ABSTRACT  Experiments were conducted to determine the relationship between poultry chilling water volume and carcass microbiology. In the first study, the volume of water used during immersion chilling was found to have a significant effect on the counts of bacteria recovered from broiler carcass halves; however, these volumes (2.1 and 16.8 L/kg) were extreme and did not reflect commercial levels. A second study using commercial chilling volumes was conducted with 3.3 L/kg (low) or 6.7 L/kg (high) distilled water in the chiller. Prechill broiler carcasses were removed from a commercial processing line, cut into left and right halves, and one-half of each pair was individually chilled in a bag containing low or high volume of water. Bags containing halves were submersed in a secondary chill tank containing approximately 150 L of an ice-water mix (0.6°C). After 45 min, halves were removed, allowed to drip for 5 min, and rinsed with 100 mL of sterile water for 1 min. Rinses were analyzed for total aerobic bacteria, *Escherichia coli*, Enterobacteriaceae, and *Campylobacter*. When the numbers of bacteria in the half-carcass rinses (HCR) were compared, counts recovered from halves chilled in a low volume of water were the same as those recovered from the halves chilled with a high volume of water (P > 0.05). Levels found in the HCR ranged from 4.0 to 4.2 log<sub>10</sub> cfu/mL for aerobic bacteria, 3.3 to 3.5 log<sub>10</sub> cfu/mL for *E. coli*, 3.6 to 3.8 log<sub>10</sub> cfu/mL for Enterobacteriaceae, and 2.4 to 2.6 log<sub>10</sub> cfu/mL for *Campylobacter*. Data were also analyzed using a paired comparison t-test, and this analysis showed that there was no difference (P > 0.05) in the numbers of aerobic bacteria, *E. coli*, Enterobacteriaceae, or *Campylobacter* recovered from paired-halves chilled in different volumes of water. The present study shows that under the conditions outlined in this experiment, doubling the amount of water during immersion chilling (3.3 vs. 6.7 L/kg) did not improve the removal of bacteria from the surfaces of chilled carcasses.

Key words: broiler, immersion chilling, carcass contamination, carcass microbiology

INTRODUCTION

In 2005, federally inspected facilities in the United States slaughtered approximately 8.9 billion chickens and produced approximately 16 billion kilograms of chilled and frozen chicken products (USDA, 2006b). These facilities use over 227 billion L of water each year at an estimated cost (water and sewer) of more than $240 million dollars (Merka, 1993; Carawan et al., 1999; Northcutt and Jones, 2004, personal communication). Because water is essential for poultry processing, there has been increased concern over the availability and quality of fresh water. Consequently, the poultry industry has increased its efforts to develop water conservation and water recycling programs, as well as conducting investigations into alternative poultry processing techniques that require less water to achieve the same results.

Nearly every stage of poultry processing (electrical stunning, scalding, defeathering, carcass washing, carcass chilling, offal removal, product movement, and equipment/facility sanitation) requires water. Merka (W. Merka, University of Georgia, Athens, personal communication) stated that 75% of a poultry processing plant’s daily water consumption was used during evisceration. Moreover, carcass washing and chilling have been found to be the 2 largest water consuming processing stages. Chilling is typically accomplished by immersion in cold water or an ice water mix; however, immersion chilling is not as popular outside the United States (Mead et al., 2000; Food Safety Inspection Service, 2001; James et al., 2005). The primary purpose for chilling poultry is to reduce the rate of growth of pathogenic and spoilage microorganisms (Brant, 1974; Thomson et al., 1974; Veerkamp, 1989; James et al., 2005). Depending upon the type of immersion chiller (auger or drag), commercial manufacturers recommend an initial fill volume of 2,000 to 5,000
L/m (Morris and Associates, 2006). In addition, the USDA suggests a minimum of 1.9 L/carcase fresh water be added during operation to minimize the build-up of bacteria and organic debris (USDA, 2006a).

A number of publications have addressed the microbiological impact of immersion chilling; however, the majority of these publications have focused on chlorine levels and carcass cross contamination (Mead and Thomas, 1973a; Blood and Jarvis, 1974; Mulder et al., 1976; Blank and Powell, 1995; Allen et al., 2000; Bilgili et al., 2002; Northcutt et al., 2003). The impact of immersion chilling using different volumes of water has also been studied (Mead and Thomas, 1973a; Blood and Jarvis, 1974; Mulder et al., 1976; Northcutt et al., 2006). Mead and Thomas (1973a) and Blood and Jarvis (1974) reported that the volume of water could be reduced during immersion chilling if the total chlorine level was maintained at 10 ppm or higher. Mead and Thomas (1973b) also suggested that the reduction in carcass contamination during immersion chilling was not due to chlorine level but rather resulted from the mechanical action of the chiller. To study this, Northcutt et al. (2006) examined the microbiology of paired broiler carcass halves after immersion chilling in different volume of distilled water. When one-half of each pair was chilled in 2.1 L/kg of water, higher counts were recovered from the half (3.7, 2.5, 2.6, and 2.1 log10 cfu/mL for total aerobic bacteria, Escherichia coli, Enterobacteriaceae, and Campylobacter, respectively) as compared with the counts recovered from the companion half (3.2, 1.7, 1.6, and 1.8 log10 cfu/mL for total aerobic bacteria, E. coli, Enterobacteriaceae, and Campylobacter, respectively) after chilling in 16.8 L/kg of water (Northcutt et al., 2006). Northcutt et al. (2006) selected an 8-fold difference in chiller volume because it represented extreme conditions, and failure to detect microbiological differences with these volumes would have precluded the need for additional research. The present study was conducted to evaluate the microbiological impact of immersion chilling in volumes of nonchlorinated water comparable with those used during commercial immersion chilling.

**MATERIALS AND METHODS**

**Broiler Carcasses**

On each of 3 different sampling days, 10 broiler carcasses were removed from a commercial poultry processing line immediately after the final inside-outside bird washer, placed into a cooler, and transported to the Russell Research Center. Four carcasses were identified and set aside to serve as prechill control carcasses. The remaining 6 carcasses were cut into left and right halves along the keel bone, and each half was tagged on the wing and weighed. One half of each pair was individually chilled in a plastic bag containing 3.3 L/kg (low) or 6.7 L/kg (high) of distilled water (4°C). No chlorine was added to the distilled water. Bags containing carcass halves and water were sealed with multiple cable ties and submersed in a secondary chill tank containing approximately 150 L of an ice-water mix (0.6°C). The secondary chill tank was agitated with compressed air. After chilling for 45 min, samples were removed from bags using clean gloves and allowed to drip for 5 min. Each half was placed into a new bag with 100 mL of sterile water and subjected to a half-carcass rinse (HCR) for 1 min. Deep breast muscle temperature was measured after the HCR. Prechill control carcasses were also subjected to a whole carcass rinse (WCR) for 1 min in 100 mL of sterile water. After shaking for 1 min, carcasses (halves or whole) were removed from the bags and discarded. The rinses (HCR and WCR) were poured into sterile specimen cups and tested for numbers of bacteria.

**Microbiological Analyses**

The HCR and WCR from each sample were analyzed for total aerobic bacteria, E. coli, Enterobacteriaceae, and Campylobacter. Serial dilutions of the each WCR and HCR were made in 0.1% peptone. The aerobic bacteria populations were enumerated on plate count agar (Becton Dickinson, Sparks, MD). One-tenth of 1 mL from the dilutions was plated in duplicate onto the surface of agar, spread, and incubated at 35°C for 48 h before counting the resulting colony-forming units. The E. coli counts were determined by plating 1 mL from each dilution onto duplicate E. coli Petrifilm plates (3M Health Care, St. Paul, MN). 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. Enterobacteriaceae were enumerated using duplicate pour plates of violent red bile glucose agar containing 1 mL from each dilution. All violent red bile glucose agar plates were overlaid with approximately 5 mL and then incubated at 35°C for 48 h. Plates with the typical presumptive Enterobacteriaceae colonies were counted and reported. Campylobacter was enumerated by plating 0.1 mL from each dilution onto Campy Blood agar (Blaser). Campy Blood agar plates were incubated 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 species using INDEX-Campy (jcl) culture confirmation test (Integrated Diagnostics, Baltimore, MD).

**Statistical Analysis**

Data were analyzed after log transformation using the GLM procedure of the SAS/STAT program (SAS, 1999). The main effects in the model were treatment (3.3 or 6.7 L/kg chilling volume) and replication. All first-order interactions were tested for statistical significance using the residual error mean squares. Bacterial counts recovered from the paired carcass halves were also analyzed.
using the paired t-test in the means procedure of SAS (SAS, 1999).

**RESULTS AND DISCUSSION**

The mean logarithmic microbial numbers for aerobic bacteria, *E. coli*, Enterobacteriaceae, and *Campylobacter* recovered from broiler carcass rinses before (prechill control) and after immersion chilling are shown in Table 1. Postchill levels ranged from 4.0 to 4.2 log_{10} cfu/mL for aerobic bacteria, 3.3 to 3.5 log_{10} cfu/mL for *E. coli*, 3.6 to 3.8 log_{10} cfu/mL for Enterobacteriaceae, and 2.4 to 2.6 log_{10} cfu/mL for *Campylobacter*. There was no significant difference (*P > 0.05*) in the numbers of aerobic bacteria, *E. coli*, Enterobacteriaceae, and *Campylobacter* recovered from postchill carcasses regardless of the volume of water (Table 1). In addition, numbers of bacteria from the paired halves were analyzed using a paired t-test, and no difference was found between the companion halves for aerobic bacteria, *E. coli*, Enterobacteriaceae, and *Campylobacter* (*P > 0.05*).

Previous research has reported that numbers of pathogenic bacteria are typically decreased by 90% or more after immersion chilling; however, in most of these studies, chlorine was used to kill bacteria (Mead and Thomas, 1973a; Izat et al., 1988; Blank and Powell, 1995; Cason et al., 1997; Bilgili et al., 2002; Northcutt et al., 2003, 2006). Mead and Thomas (1973a) suggested that chlorine has little effect on bacteria on the carcass skin and rather prevents cells in the chiller water from reattaching to another carcass (cross-contamination). Data from Smith et al. (2005) agree with idea that chlorine prevents cross-contamination. These researchers found that *Campylobacter* in feces on contaminated broiler carcass halves could be transferred to a noncontaminated carcass half during immersion chilling without chlorine (Smith et al., 2005). Without chlorine, Mead and Thomas (1973a) reported that numbers of aerobic bacteria and coliforms in chiller water equilibrated to 2 to 4 log_{10} cfu/mL. Northcutt et al. (2006) also found that numbers of aerobic bacteria, *E. coli*, Enterobacteriaceae, and *Campylobacter* equilibrate in nonchlorinated chiller water (4.3, 3.3 to 3.5, 3.2 to 3.4, and 2.7 to 3.0 log_{10} cfu/mL, respectively), and levels were consistent even when the volume of water was increased from 2.1 to 16.8 L/kg. Similar findings have also been reported for scald water with numbers of bacteria leveling off after approximately 1 h of operation (Humphrey et al., 1981).

In another study, Northcutt et al. (2006) compared bacteria recovery from broiler carcass halves after immersion chilling in 2.1 L/kg or 16.8 L/kg of distilled water. When carcass halves were chilled in 2.1 L/kg of water, numbers of aerobic bacteria, *E. coli*, Enterobacteriaceae, and *Campylobacter* were reduced from the initial, prechill counts by 1.5, 2.0, 1.2, and 2.7 log_{10} cfu/mL, respectively. A greater reduction in counts of bacteria (2.0, 2.8, 2.2, and 3.0 log_{10} cfu/mL for aerobic bacteria, *E. coli*, Enterobacteriaceae, and *Campylobacter*, respectively) on carcass halves occurred when the volume of chiller water was increased 8-fold (16.8 L/kg; Northcutt et al., 2006). The current study found higher postchill counts on carcass halves than those reported by Northcutt et al. (2006), and this may have resulted from differences in initial bird microflora (inoculated vs. noninoculated), processing conditions, or both. During the present study, carcasses were commercially processed up to the point of chilling while the former study used inoculated broilers processed in a pilot processing plant. Differences in live birds and processing conditions caused higher prechill counts for aerobic bacteria (log_{10} 5.2 cfu/mL) and *E. coli* (log_{10} 4.5 cfu/mL), along with artificially elevated levels of *Campylobacter* (log_{10} 4.8 cfu/mL; Northcutt et al., 2006). Because the effectiveness of the immersion chiller depends upon the initial carcass bacterial load, higher bacteria numbers on incoming carcasses translates into higher postchill counts (Mead and Thomas, 1973b; Blood and Jarvis, 1974; Jones et al., 1991; Northcutt et al., 2003).

Before the present study was conducted, it was suggested that doubling the amount of chill water should remove about 2 times more bacteria from the carcasses, but the difference should be within the range that is usually not significant for log values (log_{10} of 2 cfu/mL = 0.3). It was expected that postchill rinses of these carcasses would have the same log counts of bacteria. Data from the present study verified this hypothesis as paired carcass halves immersion chilled with either 3.3 or 6.7 L/kg had similar postchill counts of aerobic bacteria, *E. coli*, Enterobacteriaceae, and *Campylobacter*. In the previous study with 8 times more chilling volume (2.1 vs. 16.8 L/kg), significantly more bacteria were removed from carcass halves chilled with the higher volume, but counts per milliliter in the chill water were not different. This suggests that counts in chiller water may reach an equilibrium, and when this equilibrium occurs, bacteria remain on the carcasses (Northcutt et al., 2006).

### Table 1. Bacterial counts recovered from carcass rinses after immersion chilling with a low (3.3 L/kg) or high (6.7 L/kg) volume of water

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Prechill Control</th>
<th>High volume</th>
<th>Low volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total aerobic</td>
<td>4.7 ± 0.3</td>
<td>4.2 ± 0.5</td>
<td>4.0 ± 0.5</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>3.9 ± 0.3</td>
<td>3.5 ± 0.5</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>4.3 ± 0.3</td>
<td>3.8 ± 0.6</td>
<td>3.6 ± 0.1</td>
</tr>
<tr>
<td><em>Campylobacter</em></td>
<td>3.1 ± 0.6</td>
<td>2.6 ± 0.7</td>
<td>2.4 ± 0.6</td>
</tr>
</tbody>
</table>

1Means ± standard error.
REFERENCES


