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 ≤ 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 ≤ 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 ≤ 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

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...
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, cetlypyridinium 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]\(^{\text{NR}}\)*) and gentamycin-resistant strain of *Campylobacter coli (CG]\(^{\text{GR}}\)*) 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-cfelx 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 (SanDate 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\(_2\)SO\(_4\) (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:

$$\text{PAA concentration (%) = } \frac{[\text{Na}_2\text{S}_2\text{O}_3 (\text{mL})] \times [\text{N} \times \text{Na}_2\text{S}_2\text{O}_3 \times 3.8]}{\text{Weight of } 0.2 \text{ mL PAA}}$$

**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 

$$\text{ST}^{\text{NR}} \text{ and } \text{CC}^{\text{GR}} \text{ 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 (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 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<sub>NA</sub>). Appropriate dilutions of PW<sub>NA</sub> were plated on Petrifilm APC (Aerobic Plate Count plates) and Petrifilm ENT (Enterobacteriaceae plates). Preparing serial dilutions in PW<sub>NA</sub> 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<sub>NA</sub> and BGSNA, respectively). The plates were incubated as per the manufacturers’ instructions for Petrifilm, and the TSA<sub>NA</sub> and BGSNA 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<sup>G</sup> agar and BA<sup>G</sup> 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<sup>NA</sup>. Appropriate dilutions prepared with PW and PW<sup>NA</sup> were plated on Campy-cefex<sup>G</sup> 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<sup>G</sup> 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<sup>NA</sup>, and BGS<sup>NA</sup> 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<sup>NA</sup> (5.42 log CFU/mL), the difference may not be biologically significant. Immersion and spray application of PAA resulted in decreases ($P < 0.05$) 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<sup>NA</sup>] and nonselective media [APC, TSA<sup>NA</sup>]) 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 Enterobacteriaceae 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) agar, 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<sup>NA</sup>) and the selective medium (BGS<sup>NA</sup>) 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<sup>NA</sup> 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 peroxo acetic acid solution (500 ppm PAA, 10 min) or sprayed with PAA solution (500 ppm, 10 min).

| Medium/Treatments | Control<sup>1</sup> | Immersion | Spray |
|-------------------|---------------------|-----------|-------|
| APC<sup>2</sup>    | 5.74 ± 0.19<sup>ab</sup>a | 4.25 ± 0.14<sup>abc</sup>a | 4.47 ± 0.17<sup>b</sup>a |
| ENT<sup>3</sup>    | 5.49 ± 0.17<sup>bc</sup>a | 4.31 ± 0.49<sup>bc</sup>a | 4.18 ± 0.27<sup>b</sup>a |
| TSA<sup>NA</sup>   | 5.54 ± 0.20<sup>ab</sup>a | 4.24 ± 0.11<sup>ab</sup>a | 4.47 ± 0.19<sup>b</sup>a |
| BGS<sup>NA</sup>   | 5.42 ± 0.20<sup>bc</sup>a | 4.16 ± 0.15<sup>b</sup>a | 4.41 ± 0.24<sup>b</sup>a |

<sup>1</sup>Same superscripts<sup>a,b</sup> within the same row indicate no significant differences ($P > 0.05$) between the treatments; Same superscripts<sup>a,b</sup> within the same column indicate no significant differences ($P > 0.05$) between the media.

<sup>2</sup>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<sup>NA</sup>) 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 peroxo acetic acid solution (500 ppm PAA, 10 min) or sprayed with PAA solution (500 ppm, 10 min).

| Medium/Treatments | Control<sup>1</sup> | Immersion | Spray |
|-------------------|---------------------|-----------|-------|
| Campy-cefex<sup>G</sup> agar | 5.08 ± 0.10<sup>a,b</sup> | 3.97 ± 0.17<sup>b</sup>a | 4.09 ± 0.12<sup>b</sup>a |
| Brucella agar | 5.17 ± 0.17<sup>a,b</sup> | 4.06 ± 0.26<sup>b</sup>a | 4.10 ± 0.10<sup>b</sup>a |

<sup>1</sup>Same superscripts<sup>a,b</sup> within the same row indicate no significant differences ($P > 0.05$) between the treatments; Same superscripts<sup>a,b</sup> within the same column indicate no significant differences ($P > 0.05$) between the media.

<sup>2</sup>Gentamycin (200 ppm) was incorporated into the media before preparing petri plates.

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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 (Petri-plate) and Campy-cefex 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 a 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 ≤ 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 ≤ 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 ≤ 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 Campylobacter 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).

Perox 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/cm² 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 ≤ 0.05) were observed in Salmonella populations on breast fillets sprayed with PAA solution for 5 or 10 s.

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

| Conc. (ppm) | Immersion time (s) | Immersion^1 | Spray application time (s) | Spray^2 (resting Time) |
|-------------|--------------------|-------------|---------------------------|------------------------|
| Control     | -                  | 5.05 ± 0.10^a | -                         | 5.05 ± 0.10^a          |
| 100         | 4                  | 4.61 ± 0.08^b | 5                         | 4.37 ± 0.29^c,x       |
|             | 10                 | 4.48 ± 0.19^b | 10                        | 4.40 ± 0.15^d,x       |
|             | 30                 | 4.04 ± 0.20^c | 10                        | 4.28 ± 0.02^e,x       |
| 250         | 4                  | 4.18 ± 0.19^d | 5                         | 4.26 ± 0.09^f,x       |
|             | 10                 | 4.15 ± 0.06^e | 10                        | 4.04 ± 0.10^f,x       |
|             | 30                 | 3.72 ± 0.16^f | 10                        | 4.13 ± 0.10^g,x       |
| 500         | 4                  | 4.01 ± 0.25^g | 5                         | 4.22 ± 0.09^h,x       |
|             | 10                 | 3.89 ± 0.12^h | 5                         | 3.82 ± 0.08^i,x       |
|             | 30                 | 3.28 ± 0.05^i | 10                        | 3.70 ± 0.24^j,x       |
| 1,000       | 4                  | 3.82 ± 0.34^j | 5                         | 3.91 ± 0.09^k,x       |
|             | 10                 | 3.60 ± 0.19^k | 10                        | 3.16 ± 0.08^l,x       |
|             | 30                 | 3.13 ± 0.41^l | 10                        | 3.04 ± 0.06^m,x       |

^1 Same superscripts(x,y,z) 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 *Campylobacter* populations subsequent to 30 s of immersion treatment, whereas the utilized PAA solution (concentrations greater than 100 ppm) 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* ≤ 0.05) in *Salmonella* and *Campylobacter* populations were obtained when the inoculated breast fillets were immersed in PAA solution (concentrations greater than 100 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 (*P* ≤ 0.05) antimicrobial activity to reduce *Salmonella* and *Campylobacter* populations. An additional 30 s of resting 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.

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

| Conc. (ppm) | Immersion time (s) | Immersion | Spray application time (s) | Spray (resting Time) |
|-------------|--------------------|-----------|---------------------------|---------------------|
| Control     | -                  | -         | 0 s                       | 30 s                |
| 100         | 4                  | 4.64 ± 0.1a,b | 5                      | 4.60 ± 0.1d,b,x     |
|             | 10                 | 4.78 ± 0.2c | 10                     | 4.66 ± 0.1c,b,x     |
|             | 30                 | 4.16 ± 0.27c |                       | 4.14 ± 0.06c,y      |
| 250         | 4                  | 4.43 ± 0.05d | 5                      | 4.30 ± 0.12d,b,x    |
|             | 10                 | 4.31 ± 0.16c | 10                     | 4.20 ± 0.22b,x      |
|             | 30                 | 3.71 ± 0.02d |                       | 4.08 ± 0.18b,x      |
| 500         | 4                  | 4.09 ± 0.09d | 5                      | 4.16 ± 0.05c,x      |
|             | 10                 | 3.69 ± 0.51d | 10                     | 4.09 ± 0.01c,b,c,x  |
|             | 30                 | 3.26 ± 0.05e |                       | 3.78 ± 0.22c,x      |
| 1,000       | 4                  | 4.05 ± 0.30e | 5                      | 3.51 ± 0.13a,b,x    |
|             | 10                 | 3.71 ± 0.02e | 10                     | 3.70 ± 0.13b,c,x    |
|             | 30                 | 3.07 ± 0.64e |                       | 3.48 ± 0.58e        |

1Same superscripts(a,b,c,d,e) within the same column indicate no significant differences (*P* > 0.05) between the treatments.

2Same superscripts(x,y,z) between the 0 s and 30 s “resting” time for the spray application indicate no significant differences (*P* > 0.05).
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