the exit of the scalder toward

the entrance; and without counter-current water flow, scald water may contain higher levels of excreta. Veerkamp and Heemskerk (1992) found a reduction in numbers of Enterobacteria in scald water collected from the last tank of a three-tank, two-pass counter-flow scald system, as compared to water from a single-tank scalder in a commercial setting. Similarly, Cason et al. (1999) reported that in the third tank of a three-tank, counter-flow scalder, aerobic bacteria counts were lower than in water from a single-tank scalder; however, there were no differences in carcass bacteria counts between the two slaughter lines with different scalders. In this same study, Cason et al. (1999) found that the third tank of the counter-flow scalder had less organic and total suspended solids in the water as compared to the control scalder and the other two tanks of the three-tank counter-flow scalder.

The rate at which fresh water is added to the scald tank and the temperature of the scald water are factors affecting the extent of microbial buildup in the scald water (Mead, 1980). Currently, the USDA requires that scald tank overflow be a minimum of one quart of water per carcass (Thaxton, 2002). The temperature of the scald water varies, depending on final product specifications. A soft scald, which is typically done at approximately 52°C (Humphrey, 1981), does not remove the waxy stratum corneum layer (cuticle) of the skin on the carcasses, leaving the skin with a yellow appearance. The low temperature also poses greater risk of bacterial survival and cross contamination. Hard scald temperatures generally range from 50°C to 60°C (Dickens et al., 1999) and remove the cuticle causing a white appearance of the carcass. Hard scald temperatures may have a greater effect on reducing levels of bacteria. Enterobacteria in scald water samples were detectable at lower levels when scalded in 62.5°C water as compared to

lower temperatures (Buchli et al., 1966). Yang et al. (2001) determined at 50°C, scalding decreased numbers of *Campylobacter jejuni* significantly, but did not reduce *S.* Typhimurium. In the same study, destruction of *S*. Typhimurium was observed at 55°C or higher.

The warm environment of the scald tank is ideal for many heat-resistant mesophiles, which can impact the level of cross-contamination that may occur during scalding (McNamara, 1997). Factors such as scald water temperature may also have an impact on heat-resistance of bacteria. The ability for some serotypes of *Salmonella* to develop heat resistance demonstrates that the microorganism is able to remain viable in the scald tank water long enough to cross contaminate many other carcasses that pass through the same tank (Patrick et al., 1973; Humphrey, 1981). Some researchers have found that higher scalding temperatures cause broiler skin to lose more of the cuticle (McMeekin and Thomas, 1979; Kim et al., 1993), which could allow for easier adherence of bacteria to the skin (Kim et al., 1993). Furthermore, Notermans and Kampelmacher (1975) reported that when salmonellas have been attached to the broiler skin, the cells are more heat-resistant than bacteria that remain in suspension in the water. A more recent issue regarding scalding in the United States involves lowering scalding temperatures in order to increase yield (Russell, unpublished data). In fact, some companies have lowered the temperature of the first scald tank to 42.2°C (108°F) in order to decrease the amount of fat that is cooked off of the carcass while in the scald tank. Although these companies report an increase in yield, the warm environment is ideal for pathogens because all nutrients required for growth of these harmful pathogens are available.

Because substantial cross contamination may occur during the scalding phase of processing, bacteria may continue to spread throughout the plant, specifically during the defeathering phase. During defeathering, as the carcasses pass through automated machinery, a substantial amount of scattering of microorganisms may occur, intensifying cross contamination between carcasses (Hafez, 1999). The defeathering unit is also an area of the plant in which all carcasses pass through during processing. Additionally, when the rubber picking fingers become contaminated with bacteria, the warm, humid conditions of the defeathering unit may contribute to bacterial survival and growth (Mead, 1980). These rubber fingers are difficult to clean and disinfect and may lead to the incidence of salmonellas being transferred between processing cycles (Mead, 1980). Goksoy et al. (2004) reported that the highest incidence of *Salmonella* spp. have been found on broilers after defeathering at levels of 40% and 60% at two different plants.

Whittemore and Lyon (1994) reported that microbial loads on picker fingers build up over time and eventually level out, but the type of scald only affects this minutely. When using a marker organism, other researchers found that a single contaminated carcass could possibly cross contaminate more than 200 other carcasses during defeathering (Van Schothorst et al., 1972; Mead et al., 1975). Cason et al. (1997) reported that 23% of broiler carcasses tested positive for salmonellae when sampled after defeathering. These studies suggest that carcasses may be contaminated with pathogens before the next step of poultry processing, evisceration.

The heads and hocks of the carcasses are removed before entering the evisceration line, where both edible and inedible viscera are removed from the carcass cavity by a series of evisceration machinery (Sams, 2001). Possible cutting and tearing of the viscera

during evisceration has been noted as a source of cross contamination (Bryan and Doyle, 1995). In addition, employees, equipment, and contaminated carcasses have been reported as a means of cross contamination of carcasses during evisceration (Stewart, 1965). Other research has focused on the leakage of crop contents during processing. Hargis et al. (1995) reported that crops ruptured 86 times more frequently than ceca during processing and were more likely to be *Salmonella*-positive. Lillard (1989) reported that the incidence of *Salmonella* spp. was 19% after bleeding, decreased to 11.9% after defeathering, and increased to 14.3% after evisceration, which is indicative of cross contamination.

After evisceration, carcasses are submerged in chillers or passed through air chillers, where the primary goal is the reduction of microbial growth by rapidly reducing the temperature, and the temperature is usually 4°C or less (Sams, 2001). The chill tank is a potential source of cross contamination because it is a communal tank that all carcasses must enter. When a chill tank is operating properly, the overall microbiological quality of carcasses can be improved. On the other hand, poor maintenance of chilling systems may result in cross contamination of broiler carcasses. Waldroup et al. (1992 and 1993) reported that the incidence of *Campylobacter* spp. increases from 86.4% of pre-chill to 90.8% of post-chill carcasses, while the incidence of salmonellae increased from pre-chill to posthill by 20%. Since chill tanks act as a communal area through which all carcasses pass, chillers are often recognized as potential sources of cross contamination between carcasses.

There are many steps in which the spread of pathogens may occur, and with the higher numbers of birds processed and the level of automation, control of pathogenic

microorganisms will continue to be a major focus. Poultry processing allows opportunities for the transfer of microorganisms from one carcass to another at almost every stage (Mead, 1980). In processing plants, cross contamination may occur frequently during scalding, picking, and chilling. If microbial loads can be reduced early on such as during the scalding stage, cross contamination may be prevented further down the processing steps. Currently, intervention strategies are being implemented in various steps during processing in order to reduce the risk of cross contamination.

Intervention Strategies to Reduce Pathogens in Processing Plants

Many chemical and physical treatments have been applied during several different poultry processing steps to minimize the risk of contamination of broiler carcasses during processing. Steps such as scalding, picking, and chilling have been the focus of many intervention strategies. There are many controlling factors that determine the effectiveness of these treatments, ranging from pH and temperature to proper equipment adjustments.

During the scalding step of poultry processing, physical and chemical treatments have shown to reduce levels of bacteria on the carcasses and in the scald water. The development of counter-flow and multiple stage scalders have led to reductions of contamination both on the birds and in the scald water (Hafez, 1999). Counter-flow means that the scald water moves in the opposite direction of the birds, from the exit of the scalder toward the entrance, thereby resulting in cleaner water. In multiple stage scalders, spraying the carcasses between units is an improvement in reducing levels of contamination (Stals, 1996). Pre-scald brushes are sometimes used prior to the carcasses entering the scald tank in order to remove dirt and fecal material from the carcasses.

However, cross contamination is still a problem in the scalding and picking phases specifically due to the unique environment of the scalding tank.

Because scald water becomes so dirty and cross contamination may occur, applying intervention strategies in scald water has been suggested (Crabb and Walker, 1971). Scald water additives most often directly affect the pH of the water, and in turn, the role of suspended organic matter. However, many chemicals that could possibly be used as antimicrobials in scald water may be unacceptable because of possible changes in the quality of carcasses (Humphrey et al., 1981). Humphrey et al. (1981) added sodium hydroxide to scald water and adjusted and stabilized the pH to 9.0±0.2. From this study, they reported that there was less fecal matter adhering to the feathers on the broilers, and the total bacterial counts were reduced.

Other studies have added acidic chemicals to scald water to evaluate their antimicrobial capabilities in scald water. The addition of 0.1-0.2% acetic acid to scald water increased the death rates of salmonellas and *C. jejuni* in the water (Okrend et al., 1986). Lillard et al. (1987) added a 0.5 % acetic acid solution in scald water and found that it was effective in killing bacteria in the scald water, but it did not significantly reduce the levels of bacteria on broiler carcasses. In this same study, acetic acid produced a 100% reduction in total aerobic plate counts and enterobacteria in the scald water, but there were no significant effects on the microbial quality of the carcasses. Tamblyn et al. (1997) reported a 2 $\log_{10}$ reduction of *S*. Typhimurium on chicken skin when a 5% acetic acid solution was applied during scalding. However, it was observed that such high concentrations resulted in skin discoloration. Furthermore, Tamblyn et al. (1997) found that the addition of trisodium phosphate (TSP) and sodium metabisulfite

showed no significant reductions when utilized in a scald application. Humphrey et al. (1981) added propionic acid and glutaraldehyde to scald water and found that the death rates of *S*. Typhiumurium increased, but that unfavorable effects on plucking was observed.

Feather removal is consistently cited as a major source of cross contamination of *Salmonella* and *Campylobacter*. Proper replacement and disinfection of the rubber picking fingers is necessary to help reduce levels of cross contamination. Studies involving a separate compartment defeathering apparatus demonstrated a 100-fold reduction in cross contamination during plucking (Hinton et al., 1996; Tinker et al., 1996). However, some researchers have focused on pre- and post-picking carcass washers to help reduce microbial loads on carcasses. Shackleford et al. (1993) found that these carcass washers caused no significant reductions, but other researchers have evaluated the addition of acetic acid and hydrogen peroxide to picker sprayers. A 1% acetic acid solution significantly reduced aerobic plate counts, but 0.5, 1.0, and 1.5% hydrogen peroxide treatments in picker sprayers had no effect on the microbial quality of carcasses (Dickens and Whittemore, 1997). Data such as this further emphasizes the need to implement strategies during the scalding phase to keep pathogen contamination minimized prior to defeathering.

In many poultry processing plants in the United States, rinse waters containing disinfectants are applied in various areas throughout the plants (Byrd and McKee, 2005). Inside outside bird washers (IOBW) are used in poultry processing facilities to help reduce visible fecal contamination on broiler carcasses before entering the chill tanks. IOBW spray washers work by spraying the exterior of the carcasses, as well as the

abdominal cavity. Xiong et al. (1998) reported that a 1.0 log reduction in *Salmonella* was achieved when the washer was adjusted to 207 kPa for 30 sec. Additionally, spray washing was shown to reduce *Salmonella* by 0.4 log when carcasses were sprayed at 413 kPa for 17 sec (Yang et al., 1998). However, Brashears et al. (2001) found that high pressures may actually force bacteria into the skin of carcasses and promote cross contamination.

One of the most common chemical treatments used in poultry processing is chlorine. Chlorine has commonly been applied to immersion chillers and spray washers in processing facilities. Chlorine is very effective in removing protein residues and visible soil from contaminated surfaces, as well as physically reducing microbial loads (Byrd and McKee, 2005). When chlorine is added to water, the active antimicrobial formed is hypochlorous acid. The efficacy of chlorine is a function of pH, and at pH 5 nearly all available chlorine is in the active form of hypochlorous acid (Schmidt, 1997). However, any increase in pH from prior alkaline chemical treatments and the presence of organic matter may inactivate the chlorine, resulting in little antimicrobial activity. Although Mead and Thomas (1973) reported that chlorine at 45 to 50 ppm virtually eliminated viable bacteria from chill water, it has little effect on reducing bacteria attached to carcasses (Lillard, 1993).

Another chlorinated compound that has shown to be effective in the reduction of microorganisms in chill water is chlorine dioxide. Chlorine dioxide is soluble in water, does not react with ammonia or nitrogenous compounds, has greater oxidizing capacity, and is not affected by high pH (Byrd and McKee, 2005). Lillard (1979) found that chlorine dioxide was seven times more effective than chlorine due to greater solubility,

oxidizing capacity, and lower reactivity with organic matter. Although chlorine usage concentrations range from 1 to 10 ppm, the primary concern is safety and toxicity since the highly concentrated gas can be explosive (Schmidt, 1997).

Another chemical treatment in processing facilities is trisodium phosphate (TSP), a generally recognized as safe (GRAS) alkaline detergent approved for spray applications or carcass dips. TSP removes bacteria from carcass surfaces by means of its surfactant properties and high alkalinity. In addition, TSP kills bacteria by disrupting the cell membrane and causing leakage of cellular material (Giese, 1993). Slavik et al. (1994) found that *Campylobacter* levels were significantly reduced when poultry carcasses were dipped in a 10% TSP solution after chilling. Additionally, TSP at 8% was shown to be effective in the reduction of *S*. Typhimurium on chicken skin (Tamblyn et al., 1997). However, there are concerns about using the compound. If TSP is applied prior to carcasses entering the chiller, the high pH of TSP may result in alkalinity of the chill water, thereby decreasing the antimicrobial activity of added chlorine. Likewise, large amounts of phosphate in wastewater present environmental issues.

Because of bactericidal activity and GRAS status, organic acids have been studied for use as antibacterials in processing (Chung and Goepfert, 1970). Specifically, lactic acid has been favored because of its ability to control spoilage in fermented foods and its lack of adverse effects on sensory properties of poultry carcasses (Byrd and McKee, 2005). Lactic acid has lethal effects to *Salmonella*, *Campylobacter*, and other Gram-negative pathogens and shows delayed bacteriostatic effects during storage of treated meats (Smulders, 1987). Lactic acid treatment to broiler carcasses by immersion in a 1 to 2% solution reduced microbial contamination by 1.0 log per gram of skin (Van der Marel

et al., 1988). Concentrations of lactic acid greater than 1.5% have shown to cause discoloration of carcasses, and researchers have suggested the use of a 10% lactic acid/sodium lactate buffer (pH 3.0), which retains a decontaminating effect but avoids affecting the sensory properties (Zeitoun et al., 1999). In this same study, the lactic acid/sodium lactate treatment was used along with modified atmosphere packaging to prolong shelf-life.

In another study, Tamblyn et al. (1997) tested the effects of 5% acetic acid on chicken skin samples post-scald and post-chill. In this study, it was reported that acetic acid was effective in the chiller application, but most effective in a scalder application, with a 2.0 $\log_{10}$ reduction of *S.* Typhimurium per skin (10 cm diameter). This study suggests a synergistic affect between the acid and the application of heat. However, Tamblyn et al., (1997) observed discoloration of the skin samples treated with 5% acetic acid. Organic acids have been shown to reduce microbial levels, but the major disadvantage is the possibility of altering sensory attributes of the final product.

The implementation of various physical and chemical treatments in poultry processing plants has provided ways of reducing the microbial status of broiler carcasses. Although each treatment may have advantages and disadvantages, it is important that treatment methods are safe, effective, economical, and have no effect on the organoleptic properties of the final product. Another consideration is the step in which antimicobial treatments are applied. By implementing intervention strategies as early as possible during processing, such as during scalding, cross contamination further along the processing scheme may be minimized.

Water Quality of Scald Water

During scalding, there are many changes that occur regarding scald water quality. As the broilers enter the scald tank, blood, litter, and feces are removed from the carcasses. As a result, the scald water becomes dirty. Specifically, the pH of the water is lowered and the level of organic matter increases. Chicken feces contain ammonium urate (Miall and Miall, 1949), which dissociates in water to form uric acid and ammonium hydroxide. The pH is lowered due to the formation of uric acid (Weast and Selby, 1966). Two factors that impact the dissociation of ammonium urate and the lowering of pH are the rate the birds are killed and the turnover time of scald water (Humphrey et al., 1981). The level of organic matter in scald water may specifically affect the D-values of bacteria. The D-value refers to the amount of time that is required to kill 90% of the microorganisms (Jay, 2000). Grosklaus and Lessing (1964) suggested that the buildup of organic matter influences the death rate of salmonellas in scald water due to the increased levels of fats and proteins that offer protection to the cells. Scald water pH has been shown to affect the heat-resistance of *S*. Typhimurium. Humphrey et al. (1981) reported that the controlling factor of heat-resistance was the pH of scald water, not the level of organic matter. In this study he reported that *S*. Typhimurium was most heat-resistant at pH 6.1, the pH of the water was 5.9 to 6.0 for most of the working day, and the D52°C value of *S*. Typhimurium was 34.5 minutes. The ability of *S*. Typhimurium to survive in a scald tank water for more than a couple of minutes could allow for cross contamination to occur further down the processing scheme.

The level of organic matter in scald water may also affect the chemical oxygen demand (COD) and biological oxygen demand (BOD) of water. COD is a measure of the

total amount of oxygen required to oxidize all organic matter into carbon dioxide and water, and the COD of water increases with increasing levels of organic matter (Boyd and Tucker, 1992). Organic matter can be decomposed by microorganisms, which use oxygen in the process of decomposition. The amount of oxygen consumed by these microorganisms for oxidizing organic matter is referred to as the BOD (EPA, 1997). Oxygen is measured in its dissolved form as dissolved oxygen (DO), and if more oxygen is consumed than is produced, the DO declines. There is an existing relationship between COD and BOD; the BOD usually increases with an increasing COD (Sawyer and McCarty, 1967; Boyd, 1973). The COD of poultry processing wastewater is generally twice the level of BOD (Merka, 2001). Another common measure of water quality is total suspended solids (TSS). Dissolved organic matter, particulate organic matter, dissolved substances except gases, carbon dioxide contained in bicarbonate, and particulate inorganic substances are all solids found in water, and they can not be evaporated out (Boyd and Tucker, 1992). TSS is a measure of the particulate matter in suspension. Overall, a higher organic load in water typically results in high COD, BOD, and TSS values.

Total water hardness of scald water may impact the cleansing action of the scald water. Water hardness is the most important property that directly affects cleaning and sanitizing efficiency (Schmidt, 1997). Water hardness refers to the concentration of calcium and magnesium in the water. Although other divalent metal ions may contribute to water hardness, their concentrations are not significant in natural waters (Boyd and Tucker, 1992). In water with high levels of hardness, scaling of calcium and magnesium can occur, and the cleaning action of the water decreases (Schmidt, 1997). Therefore, it

is important from a water quality standpoint for processors to measure and control water hardness in order to improve the cleaning action of the scald water.

Wastewater from poultry processing plants generally contains 300 to 500 times more organic matter than available oxygen present in the wastewater required for microbial digestion (Merka, 2001). BOD of poultry scald water has been reported to be as high as 1,182 mg/L (EPA, 1973), which indicates that a high amount of oxygen would be required by microorganisms in the water to completely oxidize all of the organic material in the scald water. According to the EPA (2002), typical BOD and TSS levels in poultry processing wastewaters (not specifically scald water) are as high as 1,662 and 760 mg/L, respectively. Hamm (1972) reported that average COD and total solids were highest in poultry scald water and attributed the high organic load of scald water to blood, dirt, and fecal matter. TSS levels have been reported as high as 682 mg/L in poultry scald water (EPA, 1973).

Temperature, pH, BOD, COD, TSS, and water hardness are very important to scald water quality from a food safety and quality aspect. The relationships between organic matter, temperature, and pH can directly affect the survival of harmful microorganisms in scald water. The temperature and pH can be altered using scald additives, and the state of organic matter in scald water may be changed in order to overall affect the role of microorganisms in scald water. Because water quality may impact the antimicrobial efficacy of chemical treatments that are used, it is necessary to validate the effectiveness of the treatment in the conditions in which it will be applied.

Scald Water Additives

The risk of cross contamination in poultry processing plants is likely to occur during the scalding phase, and intervention strategies should be implemented as early on during the processing scheme as possible, such as during the scalding step, in order to reduce the spread of pathogens throughout the plants. The scald tank provides a unique environment in a processing plant. The scalding phase involves high temperatures, has high levels of organic material, and is the first stage in processing where all carcasses pass through a communal tank. Scalding is early on in the processing scheme, so it is a major area of concern for cross contamination, given its unique characteristics.

The chill tank is also a communal tank in which all carcasses pass through. However, unlike scald tanks, chill tanks provide cold environments and serve as one of the final stages during processing before packaging. Broiler carcasses are required by the FSIS to be cooled rapidly to prevent bacterial growth and specifically reach 4°C or below within 4 hours of chilling. Currently, chlorine is commonly added to chiller water in poultry processing due to its low cost, availability, and efficacy. Chlorine serves as an antimicrobial, and federal regulations require the addition of 20 to 50 ppm of chlorine to chill water to help prevent cross contamination between carcasses. The effectiveness of chlorine is dependent upon the environment in which it is used. The active form of chlorine is hypochlorous acid, which is formed only at pH 7 or below. Also, the presence of organic matter such as blood or feces can inactivate the effectiveness of chlorine because the organic matter binds up the chlorine. Finally, higher temperatures cause chlorine to be less soluble in water, and therefore it may not be as effective due to

evaporation out of the water. The scalding environment would not be suitable for chlorine due to high organic loads, pH, and temperature of the water.

Because scald water becomes so dirty and cross contamination may occur, applying scald water additives has been suggested (Crabb and Walker, 1971). Many studies have shown the antibacterial effects of various additives such as sodium hydroxide, propionic acid, acetic acid and glutaraldehyde on bacterial levels in scald water. Scald water additives most often directly affect the pH of the water, and in turn, the role of organic matter. However, many chemicals that could possibly be used as antimicrobials in scald water may be unacceptable because of the possible changes in the quality of carcasses (Humphrey et al., 1981). Maintaining product quality should be of great importance when selecting an intervention strategy for reducing microbial loads.

Alkaline scald additives have been used to increase the scald water pH, which has shown to reduce bacterial levels and the amount of organic matter. Humphrey et al. (1981) added sodium hydroxide to scald water and adjusted and stabilized the pH to 9.0 ± 0.2. From this study, they reported that there was less fecal matter adhering to the feathers on the broilers, and the total bacterial counts were reduced. By adding sodium hydroxide to the scald water, saponification of fats in the water improved the washing action, and there also was an increase in the total dry matter removed from the scalded carcasses (Humphrey et al., 1981).

Other studies have added acidic chemicals to scald water to evaluate their antimicrobial capabilities as scald additives. The addition of 0.1-0.2% acetic acid to scald water increased the death rates of salmonellas and *C. jejuni* in the water (Okrend et al., 1986). Lillard et al. (1987) added a 0.5 % acetic acid solution in scald water and

found that it was effective in killing bacteria in the scald water, but it did not significantly reduce the level of bacteria on broiler carcasses. In this same study, acetic acid produced a 100% reduction in total aerobic plate counts and Enterobacteria in the scald water, but there were no significant effects on the actual carcasses. Humphrey et al. (1981) added propionic acid and glutaraldehyde to scald water and found that the death rates of *S.* Typhimurium increased, but that unfavorable effects on plucking could result.

While the addition of scald additives may impact the pH and organic matter in the water, this in turn, has marked effects on other water attributes such as COD, BOD, and TSS. It may be thought that COD and TSS values may decrease with higher pH. However, a scald additive was added to scald water to raise the pH to 10, and this significantly increased total solids and COD values, as compared to scald water at pH 8.5 (Carter et al., 1975). These results indicate that higher levels of organic matter were present, and usually there are higher COD values when there are high levels of organic matter. A scald additive with a high pH may increase the washing action of the carcasses, and therefore, introduce more organic matter into the scald tank. More organic matter generally results in higher COD and TSS values. When a scald additive that altered scald water pH to 8.5 and 10 were added to scald water, the BOD values were 194 and 254 mg/L, respectively (Carter et al., 1975). In this same study, scald water at pH 6.5 had a BOD value of 394 mg/L. At a neutral pH, such as 6.5, BOD are expected to be higher due to the ability of microorganisms to survive, therefore driving up the amount of oxygen required by those microorganisms to oxidize organic matter.

RP Scald

RP Scald is a scald additive that is currently being used by commercial poultry processing plants to reduce the appearance of bruising in broilers. It has been noted by the poultry industry that this additive may also reduce microbial loads in the scald tank. The main chemical ingredient in RP Scald is sodium hydroxide, and it is believed to achieve its antimicrobial action by raising the pH of the scald water and providing an alkaline environment that is not suitable for pathogens. Sodium hydroxide is a strong alkaline compound that destroys many microorganisms, dissolves proteins, and is good at dispersing and emulsifying soils (Marriott, 1997). An alkaline environment affects the function of microbial cell enzymes and the transport of nutrients into the cell (Jay, 2000). The cytoplasmic membrane of microorganisms is relatively impermeable to H^+ and ^-OH ions, and their concentration in the cytoplasm remains constant unless variations in the surrounding environment occur (Jay, 2000).

It has been noted that the alkaline conditions created by RP Scald cause organic matter to fall out of suspension in scald water. Preliminary experiments in the current study show that the high pH caused sediment to fall out of solution in scald water samples. Since commercial scald water has been reported to have high organic loads, the efficacy of RP Scald to reduce microbial levels and to improve water quality should be considered as an effective intervention strategy in processing plants.

The recommended usage level of RP Scald is 1 gallon per 1000 gallons of water, making a 0.1% RP Scald concentration. The commercial cost of RP Scald generally ranges from $4.50 to $5.00 per gallon. Since the usage level is fairly low, RP Scald

seems to be a cost-effective intervention strategy that can be implemented in processing plants.

A major concern of strong alkalis such as sodium hydroxide is the extent of corrosiveness to processing equipment. However, scald tanks are generally constructed of stainless steel, which is usually resistant to corrosion (Marriott, 1989). At only 10% concentration of sodium hydroxide, steel and stainless steel are resistant to corrosion (<0.1 mm/year corrosion speed) (Anonymous, 2006). In commercial poultry processing plants, RP Scald is added to scald tanks through timer controlled pumping equipment. In some plants, pH controllers that monitor the system regulated the addition of RP Scald to the tanks. According to D. Sharp (personal communication, Sept. 12, 2006), a representative from Duchem Industries, there are no reports from the industry in which plants have experienced problems with corrosion in pumping systems. There have also been no complaints from members of the poultry industry that RP Scald causes any negative quality issues on the broilers. In the current study, no visible quality problems were observed with the use of RP Scald in scald water. The broilers were slightly lighter in color, which could be expected by the addition of an alkaline compound.

Little, if any, research has been conducted to validate the effectiveness of RP Scald on the reduction of *Salmonella* in a scald tank. Since *Salmonella* continues to be one of the largest causes of food-borne illness in the United States, research such as this could benefit consumers as well as the poultry industry. While many efforts have been made through consumer education and the incorporation of HACCP during poultry processing, the poultry industry should keep searching for ways to improve efforts in reducing levels of harmful pathogens in the food chain. The objective of the current

study was to assess the efficacy of RP Scald in the reduction of *Salmonella* both in vitro and in a plant setting. The efficacy of RP Scald to reduce microbial loads was evaluated through microbial analyses of carcass rinses and scald water samples. Scald water quality attributes such as temperature, pH, COD, BOD, TSS, and total hardness were investigated to better gauge the effectiveness of RP Scald in scald water. This study will provide the poultry industry with a validated intervention strategy to prevent the incidence of cross-contamination of pathogenic bacteria in processing plants.

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CHAPTER III.

USE OF A SCALD ADDITVE TO REDUCE LEVELS OF *SALMONELLA* TYPHIMURIUM AT HARD SCALD, SOFT SCALD, AND ROOM TEMPERATURES IN AN IN VITRO SETTING

ABSTRACT

The risk of cross contamination of food-borne pathogens such as *Salmonella* during poultry processing remains of great concern to the poultry industry. One of the most critical stages in which cross contamination may occur is during scalding. The goal of this study was to evaluate the efficacy of the scald additive RP Scald to reduce levels of *Salmonella* Typhimurium (ST) in various water samples and to validate the effectiveness of RP Scald as an antimicrobial. In four in vitro experiments, test tubes containing 10 ml of either tap water or autoclaved scald water were subjected to six treatment combinations consisting of scalding temperature (hard scald (56.6°C for 2 min), soft scald (50°C for 3 min), and room temperature (~25°C for 2.5 min)), additive (no RP Scald or 10 µl of RP Scald), and inoculum of a nalidixic acid-resistant strain of ST (1 ml ranging from zero to 10^6 ST). In triplicate, test tubes were placed in a water bath to mimic hard scald and soft scald conditions, while test tubes in the room temperature treatments were placed on the laboratory benchtop. Samples were direct plated for microbial analyses, and pH was measured for each treatment.

Soft scald and room temperature treatments recovered ST at the 10^4 inoculum level in the tap water only treatments, while hard scald temperatures in the tap water treatments at all inoculum levels failed to recover ST. ST was not detectable in hard scald, soft scald, and room temperature treatments containing RP Scald at all inoculum levels. In autoclaved scald water containing organic matter, ST was detectable at hard scald, soft scald, and room temperature treatments at levels of 10^4 and 10^6 ST, but after adding RP Scald, no ST was detected, regardless of temperature treatment. Average pH of treatments containing RP Scald ranged from 11.03 to 12.93 and 11.13 to 11.97 for tap water and scald water treatments, respectively. Overall, hard scald temperatures were more effective in ST reduction than soft scald and room temperatures, but the combination of hard scald temperatures and RP Scald resulted in the lowest ST levels. These in vitro experiments suggest that RP Scald may be an effective means of reducing levels of ST in scald water, and perhaps decrease the risk of cross contamination in the scheme of poultry processing.

Key Words: Scalding, scald additive, *Salmonella*

INTRODUCTION

Salmonella continues to be one of the largest causes of food-borne illness in the United States, with *S*. Typhimurium being one of the most common serotypes causing salmonellosis. According to the CDC, 76 million people will suffer from food-borne illness, and *Salmonella* infections alone are responsible for 1.4 million illnesses annually (Mead et al., 1999). Implications of food-borne illnesses to consumers and the food industry can be overwhelming. The USDA Economic Research Service (USDA ERS) estimates that costs associated with food-borne illness total $6.9 billion annually (USDA ERS, 2000).

Recently, in the United States, it was found that 33.9% of broiler carcasses tested positive for *Salmonella* over a 20-week sampling period (Simmons et al., 2003). Chickens are known natural reservoirs of many pathogenic microorganisms such as *Salmonella* and *Campylobacter*, and when broilers enter processing plants, they may carry these pathogens with them, which can then spread throughout plants during various stages of processing (Mead et al., 1994; Kotula and Pandya, 1995). Scalding and picking are two of the most critical areas in processing plants where cross contamination of *Salmonella* may occur (Okrend et al., 1986). During scalding in hot water, the feather follicles are relaxed to allow easy feather removal during picking. However, the follicles may remain open until the chilling stage, meaning that microorganisms may be retained in the follicles even after chilling (Keener et al., 2004). The scald tank provides a warm environment that may be ideal for heat-resistant mesophilic bacteria and may impact the degree of cross contamination (McNamara, 1997). Generally, scalding temperatures range from 50°C to 60°C, and higher temperatures (60°C to 62.5°C) have been shown to

reduce levels of bacteria (Buchli et al., 1966; Yang et al., 2001). In addition, the neutral pH and high level of organic matter that builds up in scald tanks may contribute to the level of cross contamination during scalding, a process in which all carcasses pass through a communal tank. Traditionally, chlorine has been added to the chiller in poultry plants, but chlorine could be ineffective in a scald tank due to the high temperature, high organic load, and pH. Adding alkaline scald additives to scald tanks may help reduce levels of bacteria, as well as reduce the risk of cross contamination between carcasses. Humphrey et al. (1981) reported that less fecal matter adhered to feathers and a reduction in total bacterial counts occurred when scald water pH was adjusted to 9.0 ± 0.2 by adding sodium hydroxide. In a study that shifted scald water to acidic conditions, the addition of 0.1-0.2% of acetic acid to scald water increased death rates of salmonellas and *C. jejuni* in scald water (Okrend et al., 1986). In another study, Lillard et al. (1987) added 0.5% acetic acid to scald water and found that it was effective in killing bacteria in water, but it had no significant effects on the reduction of bacteria on carcasses.

Currently, RP Scald (Duchem Industries, Newnan, GA) scald additive has been added to commercial scald tanks to help reduce the appearance of bruising on broilers. It is thought that the alkaline environment created by RP Scald may reduce microbial levels and reduce the risk of cross contamination between carcasses in scald tanks. The high pH created by RP Scald has also been noted to cause organic matter to precipitate out of suspension in scald water. In the poultry industry, various times and temperatures are applied during scalding, which may impact the survival of microorganisms in scald water and on broiler carcasses. Therefore, the goal of this study was to evaluate the efficacy of RP Scald in reducing levels of *S.* Typhimurium in tap water and scald water at hard scald,

soft scald, and room temperatures in an in vitro setting. In addition, the purpose of these preliminary experiments was to determine appropriate inoculum levels of ST for the in-plant experiments.

MATERIALS AND METHODS

Preliminary In Vitro Experiments

Four in vitro experiments were conducted in order to assess the efficacy of RP Scald to reduce levels of *Salmonella* in an in vitro setting. A nalidixic acid-resistant strain of *Salmonella* Typhimurium (ST) was obtained and subjected to different scalding treatments for each in vitro experiment. The experimental design consisted of combinations of 3 scalding temperature treatments, 2 scald additive treatments, and 3 inoculum level treatments. The three scalding temperature treatments consisted of hard scald (56.6°C for 2 min), soft scald (50°C for 3 min), and room temperature (~25°C for 2.5 min). The 2 additive treatments consisted of a control (water only) and one with the addition of RP Scald to water. The levels of ST inoculum that were used varied among experiments, but ranged from zero to 10^6.

Experimental Parameters

A heating water bath (Fisher Isotemp 28 L Single Chamber Water Bath Model 128, Fisher Scientific, Indiana, PA) was used to mimic the conditions of a scald tank at hard and soft scald temperatures, while room temperature treatments were evaluated on the laboratory benchtop. Test tubes of 10 ml water (either tap water or scald water) were inoculated with 1 ml of ST inoculum and 10µl of RP Scald (to reach 0.1% concentration) depending on the treatment and experiment. Typical chlorine levels of tap water ranged from 1 to 3 ppm. The combination of treatments for hard scald, soft scald, and room temperatures in each experiment is outlined in Table 1. Prepared in triplicate, test tubes were placed in the water bath for either 2 min at 56.6°C or for 3 min at 50°C for hard and

soft scald treatments, respectively. The room temperature (~25°C) test tubes rested on the laboratory benchtop for 2.5 min. At the end of the designated times, the test tubes were immediately removed from the water bath and microbial analyses were carried out by the direct plating method.

In order to optimize microbial recovery, levels of ST inoculum were increased to 10^4 and 10^6 in Experiments 2, 3, and 4. After obtaining the data from in vitro Experiments 1 and 2, Experiments 3 and 4 were conducted using scald water collected from a commercial poultry processing plant, rather than tap water. In order to determine the efficacy of RP Scald to reduce levels of *Salmonella* in scald water containing organic matter, actual scald water was collected and then autoclaved (121°C for 30 min at 30 psi) to kill any existing microorganisms. The objectives of in vitro Experiments 3 and 4 were to determine if RP Scald could perform as an intervention strategy in scald water from a commercial poultry processing plant that contained high levels of organic matter.

Additionally, the experimental design of Experiments 3 and 4 was altered in order to determine if the effectiveness of RP Scald to reduce *Salmonella* was related to the addition of RP Scald or to the length of scald time. In order to assess this question, after each minute, the scald water samples in the hard scald treatments were direct plated. For instance, for each hard scald treatment, the test tube contents were plated after each minute for 3 min.

Direct Plating Procedures and Media

In duplicate, 1 ml of sample was dispensed into a petri dish, and one ml of sample was dispensed into 9 ml Buffered Peptone Water (BPW) dilution tubes to make a 1:10 dilution. This initial dilution was thoroughly mixed and dispensed into a petri dish and 1

ml of the initial dilution was transferred to the next tube so that serial dilutions were carried out to 10^{-4} (Experiment 1) or 10^{-6} (Experiments 2, 3 and 4). After 3.78 ml of Tergitol 4 supplement and 0.8 ml of nalidixic acid were added to 800 ml bottles of Xylose Lysine Tergitol 4 (XLT4) agar, approximately 20 ml of XLT4 (~50°C) was poured into the petri dishes. The media supplement and antibiotic were added to the media in all in vitro experiments so that only the nalidixic acid-resistant marker strain of ST was recovered. The petri dishes were gently swirled to ensure proper dispersion of the bacteria. The plates incubated for 48 h at 37°C, and black colonies were counted.

pH Measurements

In Experiments 2, 3 and 4, separate sets of test tubes were prepared for each treatment, and pH measurements were recorded in order to understand the intervention mechanisms of RP Scald. In order to ensure adequate amount of sample for pH measurements, 6 test tubes for each temperature treatment contained 20 ml of tap water, 2 ml of either 10^4 or 10^6 *S.* Typhimurium, and 20 µl of RP Scald (to reach 0.1% concentration), depending on the treatment. These test tubes were also placed in the water bath for either 2 min at 56.6°C or for 3 min at 50°C for hard and soft scald treatments, respectively. The room temperature (~25°C) test tubes rested on the laboratory benchtop for 2.5 min. Following appropriate time frames, the test tubes were removed from the water bath and pH was measured using a calibrated pH meter (Thermo Orion Benchtop pH Meter, Model 720).

Statistical Analysis

Colony forming units (CFU) were multiplied by 10 (10 ml total per test tube) and then transformed to $\log_{10}$ CFU/test tube. Because "0" cannot be directly analyzed with

the statistical model, we used 0.5 log CFU for statistical analyses. The data was analyzed in a 3 x 2 x 3 factorial arrangement of scalding temperature, additive, and inoculum level. Data was analyzed using the general linear model of SAS (SAS Institute, 2003). Differences and interactions between temperature, additive, and inoculum level were compared by the Tukey test, and the values were considered statistically different at $P < 0.05$. Results are reported as least squares means with standard errors.

RESULTS AND DISCUSSION

In Vitro Experiment 1

The results of the current study were used to determine the appropriate inoculum level to use in the in vivo study, as well as to observe if RP Scald was effective as an antimicrobial in an in vitro setting. Mean $\log_{10}$ CFU per test tube (10 ml per test tube) of temperature, additive, and inoculum level interactions from Experiment 1 are summarized in Table 2. None of the treatment combinations resulted in recovery of ST when RP Scald was added. However, ST was recovered from the tap water only treatments at inoculum levels 10^2 and 10^4. Alkaline scald additives have been used to increase scald water pH, which has shown to reduce bacterial levels and the suspension of organic matter. RP Scald shifts the pH of the water to alkaline conditions, and a pH greater than 9.0 may be bactericidal to microbial cells (Jay, 2000). By adding sodium hydroxide to scald water, Humphrey et al. (1981) found total bacterial counts of the scald water to be reduced. In treatments without RP Scald, only soft scald and room temperature treatments allowed recovery of ST, when inoculated with 10^4 ST. Specifically, room temperature treatments resulted in slightly higher levels (2.61 $\log_{10}$ CFU) of ST than soft scald treatments (2.38 $\log_{10}$ CFU), due to the lack of heat treatment. Hard scald temperatures (56.6°C) caused no ST recovery, regardless of inoculum level or additive treatment. Reductions of *Salmonella* have been reported when scald temperatures are increased from 50°C to 60°C (Yang et al., 2001). It has been found that scalding at lower temperatures results in the accumulation of high numbers of viable organisms in the water, but scalding at a higher temperature (62.5°C) resulted in a decrease of viable cells (Buchli et al., 1966). Data from Experiment 1 demonstrates that

at lower inoculum levels (10^2), ST recovery was not possible when subjected to hard scald or soft scald temperatures, regardless of additive. Reasoning for this could be that tap water is a non-nutritive source and therefore, recovery was not possible. Overall results from Experiment 1 demonstrate that RP Scald was effective in hard scald, soft scald, and room temperature treatments when inoculum levels of 10^4 were used. Because the additive was effective at 10^4 inoculum levels, recovery of ST was low. As a result, higher inoculum levels were used in Experiment 2.

In Vitro Experiment 2

The inoculum levels were increased to 10^4 and 10^6 for Experiment 2 in order to optimize recovery of ST and to determine the efficacy of RP Scald in reducing microbial loads at higher inoculum levels. Table 3 summarizes the interactions among the treatment combinations for in vitro Experiment 2. ST was not recoverable when RP Scald was added to the water, most likely due to the alkaline conditions. The average pH of treatments containing RP Scald ranged from 11.03 to 12.93, while pH in treatments without RP Scald ranged from 7.30 to 7.75. At ST levels of 10^4 and 10^6, soft scald temperatures resulted in 1.87 and 4.19 $\log_{10}$ CFU ST recovery, respectively, when no RP Scald was added. Room temperature treatments at 10^6 inoculum level allowed a 3.77 $\log_{10}$ CFU ST recovery. This data is surprising because we would have expected room temperature treatments to have higher levels of ST since no heat is applied to the samples. Recovery was not possible in the room temperature treatments at the 10^4 inoculum level. Again, since tap water alone is not a nutritive source, recovery of ST may have been compromised.

Results from in vitro Experiment 2 demonstrate that RP Scald is effective in reducing microbial levels at hard scald, soft scald, and room temperatures at 10^6 inoculum level, as in the current study. Soft scald and room temperatures alone are not effective in eliminating ST cells, but the combination of hard scald temperatures and RP Scald had the most drastic effect in eliminating ST. The main intervention mechanism is attributed to the alkaline environment created by RP Scald, working synergistically with higher temperatures.

In Vitro Experiments 3 and 4

During in vitro Experiment 2, it was determined that RP Scald was effective in reducing levels of ST in tap water, particularly in the hard scald treatments. As a result, actual scald water from a commercial scald tank was used (rather than tap water) in Experiments 3 and 4, along with 10^4 and 10^6 inoculum levels in order to evaluate the efficacy of RP Scald in scald water containing high organic loads. When broilers enter a scald tank, blood, litter, fecal matter, and bacteria on the exterior of the carcasses is removed due to the agitation of the scald water (Notermans et al., 1977). The level of organic matter in scald water may specifically affect the D-value of bacteria, which is the amount of time that is required to kill 90% of the microorganisms (Jay, 2000). Grosklaus and Lessing (1964) suggested that the buildup of organic matter may influence the death rates of salmonellas in scald water due to increased levels of fats and proteins that offer protection to microbial cells. Scald water pH has been shown to affect the heat-resistance of *S.* Typhimurium. Humphrey et al. (1981) suggested the controlling factor of heat-resistance was the pH of scald water, not the level of organic matter. Humphrey et al. (1981) reported that *S.* Typhimurium was most heat-resistant at pH 6.1, the pH range of

the water was 5.9 to 6.0 for most of the working day, and the D52°C value of *S.* Typhimurium was 34.5 minutes. The ability of *S.* Typhimurium to survive in scald water for more than a couple of minutes would allow for cross contamination of carcasses to occur, resulting in cross contamination further down the processing scheme.

Significant treatment interaction effects between temperature, additive, and inoculum level show that the hard scald, soft scald, and room temperature treatments without RP Scald demonstrated recovery of ST (Table 4). In Experiment 2, when tap water was used, ST was not detected in the hard scald treatments. The fact that ST was detected in the hard scald treatments when subjected to actual scald water containing high organic loads suggests that organic matter may actually influence the death rate of salmonellas. All temperature and inoculum treatments containing RP Scald had reduced levels of ST. In treatments containing RP Scald, the average pH ranged from 11.13 to 11.97, indicative of an alkaline environment. Treatments not containing RP Scald had average pH measurements ranging from 7.19 to 7.26. The hard scald and soft scald treatments without RP Scald resulted in recovery of ST, although to a lesser extent in the hard scald treatments, which caused a 0.90 and 1.56 $\log_{10}$ CFU reduction at 10^4 and 10^6 levels, respectively. For room temperature treatments, ST was recovered only at the 10^6 inoculum level. Again, room temperature treatments would have been expected to recover ST at 10^4 inoculum level since no heat treatment was applied. The addition of RP Scald to scald water containing organic matter affected the pH of the system, by raising the pH to an alkaline system. It was also noted during this experiment that in the test tubes containing RP Scald, after just seconds, precipitation of sediment was visible in the bottom of the test tubes.

During Experiments 3 and 4, the hard scald treatments were analyzed for recovery of ST after each minute in order to determine if the reductions of ST were a function of scalding time or pH. Results indicated that in the 10^4 and 10^6 inoculum treatments without RP Scald, the average $\log_{10}$ CFU decreased over time, but the pH of the water remained neutral. These results suggest that for treatments not containing RP Scald, the amount of time to which the samples were subjected to the hard scald temperature was the major factor contributing to the reduction of ST. In the 10^4 and 10^6 inoculum treatments containing RP Scald, there was no recovery of ST, and the pH remained alkaline over the span of three minutes. This data suggests the alkaline environment created by the addition of RP Scald was the main contributing factor to ST reductions, rather than the amount of time the samples were exposed to hard scald temperatures.

Results from this experiment indicate that in scald water containing organic matter, temperature alone is not enough to reduce ST levels up to 10^6, as used in this study. The addition of RP Scald at all scalding temperatures and inoculum levels was effective in reducing ST, and therefore, RP Scald may provide an effective means of reducing levels of harmful pathogens in poultry processing plants.

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Table 1. Experimental design for in vitro experiments

Treatment[1]	Water[2]	*Salmonella*		RP Scald
		Exp 1	Exp 2, 3, & 4	
1	10 ml water	--	--	--
2	10 ml water	--	--	10 µl
3	10 ml water	10^2	10^4	--
4	10 ml water	10^4	10^6	--
5	10 ml water	10^2	10^4	10 µl
6	10 ml water	10^4	10^6	10 µl

[1]Experimental treatment design was the same for hard scald, soft scald, and room temperature.

[2]Tap water was used in Experiments 1 and 2; autoclaved scald water was used in Experiments 3 and 4.

Table 2. Mean log_{10} CFU of temperature, additive, and inoculum level interactions for in vitro Experiment 1

Log_{10} CFU/test tube[1]				
Additive	Inoculum Level	Scald Temperature		
		Hard	Soft	Room
Water	None	ND[*]	ND[*]	ND[*]
	10^2	ND[*]	ND[*]	ND[*]
	10^4	ND[*]	2.38[**]	2.61[**]
Water and RP Scald	None	ND[*]	ND[*]	ND[*]
	10^2	ND[*]	ND[*]	ND[*]
	10^4	ND[*]	ND[*]	ND[*]
SEM[2]	0.08			

[1]Values represent the mean log_{10} CFU of n=3 test tubes (10ml/tube) per treatment.
[2]Pooled SEM
ND=Not detectable; log_{10} CFU < 0.50.
[*] Means with uncommon subscripts differ significantly ($P < 0.05$).

Table 3. Mean log_{10} CFU of temperature, additive, and inoculum level interactions for in vitro Experiment 2

Log_{10} CFU/test tube[1]				
Additive	Inoculum Level	Scald Temperature		
		Hard	Soft	Room
Water	None	ND*	ND*	ND*
	10^4	ND*	1.87**	ND*
	10^6	ND*	4.19***	3.77***
Water and RP Scald	None	ND*	ND*	ND*
	10^4	ND*	ND*	ND*
	10^6	ND*	ND*	ND*
SEM[2]	0.13			

[1]Values represent the mean log_{10} CFU of n=3 test tubes (10ml/tube) per treatment.
[2]Pooled SEM
ND=Not detectable; log_{10} CFU < 0.50.
* Means with uncommon subscripts differ significantly ($P < 0.05$).

Table 4. Mean log_{10} CFU of temperature, additive, and inoculum level interactions for in vitro Experiments 3 and 4

Log_{10} CFU/test tube[1]				
Additive	Inoculum Level	Temperature		
		Hard	Soft	Room
Water	None	ND*	ND*	ND*
	10^4	0.78*	1.68**	ND*
	10^6	2.01**	3.57***	2.78**
Water and RP Scald	None	ND*	ND*	ND*
	10^4	ND*	ND*	ND*
	10^6	ND*	ND*	ND*
SEM[3]	0.13			

[1]Values represent the mean log_{10} CFU of n=3 test tubes (10ml/tube) per treatment.
[2]Pooled SEM
ND=Not detectable; log_{10} CFU < 0.05.
* Means with uncommon superscripts differ significantly ($P < 0.05$).

CHAPTER IV.

USE OF A SCALD ADDITIVE TO REDUCE LEVELS OF *SALMONELLA* TYPHIMURIUM AT HARD SCALD AND SOFT SCALD TEMPERATURES IN A PLANT SETTING

ABSTRACT

The risk of cross contamination of food-borne pathogens such as *Salmonella* during poultry processing remains of great concern to the poultry industry, and one of the most critical areas where cross contamination may occur is the scalder. The goal of this study was to evaluate the efficacy of RP Scald to reduce levels of *Salmonella* Typhimurium (ST) in scald water and to determine if the conditions of scald water quality impact the antimicrobial effectiveness of RP Scald. In two trials, experimental treatments consisted of: S1 (soft scald (50ºC for 90 s), inoculated with sterile fecal slurry); S2 (soft scald, inoculated with 10^8 ST fecal slurry); S3 (soft scald, inoculated with 10^8 ST fecal slurry, RP Scald in scald water); H1 (hard scald (56.6ºC for 45 s), inoculated with sterile fecal slurry); H2 (hard scald, inoculated with 10^8 ST fecal slurry); and H3 (hard scald, inoculated with 10^8 ST fecal slurry, RP Scald in scald water). After defeathering, carcass rinses were performed and microbial analyses were conducted. Scald water pH and temperature were monitored, and scald water samples were collected at specific intervals to measure biological oxygen demand, chemical oxygen demand, total suspended solids, water hardness, and *Salmonella* detection. Results indicated that

broilers from treatment H3 in Trial 1 resulted in the lowest level of ST (0.95 $\log_{10}$ CFU), while S2 resulted in the greatest recovery of ST (3.37 $\log_{10}$ CFU). Likewise, in Trial 2, H3 resulted in lowest levels of ST (1.00 $\log_{10}$ CFU), while S2 contained the highest levels of ST (6.00 $\log_{10}$ CFU). Overall, the combination of hard scalding with RP Scald resulted in the lowest levels of ST. Treatments containing RP Scald reached pH 11.77 ± 0.08 and 11.09 ± 0.08 in Trials 1 and 2, respectively. Regardless of scald water quality attributes, the addition of RP Scald resulted in no ST recovery. This study suggests that RP Scald may be an effective means of reducing cross contamination in poultry scalder applications**.**

Key Words: Scalding, scald additive, *Salmonella*

INTRODUCTION

Salmonella continues to be one of the largest causes of food-borne illness in the United States, with *S*. Typhimurium being one of the most common serotypes causing salmonellosis (CDC, 2006). In a recent study in the United States, 33.9% of broiler carcasses were found to be positive for *Salmonella* over a 20-week sampling period (Simmons et al., 2003). Chickens are known natural reservoirs of many pathogenic microorganisms such as *Salmonella* and *Campylobacter*. Broilers entering processing plants may carry these pathogens, which can then spread throughout plants during various stages of processing (Mead et al., 1994; Kotula and Pandya, 1995). Scalding and picking are two of the most critical areas in poultry processing plants where cross contamination of *Salmonella* may occur (Okrend et al., 1986). During scalding in hot water, the feather follicles are relaxed to allow easy feather removal during picking. However, the follicles may remain open until the chilling stage, meaning that microorganisms may be retained in the follicles even after chilling (Keener et al., 2004).

The scald tank provides a warm environment that may be ideal for heat-resistant mesophilic bacteria, and this could impact the degree of cross contamination (McNamara, 1997). In addition, the neutral pH and high level of organic matter that builds up in scald tanks may contribute to the level of cross contamination during scalding, a process in which all carcasses pass through a communal water bath. High levels of organic matter may also impact water quality attributes such as biological oxygen demand (BOD), chemical oxygen demand (COD), and total suspended solids (TSS). In general, poultry wastewater contains 300 to 500 times more organic matter than available oxygen in the water that is required for microbial digestion (Merka, 2001). Therefore, BOD, COD, and

TSS of scald water increase with increasing amounts of organic matter. Total water hardness measurements are important, because the degree of water hardness can impact the cleansing action of water. High levels of magnesium and calcium in water result in hard water, and as hardness increases, the cleaning ability decreases (Schmidt, 1997).

Adding alkaline scald additives to scald tanks may help reduce bacterial loads, as well as reduce the risk of cross contamination between carcasses. Humphrey et al. (1981) reported that less fecal matter adhered to feathers on broilers, and a reduction in total bacterial counts occurred when scald water pH was adjusted to 9.0 ± 0.2 by adding sodium hydroxide to scald water. Sodium metabisulfite and trisodium phosphate have been considered as scalder applications, but both showed no significant reductions of *S.* Typhimurium on chicken skin samples in 8% and 1% solutions, respectively (Tamblyn et al., 1997). Other studies have focused on lowering the pH of scald water. The addition of 0.1-0.2% of acetic acid to scald water increased death rates of salmonellas and *Campylobacter jejuni* in scald water (Okrend et al., 1986). In another study, Lillard et al. (1987) added 0.5% acetic acid to scald water and found that it was effective in killing bacteria in the water, but it had no significant effects on the reduction of bacteria on carcasses. Similarly, Tamblyn et al. (1997) reported that 5% acetic acid was most effective in scalders, as compared to chillers, with a 2.0 $\log_{10}$ reduction of attached *S.* Typhimurium cells per skin. However, in the same study, discoloration of skin was observed.

RP Scald (Duchem Industries, Newnan, GA) is a commercially available scald additive that has been used in commercial scald tanks to help reduce the appearance of bruising on broilers. It is thought that the alkaline environment created by RP Scald may

reduce microbial levels and reduce the risk of cross contamination between carcasses in scald tanks. The high pH may also help precipitate organic matter out of suspension in scald water. Maintaining product quality should be of great importance when using additives during processing because many chemicals may cause quality changes in the carcasses (Humphrey et al., 1981). The application of an additive during scalding may be ideal since it is the first stage in processing where all birds pass through a communal water bath. By using a scald additive that provides a practical and effective means of reducing levels of harmful pathogens such *Salmonella* in scald tank water, the risk of cross contamination occurring further along in the processing scheme could be reduced. Therefore, the objective of this study was to validate the efficacy of RP Scald in a plant setting to reduce *S*. Typhimurium at hard scald and soft scald temperatures.

MATERIALS AND METHODS

Salmonella Inoculum Preparation

Test tubes containing 10 ml of Tryptic Soy Broth (TSB) were inoculated with a frozen culture of a nalidixic acid-resistant strain of *Salmonella* Typhimurium (ST). After incubation at 37°C for 24 h, one 10 µl loopful of the ST culture was streaked onto Xylose Lysine Tergitol 4 Agar (XLT4) containing Tergitol 4 supplement and 0.1% nalidixic acid. All XLT4 plates used in this experiment contained the supplement and nalidixic acid so that the media would be selective for recovering the nalidixic acid-resistant strain of ST. The plates were incubated at 37°C for 48 h. Black, isolated colonies were picked from the XLT4 plates, and fresh TSB tubes were inoculated with one colony per test tube. The tubes were incubated 20-24 h. A stock culture of 10^8 ST was prepared.

Fecal Slurry Preparation

In order to mimic natural conditions, a fecal slurry was used as the vehicle of ST inoculation since fecal matter is a natural carrier of *Salmonella* in broilers. Approximately 500 ml of fecal matter was collected from broilers at the Auburn University Poultry Research Unit. The fecal matter was autoclaved (121°C for 30 min at 30 psi) to kill any existing microorganisms and then cooled to room temperature. Microbial analyses of 500 ml of autoclaved fecal matter were carried out to confirm that the autoclave cycle killed microorganisms in that volume of fecal matter. Approximately 150 ml of the autoclaved fecal matter was reserved for use as the sterile fecal slurry. A total of 300 ml of inoculated fecal slurry was prepared by adding 120 ml of the stock 10^8 ST inoculum to 180 ml of autoclaved fecal matter, giving 1:1.5 ratio of ST to fecal

matter. In other words, 1 ml of 10^8 ST was present in each 2.5 ml of the stock of inoculated fecal slurry.

Processing Parameters

A total of 300 broilers were obtained from a commercial grower for Trial 1, and 300 broilers were obtained from the Auburn University Poultry Research Unit for Trial 2. During each trial, broilers were processed through the defeathering stage in order to study the effects of RP Scald on reducing levels of *Salmonella*. Prior to the start of each experiment, approximately 100 broilers were commercially processed to increase the organic load (litter, blood, fecal matter, etc…) in the scalder. Specifically, the broilers were hung on shackles, electrically stunned (50 V, 20 mA, 400 Hz) and killed with a single cut to the throat, followed by a 95 s bleed out. The birds proceeded through a 2.44 m long single pass, steam injected scalder. The birds were defeathered in a 1.22 m long disk-picker and removed from the processing line.

The experimental design for each trial (Table 1) consisted of 2 replications (10 birds/treatment group/replication) and 6 treatment groups: 1) S1 (soft scald (50ºC for 90 s), inoculated with sterile fecal slurry); 2) S2 (soft scald, inoculated with 10^8 ST fecal slurry); 3) S3 (soft scald, inoculated with 10^8 ST fecal slurry, RP Scald in scald tank); 4) H1 (hard scald (56.6ºC for 45 s), inoculated with sterile fecal slurry); 5) H2 (hard scald, inoculated with 10^8 ST fecal slurry); and 6) H3 (hard scald, inoculated with 10^8 ST fecal slurry, RP Scald in scald tank). The first experimental groups to be processed consisted of the three soft scald treatment groups, beginning S1, and then continuing with S2 and S3. In order to achieve a 0.1% concentration of RP Scald, one-third of a gallon of RP Scald was added to the 300-gallon scald tank for experimental treatments containing RP

Scald (S3 and H3) in order to meet the manufacturers recommended usage level. Ten birds at a time were hung on shackles, stunned, and killed. After bleed out, 10 birds were removed from the shackles and placed on a table to be inoculated with either the sterile or ST fecal slurry. The fecal slurry inoculum was administered in 2.5 ml aliquots (10^8 ST per bird) on the breast side along the feather track so that the fecal slurry was directly on the skin of the broiler. The fecal slurries were allowed 10 minutes to dry. The birds were re-hung on the shackles and proceeded through the scald tank for either 90 s at 50°C or for 45 s at 56.6°C for soft scald and hard scald treatments, respectively. After scalding, the birds were defeathered and aseptically removed from the shackles. Whole bird rinses according to the USDA FSIS Microbiology Laboratory Guidebook (2004) were carried out on the New York dressed carcasses. The carcasses were placed into sterile carcass rinse bags and rinsed with 200 ml of Buffered Peptone Water (BPW) for one minute using a rocking motion to assure that all exterior surfaces were rinsed. The rinsate was transferred into sterile bottles and then transported to the laboratory (< 3 miles) for microbial analyses.

The scald tank was emptied, cleaned, and sanitized after the three soft scald treatments were processed. After sanitizing, the scald tank was swabbed for microbial analysis and then re-filled with water for processing of the hard scald treatments, which began with H1, and then continued with H2 and H3. The picker fingers were also cleaned and sanitized before the hard scald treatments were processed.

Microbial Analyses

No enrichment procedures were used for the microbial analyses of the carcass rinses due to the high level of ST inoculum in the fecal slurry. The direct plating method

was carried out so that microbial reductions of the high inoculum levels could be detected. Rinsate samples were direct plated in duplicate by dispensing 1 ml of rinsate into a petri dish, as well as dispensing 1 ml of rinsate into a 9 ml BPW dilution tube, giving a 1:10 initial dilution. After mixing the dilution, 1 ml was transferred to the next tube so that serial dilutions were carried out through 10^{-6}. Dilutions were direct plated into petri dishes, and approximately 20 ml of XLT4 media (~50°C) containing Tergitol supplement and 0.1% nalidixic acid was poured into the dishes. The petri dishes were gently swirled to ensure proper dispersion of the bacteria. The plates were incubated at 37°C for 48 h. Black colonies were counted.

To determine the microbial status of the scalder surfaces, three distinct areas (front, middle, and back sections) of the interior of the scald tank were swabbed for *Salmonella* detection during three time frames: before processing began, after the tank was emptied following the soft scald treatments, and after processing. A sterile sponge was moistened with 10 ml of BPW, and the tank was swabbed. The swab was placed in a sterile sample bag containing 50 ml of BPW, bringing the total volume of BPW to 60 ml. The sponge samples were incubated at 37°C for 20-24 h. Next, 0.5 ml of the sample was transferred into 10 ml of Tetrathionate broth (TT) plus iodine supplement, and 0.1 ml of the sample was transferred into 10 ml of Rappaport Vassiliadis (RV) broth. The samples were incubated at 37°C for 22-24 h and streaked for isolation on XLT4 agar plates using one 10 µl loopful of sample for each plate. The plates were incubated at 37°C for 18-24 h and well-isolated suspect colonies were picked. Triple Sugar Iron (TSI) and Lysine Iron Agar (LIA) slants were stabbed and streaked (1 TSI and 1 LIA per plate) with the suspect

positive colonies and incubated at 37°C for 24 h. Agglutination tests were used to confirm suspect colonies from the slants.

Water Sample Collection

Scald water samples were collected for chemical oxygen demand (COD), biological oxygen demand (BOD), total water hardness (Mg and Ca) measurements, total suspended solids (TSS), and microbial detection of ST in the scald water at the following stages: before processing began, after each soft scald treatment, after the scald tank was re-filled, before the second processing began, after each hard scald treatment, and after processing. Using sterile containers, three 500 ml samples of scald water were collected for COD, BOD, and TSS measurements. Water was collected from the front, middle, and end sections of the scald tank so that the water samples would be representative of the entire scald tank. In addition, three 100 ml scald water samples were collected in sterile containers from the front, middle, and end sections of the scald tank for water hardness and microbial detection of ST. Water samples were placed on ice, and COD and BOD measurements were carried out within 6 hours of sampling. COD, BOD, and TSS were measured only in Trial 2, while total hardness and microbial analysis were measured in Trials 1 and 2.

Water Quality Analyses

During processing, pH and temperature of the scald water was monitored using a portable pH meter and digital thermometer (Accumet 1000 Series Handheld pH/mV/Ion Meter, Fisher Scientific). Measurements were taken at the following time frames: before processing began, after each soft scald treatment, after the scald tank was re-filled, before

the second processing began, after each hard scald treatment, and after processing. The measurements at each time interval were taken at both ends of the scald tank.

For microbial detection of *Salmonella* in the scald water samples, 50 ml of scald water was combined with 50 ml of BPW and enriched for 24 h at 37°C. Next, 0.5 ml of the sample was transferred into 10 ml of TT broth plus iodine supplement, and 0.1 ml of the sample was transferred into 10 ml of RV broth. The samples were incubated at 37°C for 22-24 h and streaked for isolation on XLT4 agar plates using one 10 µl loopful of inoculum for each plate. The plates were incubated at 37°C for 18-24 h and well-isolated suspect colonies were picked. TSI and LIA slants were stabbed and streaked (1 TSI and 1 LIA per plate) with the suspect positive colonies and incubated at 37°C for 24 h. Agglutination was used to confirm suspect colonies from the slants.

Prepared in triplicate, 20 ml of each 500 ml water sample was used to measure COD, in which the heat of dilution technique (Boyd, 1979) was carried out. Twenty ml of the water samples and 10 ml of standard dichromate solution (0.0250 N) were dispensed into clean 125 ml Erlenmeyer flasks. Highly polluted water samples were diluted in half by combining 10 ml distilled water plus 10 ml sample to accommodate the 20 ml sample. Next, 30 ml of concentrated sulfuric acid was added, and the flask was gently swirled. The flasks were covered with clean cover glasses and allowed to sit for 30 min for digestion to occur. Next, the contents in the flasks were diluted to 75 ml with distilled water, 3 drops of ferroin indicator solution were added, and the samples were titrated with ferrous ammonium sulfate. A reagent blank prepared with 20 ml of distilled water was also carried through this procedure.

The five-day BOD (BOD_5) test was carried out, in triplicate, to measure BOD of the scald water samples. In order to measure BOD_5, water samples were diluted by half, using 200 ml of water sample plus 200 ml of distilled water. The water samples were allowed time for the water temperature to equilibrate to 20ºC. Two ml of seed inoculum solution and 1 nutrient buffer pillow were added to each BOD bottle. The diluted water samples were then transferred into BOD bottles, and the dissolved oxygen (DO) was measured on a polargraphic dissolved oxygen meter and probe (YSI Model 57). DO measurements were obtained each day for five days.

Scald water samples from each treatment were analyzed for total water hardness using a titration drop test kit (Model HA-4P, Hach Co., Loveland, CO). TSS was measured in triplicate for each water sample. Glass fiber filters were dried in an oven at 103ºC for 24 h and then weighed. The water samples were shaken thoroughly to disperse the solids within each sample. Next, 100 ml of the sample was passed through the glass fiber filters. The filters were removed and placed in an oven for 24 h at 103ºC. The filters were weighed back and TSS was calculated.

Statistical Analysis

Colony-forming units (CFU) were multiplied by 200 (200 ml carcass rinse solution used) and then transformed to $\log_{10}$ CFU/200 ml rinsate. Because "0" cannot be directly analyzed with the statistical model, we used 0.5 $\log_{10}$ CFU for statistical analyses. The data was analyzed in a 2 x 2 x 2 factorial arrangement of scalding temperature, additive, and trial. Data were analyzed using the general linear model of SAS software (SAS Institute, 2003), and a probability level of $P < 0.05$ indicated significance. Differences and interactions between temperature, additive, and inoculum

levels were compared using Duncan's multiple range tests. Results are reported as least squares means with standard errors.

RESULTS AND DISCUSSION

Differences in recovery levels of ST existed between Trial 1 and Trial 2. In Trial 1, ST was not detected on the control birds (non-inoculated) from either the S1 or H1 treatments. However, in Trial 2, the control birds (non-inoculated) tested positive for ST in both the S1 and H1 treatments. As a result, all ST recovery levels for Trial 2 were higher ($P < 0.05$) indicating that the control birds were contaminated prior to the start of the experiment. Since a selective media (XLT4) containing Tergitol 4 supplement and nalidixic acid were used, all of the *Salmonella* recovered was nalidixic acid-resistant, indicating that the experimental flock was positive for a nalidixic acid-resistant strain of *Salmonella* prior to processing. Therefore, the data from Trials 1 and 2 will be presented separately.

Results from Trial 1 are illustrated in Figure 1. Data indicated that the soft scald treatment not containing RP Scald (S2) resulted in the highest level of ST, followed by the hard scald treatment not containing RP Scald (H2). This suggests that hard scald temperatures alone had an effect on the reduction ($P < 0.05$) of ST, when RP Scald was not added to scald water. When compared to soft scald treatments, hard scald treatments resulted in a 0.75 and 0.87 $\log_{10}$ CFU reduction ($P < 0.05$) of ST in treatments without RP Scald (H2) and with RP Scald (H3), respectively. The average scald water temperature in Trial 1 was 57.39°C ± 1.95 (45 s) and 50.61°C ± 1.51 (90 s) for hard scald and soft scald treatments, respectively. Yang et al. (2001) reported that when the scalding temperature increased from 50°C to 60°C, a 2 log CFU/cm^2 reduction occurred on chicken skin samples. In the same study, destruction of *S.* Typhimurium was observed at a scalding temperature of 55°C. In other studies, scalding at temperatures of

58°C to 60°C has resulted in greater reductions of *Salmonella* and *Campylobacter*, as compared to scalding at 52°C (Notermans and Kampelmacher, 1975; Notermans et al., 1977; Mulder et al., 1978; Oosterom et al., 1983; Wempe et al., 1983).

Treatments S2 and H2 (inoculated, no RP Scald) resulted in an average of 3.37 and 2.62 $\log_{10}$ CFU recovery of ST, respectively, while broiler carcasses in treatments S3 and H3 (inoculated, addition of RP Scald) had an average of 1.82 and 0.95 $\log_{10}$ CFU recovery of ST, respectively. The addition of RP Scald resulted in a 1.55 and 1.67 $\log_{10}$ CFU reduction of ST in the soft scald and hard scald treatments, respectively. This indicates that RP Scald in scald water is effective at significantly reducing ($P < 0.05$) ST on scalded-picked broilers that have been subjected to hard and soft scald temperatures, and to a greater extent when hard scald temperatures are used. By adding RP Scald to scald water, the pH is shifted to an alkaline state, and when the pH is greater than 9.0, conditions become bactericidal to *Salmonella* spp. (Jay, 2000). In Trial 1, the addition of RP Scald to the scald water resulted in an increase of scald water pH from 7.80 ± 0.18 to 11.77 ± 0.08. The creation of this alkaline environment is considered to play an important role in the intervention mechanism of RP Scald to reduce levels of microorganisms, by altering the functioning of enzymes of microbial cells, as well as the transport of nutrients into the cells (Jay, 2000). In a study by Humphrey et al. (1981), scald water pH was stabilized at pH 9.0 ± 0.2 by adding sodium hydroxide, and the total bacterial counts in scald water were reduced. However, in the current study, pH measurements were higher than those reported by Humphrey et al. (1981), and hard scalding temperatures were utilized. Humphrey et al. (1981) also reported that there was less fecal matter adhering to the feathers on the broilers, indicating improved washing

action due to saponification of fats in scald water. When compared to all treatments in Trial 1, treatment H3 (inoculated, addition of RP Scald) resulted in the lowest recovery (0.95 $\log_{10}$ CFU) of ST. Overall, results in Trial 1 indicate that hard scald temperatures are effective in reducing ST when compared to soft scald temperatures, regardless of the additive treatment. Furthermore, the combination of hard scald temperatures and the addition of RP Scald had the greatest effect on the reduction of ST.

As stated previously, broiler rinses from Trial 2 indicated flock contamination due to the detection of ST on control birds in treatments S1 and H1. Therefore, the levels of *Salmonella* were overall higher during Trial 2. Treatments S2 and H2 (inoculated, no RP Scald) resulted in an average of 6.19 and 2.87 $\log_{10}$ CFU of ST, respectively, while broiler carcasses in treatments S3 and H3 (inoculated, addition of RP Scald) resulted in an average of 4.49 and 1.00 $\log_{10}$ CFU recovery of ST, respectively. The addition of RP Scald increased the pH of the scald water from 7.89 ± 0.10 to 11.09 ± 0.08, which contributed to a 1.51 and 1.87 $\log_{10}$ CFU reduction ($P < 0.05$) of ST in treatments S3 and H3, when compared to treatments S2 and H2. Regardless of the additive, hard scald treatments, H2 and H3, in Trial 2 resulted in a 3.13 and 3.49 $\log_{10}$ CFU reduction ($P < 0.05$) of ST, when compared to the soft scald treatments, S2 and S3. The average scald water temperature in Trial 2 was 57.29°C ± 1.12 (45 s) and 51.12°C ± 0.85 (90 s) for the hard scald and soft scald treatments, respectively. This data demonstrates the importance of high scalding temperatures in reducing microbial levels in scald water. However, the inability of RP Scald to cause a greater ST reduction on broilers in the soft scald treatment may be due to the higher level of background contamination on the broiler

flock in Trial 2. In both trials, the synergistic effect of high scalding temperatures and the addition of RP Scald resulted in the lowest levels of ST recovery (1.00 $\log_{10}$ CFU).

During the two trials, water quality measurements such as BOD (Trial 2), COD (Trial 2), water hardness (Trials 1 and 2), and TSS (Trial 2) were evaluated in order to determine if the conditions of scald water impacted the efficacy of RP Scald as an antimicrobial. In scald water samples collected before any broilers were processed or passed through the scald tank, the BOD was –2.21 and –1.07 mg/L for the hard and soft scald treatments, respectively. These extremely low BOD values were expected due to the lack of organic matter and microorganisms in the scald water at this point during the experiment. However, the average BOD measurements from scald water samples collected during processing were 12.38 and 12.80 mg/L for the hard scald and soft scald water samples, respectively. During this time, organic matter and microorganisms were being introduced into the scald water, resulting in increased BOD levels. Organic matter can be decomposed by microorganisms, which use oxygen in the process of decomposition. The amount of oxygen consumed by these microorganisms for oxidizing organic matter is referred to as the BOD (EPA, 1997). BOD of commercial poultry processing water has been reported to range from 150 to 2400 mg/L (Loehr, 1984). However, such data reflects large commercial processing plants in which thousands of birds are processed daily, and organic loads would be expected to be much higher. BOD levels in the current study were much lower due to low bird numbers and slower rate of carcass throughput.

COD is a measure of the total amount of oxygen required to oxidize all organic matter into carbon dioxide and water (Boyd and Tucker, 1992). As with BOD, COD of

water increases with increasing levels of organic matter (Boyd and Tucker, 1992). Hamm (1972) sampled seven different sites in a poultry processing plant and found that the scalder had the highest COD level (2268 mg/L). In both the soft and hard scald treatments, fresh water collected before processing began resulted in the lowest (2.11 mg/L) COD level. The low COD of the fresh scald water was expected due to the absence of organic matter in the scald water samples. The average COD of scald water samples collected during processing were 99.56 and 104.48 mg/L for hard scald and soft scald water samples, respectively.

Total water hardness, which refers to the concentration of calcium and magnesium, is one of the most important factors that may impact the efficiency of cleaning (Schmidt, 1997). In Trial 1, average water hardness was 66.39 mg/L in scald water samples. Average water hardness in Trial 2 was 81.32 mg/L. An increase in water hardness indicates elevated levels of calcium and magnesium, which can lead to scaling and decreased cleansing action of water (Schmidt, 1997).

TSS, a measure of the particulate matter in suspension (Boyd and Tucker, 1992), was measured in Trial 2. In water samples collected before processing began, TSS was 0 mg/L, which was expected because no birds had passed through the scald tank at that point. Average TSS measurements were 77.75 and 78.25 mg/L in the hard scald and soft scald water samples, respectively. This data supports the fact that there was organic matter present in the scald water during the experiment.

Scald water samples were also collected for microbial detection of ST in order to evaluate the effects of RP Scald on the survival of ST in scald water. In Trial 1, 33% of the water samples from the soft scald treatments tested positive for ST, while only 20%

of samples from the hard scald treatments were positive. Similarly, in Trial 2, 40% and 20% of scald water samples tested positive for ST in the soft scald and hard scald treatments, respectively. In Trials 1 and 2, the treatments containing RP Scald failed to detect ST, which indicates that RP Scald is effective as an antimicrobial in scald water containing organic matter.

The results from this study indicate that RP Scald could be implemented as an effective intervention strategy in poultry processing plants to reduce the incidence of *Salmonella*. Although RP Scald has shown in the current study to reduce levels of ST at soft scald temperatures, it is more effective when used in combination with hard scalding processes. RP Scald additive has the ability to reduce levels of *Salmonella*, regardless of the scald water organic loads that were reported in the current study. Therefore, RP Scald may be used as an effective intervention strategy in scalders to reduce levels of *Salmonella*, as well as reduce cross contamination that could occur further down the processing scheme.

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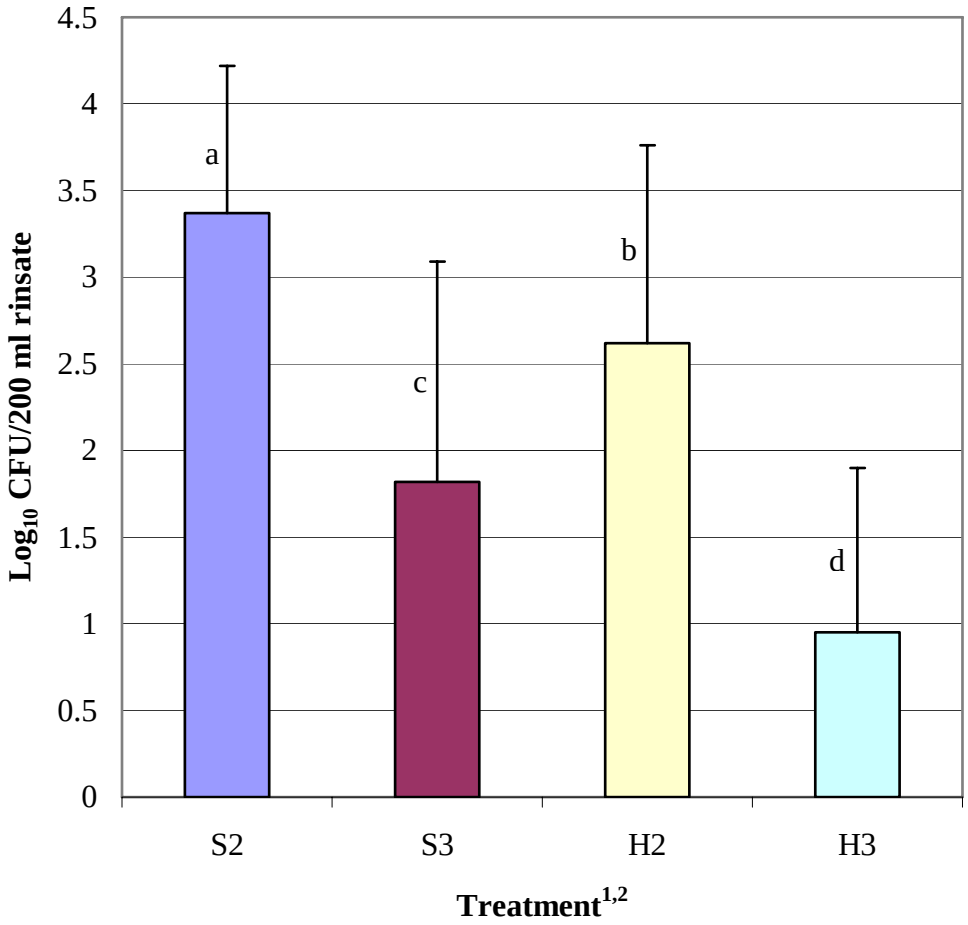
Table 1. Description of experimental treatments

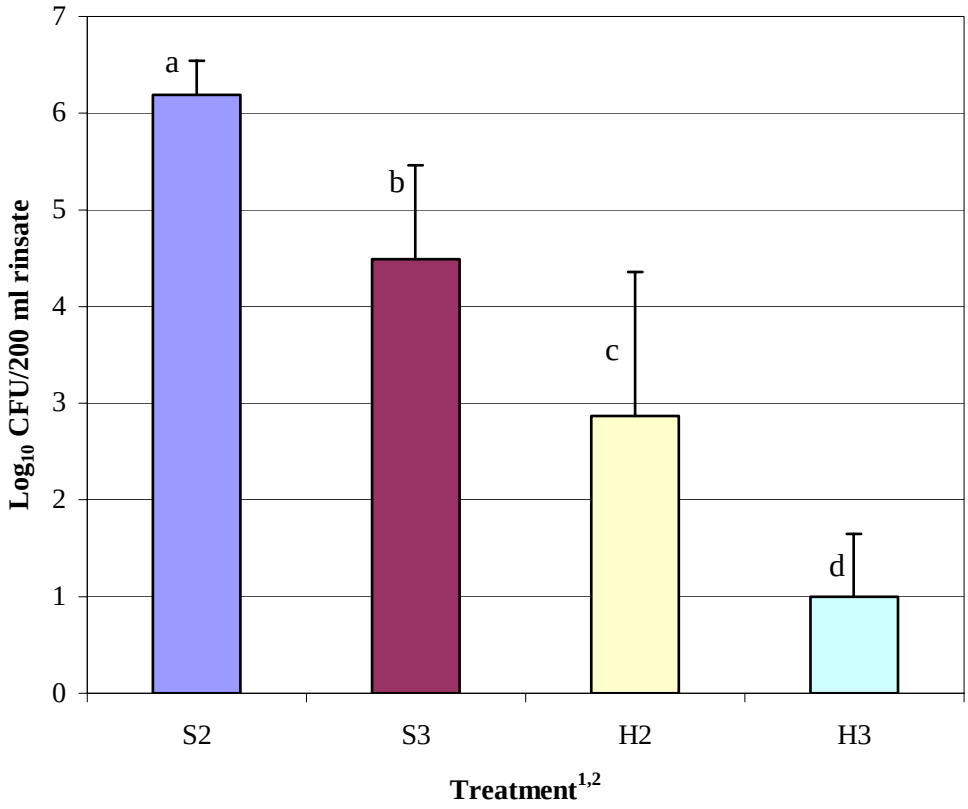
Treatment	Description
Soft Scald	50ºC for 90 s
S1	Soft scald; sterile fecal slurry
S2	Soft scald; 10^8 ST inoculated fecal slurry
S3	Soft scald; 10^8 ST inoculated fecal slurry; RP Scald in scald tank
Hard Scald	56.6ºC for 45 s
H1	Hard scald; sterile fecal slurry
H2	Hard scald; 10^8 ST inoculated fecal slurry
H3	Hard scald; 10^8 ST inoculated fecal slurry; RP Scald in scald tank

Figure 1. Mean log_{10} of *S*. Typhimurium per 200 ml rinsate for each treatment group in Trial 1



[1]Experimental treatments: S2=soft scald, inoculated fecal slurry; S3=soft scald, inoculated fecal slurry, RP Scald in scald water; H2=hard scald, inoculated fecal slurry; H3=hard scald, inoculated fecal slurry, RP Scald in scald water.
[2]Treatments S1 and H1 not included because treatments were not enumerated.
[a-d] Means with no common superscript differ significantly ($P < 0.05$).

Figure 2. Mean log_{10} of *S*. Typhimurium per 200 ml rinse for each treatment group in Trial 2



[1]Experimental treatments: S2=soft scald, inoculated fecal slurry; S3=soft scald, inoculated fecal slurry, RP Scald in scald water; H2=hard scald, inoculated fecal slurry; H3=hard scald, inoculated fecal slurry, RP Scald in scald water.
[2]Treatments S1 and H1 not included because treatments were not enumerated.
[a-d] Means with no common superscript differ significantly ($P < 0.05$).

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