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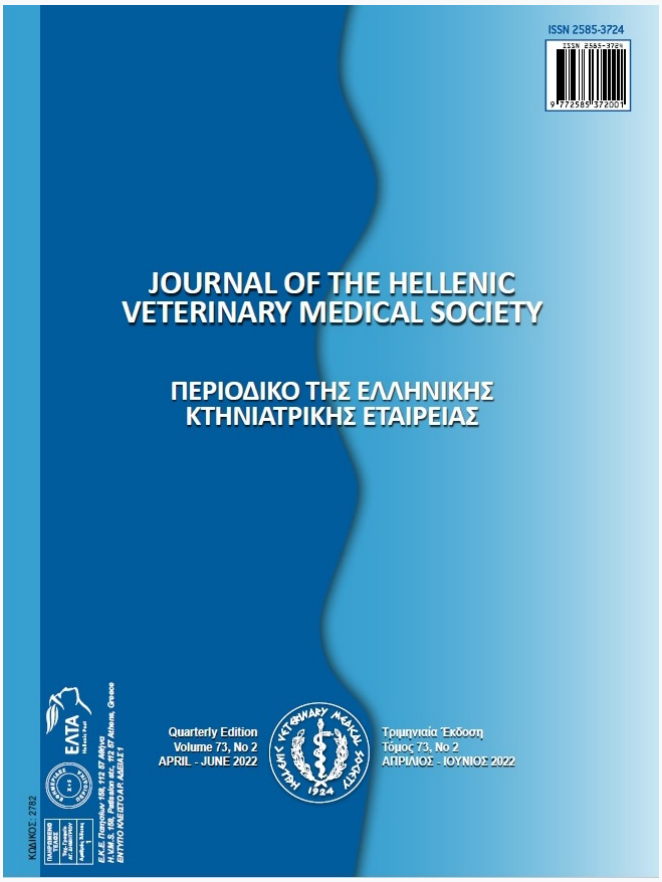

Efficacy of peroxyacetic acid against Salmonella biofilms and as a decontaminant agent in poultry meat

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Efficacy of peroxyacetic acid against *Salmonella* biofilms and as a decontamination agent in poultry meat

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ABSTRACT: This study investigated how post-chilling peroxyacetic acid (PAA) application affects the shelf life of chicken carcasses. It also evaluated the effectiveness of PAA application in chicken neck skin samples, which had been experimentally contaminated with *Salmonella* Enteritidis and *Salmonella*Typhimurium serotypes. Finally, the biofilm forming capacity of *Salmonella enterica* serovars was determined, and the activity of PAA against single and mixed *Salmonella* biofilms was examined. In all experimental groups, at least 1 log cfu/g reduction in *Salmonella* counts was observed on the day of PAA application (0 hour), and significant decreases in *Salmonella* counts were monitored in all groups after both 100 ppm and 200 ppm PAA treatments within the 6th hour. The PAA concentrations and duration of application used in this study could not appropriately reduce *S.* Typhimurium and *S.* Enteritidis counts in the chicken neck skin samples. However, these treatments could effectively extend the chicken meat's shelf life, and 200 ppm of PAA did reduce *S.* Typhimurium and *S.* Enteritidis biofilms (mean reduction: 4.8 log/ml). Statistical analysis also indicated that, when a biofilm is composed of more than one *Salmonella* strain, it is more difficult to reduce the bacterial counts with PAA treatments. However, the European Food Safety Authority (EFSA) recommends PAA as a decontamination agent for poultry production because PAA has no toxic effects on human health. In future, if this EFSA recommendation is passed into legislation, PAA studies on this subject will be of great importance.

Keywords: Biofilms; Broiler meat; Decontamination; Peroxyacetic acid; *Salmonella*

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INTRODUCTION

Various methods are used to eliminate saprophytic and pathogenic microorganisms and/or to keep the populations of those microorganisms within acceptable limits, particularly in poultry intended for human consumption. For many years, numerous countries have widely used organic acids as decontamination agents (Singh et al., 2018; Ben Braiek and Smaoui, 2021). The antimicrobial activity of organic acids is pH dependent, and it is especially evident on the surface of carcasses and meat (Anonymous, 2014). Organic acids are preferred in practice because they are inexpensive and easy to use (Ben Braiek and Smaoui, 2021). However, legal regulations for chemical decontamination technologies differ between countries. While the European Union (EU) permits the use of such methods for carcass decontamination up to a certain degree, organic acids can be used more freely for that purpose in the United States (US) (Anonymous, 2013; Anonymous, 2017a). The United States Department of Agriculture's Food Safety and Inspection Service (USDA FSIS) has determined that organic acids are generally recognized as safe (GRAS), if they do not affect the sensory qualities of the food to which they are applied. They are, therefore, approved for use as decontaminants in slaughterhouses, in water that is to be used for food production, and in the preparation stages of fresh meat and carcasses. There are also no specified daily intake limits for organic acids. Thus, these regulations facilitate the use of organic acids as decontamination agents for poultry in the US (Anonymous, 2019; Pozuelo Bonilla, 2021).

Chemical decontamination was first used in the 1960s and was reported to help control foodborne pathogens, including *Salmonella* strains (Mani-Lopez et al., 2012). Still, salmonellosis remains one of the most frequent foodborne zoonoses. Therefore, both national and international organizations have established well designed systems to control food safety and enhance food quality, especially against salmonellosis. However, implementing *Salmonella* control programs in poultry production has caused changes in the serotypes isolated from poultry. In addition to *Salmonella* Typhimurium and *Salmonella* Enteritidis, which are serious threats to public health, *Salmonella* Kentucky and *Salmonella* Infantis have become increasingly widespread (Anonymous, 2003a; Anonymous, 2016; Antunes et al., 2016).

Biofilms are collectives of bacteria growing together, and they can be found in both natural or man-made environments (e.g., food processing). Biofilms can grow on a wide variety of surfaces, including those used in the food industry (Iniguez-Moreno et al., 2018). By forming biofilms, microorganisms protect themselves against various environmental inhibitors, including disinfectants (Bialucha et al., 2021; Shatila et al., 2021). It is, thus, difficult to destroy biofilms via standard hygienic procedures (Carrascosa et al., 2021; Dula et al., 2021; Jimenez-Pichardo et al., 2021; Shatila et al., 2021). Biofilms reduce production efficiency in the food processing industry and cause equipment malfunction and unpleasant odors. The structural components of *Salmonella enterica* serovars play a role in biofilm formation on different surfaces.

In recent years, it has been frequently emphasized that peroxyacetic acid (PAA), which contains acetic acid, 1-diphosphonic acid, 1-hydroxyethylidene-1, and hydrogen peroxide, can be used as a decontaminating agent in poultry meat, inhibiting the growth of many pathogenic microorganisms and their biofilms (Anonymous, 2014; Sukumaran et al., 2015). PAA has been used for this purpose in spray treatment methods, short-term immersion applications, or cooling tanks at ambient temperatures (Gonzales Sanchez, 2020). According to the European Food Safety Authority (EFSA), PAA does not have any toxic effects on humans when it is applied as a short-term treatment in poultry meat. When PAA is used in short-term immersion applications, it does not leave peroxyacid residues, does not form hydrogen peroxide reactions with proteins and fats, and does not create public health risks in the poultry carcasses or in the water used in poultry production (Anonymous, 2014; Gonzales Sanchez, 2020). However, though PAA is approved for use in poultry, there is a lack of data demonstrating its rational antimicrobial efficacy.

Within this context, this research analyzed the effects of PAA applications on the shelf life of chicken carcasses. It also evaluated the effectiveness of PAA application in chicken neck skin samples that were experimentally contaminated with *S.* Enteritidis and *S.* Typhimurium serotypes. Finally, the biofilm forming capacity of *S. enterica*serovars was determined, and the activity of PAA against single and mixed *Salmonella* biofilms was examined.

MATERIALS AND METHODS

Experimental design

This study was performed on chicken carcasses

and chicken neck skins collected from healthy animals in a clean section of a broiler slaughterhouse in Bolu Province, northwest Turkey, between May and July 2019. The slaughterhouse samples were collected at separate times for each replicate experimental analysis. A total of 125 chicken carcasses and 120 neck skins were collected aseptically, taken to the Ankara University Faculty of Veterinary Medicine, Laboratory of Department of Food Hygiene and Technology under 4°C conditions on the same day, and analyzed. The study was carried out in three stages. In the first stage, the effects of PAA application post-chilling on the shelf life of chicken carcasses were analyzed. In the second stage, the effectiveness of PAA application was investigated in chicken neck skin samples, which were experimentally contaminated with *S.* Typhimurium and *S*. Enteritidis serotypes. In the third stage, the biofilm-forming capacities of *S.*Enteritidis ATCC 13076, *S.* Typhimurium ATCC 14028, *S*. Enteritidis, *S*. Typhimurium, *S*. Kentucky, and *S*. Infantis were determined, and the PAA activity against single and mixed *Salmonella enterica* biofilms was evaluated. All experimental analyses were conducted in triplicate.

Bacterial isolates

A total of four different wild *Salmonella enterica* serovars (i.e., *S.* Enteritidis and *S.* Typhimurium isolated from packaged carcasses and *S.* Kentucky and *S.* Infantis isolated from scalding tank water) and two different reference *S.enterica* serovars (i.e., *S.*Enteritidis ATCC 13076 and *S.*Typhimurium ATCC 14028) were used in this study.

Preparation of PAA solutions

Four different concentrations (100, 200, 230, and 690 ppm) of PAA were prepared from a stock solution (38%-40% purity) (Merck 1.07222.1000) for all experimental analyses. To prepare 100, 200, 230, and 690 ppm concentrations of PAA, 0.25, 0.50, 0.58, and 1.73 mL of stock solution were added to 1 L of sterile distilled water at 20°C, respectively. The peroxyacetic acid concentrations applied began with the lowest to the highest doses recommended by EFSA in poultry meat. All dilutions were prepared fresh 1 h before each experiment (Anonymous, 2014).

Determination of the effect of post-chilling PAA application on the shelf life of chicken carcasses

A total of 125 chicken carcasses were collected aseptically from a slaughterhouse and taken to the laboratory under 4°C conditions on the same day and analyzed. All carcasses were from the same healthy farm and slaughtered under veterinary control. For the application of PAA, 25 chicken carcass samples were used in each of the four groups of treatments: 1) 230 ppm/15 s, 2) 230 ppm/30 s, 3) 690 ppm/15 s, and 4) 690 ppm/30 s. The control groups were formed to determine the microbiological quality differences between chicken carcasses with and without the post-chilling PAA application. The samples were analyzed for aerobic mesophilic count (AMC), psychrophilic count (PC), *Enterobacteriaceae* (E) and coliform group (CG) microorganisms. The carcasses were prepared using the rinse method. The carcasses in sterile sample bags were rinsed with 500 mL of sterile peptone water (PS, Oxoid CM009) for 2 min, and dilutions were prepared using 9 mL of PS sterile tubes (Anonymous, 2003b). For the evaluation of AMC and PC, tryptic soy agar (TSA, Oxoid CM0131) was used, and the plates were incubated for up to 72 h at 30°C and 4°C, respectively (Anonymous, 2003c). For the *Enterobacteriaceae* and coliform bacteria counts, violet red bile glucose agar (Oxoid CM1082) and violet red bile lactose agar (Oxoid CM0968) were used, and the plates were incubated at 37°C for 24 h (Anonymous, 1991; Anonymous, 2004). Microbiological analyses were performed on the zero hour and on the first, third, fifth, and seventh days of shelf life.

***Salmonella* control with PAA applied to chicken neck skin**

Neck skins of healthy chickens were used as materials. After the removal of internal organs, the neck skins taken from the slaughter line were immediately brought to the laboratory under a cold chain on the same day and analyzed. For this purpose, 120 neck skins were used. Before the experimental contamination with *S.*Typhimurium and *S.*Enteritidis serovars, the neck skin samples were prepared as 5 × 5 cm^2 pieces and analyzed for the presence of *Salmonella* spp. Prior to the experiment, each of the *S.enterica*-serovars was reactivated in tryptone soy broth (TSB, Oxoid CM0876) and incubated at 37°C for 18-24 h. The serotypes were then diluted with buffered peptone water (BPW, Oxoid CM0509) to achieve an inoculum containing approximately 10^3 and 10^5 log cfu/mL. Subsequently, the neck skin samples were contaminated for 1 min in 500 mL solutions containing *S.enterica* serovars at concentrations of 10^3 and 10^5 log cfu/mL for each *Salmonella* group and preserved at room temperature for 30 min for adhesion of the

microorganisms (Meredith et al., 2013; Anonymous, 2017b).

After the neck skin samples were experimentally contaminated with *S.enterica* serovars (*S.* Typhimurium 10^3, *S.* Typhimurium 10^5, *S.* Enteritidis 10^3, and *S.*Enteritidis 10^5), 25 chicken carcass samples were used in four groups of PAA treatments: 1) 100 ppm/10 s, 2) 100 ppm/30 s, 3) 200 ppm/10 s, and 4) 200 ppm/30 s. The control groups were separated to determine the adhesion levels of the serotypes that would not be decontaminated. Microbiological analyses were performed on the zero and sixth hours and on the first, third, and fifth days of the study.

pH measurement

Skin samples treated only with PAA concentrations were classified as the pH group in which pH would be analyzed (Meredith et al., 2013). The pH group contained 20 neck skin samples. An electronic pH meter (HI-2221 Hanna pH meter) was used for the measurement.

PAA activity against *Salmonella* biofilms

Prior to the experiment, each of the *S.enterica* serovars was reactivated in TSB and incubated at 37°C for 18-24 h. The serotypes were then diluted with BPW to achieve an inoculum containing approximately 10^3, 10^5, and 10^8 log cfu/mL. A total of 22 experimental groups were created according to different dilutions of different microorganisms used in the experiment. Microorganisms were classified as A (*S.* Typhimurium wild type), B (*S.* Enteritidis wild type), C (*S.* Typhimurium ATCC 14028), D (*S.* Enteritidis ATCC 13076), E (*S.* Kentucky wild type), F (*S.* Infantis wild type), and G (cocktail of the wild-type serovars) groups. The negative control group was coded K.

Microplates (96-well Mikroplate, Thermo Scientific ™ 15041) were used in sterile conditions to evaluate biofilm formation. First, 230 µL of TSB was inoculated into each well of the microplates, and then 20 µL of the liquid cultures of the pre-enriched serotypes were added to each well. The wells used for the negative control were provided to contain only 230 µL of TSB. The microplates were incubated at 35°C for 48 h in aerobic conditions. After incubation, the microplate contents were poured, and the wells were washed with 300 µL of sterile distilled water. To fix the microorganisms attached to the surface of the microplates, 250 µL of methanol were added to each well and maintained for 15 min (Stepanovicet al., 2004). Afterward, the contents of the microplates were evacuated and air-dried. Then, 250 µL of 33% glacial acetic acid (Thermo Scientific FLA38S500) was added (Stepanovicet al., 2004). The microorganisms dissolved from the microplate surfaces with the help of acetic acid were transferred to 100 mL of physiological saline (sterile 0.85% NaCl) using sterile cotton swabs, and TSA was used for the counting of biofilm-forming microorganisms. The plates were incubated for 48 h at 35°C, and all colonies were counted (Wang et al., 2013).

To determine the susceptibility of *S.*Typhimurium and *S.*Enteritidis biofilms to PAA, the procedure in which biofilm formation was achieved in the previous stage was repeated with the addition of PAA solution instead of 33% glacial acetic acid. In this context, 250 and 500 µL of PAA were used for 100 and 200 ppm PAA applications, respectively. The procedures were repeated three times for each serotype (Joseph et al., 2001).

Statistical analysis

Descriptive analysis was used to summarize the data and to check whether the assumptions were met. The results were evaluated using the Shapiro-Wilk test for normality, Levene's test for homogeneity of variances, and Mauchly's test for sphericity. The Greenhouse-Geisser correction was used in terms of violation of sphericity. A two-way repeated measures analysis of variance (ANOVA) was used to determine the similarities and differences between the experimental groups. Measurements from different intervals were used as equivalents for the within-subject and distinct bacteria groups defined as between-subjects. One-way repeated measures ANOVA was used to determine the PAA treatments for biofilms. All data were analyzed using SPSS 14.01 (SPSS, Inc., USA). SPSS for Windows License No.: 9869264 version 14.01 (computer software) Chicago, Illinois, USA, SPSS Inc. A p value of 0.05 was considered significant for all analyses.

RESULTS

Determination of the effect of post-chilling PAA application on the shelf life of chicken carcasses

Figure 1 presents the microbiological analyses of chicken carcasses after the PAA application. According to the results, the number of microorganisms analyzed in all the experimental groups had at least 1

log cfu/g reduction on the day of the PAA application (zero hour). In the 690 ppm/15 s and 690 ppm/30 s group samples, only a 1 log cfu/g increase was observed in terms of the AMC counts until the end of the seventh day. In the 690 ppm/30 s group, 2 log decreases were detected in all microbiological values at the end of the seventh day.

Salmonella control with PAA applied to chicken neck skin

The microbiological analysis results of the zero and sixth hours and the first, third, and fifth days after the application of 100 and 200 ppm PAA concentrations were evaluated by considering the average of the three applications. Figure 2 shows the analysis of the chicken neck skin samples after decontamination with the PAA treatments. Before the experimental analyses, all neck skin samples were examined to determine if they were contaminated with *Salmonella* spp. or not. No sample was detected as positive.

We found significant decreases in all experimental groups after the 100 and 200 ppm PAA treatments on the sixth hour ($p < 0.05$). Figure 3 shows the bacterial counts after the treatment procedure as an example of the *S*. Enteritidis ATCC 14028 treatment.

pH measurement

The analysis of the pH results showed a slight decrease until the third day, and increased pH values were observed after the third day. The pH levels of the samples are shown in Figure 4. The results from the statistical analyses interpreted as time intervals, excluding the 0-6 hours, were significantly different from others. All experimental groups had significantly different pH levels ($p < 0.05$). No interaction was observed between the time and group subjects in terms of pH.

PAA activity against *Salmonella* biofilms

The experimental analysis results showed that biofilm formation was observed only in the 10^8 log cfu/mL bacteria groups. The biofilm counts were < 2.0 log cfu/mL in the 10^3 and 10^5 log cfu/mL bacteria groups. According to the statistical analyses of PAA treatment, the 200 ppm treatments were effective in reducing *S*. Enteritidis ATCC 13076, *S*. Typhimurium ATCC 14028, *S*. Enteritidis, and *S*. Typhimurium wild-type biofilms at a 10^8 log cfu/mL concentration ($p = 0.02$, $p < 0.001$, $p < 0.00$ $p = 0.001$). However, the 100 ppm treatments were not significantly effective against any biofilms, as a mean of 4.8 log/mL reduction was detected against *S*. Enteritidis and *S*. Typhimurium biofilms. In addition, the biofilms of *S*. Kentucky, *S*. Infantis, and the cocktail of the wild-type serovars were not affected by the PAA treatment. The bacterial counts of biofilm formation and the counts after the PAA treatments are shown in Figure 5.

DISCUSSION

Different organic acids can be used to increase the shelf life of poultry carcasses and meat parts or to inhibit pathogenic microorganisms. Among the organic acids used for this purpose, PAA is preferred because it is effective in cold water and the environment, is not affected by the presence of organic matter, and does not need to be rinsed after application. PAA has been previously shown to effectively lessen the bacterial contamination of poultry meat parts and carcasses (Anonymous, 2014; Park et al., 2017; Ramirez-Hernandez et al., 2018; Walsh et al., 2018; Bourassa et al., 2021). The current data clearly demonstrate that despite the recommended PAA concentrations, PAA does not seem sufficient in controlling for *S*. Typhimurium and *S*. Enteritidis in chicken neck skin, but it affects decontamination in broiler meat when used in efficient doses. Our data showed that the recommended PAA concentrations might not be sufficient for the control of *S.*Typhimurium and *S.*Enteritidis in chicken neck skin. However, when used in these doses, PAA has a significant effect on general decontamination in broiler meat and a positive effect on shelf life. We determined that PAA treatment was effective in microbial reduction in chicken carcasses, even at very low doses and short application times.

In our study, PAA application on aerobic mesophilic bacteria, psychrophilic bacteria, *Enterobacteriaceae*, and coliform bacteria was shown to be effective in terms of carcass decontamination at 230 and 690 pmm concentrations. The highest concentration and longest time parameters had the most common microbiological reduction effects among the experimental groups. The dose applied in the A and B groups was the lowest dose recommended by the EFSA (Anonymous, 2014). Similar to our study, Purnell et al. (2014) analyzed a 15-s spray treatment of a commercial PAA solution containing 400 ppm peracetic acid and found it to be efficient for 0.80 log cfu/g *Enterobacteriaceae* reduction in neck skin and 0.19 log cfu/g in breast skin.

PAA solutions caused a slight change in the pH of chicken skins (Figure 4). Related studies have simi-

larly shown that PAA applications did not change the meat pH, which is important and desired evidence of meat quality (Young et al., 2004; Park et al., 2017). Nagel et al. (2013) reported that there was no difference in terms of quality problems with 400 and 1000 ppm PAA treatments at a short dwell time in a post-chill immersion tank. According to Bauermeister et al. (2015), PAA could extend product shelf life when carcasses were treated with >150 ppm PAA in the primary chiller, and no quality defects related to PAA applications were observed. Similarly, in their study, 200 ppm PAA treatments showed a 1.1 log cfu/g reduction in coliform and psychrotrophic bacteria and a 1.4 log cfu/g reduction in aerobic bacteria at the end of the first day.

The *S.enterica* serovars used in this study for chicken neck skin decontamination were selected according to Commission Regulation (EC) No. 200/2012 (Anonymous, 2012). The application concentrations in chicken skins recommended by EFSA were taken as the basis (Anonymous, 2014). PAA was effective in the control of *Salmonella* on chicken meat parts, but PAA concentrations of 100 and 200 ppm, as recommended by EFSA (Anonymous, 2014), for the specific decontamination of *S.enterica* serovars, were considered insufficient.

According to our results, a 1.0 log cfu/g reduction was achieved on the sixth hour following the PAA applications, but the growth curve began to increase after the sixth hour (Figure 3). This shows that the applications of 100 and 200 ppm PAA in the study for 10 and 30 s were insufficient for *S*. Typhimurium and *S*. Enteritidis decontamination. This may be due to the organic acid concentration used during the applications not being able to provide sufficient lethal effects and the microorganisms continuing to multiply more resiliently, adapting to the acidic environment (Mani-Lopez et al., 2012; Martiny et al., 2017). Moreover, there was no significant difference in microbial reduction between the *S*. Typhimurium and *S*. Enteritidis serotypes in terms of the PAA decontamination applications on chicken neck skin samples ($p < 0.5$).

Similar to our study, Ramirez-Hernandez et al. (2018) examined PAA applications at different concentrations for *Salmonella* reduction in poultry meat parts under simulated commercial processing conditions and found that even a PAA concentration of 800 ppm failed to achieve more than a 1.0 log cfu/g *Salmonella* reduction, which we achieved. In another study, *S.*Typhimurium reductions of 2.02 and 2.14 log cfu/g were achieved in broiler carcasses as a result of 400 and 1000 ppm PAA treatments in post-chill decontamination tanks, respectively (Nagel et al., 2013). Vaddu et al. (2021) compared 50, 250, and 500 ppm PAA applications for 10 s and 60 min in chicken wings contaminated with 6.24 log cfu/mL *Salmonella*. According to the statistical analysis, when chicken wings were immersed for 10 s, increasing the PAA concentration from 50 ppm to 250 and 500 ppm did not affect *Salmonella* reduction ($p > 0.05$). *Salmonella* reduction was higher (2.56 log cfu/mL) when chicken wings were immersed for 60 min at a PAA concentration of 500 ppm ($p < 0.05$) (Vaddu et al., 2021). Studies related to the present work have found that PAA treatments at concentrations of 1,200-2,000 ppm could provide the necessary lethal effect (Park et al., 2017; Walsh et al., 2018). These findings do not contradict our result that PAA concentrations of 100-200 ppm are insufficient.

Other studies have achieved *Salmonella* reduction similarto ours, despite their use of high concentrations of PAA. Kumar et al. (2020) reported that immersion of breast fillets in 500 ppm PAA solution for 10 s resulted in a 1.16 log cfu/mL reduction. Chen et al. (2014) and Zhang et al. (2018) found that poultry parts treated with 700 and 1000 ppm of PAA achieved a 1.5 log cfu/g *Salmonella* reduction in a post-chill decontamination tank. Scott et al. (2015) examined the efficiency of 700 ppm for a 20-s PAA treatment in chicken wings samples and again found a 1.5 log cfu/g reduction of *Salmonella* populations. However, unlike our 1.0 log cfu/g reduction on the sixth hour, they achieved a 0.6-log cfu/g reduction in *Salmonella* populations up to the 24th hour. These findings show that 200 ppm of PAA is effective only for 6 h for *Salmonella* reduction, but higher amounts of PAA concentrations can be effective for one day. Moore et al. (2017) analyzed six antimicrobial agents for the reduction of *Salmonella* in ground chicken frames and found that among the agents, 1,000 ppm of PAA had one of the highest reductions (0.9 log cfu/g) in *Salmonella* counts ($p \leq 0.05$), consistent with our result.

The inactivation of *Salmonella* biofilms in the food industry is crucial in public health. According to our PAA treatment experiments against *Salmonella* biofilms, 200 ppm was effective in reducing *S*. Typhimurium and *S*. Enteritidis biofilms but not *S*. Kentucky, *S*. Infantis, and the cocktail of the wild-type serovars. There was no difference in the PAA efficiencies in the wild-type serovars, *S.*Enteritidis ATCC

13076, and *S.*Typhimurium ATCC 14028. Thus, we can conclude from the statistical analysis that when *Salmonella* biofilms are formed with another strain, it is more difficult to reduce their counts with PAA treatments. There was no significant reduction of the cocktail of the wild-type serovars, whereas a significant reduction was observed in the countsof *S*. Typhimurium and *S*. Enteritidis biofilms alone ($p<0.001$) (Figure 5). Therefore, PAA treatments have dose- and species-dependent effects against *Salmonella* biofilms.

Similar to our 4.8 log/mL reduction of *S*. Typhimurium and *S*. Enteritidis biofilms, Iniguez-Moreno et al. (2018) reported a 5 log cfu/cm^2 biofilm reduction of *S.*Enteritidis ATCC 13076, despite a high concentration of 3,500 ppm of PAA treatment. Chylkova et al. (2017) found that 230 ppm PAA treatments were not significantly effective against *Salmonella* Heidelberg and *Salmonella* Senftenberg biofilms, with mean reductions of 2.8 and 3.9 log cfu/mL, respectively. From these findings, we can conclude that the efficacy of PAA treatments against *Salmonella* biofilms changes in a strain-dependent manner.

An interesting result of our study is that the application of a 200 ppm PAA concentration in a chicken neck skin decontamination experiment was insufficient to inactivate *S*. Typhimurium and *S*. Enteritidis, whereas 200 ppm was effective in reducing the same serotypes in the biofilm experiment. This may be due to the microorganisms attached to the follicles on the skin surface, which are easily protected against acid applications. Many studies about this issue have indicated that follicles, in which microorganisms are well protected, are the greatest threat to skin contamination and that the accumulation of more fat in the skin prevents sanitizers from contacting the surface, thus significantly inhibiting the effectiveness of decontamination (Duan et al., 2017; Vaddu et al., 2021; Vetchapitak et al., 2021).

CONCLUSIONS

The combination of PAA concentrations and duration of applications used in the study was insufficient in controlling for *S*. Typhimurium and *S*. Enteritidis in chicken neck skin samples, but these treatments were effective in extending the shelf life of chicken carcasses. A PAA concentration of 200 ppm was efficient in reducing *S*. Typhimurium and *S*. Enteritidis biofilms but not *S*. Kentucky, *S*. Infantis, and the cocktail of the wild-type serovars. We believe that PAA applications can be used to extend the shelf life of chicken carcasses and to control *Salmonella* in poultry meat when used in higher concentrations. To prevent the development of stress adaptation and acid resistance in pathogenic microorganisms, the target microorganisms should be completely inactivated during decontamination. In this context, the use of organic acids at an appropriate concentration and pH control are crucial in the framework of good manufacturing practices. Therefore, the hazard analysis critical control point plans for poultry production may be included in the PAA treatment. More studies are needed to ensure the effectiveness of PAA in the poultry meat production chain. There will always be a need for microbial load reduction in poultry production within the context of farm-to-fork food safety and, thus, public health.

CONFLICT OF INTEREST

None declared by the authors.

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