Microbiology of Broiler Carcasses and Chemistry of Chiller Water as Affected by Water Reuse

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ABSTRACT A study was conducted to determine the effects of treating and reusing poultry chiller water in a commercial poultry processing facility. Broiler carcasses and chiller water were obtained from a commercial processing facility which had recently installed a TOMCO Pathogen Management System to recycle water in sections 2 and 3 of two 3-compartment chillers. In this system, reused water is blended with fresh water to maintain the chiller volume. Carcasses were sampled prechill and postchill (final exit), and chiller water was sampled from the beginning and end of each of the 3 sections. Carcasses were subjected to a whole carcass rinse (WCR) in 0.1% peptone. Numbers of Escherichia coli (EC), coliforms (CF), and Campylobacter (CPY) were determined from the WCR and chiller water samples. Prevalence of Salmonella (SAL) was also determined on the WCR and chiller water samples. On average, prechill levels of bacteria recovered from rinses were 2.6, 2.9, and 2.6 log10 cfu/mL for EC, CF, and CPY, respectively. Ten out of 40 (25%) prechill carcasses were positive for SAL. After chilling, numbers of EC, CF, and CPY recovered from carcass rinses decreased by 1.5, 1.5, and 2.0 log10 cfu/mL, respectively. However, 9 out of 40 (22%) postchill carcasses were positive for SAL. When the chiller water samples were tested, counts of EC, CF, and CPY were found only in water collected from the first section of the chiller (inlet and outlet). Two of 4 water samples collected from the inlet of the first section tested positive for SAL. This study shows that fresh and reused water can be used to cool poultry in chiller systems to achieve a reduction in numbers of bacteria (EC, CF, and CPY) or equivalent prevalence (SAL) of bacteria recovered from broiler carcasses.

Key words: broiler, chiller water microbiology, carcass microbiology, chlorine

INTRODUCTION

Rapid cooling of poultry carcasses during processing reduces the growth of pathogenic bacteria, improves carcass appearance and increases overall product shelf-life (Brant, 1974; Thomson et al., 1974; Veerkamp, 1989; James et al., 2005). The most common methods for chilling poultry include water immersion or cold-air blast with or without an intermittent water spray (Veerkamp, 1989; Mead et al., 2000; USDA, 2001; James et al., 2005). Historically, there has been controversy surrounding poultry chilling. In 1971, the European Economic Council (EEC) issued a directive that banned the use of “spin” or auger immersion chillers in its member states (EEC, 1971). According to the EEC directive, immersion chilling was viewed as an unhygienic operation that could lead to carcass cross-contamination with microorganisms and uptake of pathogenic bacteria in contaminated chiller water. Although the ban on immersion chilling was removed in 1993 (EEC, 1993), it resulted in a significant amount of research on poultry chilling and ultimately produced process modifications that optimized immersion chilling (Brant, 1963, 1974; Thomson et al., 1974; Lillard, 1982; James et al., 2005). Much of the research on poultry immersion chilling has demonstrated that postchill carcass microbiological populations depend upon prechill carcass bacteria and chiller conditions such as the amount of fresh water overflow and the ratio of carcasses to water. Mead and Thomas (1973) and Blood and Jarvis (1974) reported that poultry cross-contamination during immersion chilling could be prevented by maintaining a balance between the carcass-to-water ratio and residual concentrations of total chlorine. Mead and Thomas (1973) also found that the effectiveness of chlorine as an antimicrobial treatment during poultry processing is dependent upon the initial chlorine concentration, contact time, temperature, pH, and the chemical composition of the water. Reductions in bacteria recovered from postchill broiler carcasses were reported to result from the mechanical action of the immersion chiller rather than the disinfectant properties of chlorine.
(Mead and Thomas, 1973). When immersion chiller chlorine levels were maintained between 45 and 50 mg/L, most of the bacteria in chiller water were destroyed (Mead and Thomas, 1973), and levels of chlorine above 30 mg/L were found to prevent bacteria in chiller water from reattaching to uninfected carcasses (Mead et al., 1994). Bacterial populations in poultry immersion chiller water were reported to equilibrate when antimicrobial treatments were omitted and populations were constant (cells per mL) irrespective of the carcass-to-water ratio—the rate of bacterial removal was equivalent to the rate of bacterial reattachment, dying, or both (Northcutt et al., 2006).

To minimize the build-up of bacteria and organic debris during immersion chilling, the USDA suggests adding a minimum of 1.9 L per carcass of fresh water during operation (USDA, 2006). Traditional immersion systems (3 sections of chiller; length 3 m per section) that follow the USDA recommendation require an estimate of 2.3 to 2.6 L of fresh water per carcass. Immersion chilling of all of the chickens produced in the United States on an annual basis (9 billion chickens) would require more than 23 billion L each year. Ongoing and widespread drought conditions have resulted in water conservation and recycling efforts in the poultry industry, including alternative procedures for chilling. According to the USDA, reused poultry chiller water or “red” water may be placed back in the chiller as make-up water to maintain carcass-to-water ratios provided the red water contains “no more than 5 ppm free available chlorine” (USDA, 2003). However, little is known about chiller water reuse or the effects of reusing poultry processing water on carcass bacteriology and chiller water microbiology or chemistry. The purpose of the present study was to investigate broiler carcass microbiology, chiller water bacteriology, and chiller water chemistry in a commercial immersion system that reuses chiller water.

**MATERIALS AND METHODS**

**Chilling Treatment and Sampling Plan**

This research was carried out in a commercial processing facility that operates 2 separate but identical processing lines (144 birds per min with two 8-h shifts). Defeathered, eviscerated poultry carcasses from each line were continually delivered into 2 separate “auger-type” counter-current poultry chiller systems. Each chilling system consisted of 3 sections, and the last 2 sections for each system received reused chiller water. In the last 2 sections, water was introduced in one end (inlet end) and allowed to flow progressively to the other end (outlet end) where it was collected for recycling. Approximately 2,300 L/min were collected from each section (4,600 L/line), recirculated through a heat exchanger, mixed with clean water, injected with carbonic and hypochlorous acids (Pathogen Management System, TOMCO Equipment Company, Loganville, GA) to maintain a total chlorine concentration of 20 to 50 mg/L, and delivered to the inlet end of the section.

For each of 4 replications, 10 randomly selected carcasses were removed from the processing line before (prechill) and after (postchill) immersion chilling (Figure 1). Carcasses were individually placed into sterile plastic bags, packed into coolers and transported to the laboratory for analyses. Two liters of chiller water was also collected using sterile bottles submerged at the inlet and outlet end of each chiller section (Figure 1). Chiller water was placed on ice in a cooler separate from the carcasses and transported back to the laboratory for analyses. Sampling was alternated between the 2 processing lines. A preliminary experiment showed no difference in carcass microbiology due to processing shift (first and second shift were equivalent); therefore, subsequent samples were collected during second shift only (−0730 h).

**Water Analyses**

Water samples were analyzed for pH, free residual chlorine, total solids, total Kjeldahl nitrogen (TKN), chemical oxygen demand (COD), total aerobic bacteria, coliforms, *E. coli*, *Campylobacter* and *Salmonella*. The pH was measured with a hand-held probe calibrated at pH 4.0 and 7.0 (model number AP5, Denver Instruments, Denver, CO). Chlorine was determined by colorimetric reaction with *N,N*-diethylphenylenediamine using a Single Analyte Photometer Kit (CHEMetrics Inc., Calverton, VA; APHA, 1998). Total solids were determined using standard methods and reported as milligrams per liter (APHA, 1998). The TKN was determined using the micro-Kjeldahl procedure and was reported as milligrams per liter (APHA, 1998). The COD was determined using a Hach Manganese III digestion procedure with chloride removal and levels in milligrams per liter were determined using a hand-held Hach DR 870 colorimeter (Hach Company, Loveland, CO).

**Microbiology**

Carcasses were subjected to a low volume whole carcass rinse (WCR) procedure (Lillard, 1988). For the WCR, carcasses were placed in a bag with 100 mL of 0.1% peptone solution and shaken in an automated carcass shaker for 1 min. After shaking, carcasses were removed aseptically and the rinse was sampled for bacteria recovery. Carcass rinses and chiller water were analyzed for *Escherichia coli* (EC), coliforms (CF), *Campylobacter* (CPY), and *Salmonella* (SAL). Serial dilutions of the rinses and chiller water were prepared in 0.1% peptone. The EC and CF counts were made by plating 1 mL from a serial dilution of the rinse diluent onto duplicate *E. coli* /coliform Petrifilm plates (3M Health Care, St. Paul, MN). Petrifilm plates were incubated at 35°C for 24 h. After incubation, blue colonies closely associated with entrapped gas were counted as EC, whereas blue and red colonies closely associated with entrapped gas were counted as CF. The CPY was enumerated by plating 0.1 mL from the serial
dilutions onto *Campylobacter* Blood agar (Blaser; Difco Laboratories, Detroit, MI) and incubating the plates at 42°C for 48 h in a microaerophilic environment (5% O₂, 10% CO₂, and balance N₂) in a BBL GasPak Jar (Becton, Dickinson and Co., Sparks, MD). Colony forming units characteristic of CPY were counted. Each colony type identified as CPY 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 kit (Integrated Diagnostics, Baltimore, MD). For *Salmonella* enrichments, 30 mL the WCR was removed and incubated for 24 h at 37°C. After incubation, 0.1 mL of the rinse was transferred to 10 mL of Rappaport-Vassiliadis broth (Difco Laboratories) and 0.5 mL of the rinse was transferred to 10 mL of tetrathionate (HAJNA; Becton, Dickinson and Co.) broth and incubated for 24 h at 42°C. Each broth was then streaked onto Brilliant Green Sulfa (BGS, Becton, Dickinson and Co.) agar plates and modified lysine iron agar (Oxoid, Basingstoke, Hampshire, UK) and incubated for 24 h at 35°C. Suspect colonies were inoculated into triple sugar iron and lysine iron agar slants by stabbing. Slants were incubated for 24 h at 35°C. PolyO (Becton, Dickinson and Co.) and PolyH (Microgen, Camberly, Surrey, UK) agglutination tests were used to confirm presumptive positives.

**Statistical Analysis**

Bacterial numbers in WCR or chiller water were converted to log colony-forming units (cfu) per milliliter for statistical analysis using the ANOVA procedure of the general linear model of SAS software (SAS, 1999). Sources of variation in data were sampling site (pre- and post-chill), sampling time (start or end of the shift), and replications. Main effects and their interactions were tested for statistical significance (P < 0.05) using the residual error. When the interaction between sampling time and replication was found to be significant, it was used as the error term for the main effects. *Salmonella* and *Campylobacter* prevalence was analyzed using the chi-squared test for equal proportions (SAS, 1999; P < 0.05).

**RESULTS AND DISCUSSION**

Table 1 shows the pH, free available chlorine, COD, TKN, and TSS recovered from chiller water. Optimal pH for disinfectant properties of chlorine has been reported to be pH 6.5 to 7.5 (White, 1998), and pH values measured during this study were within optimal range. Chiller water pH values ranged from approximately 6.8 (section 1 inlet) to 6.4 (section 3 outlet), and values were not affected by sampling site. Highest values for free available chlorine were found in the first section of the chiller where the organic load (COD, TKN, and TSS) was the greatest; however, reused chiller water was not introduced in this section. Concentrations of free available chlorine in all of the water samples were below the USDA guidelines (≤5 ppm) for reused water (USDA, 2003).

Overall, a reduction was observed for COD, TKN, and TSS concentrations in chiller water from sections 1 to 3 of the chiller system. Merka (2001) reported that the average concentration of contaminates in broiler processing wastewater were 3,770 mg/L of COD, 1,440 mg/L of TSS, and 130 mg/L of TKN. Organic matter in chiller water, as measured by COD, was less than half of that traditionally recovered from chicken processing wastewater (range of 428 to 1,693 mg/L). Protein concentrations in chiller water (TKN) ranged from 42 to 123 mg/L, below traditional wastewater levels of 130 mg/L. Proteins composition of chiller water is similar to that found in muscle exudate or drip, which Offer and Knight (1988) reported to consist of myoglobin, amino acids, vitamins, and glycolytic proteins. Concentrations of particulate matter in chiller water (TSS) collected from section 1 of the system were 60 to 70% higher than TSS levels found in traditional broiler.
processing wastewater, but these levels decreased in sections 2 and 3.

Bacteria were recovered from chiller water, but only in water samples collected from section 1 of the chiller system (Table 2). The EC, CF, and CPY populations were 1.4, 1.8, and 0.7 log10 cfu/mL at the inlet of section 1 (Table 2). The EC, CF, and CPY populations were found to be positive for contamination (site 1). Two of the 4 water samples collected where carcasses are considered to have the highest contamination (site 1). Two of the 4 water samples collected from section 1 outlet (site 2). Bacteria were not detected in sections 2 or 3 of the chiller system (sites 3 to 6). Previous research has shown that bacteria accumulate in chiller water and achieve equilibrium based on fresh water input and antimicrobial levels (Mead and Thomas, 1973; Northcutt et al., 2006). Allen et al. (2000) evaluated the hygienic aspects of 6 different commercial poultry chilling systems in the United Kingdom and concluded that few microorganisms are present in chiller water beyond the entry point of carcasses if chlorination is maintained at 45 mg/L. Data from the present study support this conclusion.

Bacterial populations recovered from broiler carcasses before and after immersion chilling are shown in Table 3. Overall, immersion chilling reduced levels of EC, CF, and CPY by 1.5, 1.5, and 2.0 log10 cfu/mL, respectively. Sampling time (beginning or end of processing shift) had no effect on carcass bacteria recovery ($P < 0.05$). These data agree with previous research that found reductions of at least 1.0 log10 cfu/mL for EC and CPY after immersion chilling of broiler carcasses (Mead and Thomas, 1973; Izat et al., 1988; Blank and Powell, 1995; Cason et al., 1997; Bilgili et al., 2002; Northcutt et al., 2003, 2006).

Prevalence of SAL on carcasses was not affected by chilling and was found to be 25% (prechill) and 22% (postchill) SAL positive. Similar findings have been reported by James et al. (1992a,b) who found that SAL prevalence increased after immersion chilling without chlorine (48 increased to 72% positive) or remained the same with 25 mg/L of chlorine added to the chillers (43 and 46% SAL positive for pre- and postchill, respectively). Manufacturers of the chiller water reuse system suggest adding chlorinated sprays with reused chiller water to prechill carcasses to decrease SAL prevalence. During a personal communication with the plant’s HACCP coordinator, she indicated that chlorinated sprays with reused water were installed as a result of this study (specifically on the picking rails). After installation of the chlorinated

<table>
<thead>
<tr>
<th>Site</th>
<th>pH</th>
<th>Free chlorine (mg/L)</th>
<th>COD (mg/L)</th>
<th>TKN (mg/L)</th>
<th>TSS (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.79 ± 0.07</td>
<td>2.73 ± 0.50</td>
<td>1592.9 ± 172.6</td>
<td>122.6 ± 7.3</td>
<td>3405.6 ± 470.4</td>
</tr>
<tr>
<td>2</td>
<td>6.71 ± 0.08</td>
<td>2.01 ± 0.38</td>
<td>1150.0 ± 119.8</td>
<td>86.5 ± 9.0</td>
<td>2557.0 ± 341.9</td>
</tr>
<tr>
<td>3</td>
<td>6.58 ± 0.08</td>
<td>1.31 ± 0.26</td>
<td>628.8 ± 120.6</td>
<td>64.3 ± 4.6</td>
<td>1408.4 ± 172.4</td>
</tr>
<tr>
<td>4</td>
<td>6.42 ± 0.11</td>
<td>1.22 ± 0.27</td>
<td>596.4 ± 106.6</td>
<td>60.0 ± 4.1</td>
<td>1390.8 ± 221.6</td>
</tr>
<tr>
<td>5</td>
<td>6.55 ± 0.13</td>
<td>0.87 ± 0.18</td>
<td>465.4 ± 107.5</td>
<td>46.8 ± 2.9</td>
<td>1000.4 ± 93.1</td>
</tr>
<tr>
<td>6</td>
<td>6.45 ± 0.08</td>
<td>1.40 ± 0.41</td>
<td>427.8 ± 79.9</td>
<td>41.7 ± 3.1</td>
<td>975.27 ± 101.3</td>
</tr>
</tbody>
</table>

1 Mean ± standard error.
2 Free chlorine refers to available chlorine consisting of chlorine, hypochlorous acid, and hypohypochlorite ions;
   COD refers to chemical oxygen demand; TKN refers to total Kjeldahl nitrogen; TSS refers to total suspended solids.
3 Probability values for significance of sampling site from the ANOVA procedure of SAS (1999); $n = 4$.

### Table 2. Chilling water microbiological1,2 analyses per sampling site

<table>
<thead>
<tr>
<th>Site</th>
<th>Escherichia coli</th>
<th>Coliform</th>
<th>Campylobacter</th>
<th>Salmonella</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.4 ± 0.6</td>
<td>1.8 ± 0.6</td>
<td>0.7 ± 0.5</td>
<td>2/4</td>
</tr>
<tr>
<td>2</td>
<td>0.4 ± 0.2</td>
<td>0.8 ± 0.4</td>
<td>0.0</td>
<td>0/4</td>
</tr>
<tr>
<td>3</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0/4</td>
</tr>
<tr>
<td>4</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0/4</td>
</tr>
<tr>
<td>5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0/4</td>
</tr>
<tr>
<td>6</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0/4</td>
</tr>
</tbody>
</table>

1 Mean ± standard error.
2 Microbiological counts are represented as log10 cfu/mL chiller water.
3 Sampling sites 1 and 2 represent the inlet and outlet of section 1 of the chiller system, respectively; sampling sites 3 and 4 represent the inlet and outlet of section 2 of the chiller system, respectively; sampling sites 5 and 6 represent the inlet and outlet of section 3 of the chiller system, respectively.
4 Probability values for significance of sampling site for E. coli, coliforms, and Campylobacter from the ANOVA procedure; $n = 4$. Salmonella probability values for significance of sampling site from chi-square test.

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**POULTRY CHILLER WATER REUSE**

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sprays, postchill SAL prevalence decreased to 12%. However, it is unlikely that the chlorination was the sole contributor to the SAL reduction. Previous research conducted by Northcutt et al. (2005) demonstrated that there was no difference in reductions of nalidixic-acid resistant SAL applied to broiler carcasses that were washed with tap water (~0.5 mg/L of chlorine) or chlorinated water (50 mg/L) in an inside-outside bird washer (552 kPa, 5 s). Both tap and chlorinated water were found to reduce SAL from approximately 5 to 3 log10 cfu/mL rinse (Northcutt et al., 2005). The application of the additional spray, regardless the chlorine level, or another unforeseen factor, may have contributed to a lower SAR prevalence (22% positive reduced to 12% positive).

Data collected during the present study demonstrates that immersion chiller water may be reused to cool carcasses without compromising numbers of bacteria recovered on postchill carcasses. The water reuse system evaluated in this study recycles approximately 9,200 L/min (2 processing lines with 4,600 L/min per line) or 8.8 million liters per day for two 8-h shifts. On an annual basis, this system could save the processing plant over $364,000.

### Table 3. Carcasses microbiological1 profile and statistical analysis2 of data

<table>
<thead>
<tr>
<th>Source</th>
<th>E. coli</th>
<th>Coliform</th>
<th>Campylobacter</th>
<th>Salmonella</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep</td>
<td>0.0983</td>
<td>0.1069</td>
<td>0.6634</td>
<td>0.1890</td>
</tr>
<tr>
<td>Site</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.7928</td>
</tr>
<tr>
<td>Time</td>
<td>0.6483</td>
<td>0.4145</td>
<td>0.7243</td>
<td>0.7928</td>
</tr>
<tr>
<td>Rep x site</td>
<td>0.7505</td>
<td>0.5763</td>
<td>0.0136</td>
<td></td>
</tr>
<tr>
<td>Rep x time</td>
<td>0.1314</td>
<td>0.1189</td>
<td>0.0531</td>
<td></td>
</tr>
</tbody>
</table>

1Microbiological counts are reported as log10 cfu/mL rinse.
2ANOVA model includes rep (replication), site (pre- or postchill), and time (beginning or end of processing shift).
3P-values refer to probability values; n = 40 carcasses.

### REFERENCES


