MICROBIOLOGY AND FOOD SAFETY

Efficacy of peroxy acetic acid in reducing *Salmonella* and *Campylobacter* spp. populations on chicken breast fillets

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ABSTRACT Poultry processors use antimicrobials to reduce the risk of pathogens on poultry and poultry products. The efficacy of selective and nonselective plating media to enumerate injured *Salmonella* (selective media—brilliant green sulfa agar and Petrifilm Enterobacteriaceae Plate Count; nonselective media—tryptic soy agar and Petrifilm Aerobic Plate Count) and *Campylobacter* (selective medium–Campy cefex agar and nonselective medium—Brucella agar) populations and the efficacy of peroxy acetic acid (PAA) to reduce *Salmonella* and *Campylobacter* populations on chicken breast fillets were evaluated. All plating media for *Salmonella* and *Campylobacter* contained nalidixic acid (200 ppm) or gentamycin (200 ppm), respectively. Breast fillets were sprayed or immersed in PAA (500 ppm) for 10 min for evaluation of the plating media. Breast fillets inoculated with a mixed *Salmonella* and *Campylobacter* cocktail were sprayed (5 or 10 s) or immersed (4–30 s) in PAA (100, 400, 500, or 1,000 ppm) for evaluation of PAA efficacy. *Salmonella* populations were higher ($P \leq 0.05$) when plated on nonselective media compared with the selective media for the non-PAA treated fillets, although the differences in populations were low (<0.32 log CFU/mL). For both the microorganisms, populations on PAA treated (immersion or spray) fillets were similar when enumerated on nonselective or selective media within each treatment (PAA immersion or spray). Both immersion and spray applications reduced ($P \leq 0.05$) the *Salmonella* and *Campylobacter* populations compared with the control. Increasing the PAA concentration to 250, 500, and 1,000 ppm resulted in greater reductions ($P \leq 0.05$) in *Salmonella* and *Campylobacter* populations. Immersion of the inoculated breast fillets in 1,000 ppm PAA solution for 30 s resulted in *Salmonella* and *Campylobacter* population reductions of 1.92 and 1.87 log CFU/mL, respectively. Method of antimicrobial application (immersion and spray) did not affect the reductions in *Salmonella* and *Campylobacter* populations. Either immersion or spray application can be used to improve microbial safety of chicken breast fillets in a poultry processing plant.

Key words: peroxy acetic acid, salmonella, campylobacter, chicken breast fillet, antimicrobial

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INTRODUCTION

Poultry meat and other poultry products are some of the most popular sources of meat consumed worldwide (Kearney, 2010). However, during preharvest and postharvest processing, poultry and poultry products can be contaminated by pathogenic bacteria such as *Salmonella* and *Campylobacter*. Presence of these pathogens has been frequently implicated in outbreaks associated with consumption of chicken and poultry products. Therefore, these pathogens are major concern of consumers, the poultry industry as well as the regulatory agency, U.S Department of Agriculture Food Safety and Inspection Service (Kramer et al., 2000; USDA FSIS, 2019; Sahin et al., 2002; Bauermeister et al., 2008; Park et al. 2014). Foodborne Disease Active Surveillance Network (FoodNet) reported 25,606 foodborne illness cases in 2018 from *Campylobacter, Cyclospora*, *Listeria*, *Salmonella,* Shiga toxin-producing *Escherichia coli, Shigella, Vibrio, and Yersinia.* Of these pathogens, *Salmonella* and *Campylobacter* caused 9,084 and 9,723 cases, respectively in 2018 (Tack et al., 2019). The USDA FSIS has proposed pathogen reduction performance standards for *Campylobacter* and *Salmonella* that should result in 37% and 30% reduction in salmonellosis and campylobacteriosis cases, respectively (USDA FSIS, 2015).

Poultry processing plants differ in the number and types of antimicrobial interventions applied, including

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application of antimicrobials at multiple inside-outside bird washers, brush washers, cabinet washers, and/or immersion tanks before and after chilling (Keener et al., 2004). During immersion chilling, poultry carcasses are exposed to antimicrobials most prominently, chlorine. Other antimicrobials such as acidified sodium chlorite, cetylpyridinium chloride, chlorine dioxide, gamma irradiation, ozone, sodium hypochlorite, trisodium phosphate, citrilow, poultry pHresh, etc. have been used as sprays or dips (Park et al., 2002; Oyarzabal, 2005; Bauermeister et al., 2008; Chen et al., 2014; Smith et al., 2015; Kim et al., 2017; Landrum et al., 2017). However, use of these antimicrobials is limited because of the residual adverse effects such as chicken skin discoloration, consumer awareness, corrosiveness to equipment, cost, or limited effectiveness. While the application of antimicrobials has been limited to whole broilers in poultry processing previously, the poultry processors have incorporated antimicrobial interventions for chicken parts as well to reduce the prevalence of *Salmonella* and *Campylobacter*.

Peroxy acetic acid (**PAA**), in a mixture of acetic acid and hydrogen peroxide, has been used as an antimicrobial in the chiller, although some processors apply it as in a postchill immersion or in a finishing chiller. Peroxy acetic acid decomposes spontaneously to acetic acid, water, and oxygen (Swern, 1970). Further, being soluble in water, it can be easily used in the food and poultry processing industries (Bauermeister et al., 2008). The use of PAA in poultry has been approved by the U.S. Food and Drug Administration (US FDA, 2019) and classified as generally regarded as safe by FDA and is considered a processing aid by USDA FSIS.

Recent USDA-FSIS postchill regulations and performance standards are the focus of many food safety researchers studying the microbiological analysis of foodborne pathogens during general poultry processing. Peroxy acetic acid has been approved as an antimicrobial for use during immersion chilling of broiler carcasses and was shown to be effective in reducing *Salmonella* and *Campylobacter* populations and prevalence (Chen et al. 2014; Smith et al., 2015; Kim et al. 2017). While research on reduction of these organisms on poultry parts has been reported (Stopforth et al., 2007; Scott et al., 2015; Sukumaran et al., 2015; Ramirez-Hernandez et al., 2017; Zhang et al., 2018), range of effective concentrations and application methods (spray vs. immersion) have not been evaluated. More specifically, the efficacy of antimicrobial treatments in reducing *Salmonella* and *Campylobacter* on poultry or poultry products is of a particular interest.

Exposure of organisms to antimicrobials causes destruction of microorganisms as well as injury to a proportion of the microorganisms. Selective media that are traditionally used to inhibit or prevent the growth of background flora cannot be used in this case as the selective agents used in the media can prevent or inhibit the recovery and growth of the injured cells. Use of antibiotic resistant microorganisms for microbial challenge testing allows for accurate enumeration of noninjured as well as injured surviving populations while using nonselective media (Landrum et al., 2017). However, the efficacy of the media (selective as well as non-selective) needs to be carefully evaluated before their use. The objectives of the research were to (1) evaluate the efficacy of different media (selective and nonselective) to enumerate healthy and stressed (injured) *Salmonella* (Nalidixic acid-resistant) and *Campylobacter* (Gentamycin-resistant) population subsequent to exposure to PAA and (2) evaluate efficacy of PAA in reducing *Salmonella* spp. and *Campylobacter* populations on chicken breast fillets using both postchill immersion or a spray application.

MATERIALS AND METHODS

Bacterial Strains

The nalidixic acid–resistant strain of *Salmonella* Typhimurium (ST^{NR}) and gentamycin-resistant strain of *Campylobacter coli* (CC^{GR}) were obtained from the U.S. National Poultry Research Center, United States Department of Agriculture, Athens, GA. The marker strain of *Salmonella* was streaked onto Trypticase soy agar (Becton, Dickinson and Company, Franklin Lakes, NJ) supplemented with 200 ppm of nalidixic acid (Sigma-Aldrich; St. Louis, MO) and incubated for 24 h at 35 ± 1°C. The *Campylobacter* strain was streaked onto Campy-cefex agar (Neogen Corporation, Lansing, MI) without the antimicrobial supplements (cefoperazone), but containing 200 ppm of gentamycin (CCGA; Sigma-Aldrich) and incubated for 48 h at 42 ± 1°C in a re-sealable plastic bag flushed with a microaerobic environment having 5% O_2, 10% CO_2, and 85% N_2. Stock cultures of *Campylobacter* and *Salmonella* were maintained at −80°C in *Campylobacter* Bolton's broth (Oxoid Ltd. Basingstoke, Hampshire) without supplements and Trypticase Soy broth with 15% glycerol. The bacterial cocktail was prepared by combining equal portions of each strain (ca. 6-log CFU/mL) for inoculation on split chicken breast fillets. *Salmonella* and *Campylobacter* populations in the cocktail were enumerated by plating serial dilutions using appropriate media and incubation conditions.

Preparation of Peroxy Acetic Acid Solutions

Peroxy acetic acid solutions (SaniDate FD Plus, BioSafe Systems LLC, East Hartford, CT) of various concentrations (100, 250, 500, and 1,000 ppm) were prepared using chilled (≤4°C) water and mixed. The PAA concentration in each treatment was confirmed using the standard titration protocol for PAA. Briefly, 200 µL of PAA was transferred to a tared, clean, glass beaker and weighed. The beaker was placed in an ice bath, and PAA was diluted with 50 mL of 5% H_2SO_4 (Ward's Science, Rochester, NY). Ferroin indicator (3 drops; Tintometer Inc., Sarasota, FL) was added to the solution until the salmon color appeared, and the solution was titrated with 0.1 N ceric ammonium sulfate (Ricca Chemical Company, Arlington, TX) until the salmon color disappeared. Potassium iodide (10 mL of 10% solution; Fisher

Scientific International Inc., Fairlawn, NJ) was added to the beaker, followed by 3 mL of starch indicator solution (Ricca Chemical Company, Arlington, TX) and swirled to mix. The mixed solution was titrated with 0.1 N sodium thiosulfate (Macron Fine Chemicals, Center Valley, PA) until the blue color solution turned into orange, and volume of sodium thiosulfate was recorded. The concentration of peroxy acids was calculated as follows:

$$\text{PAA concentration (\%)} = \frac{[Na_2S_2O_3\ (\text{mL})]\text{x}\ [\text{N}\ Na_2S_2O_3]\ \text{x}\ 3.8}{(\text{Weight of 0.2 mL PAA})}$$

Inoculation of Chicken Breast Fillets

Fresh chicken breast butterflies (before any antimicrobial application) were obtained from a local poultry processor on the day of the experiment. For each treatment, 1 split skinless, boneless chicken breast fillet (ca. 0.45 kg) was inoculated on the surface (skin-side) with a cocktail of ST^{NR} and CC^{GR} strains (ca. 6 log CFU/mL, each) using a handheld sprayer (5 mL) and were allowed to attach for 30 min at ambient temperature (21°C). While shorter attachment time (5 min) was used in previous research (Thanissery and Smith, 2014), the longer attachment time provided adequate time for drying of the surface from additional moisture (inoculum) from the inoculum.

Treatment Application

For evaluation of the efficacy of the selective and nonselective media to enumerate nonstressed and stressed (because of exposure to PAA solutions) *Salmonella* and *Campylobacter*, an inoculated breast fillet (n = 1 for each replication) was immersed (500 ppm for 10 min) or sprayed (500 ppm, 10 min) and transferred to stomacher bags containing chilled buffered peptone water (100 mL) containing sodium thiosulfate (0.1%; Macron Fine Chemicals, Center Valley, PA) to neutralize the antimicrobial action of PAA and rinsed for 1 min. An inoculated breast fillet, that is not treated with the antimicrobial was used as a control. The rinsate was used for preparation of further serial dilutions and plated on appropriate media.

For evaluation of the efficacy of antimicrobial treatments, each inoculated split chicken breast sample (n = 1 for each replication) was treated with PAA using either immersion (0, 4, 10 and 30 s) or spray (5 and 10 s) methods. For immersion treatment (without agitation), inoculated breast fillet was placed on a sterile stainless steel mesh basket (Model DND-095RND120-C04S, Anysizebasket, York, Pa) and immersed in 3.5 L of PAA solution in a high-density plastic container (18.9 L; Encore Plastics, Forsyth, GA). Fresh antimicrobial solution as prepared for each of the PAA concentrations and used for all the treatments (0, 4, 10 and 30 s). After treatment, each chicken breast sample was immediately placed in chilled BPW (100 mL) containing sodium thiosulfate (0.1%; Macron Fine Chemicals, Center Valley, PA) to neutralize the antimicrobial action of PAA and rinsed for 1 min. For spray treatment, each sample was placed on sterile stainless-steel mesh, and each side of the split chicken breast was sprayed with PAA solution for 5 or 10 s and immediately rinsed. In a parallel set (after 5 or 10 s of spray), the samples were allowed to rest for 30 s before rinsing to simulate the time needed for the product on the conveyor to reach the packaging station for packaging. For spray application, the antimicrobial of appropriate concentration was transferred (5 L) to a stainless steel ball lock keg (11.4 L; NSF Model 29748 PS; Italy). The tank was fitted with a 100 psi pressure gauge (1.27 cm I.D.) and a 690 kPa (100 psi) pressure relief valve (1.27 cm I.D). The tank was attached to an air pressure hose (862 kPa; [125 psi], PVC Braid) with a flat, fan spray nozzle (0.635 cm; NF0530303; Bete Fog Nozzle, Inc., Greenfield, Ma) and a quick connect plug for rapid pressurization with an air compressor (1.6 HP Continuous, 1,380 kPa [200 PSI], 17 L [4.5 Gallon]; Dewalt Manufacturing Company, Baltimore, MD). The spray volume was 15 mL/s of continuous spraying. The fan nozzle covered an area approximately 20 cm wide, and the distance between the breast fillet and spray wand was held constant at ca. 60 cm and was moved from side to side to cover the breast fillet surface (lengthwise). An inoculated breast fillet that is not treated with the antimicrobial was used as a control. Three individual replications (n = 3 for each treatment) were performed for each treatment. The concentration of final PAA solution was determined using the LaMotte Test Kit (LaMotte Company, Ocean city, MD).

Microbial Enumeration

For evaluation of the efficacy of the selective and nonselective media to enumerate nonstressed and stressed (exposure to PAA solutions), the rinsate was serially diluted in either peptone water (0.1%; PW) or peptone water supplemented with nalidixic acid (100 μg/mL; PW^{NA}). Appropriate dilutions of PW^{NA} were plated on Petrifilm APC (Aerobic Plate Count plates) and Petrifilm ENT (Enterobacteriaceae plates). Preparing serial dilutions in PW^{NA} allows for dispensing appropriate concentration of nalidixic acid in the Petrifilm and prevents the growth of background flora. Similarly, appropriate dilutions of PW were spread plated on TSA and Brilliant Green Sulfa agars, each containing 100 μg of nalidixic acid/mL of the respective agars (TSA^{NA} and BGS^{NA}, respectively). The plates were incubated as per the manufacturers' instructions for Petrifilm, and the TSA^{NA} and BGS^{NA} were incubated at 35°C for 24 h and enumerated. For evaluation of the media for enumeration of nonstressed and stressed *Campylobacter*, serial dilutions prepared with PW were spread plated on Campy-cefex agar (selective medium) or Brucella agar (nonselective medium) containing

200 μg of gentamicin/mL of the respective agars (Campy-cefex^{G} agar and BA^{G} agar, respectively).

For evaluation of the efficacy of PAA treatments, the rinsates collected from each treated split chicken breast were serially diluted in PW and PW^{NA}. Appropriate dilutions prepared with PW and PW^{NA} were plated on Campy-cefex^{G} agar and Aerobic Plate Count Petrifilm (3M Food Safety, St. Paul, MN), respectively, for the enumeration of *Campylobacter* and *Salmonella*. Petrifilm APC plates were incubated for 24 h at 35°C, while Campy-cefex^{G} agar plates were incubated microaerobically in re-sealable Ziploc bags for 48 h at 42°C. Typical colonies were counted and expressed as log CFU/mL.

Statistical Analysis

Three independent replications were performed for each treatment combination, on a separate day using freshly prepared solutions of PAA and the inocula. Data were analyzed by analysis of variance using the general linear model procedure of the Statistical Analysis System (Release 8.01, SAS Institute, Inc., Cary, NC; SAS Institute Inc., 2004). Fisher's least significant difference was used to separate means of the residual *Salmonella* spp. or *Campylobacter* populations (log CFU/mL) for the samples.

RESULTS AND DISCUSSION

Multiple intervention strategies are used to reduce the population of foodborne pathogens *Campylobacter* and *Salmonella* spp. or eliminate them from the products during poultry processing. The recent USDA FSIS performance standards for poultry parts has provided the impetus for poultry processors to incorporate antimicrobial interventions for the poultry parts to reduce the prevalence of foodborne pathogens on poultry parts.

Plating of breast fillet rinsates (no antimicrobial treatment) on APC, ENT Petrifilm and TSA^{NA}, and BGS^{NA} plates resulted in *Salmonella* populations of 5.74, 5.49, 5.54, and 5.42 log CFU/mL, respectively (Table 1). Although the *Salmonella* population (for control) enumerated on APC (5.74 log CFU/mL) was higher ($P > 0.05$) by ca. 0.30 log CFU/mL compared the populations enumerated on BGS^{NA} (5.42 log CFU/mL), the difference may not be biologically significant. Immersion and spray application of PAA resulted in decreases ($P \leq 0.05$) in mean *Salmonella* populations by 1.31 and 1.17 log CFU/mL, respectively. For treatments that received PAA application, the plating methods (selective [ENT, BGS^{NA}] and nonselective media [APC, TSA^{NA}]) were equally effective ($P > 0.05$) in enumerating *Salmonella* populations, regardless of the antimicrobial treatment methods (immersion or spray). It is possible that PAA application did not result in significant injury to the cells, although *Salmonella* reductions (ca. 1.5 log CFU/mL) were observed. The ENT Petrifilm utilizes violet red bile glucose agar (**VRBGA**) for enumeration of Enterobacteriaeae population. The VRBGA contains bile salts #3 (1.5 g/L) as an inhibitory agent for Gram positive microorganisms and sodium chloride (5 g/L), which was shown to inhibit injured *Salmonella*. Kang and Fung (2000) reported ca. 1 log CFU/mL difference between *Salmonella* populations (heat treated at 55°C for 10 min) enumerated on tryptic soy agar and xylose lysine deoxycholate (**XLD**) agars, indicating the inhibition of injured cells by selective agents in XLD agar. The XLD agar contains sodium deoxycholate (bile acid; 1.0 g/L) and sodium chloride (5 g/L), similar to that of VRBGA used in ENT Petrifilm. However, XLD contains other salts, ferric ammonium citrate, and sodium thiosulfate to aid in differentiating *Salmonella* from other nonhydrogen sulfide producers. It is possible that these other ingredients, along with acid production from sugars (xylose, lactose, and sucrose in XLD agar) may inhibit recovery of injured cells. Also, the BGS agar consists of sodium sulfapyridine and brilliant green as inhibitors for gram-positive microorganisms and does not include any bile salts. The lack of differences between the nonselective media (APC and TSA^{NA}) and the selective medium (BGS^{NA}) may be because of the absence of bile salts which are shown to inhibit recovery of cells from injury. Restaino et al. (1980) reported significant injury (>99%) in *Yersinia enterocolitica* serotypes subjected to sublethal heat injury (47°C for 70 min in 0.1 Mol phosphate buffer) in TSA containing 0.6% bile salts compared with the populations on TSA. It is possible that BGS^{NA} could have

Table 1. *Salmonella* populations (log CFU/mL; Mean ± S.D.) on chicken breast fillets (n = 15) inoculated with *Salmonella* and either immersed in peroxy acetic acid solution (500 ppm PAA, 10 min) or sprayed with PAA solution (500 ppm, 10 min).

Medium/Treatments	Control[1]	Immersion	Spray
APC[2]	$5.74 \pm 0.19^{a,x}$	$4.25 \pm 0.14^{b,x}$	$4.47 \pm 0.17^{b,x}$
ENT	$5.49 \pm 0.17^{a,y}$	$4.31 \pm 0.49^{b,x}$	$4.18 \pm 0.27^{b,y}$
TSA^{NA}	$5.54 \pm 0.29^{a,x,y}$	$4.24 \pm 0.11^{b,x}$	$4.47 \pm 0.19^{b,x}$
BGS^{NA}	$5.42 \pm 0.20^{a,y}$	$4.16 \pm 0.15^{b,x}$	$4.41 \pm 0.24^{b,x}$

[1]Same superscripts$^{(a,b)}$ within the same row indicate no significant differences ($P > 0.05$) between the treatments; Same superscripts$^{(x,y)}$ within the same column indicate no significant differences ($P > 0.05$) between the media.

[2]APC–Petrifilm APC; ENT—Petrifilm Enterobacteriaceae; TSA, tryptic soy agar; and BGS, BG Sulfa agar. Samples plated on APC and ENT were diluted in peptone water containing nalidixic acid (200 ppm; PW^{NA}) and nalidixic acid (200 ppm) was incorporated into the medium before preparing TSA and BGS petri plates.

Table 2. *Campylobacter* populations (log CFU/mL; Mean ± S.D.) on chicken breast fillets (n = 15) inoculated with *Campylobacter* and either immersed in peroxy acetic acid solution (500 ppm PAA, 10 min) or sprayed with PAA solution (500 ppm, 10 min).

Medium/Treatments	Control[1]	Immersion	Spray
Campy-cefex agar[2]	$5.08 \pm 0.10^{a,x}$	$3.97 \pm 0.17^{b,x}$	$4.09 \pm 0.12^{b,x}$
Brucella agar	$5.17 \pm 0.17^{a,x}$	$4.06 \pm 0.26^{b,x}$	$4.10 \pm 0.10^{b,x}$

[1]Same superscripts$^{(a,b)}$ within the same row indicate no significant differences ($P > 0.05$) between the treatments; Same superscripts$^{(x,y)}$ within the same column indicate no significant differences ($P > 0.05$) between the media.

[2]Gentamycin (200 ppm) was incorporated into the media before to preparing petri plates.

Table 3. *Salmonella* populations (log CFU/mL; Mean ± S.D.) on chicken breast fillets (n = 3) either immersed in peroxyacetic acid solution (PAA) or application of PAA spray for various time periods.

				Spray[2] (resting Time)	
Conc. (ppm)	Immersion time (s)	Immersion[1]	Spray application time (s)	0 s	30 s
Control	-	5.05 ± 0.10^{a}	-	5.05 ± 0.10^{a}	
100	4	4.61 ± 0.08^{b}	5	$4.37 \pm 0.29^{b,x}$	$4.39 \pm 0.12^{b,x}$
	10	4.48 ± 0.19^{b}	10	$4.40 \pm 0.15^{b,x}$	$4.28 \pm 0.02^{b,x}$
	30	$4.04 \pm 0.20^{c,d}$			
250	4	4.18 ± 0.19^{c}	5	$4.26 \pm 0.09^{b,c,x}$	$4.32 \pm 0.10^{b,x}$
	10	4.15 ± 0.06^{c}	10	$4.04 \pm 0.10^{c,d,x}$	$4.13 \pm 0.10^{b,x}$
	30	3.72 ± 0.16^{e}			
500	4	4.01 ± 0.25^{c}	5	$4.22 \pm 0.09^{b,c,x}$	$3.99 \pm 0.13^{b,d,x}$
	10	$3.89 \pm 0.12^{c,e}$	10	$3.82 \pm 0.08^{d,x}$	$3.70 \pm 0.24^{d,x}$
	30	3.28 ± 0.05^{f}			
1,000	4	$3.82 \pm 0.34^{d,e}$	5	$3.91 \pm 0.09^{d,x}$	$3.11 \pm 0.08^{d,y}$
	10	3.60 ± 0.19^{e}	10	$3.16 \pm 0.08^{e,x}$	$3.04 \pm 0.06^{e,x}$
	30	3.13 ± 0.41^{f}			

[1]Same superscripts$^{(a,b,c,d,e)}$ within the same column indicate no significant differences ($P > 0.05$) between the treatments.
[2]Same superscripts$^{(x,y,z)}$ between the 0 s and 30 s "resting" time for the spray application indicate no significant differences ($P > 0.05$).

been a suitable medium for enumerating potentially injured *Salmonella* populations. Similarly, *Campylobacter* populations were similar ($P > 0.05$) on both nonselective (BA^{G}) and selective (Campy-cefex^{G}) media, regardless of the antimicrobial treatment method (Table 2). For ease of use, we selected the APC (Petrifilm) and Campy-cefex^{G} as plating media for *Salmonella* and *Campylobacter*, respectively for evaluating the efficacy of antimicrobial treatments.

The mean pH values of the PAA solutions (100, 250, 500, and 1,000 ppm) were 2.6 ± 0.3, 2.7 ± 0.4, 2.8 ± 0.2, and 3.0 ± 0.1, respectively and the temperatures were 4.20 ± 0.02°C, 3.80 ± 0.04°C, 3.60 ± 0.04°C, and 3.20 ± 0.02°C, respectively, for the same PAA solutions used during the experiment. Inoculation of the breast fillets resulted in *Salmonella* and *Campylobacter* population of 5.05 and 4.94 log CFU/mL, respectively, in the rinsate (Tables 3 and 4). Immersion of the inoculated breast fillets in PAA solution (100 ppm) for 4, 10, and 30 s resulted in *Campylobacter* reductions of 0.20, 0.39 and 0.78 log CFU/mL, respectively. Statistically significant reductions ($P \leq 0.05$) in *Campylobacter* and *Salmonella* were observed when the breast fillets were immersed in PAA solution (100 ppm) for at least 30 s or in PAA solutions of higher concentrations for any immersion times (4, 10 or 30 s). Greater reductions ($P \leq 0.05$) in *Salmonella* populations were observed by immersion of inoculated chicken breast fillets in PAA solution (100 ppm) for the same immersion times (0.44, 0.57, and 1.00 log CFU/mL after to immersion for 4, 10 and 30 s, respectively). Increasing the PAA solution concentrations to 250, 500, and 1,000 ppm resulted in increasingly greater reductions ($P \leq 0.05$) in *Salmonella* and *Campylobacter* populations (Table 3 and 4). Immersion of the inoculated breast fillet in 1,000 ppm PAA solution for 30 s resulted in *Salmonella* and *Campylobacter* population reductions by 1.92 and 1.87 log CFU/mL, respectively. Chen et al. (2014) reported 1.5 and 1.3 log CFU/mL reductions in *Salmonella* and *Campylobacter* on ground chicken treated with 700 and 1,000 ppm of PAA at 4°C, respectively. Recently, Kim et al. (2017) showed that postchill application with 750 ppm of PAA for 15 s immersion resulted in 4.08 and 2.23 log CFU/chicken reduction of *Campylobacter* and *Salmonella*, respectively. Smith et al. (2015) reported reduction in Cam*pylobacter jejuni* population after immersion in a PAA solution (100 and 200 ppm) maintained at 10°C. Greater reductions in populations of *Salmonella* and *Campylobacter* were observed with higher concentration of PAA may be attributed to the lower pH value with the higher concentrations of PAA, as lower pH enhances the oxidizing effect of the PAA solution (Bell et al., 1997).

Peroxy acetic acid may have variable effects, depending on the susceptibility of microorganisms and their adherence properties. King et al. (2005) reported that PAA spray application on beef carcasses had no effect on *E. coli* O157:H7 and *S. typhimurium*. Our study suggests that PAA was slightly more effective against *Salmonella* than *Campylobacter* (Table 3 and 4). The mode of action of PAA is still unclear, although PAA, like other oxidizing agents, disrupts or oxidizes sulfhydryl and sulfur bonds in the proteins and lipids of the microorganism (Middleton et al., 1997; Block, 2011). Rio et al. (2007) reported significant reductions (0.5 to 2.0 log CFU/g skin) of various microflora, including Enterobacteriaceae, Micrococcaceae, Enterococci, *Brochothrix thermosphacta*, pseudomonads, lactic acid bacteria, molds, and yeasts on inoculated chicken legs immersed in 220 ppm PAA for 15 min. In a similar study, a 1.0 log CFU/cm^2 reduction of *S. typhimurium* was observed when beef trimmings were immersed in 200 ppm PAA treatments for 15 s (Ellebracht et al., 2005).

Spraying PAA solution (100 ppm) onto inoculated breast fillets for 5 s resulted in *Campylobacter* population reductions of 0.34 and including a 30 s resting time subsequent to application of PAA spray resulted in 0.58 log CFU/mL reduction (Table 4). Slightly greater reductions (0.68 and 0.65 log CFU/mL; $P \leq 0.05$) were observed in *Salmonella* populations on breast fillets sprayed with PAA solution for 5 or 10 s

Table 4. *Campylobacter* populations (log CFU/mL; Mean ± S.D.) on chicken breast fillets (n = 3) either immersed in peroxy acetic acid solution (PAA) or application of PAA spray for various time periods.

				Spray[2] (resting Time)	
Conc. (ppm)	Immersion time (s)	Immersion[1]	Spray application time (s)	0 s	30 s
Control	-	4.94 ± 0.11^{a}	-	4.94 ± 0.11^{a}	
100	4	$4.74 \pm 0.08^{a,b}$	5	$4.60 \pm 0.14^{a,b,x}$	$4.36 \pm 0.26^{b,x}$
	10	$4.55 \pm 0.13^{a,b}$	10	$4.66 \pm 0.11^{a,b,x}$	$4.14 \pm 0.06^{b,y}$
	30	4.16 ± 0.27^{b}			
250	4	4.43 ± 0.05^{b}	5	$4.30 \pm 0.12^{b,x}$	$4.38 \pm 0.33^{b,y}$
	10	4.23 ± 0.16^{b}	10	$4.20 \pm 0.22^{c,x}$	$4.08 \pm 0.18^{b,x}$
	30	3.71 ± 0.02^{c}			
500	4	4.09 ± 0.09^{b}	5	$4.16 \pm 0.05^{c,x}$	$3.71 \pm 0.22^{b,c,y}$
	10	3.69 ± 0.51^{c}	10	$4.09 \pm 0.01^{c,d,x}$	$3.78 \pm 0.22^{b,c,x}$
	30	3.26 ± 0.05^{d}			
1,000	4	4.05 ± 0.30^{b}	5	$3.51 \pm 0.13^{e,x}$	$3.31 \pm 0.08^{d,x}$
	10	3.71 ± 0.02^{c}	10	$3.70 \pm 0.13^{d,e,x}$	$3.48 \pm 0.58^{c,d,y}$
	30	3.07 ± 0.64^{e}			

[1]Same superscripts$^{(a,b,c,d,e)}$ within the same column indicate no significant differences ($P > 0.05$) between the treatments.
[2]Same superscripts$^{(x,y,z)}$ between the 0 s and 30 s "resting" time for the spray application indicate no significant differences ($P > 0.05$).

(Table 3). Greater *Campylobacter* reductions (1.71 log CFU/mL) were achieved with spraying higher concentrations of PAA solution on the chicken breast fillets. Relatively greater reductions in *Campylobacter* populations was achieved by immersion of the inoculated breast fillets in PAA solution compared with spraying for the same period of time (10 s; e.g., 0.39 vs. 0.28 log CFU/mL at PAA concentration of 100 ppm), for all the concentrations of PAA, except 1,000 ppm. Smith et al. (2015) reported greater reductions in *C. jejuni* populations (0.46 log CFU/mL) after PAA spray (100 ppm) on broiler carcasses for 60 s compared with the study (0.28 log CFU/mL at 100 ppm sprayed for 10 s). Further, they showed that immersion treatment of inoculated samples was more effective compared with the spray method (Smith et al., 2015). Our results obtained are in contrast to findings of Smith et al. (2015) and Nagel et al. (2013), where the difference in *Salmonella* and *Campylobacter* reductions were similar, for both spray and immersion application. This difference can be attributed to the better adherence of the bacteria to the whole carcass (containing the skin) compared with the skin-off breast fillets.

During normal operations, the PAA solution on the surface of the chicken breast fillets following immersion or spray treatment will remain until the activity dissipates. In the current research, the antimicrobial activity of the PAA solution was terminated by immersion of the treated breast fillets in dilution buffer (for sampling) that was supplemented with a neutralizing chemical (sodium thiosulfate) to determine the immediate antimicrobial effect on *Salmonella* and *Campylobacter* populations. However, in typical poultry processing, subsequent to immersion or spray, the breast fillets are conveyed to the packaging station and packaged. This additional time may result in additional antimicrobial activity and further reduction of *Salmonella* and *Campylobacter* populations. Thus, we evaluated the reduction in pathogen population subsequent to 30 s of "resting time" to simulate processing conditions and estimate the additional lethality achieved. The reductions in *Salmonella* and *Campylobacter* populations on breast fillets subsequent to the "resting period" were similar ($P > 0.05$) to the reductions observed immediate to the spray treatment (for similar immersion times) for majority of the concentration-spray application time combinations (11 of 16). This additional resting time may not have provided adequate contact time for the antimicrobial activity to reduce *Salmonella* and *Campylobacter* populations consistently, regardless of the PAA concentration. However, consistent and significant reductions ($P \leq 0.05$) in *Salmonella* and *Campylobacter* populations were obtained when the inoculated breast fillets were immersed in PAA solution (concentrations greater than100 ppm) for 30 s compared with 4 s, indicating the longer contact with the PAA solution during the immersion treatment compared with the spray treatment. This difference was probably because of replenishment of fresh PAA solution during immersion treatment, whereas the utilized PAA was not replenished during "resting" time subsequent to the spray treatment.

Application of PAA either as an immersion or a spray treatment was effective in reducing populations of *Salmonella* and *Campylobacter* on chicken breast fillets. While immersion treatment of inoculated breast fillets in PAA solutions for 4 and 10 s resulted in similar reductions of *Salmonella* and *Campylobacter*, longer immersion time (30 s) resulted in greater ($P > 0.05$) reductions in populations of both microorganisms. In general, higher concentrations of PAA resulted in greater *Salmonella* and *Campylobacter* reductions. An additional 30 s of rest time after spray application of PAA did not result in additional reductions in either *Salmonella* or *Campylobacter* populations. The results of this study indicate that PAA can be used as an antimicrobial application for chicken breast fillets to reduce populations of *Salmonella* and *Campylobacter*. Future research on a large scale in a commercial processing plant should be conducted to validate the viability of PAA to reduce *Salmonella* and *Campylobacter* on skin-on poultry parts/carcasses in a commercial setting.

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