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Research Note

Validation of a 2 Percent Lactic Acid Antimicrobial Rinse for Mobile Poultry Slaughter Operations

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ABSTRACT

Poultry processing antimicrobial interventions are critical for pathogen control, and organic, mobile operations in Washington seek alternatives to chlorine. Laboratory and field studies (three replications each) evaluated lactic acid efficacy as a chlorine alternative. For the laboratory study, retail-purchased, conventionally processed chicken wings inoculated with *Salmonella* were randomly assigned to the following treatments: *Salmonella* inoculation followed by no treatment (10 wings) or by 3-min rinses of water, 50 to 100 ppm of chlorine, or 2% lactic acid (20 wings for each rinse treatment). Wings were sampled for *Salmonella* enumeration on xylose lysine desoxycholate agar. During pastured poultry processing at mobile slaughter units for each field study replication, 20 chicken carcasses were randomly assigned to each treatment: untreated control or 3-min immersion in lactic acid or chlorine. Whole-carcass rinses were examined for aerobic plate count (APC) on tryptic soy agar and coliforms on violet red bile agar. Untreated controls were also examined for *Salmonella*. In the laboratory study, lactic acid produced a significant ($P < 0.01$) *Salmonella* reduction compared with the inoculated no-rinse, water, and chlorine treatments, which were statistically similar to each other. In the field study, no *Salmonella* was detected on untreated controls. Lactic acid produced significant >2-log ($P < 0.01$) reductions in APC and coliforms, whereas chlorine resulted in slight, but significant 0.4-log reductions ($P < 0.01$) and 0.21-log reductions ($P < 0.05$) in APC and coliforms compared with untreated controls. Considering laboratory and field studies, lactic acid produced greater reductions in *Salmonella,* APC, and coliforms, validating its effectiveness as a chlorine alternative in mobile poultry slaughter operations.

Interest in pastured poultry production and on-farm poultry slaughter has increased over the last 20 years. Through exemptions in the Poultry Products Inspection Act, many states offer opportunities for individual farms to raise and process up to 1,000 broilers per year for direct sale to consumers to supply local and intrastate food systems *(3, 11)*. Furthermore, marketing organic poultry can result in increased profitability for producers *(26)*. On-farm processing of poultry varies depending on equipment availability (such as scalders and pluckers), producer resources, and facilities (indoor versus outdoor processing). The slaughter and carcass processing steps are performed with more manual input than in automated commercial processing. Data addressing typical microbial levels on on-farm processed poultry carcasses are currently unavailable. Food safety has been identified as a processing issue faced by mobile processors *(11),* but discussion of antimicrobial interventions other than a final ice water chilling step is limited *(3, 12)*.

Several interventions have been examined, either alone or in combination, to control and reduce foodborne pathogens on poultry carcasses, including water, chlorine, organic acids, ozone, bacteriocins, and hydrogen peroxide (reviewed by Bolder *(6)* and Hugas and Tsigarida *(16)*). Chlorine is the most frequently used antimicrobial intervention in commercial poultry processing due to its availability, low cost, and efficacy *(20)*. However, chlorine reacts with organic materials relatively easily and can quickly lose effectiveness, thus requiring careful monitoring for appropriate replenishment *(4, 7)*. Several organic acids, including acetic acid and citric acid, have been studied in regard to their effectiveness as antimicrobial interventions in meat and poultry processing *(2, 9, 10, 13)*. Lactic acid has a thoroughly studied mechanism of action and generally regarded as safe status; several studies demonstrated the effectiveness of lactic acid as an antimicrobial intervention in red meat processing *(14, 15),* and lactic acid is commonly used in commercial beef slaughter operations. Other studies have evaluated lactic acid as a poultry processing intervention *(1, 5, 17, 22)*. However, most antimicrobials have not been studied under mobile poultry slaughter conditions.

In Washington, some organic poultry growers and operators of mobile poultry slaughter units utilize chlorine as an antimicrobial rinse but were interested in identifying an alternative that would meet U.S. Department of Agriculture standards for organic labeling *(28)*. These processors also expressed interest in identifying antimicrobial alternatives with greater consumer acceptance. Lactic acid is an appealing option for production of organic poultry

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products because the U.S. Department of Agriculture–National Organic Program *(28)* states that lactic acid is an allowed substance in or on processed products labeled as organic. Laboratory and field studies were performed to validate a 3-min, 2% lactic acid antimicrobial rinse for poultry carcasses as an alternative to chlorine. A laboratory inoculation study was conducted to examine the effectiveness of water, 50 to 100 ppm of chlorine, and 2% lactic acid for *Salmonella* spp. reduction on chicken wings. A field study examined a 50- to 100-ppm chlorine rinse, currently used by some processors, and a 2% lactic acid rinse for whole chicken carcasses under mobile poultry slaughter conditions. The incidence of *Salmonella* on organic poultry carcasses in western Washington was also examined. This study provides the first validation data for antimicrobial interventions under mobile poultry slaughter conditions.

MATERIALS AND METHODS

Laboratory study: strain activation and cocktail and inoculation solution preparation. Four isolates of *Salmonella* were utilized: *Salmonella* Enteritidis ATCC 13076 (acquired from ATCC), *Salmonella* Typhimurium 14028 (an ATCC strain, acquired from a culture collection at Texas Tech University, Lubbock), and *Salmonella* Heidelberg S9481 and *Salmonella* Kentucky S94611 (poultry isolates obtained from the Washington State University Veterinary Microbiology and Pathology laboratories, Pullman). Frozen cultures were activated with two successive passes in 9 ml of tryptic soy broth (TSB; Hardy Diagnostics, Santa Maria, CA) and incubated at 37°C for 18 to 24 h. Then, for each individual culture 1 ml of the stock inoculum was added to 100 ml of TSB and incubated with shaking for 18 to 24 h at 37°C.

On the day of the study, the four 100-ml *Salmonella* cultures were combined and mixed thoroughly. An inoculation solution was prepared by combining the *Salmonella* cocktail to 3 liters of TSB. The concentration of the inoculation solution was 8.0 log CFU/ml, as determined by plating serial dilutions on xylose lysine desoxycholate agar (XLD; Hardy Diagnostics).

Laboratory study: antimicrobial rinse preparation. For the water rinse, tap water (7.57 liters) was utilized to reflect potable water sources available to mobile poultry slaughter operations. For the chlorine rinse, 18.75 ml of chlorine bleach (5.7% available chlorine; The Clorox Company, Oakland, CA) was added to 7.57 liters of tap water. The free available chlorine concentration (in parts per million) was measured with a chlorine test strip (pHydrion Micro Chlorine, Micro Essential Laboratory, Brooklyn, NY) and was between 50 and 100 ppm; chlorine test strips were used because this method would be the most accessible to mobile poultry processors for monitoring chlorine concentrations. The initial pH of the chlorine rinse was 8.4, and pH declined to 7.4 by the end of the sampling. During each replication, the chlorine concentration remained stable, between 50 and 100 ppm, and did not require a fresh solution. The 2% lactic acid rinse was prepared by adding 178.1 ml of 85% lactic acid (Purac FCC 88, Purac America, Lincolnshire, IL) to 7.57 liters of tap water, and the pH of the solution was 2.4.

Laboratory study: sampling and microbial analysis. The purpose of the laboratory study was to evaluate pathogen reduction by the antimicrobial treatments; however, inoculation of whole chicken carcasses was not feasible in the available facilities for safety considerations. A preliminary study was conducted to determine the microbial load on chicken wings, legs, breasts, and thighs to select a chicken cut to represent the microbial load similar to whole chicken carcasses. Chicken wings were selected as the most appropriate cut (data not shown).

Three replications were performed. For each replication, chicken wings (conventionally processed) were purchased at a local retail store, and 90 wings were randomly assigned to the following treatments: no inoculation (20 wings), inoculation and no rinse (10 wings), inoculation and water rinse (20 wings), inoculation and chlorine rinse (20 wings), and inoculation and lactic acid rinse (20 wings). Chicken wings were inoculated by being placed three at a time for 20 s in the inoculation solution followed by drying under a hood at least 20 min to allow *Salmonella* attachment *(1, 8, 13, 18, 30, 31)*.

For rinse treatments, individual chicken wings were placed in the rinse solution (at ambient temperature) for 3 min. A 3-min rinse was selected because processors indicated that although most carcasses would receive a longer application time, 3 min would be the least amount of time a carcass would remain in a rinse solution. After treatment, wings were placed in a stomacher bag (VWR, West Chester, PA) with 99 ml of 0.1% peptone (Becton Dickinson, Sparks, MD) water and massaged by hand for 2 min. Serial dilutions were prepared, spread plated in duplicate on XLD, and incubated at 35°C for 24 h. Samples from uninoculated wings were also plated in duplicate on tryptic soy agar (TSA; Hardy Diagnostics) and incubated at 35°C for 48 h. Colonies were enumerated manually, and the CFU per ml (CFU per wing) of the rinse solution was calculated.

Field study: antimicrobial rinse preparation. The 50- to 100-ppm chlorine rinse was prepared with approximately 15.1 liters of water and 37.5 ml of chlorine bleach (5.7% available chlorine; The Clorox Company). Chlorine levels were monitored by chlorine test strips, and fresh solutions were prepared when concentrations measured below 50 ppm. The need to prepare a fresh chlorine rinse solution differed between replications, varying from one to three additional preparations. The 2% lactic acid rinse (pH 2.4 throughout the sampling) was prepared by measuring 356.2 ml of 85% lactic acid (Purac FCC 88, Purac America) into approximately l5.1 liters of water. The pH of the lactic acid solution did not indicate that fresh solutions were needed; however, for each replication, the solution was changed at least once to maintain aesthetic appearance and sanitary processing conditions due to accumulation of materials such as feathers and blood in the solution.

Field study: sampling. Three replications were performed on three different days of production over a 1-year period, utilizing the same mobile poultry slaughter process and personnel. For each replication, the carcasses (60 carcasses per replication for a total of 180) were processed on the farm where the chickens were raised as pastured poultry. For each replication, 20 carcasses were randomly assigned to each of the following treatments: no treatment, 50- to 100-ppm chlorine rinse, and a 2% lactic acid rinse.

The untreated carcasses were sampled immediately after evisceration and a water spray wash. For chlorine and lactic acid treatments, after evisceration and a water spray wash, individual carcasses were immersed in either the chlorine or lactic acid rinse at ambient temperature for 3 min and then sampled. A whole-carcass rinse method was used for sampling. Each carcass was placed in a poultry rinse bag with 200 ml of 0.1% peptone water and massaged by hand for 2 min. The carcass rinse was collected in a sterile 50-ml centrifuge tube. The tubes were immersed in ice for

at least 15 min to rapidly chill to 4°C. The carcass rinse samples were transported to Pullman, WA, at 4°C for further laboratory analysis.

Field study: microbiological analyses. All samples were examined for aerobic plate counts and coliforms. Untreated carcasses were also examined for the incidence of *Salmonella*. The carcass rinses were serially diluted and plated in duplicate on TSA (Hardy Diagnostics) for determination of aerobic plate count, and on violet red bile agar (Hardy Diagnostics) for examination of coliforms. Plating was performed by an automated spiral plater (Autoplate 4000, Spiral Biotech Inc., Norwood, MA). TSA and violet red bile agar plates were incubated at 35°C for 48 and 24 h, respectively. The colonies were enumerated by an automated counting system (Q-count, Spiral Biotech Inc., Norwood, MA), and the CFU per carcass was calculated.

For *Salmonella* isolation, 11 ml of each carcass rinse (60 total) was preenriched in 99 ml of buffered peptone water (HiMedia Laboratory Inc., Mumbai, India) and incubated at 37°C for 24 h. This was followed by selective enrichment with Rappaport-Vassiliadis broth (Becton Dickinson) incubated at 42°C for 24 h and with tetrathionate broth (Becton Dickinson) incubated at 35°C for 24 h. After selective enrichment, samples were streaked for isolation on XLD agar incubated at 35°C for 24 h (Hardy Diagnostics) and bismuth sulfite agar (Hardy Diagnostics) and incubated at 35°C for 48 h. Presumptive-positive colonies were examined for biochemical and serological reactions by using triple sugar iron agar (Hardy Diagnostics), lysine iron agar (Acumedia Manufacturers, Lansing, MI), and a *Salmonella* latex agglutination test (Oxoid Ltd., Basingstoke, England).

Statistical analysis. Both the laboratory and field studies were a randomized complete block design with blocking by replication. Data were analyzed by using the mixed procedure from SAS software (release 9.1, 2003, SAS Institute, Cary, NC) after logarithmic transformation. Means were separated by Fisher's least significant difference test, and significance was tested at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Laboratory study. Prior to inoculation with *Salmonella,* chicken wing aerobic plate count (APC) levels were 4.09 log CFU per wing and levels of hydrogen sulfide (H_2S)–producing bacteria were 3.78 log CFU per wing. The observed APC levels were comparable to the levels (range, 3.4 to 4.7 log CFU per wing) reported immediately after inside-outside bird washing at commercial plants *(19)*. In the present study, inoculation with *Salmonella* significantly ($P < 0.01$) increased levels of H_2S-producing bacteria to 5.78 log CFU per wing (Table 1), so that *Salmonella* organisms were the predominant microflora present on the chicken wings.

Salmonella counts for the water rinse treatment (5.81 log CFU per wing) were similar ($P = 0.76$) to that of the inoculated control (5.78 log CFU per wing) (Table 1). Although Thomson et al. *(25)* observed significant reductions (0.69 to 1.19 log CFU/cm^2) with water spray washing using 56.6 to 71.1°C water, other studies reported that water spray washes did not result in significant microbial reductions on chicken carcasses *(10, 21, 30)*.

Salmonella counts for the chlorine rinse treatment (5.69 log CFU per wing) were similar ($P = 0.32$) to those of the inoculated control (5.78 log CFU per wing) (Table 1). This aligned with other observations that chlorine rinses had limited ability to reduce bacterial populations on poultry. Northcutt et al. *(21)* observed that 50 ppm of chlorine did not significantly alter APC or *Salmonella* populations on spray-washed broiler carcasses. Similarly, Bautista et al. *(5)* reported that chlorine treatments ranging from 7.32 to 50 ppm did not significantly ($P > 0.20$) reduce *Salmonella* spp. or total counts on turkey carcasses when compared with a water spray.

TABLE 1. Salmonella *counts from chicken wings inoculated with* Salmonella *and then either untreated or exposed to a 3-min rinse with water, 50 ppm of chlorine, or 2% lactic acid*[a]

Treatment	*Salmonella* population (mean log CFU/wing ± SE)
None	5.78 ± 0.09 A
Water rinse	5.81 ± 0.07 A
Chlorine rinse	5.69 ± 0.07 A
Lactic acid rinse	0.39 ± 0.07 B[b]

[a] Values without a common letter differ ($P < 0.05$).
[b] Estimated count below the detection limit.

Lactic acid achieved a significant ($P < 0.01$) reduction (below the detection limit) in *Salmonella* levels compared with the inoculated control and the water and chorine rinses (Table 1). Several studies investigating lactic acid for poultry utilized a spray treatment with application times ranging from 17 to 180 s rather than a rinse and observed *Salmonella* reductions ranging from 0.73 to 2.2 log *(18, 29–31)*. Anang et al. *(1)* utilized a 10-min 2% lactic acid dip and observed a 1.17-log reduction of *Salmonella* Enteritidis on chicken breasts; the study by Anang et al. *(1)* decontaminated the chicken breasts with an ethanol dip followed by passing the poultry through a flame, whereas the present study did not utilize a decontamination step. Differences in surface characteristics of the inoculated chicken may have contributed to the observed efficacy of lactic acid. The studies discussed above did not yield results near the detectable limit of the method used for *Salmonella* enumeration, and most did not utilize a methodology to recover injured *Salmonella*.

Field study. For untreated carcasses, APC levels were 4.16 log CFU per carcass and coliform levels were 3.15 log CFU per carcass; *Salmonella* was not detected on the untreated carcasses. Overall, initial microbial levels on untreated chicken carcasses in this study were similar to or lower than those typically observed on commercially processed chickens. Northcutt et al. *(21)* reported 4.4 log CFU/ml (APC) and 3.8 log CFU of *Escherichia coli* on washed chicken carcasses. In another study, higher APC levels (5.1 log CFU/g) and lower coliform levels (2.86 log CFU/g) were observed on chicken legs harvested immediately after evisceration *(10)*. In a 2005 study of U.S. poultry processing plants, 16.3% of chickens were contaminated with *Salmonella (27)*. Furthermore, Parveen et al. *(23)* observed an average *Salmonella* prevalence of 80% on whole chicken carcasses prior to and after chilling, and Stopforth et al. *(24)* observed *Salmonella* incidence ranging from 4 to 36% during processing.

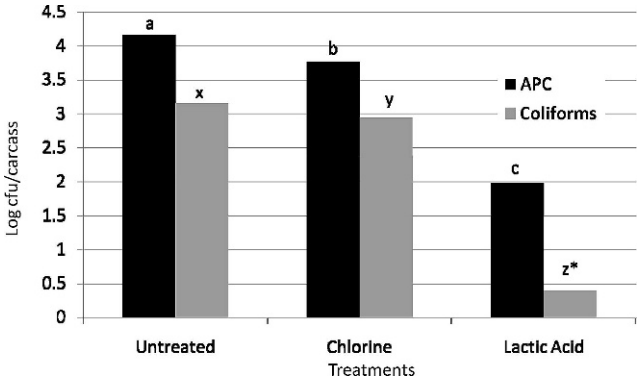

FIGURE 1. *Aerobic plate counts (APC) and coliform counts reported in log CFU per carcass from poultry carcass rinses collected from untreated controls and chlorine and lactic acid treatments from mobile slaughter operations. For APC, means without a common letter (a, b, and c) differ ($P < 0.05$). For coliforms, means without a common letter (x, y, and z) differ ($P < 0.05$). *, estimated count below the detection limit. Standard error = 0.08.*

The 2% lactic acid rinse was the most effective treatment in the field study, producing a significant >2-log reduction ($P < 0.01$) in APC and coliform levels in comparison with the untreated carcasses (Fig. 1). Coliform levels for the lactic acid–rinsed carcasses were <0.39 log CFU per carcass, below the detectable limit of the method. Bautista et al. *(5)* reported that 1.24% lactic acid treatment reduced APC by 2.4 log cycles and coliforms by at least 1.5 log compared with initial inoculation levels on turkey carcasses. Okolocha and Ellerbroek *(22)* observed a 0.6-log CFU/ml reduction in APC and a 1.1-log CFU/ml reduction in *Enterobacteriaceae* when poultry carcasses were dipped in 1% lactic acid. Furthermore, in this study, the APC and coliform levels for the lactic acid–rinsed carcasses were significantly ($P < 0.01$) lower than those of the chlorine-rinsed carcasses (Fig. 1).

The chlorine rinse resulted in significant ($P < 0.01$) but much smaller reductions in APC and coliforms (Fig. 1). The 0.2-log reduction for coliforms may not be considered biologically significant for a processing antimicrobial intervention. Stopforth et al. *(24)* observed significant reductions of a similar magnitude in APC (0.5 log CFU/ml) and coliforms (0.4 log CFU/ml) on poultry carcasses processed in a commercial chlorine chiller. Bautista et al. *(5)* reported no significant effect of a 50-ppm chlorine treatment on total counts and coliform counts on turkey carcasses compared with the uninoculated control.

Lactic acid achieved significant reductions in *Salmonella* in the laboratory study and in APC and coliforms in the field study. Chlorine achieved significant but small reductions in APC and coliforms in the field study but did not reduce *Salmonella* levels on chicken wings in the laboratory study. Results of this study indicate that including methodology for recovery of injured cells from poultry treated with acidic interventions is critical to obtain accurate, measurable results. This study shows that a 2% lactic acid rinse for 3 min is an effective antimicrobial intervention for mobile poultry slaughter operations. Given the significant microbial reductions observed and stability of lactic acid during processing, it is an attractive and effective alternative for mobile processors to chlorine, which requires careful monitoring to maintain antimicrobial activity.

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