

Microbiological Impact of Spray Washing Broiler Carcasses Using Different Chlorine Concentrations and Water Temperatures

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ABSTRACT A study was conducted to investigate the microbiological impact of spray washing broiler carcasses with chlorinated water (0 or 50 ppm) at different temperatures (21.1, 43.3, or 54.4°C). A whole carcass rinse (WCR) was performed on each carcass before (control) and after spray washing (final). After the control WCR, carcasses were inoculated with 0.1 g of cecal material containing 2×10^5 cells per gram of *Campylobacter* and 2×10^5 cells per gram of nalidixic acid-resistant *Salmonella*. Carcasses were held at room temperature for 12 min before washing in an inside-outside bird washer (80 psi for 5 s). Chlorine level and water temperature had no effect on total aerobic bacteria, *Escherichia coli*, or *Campylobacter* numbers recov-

ered from the final WCR. Levels of bacteria found on carcasses before and after washing were 4.6, 3.6, and 3.5 log₁₀ cfu/mL rinse for total aerobic bacteria, *E. coli*, and *Campylobacter*, respectively. Average counts for nalidixic acid-resistant *Salmonella* after washing were 3.1 log₁₀ cfu/mL rinse irrespective of water temperature or chlorine level ($P < 0.05$). In addition, chlorine level and water temperature had no effect on the breast skin color, with average values of $L^* = 66.6$; $a^* = -0.09$; $b^* = -0.05$ ($P < 0.05$). Under the conditions outlined in the present study, adding chlorine and/or elevating the water temperature during spray washing in an inside-outside bird washer did not enhance the removal of bacteria from broiler carcasses and had no effect on carcass skin color.

(Key words: broilers, carcass contamination, bird washers, carcass microbiology)

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INTRODUCTION

Consumption of contaminated or undercooked food has been estimated to cause 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths in the United States each year (Mead et al., 1999). Of those illnesses, 2.4 million and 1.4 million cases may be attributed to campylobacteriosis and salmonellosis, respectively (Mead et al., 1999). The USDA Economic Research Service has predicted that the annual costs associated with either salmonellosis or campylobacteriosis are several billion dollars (USDA, 2004). Whereas salmonellosis and campylobacteriosis can be transmitted in a variety of foods, both illnesses have been closely associated with the consumption of raw or undercooked poultry and poultry products (Altekruse et al., 1999; Kimura et al., 2004).

During poultry processing, bacteria are removed, destroyed, or controlled using a combination of heat treatments, water, chemical additives (antimicrobials), and mechanical methods. Traditionally, chlorinated water has been used in carcass cabinet washers, immersion chillers, or equipment sprays to reduce microbial contamination

and cross-contamination (Walker and Ayres, 1956; May, 1961; Wilkerson et al., 1961; Sanders and Blackshear, 1971; Mead et al., 1975; Lillard, 1990; Morrison and Fleet, 1985; Northcutt et al., 2003; Bashor et al., 2004). However, the implementation of the USDA's Pathogen Reduction, Hazard Analysis and Critical Control Point System Final Rule (USDA, 1996) has sparked renewed interest in alternatives to chlorine or chemical additives which enhance the efficacy of chlorine on pathogenic bacteria. Research has investigated the antimicrobial effects of chlorine dioxide (Lillard, 1990), sodium chloride (Li et al., 1997), trisodium phosphate (Kim et al., 1994; Lillard, 1994; Hwang and Beuchat, 1995; Li et al., 1997; Xiong et al., 1998a,b), cetylpyridinium chloride (Kim et al., 1996; Li et al., 1997; Xiong et al., 1998a,b), ozone (Yang and Chen 1979; Sheldon and Brown, 1986), hydrogen peroxide (Hwang and Beuchat, 1995); lactic acid (Mulder et al., 1987; Izat et al., 1990; Hwang and Beuchat, 1995; Li et al., 1997; Xiong et al., 1998a,b), and acidified sodium chlorite (Kemp et al., 2000, 2001) in poultry processing water. Other chemicals that currently are being used by the poultry industry for online reprocessing include sodium metasilicate, peroxyacetic acid, citric acid blended with hydrochloric and phosphoric acid, monochloramines and solutions of hypochlorous acid (chlorine) with controlled pH (Volk, 2004). To date, chlorine still remains the most widely used antimicrobial chemical by the poultry industry (Northcutt and Jones, 2003).

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Some poultry processing establishments have reported using hot chlorinated water in cabinet washers to enhance removal of carcass fecal material and the associated bacteria (Willis, 2002; Bashor et al., 2004). Previous research has shown that treatment of poultry carcasses with non-chlorinated hot water by immersion (Pickett and Miller, 1966; Avens and Miller, 1972; Teotia and Miller, 1972) or spray (Thomas et al., 1974; Bashor et al., 2004) will effectively reduce the surface microbial counts. However, there is little information regarding the microbiological implications of combining chlorine with hot water in a bird washer. Thomas et al. (1974) reported significant reductions (0.7 to 1.2 log₁₀ per cm²) in counts of total aerobic bacteria on broiler carcasses washed for 12 s with 54.4 to 71.1°C water compared with counts on carcasses washed for 12 s with 21.1°C water. These authors suggested that the higher water temperatures destroy the bacteria and improve the efficiency of removing the bacteria from the skin by softening the fat and lowering the surface tension (Thomas et al., 1974). Bashor et al. (2004) found that the use of hot water (63°C) in a cabinet washer increased the washer efficiency for removing bacteria from broiler carcasses (reduction in *Campylobacter* counts per volume of water) by 45% (efficiency of 16,265 vs. 8,989). Thomas et al. (1974) and Bashor et al. (2004) suggest that additional research is needed to determine the effects of hot water treatments as a means of destroying bacteria on poultry carcasses during processing. Thus, the objective of this project was to evaluate the microbiological effects of spray washing broiler carcasses with chlorinated or nonchlorinated water at 21.1, 43.3, or 54.4°C.

MATERIALS AND METHODS

Sampling and Treatment

On each of four different sampling days, twenty-six carcasses were removed from a commercial processing line after evisceration and before the inside-outside bird washer (IOBW). Carcasses were placed into coolers, transported to the laboratory and subjected to a whole carcass rinse (WCR). Microbiological analyses of this initial WCR were reported as the "control" treatment. After the initial WCR, CIE (1978) L*, a*, and b* color values for breast skin were measured in 4 locations with a handheld Minolta Colorimeter,² which was previously standardized with a white tile.³ Ten ceca were also collected from the processing line, placed into a single bag, and returned to the laboratory. The 10 ceca were opened, emptied into a weigh boat, and blended with a spatula for approximately 1 min. After blending, cecal contents (4.95 g) were inocu-

TABLE 1. Temperature, chlorine levels and average pH¹ of water used during spray washing

Treatments	Water temperature (°C)	Chlorine concentration (ppm)	Average pH
1	21.1	0	7.4 ± 0.15
2	21.1	50	8.3 ± 0.41
3	43.3	0	7.4 ± 0.12
4	43.3	50	8.2 ± 0.06
5	54.4	0	7.6 ± 0.11
6	54.4	50	8.2 ± 0.04

¹Means for pH ± standard error.

lated with a cosuspension (0.1 mL) containing 10⁷ cells of *Campylobacter* and nalidixic acid-resistant *Salmonella*. The inoculated cecal material (0.1 g) was applied to each carcass, and the carcasses were held at room temperature (27°C) for 12 min before washing. Twelve minutes was selected as the holding time to simulate the longest potential contamination time in a commercial facility based on line speed. After the 12-min holding time, 24 carcasses were washed in a spray cabinet (80 psi for 5 s) using the treatment scheme shown in Table 1. The two remaining carcasses were subjected to a WCR without washing, and these data were designated as the fecally contaminated control data.

Water used to wash the carcasses was heated in a previously sanitized prototype scalding tank. Heated water was pumped to a secondary sanitized container containing an external, stainless steel temperature probe that was attached to a Cox Tracer data logger.⁴ After the heated water was transferred to the secondary container, an appropriate amount of chlorine (6.15% sodium hypochlorite) was added (0 or 50 ppm). Tap water averaged 0.5 ppm chlorine. This water was used to wash carcasses in the 0 ppm chlorine treatment. After the chlorine was added, the water was mixed and the concentration of total chlorine was measured immediately before treating carcasses using a colorimetric reaction with *N,N*-diethyl-*p*-phenylenediamine from the CHEMetrics 2 SAM test kit.⁵ The pH of the water also was measured with a handheld pH meter before it was sprayed onto the carcasses.⁶

The spray cabinet was an individual carcass unit consisting of a pressure pump and a pressure regulator attached to a metal frame with Plexiglas sides. Cabinet dimensions were 91 × 91 × 76 cm (length × width × height). The center of the cabinet consisted of a wire cone with a spray nozzle in the center of the cone. There were 3 additional nozzles on each of 4 sides to wash the outside of the carcasses. During the 5-s spray wash, each carcass received 3 L of water. Immediately after washing, carcasses were drained of the excess wash water, subjected to a final WCR, and breast skin color was measured in approximately the same 4 locations.

Microbiological Analyses

Each carcass was subjected to 2 WCR. The initial WCR was conducted before inoculation and spray washing

²Minolta Chroma Meter CR-2000, Minolta Camera Co., Ltd., Kagashi-Ku, Japan.

³Reference number 13533123. Y = 92.7, x = 0.3133, y = 0.3193.

⁴Cox Technologies, Inc. Belmont, NC.

⁵CHEMetrics Inc., Calverton, VA.

⁶Model AP5, Denver Instruments, Denver, CO.

(Control), whereas the final WCR was conducted after spray washing. During each replication, 2 additional carcasses were subjected to an initial WCR, inoculated, and given a second WCR without washing (fecally contaminated control). For the WCR, carcasses were individually placed into clean plastic bags with 100 mL of sterile water and shaken vigorously in a 1-ft arc for 60 s. After shaking, carcasses were aseptically removed from the bag, allowed to drain briefly into the bag and then discarded. Serial dilutions of the rinse were made in 1% peptone. Total aerobic bacterial populations were enumerated on plate count agar.⁷ A 0.1-mL sample from a serial dilution of the rinse diluent was plated in duplicate on the surface of the agar, spread, and incubated at 35°C for 48 h prior to counting the resulting colony forming units. *Escherichia coli* counts were made by plating 1 mL from a serial dilution of the rinse diluent onto duplicate *E. coli* Petrifilm plates.⁸ Petrifilm plates were incubated at 35°C for 24 to 48 h and blue colonies closely associated with entrapped gas were counted as *E. coli*. Nalidixic acid-resistant *Salmonella* counts were made by plating 0.1 mL from a serial dilution of the rinse diluent onto duplicate Brilliant Green Sulfa plates containing 200 mg/L nalidixic acid and 25 mg/L novobiocin.⁹ *Campylobacter* was enumerated by plating 0.1 mL from the serial dilutions onto Campy Blood agar (Blaser¹⁰) and incubating the plates at 42°C for 36 h in a microaerophilic environment (5% O₂, 10% CO₂ and balance N₂). Colony forming units characteristic of *Campylobacter* were counted. Each colony type identified as *Campylobacter* was confirmed for genus by examination of cellular morphology and motility on a wet mount under phase contrast microscopy. Each colony type was further identified as *Campylobacter* spp. using INDX-Campy (jcl) culture confirmation test.¹¹

Statistical Analysis

Data were analyzed by the ANOVA option of the GLM procedure of the SAS/STAT program using chlorine concentration, water temperature, and replicate as main effects. All first-order interactions were tested for statistical significance ($P < 0.05$) using the residual error mean squares. Analyses were performed on the data after logarithmic transformation and included analyzing the change in the counts due to washing (counts on inoculated washed carcasses minus counts on control carcasses). Means were separated using the least squares means option and reported along with the standard error (SAS, 1999).

RESULTS AND DISCUSSION

Breast skin color was measured on all carcasses before and after spray washing to examine the effects of the

TABLE 2. Levels of bacteria in the whole carcass rinse of broiler carcasses before washing in the inside-outside bird washer¹

Bacteria	Control ²	Fecally contaminated control ³
Total Aerobic	4.6 ± 0.7	6.7 ± 0.5
<i>Escherichia coli</i>	3.6 ± 0.7	5.9 ± 0.2
<i>Salmonella</i>	NA	4.0 ± 0.7
<i>Campylobacter</i>	3.5 ± 0.8	6.3 ± 0.8

¹Means for log₁₀ colony-forming units per milliliter of rinse ± standard error.

²Control refers to carcasses that have not been washed or inoculated.

³Fecally contaminated control refers to carcasses that have not been washed but have been inoculated.

washing on carcass appearance. The time of exposure to the spray (5 s) was short, and therefore neither chlorination nor water temperature had an effect on carcass appearance ($P < 0.05$). On average, the skin color was found to have the following parameters: $L^* = 66.6$; $a^* = -0.09$; $b^* = -0.05$. Similar findings were reported by Thomson et al. (1974) when they subjectively evaluated broiler skin color of carcasses spray washed with water at 21.1, 54.4 or 60.0°C for 12 s. These same researchers reported an "inferior appearance" for broiler carcasses washed with 71.1°C water for 12 s, but they suggested that the magnitude of color difference was small (Thomas et al., 1974).

The mean logarithmic microbial counts (log₁₀) for total aerobic bacteria, *E. coli*, nalidixic acid-resistant *Salmonella*, and *Campylobacter* on carcasses before (control) and after (fecally contaminated control) inoculation are shown in Table 2. Carcasses were inoculated with bacteria to increase the likelihood of seeing subtle differences due to washing. Researchers evaluating the effects of chlorinated spray washing (200 ppm) on the recovery of bacteria from beef carcasses have indicated that washing did not cause an additional reduction in the bacterial load because the initial levels of bacteria were too low (Stevenson et al., 1978). By inoculating carcasses, levels of bacteria were increased by 2 to 3 log units for total aerobic bacteria, *E. coli*, and *Campylobacter*, and by 4 log units for *Salmonella* (Table 2).

Counts of total aerobic bacteria (log₁₀ 4.4 to 4.6 cfu/mL), *E. coli* (log₁₀ 3.8 to 4.1 cfu/mL) and *Campylobacter* (log₁₀ 3.5 to 4.2 cfu/mL) found on carcasses after washing were similar to those counts found on the control carcasses (log₁₀ 4.6, 3.6, and 3.5 cfu/mL, respectively) before washing (Table 3). Neither water temperature (21.1, 43.3 or 54.4°C) nor chlorine level (0 or 50 ppm) were found to have a significant effect on the counts of total aerobic bacteria, *E. coli*, *Salmonella* or *Campylobacter* recovered from the carcasses ($P < 0.05$). This suggests that the added contamination (inoculated feces) was removed during washing. Dickson and Anderson (1992) reported that bacterial attachment to surfaces is typically considered to be a two-step process, consisting of an initial reversible phase involving physical forces followed by an irreversible second phase involving extracellular polysaccharides. Notermans and Kampelmacher (1974) found that bacterial

⁷Becton Dickinson, Sparks, MD.

⁸3M Health Care, St. Paul, MN.

⁹Sigma-Aldrich, St. Louis, MO.

¹⁰Difco Laboratories, Detroit, MI.

¹¹Integrated Diagnostics, Baltimore, MD.

TABLE 3. Levels of bacteria in the whole carcass rinse of broiler carcasses after washing in an inside-outside bird washer¹ with 21.1, 43.3, or 54.4°C water containing 0 or 50 ppm chlorine

Bacteria	0 ppm Chlorine			50 ppm Chlorine		
	21.1°C	43.3°C	54.4°C	21.1°C	43.3°C	54.4°C
Total Aerobic	4.4 ± 0.1	4.6 ± 0.2	4.6 ± 0.2	4.4 ± 0.2	4.5 ± 0.2	4.6 ± 0.2
<i>Escherichia coli</i>	3.8 ± 0.1	4.1 ± 0.1	4.0 ± 0.2	3.8 ± 0.1	4.0 ± 0.2	3.9 ± 0.2
<i>Salmonella</i>	3.2 ± 0.2	3.3 ± 0.1	2.9 ± 0.2	3.1 ± 0.1	3.0 ± 0.2	2.9 ± 0.2
<i>Campylobacter</i>	3.5 ± 0.3	4.2 ± 0.2	3.9 ± 0.2	3.8 ± 0.2	3.8 ± 0.2	3.7 ± 0.2

¹Means for log₁₀ colony-forming units per milliliter of rinse ± standard error.

attachment to broiler skin was dependent on temperature, pH, and the presence and activity of flagella. Optimal conditions for bacterial attachment to broiler skin were found to be 21°C and pH 8.3 to 8.4 (Notermans and Kampelmacher, 1974). Flagellated bacteria, similar to those evaluated in the present study, were reported to attach to the skin at a much faster rate (Notermans and Kampelmacher, 1974). This suggests that the 12-min holding time used in the present study was sufficient to allow the bacteria to become attached, yet washing was sufficient to remove bacteria.

After washing, levels of *Salmonella* recovered from carcasses ranged from 2.9 log₁₀ cfu/mL (54.4°C) to 3.3 log₁₀ cfu/mL (43.3°C). This was a 0.7 to 1.1 log₁₀ reduction in the *Salmonella* levels compared with the levels of *Salmonella* on the fecally contaminated control carcasses (4.0 log₁₀ cfu/mL; Table 2). Xiong et al. (1998b) reported similar findings of a 1 log reduction in *Salmonella* counts with a 30-s water spray at 207 kPa (6.5 log₁₀ cfu/mL vs. 5.5 log₁₀ cfu/mL). Yang et al. (1998) found that spray washing with water for 17 s at 413 kPa lowered *Salmonella* counts by 0.4 log₁₀ cfu/mL compared with the unwashed control. Thomas et al. (1974) compared levels of total aerobic bacteria on unwashed carcass (3.6 log₁₀ cfu/cm²) to those levels found on carcasses washed with 21.1 or 54.4°C water for 12 s and found that washing reduced the counts by 0.32 or 0.69 log₁₀ cfu/cm², respectively (Thomas et al., 1974). Greater reductions in counts occurred with warmer water (71.1°C).

Data collected during the present study shows that the efficiency of the IOBW was due solely to washing (3 L/carcass). In their report on commercial bird washers, Bashor et al. (2004) found that the volume of water used per carcass in commercial IOBW ranged from 2.2 to 9.1 L, and efficiency of washing was not related to the volume of water. These authors also found that the washer pressure in commercial IOBW ranged from 40 to 180 psi, with an overall average pressure approaching 80 psi (Bashor et al., 2004). The only difference between commercial IOBW and the one used in the present study involves the orientation of the carcasses. The IOBW used in the present study orients the carcass in the opposite position from that used in commercial IOBW, and this allowed water to drain from the body cavity. This orientation seemed to prevent internal water entrapment, which could improve the efficiency of the wash.

Previous research conducted on spray washing poultry, beef, or lamb carcasses shows that the effects of water

temperature and chlorine concentration for reducing bacterial counts are additive (Thomas et al., 1974; Kotula et al., 1974; Kelly et al., 1981). The results of the present study show that this may not be true for poultry carcasses.

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