Application of Amplon in combination with peroxyacetic acid for the reduction of nalidixic acid–resistant *Salmonella* Typhimurium and *Salmonella* Reading on skin-on, bone-in tom turkey drumsticks

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ABSTRACT Peroxyacetic acid (**PAA**) has become an important component of pathogen reduction in poultry processing, but there are potential concerns for continued exposure. The objective was to evaluate the effects of PAA and Amplon (**AMP**) used alone or in the combination. Bone-in tom turkey drumsticks (N = 100, n = 10, k = 5, 0 and 24 h) per study were obtained and inoculated with either nalidixic acid–resistant *Salmonella* Typhimurium or *Salmonella* Reading (64 μg/mL). The inocula were allowed to adhere to the drums at 4°C for 60 min for a final attachment of 10^8 and 10^7 cfu/g per *S.* Typhimurium and *S.* Reading, respectively. Drumsticks were treated with a no-treatment control; tap water, pH 8.5 (**TW**); TW+500 ppm PAA, pH 3.5 (PAA); TW+500 ppm AMP, pH 1.3 (AMP); TW + PAA + AMP (PAA + AMP). Treatments were applied as short duration dips (30 s) and allowed to drip for 2 min. After treatment, drums were stored at 4°C until microbial analyses at 0 and 24 h. Drums were rinsed in neutralizing buffered peptone water and spot plated for total aerobes and *Salmonella.* Bacterial counts were $\log_{10}$ transformed and analyzed using n-way ANOVA. All treatments reduced *S.* Reading on turkey legs at both 0 and 24 h ($P < 0.0001$; $P < 0.0001$). At 24 h, drums treated with PAA + AMP (3.92 $\log_{10}$ cfu/g) had less *S.* Reading than no-treatment control, TW, and AMP. Treatment by time interactions were observed for total aerobes among drums in both studies ($P < 0.0001$, $P < 0.0001$) and *Salmonella* among drums inoculated with *S.* Typhimurium ($P < 0.0001$). During the *S.* Reading and *S.* Typhimurium study, all treatments reduced *Salmonella* and total aerobes on drums. During the *S.* Typhimurium study, drums treated with PAA + AMP had the lowest numerical load of *S.* Typhimurium and total aerobes. The combination of AMP + PAA may exhibit a synergistic effect in reducing *Salmonella* on turkey drums, thus increasing the safety of turkey products for consumers.

Key words: Turkey, peroxyacetic acid, Amplon, pathogen reduction, *Salmonella*

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INTRODUCTION

Nearly fifty million people are affected by foodborne illness in the United States yearly, with some cases being fatal (CDC, 2019a). *Salmonella* is the etiological agent of Salmonellosis and results in significant bacterial foodborne disease outbreaks annually (CDC, 2019a). *Salmonella* is most commonly transmitted through the consumption of poultry products with approximately 4 of 5 *Salmonella* outbreaks associated with meat products in 2018 in poultry-related products (Chai et al. 2017; CDC, 2019b). Thus, federal regulatory oversight agencies that safeguard the US food supply, such as the Center for Disease Control, have set objectives for reducing *Salmonella* related infections. Specifically, the Center for Disease Control aimed to lower *Salmonella*-related infections to 11.4 cases per 100,000 people by 2020, while currently developing more stringent goals for 2030 (Healthy People, 2020). One tool the poultry industry uses to help reduce *Salmonella* and improve food safety on poultry products is antimicrobials applied at the processing plant.

Because different chemicals have various mechanisms that they exhibit on particular bacteria, the combination of the sanitization agents may ultimately have a better outcome for reducing pathogens (Bacon et al., 2000;

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Stopforth et al., 2007). Furthermore, the combination of various processing antimicrobials such as organic and inorganic acids may benefit processors by eliminating the potential for noxious residues and carcass discoloration as compared with other antimicrobials (Kim et al., 2017; Landrum et al. 2019). The industrial use of up to 2,000 ppm of peroxyacetic acid (**PAA**) in poultry manufacturing has been officially permitted by the USDA and Food Safety and Protection Services and is regarded as generally recognized as safe (21 CFR 173.370; FSIS, 2019; Kim et al., 2017; Moore et al., 2017). The antimicrobial PAA is an organic acid and a combination of acetic acid and hydrogen peroxide that degrades into acetic acid, oxygen, and water without imparting a reduction in meat quality (Kim et al., 2017; Moore et al., 2017). Although organic acids are efficient and function by reducing the pH in the immediate bacterial environment, gram-negative microorganisms can develop resistance (Dittoe et al., 2019).

Salmonella is capable of adapting to and surviving acidification from organic acids owing to the presence of lipopolysaccharides in the outer membrane as well as other mechanisms (Ricke, 2003; Dittoe et al., 2019). However, acid resistance acquired by bacteria from organic acid acidified environments does not exhibit resistance to inorganic acids, as *Salmonella* requires the production of additional proteins to control the pH homeostasis (Foster et al., 1991; Dittoe et al., 2019). Consequently, inorganic acids remain strong sanitization agent alternatives to PAA (Dittoe et al., 2019). A registered inorganic mix of sulfuric acid and sodium sulfate sold as Amplon by Zoetis (Florham Park, NJ) is used as an accepted antimicrobial, acidifier, and processing aid in meat manufacturing that shows the ability to reduce pathogen contamination in poultry-associated products while keeping its organoleptic properties (Kim et al., 2017). Similar to PAA, the constituents of Amplon are considered generally recognized as safe under the Food and Drug Administration (FSIS 7120.1), and are registered for the antimicrobial use throughout poultry processing via spray, wash, or dip application (Kim et al., 2017; Moore et al., 2017).

While extensive research has been conducted with chicken processing and potential interventions, less is known for turkey processing. Therefore, it is an objective of the present study to examine the potential of Amplon as an antimicrobial intervention in turkey production by determining the efficacy of Amplon alone (500 ppm) or in combination with PAA (500 ppm) on the frequency of *Salmonella* Reading and *Salmonella* Typhimurium on skin-on, bone-in turkey drumsticks. Commonly, processing aids and antimicrobials are tested against *S.* Typhimurium as an industry standard. However, *S.* Reading is becoming a significant problem linked to raw turkey products (Tanguay et al., 2017; CDC, 2019b). Consequently, we evaluated the efficacy of Amplon-acidified PAA as a short-duration antimicrobial part dip by demonstrating the reduction of *S.* Typhimurium and *S.* Reading. The study aimed to provide the industry with further intervention strategies to mitigate poultry-associated Salmonellosis.

MATERIALS AND METHODS

Drum Leg Procurement and Indigenous Pathogen Screening

One hundred bone-in, skin-on drum tom turkey legs were obtained for the study evaluating *S.* Typhimurium (study 1; N = 100, n = 10, k = 5, 0 and 24 h) and another 100 for the studies evaluating *S.* Reading (study 2; N = 100, n = 10, k = 5, 0 and 24 h). The turkey drumsticks, with an average weight of 1,023.59 g (SEM = 15.20) and 1,118.12 (SEM = 92.16), were obtained from a poultry facility immediately after processing. These turkey legs were acquired from a commercial partner and were killed as a component of normal industrial turkey processing and were, therefore, institutional animal care and use committee exempted. All industry and federal guidelines were followed. Immediately after processing, the drum turkey legs were shipped to the University of Arkansas Center for Food Safety, where 1 turkey leg was screened for the presence of indigenous *Salmonella* per study. The rest were stored at 4°C refrigeration for 3 to 4 h (4 h maximum) until the onset of the study.

Inoculum Preparation and Inoculation

To assure the validity of the antimicrobials against both *Salmonella* species, the studies were performed on separate d: In study 1, only *S.* Typhimurium was used; in study 2, only *S.* Reading was used. Between 12 and 16 h before each study started, the isolated cultures were inoculated using the following methodology and grown overnight. For simplification purposes, all methodologies in the following are described uniformly, as the methods used were the same across both studies.

Preparation of Nalidixic Acid–Resistant *Salmonella* Species

Frozen and pure stock cultures of *S.* Typhimurium (ATCC 19585) and *S.* Reading were streaked to isolation under aerobic conditions at 37°C for 24 h on Xylose Lysine Dextrose (**XLD**; HiMedia, West Chester, PA) media before the study. The *S.* Reading culture was part of the Center for Food Safety's collection of stock cultures, and the culture was initially isolated from turkey. To prepare 64 μg/mL nalidixic acid (**NA**)–resistant strains, 10 mL of sterile 1X PBS (8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4, and 0.24 g of KH_2PO_4 per 1 L, with the pH adjusted to 7.4 with HCl) and 64 mg/mL of NA (higher than the breaking point for resistance) were combined in a sterile 15-mL conical tube to prepare 64 μg/mL stock solution. The solution was then serially diluted (1:10) in 9 mL of sterile Mueller Hinton Broth (Hardy Diagnostics, Irving, TX). These tubes were then inoculated with either *S.* Typhimurium or *S.* Reading and grew overnight at 37°C. The following day, serial dilutions were performed again, with the highest dilution with NA-resistant surviving strains

inoculated into higher NA dilutions and incubated overnight at 37°C. The method was repeated until the cultures survived in 64 μg/mL of NA.

Once cultures grew in Mueller Hinton Broth with 64 μg/mL of NA, the resistance was confirmed by streaking on XLD plates prepared with 64 μg/mL of NA (XLD + NA). One colony was isolated from the incubated plates and streaked to isolation again using the methods described previoulsy. This was repeated once more to confirm the presence of *Salmonella*.

Inoculation

Isolated colonies from each plate were transferred to 10 capped, sterile 500-mL bottles containing 400 mL (for a total of 4 L for each *Salmonella* serovar) each of Mueller Hinton Broth (Hardy Diagnostics, Irving, TX). The cultures were placed in a shaking incubator at 37°C for 12 to 16 h at 200 rpm. Immediately after the overnight incubation of the cultures, 40 mL of the cultures aliquoted to separate 50-mL conical tubes and centrifuged at 18,000 *g* for 3 min to collect the pellet, decanted, washed, and resuspended in 1X PBS (8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4, and 0.24 g of KH_2PO_4 per 1 L, with the pH adjusted to 7.4 with HCl) and recentrifuged. A total of 2 washes were performed. After the final wash, the pellets were resuspended in 40 mL of sterile PBS.

The drum turkey legs were inoculated using 550 mL of inoculum per 10 legs (1 mL of inocula per 20 g of turkey). To determine the effects of the treatments against both strains, the first 100 legs were inoculated with only *S.* Typhimurium (study 1), while the remaining 100 legs were inoculated with *S.* Reading (study 2). The inocula were allowed to adhere at 4°C for 60 min for a final attachment level of 10^8 cfu/g. After the attachment period, the weights of each leg were recorded, and the treatments were administered. In poultry rinse bags, a drum turkey leg and 500 mL of the corresponding treatment were transferred and agitated to allow full coverage for 30 s by shaking using a 90° arcing motion. The treatments were decanted, and the legs were allowed to drip for 2 to 3 min before proceeding to the next step. To reduce the risk of cross contamination, each step of the treatment occurred in individual sterile poultry rinse bags. The treatments used were a no-treatment control (**NTC**); tap water (TW, pH 8.5); **TW** + 500 ppm PAA (PAA, pH 3.5); TW + 500 ppm Amplon (**AMP**, pH 1.3); TW + PAA + AMP (PAA + AMP). The commercial PAA used in the present study was Actrol Max (22% PAA; Zoetis, Florham Park, NJ).

Microbial Analysis

After the allotted resting period, 400 mL of neutralizing Buffered Peptone Water (pH 7.7; 20.0 g of buffered peptone, 7 g of refined soy lecithin or equivalent, 1.0 g of sodium thiosulfate, 12.5 g of sodium bicarbonate, per 1 L of DI water; USDA Food Safety and Inspection Service, 2016) was poured into each bag on top of the drum turkey legs. The legs were manually agitated for 1 min using a 180° arcing motion. The legs were aseptically removed from the bags and discarded, while the rinsates were used for downstream analysis. Exactly 1 mL of each biological replicate was aliquoted to 2-mL microcentrifuge tubes with subsequent 20 μL being serially diluted (1:10) to 10^{-7} in 180 μL of 1X PBS in a flat-bottom 96-well plate. Using the drop method for plating, 10 μL of the rinsate was spot plated on XLD + NA and Tryptic Soy Agar (EMD Millipore Corporation, Billerica, Massachusetts) and allowed to dry completely before inverting (Thomas et al., 2015). The plates were inverted and incubated aerobically at 37°C for 24 h. On XLD + NA, only black colonies were identified as *Salmonella* isolates.

Statistical Analysis

Each turkey leg was randomly assigned to a treatment and a time point before analyses. Duplicate plates were averaged before analyses. CFU/mL was transformed on a cfu/g basis with the following equation as described by Dittoe et al. (2019).

$$\frac{\left(\frac{Number\ of\ Colonies}{0.01\ mL\ plated}\right) * Dilution\ Factor}{\left(\frac{Drumstick\ Weight\ (g)}{Original\ Homogenate\ (mL)}\right)} = CFU\ /\ g\ of\ Drumstick$$

The cfu/g of *Salmonella* were $\log_{10}$ transformed and reported on a $\log_{10}$ cfu of *S.* Typhimurium and *S.* Reading per g of turkey drumstick ($\log_{10}$ CFU/g of drumstick). Data were analyzed using JMP 14 (Cary, NC) and analyzed using a two-way ANOVA (treatment and time). If no interaction existed between treatment and time, 1-way ANOVA was performed to evaluate the effect of treatment and time, separately. In addition, a 1-way ANOVA of treatments at each time (0 and 24 h) was performed if no interaction existed (treatment and time). Data were considered significant at a P value of less than or equal to 0.05.

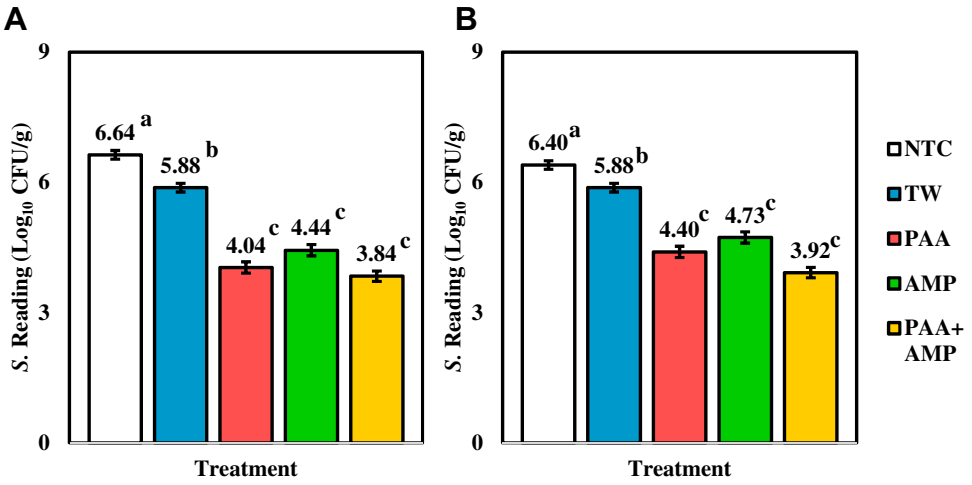

Figure 1. The effect of treatment at 0 (A) and 24 (B) h on *Salmonella* when 64 μg/mL nalidixic acid–resistant *S.* Reading was artificially inoculated on tom turkey drumsticks. Drumsticks were treated with the following short-duration dip treatments: no -reatment control (NTC), tap water (TW); TW + 500 ppm PAA (PAA); TW + 500 ppm AMP, pH 1.3 (AMP); TW + PAA + AMP (PAA + AMP). Drumsticks were agitated in 500 mL of the treatments for 30 s and allowed to drip for 2–3 min before microbial analysis (A: $P < 0.0001$, N = 50, n = 10, k = 5; B: $P < 0.0001$, N = 50, n = 10, k = 5). Baseline attachment of *Salmonella* Reading at 10^7 cfu/g or 6.64 $\log_{10}$ cfu/g as represented by NTC at 0 h. Abbreviations: AMP, Amplon; PAA, peroxyacetic acid.

RESULTS

In the present study, we evaluated whether or not Amplon-acidified PAA reduces NA-resistant *S.* Typhimurium and *S.* Reading on poultry drumsticks when applied as a short-duration dip. Results of the present study demonstrated the efficacy of combining AMP and PAA. As such, all treatments were capable of reducing *Salmonella* on turkey drums inoculated with NA-resistant *S.* Reading at 0 h (Figure 1A: $P < 0.0001$) and 24 h (Figure 1B: $P < 0.0001$). Drums treated with PAA, AMP, and PAA + AMP were not different from one another at 0 h (4.04, 4.44, and 3.84 $\log_{10}$ cfu/g, respectively). Although not significantly different, at 24 h, PAA + AMP resulted in the lowest recoverable load of NA-resistant *S.* Typhimurium than AMP (3.92 and 4.73 $\log_{10}$ cfu/g, respectively), as well as PAA when used alone (4.40 $\log_{10}$ cfu/g). However, all drums treated with the 3 treatments were different than the controls, NTC, and TW at 0 and 24 h (6.63 and 5.87 $\log_{10}$ cfu/g at 0 h and 6.40 and 5.88 at 24 h $\log_{10}$ cfu/g). Therefore, treatments had an effect overall, but that effect was not unique for each treatment group over time. The lowest load of NA-resistant *Salmonella* on treated drumsticks was observed among those treated with PAA + AMP at both 0 and 24 h (3.84 and 3.92 $\log_{10}$ cfu/g) compared with the controls (6.63 and 6.40 $\log_{10}$ cfu/g).

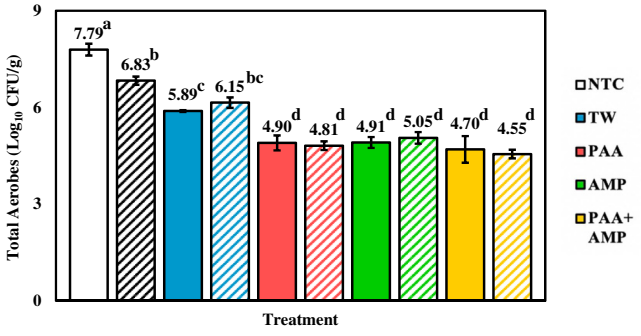

Figure 2. The interaction of treatment and time (0 and 24 h) on the load of total aerobes when 64 μg/mL nalidixic acid–resistant *Salmonella* Reading was artificially inoculated on tom turkey drumsticks. Drumsticks were treated with the following short-duration dip treatments: no-treatment control (NTC), tap water (TW); TW + 500 ppm PAA (PAA); TW + 500 ppm AMP, pH 1.3 (AMP); TW + PAA + AMP (PAA + AMP). Drumsticks were agitated in 500 mL of the treatments for 30 s and allowed to drip for 2–3 min before microbial analysis ($P = 0.02$, N = 100, n = 10, k = 5). Solid colored bars represent the mean $\log_{10}$ cfu of aerobes per g at 0 h, whereas diagonal hashed bars represent the mean $\log_{10}$ cfu of aerobes per g at 24 h. Abbreviations: AMP, Amplon; PAA, peroxyacetic acid.

To monitor the overall bacterial load, aerobic plate counts, were taken alongside NA-resistant *S.* Reading. All treatments reduced bacterial load on inoculated drumsticks (Figure 2, $P = 0.02$). Although there was not an interaction between time and treatment for *Salmonella* load ($P > 0.05$), overall bacterial load was significantly different ($P = 0.02$) for drums inoculated with NA-resistant *S.* Reading. Drumsticks rinsed with TW at 0 h (5.89 $\log_{10}$ cfu/g) had less aerobic bacteria than those not treated, NTC, at 0 and 24 h (7.79 and 6.83 $\log_{10}$ cfu/g). Drums treated with PAA + AMP had the lowest load of total aerobic bacteria (4.70 and 4.55 $\log_{10}$ cfu/g at 0 and 24 h) with over a 2 $\log_{10}$ reduction as compared with the controls (7.79 and 6.83 $\log_{10}$ cfu/g at 0 and 24 h). Drums treated with PAA, AMP, and PAA + AMP were not different from one another (4.90 and 4.81; 4.91 and 5.05; 4.70 and 4.55 $\log_{10}$ cfu/g, respectively).

A treatment by time interaction was observed for the load of *Salmonella* on turkey drums inoculated with NA-resistant *S.* Typhimurium where all treatments reduced *Salmonella* (Figure 3, $P < 0.0001$). The lowest load of NA-resistant *S.* Typhimurium was observed among

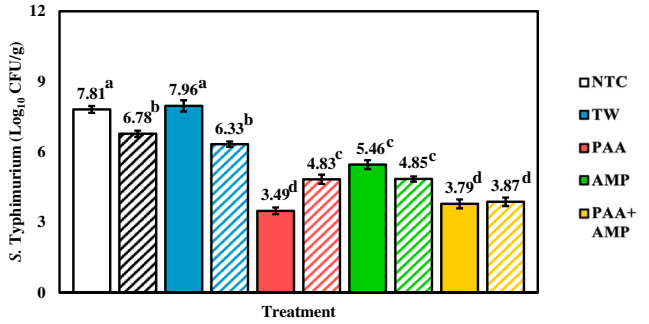

Figure 3. The interaction of treatment and time (0 and 24 h) on the load of *Salmonella* when an antibiotic resistant *Salmonella* Typhimurium was artificially inoculated on tom turkey drumsticks. Drumsticks were treated with the following short-duration dip treatments: no-treatment control (NTC), tap water (TW); TW + 500 ppm PAA (PAA); TW + 500 ppm AMP, pH 1.3 (AMP); TW + PAA + AMP (PAA + AMP). Drumsticks were agitated in 500 mL of the treatments for 30 s and allowed to drip for 2–3 min before microbial analysis ($P < 0.0001$, N = 100, n = 10, k = 5). Baseline attachment of *S.* Typhimurium at 10^8 cfu/g or 7.81 $\log_{10}$ cfu/g as represented by NTC at 0 h. Solid colored bars represent the mean $\log_{10}$ cfu of *Salmonella* per g of drumstick at 0 h, whereas diagonal colored hashed bars represent the mean $\log_{10}$ cfu of *Salmonella* per g of drumstick at 24 h. Abbreviations: AMP, Amplon; PAA, peroxyacetic acid.

turkey legs treated with PAA + AMP and PAA at 0 h (3.79 and 3.49 $\log_{10}$ cfu/g, respectively) compared with those treated with NTC (7.81 $\log_{10}$ cfu/g). Compared with the mean of *Salmonella* for the inoculated untreated control turkey legs, NTC and TW, all experimental treatments reduced the total load of *Salmonella*. The drums treated with PAA had the lowest recoverable load of NA-resistant *S.* Typhimurium at 0 h (3.49 $\log_{10}$ cfu/g), but those treated with PAA + AMP exhibited the sustained mitigation of *Salmonella* (3.79 and 3.87 $\log_{10}$ cfu/g at 0 and 24 h, respectively), which was not different than the reduction that drums treated

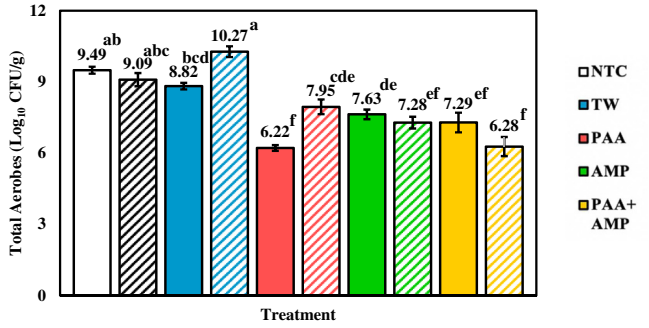

Figure 4. The interaction of treatment and time (0 and 24 h) on the load of total aerobes when 64 µg/mL nalidixic acid–resistant *Salmonella* Typhimurium was artificially inoculated on tom turkey drumsticks. Drumsticks were treated with the following short-duration dip treatments: no-treatment control (NTC), tap water (TW); TW + 500 ppm PAA (PAA); TW + 500 ppm AMP, pH 1.3 (AMP); TW + PAA + AMP (PAA + AMP). Drumsticks were agitated in 500 mL of the treatments for 30 s and allowed to drip for 2 to 3 min before microbial analysis ($P < 0.0001$, N = 100, n = 10, k = 5). Solid colored bars represent the mean $\log_{10}$ cfu of aerobes per gram of drumstick at 0 h, whereas diagonal hashed bars represent the mean $\log_{10}$ cfu of aerobes per gram of drumstick at 24 h. Abbreviations: AMP, Amplon; PAA, peroxyacetic acid.

with PAA exhibited at 0 h. Therefore, PAA + AMP potentially exhibited a synergistic effect.

There was an interaction between time and treatment on the load of total aerobic bacteria on artificially inoculated turkey drums in the current experiment (Figure 4, $P < 0.0001$). Drums treated with PAA had the lowest load of aerobic bacteria at 0 h (6.22 $\log_{10}$ cfu/g) compared with the controls, NTC and TW (9.49 and 8.82 $\log_{10}$ cfu/g, respectively) and was not statistically different from the PAA + AMP treatment (7.29 $\log_{10}$ cfu/g). Although PAA showed the greatest bacterial reduction at 0 h for both NA-resistant *S.* Typhimurium and associated aerobes (3.49 and 6.21 $\log_{10}$ cfu/g, respectively), it exhibited a rebound in numbers of *S.* Typhimurium and aerobic bacteria at 24 h (4.83 and 7.95 $\log_{10}$ cfu/g). However, the PAA + AMP treatment was able to sustain the aerobic bacterial populations from 0 h (7.28 $\log_{10}$ cfu/g) to 24 h (6.27 $\log_{10}$ cfu/g).

DISCUSSION

In the present study, Amplon-associated treatments reduced the concentration of NA-resistant *S.* Typhimurium and *S.* Reading lower than the representative infectious dose, 10^6 to 10^8 cfu (Wickham et al., 2007; Chen et al., 2013; Dittoe et al., 2019; FSIS, 2019). The $\log_{10}$ reduction of 4.02 cfu/g of *S.* Typhimurium and 2.79 cfu/g of *S.* Reading in this research demonstrated the feasibility of Amplon-acidified PAA to decrease the number of such *Salmonella* serovars. Thus, the data presented in this study suggest further research into whether or not treated poultry parts with Amplon-acidified PAA could reduce *Salmonella* contamination in raw turkey parts.

The effects of antimicrobials depend on their dilution, length of interaction with the surface area of the product, and its environmental conditions to demonstrate the antimicrobial effects (Kim et al. 2017; Moore et al., 2017; Landrum et al., 2019). Poultry manufacturers target a 1 $\log_{10}$ cfu/mL bacterial decline throughout each processing step (Mixon, 2020). In the study performed by Scott et al. (2015) on the advantage of Amplon and its antimicrobial effects on chicken wings, the use of Amplon (pH 1.1, 20 s) produced approximately 1 $\log_{10}$ reduction of *Salmonella*. An analogous study performed with sulfuric acid and sodium sulfate mix (pH 1.5 and 1.0) applied on beef surfaces exhibited significant antimicrobial effects against various strains of *Escherichia coli* and *Salmonella*, displaying its effects against various bacterial genera as well as diverse protein matrices, with a significant impact of solution pH on *Salmonella* load (Scott-Bullard et al., 2017). Alternatively, Bauermeister et al. (2008) investigated the use of PAA (85 ppm) alone and observed the reduction of *Salmonella*-positive chicken carcasses. In another study carried out by Nagel et al. (2013), the application of PAA in postchill tanks resulted in the most reduction of *S.* Typhimurium (2 $\log_{10}$ cfu/mL) on whole chicken carcasses than other antimicrobials used in the study.

Furthermore, using any antimicrobials that can produce a 2-$\log_{10}$ decrease should successfully reduce pathogens persisting on carcasses after chilling (Nagel et al., 2013). Rosenquist et al. (2003) determined that a 2 $\log_{10}$ reduction of *Campylobacter* on poultry carcasses results in a 30-fold decrease in the risk associated with foodborne disease. These findings suggest a possibility that the combination of the 2 antimicrobials might lead to greater results in sustained reduction of bacterial pathogens, thus averting foodborne-related illness.

Amplon and PAA exhibited the same trend throughout the study and were not different from one another. Peroxyacetic acid had an initial reduction but in most cases resulted in a rebounding of aerobic plate counts. *S.* Reading and *S.* Typhimurium were reduced differently, although PAA + AMP, AMP alone, and PAA were all effective. The additive effect of PAA may be due to the increased acidification of PAA owing to the combination with Amplon as was seen by Dittoe et al. (2019) who acidified PAA with an inorganic acid, sodium bisulfate. The grouping of Amplon with PAA exhibited analogous trends in reducing *Salmonella* as previous studies investigating the use of PAA alone (Scott et al., 2015). Similarly, the use of Amplon alone has been demonstrated to be an effective antimicrobial agent against *Salmonella* in former studies (Schmidt et al., 2012; Scott et al., 2015). The use of the sulfuric acid and sodium sulfate mix on beef trimmings produced a reduction of *S.* Typhimurium and *Salmonella* Newport, showing the antimicrobial mixture does not appear to be serovar specific (Geornaras et al., 2012). Dittoe et al. (2019) noted that the combination of PAA and SBS produced the most sustained reduction of Salmonella Enteritidis compared with the use of either PAA or SBS alone. A simultaneous multihurdle approach may hinder the ability of *Salmonella* to develop resistance to the mixture of inorganic and organic compounds. As determined in the present research, the significant decrease of pathogenic counts produced by Amplon-acidified PAA has the potential to successfully moderate foodborne pathogens. However, its use may be impeded owing to the complex composition of the poultry integument.

Although Amplon-acidified PAA proved a plausible antimicrobial combination in the present study, there is a potential shielding influence of poultry meat and skin that may obstruct the capability of sanitizer on poultry cuts (Dittoe et al., 2019). Poultry integument exhibits a greater buffering effect than its offcuts or the fat tissue itself (Tan et al., 2014). Consequently, the ability of Amplon-acidified PAA may be restrained. In future studies, the application of Amplon-acidified PAA should be investigated using ground meat products to explore the potentially beneficial reduction of bacterial counts owing to the lack of integument.

CONCLUSIONS

As determined in the present research, the significant and sustainable decrease of *S.* Reading counts produced by Amplon-acidified PAA has the potential to successfully moderate foodborne pathogens. Turkey parts treated with Amplon (500 ppm)-acidified PAA (500 ppm) resulted in a 4.02 and 2.79 $\log_{10}$ cfu/g decrease in *Salmonella* load (*S.* Thyphimurium and *S.* Reading), compared with controls. The application of Amplon and PAA alone showed a 2 $\log_{10}$ cfu/g reduction of *S.* Reading, which nevertheless suggests the potential to be an advantageous tool to further regulate the contamination of poultry parts.

The combinatorial application of these antimicrobial compounds could restrict the ability of *Salmonella* to adapt and develop resistance. To establish if the antimicrobial activity is similar throughout all general poultry-associated serovars, Amplon-acidified PAA must be tested with other *Salmonella* serovars. Although the present study did not evaluate the change of pH in the solutions, it will be necessary and economically beneficial for the industry to know the effect of the pH to formulate the most cost-effective and safe antimicrobial mixture. Finally, studies that improve the function of Amplon to reduce *Salmonella* and establish other possible synergistic mixtures need elucidation. With further studies, continued advances in antimicrobial development for use in poultry processing can be conducted that will diminish the spread of pathogens to the food supply.

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SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.psj.2020.08.078.

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