

Research Paper

Efficacy of Lactic Acid, Lactic Acid–Acetic Acid Blends, and Peracetic Acid To Reduce *Salmonella* on Chicken Parts under Simulated Commercial Processing Conditions

ALEJANDRA RAMIREZ-HERNANDEZ, MINDY M. BRASHEARS, AND MARCOS X. SANCHEZ-PLATA*

Department of Animal and Food Sciences, Texas Tech University, Lubbock, Texas 79409, USA

MS 17-087: Received 17 February 2017/Accepted 24 August 2017/Published Online 13 December 2017

ABSTRACT

The poultry processing industry has been undergoing a series of changes as it modifies processing practices to comply with new performance standards for chicken parts and comminuted poultry products. The regulatory approach encourages the use of intervention strategies to prevent and control foodborne pathogens in poultry products and thus improve food safety and protect human health. The present studies were conducted to evaluate the efficacy of antimicrobial interventions for reducing *Salmonella* on inoculated chicken parts under simulated commercial processing conditions. Chicken pieces were inoculated by immersion in a five-strain *Salmonella* cocktail at 6 log CFU/mL and then treated with organic acids and oxidizing agents on a commercial rinsing conveyor belt. The efficacy of spraying with six different treatments (sterile water, lactic acid, acetic acid, buffered lactic acid, acetic acid in combination with lactic acid, and peracetic acid) at two concentrations was evaluated on skin-on and skin-off chicken thighs at three application temperatures. Skinless chicken breasts were used to evaluate the antimicrobial efficacy of lactic acid and peracetic acid. The color stability of treated and untreated chicken parts was assessed after the acid interventions. The lactic acid and buffered lactic acid treatments produced the greatest reductions in *Salmonella* counts. Significant differences between the control and water treatments were identified for 5.11% lactic acid and 5.85% buffered lactic acid in both skin-on and skin-off chicken thighs. No significant effect of treatment temperature for skin-on chicken thighs was found. Lactic acid and peracetic acid were effective agents for eluting *Salmonella* cells attached to chicken breasts.

Key words: Chicken parts; Interventions; Organic acids; *Salmonella*

Salmonella infection results in a greater disease burden than that caused by any other foodborne pathogen, and according to FoodNet surveillance data, *Salmonella* is one of the few pathogens responsible for foodborne illness that has not significantly declined over the past years in the United States (3). A list of the top 10 pathogen-food combinations in terms of annual disease burden was published by Batz et al. (3). *Salmonella* in poultry was ranked fourth at that time, with a total of 221,045 illnesses (at an estimated cost of \$712,000 in 2009 U.S. dollars) resulting in 4,159 hospitalizations and 81 deaths. Hence, preventive strategies for controlling *Salmonella* is a priority aim for food producers and processors.

On 26 January 2015, U.S. Department of Agriculture, Food Safety and Inspection Service (FSIS) (28) published new performance standards for reducing *Salmonella* and *Campylobacter* in poultry products, based on a preventive approach with a scientific risk assessment. The new standards focused on whole poultry carcasses sampled after the chilling process, on chicken and turkey parts in the cut-up and deboning room, and on comminuted poultry

derivatives. Because more than 85% of poultry meat in the United States is consumed as parts instead of whole carcasses, the FSIS recommendations were aimed at controlling pathogen prevalence in products that are most often purchased by consumers. The previous standards revised in 2011 (25) included *Salmonella* and *Campylobacter* prevalence in whole carcass rinses collected at the end of the chilling processing step. The new standards include sampling for both pathogens at the end of the chilling line and in both chicken and turkey parts in the cut-up room. The FSIS concern is that despite an overall prevalence of less than 7.5% for *Salmonella* on whole carcasses after chilling, the new baseline in poultry parts indicates a national prevalence in chicken parts of 24% for *Salmonella* and 21% for *Campylobacter* (26). Consequently, processors need to demonstrate that their food safety programs will reduce *Salmonella* to levels in chicken parts below eight positive samples over 52 weeks (15.4%) of a moving window sampling program (Table 1). The FSIS is using various categories to classify poultry establishments that meet the pathogen reduction performance standards applied to chicken parts.

Alternatives to complement antimicrobial interventions in primary processing must include potential

* Author for correspondence. Tel: 806-835-6503; Fax: 806-742-4003; E-mail: marcos.x.sanchez.ttu.edu.

TABLE 1. *Salmonella* and *Campylobacter* performance standards for poultry products from FSIS directive 10250.1

Product	Prevalence (%)		Maximum acceptable percent positive ^a		Performance objective (no. of positive samples)	
	<i>Salmonella</i>	<i>Campylobacter</i>	<i>Salmonella</i>	<i>Campylobacter</i>	<i>Salmonella</i>	<i>Campylobacter</i>
Whole chicken carcass	7.5	10.4	9.8	15.7	5 of 51	8 of 51
Whole turkey	1.7	0.79	7.1	5.4	4 of 56	3 of 56
Ground chicken (325 g)	49	3.4	25	1.9	13 of 52	1 of 52
Ground turkey (325 g)	19.9	12	13.5	1.9	7 of 52	1 of 52
Chicken parts (4-lb portions)	28	15.5	15.4	7.7	8 of 52	4 of 52

^a Maximum percentage of samples positive for *Salmonella* and *Campylobacter* under the performance standards for young chicken and turkey carcasses listed in FSIS directive 10250.1 (27).

interventions in the cut-up and deboning room so that the new standards can be met. Among potential interventions, blends of organic acid solutions and oxidizing agents have been considered and tested in poultry products with variable results. Lactic, acetic, and peracetic acid formulations could be effective for reducing pathogen prevalence and levels in poultry parts if applied in the cut-up and deboning room. However, challenge studies with actual poultry pathogens in chicken parts are few. Results reported with lactic acid (LA) treatments in poultry range from 0.73- to 2.2-log reductions and sometimes greater (12). Few studies have been conducted under simulated commercial processing conditions. Chlorine has been one of the most common antimicrobials used in poultry processing plants for carcass decontamination; however, its effect decreases with increasing organic loads and increasing pH (6, 16). Peracetic acid (PAA) has been used to replace chlorine in some facilities and now is widely used in carcass rinses and water chilling stations at various concentrations, with a maximum of 2,000 ppm in aqueous solution (30).

However, when these antimicrobials are used as decontamination interventions, undesired color and texture effects and development of off-flavors can occur in treated tissues (13, 17). The color and uniformity of chicken skin and meat are important attributes that can be affected by slaughtering and further processing. Acid and/or heat interventions may change the final color of chicken products, potentially affecting consumer acceptance.

The objective of this study was to evaluate the efficacy of commercially available LA solutions, LA-acetic acid (AA) blends, and PAA formulations for reducing loads of *Salmonella* in chicken parts when applied at different solution concentrations and temperatures. The color stability of chicken parts was also evaluated to identify changes after the acid intervention.

MATERIALS AND METHODS

Samples. Commercially processed bone-in, skin-on (with skin) and skin-off (without skin) chicken thighs ($n = 1,080$; weight = 0.5 lb [0.23 kg] \pm 4%) and boneless skin-off chicken breasts ($n = 108$; 0.6 lb [0.27 kg] \pm 5%) were collected and procured fresh from a local grocery store in Lubbock, TX. Samples were held in insulated containers at $\leq 4^{\circ}\text{C}$ (39.2°F) and transported to the pathogen laboratory at Texas Tech University where they were processed for the study.

Bacterial culture. A cocktail of five strains of *Salmonella* was used in this study: *Salmonella* Enteritidis ATCC 13076, *Salmonella* Typhimurium ATCC 14028, *Salmonella* Typhimurium ATCC 13311, *Salmonella* Heidelberg ATCC 3347-1, and a wild-type *Salmonella* isolated from chicken thighs that were stored in the culture collection at Texas Tech University. 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 12 h. For each activated culture, 1 mL of the stock culture was added to 100 mL of TSB and incubated in a shaking incubator for 12 h at 37°C. On the day of the study, the five 100-mL *Salmonella* cultures were combined and mixed thoroughly. An inoculation solution was prepared by adding the *Salmonella* cocktail to 5 L of sterile TSB. The *Salmonella* level in this inoculation solution was determined by plating serial dilutions on xylose lysine desoxycholate agar (XLD; Hardy Diagnostics) and incubated for 24 h at 37°C.

Inoculation. Chicken thighs and chicken breasts were submerged the *Salmonella* cocktail solution at 6 log CFU/mL for 30 s and then placed onto racks with the skin side up for 20 min to allow for bacterial attachment.

Organic acid treatments. Three replications of each treatment were performed on three days. For each treatment replication, five chicken thighs with and without skin were inoculated with the *Salmonella* cocktail and then subjected to a spray treatment at one of the following temperatures: 21°C (70°F), 38°C (100°F), and 54°C (130°F). Four commercial organic acids were used in this study at the following concentrations: LA (FCC 88, Corbion Purac America, Lincolnshire, IL) solutions prepared at 2.84 and 5.11% (v/v, pH 2.3); LA+AA blend (CL21/80, Corbion Purac America) solutions prepared at 2.0 and 2.5% (v/v, pH 2.8); buffered LA spray (BLA; Spray 80, Corbion Purac America) solutions prepared at 3.25 and 5.85% (v/v, pH 3.0); and PAA (Peracet 15, CraftChem, Lawrenceville, GA) solution at 200 and 400 ppm (v/v, pH 7.5). All solutions were prepared according to the manufacturers' instructions and heated to the specific temperatures of application. Concentrations were selected based on common commercial uses and the maximum concentrations permitted for processing of poultry products (30).

For the positive control, chicken thighs with and without skin were sampled after inoculation with the *Salmonella* cocktail. For the negative control, chicken thighs with and without skin were inoculated with the *Salmonella* cocktail and then spray treated with sterile water. Concentrations and pH (model 550 A Orion pH meter, Thermo Fisher Scientific, Waltham, MA) of each intervention solution were evaluated just before application to determine concentrations and pH before treatment. The same

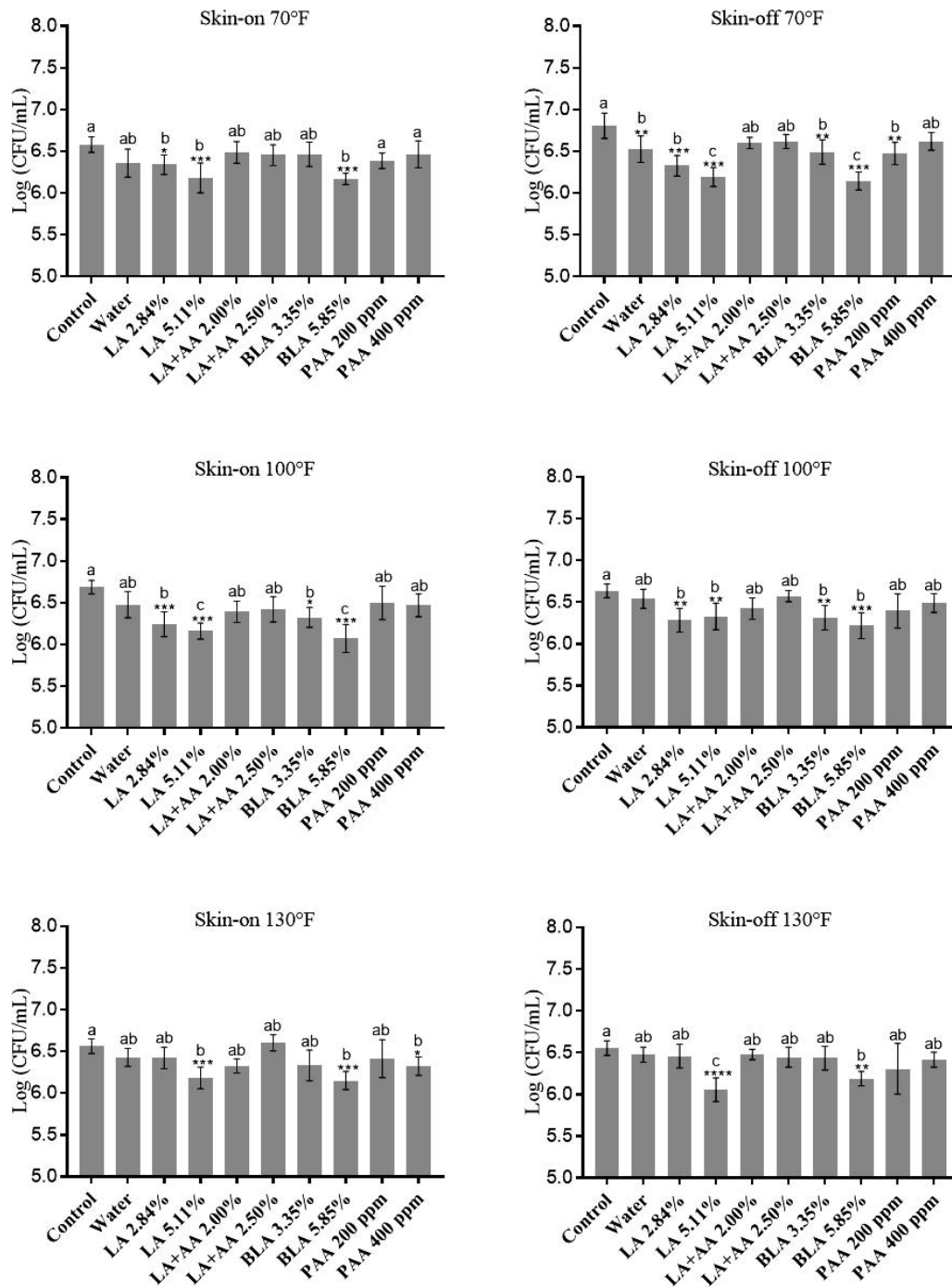


FIGURE 1. *Salmonella* counts (log CFU per milliliter) in chicken thigh rinsate collected from untreated controls and water and chemical intervention treatments. Bars with the same letter are not significantly different according to a one-way ANOVA followed by Dunnnett's multiple comparison test. Significant effects between the control and the treatment are indicated (* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; **** $P \leq 0.0001$).

protocol was followed for the second study with the chicken breasts. These were treated with LA and PAA, but the concentration of PAA was increased to 800 ppm.

All interventions were sprayed for 15 s in a commercially equivalent spray cabinet (Chad Co., Olathe, KS) equipped with four spray bars with six nozzles each and a conveyor belt system (series 800, Intralox, Harasham, LA) in the Texas Tech University pathogen laboratory. The spray solution was applied to both sides of the chicken parts with nozzles located 15.2 cm above and 5.1 cm below the chicken parts passing through the stainless steel mesh

conveyor belt at a flow rate for each nozzle of 0.421 L/min, and a pressure of 138 kPa.

Microbiological analysis. After treatment, the samples were transported to the food microbiology laboratory at Texas Tech University, where they were placed in a stomaching bag with 99 mL of 0.1% peptone water (BD, Sparks, MD) and homogenized for 2 min by hand massaging (11). Serial dilutions were made in buffered peptone water (Difco, BD, Detroit, MI). Dilutions were spread plated onto XLD with a 14-mL overlayer of Trypticase soy

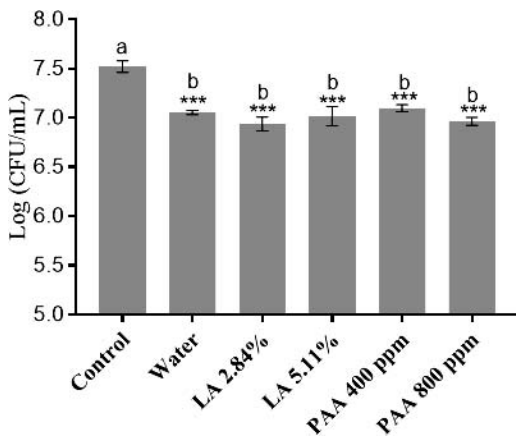


FIGURE 2. *Salmonella* counts (log CFU per milliliter) in chicken breast rinsate collected from untreated controls and water and chemical intervention treatments. Bars with the same letter are not significantly different according to a one-way ANOVA followed by Dunnett's multiple comparison test. Significant effects between the control and the treatment are indicated (*** $P \leq 0.001$).

agar (BD) to recover surviving and injured cells (5). The plates were incubated for 24 h at 37°C, bacterial colonies were counted manually, and populations were converted to log CFU per milliliter of rinse per chicken part before statistical analysis.

Color analysis. Separate sets of skin-on and skin-off chicken thighs and skin-off chicken breasts were prepared on each treatment day as previously described. Chicken thighs ($n = 216$) and chicken breasts ($n = 15$) were subjected to color analysis for L^* , a^* , and b^* (lightness, redness, and yellowness; colorimeter 200b, Minolta Camara Co., Osaka, Japan). The ΔE value was calculated to determine the color differences between all coordinates tested based on the CIELAB system:

$$\Delta E = \sqrt{(L_c - L_t)^2 + (a_c - a_t)^2 + (b_c - b_t)^2}$$

where L_c and L_t are the mean L^* values for lightness of chicken parts without (control) and with acid treatment, respectively; a_c and a_t are the mean a^* values on the red-green axis of chicken parts without (control) and with acid treatment, respectively; and b_c and b_t are the mean b^* values on the yellow-blue axis of chicken parts without (control) and with acid treatment, respectively.

Scanning electron microscopy. Chicken thigh skin and meat pieces were examined by scanning electron microscopy following the protocol described by Thomas and McMeekin (23). Control and treated skin and meat pieces ($\sim 1 \text{ cm}^2$) were fixed overnight at 4°C in glutaraldehyde solution (5%, v/v), and then pieces were rinsed in cold phosphate buffer, dehydrated in a graded ethanol series (30 to 100%), and critical point dried. The dried skin pieces were glued to scanning electron microscopy stubs and coated with 27.0 nm of gold in a sputter coating unit (SDC-050, BalTec, Canonsburg, PA) and examined in a S-4700 field emission scanning electron microscope (Hitachi, Tokyo, Japan).

Statistical analysis. All studies were performed in triplicate, and data were subjected to statistical analysis using Prism 7.01 trial statistical software (GraphPad, San Diego, CA). The sample, treatment, and day of treatment were fixed effects, and the replication was a random effect. A one-way analysis of variance (ANOVA) was performed followed by Dunnett's multiple

comparison test to analyze differences between treatment means and control means. Tukey's test was used to determine the difference between treatment means at different application temperatures. A two-way ANOVA followed by Dunnett's multiple comparison test was used to determine differences in the color parameters of chicken parts subjected to the acid interventions. Differences were considered significant at $P < 0.05$.

RESULTS

***Salmonella* reductions on chicken thighs by acid interventions.** When applied at 21°C, LA at both the low and high concentrations and BLA at the low concentration were the most effective treatments for reducing bacterial levels on chicken thighs (Fig. 1). Greater reductions were observed in the skin-off than the skin-on samples at the same temperature; however, the differences were not significant ($P > 0.05$). Similar results were observed at higher temperatures. At 38°C, LA+AA produced a significant reduction in bacterial levels on skin-on thighs compared with the control. Overall, there were no significant differences between the high and the low temperature treatments. Treatments with 5.11% LA and 5.85% BLA resulted in greater bacterial reductions compared with the control. For skin-on chicken thighs at 38°C, 0.53-log (95% confidence interval [CI]: 0.26 to 0.79) and 0.59-log (95% CI: 0.35 to 0.87) reductions, respectively, were obtained. For skin-off chicken thighs at 21°C, 0.69-log (95% CI: 0.26 to 0.79) and 0.66-log (95% CI: 0.26 to 0.79) reductions, respectively, were obtained. There were no significant differences between the skin-on and skin-off samples among acid treatments and treatment concentrations.

***Salmonella* reductions on chicken breasts by acid interventions.** In general, acid treatments reduced *Salmonella* populations on chicken breasts compared with the control. Significant differences were found for all treatments ($P < 0.05$). However, differences between the acid treatments and water were not significant, with a reduction of 0.46 log CFU/mL of chicken rinse (95% CI: 0.36 to 0.57) (Fig. 2).

Color analysis of treated chicken parts. No significant effects ($P < 0.05$) were found on the color parameters (L^* , a^* , and b^*) of chicken thighs treated with any of the acid treatments at the three temperatures (Table 2), and no changes in color attributes were identified in chicken breasts after the acid treatment (Table 3). ΔE values of 1 to 2 mean that color change is perceived through close observation, and values of 3 to 10 mean change is perceived at a glance. The ΔE values in this study all were less than 10. The skin-off chicken thighs treated with PAA at 400 pm had a slight increase in L^* values (lightness).

DISCUSSION

A variety of postharvest interventions have been implemented by the poultry processing industry to reduce the presence of pathogens such as *Salmonella* and *Campylobacter* in the final product (15). However, with the new standards published by the FSIS for chicken parts

TABLE 2. Hunter-Lab color scores after treatments of skin-on and skin-off chicken thighs^a

Treatment	70°F (21°C)				100°F (38°C)				130°F (54°C)			
	L*	a*	b*	ΔE ^b	L*	a*	b*	ΔE	L*	a*	b*	ΔE
Skin on												
Control	89.25	13.19	32.87		89.25	13.19	32.87		89.25	13.197	32.87	
	±3.18	±2.37	±1.22		±3.18	±2.37	±1.22		±3.18	±2.37	±1.22	
Water	88.27	14.56	34.90	2.63	87.65	10.18	36.65	3.68	87.65	10.185	36.65	5.09
	±2.80	±4.47	±1.80		±2.26	±1.90	±2.80		±2.26	±1.90	±2.79	
LA 2.84%	86.18	9.28	31.6	6.23	91.20	14.19	36.34	4.10	84.89	11.026	31.33	2.96
	±4.37	±0.58	±1.99		±1.88	±1.99	±2.70		±1.24	±4.33	±0.93	
LA 5.11%	90.611	11.71	35.82	3.56	94.12	11.03	37.09	3.18	84.07	13.798	31.00	5.10
	±1.57	±2.44	±2.94		±0.60	±1.96	±1.01		±3.61	±1.80	±4.195	
LA+AA 2.00%	85.82	12.69	33.52	3.96	86.03	12.18	32.55	3.39	84.16	12.49	31.48	5.52
	±5.27	±0.99	±4.25		±1.70	±1.66	±1.95		±0.68	±0.77	±1.35	
LA+AA 2.50%	89.39	14.419	34.30	1.88	85.13	9.97	30.05	5.93	85.136	9.97	30.05	5.32
	±5.70	±3.46	±6.39		±3.57	±1.99	±2.77		±3.57	±1.99	±2.77	
BLA 3.35%	81.11	11.51	33.28	8.70	87.03	9.13	37.39	6.47	88.509	7.46	33.59	5.82
	±8.36	±6.18	±2.16		±1.21	±4.17	±4.15		±5.55	±1.68	±2.99	
BLA 5.85%	86.60	12.34	31.85	2.96	83.29	11.23	31.57	6.40	85.764	8.49	29.62	6.69
	±2.97	±0.92	±0.41		±2.77	±1.04	±1.84		±1.77	±1.52	±0.96	
PAA 200 ppm	89.88	14.94	35.13	2.37	91.52	13.73	34.77	3.00	92.327	13.98	35.12	3.88
	±2.61	±1.38	±2.29		±2.77	±2.69	±1.32		±1.29	±2.49	±1.10	
PAA 400 ppm	87.25	11.47	27.37	6.10	88.38	15.59	35.53	3.68	87.185	14.11	32.31	2.33
	±1.88	±2.23	±5.24		±1.01	±2.08	±1.48		±3.62	±3.80	±2.90	
Skin off												
Control	62.53	13.20	22.38		62.53	13.20	22.38		62.53	13.20	22.38	
	±6.60	±2.53	±2.60		±6.60	±2.53	±2.60		±6.60	±2.53	±2.60	
Water	64.50	11.74	23.04	2.53	59.53	14.67	20.12	4.04	61.023	14.02	21.80	1.82
	±1.09	±1.51	±1.40		±4.78	±2.29	±0.80		±3.03	±5.97	±1.75	
LA 2.84%	65.80	13.49	25.86	4.77	61.86	12.45	21.70	1.23	66.22	10.93	21.76	4.36
	±5.07	±1.81	±2.08		±3.74	±3.54	±1.28		±5.14	±1.46	±0.86	
LA 5.11%	67.46	10.18	22.62	5.79	74.50	12.57	28.28	13.35	64.15	17.73	23.76	3.65
	±4.40	±1.68	±2.93		±10.93	±1.99	±4.31		±2.33	±5.00	±1.28	
LA+AA 2.00%	62.71	12.10	23.63	5.79	53.72	14.06	17.98	9.89	65.05	13.14	19.24	4.03
	±2.46	±2.36	±6.33		±0.46	±1.36	±2.25		±7.17	±1.19	±3.21	
LA+AA 2.50%	56.71	14.46	19.35	1.89	68.05	11.90	24.74	6.16	67.74	13.66	20.35	5.61
	±3.31	±3.94	±0.21		±9.69	±3.37	±5.32		±10.56	±4.13	±8.79	
BLA 3.35%	54.88	5.76	19.89	10.97	70.10	9.86	23.73	8.37	84.60	14.33	29.70	3.04
	±1.05	±0.57	±0.90		±3.26	±2.60	±0.71		±2.71	±2.04	±0.88	
BLA 5.85%	60.11	13.59	21.28	2.70	65.42	11.75	22.32	3.22	85.764	8.498	29.623	3.65
	±2.82	±2.58	±1.28		±3.80	±0.80	±1.38		±1.77	±1.52	±0.96	
PAA 200 ppm	60.55	14.83	21.01	2.69	64.78	12.66	22.27	2.31	64.15	17.73	23.76	5.00
	±0.20	±0.81	±1.94		±3.45	±1.46	±1.38		±2.33	±5.00	±1.28	
PAA 400 ppm	75.03	13.91	26.72	13.25	61.28	12.30	21.48	1.79	65.26	14.96	23.09	3.33
	±1.01	±1.40	±0.15		±6.04	±1.10	±1.53		±5.78	±2.65	±4.02	

^a Values are mean ± 95% confidence interval ($n = 216$). In the CIELAB system, L* (lightness of color) ranges from black to white (0 to 100); for a* (redness), positive numbers are in the red direction and negative numbers are in the green direction; and for b* (yellowness), positive numbers are in the yellow direction and negative numbers are in the blue direction.

^b Difference between the control and treatment samples in the L*a*b* color space.

(28), it is critical to identify potential interventions to meet the bacterial load reduction requirements. The goal of this study was to identify the effectiveness of commonly used organic acid blends applied by spraying to reduce *Salmonella* in chicken thighs and chicken breasts in a simulated commercial setting.

The decontamination of poultry carcasses in chicken processing facilities occurs mostly at the carcass rinse and water chilling steps and by spray or drench after the chilling

process (7). Organic acids such as LA and AA have been widely used on chicken surfaces because of the availability, cost-effectiveness, ease of use, decontamination potential, and generally recognized as a safe status of these acids. Trisodium phosphate, cetylpyridium chloride, acetic acid, ozonated water, and hydrogen peroxide treatments are alternatives for carcass rinse or chill water with variable efficacy against pathogens (20). The mode of action of LA occurs with the undissociated form, which penetrates the

TABLE 3. Hunter-Lab color scores after treatment of chicken breasts^a

Treatment	L*	a*	b*	ΔE^b
Control	69.91 ± 3.3	6.02 ± 1.2	34.11 ± 1.7	
Water	68.50 ± 2.7	7.70 ± 1.1	33.38 ± 1.6	2.308
PAA 400 ppm	67.44 ± 1.1	5.77 ± 1.8	30.46 ± 2.5	4.408
PAA 800 ppm	74.33 ± 2.0	6.83 ± 1.1	35.47 ± 1.7	4.699
LA 2.84%	71.80 ± 3.4	6.74 ± 1.1	34.44 ± 1.2	2.050
LA 5.11%	69.93 ± 1.5	6.11 ± 1.4	32.48 ± 3.0	1.625

^a Values are mean ± 95% confidence interval ($n = 15$). In the CIELAB system, L* (lightness of color) ranges from black to white (0 to 100); for a* (redness), positive numbers are in the red direction and negative numbers are in the green direction; and for b* (yellowness), positive numbers are in the yellow direction and negative numbers are in the blue direction.

^b Difference between the control and treatment samples in the L*a*b* color space.

cytoplasmic membrane, reduces intracellular pH, and disrupts the outer membrane (1, 18). AA causes cytoplasmic acidification, which results in malfunction of energy and regulation parameters and accumulation of free acid anions that kill or retard microbial growth (20). PAA is an oxidizing agent that disrupts the sulfhydryl (SH) and sulfur (S-S) bonds of enzymes and cells walls (2, 11). The fact that LA and PAA may elute *Salmonella* attached to skin and meat surfaces suggests that the attachment process is mediated by physicochemical relationship with the collagen or mucopolysaccharide matrix between the individual collagen fibrils present in chicken skin (24).

Many bacterial species employ a survival strategy response to adverse environmental conditions; these bacterial cells enter into a viable but nonculturable state, in which they can retain their infectious and pathogenic potential (11). In previous studies, *Salmonella* was able to enter and

recover from the viable but nonculturable stage after PAA treatment (11). However, little information is available on the effect of organic acid treatment and the ability of *Salmonella* to recover from a dormant persistent state after acid interventions. In the present study, we used scanning electron micrographs (Fig. 3) to reveal bacterial attachment to skin and meat surfaces before and after the LA and PAA interventions. Changes in skin and meat microtopography can be caused by the exposure to commercial processing procedures and can have a significant influence on the contamination of carcasses by bacteria during processing and the recovery of microorganisms during sampling.

During the chicken carcass processing stages, removal of the outer skin layer results in removal of attached microorganisms; however, dermal skin tissue provides a new surface niche for bacterial colonization during further processing (23). After chilling, chicken skin swells, opening and exposing channels and crevices to contaminants present in the water, and these crevices can protect bacteria from the effects of subsequent antimicrobial interventions (22). Results obtained in this study indicated greater *Salmonella* reduction on chicken meat surfaces. Although chicken meat has a smoother and less hydrophobic microtopography, scanning electron micrographs revealed more bacteria attached to the muscle fibers than to the chicken skin. Bacteria can be entrapped in tissue crevices, which provide a level of protection against antimicrobial treatments (14, 21, 23).

One important concern of food safety authorities has been the potential carryover of antimicrobial residues to the chicken rinse used for bacterial sampling postchilling. In response, a new neutralizing buffered peptone water rinse for verification sampling has been proposed by the FSIS (9, 29). The reason for utilizing this rinsate solution is to reduce false-negative results due to carryover of the active sanitizer in the rinsate solution. In this study, chicken rinses were

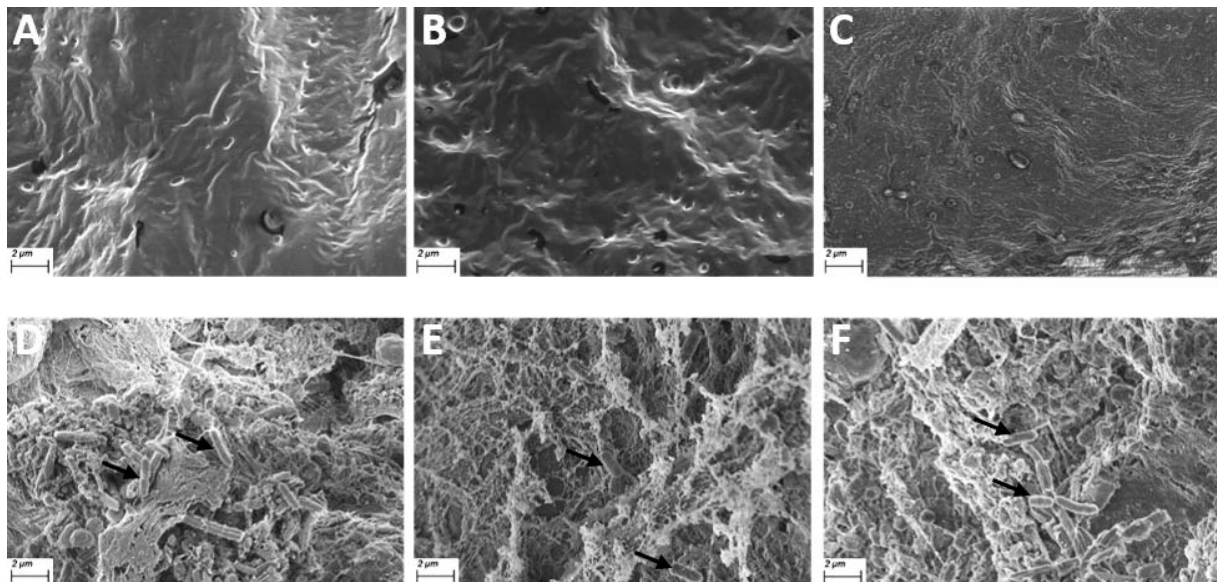


FIGURE 3. Representative scanning electron micrographs ($> \times 1,000$ magnification) of *Salmonella* cells adhered to the surface of chicken thigh skin and meat surfaces. (A) Control skin; (B) skin treated with 5.11% LA; (C) skin treated with 400 ppm of PAA; (D) control meat surface; (E) meat treated with 5.11% LA; (F) meat treated with 400 ppm of PAA.

collected and processed within 1 h after intervention to minimize antimicrobial carryover and extended contact time with the sample; however, future work will require the use of a neutralizing agent to account for the potential carryover and enable the recovery of injured cells.

The color of fresh chicken meat is an important quality attribute that can influence consumer purchasing decisions (19). Changes in carcass appearance such as bleaching and darkening after treatment with organic acids has been reported (4). However, data collected in this study indicate no significant treatment-related discoloration. Muscle pH and meat color are highly correlated; higher pH is correlated with darker than normal color ranges (8). A variety of factors such as environmental variables, field management practices, and stress can alter the color parameters of chicken carcasses and parts during production and processing (10).

The results of this study suggest that LA and BLA achieved significant reductions in *Salmonella* under the conditions evaluated in this study, and these acids may be effective antimicrobials for applications with poultry parts. However, the *Salmonella* reductions were relatively small and have minimal biological significance as a single intervention option. A multiple, sequential application approach may be needed to achieve greater reductions during the cutting and deboning processing stages. The acid treatments did not affect physical properties such as color in skin-on and skin-off chicken parts. Further research is needed with other chicken parts to evaluate the effect of sequential interventions when applied with different or combined methods, such as drenching and immersion, that could potentially enhance overall antimicrobial effectiveness.

ACKNOWLEDGMENTS

The researchers thank Corbion Purac for providing organic acid concentrates and CraftChem for providing PAA treatments. We also thank the Food Microbiology Laboratory staff at Texas Tech University for help and support during this study.

REFERENCES

- Alakomi, H.-L., E. Skyttä, M. Saarela, T. Mattila-Sandholm, K. Latva-Kala, and I. M. Helander. 2005. Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. *Appl. Environ. Microbiol.* 66:2001–2005.
- Baldry, M. G. C., and J. A. L. Fraser. 1988. Disinfection with peroxygens, p. 91–116. In K. R. Payne (ed.), *Industrial biocides*. Society of Chemical Industry, Wiley and Sons, London.
- Batz, M. B., S. Hoffmann, and J. G. Morris, Jr. 2011. Ranking the risk: the 10 pathogen-food combinations with greatest burden on public health. Emerging Pathogens Institute, University of Florida, Gainesville.
- Bilgili, S. F., D. E. Conner, J. L. Pinion, and K. C. Tamblyn. 1997. Broiler skin color as affected by organic acids: influence of concentration and method of application. *Poult. Sci.* 77:752–757.
- Brashears, M., A. Amezquita, and J. Stratton. 2001. Validation of methods used to recover *Escherichia coli* O157:H7 and *Salmonella* spp. subjected to stress conditions. *J. Food Prot.* 64:1466–1471.
- Byrd, J. A., and S. R. McKee. 2005. Improving slaughter and processing technologies, p. 310–327. In G. C. Mead (ed.), *Food safety control in the poultry industry*. CRC Press, Boca Raton, FL.
- Chen, X. I., L. J. Bauermeister, G. N. Hill, M. Singh, S. F. Bilgili, and S. R. McKee. 2014. Efficacy of various antimicrobials on reduction of *Salmonella* and *Campylobacter* and quality attributes of ground chicken obtained from poultry parts treated in a postchill decontamination tank. *J. Food Prot.* 77:1882–1888.
- Fletcher, D. L., M. Qiao, and D. Smith. 1997. The relationship of broiler breast meat color and pH to shelf-life and odor development. *Poult. Sci.* 76:1042–1046.
- Gamble, G. R., M. E. Berrang, R. J. Buhr, A. Hinton, D. V. Bourassa, J. J. Johnston, K. D. Ingram, E. S. Adams, and P. W. Feldner. 2016. Effect of simulated sanitizer carryover on recovery of *Salmonella* from broiler carcass rinsates. *J. Food Prot.* 79:710–714.
- Gotardo, L. R. M., P. B. Vieira, C. F. P. Marchini, M. R. B. de Mattos Nascimento, R. C. Antunes, E. C. Guimarães, J. P. R. Bueno, and D. B. Santos. 2015. Cyclic heat stress in broilers and their effects on quality of chicken breast meat. *Acta Sci. Vet.* 43:1325.
- Jolivet-Gougeon, A., F. Sauvager, M. Bonnaure-Mallet, R. R. Colwell, and M. Cormier. 2006. Virulence of viable but nonculturable *S. Typhimurium* LT2 after peracetic acid treatment. *Int. J. Food Microbiol.* 112:147–152.
- Killinger, K. M., A. Kannan, A. I. Bary, and C. G. Cogger. 2010. Validation of a 2 percent lactic acid antimicrobial rinse for mobile poultry slaughter operations. *J. Food Prot.* 73:2079–2083.
- Kross, R. September 2004. Methods for reducing meat discoloration. U.S. patent 20040175476 A1.
- Lillard, H. S. 1986. Distribution of “attached” *Salmonella* Typhimurium cells between poultry skin and a surface film following water immersion. *J. Food Prot.* 49:449–454.
- Loretz, M., R. Stephan, and C. Zweifel. 2010. Antimicrobial activity of decontamination treatments for poultry carcasses: a literature survey. *Food Control* 21:791–804.
- Nagel, G. M., C. L. Bauermeister, L. J. Bratcher, M. Singh, and S. R. McKee. 2013. *Salmonella* and *Campylobacter* reduction and quality characteristics of poultry carcasses treated with various antimicrobials in a post-chill immersion tank. *Int. J. Food Microbiol.* 165:281–286.
- Qiao, M., D. L. Fletcher, J. K. Northcutt, and D. P. Smith. 2002. The relationship between raw broiler breast meat colour and composition. *Poult. Sci.* 81:422–427.
- Ray, B., and W. E. Sandine. 1992. Acetic, propionic, and lactic acids of starter culture bacteria as biopreservatives, chap. 5. In B. Ray and M. Daeschel (ed.), *Food biopreservatives of microbial origin*. CRC Press, Boca Raton, FL.
- Sanchez-Escalante, A., D. Djenane, G. Torrescano, A. Beltran, and P. Roncales. 2001. The effects of ascorbic acid, taurine, carnosine and rosemary powder on colour and lipid stability of beef patties packaged in modified atmosphere. *Meat Sci.* 58:421–429.
- Sohaib, M., F. M. Anjum, M. S. Arshad, and U. U. Rahman. 2016. Postharvest intervention technologies for safety enhancement of meat and meat based products; a critical review. *J. Food Sci. Technol.* 53:19–30.
- Stivarius, M. R., F. W. Pohlman, K. S. McElyea, and A. L. Waldroup. 2002. Effects of hot water and lactic acid treatment of beef trimmings prior to grinding on microbial, instrumental color and sensory properties of ground beef during display. *Meat Sci.* 60:327–334.
- Tan, S. M., S. M. Lee, and G. A. Dykes. 2014. Buffering effect of chicken skin and meat protects *Salmonella enterica* strains against hydrochloric acid but not organic acid treatment. *Food Control* 42:329–334.
- Thomas, C. J., and T. A. McMeekin. 1980. Contamination of broiler carcass skin during commercial processing procedures: an electron microscopy study. *Appl. Environ. Microbiol.* 40:133–144.
- Thomas, C. J., and T. A. McMeekin. 1981. Attachment of *Salmonella* spp. to chicken muscle surfaces. *Appl. Environ. Microbiol.* 42:130–134.
- U.S. Department of Agriculture, Food Safety and Inspection Service. 2011. New performance standards for *Salmonella* and *Campylobacter* in young chicken and turkey slaughter establishments: response to comments and announcement of implementation schedule. Docket FSIS-2010-0029. *Fed. Regist.* 76:15282–15290.
- U.S. Department of Agriculture, Food Safety and Inspection Service. 2012. The nationwide microbiological baseline data collection program: raw chicken parts survey. January 2012–August 2012.

Available at: http://www.fsis.usda.gov/shared/PDF/Baseline_Data_Raw_Chicken_Parts.pdf. Accessed 2 November 2016.

27. U.S. Department of Agriculture, Food Safety and Inspection Service. 2013. *Salmonella* and *Campylobacter* verification program for raw meat and poultry products. Directive 10250.1. U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, DC.
28. U.S. Department of Agriculture, Food Safety and Inspection Service. 2015. Changes to the *Salmonella* and *Campylobacter* verification testing program: proposed performance standards for *Salmonella* and *Campylobacter* in not-ready-to-eat comminuted chicken and turkey products and raw chicken parts and related agency verification procedures and other changes to agency sampling. 26 January 2015. Docket FSIS-2014-0023. *Fed. Regist.* 80:3940–3950.
29. U.S. Department of Agriculture, Food Safety and Inspection Service. 2016. New neutralizing buffered peptone water to replace current buffered peptone water for poultry verification sampling (FSIS Notice 41-16). Available at: <http://www.fsis.usda.gov/wps/wcm/connect/2cb982e0-625c-483f-9f50-6f24bc660f33/41-16.pdf?MOD=AJPERES>. Accessed 2 November 2016.
30. U.S. Department of Agriculture, Food Safety and Inspection Service. 2017. Safe and suitable ingredients used in the production of meat, poultry, and egg products. Directive 7120.1. U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, DC.