Quantitative Investigation of the Effects of Chemical Decontamination Procedures on the Microbiological Status of Broiler Carcasses during Processing

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ABSTRACT

The effects of elevated chlorine concentrations (25 ppm) added to water in the final carcass washing equipment on total viable counts (TVCs) and 

Escherichia coli and Enterobacteriaceae levels on poultry carcasses were investigated. Mean TVC counts on neck skin samples were significantly reduced when pre-evisceration and postwash samples were compared with log_{10} 4.98 to 4.52 CFU/g recovered, respectively (P ≤ 0.05). No significant reductions in TVC counts were observed in control samples at corresponding sampling points subjected to wash water containing 1 to 2 ppm chlorine. E. coli and Enterobacteriaceae counts were not significantly altered following final carcass washing in the processing plant. A second trial assessed the microbial decontamination capabilities of sodium tripolyphosphate (TSP) on broiler carcasses. Neck skin samples from carcasses were obtained before final washing (control), following a 15-s dip in potable water and after dipping in a 10% TSP solution (pH 12) for 15 s. Reductions in E. coli and Enterobacteriaceae counts were all statistically significant for both water and TSP-treated samples when compared with corresponding controls (P ≤ 0.01). The TSP treatment resulted in higher reductions of log_{10} 1.95 and 1.86/g for E. coli and Enterobacteriaceae, respectively. In contrast, reductions of log_{10} 0.37 and 0.31/g were observed for E. coli and Enterobacteriaceae counts when water-dipped carcasses were compared with corresponding controls. Significantly, Salmonella was not detected in any of the TSP-treated carcasses, while log_{10} 1.92 and 1.04/g were found in control and water-dipped samples, respectively. Thermophilic Campylobacter counts were significantly lower in both treatment groups when compared with corresponding controls resulting in log_{10} 0.55 and 1.71/g reductions for water- and TSP-dipped carcasses, respectively (P ≤ 0.01).

Live poultry and processed poultry products have often been associated with pathogenic microorganisms including Salmonella spp. and thermophilic Campylobacter spp. (3, 11, 13, 17, 24, 27). The infection and contamination of poultry by pathogenic bacteria during production and processing has been epidemiologically linked to foodborne illness in humans. The Centers for Disease Control reported that contaminated chicken and turkey meat products accounted for 1.5%, 1.9%, and 2.4% of foodborne disease outbreaks in the United States during 1995, 1996, and 1997, respectively (4).

Previous investigations have shown that, in general, bacterial counts of indicator organisms including total viable counts (TVCs) and Enterobacteriaceae, together with Escherichia coli, decrease during commercial slaughter processes with concurrent increases in the prevalence of Salmonella spp. and Campylobacter spp. on carcasses (11, 29). The effectiveness of potable water in carcass-washing systems for the removal of pathogens is limited. This was found to be a result of bacterial entrapment in skin ridges or crevices and feather follicles (15). The addition of multiple spray-washing stages during evisceration was found to reduce Enterobacteriaceae and TVCs on carcasses by approximately 1.0 and 0.5 (log_{10}), respectively (22). The addition of chlorine to poultry chill water, at concentrations of 25, 50, and 20 ppm, respectively, was demonstrated to reduce cross-contamination by Salmonella spp. (10, 20, 31). However, the performance of chlorine in carcass-washing systems has been less conclusive. It has been reported that the addition of chlorine to rinse water at a concentration of 25 ppm resulted in significant reductions in TVCs (18); however, other studies found insignificant decreases in both total aerobes and coliform organisms when 30 to 70 ppm of chlorine was added (19). Trisodium phosphate (TSP) used at concentrations of between 8 and 12% (pH > 11.5) has been shown to be an effective decontaminant of poultry carcasses and has been approved for use in the United States by the U.S. Department of Agriculture Food Safety and Inspection Service (7). TSP has been reported to remove significant numbers of E. coli, Enterobacteriaceae, thermophilic Campylobacter spp., Salmonella spp., and total aerobes from poultry carcasses by more than 2 log_{10} cycles when concentrations of between 10 and 12% (pH 11.5 to 13) were used (5, 6, 25, 30).

In Ireland, current legislation enforced by the Department of Agriculture and Food prohibits the addition of high levels of chlorine or other bactericidal agents in processing

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TABLE 1. Mean TVCs (22°C) E. coli and Enterobacteriaceae from neck skin samples recovered at three sampling points and exposed to two chlorine concentrations during the final carcass wash

<table>
<thead>
<tr>
<th>Sample point</th>
<th>Chlorine concentration (ppm)</th>
<th>TVC (22°C)</th>
<th>E. coli</th>
<th>Enterobacteriaceae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1–2</td>
<td>25</td>
<td>1–2</td>
<td>25</td>
</tr>
<tr>
<td>1</td>
<td>4.81 ± 0.31 A</td>
<td>3.30 ± 0.13 A</td>
<td>3.32 ± 0.46 A</td>
<td>3.44 ± 0.12 A</td>
</tr>
<tr>
<td>2</td>
<td>4.67 ± 0.36 A</td>
<td>3.48 ± 0.38 A</td>
<td>3.20 ± 0.28 A</td>
<td>3.53 ± 0.2 A</td>
</tr>
<tr>
<td>3</td>
<td>4.76 ± 0.27 A</td>
<td>3.27 ± 0.35 A</td>
<td>3.22 ± 0.29 A</td>
<td>3.48 ± 0.21 A</td>
</tr>
</tbody>
</table>

* No statistical significance was observed between sampling points within the same treatment group (P > 0.05). Values with different letters denote statistical significance between sampling points when treatment groups were compared (P ≤ 0.05 ± standard deviation. Sampling point 1, after defeathering; 2, after final carcass wash; 3, after air chilling.

water supplied to poultry plants. Sufficient chlorine may only be added in order to ensure that a plant’s water supply complies with potable water standards. The current studies were carried out in order to investigate the effects of altering chlorine concentrations in a carcass-washing system on the microbiological status of poultry carcasses processed under commercial conditions. In addition, the performance of TSP as a chemical decontaminant of poultry carcasses was also evaluated as a possible means of improving end product safety should current legislation be altered.

MATERIALS AND METHODS

Description of poultry plant. Sampling for chlorine and TSP evaluations were each carried out as two separate experiments. The broiler processing plant used in this study was located in Ireland and had a throughput of approximately 60,000 birds per day. All broilers were subjected to an automated inside and outside carcass rinse and air chilling immediately following evisceration. A separate evisceration water supply was used in the plant that normally contained a 25-ppm residual chlorine concentration in the final carcass-washing system. Chlorine was added in the form of a sodium hypochlorite (14% vol/vol) solution to the water supply by means of an in-line electric pump. The pulse-type pump added a fixed volume of hypochlorite to the supply during the plant’s daily production schedule.

Sampling procedures for chlorine wash evaluation (trial 1). The chlorine concentration of water at the final carcass washer was set and confirmed at 1 to 2 ppm before sampling commenced. Five neck skin samples were aseptically removed each day from birds immediately following defeathering, final washing, and air chilling over five visits to the plant. This procedure was repeated each day on birds within the same flocks when the evisceration water supply contained 25 ppm chlorine.

The concentrations of chlorine in the water supply were determined using a colorimetric comparison technique (Lovibond 2000 Mk II Tintometer; Tintometer Ltd., Salisbury, UK). Elevated chlorine concentrations in the range 5 to 50 ppm were monitored by dissolving a 300-mg potassium iodide tablet in a 10-ml sample. The color of the sample was then compared using a suitable disc in the apparatus against an untreated control sample. Chlorine concentrations in the range of 0.25 to 5 ppm were determined by dissolving a DPD 1 (Tintometer Ltd.) tablet in 10 ml of water and comparing color intensity as before, using an appropriate disc.

Sampling procedures for TSP evaluation (trial 2). Samples analyzed in this investigation were obtained from a preconfirmed Salmonella-infected flock. Initially, a 10% (wt/vol) TSP solution was prepared by dissolving 4 kg of Avgard (Rhône-Poulenc, France) in 40 liters of water obtained from the plant’s main water supply. The solution was mixed thoroughly in a 70-liter plastic container to give a final pH of 12. The TSP solution was stored overnight at 20°C to allow temperature equilibration. A similar volume (40 liters) of untreated water was also added to a separate container and its temperature was adjusted to 20°C. The following day, 30 carcasses were removed from the evisceration line immediately before the final carcass wash and a further 60 carcasses following washing. A minimum of 25 g of neck skin was aseptically removed from each of the initial 30 carcasses and placed in labeled sterile sample bags (control group A). Thirty of the carcasses subjected to the final wash were dipped individually in the vessel containing 40 liters of water for 15 s, allowed to drip dry before neck skin samples were removed, and placed in bags (treatment group B). The remaining 30 carcasses were dipped in the 10% TSP solution (40 liters) for 15 s each prior to neck skin removal and storage.

All samples from trials 1 and 2 were transported at ≤ + 4°C to the laboratory for microbiological analysis.

Microbiological analysis: trial 1. A 20-g sample from each neck skin was placed in 180 ml of 0.10% peptone water and homogenized using a stomacher (Lab Blender 400 series, Seward Medical, London, UK) for 1 min, after which serial dilutions were prepared. All samples were subsequently enumerated for TVCs (22°C), E. coli, and Enterobacteriaceae following carcass washing in water containing 1 to 2 ppm and 25 ppm chlorine. TVCs were determined using standard plate count agar with plates incubated for 72 h (1). E. coli were enumerated by spreading inocula onto tryptone bile agar plates that had previously been overlaid with membrane filters (21). Plates were incubated at 44°C and suspect colonies presumptively confirmed by detection of indole, as previously described. Enterobacteriaceae were cultured using violet red bile glucose agar and incubated for 24 h at 37°C (8). All pour and spread plates used for quantitative analysis were carried out in duplicate at each relevant dilution.

Trial 2. Each neck skin sample was aseptically trimmed to give a weight of 25 g and placed in 225 ml of 0.10% peptone water from which serial dilutions were prepared. The rinsing of samples prior to microbiological analysis was considered unnecessary as it has been previously reported that E. coli, Campylo-
TABLE 2. Mean counts of thermophilic campylobacters and Salmonella enumerated from neck skin samples subjected to immersion in TSP and potable water compared with untreated controls

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Salmonella (MPN)</th>
<th>Campylobacter</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.92 ± 0.46</td>
<td>3.59 ± 0.37</td>
</tr>
<tr>
<td>B</td>
<td>1.04 ± 0.31</td>
<td>3.04 ± 0.58</td>
</tr>
<tr>
<td>C</td>
<td>&lt;0.48 ± 0.03</td>
<td>1.88 ± 0.40</td>
</tr>
</tbody>
</table>

a Treatment: A, control; B, carcasses dipped in potable water (20°C) for 15 s; C, carcasses dipped in 10% TSP (20°C) for 15 s.
b Salmonella most probable number.
c Letters denote statistical significance between different treatment groups (P ≤ 0.01) ± standard deviation.

Statistical analysis. All bacterial counts obtained in trial 1 were transformed to log_{10} for subsequent data analysis. The effect of sampling point within the same treatment group was compared statistically using unpaired Student’s t-tests with significance defined at the 95% level (P ≤ 0.05). Corresponding sampling points between the two treatments were also compared using unpaired Student’s t-tests and identical confidence intervals.

Statistical analysis in trial 2 applied unpaired Student’s t-tests to compare TVCs, Enterobacteriaceae, E. coli, and Campylobacter spp. levels on skin samples subjected to the three treatments. Salmonella counts obtained with the most probable number technique were compared using analysis of variance as previously described (9). Statistical significance was defined at the 99% level (P ≤ 0.01).

RESULTS AND DISCUSSION

Carcasses washed in water containing 1 to 2 ppm residual chlorine generally demonstrated little improvement in microbial counts in trial 1. Mean total counts (22°C) along with E. coli and Enterobacteriaceae counts expressed as log_{10} per gram for washed carcasses exhibited statistically insignificant reductions of 0.14, 0.18, and 0.09, respectively, when compared to corresponding pre-evisceration samples (P ≤ 0.05). No significant changes in counts of the organisms tested were observed when samples taken after the final carcass washing and following air chilling were compared (Table 1). This is in contrast with previous studies that have reported significant decreases in TVCs, E. coli, and Enterobacteriaceae when carcasses sampled prior to evisceration and following chilling were compared (11, 16). Other investigators have reported insignificant reductions in TVCs on poultry carcasses after final washing (18, 22), while Enterobacteriaceae levels increased by 1 log_{10} during evisceration and washing (22).

Carcasses subjected to final washing in 25 ppm chlorinated water exhibited more substantial reductions in bacterial counts than those washed in 1 to 2 ppm. A statistically significant reduction (P ≤ 0.05) in TVCs of log_{10} 0.46/g was obtained between pre-evisceration and postwash samples. E. coli and Enterobacteriaceae counts were not significantly altered between the two sampling points. Total viable and Enterobacteriaceae counts were significantly different for postwashing and chilling samples when both treatments were compared. Overall, the application of elevated chlorine concentrations in the final carcass wash water reduced the levels of the organisms. However, the effectiveness of chlorine as a microbial decontaminant is questionable, as the reductions in public health terms were not substantial. The findings of the present study concurred with those of previous studies where modest microbial reductions on poultry carcasses after exposure to similar chlorine concentrations were observed (19, 31).

Levels of Salmonella were statistically different for both treatment groups in trial 2, when each was compared with corresponding controls (P ≤ 0.01). The mean count of log_{10} 1.04/g obtained from water-dipped carcasses suggested that water was not as effective in removing or destroying Salmonella spp. from carcasses as the TSP solution, in which case the organism was not detected (Table 2). These results were in agreement with previous studies

TABLE 3. Mean TVCs (30°C) and E. coli and Enterobacteriaceae counts recovered from neck skin samples subjected to immersion in TSP and potable water compared with untreated controls

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TVC (30°C)</th>
<th>E. coli</th>
<th>Enterobacteriaceae</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4.59 ± 0.25</td>
<td>3.10 ± 0.31</td>
<td>3.58 ± 0.22</td>
</tr>
<tr>
<td>B</td>
<td>4.53 ± 0.25</td>
<td>2.73 ± 0.32</td>
<td>3.27 ± 0.24</td>
</tr>
<tr>
<td>C</td>
<td>3.70 ± 0.26</td>
<td>1.15 ± 0.66</td>
<td>1.41 ± 0.53</td>
</tr>
</tbody>
</table>

a Treatment: A, control; B, carcasses dipped in potable water (20°C) for 15 s; C, carcasses dipped in 10% TSP (20°C) for 15 s.
b Different letters denote statistical significance between different treatment groups (P ≤ 0.01) ± standard deviation.
on the performance of TSP in the decontamination of Salmonella on poultry carcasses (12, 14, 26).

Thermophilic Campylobacter counts were significantly reduced in both treatment groups compared to samples in control group A (\(P \leq 0.01\)). Reductions of \(\log_{10} 0.55\) and 1.71/g were observed in treatments B and C, respectively, in comparison to untreated equivalents. Decontamination rates for Campylobacter spp. of \(\log_{10} 1.5\) and 1.3/g, respectively, have previously been reported for carcasses dipped in 10% TSP solutions (6, 28).

TVCs were significantly reduced by \(\log_{10} 0.89/g\) on the TSP-treated carcasses alone (\(P \leq 0.01\)). Both \(E. coli\) and Enterobacteriaceae counts were significantly reduced for treatments B and C, when compared with corresponding controls (\(P \leq 0.01\)). Again, TSP-dipped samples exhibited the greatest reductions in \(E. coli\) and Enterobacteriaceae, with \(\log_{10} 1.95\) and 2.17/g observed, respectively (Table 3). Significant reductions in counts of these organisms were previously reported for TSP-treated carcasses by other authors (25, 26). The incorporation of a short incubation period in nonselective media prior to selective subculturing may have resulted in the recovery of higher numbers of sublethally injured microorganisms. However, a previous investigation using electron micrographs of TSP-treated chicken skin samples inoculated with Salmonella Typhimurium suggests that the decontaminant’s principal mode of action is based on the physical detachment of cells from poultry skin surfaces (12).

The present study illustrates the effectiveness of TSP as a decontaminant of pathogenic microorganisms on poultry carcasses. It is suggested that the application of TSP on carcasses originating from Salmonella- or Campylobacter-infected flocks would significantly reduce risks to public health associated with the consumption of pathogen-contaminated poultry products.

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REFERENCES


