Efficacy of Selected Chemicals on the Attachment and Survival of *Campylobacter jejuni* on Chicken Breast Skin

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EFFICACY OF SELECTED CHEMICALS ON THE ATTACHMENT AND SURVIVAL OF CAMPYLOBACTER JEJUNI ON CHICKEN BREAST SKIN

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(ABSTRACT)

Campylobacter is considered to be the leading cause of acute bacterial gastroenteritis in humans in the United States with *Campylobacter jejuni* being responsible for 80-90% of those infections. Many cases of *Campylobacter* gastroenteritis have been linked to the consumption of raw or undercooked chicken. The population of bacteria on the breast skin has been reported to be greater than on other edible portions of the chicken carcass making this an important site to control the organism and to study bacterial attachment properties. This research examined the efficacy of trisodium phosphate (TSP)(10%), cetylpyridinium chloride (CPC)(0.1% & 0.5%), acidified sodium chlorite (ASC)(0.1%), Tween 80 (polysorbate 80) (1%) and water (50°C) for reducing the number of viable *Campylobacter jejuni* on inoculated chicken breast skin. All chemicals were evaluated using contact times of 30 sec., 3 min. or 10 min. Statistically significant ($p \leq 0.05$) differences in the reduction of *C. jejuni* populations were observed across chemical treatments and contact time. When bacteria were applied before treatment, a reduction of >1.0 $\log_{10}$ CFU/skin was achieved with 0.5% CPC (2.89), 10% TSP (1.63), 0.1% ASC (1.52), and 0.1% CPC (1.42). When bacteria were applied after treatment, a reduction of >1.0 $\log_{10}$ CFU/skin was achieved with 0.5% CPC (4.67) and 10% TSP (1.28). The main effects of contact time were statistically significant ($p=0.02$) only when bacteria were applied after treatment.

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Campylobacter *spp.*

Bacteriology

Campylobacter enteritits is caused by two closely related species, *Campylobacter jejuni and C. coli*. Within these two species there are more than 100 serotypes based on lipopolysaccharide (O) and protein (H) antigens (Perez-Perez and Blaser, 1996). *C. jejuni* is responsible for 80-90 % of infections world wide but the difference in the two species is epidemiological in nature since the disease each species produces is nearly the same. It has been suggested that *C. upsaliensis, C. laridis,* and *C. hyointestinalis* can also be isolated from patients' diarrhea. *C. fetus* is typically associated with systemic campylobacteriosis, but other *Campylobacter* spp. have been implicated (Skirrow, 1990).

Structure

Campylobacter jejuni is a small Gram-negative rod approximately 0.2 to 0.5 microns long and 0.2 microns across. In young cultures, typically no older than forty-eight hours, the cells are comma, spiral, gull-wing, or S-shaped. If exposed to any stresses including age, atmosphere, and temperature, coccoid or round forms appear. They are motile with a single polar flagellum at one or both ends. The lipopolysaccharide layer has endotoxin activity similar to that of other Gram-negative bacteria (Perez-Perez and Blaser, 1996). Some isolates have been reported to produce an enterotoxin that appears functionally similar to cholera toxin. (Ruiz-Palacios, et al., 1993).

Pathogenesis

Pathogenesis of *Campylobacter jejuni* is dependent on pathogen-specific and host related factors including age, health, and immune response. Due to these factors, the minimum infectious dose has not been established although in a volunteer study, 500 to 800 cells was enough to cause illness (Black, et al.,1988; Robinson, 1981). Within 1-7 days of infection by *C. jejuni* (Skirrow, 1990) (average incubation is 3 days) the clinical response can be broad (Blaser and Reller, 1981). On the mild side, the infection can be a transient asymptomatic colonization or symptoms lasting about 24 hours, resembling viral gastroenteritis. At the other end of the spectrum it may cause relapsing colitis (The National Advisory Committee on Microbiological Criteria for Foods, 1994). Typically, illness includes diarrhea, fever, malaise, and abdominal cramping (at times confused for acute appendicitis) (Archer, 1988). The illness last about a week and can also include nausea and vomiting. Having at least 1 day with eight or more bowel movements is not uncommon (Black,et al., 1988). Diarrhea can be bloody due to the penetration and proliferation of *Campylobacter* within the intestinal epithelium. Chronic illness can occur within 1-2 weeks in approximately 1% of patients (Skirrow, 1990) appearing in the form of reactive arthritis (Archer, 1988; Skirrow, 1990) and myo-and pericarditis

(Archer, 1988). An estimated one in every 1,000 (Allos, 1997) patients contract Guillain-Barre syndrome (peripheral polyneuropathy) a more serious side effect (Archer, 1988; Kaldor and Speed, 1984; Winer, et al.,1988). A *Campylobacter* infection has been implicated in up to 40% of the syndrome cases (Allos, 1997). Seasonal trends in campylobacteriosis show a rise in May peaking in July and leveling off to base levels by December. The summer rise occurs about eight weeks before the summer rise of *Salmonella* infections. A small secondary peak is seen in the autumn (Skirrow, 1990).

Ecology

Unlike many bacteria, they are non-fermentative and use amino acids as an energy source. They are strictly microaerophilic and capnophilic requiring about 5% oxygen, 10% carbon dioxide, and 85% nitrogen. Optimum growth temperature is between 37 and 42^{o} C (Nachamkin, 1995; Perez-Perez and Blaser, 1996). These organisms survive better at refrigeration temperatures than they do at room temperature (Barrell, 1981; Christopher, et al., 1982). Consequently they appear in refrigerated foods (Koidis and Doyle, 1983; Koidis and Doyle, 1984), but may be sublethally injured during refrigeration at 4^{o}C or freezing at –20^{o}C or below (Ray and Johnson, 1984). Rapid inactivation occurs in milk and meat at between 47 and 60^{o}C (The National Advisory Committee on Microbiological Criteria for Foods, 1994).

Factors affecting growth or survival

In addition to temperature *C. jejuni* is also very sensitive to changes in pH, salinity, preservatives, irradiation, and disinfectants, and desiccation. At a pH of 6.5 to 7.5 growth is optimal. Growth inhibition has been observed at a pH of less than 5.1 (Gill and Harris, 1983). A NaCl concentration of 0.5% is optimal for growth, but if the concentration gets above 1%, death rate increases (Doyle and Roman, 1981). When combined with temperatures of 30, 35 (Hanninen, 1981), or 42^{o}C(Abram and Potter, 1984) a concentration of 2% NaCl is bactericidal. Ascorbic acid is also bactericidal at concentrations of 0.09% (Fletcher, et al., 1983) and 0.05% (Juven and Kanner, 1986). Sage, cloves, and oregano are inhibitory at the 0.05% concentration (Deibel and Banwart, 1984). Most common disinfecting agents at standard concentrations affect the organism negatively (Wang, et al., 1983). Viable, non-culturable and attached culturable cells are more resilient to disinfectants than culturable cells in suspension (The National Advisory Committee on Microbiological Criteria for Foods, 1994). It has been shown that if *C. jejuni* is attached to chicken skin before chlorine treatment, chlorine has no significant effect (Yogasundram, et al., 1987). In addition, chlorine can act as a corrosive on plant machinery and combine with organic materials to generate potential mutagens (Masri, 1986). During gamma irradiation not only has it been shown that *C. jejuni* is more susceptible than *Salmonella* (Lambert and Maxcy, 1984; Tarjan, 1981), but it cannot survive a dose of 1 kGy in chicken paste (Tarjan, 1981).

Attachment

The attachment mechanism initially involves retention of bacteria in a liquid film on the skin from which they migrate and become entrapped in ridges and crevices (Lillard, 1988; Thomas and McMeekin,1980). The process of retention begins with the live birds and is exacerbated during scalding. It continues during processing, and the level of contamination is directly related to the microbial concentration in the processing water (McMeekin and Thomas, 1978). The scalding operation opens feather follicles to aid feather removal, and the follicles remain open throughout processing until chilling where they close thereby retaining microorganisms. Later, because of water uptake during immersion, certain microorganisms (e.g., *Campylobacter*) adhere to polysaccharide material and material surrounding collagen fibers (Firstenberg-Eden, 1981; Thomas and McMeekin,1981). Lillard (1985), proposed that adherence is rapid, within 15 s of exposure, but attachment is a time dependant process. On the other hand McMeekin and Thomas (1978) reported that time did not influence attachment but bacterial population did. Finally, Conner and Bilgili (1994) stated that there was no significant difference for attachment concerning culture temperature (23 C or 37 C), inoculum level (100 cells/skin, 1,000 cells/skin, or 10,000 cells/skin), or contact time (10 min, 20 min, or 30 min). These conflicting results indicate that the nature of bacterial attachment to skin is complex and involves many elusive factors.

Chicken breast skins allowed for the area of greatest attachment on carcasses (Kotula and Pandya, 1995; Kotula and Davis, 1999). Several researchers have used room temperature conditions for attachment studies on *Salmonella* (Breen, et al., 1995; Kim and Slavik, 1995; Li, et al., 1997; Wang, et al., 1997; Xiong, et al. 1998). Even though many commercial spray systems use high water pressure, it was determined that they could not be used in order to achieve true estimates of firmly attached organisms. The pressure itself could forcefully remove organisms or drive them deeper into the skin or carcass surface.

Bactericidal Chemicals

It is important to identify post-mortem processes, including chemical spray rinses that would help reduce or eliminate *Campylobacter* from our poultry product supply. Some antimicrobial sprays have been shown to be effective against several common pathogens, however, limited research has been conducted to show the effectiveness of using a chemical spray during processing to inactivate or reduce the attachment of *Campylobacter* sp.. The use of an effective antimicrobial chemical in a carcass spray may allow processors to reduce the volume and pressure of water used to wash carcasses.

Trisodium phosphate (TSP)

Chemical description and approval

Trisodium phosphate, trisodium phosphate dodecahydrate, or sodium orthophosphate dodecahydrate with a CAS regulation number 10101-89-0 has the molecular formula of $(Na_3PO_4{*}12H_2O)_4NaOH$. If the chemical is consumed in pure form

orally LD_{50} is 7400 mg/kg (rat) (Rhodia Inc., 1996). USDA/FSIS has approved its use with poultry in concentrations of 8 to 12 % when maintained at a temperature between 45 and 55°F. It is typically used in concentrations of 10% due to its proven effectiveness and reduced phosphate waste at this level. Application method must be by spraying or dipping for up to fifteen seconds in accordance with 21 CFR 182.1778.

Mechanism of action

Once mixed, a solution of TSP achieves a pH of 12 to 13. This high alkalinity can help remove fat films to allow the chemical to contact more bacteria as well as disrupt fatty molecules in the cell membrane causing the cells to leak intracellular fluid (Giese, 1993). This chemical also acts as a surfactant to help facilitate the removal of bacteria. Due to the necessary high pH of the chemical potential problems arise. First, it can be corrosive to plant equipment after extended exposure. It has been observed that the chill tank water pH increases with TSP use allowing a higher aerobic SPC. This can be counteracted by adding phosphoric acid to the chill tank water but at an extra cost to the plant. In addition, TSP use increases phosphate waste that may or may not affect the water treatment facility. TSP also carries a level 3 health hazard rating being capable of causing severe or permanent damage to the eyes, skin, and lungs.

Previous work

Trisodium phosphate (TSP) was studied by Slavik, et al. (1994) whereby post-chill chicken carcasses were dipped into a 10% solution of TSP at 50°C for 15 sec. Treated carcasses were stored at 4°C for 0, 1, or 6 days before they were analyzed for *Campylobacter*. At day zero there was no reduction in *Campylobacter* numbers as compared to the controls. After one day of storage, the incidence for *Campylobacter* in the control group was between 96-100%, but only 24-28% for treated carcasses when a nitrocellulose membrane lift method was used. When a standard culture method was used only a 4-36% reduction was found. In a third trial, *Campylobacter* was quantified using the MPN method, which resulted in a reduction of 1.5 and 1.2 $\log_{10}$ CFU in 1- and 6-day stored TSP- treated carcasses. Salvat, et al. (1997) also reported a reduction in the incidence and concentration of *Campylobacter* sp. from chicken neck skin samples after treatment with a 10% TSP solution. Hwang and Beuchat (1995) reported a reduction of 1.7 $\log_{10}$ CFU/ml for *Salmonella* spp., 1.9 $\log_{10}$ CFU/ml for *Listeria monocytogenes*, and a 1.8 $\log_{10}$ CFU/ml for psychotrophs with a 1% TSP solution. An 8% solution of TSP was sprayed on chicken breast skin with a coarse sprayer which produced a 1.48 $\log_{10}$ CFU/skin reduction in loosely attached cells and a 1.60 $\log_{10}$ CFU/skin reduction in firmly attached cells for *Salmonella typhimurium* (Tamblyn, et al. 1997). Wang, et al. (1997) used a 10% solution of TSP with varying spray pressures to reduce the amount of *S. typhimurium* on breast skin from 1.5 to 2.3 $\log_{10}$ CFU. It was found that as pressure increased from 620.5 to 1034.2 kPa the amount of reduction did also with the best reduction at 827.4 kPa. The difference in the reductions for the spray pressures was not always statistically different. Xiong, et al. (1998) also used chicken breast skins, a spray system, and a 10% TSP solution but with conflicting results on spray pressure. The $\log_{10}$ reductions of *S. typhimurium* were 1.2 to 1.9 but with no statistical difference between

the spray pressures of 207 and 1034 kPa. It was also noted that reductions increased for exposure times up until 90 sec. but were not significant afterward. Whole carcasses were used to test another 10% solution of TSP for reduction of *S. typhimurium* and total aerobes by Yang, et al. (1998) with net losses of 2.0 log $_{10}$ CFU and 0.74 log $_{10}$ CFU, respectively. Li et al. (1997) also compared spray pressure on whole carcasses inoculated with *S. typhimurium* with a chemical concentration of 10%. It was found that there was not statistical difference between 207 and 345 pKa but there was with a 827 pKa pressure. At a 30 sec. spray time the chemical reduced the bacteria by 1.82 log $_{10}$ CFU/bird and at a 90 sec. spray time that number increased to 3.84 log $_{10}$ CFU/bird.

Cetylpyridinium chloride (CPC)

Chemical description and approval

Cetylpyridinium chloride, or 1-hexa-decyl pyridinium chloride, is a quaternary ammonium compound with antimicrobial properties against many microorganisms including viruses (FDA, 1998). "It is classified as a cationic surface-active agent and contains a cetyl radical substituted for hydrogen atom on position 1. In hydrochloric acid it forms a chloride salt. The cetyl radical renders the molecule lipophilic, contributing to the lipophilic/hydrophilic balance which is necessary for the antimicrobial activity of such quaternary nitrogenous compounds." (FDA, 1998). Currently it is used in some commercial mouthwashes to prevent the formation of dental plaque (Ashley, et al, 1984; Barnes, et al. 1976; Ciancio, et al. 1978; Frost and Harris, 1994). A maximum concentration of 0.1% is permitted for use in several dental products by the U.S. Food and Drug Administration. It has also been approved for limited human consumption in forms such as Cepacol.RTM. Lozenges (Lattin, et al.,1994). Currently, it is not approved for use in poultry processing. The use of CPC in spraying or dipping poultry products commercially is disclosed in a patent application (Lattin, et al. 1994). The LD_{50} for rats is 20 mg/kg given orally as a pure compound (FDA, 1998).

Mechanism of action

Antimicrobial activity is dependent on the charged cetyl radical being positioned with a bacterial cell possessing a net negative charge. This allows the hydrophilic portion of the molecule to react with the cell membrane resulting in the leakage of the cellular components, disruption of cell metabolism, prevention of growth and replication, and cell death (FDA, 1998). At a pH of approximately 7.2 CPC is not corrosive to metal, does not add to phosphate waste, and is not a severe health hazard.

Previous work

Even though CPC has been successful in reducing bacterial counts of *Salmonella typhimurium*, there has been no published research to recognize the effects this chemical may have on *Campylobacter*. Wang, et al. (1997) showed that with a temperature of 10, 35, or 60^{o}C, spray pressure from 0 to 1034.2 kPa, and spray duration 30 sec., *Salmonella* reductions with 0.1% CPC were 1.5 to 2.5 log $_{10}$ CFU. Several other papers used a concentration of 0.1% to reduce *Salmonella typhimurium*. With spray times of 30 and 90

sec. a reduction of 0.59 to 0.85 log $_{10}$ CFU and 1.2 to 1.63 log $_{10}$ CFU respectively was observed (Li, et al., 1997). In another study with variable spray temperatures, pressures, times, and setting times the bacterial reduction was 1.45 to 1.85 log $_{10}$ CFU. Reductions increased as exposure time surpassed 90 sec. (Xiong, et al. 1998). When 10 cm^2 of chicken breast skin was drip inoculated then treated with 0.1% CPC for 1 min. *Salmonella* was reduced by 87 to 98% (Kim and Slavik, 1995). A 0.5% CPC solution maintained a pH of 7.6 and reduced *Salmonella* by 2.16 log $_{10}$ CFU (Yang, et al. 1998). Reaction times of 1, 3, and 10 min. at CPC levels of 1, 2, 4, and 8 mg/ml resulted in *Salmonella* reductions of 0.59 to 4.91 log $_{10}$ CFU (Breen, et al. 1997). At the higher levels and times there was complete elimination of the bacterium. Due to its ability to prevent attachment (Breen, et al. 1995), no visible effects to the physical appearance of the poultry product (Breen, et al. 1997), and success with *Salmonella*, CPC was tested for use against *Campylobacter* in the current study.

Acidified Sodium Chlorite (ASC)

Chemical description and approval

This additive approved under 21 CFR 173.325 is produced by mixing an aqueous solution of sodium chlorite (CAS regulation number 7758-19-2) with a generally recognized as safe (GRAS) acid (USDA, 1982). Citric acid has been used because it may be more effective than phosphoric acid in eliminating Campylobacter (Kemp, et al.,2000). When used in a pre-chiller or chiller tank application, sodium chlorite levels must be maintained between 50 and 150 ppm with a final pH concentration, including the GRAS acid, of between 2.8 and 3.2 (USDA, 1982). As a spray or dip, sodium chlorite concentrations should be between 500 and 1200 ppm with GRAS acid levels high enough to produce a pH solution of 2.3 to 2.9 (Anonymous, 1982). "ASC has been successfully used as a sterilant for non-porous surfaces and devices in the medical and pharmaceutical industries, as a disinfectant in automobile air conditioning systems, and as a skin antiseptic in the dairy industry." (Kemp, et al., 2000).

Mechanism of action

The ASC chemical combination is the result of $NaClO_2$ acidification which when applied to organic matter a number of organic intermediates are formed (Gordon, et al., 1972). These intermediates cause a disruption of the oxidative bonds on the cell membrane surface making them broad-spectrum germicides (Kross, 1984). Due to this non-specific chemical action, it is thought that the common formation of resistant strains following long exposure is reduced (Kemp, et al., 2000). Using a Food and Drug Administration defined test procedure (Anonymous, 1999) a recent study of ASC showed that none of 10 test microbe strains formed any resistance to the chemical after 100 divisions of subinhibitory doses (Kemp, 2000). At such a low pH, ASC can be corrosive to plant equipment as well present a health hazard individuals working with it.

Previous work

The affects of acidified sodium chlorite on *Escherichia coli* attached to broiler skin have been examined by Conner and Bilgili, (1996). For loosely attached cells, ASC

made with phosphoric acid possessing a high buffering capacity, reductions ($\log_{10}$ skin/skin) of 0.16, 0.04, and 0.46 for 500, 850, and 1200 ppm respectively were observed. Considering the same situation but lowering the buffering capacity the reductions are 0.06, 0.03, and 0.24 approximately. The same experiment produced different results for citric acid. If the acid possessed a high buffering capacity reductions were 0.06, 0.18, and 0.55, but if the buffering capacity was low reductions were 0.14, 0.17, and 0.39. None of these reductions were considered significant. In the case of firmly attached *E. coli*, larger reductions were observed. Considering only citric acid due to its suspected performance against *Campylobacter*, it was observed that for a lower buffering capacity in 500, 850, and 1200 ppm the reductions were 0.25, 1.4, and 0.5, respectively. When the acid solution had a high buffering capacity the reductions were 0.15, 0.5, and 1.94. These numbers were averages of 12 treatments. It is also important to note that the final log reductions for these treatments would have been reported as higher due to spray and water effect, but those factors have been subtracted to give pure bactericidal figures (Conner and Bilgili, 1996).

Alcide also released some of their own data on whole carcasses compiled from 5 different commercial plant studies in which each plant participated for 12 weeks. Using the Sanova continuous on-line protocol there was a 2.3 $\log_{10}$ ML/ml reduction in total *E. coli* counts as well as a 27% reduction in *Salmonella* spp. incidence. For *Campylobacter* spp. there was a 2.6 $\log_{10}$ CFU/ml reduction when the protocol was used. The protocol was typical of a slaughtering facility with the exception of a Sanova anti-microbial system added before the chill step (Alcide Corporation, 1998).

Kemp, et. al. (2000) reported using a 1200 ppm spray of ASC on whole broiler carcasses to produce a reduction of 0.52, 0.77, and 0.52 $\log_{10}$ ML/ml in aerobes, *E.coli*, and total coliform counts. For the same concentration of ASC a 5 sec. dip was used to compare phosphoric acid and citric acid as the activator. Phosphoric acid produced 0.21, 1.20, and 1.03 $\log_{10}$ ML/ml reductions compared to citric acid with 0.20, 1.18, and 1.00 $\log_{10}$ ML/ml reductions for aerobes, *E.coli*, and total coliform counts. Experiments were also performed with 500 and 850 ppm concentrations but as in other studies, none were as effective as the 1200 ppm solution (Kemp, 2000). As aforementioned it is felt that citric acid is more effective than phosphoric acid on *Campylobacter* when used to activate ASC.

Surfactant

Tween 80

Tween 80 (polysorbate 80) is a surfactant that has been approved for use in poultry processing up to a concentration of 1%. It has been shown that when used in conjunction with other chemicals it aids in the prevention of attachment by bacteria on chicken skin (Hwang and Beuchat, 1995). When applied as a spray or dip, this chemical may be useful for preventing the attachment of microorganisms that may contaminate a poultry carcass during washing, cutting and handling operations.

CHAPTER II
INTRODUCTION

Raw chicken and turkey are significant sources of the bacterial pathogens *Salmonella* sp. and *Campylobacter* sp.(Deming, et al. 1987). There are many points in the growing, processing, and preparation of poultry for consumption where carcasses and products can become newly contaminated or cross-contaminated with organisms not found on the meat at an earlier time. Some studies have suggested that due to current poultry plant procedures cross-contamination is virtually impossible to eliminate (Baker, et al. 1987; Izat, et al. 1988).

Campylobacter infections have been linked to poultry in many outbreaks (Blaser, et al. 1983; Finch and Blake, 1985; Tauxe, et al. 1988), primarily due to the consumption of raw or undercooked chicken. Because of the optimal growth temperature of *C. jejuni,* the association might be in part due to the naturally high body temperature (42^{o}C) of these birds (Luechtefeld, et al. 1981). The prevalence of *Campylobacter* on raw poultry products varies from 0 to 100% with an average of 62% (Bryan and Doyle, 1995). Fresh eviscerated carcasses can have populations as high as one million organisms per carcass (Stern, et al. 1985; Tokumaru, et al. 1990). A USDA/FSIS nationwide broiler chicken microbiolgical baseline data collection program sampled 1,297 carcasses and found that 1,144 (88.2%) of the carcasses were contaminated with *C. jejuni*. An average of 21 cells per ml of rinse or $8.4x10^{3}$ cells per carcass were recovered (USDA, 1996). It has also been noted that there is a greater population of bacteria on the breast skin than other edible portions of the chicken carcass (Kotula and Davis, 1999; Kotula and Pandya, 1995) making this an important site to control the organism and to study bacterial attachment properties.

Campylobacter jejuni contamination in chicken processing plants comes almost exclusively from the viscera. The intestinal tract, particularly the lower parts (ileum, caecum, and colon), are frequently contaminated with *C. jejuni*. The lungs are also regularly contaminated with a small number of birds possessing contamination in the liver (Oosterom, et al. 1983).

In typical processing there is a significant decrease in the contamination of *C. jejuni* on skin during scalding followed by a significant increase during defeathering (Izat, et al.1988). If the scald tank temperature is at 58^{o}C or above the reduction is significant. If the scald water temperature is done at 51.8^{o}C the decrease is not as dramatic. The marked increase in defeathering is caused by the mechanical action of the rubber fingers designed to remove the feathers. The pressure applied on the carcasses is sufficient enough so that fecal material is pressed out (Oosterom, et. al, 1983). After this point the levels of *C. jejuni* remain relatively constant, but the amount of contamination may go up or down dependant on the actual process and the amount of handling.

Equipment surfaces also are a likely source for contamination and recontamination of the product (Izat, et al. 1988).

Improving detection methodology and additional surveys for the presence and level of *Campylobacter* contamination on processed poultry will lead to a better understanding and control of this pathogen. Many poultry processors have increased their usage of chlorine and rinse water volumes to reduce *Salmonella* and *Escherichia coli* on carcasses. These strategies may have little effect for reducing *Campylobacter* populations (Morrison and Fleet, 1985). Limited research has been done to demonstrate interventions that are effective for reducing both the prevalence and level of contamination by this pathogen.

The objective of this research was to determine the ability of several chemical spray solutions to inactivate *Campylobacter jejuni* applied to chicken breast skin. The commercial use of an effective chemical may help to reduce the level of *Campylobacter* on carcasses and reduce water usage if it can replace existing spray applications. Furthermore, the ability of these same chemicals to prevent the attachment of *Campylobacter* to chicken breast skin was studied. For this research, the chemical sprays were applied prior to inoculation with the pathogen. Some of these chemicals may also be effective for limiting cross-contamination in the plant by hindering the attachment of the organism to skin.

MATERIALS AND METHODS

Chicken breast skins

Chicken breast skin samples were collected from a poultry plant within 20 minutes of being removed by a breast-deskinning machine. The pieces were selected only if there was no visible damage on the skin surface. The skins were transported in a cooler and refrigerated that same day. Skins were trimmed into round pieces approximately 28.3 cm^2 by tracing with a scalpel the diameter of a 150 ml biohazard specimen cup (Fisher Scientific Cat. # 14-375-221). The cups used were identical to those used later for spray applications. Following excision, the samples were vacuum packaged in sterile oxygen impermeable 3 X 5" Whirl-Pak retain bags, then frozen.

Gamma irradiation was used to eliminate any background organisms on the skin. The packaged skins were shipped frozen, by overnight courier, to Auburn University and sterilized with 12 kGy of ionizing radiation using a Cobalt-60 source. This dose was identical or more than what has been shown to be effective on similar pieces of chicken breast skin (Tamblyn et al. 1997; Conner and Biligli, 1994). As little as 5 kGy has been

used to successfully destroy residual bacteria under these conditions (Kim and Doores, 1993). Once returned the skins were immediately frozen to –20^{o}C and maintained at that temperature until testing (Conner and Biligli, 1994; Kim and Doores, 1993). The sterility of the skin pieces was confirmed after the sterilization process on some randomly selected skins. The confirmation procedure used the same conditions as the spray application procedure followed by enumeration of a rinse solution, as though they had been inoculated.

Inoculum and Media

Skins were thawed and drip inoculated with a 1cc syringe with approximately 0.1 ml of an equal mixture of four strains of Campylobacter jejuni. The final inoculum mixture was approximately 10^{8} cells/ml. The actual concentraton was determined at the beginning of each analysis session by serially diluting and plating the inoculum. The strains used were ATCC 3444, ATCC 29428, ATCC 33291, and a typed strain 61-784 (provided by Dr. Noel Krieg, Department of Biology, Virginia Polytechnic Institute and State University). Each strain was maintained on separate Brucella agar (Difco, Detriot,MI) FBP (ferrous sulfate, sodium metabisulfate, and pyruvic acid; Sigma Chemical) slants (Ransom and Rose, 1998). Inoculated slants were incubated under microaerophilic conditions at 42^{o}C for 48 hours. Following incubation, each slant was filled with 6 ml of sterile buffered peptone (Difco, Detriot, MI) and fresh cells were scraped off of the medium into the liquid. This was accomplished by using a gentle up and down motion with the 10 ul end of a plastic disposable inoculating loop (Fisher Scientific Cat. # 13-075-3). The resulting suspensions were withdrawn via 5 ml pipette and combined in a 16 X 100 mm sterile screw capped tube.

Chemical treatment preparation

Chemical treatments including either 10% trisodium phosphate (TSP) (Rhone-Poulenc, Cranbury, NJ), 1% Tween 80 (polysorbate 80)(Sigma), 0.1 or 0.5 % cetylpyridinium chloride (CPC)(Sigma), 1000 ppm (0.1%) solution of acidified sodium chlorite (ASC)(Alcide Corporation), and water at 50^{o}C were applied to separately inoculated skin samples. A control was performed in which approximately 21^{o}C water was used in order to establish a baseline on survival and attachment under these conditions. All chemical treatments were prepared by first pre-dissolving the chemical with sterile distilled water in a 50 ml beaker with the exception of TSP which required a 150 ml beaker. After the chemical was completely dissolved the contents were placed into a 250 ml volumetric flask. The interior of the beaker was then rinsed three times with additional sterile distilled water and that rinse was also added to the flask. The flask was then filled to the proper 250 ml mark with the appropriate volume of distilled sterile water. Finally the chemicals were then filter sterilized with 0.2 micron filters (Difco, Detriot, MI) and sealed into separate 500 ml pyrex screw capped sterile containers. ASC was prepared and used within five minutes of mixing. All other chemicals were made fresh when experiments were performed.

Use of the skin attachment model (SAM)

The protocol for irradiation, inoculation, spray application, and enumeration used was the skin attachment model (SAM) as adopted by Conner and Bilgili (1994), Conner and Biligi (1996), Tamblyn and Conner (1997), and Tamblyn, et al. (1997). It was designed for consistency in testing of antimicrobial agents against firmly attached and loosely attached bacteria without the interference of background bacteria while using relatively non-selective media. A dip method was discounted for inoculation due to water uptake, contamination of the cell suspension, and uneven distribution of cells in the suspending medium (Conner and Biligi, 1994). The common carcass rinse for bacterial enumeration has been questioned due to evidence that indicates bacteria can still be removed after 41 washes (Benedict et al. 1990). Only under this method can the effects of the chemical on both loosely and firmly attached bacteria be examined. This method allows for the largest amount of data to be extrapolated from an antimicrobial spray analysis.

Spray application method for skin samples

The application instrument was a hand held 1.5-liter KGRO all purpose plant sprayer (Kmart Corp., Troy, MI; code # 0-436792-133). The interior of the sprayer was sanitized with 200ppm chlorine solution then rinsed 3 times with sterile water. The test chemical was aseptically placed and sealed into the plant sprayer. The irradiated sample bags were ethanol (75%) sanitized on the exterior and then rinsed with sterile water to remove any residual sanitizer. The bags were then cut open with a pair of flame-sterilized scissors. Skin samples were removed with flame-sterilized tweezers and placed to the inside lid of a 150-ml sterile biohazard specimen cup (Fisher Scientific Cat. # 14-375-221). The entire exterior surface of the skin was visible for each sample. The skin remained there, held by surface tension, even during inversion. The lid was tightly capped onto the cup to prevent any accidental contamination. A hole was carved into the bottom of the cup with a red-hot sterile dissecting knife (Variation of Lattin, et al. 1994). This penetration into the sterile environment was immediately covered with flame sterilized aluminum foil until the spray application procedure. The sprayer nozzle was sanitized with ethanol (75%) then rinsed with sterile water and inserted into the bottom of the cup when the spray application was required. The chemical application was misted on at a rate of 2ml per second for three seconds. The spray pressure (8 psi) was held constant throughout the study. Before any application the amount of pumps required to build the appropriate pressure was eight. The nozzle was adjusted to allow for the proper amount of pressure and liquid to be released. In addition, before any of the test chemical applications the top of the sprayer was loosened to release any pressure that was built up from any previous application.

C. jejuni inoculation before chemical treatment application

Skins were thawed and drip inoculated with a 1cc syringe with approximately 0.1 ml of an equal mixture of four strains of Campylobacter jejuni. Skin samples were

placed and remained undisturbed at approximately 21^{o}C for 10 minutes to allow for bacterial attachment. Lillard (1985), proposed that adherence is rapid, within 15 s of exposure. Conner and Bilgili (1994) stated that there was no significant difference for attachment concerning contact time (10 min, 20 min, or 30 min). The eluate from the skin spray application was collected by tilting the sample cup away from the skin and removing the material with a pipette. A one ml aliquot of the pipetted liquid was neutralized in 9ml of 0.1% peptone buffer (Difco, Detroit, MI) to prevent any further action of the chemical. The samples remained undisturbed at room temperature for 30 seconds, 3 min, or 10 min. prior to analysis. A 30 sec. contact time for chemicals was used by Li, et al. (1997) and Wang, et al. (1997). Reaction times of 1, 3, and 10 min. were used by Breen, et al. (1997) in addition to a 1 min. contact time used by Kim and Slavik, (1995).

Next, 20 ml of neutralizing peptone buffer was added to the samples in the cup where they were rinsed for 30 arch rotations similar to the FSIS sampling protocol for carcass rinses. During rinsing, fluid was kept from escaping by sealing up the spray nozzle hole that was previously bored in the bottom of the cup by firmly placing ones thumb over the opening. A pair of sterile gloves must be aseptically placed on the hand to prevent any possible contamination. The rinse and the collected eluate from the spray application were serially diluted and plated onto Brucella-FBP agar. The numbers of cells counted on these two separate sets of plates are representative of loosely attached cells. The cup was then removed from its lid and the skin sample was transferred from its location into a small sterile stomacher bag with a pair of flame-sterilized tweezers. Next, the sample was blended in a laboratory stomacher for 2 min. with 20 ml of fresh neutralizing peptone buffer. This solution was also serially diluted and plated onto Brucella-FBP agar. These remaining cells were the firmly attached cells. The difference between the number of cells inoculated and the loosely attached plus firmly attached counts indicate cell death and non-viable cells. At any given time when fluid was being placed inside or being removed from the sterile environment of the specimen cup it was being done through the sprayer nozzle opening in the bottom of the cup. This opening was always covered with sterile aluminum foil unless a particular task was being performed to prevent contamination.

C. jejuni inoculation after chemical treatment application

The same chemical sprays were also applied before the skin was inoculated to test their ability to prevent attachment. Sprays were allowed to contact the skin for 10 minutes prior to inoculation with *C. jejuni*. Skins then remained undisturbed for 30 seconds, 3 minutes, or 10 minutes after inoculation and prior to analysis. All of the chemical concentrations, temperatures, and analytical methods were the same as the pretreatment inoculation study. In all cases two replicates were performed.

Incubation and enumeration methods

All plates were placed into anaerobic containers and flushed with a microaerobic gas mixture of approximately 5% oxygen, 10% carbon dioxide, and 85% nitrogen. Initial

inoculum slants were heat sealed in sterile oxygen impermeable retain bags with the same gas mixture. Incubation time for the Brucella-FBP plates and slants was for 48 hours at 42^{o}C. All possible operations were performed in a hood to prevent contamination. Colonies counted were typical of *C. jejuni* on this particular medium being small (>1mm), round, convex, red, and possessing a greenish metallic sheen. Confirmation tests included negative gram-stains, wet-mounts, positive catalase tests, agglutination tests, and API Campy strips (Bio-Mereiux). These tests were performed at random intervals during the experiments and immediately if any outside contamination or abnormal colony growth was suspected.

Statistical analysis

When the bacterial inoculum was applied before the chemical spray, the effects of the spray (7 test chemicals, contact time 0.5, 3.0, or 10 min.), and their interactions on log reduction were analyzed by 2-way ANOVA of a completely randomized factorial design. When the chemical spray was applied before the bacterial inoculum, the effects of the spray (6 levels, contact time 0.5, 3.0, or 10 min.), and their interactions on log reduction were again analyzed by 2-way ANOVA of a completely randomized factorial design (Sall and Lehman, 2001).

RESULTS AND DISCUSSION

Statistical significance

Bacterial inoculum application before chemical treatment

For the purposes of statistical analysis the experiment was examined on an individual basis determined by order of application. When *Campylobacter jejuni* was applied to the skin before the chemical treatment the interactions were not statistically significant (p=0.12) nor were the main effects of contact time (p=0.9). There was no statistical difference in $\log_{10}$ reduction between 0.5, 3, and 10 minutes of contact time. The main effects of the chemical sprays were highly significant (p<0.0001) as described in Table 2. This table uses cumulative results for all three contact times and replications. The chemicals are listed in order of chemical effectiveness as it relates to $\log_{10}$ reduction. 0.5% CPC was the most effective and statistically different from all other chemical treatments with a $\log_{10}$ reduction of 2.89. 10% TSP, 0.1% ASC, 0.1% CPC, 1% Tween, and Hot water (50^{o}C) followed in effectiveness with $\log_{10}$ reductions of 1.64, 1.52, 1.42, 0.56, and -0.28 respectively. In figure 1 the data is displayed in a graph form with differentiation between the three contact times for each chemical. Individual numerical results are listed for each contact time at the bottom of the chart.

Bacterial inoculum application after chemical treatment

The second phase of the study involved the inoculation of *Campylobacter jejuni* on the chicken skin after the chemical treatment was applied by spray. The interactions between contact times and chemical treatments were not statistically significant (p=0.4). The main effects of contact time were statistically significant (p=0.01)(Table 5). When all data was pooled to consider contact time alone it was shown that as time increased so did $\log_{10}$ reductions. 0.5 minutes was significantly different from 10 minutes but not the 3 minute contact time. The 3 minute contact time was not statistically different from either of the two other contact times. The main effects of the chemical treatments were highly significant (p<0.0001) as described in Table 4. The chemicals are listed in order of chemical effectiveness as it relates to $\log_{10}$ reduction. Once again 0.5% CPC is considerably more effective and statistically different from all other chemical treatments with an average $\log_{10}$ reduction of 4.67. 10% TSP, 0.1% ASC, 0.1% CPC, Water (50^{o}C), and 1% Tween followed in chemical effectiveness with mean $\log_{10}$ reductions of 1.28, 0.94, 0.77, -0.11, and -0.21 respectively.

Data from both phases combined

When considering the data as a whole by not differentiating between the order of application and contact time there emerges three different categories of chemical effectiveness. Chemical effectiveness under these circumstances is being defined as the amount of bacterial inactivation caused by the chemical. The only chemical that could be comparatively defined as very effective was 0.5% CPC due to it being over 2.5 times more effective than the nearest chemical with an average $\log_{10}$ reduction of 3.78. In the presence of 10% TSP, 0.1 % ASC, and 0.1%CPC were mediocre with average $\log_{10}$ reductions of 1.46, 1.23, and 1.10 respectively. Water (50^{o}C) and 1% Tween 80 both had little to no effect as compared to the control. Chemical applications were more effective if applied after the bacterial contamination with the exception of 0.5 % CPC which appeared to have an increased bactericidal effect when applied before the bacteria. 0.1 % ASC and 10% TSP were the only two chemicals which seemed to be affected by contact time. As time increased from 0.5 min. to 10 min. the amount of reduction increased approximately one $\log_{10}$ CFU/skin.

Comparison to literature

Trisodium phosphate (TSP)

Trisodium phosphate (TSP) was studied by Slavik, et al. (1994) whereby post-chill chicken carcasses were dipped into a 10% solution of TSP at 50^{o}C for 15 sec. Treated carcasses were stored at 4^{o}C for 0, 1, or 6 days before they were analyzed for *Campylobacter*. At day zero there was no reduction in *Campylobacter* numbers as compared to the controls. *Campylobacter* was quantified using the MPN method, which resulted in a reduction of 1.5 and 1.2 $\log_{10}$ CFU in 1- and 6-day stored TSP- treated carcasses. These results were similar to the results achieved in this study. Bacterial inoculation before chemical treatment spray produced $\log_{10}$ reductions of 1.64 while

bacterial inoculation after chemical treatment spray resulted in a 1.28 $\log_{10}$ reduction. Combining the data the average $\log_{10}$ reduction equaled 1.46. During the 10 min. contact time it was observed that the $\log_{10}$ reduction when the bacterial inoculum was applied before the chemical treatment was 1.64 while the $\log_{10}$ reduction when the bacterial inoculum was applied after the chemical treatment was 2.20. This would suggest that at this contact time the chemical was exhibiting attachment prevention properties.

Cetylpyridinium Chloride (CPC)

There has been no published research to recognize the effects this chemical may have on *Campylobacter*. In all of the following results comparisons must be considered relative. The reductions listed are those of another genus and species as well as other sampling protocols, procedures, and variables. Each bacterial strain has its' own attachment properties and unique interactions to the same treatment depending upon cellular structure, conditions of application, survival mechanisms, inoculum fitness, etc. Salmonella was chosen because of is similar attachment properties, pathogenicity, and association with poultry.

0.1% CPC

Wang, et al. (1997) showed that with a temperature of 10, 35, or 60^{o}C, spray pressure from 0 to 1034.2 kPa, and spray duration 30 sec., Salmonella reductions with 0.1% CPC were 1.5 to 2.5 $\log_{10}$ CFU. When compared to the current studies log reductions of 0.77 to 1.42 spray pressure and duration must be considered. Wang, et al. (1997) used spray pressure as a variable and 30 sec. spray duration as compared to 3 sec. spray duration and a negligible spray pressure. With a 0.1 % concentration and spray times of 30 and 90 sec. a reduction of 0.59 to 0.85 $\log_{10}$ CFU and 1.2 to 1.63 $\log_{10}$ CFU respectively was observed (Li, et al., 1997). These results must also be compared relative to the spray times.

0.5 % CPC

A 0.5% CPC solution maintained a pH of 7.6 and reduced Salmonella by 3.62 $\log_{10}$ CFU with a 17 sec. spray time and a 60 sec. reaction time (Yang, et al. 1998). Once again the spray duration was almost 6 times longer than in the current study where the $\log_{10}$ reduction for was from 2.77 to 3.13 when bacterial inoculation occurred before chemical treatment and 4.50 to 5.18 when the bacterial inoculation occurred after the chemical treatment.

Reaction (contact) times of 1, 3, and 10 min. at CPC levels of 1, 2, 4, and 8 mg/ml resulted in Salmonella reductions of 0.59 to 4.91 $\log_{10}$ CFU when the bacteria was applied before the treatment (Breen, et al. 1997). At the higher levels and times there was complete elimination of the bacterium. More specifically when the bacteria was applied before the treatment the average $\log_{10}$ reductions for the three time periods were 1.05 for 1 mg/ml or 0.1% and 3.63 for 4 mg/ml or 0.4%. The current study produced $\log_{10}$ reductions of 1.42 for 0.1% and 2.89 for 0.5%. For the Salmonella study the $\log_{10}$ reductions were also higher because of the differences in treatment application technique.

The inoculated skins were set in a well with 5 ml of chemical treatment and incubated with shaking at 100 rpm. This movement of liquid may have removed some additional bacteria. Breen, et al. (1997) also applied the bacteria after the treatment where the average $\log_{10}$ reductions for the three time periods were 0.91 for 0.1% and 2.74 or 0.4%. The current study resulted in $\log_{10}$ reductions of 0.77 for 0.1% and 4.67 for 0.5%.

The potential for 0.1% CPC to prevent attachment and generate $\log_{10}$ reductions appears to be the same for *Campylobacter* and *Salmonella*. However, when the concentration increases to 0.5% CPC more significant $\log_{10}$ reductions and higher attachment prevention potentials occur for *Campylobacter*.

Acidified Sodium Chlorite (ASC)

When citric acid was used as an activator it was observed that for a lower buffering capacity in 500, 850, and 1200 ppm the $\log_{10}$ reductions were 0.25, 1.40 , and 0.50, respectively for *E. coli*. When the acid solution had a high buffering capacity the $\log_{10}$ reductions were 0.15, 0.50, and 1.94. It is also important to note that the final $\log_{10}$ reductions for these treatments would have been reported as higher due to spray and water effect, but those factors have been subtracted to give pure bactericidal figures (Conner and Bilgili, 1996). In the current study when bacteria was applied before the chemical treatment the average $\log_{10}$ reduction was 1.42. When considering the 1000 ppm or 0.1% solution was used these reductions are equivocal.

Alcide also released some of their own data for *Campylobacter* spp. They reported a 2.6 $\log_{10}$ CFU/ml reduction when their protocol was used. The protocol was typical of a slaughtering facility with the exception of a Sanova anti-microbial system added before the chill step (Alcide Corporation, 1998). The report did not mention contact time, spray pressure, or spray duration. This study achieved a 1.79 $\log_{10}$ reduction with a 10 min. contact time.

Attachment

The difficulty of this experiment was not in quantifying the antimicrobial abilities of each chemical but the mechanism by which the reduction was accomplished. By performing two phases during this research we attempted to decipher which chemical was bactericidal, preventing attachment, or both. It is important to recognize these traits and separate them in order to have a complete understanding of the chemical once it is in use. With this knowledge a plant can correctly make adjustments in their plant procedures in order to use the chemical in the most effective application possible. These aspects must also be considered when the decision is made to adjust the concentration or recycle the chemical to become more cost effective.

Although the attempt was made it was still very difficult to say with certainty which one of the chemicals had which trait. It is impossible to compare chemical to chemical due to the bactericidal nature of some of the chemicals. One cannot say if the

chemical prevented attachment or just destroyed the cells. One can make some generalizations based on the separate phases if reductions are compared within each chemical. It appears that there is some difference between each order of application. The two most successful antimicrobial treatments (10 % TSP and 0.5 % CPC) showed marked increase in performance when applied before inoculation of bacteria suggesting that this may be a more effective method of reducing bacteria load (as mentioned earlier in this section) under each chemical. These chemicals when used in a plant may be more efficient if applied before evisceration. In this application procedure they may either prevent attachment or have a longer period of time to be in contact with potentially hazardous bacteria. It was felt that 1% Tween 80 would be the standard by which the other chemicals would be measured. Unfortunately, there was not a significant difference in Tweens' performance between the two phases. There is a lack of sufficient data to conclusively suggest any alterations in the current process. More experiments are needed to fully understand these chemicals in this particular aspect.

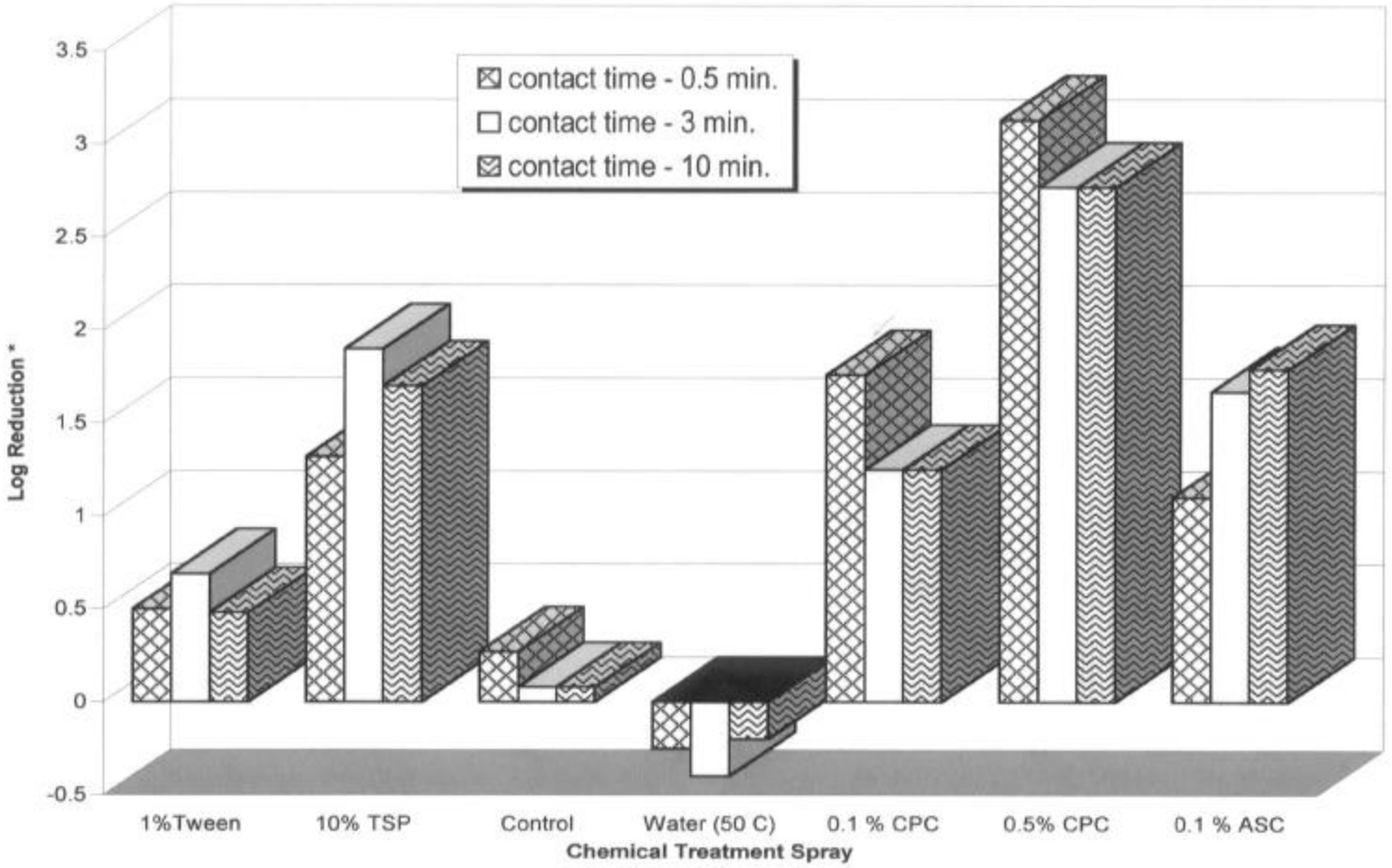
After reviewing these results adaptations could be made to the project design to more accurately depict the poultry process as well as maximize to potential for each chemical. Whole carcasses should be used in addition to a greater repetition of analysis. Procedures for artificially inoculating an entire carcass consistently with a known level of *C. jejuni* would have to be developed. Carcass washers similar to those already in use in the industry should be introduced and equipped so that the pressures could be adjusted to study the potential effects of chemical spray pressure. Perhaps a standard spray pressure could be used first followed by a comparatively low and high pressure. Dependant on the results one could adjust the pressure to achieve the optimal conditions.

More research encompassing the full capabilities of CPC in various concentrations on this bacterium as well as other pathogens should be imperative. This research in addition to research previously reviewed indicates a great potential for this chemical. Its' abilities should be exploited as many others before it have been. Additional chemicals in other industries and procedures may have the same or increased potential for reducing pathogenic load. This particular method has proven effective and should be applied in the future to test the productiveness of newly created or potentially applicable chemicals. One must also consider the combination of some of these chemicals with different mechanisms to achieve a superior "cocktail" of chemicals. Not only should one notice a greater reduction but also it may prevent the formation of resistant mutagens. In actuality the number of combinations and potentially successful chemicals is infinite.

As compared to other foodborne pathogens, studies with *Campylobacter* are limited. None of the chosen sprays had been tested to see if they could be effective both before and after inoculation of *Campylobacter.* The poultry processing industry will need to develop options for controlling *Campylobacter* populations in their plants and products. *Campylobacter* will continue to be a problem until methods of control are

tested and approved. Both methods are effective but as described before it is important to know which way it occurred.

Figure 1: Average log reduction of C. jejuni when bacterial inoculum applied before chemical treatment



TSP - Trisodium Phosphate
CPC - Cetylpyrinidium Chloride
ASC - Acidified Sodium Chlorite
* - reductions in log (base 10) CFU/skin

Table 1: Log_{10} reductions* produced as a function of chemical treatment and contact time when *C. jejuni* was applied before chemical treatment sprays

Chemical treatment	Contact time (min.)	log_{10} reduction	Std. Err. log_{10} reduction
0.1% CPC	0.5	1.76	0.36
0.1% CPC	3	1.25	0.01
0.1% CPC	10	1.25	0.06
0.5% CPC	0.5	3.13	0.14
0.5% CPC	3	2.77	0.35
0.5% CPC	10	2.77	0.06
1% Tween	0.5	0.50	0.11
1% Tween	3	0.69	0.26
1% Tween	10	0.48	0.10
10% TSP	0.5	1.32	0.24
10% TSP	3	1.86	0.04
10% TSP	10	1.70	0.29
0.1% ASC	0.5	1.09	0.12
0.1% ASC	3	1.67	0.32
0.1% ASC	10	1.79	0.09
Water(50°C)	0.5	-0.25	0.21
Water(50°C)	3	-0.40	0.03
Water(50°C)	10	-0.20	0.18
Control	0.5	0.26	0.02
Control	3	0.08	0.13
Control	10	0.08	0.00

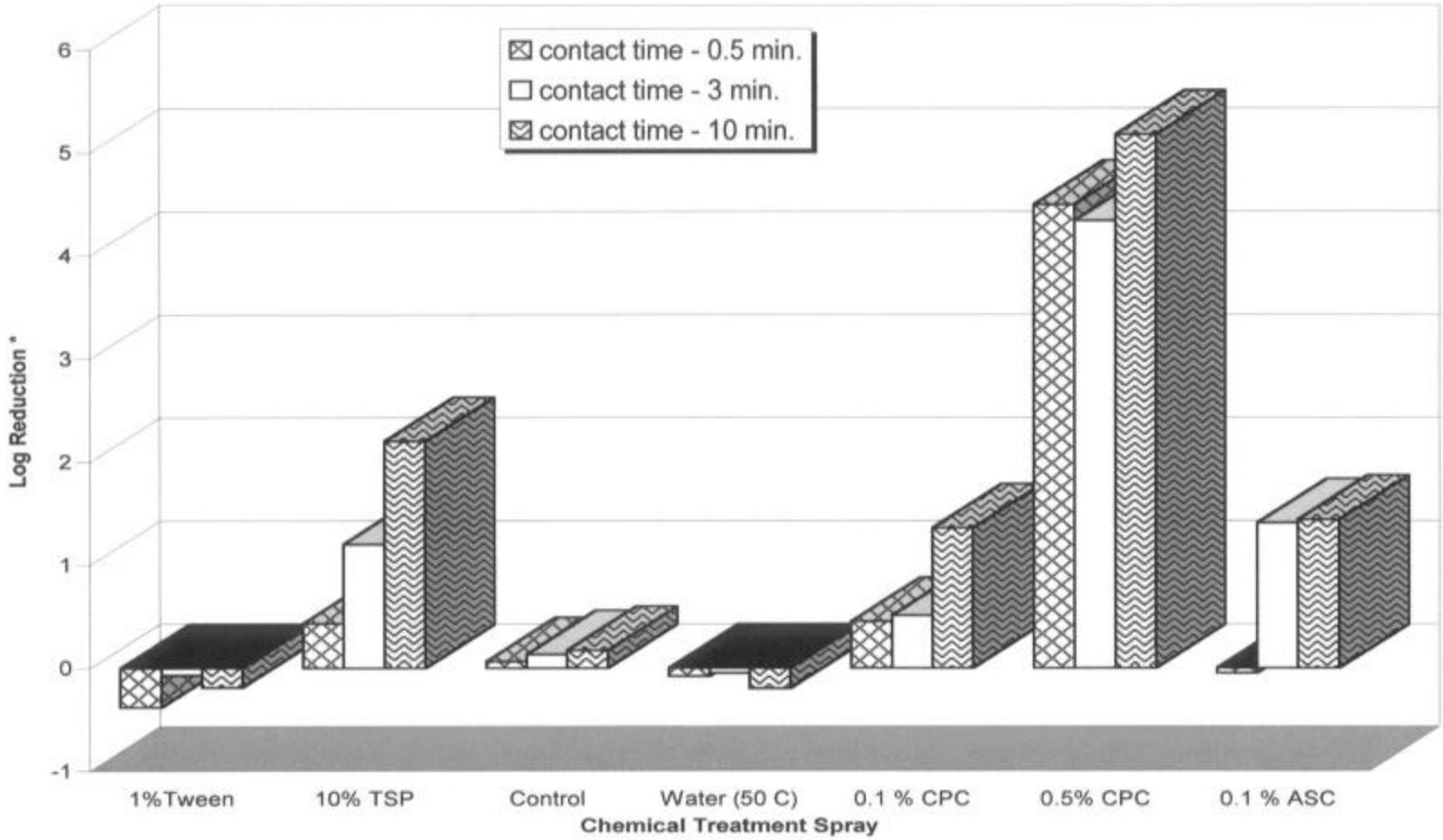
CPC – Cetylpyridinium Chloride
TSP – Trisodium Phosphate
ASC – Acidified Sodium Chlorite
* - Reductions given in log $_{10}$ CFU/skin

Table 2 : Means and standard errors of log reductions* when *C. jejuni* was applied before chemical treatment spray

Chemical Spray	Mean **	Std. Err.
0.5 % CPC	2.89 [a]	0.12
10 % TSP	1.63 [b]	0.14
0.1 % ASC	1.52 [b]	0.16
0.1 % CPC	1.42 [b]	0.14
1 % Tween 80	0.56 [c]	0.09
Control	0.15 [c d]	0.05
Water (50^{o}C)	-0.28 [d]	0.08

CPC – Cetylpyridinium Chloride
TSP – Trisodium Phosphate
ASC – Acidified Sodium Chlorite
* - Reductions given in $\log_{10}$ CFU/skin
** - Means followed by a different letter are significantly different ($P \leq 0.05$) experiment wise using Tukey's HSD (Sall and Lehmann, 2001).
N = 6

Figure 2 : Average log reduction of *C. jejuni* when bacterial inoculum applied after chemical treatment



TSP - Trisodium Phosphate
CPC - Cetylpyridinium Chloride
ASC - Acidified Sodium Chlorite
* - reductions in log (base 10) CFU/skin

Table 3: Log_{10} reductions* produced as a function of chemical treatment and contact time when *C. jejuni* was applied after chemical treatment sprays

Chemical treatment	Contact time (min.)	log_{10} reduction	Std. Err. log_{10} reduction
0.1% CPC	0.5	0.44	0.43
0.1% CPC	3	0.51	0.46
0.1% CPC	10	1.36	0.94
0.5% CPC	0.5	4.50	0.59
0.5% CPC	3	4.34	0.51
0.5% CPC	10	5.18	0.59
1% Tween	0.5	-0.38	0.02
1% Tween	3	-0.07	0.18
1% Tween	10	-0.19	0.13
10% TSP	0.5	0.43	0.14
10% TSP	3	1.20	0.98
10% TSP	10	2.20	0.10
0.1% ASC	0.5	-0.05	0.35
0.1% ASC	3	1.41	0.24
0.1% ASC	10	1.44	0.00
Water(50°C)	0.5	-0.08	0.02
Water(50°C)	3	-0.05	0.04
Water(50°C)	10	-0.20	0.05
Control	0.5	0.06	0.13
Control	3	0.13	0.11
Control	10	0.17	0.01

CPC – Cetylpyridinium Chloride
TSP – Trisodium Phosphate
ASC – Acidified Sodium Chlorite
* - Reductions given in $\log_{10}$ CFU/skin

Table 4 : Means and standard errors of log reductions* when *C. jejuni* was applied after chemical treatment spray**

Chemical Spray	Mean ***	Std. Err.
0.5 % CPC	4.67 [a]	0.30
10 % TSP	1.28 [b]	0.41
0.1 % ASC	0.93 [b c]	0.33
0.1 % CPC	0.77 [b c d]	0.35
Control	0.12 [c d]	0.05
Water (50^{o}C)	-0.11 [c d]	0.03
1 % Tween 80	-0.21 [d]	0.08

CPC – Cetylpyridinium Chloride
TSP – Trisodium Phosphate
ASC – Acidified Sodium Chlorite
* - Reductions given in $\log_{10}$ CFU/skin
** - N=6
*** - Means followed by a different letter are significantly different ($P \leq 0.05$) experiment wise using Tukey's HSD (Sall and Lehmann, 2001).

Table 5 : Means and standard errors of log reductions* as a function of contact time when *C. jejuni* was applied after chemical treatment spray **

Contact time (minutes)	Mean ***	Std. Err.
0.5	0.70 [a]	0.44
3	1.07 [a b]	0.42
10	1.42 [b]	0.50

* - Reductions given in $\log_{10}$ CFU/skin
** - N = 14
*** - Means followed by a different letter are significantly different ($P \leq 0.05$) experiment wise using Tukey's HSD (Sall and Lehmann, 2001).

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