Antibacterial Activity of a Sodium Acid Pyrophosphate Product in Chiller Water Against Selected Bacteria on Broiler Carcasses

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ABSTRACT

Bactericidal activity of Brifisol KTM (a commercial blend of sodium acid pyrophosphate and orthophosphoric acid) (BK Ladenburg, Ladenburg, Germany) was evaluated during chilling of broiler carcasses. Brifisol KTM (1.5% at 1°C for 60 min) significantly reduced Escherichia coli, coliforms and aerobic plate counts (APC) on postchill broilers and increased shelflife by 1 to 2 days when stored at 4.4°C. Reductions in incidence and levels of Salmonella were directly related to successful neutralization of the carcass rinse solutions. Effective neutralization of all phosphate applications, whether the treatment is alkaline or acidic-based, is extremely important for accurate quantification of bactericidal efficacy.

Key words: Broilers, pyrophosphates, chilling, antibacterial activity

Phosphates are added to meat and poultry products as curing agents and to increase water holding capacity (9). Recently, U.S. Department of Agriculture (USDA) approved the use of trisodium phosphate (TSP) as a postchill processing aid to reduce bacteria on raw poultry carcasses (1). The treatment solution has a pH between 11.6 and 13.0. In studies supportive of TSP, broiler carcasses have been artificially inoculated with nalidixic acid-resistant Salmonella typhimurium. Significant reductions in numbers of this organism and others with a 10% TSP postchill dip solution have been reported (1). Investigators have speculated that TSP works by removing a thin layer of fat on the surface of poultry skin allowing for removal of bacteria from the surface of the carcass (7).

Prior to approval of TSP, the use of pyro- and polyphosphates to control bacteria on poultry carcasses was investigated. Researchers chilled broiler carcasses in 3% and 8% solutions of sodium tripolyphosphate (STPP)/tetra sodium pyrophosphate (TSPP) blend for 20 to 24 h (6). Increases in shelf-life of 17% and 25%, respectively, were reported. In a similar study, researchers chilled broilers in an 8% solution of the same phosphate blend for 6 h (16), and shelflife was increased by 1 to 2 days.

Researchers have investigated the addition of various phosphates to ground meat (10,12). During temperature abuse, sodium acid pyrophosphate (SAPP) was the most effective for inhibiting the growth of microorganisms compared to longer chain phosphates and TSPP (10). In other studies, SAPP was compared to TSPP and a neutral pyrophosphate (trisodium pyrophosphate) in ground meat. SAPP was the most effective in inhibiting growth of microorganisms during temperature abuse. The influence of phosphates on selected spoilage bacteria on vacuum-packed sliced bologna has also been investigated (13). Addition of STPP alone had little effect on bacteria that were inoculated onto bacon. However, an acidic phosphate blend (sodium pyrophosphate + STPP + sodium polyphosphate) strongly inhibited growth of the inoculated organisms.

Preliminary studies treating poultry carcasses with a commercial blend of SAPP and orthophosphoric acid, Brifisol KTM, have produced favorable results with regard to bacterial reductions (3). This was the basis for the following series of experiments in which the use of this SAPP blend as a bactericide for raw poultry was evaluated.

MATERIALS AND METHODS

Experiment 1 (trials 1 and 2)

In Experiment 1, the bactericidal effects of chilling carcasses in Brifisol KTM solutions were evaluated in two replicate trials. In each trial, commercial broilers from a local hatchery were raised on a research poultry farm. Birds were gavaged with 0.5 ml of nutrient broth containing 2.0 x 10⁹ CFU/ml of a 24-h culture of a nalidixic acid-resistant (NAL) Salmonella typhimurium on days 3, 7, 14 and 21. Eighty broilers in each of the two replicate trials were processed in a pilot processing facility at 42 (Trial 1) and 49 (Trial 2) days of age.

Following evisceration, carcasses were assigned to one of four immersion chilling treatments. The first group of 20 carcasses was chilled for 1 h in 20 L of ice water. The second, third and fourth groups of 20 carcasses were chilled for 1 h in 20 L of ice water containing 0.5, 1.0 or 1.5% Brifisol KTM. The pH of all treatment solutions was measured and recorded; however, in these trials no attempt was made to neutralize the pH of the recovered rinse solutions.

After chilling, carcasses were transferred to clean containers containing 20 L of ice water, where they were continuously agitated for 10 min. Carcasses were removed from the ice water
and allowed to drain for 15 min. After draining, carcasses were
placed in sterile bags to which 100 ml of 0.1% peptone was added (4). Carcasses were shaken in a mechanical shaker (5) for 1 min. Bags were aseptically opened and the rinse fluid was poured into sterile bottles, which were placed on ice until microbiological evaluations were performed (<1 h) After bacteriological evaluations were initiated, five rinse samples were pooled together from each treatment and the pH of the solution was measured.

The recovered rinse solution samples were plated in duplicate on APC and E. coliicoliform petrifilm™ (Medical-Surgical Division, 3M, St. Paul, MN). All samples were serially diluted and added to tetrasionate Hajna broth (Difco Laboratories, Inc., Detroit, MI) to determine most probable number (MPN) NAL Salmonella, using a 3-tube MPN procedure (15). Tubes were incubated at 42°C for 24 h and then streaked onto brilliant green and MacConkey agar plates. Both media contained 200 ppm nalidixic acid. Plates were incubated at 37°C for 24 h. Any presumptive positive colonies were assumed to be NAL S. typhimurium.

**Experiment 2 (trials 1 and 2)**

It was determined in Experiment 1 that 1.5% Brifisol K™ was the optimum level for a chill water application; higher levels resulted in skin discoloration. Thus, in two similar trials in Experiment 2, 1.5% Brifisol K™ was again evaluated as a chill water treatment. However, in these two trials there were some procedure modifications.

On two occasions, 20 pre-chill carcasses were obtained from a local processing plant. Upon arrival at a pilot processing plant, carcasses were split longitudinally and each carcass half was wing-banded to reduce variation between treatments by comparing corresponding halves (8). The right halves were designated as the treatment and the left halves were used for the untreated control.

In each of the two replicate trials, 20 right halves were chilled for 1 h in 40 L of a 1.5% Brifisol K™ ice water solution (most effective treatment from Experiment 1). The 20 corresponding left halves were chilled at the same time in 40 L of ice water. The chill water in both tanks was continually circulated using a commercial aquarium pump.

In each of the two similar trials in Experiment 2, carcass halves were rinsed with 100 ml 0.1% peptone plus 1.0% Tween 80 (a surfactant). Tween was used in these trials because the manufacturer of the phosphate blend felt that addition of a surfactant might improve the antibacterial properties of their product. Five milliliters of 1.0% (wt/vol) Tris hydrochloric acid (HCl) buffer was added to the rinse fluid immediately following the rinse procedure to neutralize the recovered rinse solution. This is similar to the procedure used to neutralize whole carcass rinse samples following TSP treatment (2), where dilute HCl is added to recovered rinse solutions. In both of these trials, the pH of the recovered carcass rinse solutions was measured before and after addition of the Tris HCl buffer to ensure neutralization of the phosphate treatment.

Coliforms, E. coli and APC per milliliter of rinse fluid were determined as described in Experiment 1. Tetrasionate Hajna broth (10x) (Difco) was added to the remaining rinse solution to determine incidence of indigenous Salmonella. Samples were incubated at 42°C for 24 h and then streaked on modified lysine iron agar (MLIA) (Oxoid, Ogdenburg, NY) and xylose lysine tergitol-4 agar (XLT-4) (FMTI, Riviera Beach, FL). Plates were incubated at 42°C for 24 h. Presumptive colonies were picked, and then streaked and stabbed on lysine iron agar slants (LIA) and triple sugar iron slants (TSI) (Difco) at 37°C for 24 h. Presumptive colonies were confirmed using somatic antiserum poly A-I and Vi (Difco).

**Experiment 3 (trials 1, 2 and 3)**

In Experiment 3, the effect of 1.5% Brifisol K™ in poultry chill water on the shelf-life of broiler carcasses was evaluated in three replicate trials. Attempts were made using Tris HCl buffer to neutralize the rinse solution pH during the whole carcass rinse procedure as opposed to neutralization following the recovery of the rinse solution as was done in the previous two experiments (Table 3).

All broilers for this study were raised on a research farm to 6 weeks of age. Birds were gavaged with 0.5 ml of nutrient broth containing 2.0 x 10⁶ CFU/ml of a 24-h culture of S. typhimurium (ATCC 14028) on days 0, 3, 7, 14 and 21. It was discovered in unrelated studies that this strain of S. typhimurium, which is not resistant to nalidixic acid, was more capable of colonizing young chicks (17).

In each of three trials, 20 birds were processed in a pilot processing facility at 42 days of age. Carcasses were split and wing-banded. Twenty right halves were chilled 1 h in 40 L of 1.5% Brifisol K™ in ice water. The 20 corresponding left halves were chilled at the same time in 40 L of ice water. The chill water in both tanks was circulated with a commercial aquarium pump.

Following the chill water treatments, carcasses to be sampled on Day 0 (10/trial) were rinsed with 100 ml buffered peptone water (BPW) (Difco). The remaining halves were held at 4.4°C for shelf-life determination. On each sampling day, two treated halves and the corresponding control halves were removed from refrigerated storage and were rinsed with 100 ml BPW on days 2, 4, 6, 8, 10 and 20. Rinse samples were evaluated for E. coli, coliforms and APC as previously described. The remaining part of the rinse solution was serially diluted to determine MPN Salmonella as previously described.

**Statistical analyses**

All quantitative microbiological data were transformed to log₁₀ prior to statistical analysis. For Salmonella-negative samples one-third of the lower detection level was used for statistical analysis. Treatment effects were analyzed using the General Linear Model procedure (SAS Institute, Inc., Cary, NC). Contrast statements were used to determine the linear, quadratic and cubic trends of the treatment means with the trial by treatment mean square as the error term for E. coli and Salmonella. Trial by treatment on APC and coliform counts was tested with the subsampling mean square. The subsampling mean square was pooled with the trial by treatment mean square for the error term for analyzing the main effect of treatment on APC and coliforms. Treatment means were separated using Least Square Means with the same error term as the contrast statements to obtain standard errors. In Experiment 2, incidence of Salmonella was not analyzed because treatment and control incidences were identical. In Experiment 3, the effects of storage time (day) were analyzed using the linear model of mean square of trial by day as the error term. The interaction of day and treatment for APC was analyzed using trial by day by treatment as the error term. The effects of phosphate treatment on APC were analyzed using trial by treatment as the error term for each day separately. Escherichia coli, coliforms and Salmonella were not analyzed separately by day, since the day by treatment interaction was not significant. For these organisms, trial by treatment was tested using trial by day by treatment as the error term.

**RESULTS AND DISCUSSION**

Treatment of broiler carcasses during chilling with the SAPP/orthophosphoric acid blend, Brifisol K™, was the first application investigated. Chill water treatments are a good way to evaluate potential bactericidal activity of a
product because of the relatively long exposure period (45 to 60 min). Preliminary experiments conducted to evaluate the effects of Brifisol on the appearance of treated broiler carcasses indicated that greater than 1.5% Brifisol K™ altered the appearance of postchill broilers.

Experiment 1 (trials 1 and 2)
There was no trial by treatment interaction in Experiment 1; therefore, the results from trials 1 and 2 have been combined for presentation and discussion (Table 1). The addition of 1.5% Brifisol K™ to poultry chill water significantly (P<0.05) reduced the levels of all organisms evaluated; however, the reduction in MPN NAL Salmonella was not linear in regard to level of Brifisol K™. The elevated level (log_{10} 1.23) for carcasses treated with 0.5% Brifisol K™ was due to one carcass that had an unusually high level of NAL Salmonella. Only 1 of 40 carcasses was positive for NAL Salmonella when carcasses were treated with 1.5% Brifisol K™, and no carcasses tested positive when treated with 1.0% Brifisol K™. No detrimental effects on appearance of the treated carcasses were noted.

The pH values of the treatment solutions were much lower than those reported for meat products containing SAPPs. The chill water pH values were 3.0, 2.9 and 2.8 for the 0.5, 1.0 and 1.5% phosphate solutions, respectively. The 0.1% peptone rinse solution did not completely neutralize the acid in the whole carcass rinse fluid. The pH values of the recovered rinse samples were 5.7, 5.0, 4.8 and 6.8 for the 0.5, 1.0, 1.5 and 0.0% phosphate treatments, respectively. Bacterial reductions may not have been as great as those reported if the rinse solution had been neutralized.

Experiment 2 (trials 1 and 2)
There was no trial by treatment interaction; therefore, the results from trials 1 and 2 have been combined for presentation and discussion (Table 2). In these trials pH values of the recovered carcass rinse solutions were closer to neutrality than those noted in Experiment 1. Before addition of Tris HCl, the pH of the recovered rinse solutions was 4.8; the pH increased to 6.8 following the addition of the Tris HCl buffer. Fewer microorganisms were recovered from the phosphate-treated carcasses but reductions were statistically significant for coliforms and E. coli only (Table 2). Incidence of indigenous Salmonella was 29/40 (72.5%) in both the treatment and control group. The disagreement in Salmonella incidence data between experiments 1 and 2 suggests that either the NAL Salmonella used in Experiment 1 is more sensitive to the phosphate treatment than are indigenous Salmonella, or successful neutralization of the recovered carcass rinse solution is extremely important in regard to determination of bactericidal potential.

Experiment 3 (trials 1, 2 and 3)
There was no treatment by trial by day interaction; therefore data from the three trials have been combined for presentation (Table 3). Buffered peptone water neutralized the pH of the recovered rinse fluid in these trials. The pH of rinse solutions recovered from phosphate-treated carcasses was in the range of 6.0 to 6.3. There was a day by treatment interaction for APC (P=0.003), so treatment effects were analyzed by day. Aerobic plate counts on days

<table>
<thead>
<tr>
<th>% Brifisol K™</th>
<th>log_{10} CFU/ml</th>
<th>( \log_{10} ) CFU/ml</th>
<th>log_{10} CFU/ml</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>APC(^1)</td>
<td>E. coli</td>
<td>Coliforms</td>
<td>NAL Salmonella</td>
</tr>
<tr>
<td>0.0</td>
<td>3.37</td>
<td>1.80</td>
<td>1.99</td>
<td>0.32</td>
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<td>0.5</td>
<td>3.26</td>
<td>1.08</td>
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<td>1.23</td>
</tr>
<tr>
<td>1.0</td>
<td>3.12</td>
<td>1.05</td>
<td>1.56</td>
<td>0.00</td>
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<td>1.5</td>
<td>3.03</td>
<td>0.83</td>
<td>0.84</td>
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<tr>
<td>PSEM(^2)</td>
<td>0.07</td>
<td>0.22</td>
<td>0.01</td>
<td>0.45</td>
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</table>

\(^a\) Row means with different superscripts differ significantly (P<0.05).
\(^b\) S. typhimurium resistant to 200 ppm nalidixic acid; chickens were gavaged at 3, 7, 14 and 21 days of age.
\(^c\) MPN is three-tube most probable number; lower detection level is three organisms/100 ml.
\(^d\) APC is aerobic plate count at 30°C.
\(^e\) PSEM is pooled standard error of the mean.

TABLE 1. Effects of Brifisol K™ in poultry chill water on selected bacteria including NAL Salmonella on broiler carcasses (Experiment 1, n=40).

<table>
<thead>
<tr>
<th>% Brifisol K™</th>
<th>log_{10} CFU/ml</th>
<th>log_{10} MPN/100 ml</th>
<th>( \log_{10} ) MPN/100 ml</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>APC(^1)</td>
<td>E. coli</td>
<td>Coliforms</td>
<td>NAL Salmonella</td>
</tr>
<tr>
<td>0.0</td>
<td>3.46(^a)</td>
<td>2.08(^a)</td>
<td>2.09(^a)</td>
<td>(29/80)(^a)</td>
</tr>
<tr>
<td>1.5</td>
<td>3.08(^a)</td>
<td>1.22(^b)</td>
<td>1.32(^b)</td>
<td>(29/80)(^a)</td>
</tr>
<tr>
<td>PSEM(^2)</td>
<td>0.05</td>
<td>0.12</td>
<td>0.11</td>
<td></td>
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</tbody>
</table>

\(^a\) Row means with different superscripts differ significantly (P<0.05).
\(^b\) APC is aerobic plate count at 30°C.
\(^c\) PSEM is pooled standard error of the mean.

TABLE 2. Effects of Brifisol K™ in poultry chill water or selected indigenous bacteria on broiler carcasses (Experiment 2, n=40).

| Incidence |
|-----------|--------|--------|--------|
| NAL Salmonella | (17/40)\(^a\) | (5/40)\(^a\) | (0/40)\(^a\) |
| Incidence Salmonella | (1/40)\(^a\) | | |

\(^a\) Column means with different superscripts differ significantly (P<0.05).
TABLE 3. Effects of Brifisol K™ in poultry chill water on selected organisms during storage at 4.4°C (n=30 on Day 0, n=6 on all other days).

<table>
<thead>
<tr>
<th>Organism evaluated</th>
<th>% Brifisol K™</th>
<th>Days of storage at 4.4°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>log₁₀ APC CFU/ml</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>log₁₀ E. coli CFU/ml</td>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
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<td></td>
<td>x</td>
</tr>
<tr>
<td>log₁₀ Coliforms CFU/ml</td>
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<td>0.0</td>
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<td></td>
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<td>1.5</td>
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<tr>
<td>log₁₀ Salmonella ³ MPN/100 ml</td>
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</tr>
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<td></td>
<td></td>
<td>1.5</td>
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<td>x</td>
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</tbody>
</table>

¹ Column means with different superscripts differ significantly (P≤0.05).
² Row means with different superscripts differ significantly (P≤0.05).
³ APC is aerobic plate count at 30°C.
⁴ PSEM is pooled standard error of the mean.
⁵ Chickens were gavaged at 0, 3, 7, 14 and 21 days of age. Results include indigenous Salmonella as well as S. typhimurium (ATCC 14028).
⁶ MPN is three-tube most probable number; lower detection level is three organisms/100 ml.

0 and 2 were not affected by treatment; however, the phosphate treatment reduced APC on days 4 and 6. Control carcasses reached spoilage levels (10⁵ CFU/ml) by Day 6. Treated carcasses were not spoiled until sometime between Day 6 and Day 8. Others have also noted a slight increase in shelf-life of raw poultry treated with acid phosphates (16).

Coliform and E. coli populations were reduced during refrigerated storage (Table 3). Phosphate treatment significantly lowered both E. coli and coliform counts on Day 0 and counts remained lower (P<0.05) throughout the storage period. There was no day by treatment interaction, although treatment and control levels of coliforms were the same by Day 6. Levels of E. coli and coliforms could not be determined on Day 8 because the growth of background organisms on the E. coli /coliform plates interfered with colony counting and interpretation of results.

Salmonella does not grow below 7°C (14), and did not grow, but remained at a constant level in both groups of carcass halves, during refrigerated storage (Table 3). The phosphate treatment had no effect on numbers of Salmonella recovered from the carcass halves on any of the sampling days.

The use of a substance that helps detach bacteria from broiler skin in conjunction with Brifisol K™ might improve the effectiveness of a chill water application. Organisms in solution are typically much more susceptible to bactericides than organisms that are attached to the surface of the carcass. A pre-chill foam treatment might improve the effectiveness of Brifisol K™ by allowing the phosphate product to penetrate the lipid layer on the surface of the skin.

REFERENCES
3. BK Ladenburg, Ladenburg, Germany. (Unpublished data).