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ORIGINAL ARTICLE

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Efficacy of organic acids, lactic and formic acid, and peracetic acid in decontaminating process water and carcasses in chicken slaughter

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Abstract

Foodborne pathogens pose a persistent threat in broiler chicken production, particularly during the slaughter process, where contamination with zoonotic pathogens remains a concern. This study focuses on the potential of organic acids, such as formic and lactic acid, and the oxidizing agent peracetic acid, to decontaminate scalding water and enhance the hygiene of chicken carcasses. We conducted suspension tests introducing various organic loads to mirror the conditions of practical scalding water. Additionally, the surface tests were performed on chicken skin. Both methods were further tested in an experimental slaughtering facility. In suspension tests, the organic acids achieved impressive decontamination, with a 5-$\log_{10}$ reduction of the test organisms *Enterococcus hirae*, *Salmonella Typhimurium* and *Campylobacter jejuni* at minimal concentrations (between 0.04% and 2% for formic acid; between 0.1% and 4.5% for lactic acid). Peracetic acid also effectively sanitized model water and chicken skin, even when used in low concentrations (between 0.001% and 0.1%), both in the laboratory-based testing and in the experimental slaughtering facility. These results suggest that the tested disinfectants can effectively sanitize process water, even under conditions mimicking practical scalding water with organic matter. Peracetic acid, in particular, proved highly effective in improving chicken skin hygiene even at low concentrations.

KEYWORDS
Campylobacter, chicken, disinfection, *Enterococcus*, *Salmonella*, slaughter hygiene

1 | INTRODUCTION

Foodborne diseases, particularly infections caused by pathogens such as *Campylobacter* and *Salmonella*, remain an essential global public health concern (WHO, 2015). Within the European Union (EU) and worldwide, these diseases continue to exert a substantial impact. An example of this issue is the high prevalence of human campylobacteriosis, which was highlighted in the EU One Health Zoonoses Report (EFSA, 2023) for the year 2022, reporting 137,107 cases. Notably, *Campylobacter* monitoring data revealed that 38.3% of 7905 neck skin samples from chilled broiler carcasses collected at EU slaughterhouses tested positive for *Campylobacter*. Furthermore, Salmonellosis, another foodborne gastrointestinal infection, ranked as the second most common infection in humans within the EU, with 65,208 confirmed cases reported in 2022.

Beyond the critical implications for public health, foodborne diseases also impose significant healthcare costs. A study by Schorling et al. (Schorling et al., 2023) estimated the costs associated with patients suffering from *Campylobacter* enteritis in Germany to be a substantial €95.19 million over a 12-month period.

Numerous measures are in place to reduce the presence of these pathogens in chicken meat products, spanning the entire production chain from pre-harvest to post-harvest stages. The European Food Safety Authority (EFSA) conducted a comprehensive review of control options for *Campylobacter* in broilers during primary production (EFSA, 2020). Their findings underscored the importance of interventions such as vaccination, the use of feed or water additives, and the employment of few and well-trained staff in reducing *Campylobacter* colonization in broiler chickens. Additionally, research has revealed similar outcomes for on-farm interventions targeting CTX-resistant *E. coli* (M. Projahn et al., 2021). However, it is worth noting that many of these studies primarily focus on pre-harvest interventions.

Throughout the chicken slaughter process, there exists a persistent risk of bacterial cross-contamination via process waters and surfaces at various stages, including scalding and defeathering (Olsen et al., 2003; Projahn et al., 2019). Moreover, studies have shown that carcass contamination, particularly with intestinal contents, can occur during the evisceration process (Hue et al., 2010; Rivera-Pérez et al., 2014).

Research has demonstrated that the introduction of chemical decontamination procedures at various stages within broiler slaughterhouses can effectively reduce the bacterial load on chicken carcasses (Loretz et al., 2010). Organic acids and oxidizing agents are well-known for their potent antimicrobial properties. Organic acids, such as formic or lactic acid, exert their antimicrobial effects primarily by lowering pH levels (Ricke, 2003). Conversely, oxidizing agents like peracetic acid or hydrogen peroxide operate by oxidizing and disrupting components of bacterial cell walls (Maris, 1995). Disinfectants based on these organic acids and oxidizing agents are widely utilized across diverse industries, including food processing, animal husbandry, and healthcare, owing to their proven effectiveness against bacteria.

We sought to investigate whether adding organic acids and oxidizing agents into the scalding water and a pre-cooling treatment in the slaughter process effectively reduces bacterial concentrations in both the water used and on the carcasses themselves.

To address this question, we conducted a series of laboratory-based experiments to evaluate the efficacy of two organic acids and one oxidizing agent in suspension tests, using different organic loads to mimic the conditions in scalding water, and in surface tests on chicken skin. Additionally, in an experimental slaughtering facility setting, we assessed the impact of these interventions on chicken carcasses under conditions very similar to the real-world scenarios in commercial slaughterhouses. This research aims to highlight the effectiveness of these disinfection strategies in reducing bacterial contamination during chicken processing, with potential implications for improving food safety and public health irrespective of pre-harvest conditions.

2 | MATERIALS AND METHODS

2.1 | Study design

This study seeks to evaluate the effectiveness of using organic acids and oxygen-releasing agents for improving hygiene within the chicken slaughter process. Initially, we conducted laboratory-based suspension tests, with a primary focus on the decontamination of scalding water. Additionally, surface tests involving chicken skin germ carriers to assess the decontamination of chicken carcasses via a pre-cooling treatment were performed. To evaluate the practical application of these interventions, both methodologies were implemented in an experimental slaughtering facility at the German Federal Institute for Risk Assessment.

2.2 | Laboratory-based testing

2.2.1 | Strains

For the laboratory-based tests, we selected *Enterococcus hirae* (*E. hirae*) ATCC 10541, *Salmonella Typhimurium* (*S. Typhimurium*) ATCC 13311, and *Campylobacter jejuni* (*C. jejuni*) reference strain BfR-CA-14430, a field strain from chicken meat provided by the German Federal Institute for Risk Assessment, as the test organisms. *E. hirae* ATCC 10541 and *S. Typhimurium* ATCC 13311 were selected as test organisms in accordance with the criteria outlined in DIN EN 1276 and DIN EN 13697. According to our laboratory's previous experiences and pre-trials conducted for this study, *E. hirae* was shown to be the most resilient one, which is the reason why this strain was included in our study. As previously mentioned, *Salmonella* and *Campylobacter* are frequently associated with foodborne illnesses linked to chicken meat consumption, which is the reason we selected these strains for our study.

2.2.2 | Preparation of bacterial suspension

For the laboratory-based tests, both the suspension tests and the surface tests on chicken skin, a bacterial suspension with a density of 1.5–5 x 10^8 colony-forming units per milliliter (CFU/mL) needs to be prepared.

Stocks of *E. hirae* and *S. Typhimurium* were stored at −80°C on Cryobank beads (Mast Diagnostica GmbH, Reinfeld, Germany) and cultured on tryptone soy agar (TSA) plates (bioMérieux SA, Marcy l'Etoile, France) at 37°C for 24 h under aerobic conditions. The recovered colonies were stored at 4°C for up to 4 weeks and passaged on TSA plates for new working cultures the day before each test.

For preparing the bacterial suspension, the colonies were suspended in 10 mL of a Tryptone-NaCl solution, containing 1 g of Tryptone and 8.5 g of NaCl per liter. To obtain a homogenous suspension, the centrifuge tube with 5 g of glass beads was vortexed for 3 min.

The optical density was measured using a McFarland densitometer (BioSan, Riga, Latvia) and adjusted to 2.0–2.5 McFarland units, resulting in a density of 1.5–5 x 10^8 CFU/mL. For confirmation, a 10-fold serial dilution was prepared and plated on TSA plates for each test.

C. jejuni was stored and recovered following the protocol of Rollins et al. (Rollins et al., 1983). Stocks of *C. jejuni* were prepared in brain heart infusion (BHI; Oxoid, Hampshire, UK) and stored at −80°C. 25 cm^2 cell culture flasks were previously equipped with 7 mL of BHI agar and 4 mL of BHI broth containing 4 *Campylobacter* growth supplements (SR 0232E; Oxoid, Hampshire, UK) per liter. The prepared cell culture flasks were spiked with 20 µL of the thawed *Campylobacter* suspension and incubated at 42°C in a microaerobic atmosphere (N_2: 85%, CO_2: 10%, O_2: 5%) for 18–24 h. From this overnight culture, 1 mL was transferred into a centrifuge tube containing 9 mL of BHI broth and vortexed to homogenize. The cell density of 1.5–5 x 10^8 CFU/mL was confirmed by preparing a 10-fold serial dilution and plating on modified *Campylobacter*-selective charcoal cefoperazone deoxycholate agar (mCCDA) plates (Roth, Karlsruhe, Germany) supplemented with CCDA selective supplement (4857.1; Roth, Karlsruhe, Germany). The plates were incubated for 24 h at 42°C under microaerobic conditions.

2.2.3 | Disinfectants

We examined the efficacy of two organic acids, formic acid (FA; Roth, Karlsruhe, Germany) and lactic acid (LA; VWR International, Paris, France), and one oxidizing agent, peracetic acid (PAA; PanReac AppliChem, Darmstadt, Germany), in their pure forms. The substances were diluted with water of standardized hardness at a pH of 7.0 ± 0.2 to attain the required concentrations. The different concentrations that were tested in each experiment are further described in the respective chapters about each testing setup. To terminate the action of the disinfectants after the designated reaction time, a specific neutralizing agent for the respective disinfectant was used.

2.2.4 | Suspension test

To assess the decontamination of scalding water, we conducted the quantitative suspension test following DIN EN 1276–2019 guidelines. The test was carried out at a temperature of 52°C to simulate chicken abattoir scalding conditions. The aim of this test is to find the concentration that achieved a 5-log_{10} reduction of the respective tested pathogen in suspension.

The bacterial suspension was prepared as previously described, and 1 mL of it was combined with 1 mL of an organic load. After a pre-mixing for 2 min, 8 mL of the respective disinfectant solutions at various concentrations were added. Following a contact time of 3 min, reflecting the scalding duration at the chicken abattoir, 1 mL of the mixture was transferred into 8 mL of a suitable neutralizing medium specific to the disinfectant, along with 1 mL of water, for a 10 ± 1 s neutralizing period to stop the disinfectant's reaction. The neutralized solution was then plated on TSA plates for *E. hirae* and *S. Typhimurium* or mCCDA plates for *C. jejuni* using a dual approach, followed by incubation at 37°C for 24 h in aerobic conditions or incubation at 42°C under microaerobic conditions, respectively.

After incubation, the colonies on all plates were counted manually, and the log_{10} reduction was calculated. The lowest disinfectant concentration resulting in a 5-log_{10} reduction was deemed effective for the testing.

To validate the testing, three different controls were performed. Control A was carried out to verify the absence of germicidal effects under the test conditions. Control B tested the absence of germicidal effects of the neutralizer. Finally, control C was performed to validate the process.

TABLE 1 Acronyms and explanations of the three tested organic loads.

Organic load (acronym)	Explanation
BSA	3 g/l BSA, according to DIN EN 1276, volume introduced in suspension test: 1 mL
SW1	Autoclaved scalding water from a chicken abattoir, volume introduced in suspension test: 1 mL
SW2	Autoclaved scalding water from a chicken abattoir, practice-oriented approach with a higher volume introduced in suspension test: 8.2 mL

2.2.5 | Organic loads

Given the presence of substantial organic matter in scalding water, three distinct organic loads were investigated in the suspension tests (Table 1).

In the initial approach, two diverse organic loads were examined: the high-level organic load prepared in accordance with DIN EN 1276 (BSA). This suspension contained 30 g/L bovine serum albumin (BSA; Sigma, St Louis, Missouri), resulting in a final BSA concentration of 3 g/L. And a sample of scalding water obtained from a chicken abattoir's scalding tank after 7 h of processing, which underwent subsequent autoclaving (SW1).

In the subsequent approach, our goal was to accurately replicate conditions within a chicken abattoir. To achieve this, we introduced an increased volume of autoclaved scalding water into the experiment. Accordingly, the tested disinfectant concentrations were pre-concentrated tenfold, and only one-tenth of this volume (0.8 mL disinfectant) was introduced. The remaining volume (7.2 mL) was then supplemented with autoclaved scalding water (SW2).

The scalding water utilized for the suspension tests in the laboratory remained unchanged throughout all experiments, drawn from the same collection. It was collected from the slaughterhouse prior to starting the experiments and, after being autoclaved, was stored in small quantities at −20°C. For comparison with the other organic load using bovine serum albumin, the protein level of the collected scalding

water was determined using the RC DC™ Protein Assay, which is based on the Lowry protocol. It resulted in a protein level of approximately 1 g/L in the autoclaved scalding water.

2.2.6 | Surface test

To assess the efficacy of chemical disinfectants on chicken skin, we developed a method based on the principles of the surface test described in DIN EN 13697-2019 for steel surfaces. The carriers used in this method were obtained by punching 2.5 x 2.5 cm sections from the skin of commercially available, fresh chicken drumsticks. For each disinfectant, three different concentrations were tested: PAA at 0.1%, 1%, and 2%; FA at 10%, 20%, and 30%; and LA at 10%, 20%, and 40%. These concentrations were chosen based on preliminary trials to demonstrate a broad spectrum of reduction rates across various concentration levels while achieving at least a reduction by approximately one $\log_{10}$ level for all tested pathogens. The testing was conducted with five biological replicates for each concentration to account for potential variation and enhance statistical robustness.

Each chicken skin carrier was placed in a sterile petri dish and inoculated with 50 µL of the prepared bacterial suspension. The bacterial suspension was evenly distributed on the carrier using a plating spatula. After a 5-min contact time, 100 µL of the respective disinfectant concentration were applied to the carrier and distributed using a plating spatula. An additional carrier was moistened with 100 µL of water to serve as a baseline for comparison. The carriers were then stored for a contact time of 2 h at 4°C, simulating the cooling conditions in chicken slaughterhouses.

Following the contact time, each carrier was placed in a centrifuge tube containing 10 mL of the respective neutralizing medium, designed to stop the disinfectant reaction, and 5 g of glass beads. The tubes were vortexed for 1 min to transfer the bacteria from the chicken skin germ carrier into the medium and left undisturbed for a neutralization period of 5 min. The suspension was subsequently diluted and plated in duplicate. A selective agar was used for each tested organism in order to assure that the background bacterial load on the chicken skin does not affect the result. For *E. hirae*, the suspensions were plated on CHROMagar™ Orientation (CHROMagar, Paris, France) plates and for *S. Typhimurium*, on Xylose Lysine Deoxycholate (XLD) agar (Merck, Darmstadt, Germany) plates, both incubated at 37°C for 24 h under aerobic conditions. For *C. jejuni*, the suspensions were plated on mCCDA agar plates and incubated at 42°C for 24 h under microaerobic conditions.

To validate the testing, two additional controls were performed in addition to the water control. The absence of germicidal effects of the neutralizers was tested by adding 100 µL of water, and the process validation was tested by adding 100 µL of the highest tested disinfectant concentration to 10 mL of the neutralizer and allowing a neutralization period of 5 min. After that time, one inoculated chicken skin germ carrier was transferred into the neutralizing medium, and the tube was vortexed for 1 min. From these suspensions, a 10-fold serial dilution was prepared and plated on the respective agar.

After incubation, colonies on all plates were counted manually, and the $\log_{10}$ reduction was calculated based on the difference between the number of bacteria on the chicken skin carrier treated with water and those treated with the different disinfectant.

2.3 | Experimental slaughtering plant

To evaluate the practicability of the two measures, we conducted tests in an experimental chicken slaughtering facility at the German Federal Institute for Risk Assessment. The objective was to assess the effectiveness of scalding water hygienization and pre-cooling dip in reducing bacterial contamination on the carcasses during chicken processing. In these experiments, we exclusively examined the impact of introducing the disinfectant PAA, based on its favorable outcomes in the laboratory-based trials. Our focus was on assessing how the inclusion of PAA influenced the total aerobic colony counts on commercial chicken carcasses. We evaluated the total aerobic colony count on the neck skin samples both before and after the respective treatment. Calculating the reduction between the colony counts allowed for effective comparison across the different groups.

2.3.1 | Scalding water

For testing the hygienization of scalding water, chicken carcasses were collected from a slaughterhouse just before scalding. Each carcass was transported in a sterile plastic bag to the experimental slaughtering facility where we conducted the experiment on the same day. The treatment group ($n = 22$) was scalded in water containing a 0.03% concentration of PAA at 52 ± 2°C for 3 min. This concentration was chosen based on the results of preliminary laboratory-based suspension tests. The control group ($n = 22$) was scalded in water that did not contain peracetic acid or any other additives. The scalding water tank had a capacity of 350 L. Neck skin samples of approximately 2 g were taken from the side of each carcass neck using sterile scissors and forceps, both before and after scalding. The skin samples were each placed in sterile homogenizing bags and immediately cooled at 4°C after sampling.

Additionally, samples of the scalding water were collected before starting the experiment and after every 3–9 carcasses to determine the bacterial count in the water during the experimental procedure.

2.3.2 | Pre-cooling treatment

To assess the effect of carcass hygienization through a pre-cooling dip or spray, carcasses were procured from the slaughterhouse post evisceration and carcass washer. Treatment Group 1 ($n = 25$) underwent a dip for 2 s in a tank containing 90 L of water at 14–18°C, with a 0.1% concentration of PAA, followed by cooling at 4°C for 2 h. The concentration of 0.1% PAA was chosen based on the results of the previously conducted laboratory-based surface tests. Treatment Group 2 ($n = 25$) was subjected to a spray of 0.1% PAA solution,

followed by cooling. The carcasses were sprayed with a conventional spray bottle from all four sides with six pumps per side, delivering a total of 24 mL of the PAA solution per carcass. The control group ($n = 25$) was dipped in a 15-L water tank without PAA addition, with water renewal prior to each carcass, followed by cooling. Neck skin samples were extracted from each carcass before treatment and after cooling, resulting in two neck skin samples per chicken carcass. The skin samples were placed in sterile homogenizing bags and immediately cooled at 4°C after sampling.

2.3.3 | Sample processing

All samples, neck skin and collected water samples, were transported to our laboratory and processed within 2 h. The neck skin samples were weighed, suspended at a 1:10 ratio in phosphate-buffered saline (PBS; Oxoid, Hampshire, UK), and homogenized using a paddle blender (AES Laboratoire, Combourg, France) in fast mode for 2 min. Subsequently, 1 mL of this solution was extracted into a 2 mL reaction tube, followed by a 10-fold dilution, and then plated onto TSA plates. The collected water samples were plated onto TSA plates. A dual approach was adopted for plating all samples.

All plates were incubated at 37°C for 24 h under aerobic conditions. Following incubation, colonies on all plates were counted manually, and the aerobic colony count, measured in CFU/g or CFU/mL, depending on the sample type, was calculated.

2.4 | Statistical analysis

All statistical analyses were conducted using IBM SPSS Statistics software version 27 for Windows (SPSS Inc., Chicago, IL, United States).

The data of the suspension and surface tests was not normally distributed. For the suspension tests, the effective concentrations of each disinfectant for the various organic loads and tested pathogens were compared using the Kruskal–Wallis test for non-parametric data with the Bonferroni correction. In the surface tests, the $\log_{10}$ reductions of the different tested pathogens with the three disinfectants were also compared using the non-parametric Kruskal–Wallis test with the Bonferroni correction.

The data from the experiments in the slaughtering plant was normally distributed. To compare the two groups of scalding treatment, an independent samples *t*-test was used. Additionally, for comparing the three groups of pre-cooling treatment, a one-way ANOVA was selected. Given the absence of variance homogeneity, the Games Howell test was applied as a post hoc test.

3 | RESULTS

3.1 | Suspension tests

Within each experiment, the minimum concentration that led to a 5-$\log_{10}$ reduction (CFU/mL) was determined. Each experiment involved testing 3 and 4 successive disinfectant concentrations to establish the threshold between ineffective and effective concentrations and had three biological replicates. When comparing the three replicates, the highest concentration was selected as the effective concentration for the respective test organism.

3.1.1 | Peracetic acid

The PAA tests (Figure 1a) involving the scalding water-based practice-oriented organic load (SW2) tests indicated a significantly higher effective concentration of 0.03% for all tested pathogens compared to the organic loads lower concentrated scalding water (SW1) and 3 g/L BSA. For *S. Typhimurium*, the effective concentration even increased 15-fold from 0.002% to 0.03% when comparing *Salmonella* inactivation in 3 g/L BSA and in the practice-oriented scalding water (SW2). When comparing the effective concentrations of the three tested pathogens, the tests revealed no significant difference between those.

3.1.2 | Formic acid

The tests involving FA (Figure 1b) revealed *E. hirae* as the most resilient pathogen across all test setups. *E. hirae* exhibited significantly higher effective concentrations compared to *S. Typhimurium* ($p = 0.002$) and *C. jejuni* ($p = 0.029$) for all tested organic loads. In the case of organic load SW2, the effective concentrations were 2% for *E. hirae* and 0.4% for both *S. Typhimurium* and *C. jejuni*. Concerning the different organic loads, the tests involving the practice-oriented organic load SW2 displayed a significant difference in the effective concentrations, which were up to 10-fold higher than those associated with the organic load BSA ($p < 0.05$).

3.1.3 | Lactic acid

Similar to the results of the FA tests, in the suspension tests involving LA (Figure 1c), the effective concentrations of the *E. hirae* tests were significantly higher (SW2: 4.5%), exceeding those for *S. Typhimurium* ($p < 0.001$; SW2: 1%) and *C. jejuni* ($p = 0.002$; SW2: 1%). Moreover, concerning the different organic loads, the practice-oriented SW2 displayed significantly higher effective concentrations compared to both SW1 and BSA for all tested pathogens.

3.2 | Surface tests

The surface tests as mentioned before included three different concentrations for every disinfectant and were executed with five biological replicates each. Across all tested disinfectants, the pathogen *E. hirae* was the most resilient one and consistently exhibited the lowest $\log_{10}$ reductions. The results in Figure 2a–c show the mean ± standard deviation.

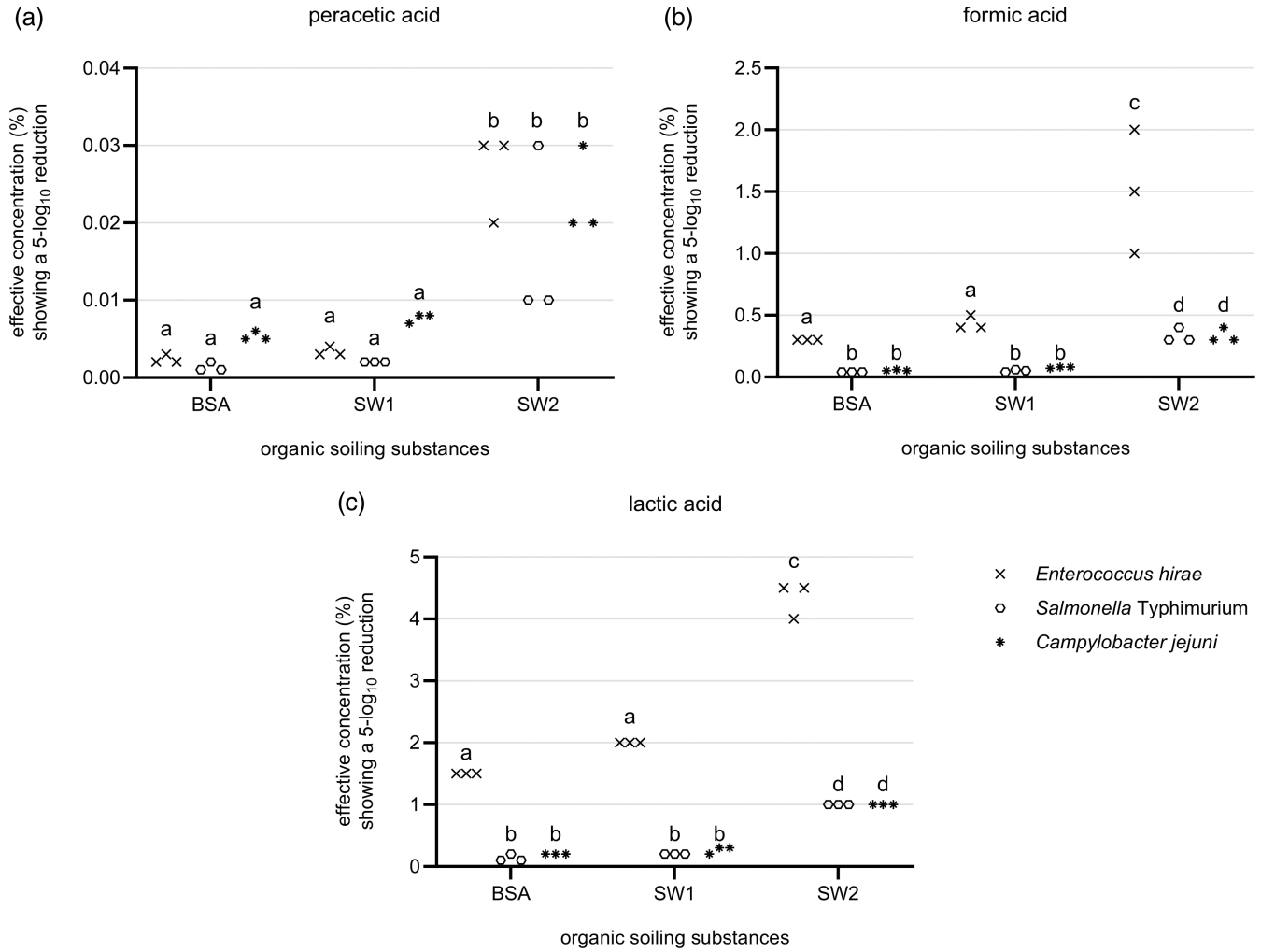

FIGURE 1 (a-c) Results of the suspension tests with oxidizing agent peracetic acid and organic acids formic acid and lactic acid; the y-axis showing the effective concentration resulting in a 5-$\log_{10}$ reduction (CFU/mL); the x-axis showing the three different organic loads: 3 g/L BSA (BSA), scalding water (SW1), scalding water in a practice-oriented higher concentration (SW2); the symbols mark the three replicates of each pathogen; different letters indicate statistically significant differences.

3.2.1 | Peracetic acid

In the surface tests using PAA (Figure 2a), the lowest tested concentration was 0.1%. For this low concentration, the $\log_{10}$ reduction was 0.88 ± 0.11 CFU/mL for *E. hirae*, 1.58 ± 0.07 CFU/mL for *S. Typhimurium*, and 1.24 ± 0.24 CFU/mL for *C. jejuni*. For the 1% PAA tests, the $\log_{10}$ reduction ranged between 1.56 ± 0.16 CFU/mL and 2.30 ± 0.21 CFU/mL for the pathogens, while the 2% PAA tests resulted in a $\log_{10}$ reduction spanning 2.32 ± 0.33 to 2.62 ± 0.14 CFU/mL. Notably, no significant differences were observed in the $\log_{10}$ reductions among the tested pathogens.

3.2.2 | Formic acid and lactic acid

In the chicken skin surface tests conducted with the lowest tested concentration of FA (10%; Figure 2b), the $\log_{10}$ reduction was 0.84 ± 0.14 CFU/mL for *E. hirae*. Remarkably, this $\log_{10}$ reduction more than doubled for *S. Typhimurium* to 2.36 ± 0.40 CFU/mL and more than tripled for *C. jejuni* to 3.12 ± 0.55 CFU/mL. Tests involving 20% FA concentration exhibited a $\log_{10}$ reduction of 1.38 ± 0.32 CFU/mL for *E. hirae*, while the same concentration reduced both *S. Typhimurium* and *C. jejuni* on the chicken skin by over 3 $\log_{10}$ levels. All tested FA concentrations led to a significantly higher $\log_{10}$ level reduction for *S. Typhimurium* and *C. jejuni* in comparison to *E. hirae* ($p < 0.001$).

The lowest tested LA (Figure 2c) concentration of 10% displayed a $\log_{10}$ reduction of 0.92 ± 0.28 CFU/mL for *E. hirae*, 1.64 ± 0.34 CFU/mL for *S. Typhimurium*, and 1.66 ± 0.20 CFU/mL for *C. jejuni*. Notably, *C. jejuni* exhibited the highest $\log_{10}$ reductions with 1.94 ± 0.29 $\log_{10}$ levels CFU/ml for 20% LA and 2.70 ± 0.47 $\log_{10}$ levels CFU/ml for 40% LA. Even at 40% LA, *E. hirae* showed a $\log_{10}$ reduction by only 1.5 ± 0.18 CFU/mL.

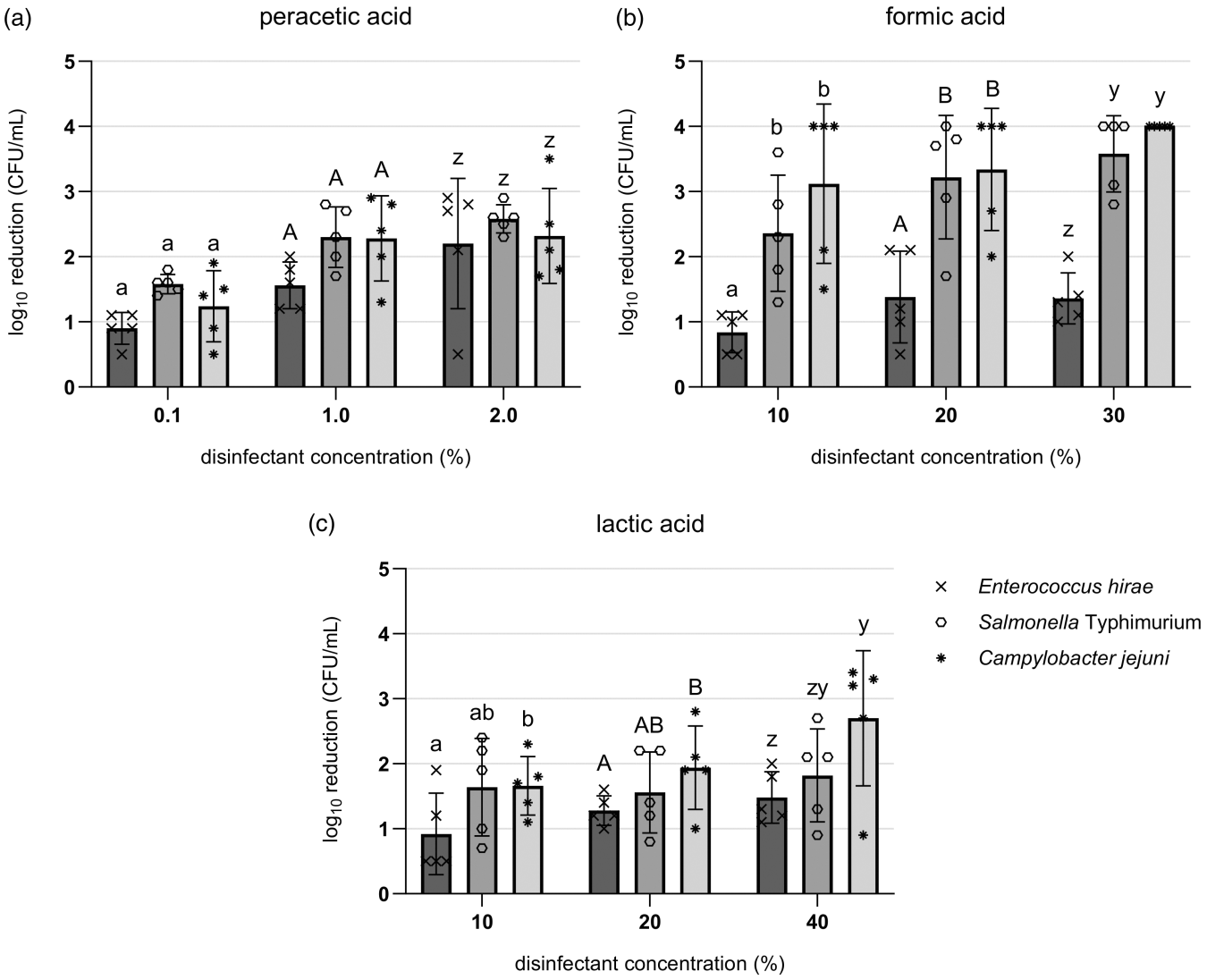

FIGURE 2 (a-c): Results of the surface tests on chicken skin with oxidizing agent peracetic acid and organic acids formic acid and lactic acid; y-axis showing the $\log_{10}$ reductions (CFU/mL) compared to the water control; x-axis showing the tested concentrations for each pathogen; bars indicate the mean, error bars the standard deviation; different letters indicate statistically significant differences between different test organisms.

3.3 | Experimental slaughtering plant

3.3.1 | Scalding treatment

The control group ($n = 22$) of the scalding treatment (Figure 3) underwent scalding for 3 min at 52°C without any additives to the scalding water, resulting in a $\log_{10}$ reduction of 1.10 ± 0.14 CFU/g when comparing the total colony counts on the neck skin before and after scalding. Notably, the group treated with a 0.03% concentration of PAA ($n = 22$) in the scalding water exhibited a reduction of 2.09 ± 0.15 $\log_{10}$ levels CFU/g, which is a significantly higher reduction than that observed in the non-treated group ($p < 0.001$).

The results of the scalding water samples revealed a continuous increase in the total colony count over time within the control group. Initially, the samples showed a total colony count of 20 CFU/mL before the scalding process commenced. Following the scalding of 12 carcasses, this count increased to 4.3 x 10^2 CFU/mL. Towards the experiment's end, after scalding 22 chicken carcasses, the scalding water sample recorded the highest total colony count, measuring 1.7 x 10^3 CFU/mL. Conversely, the water samples in the treatment group containing 0.03% PAA were below detection limit throughout the entire experiment.

3.3.2 | Pre-cooling treatment

In the control group ($n = 25$) of the pre-cooling treatments (Figure 4), the carcasses were individually dipped in fresh water, exhibiting no notable effect on the total colony count of the skin samples ($\log_{10}$ difference between groups: 0.05 ± 0.11 CFU/g). The group subjected to spray treatment (n = 25) with 0.1% PAA displayed a $\log_{10}$ reduction of 0.51 ± 0.13 CFU/g ($p = 0.024$), whereas the dipping treatment

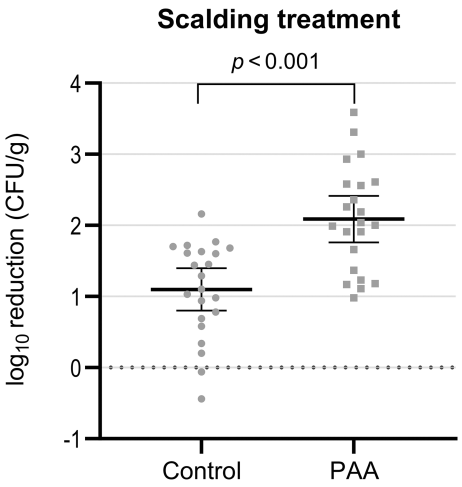

FIGURE 3 Results of the scalding water treatment in the experimental slaughtering facility showing the $\log_{10}$ reductions (CFU/g) of the total colony counts on the carcass neck skin between before and after the treatment with (PAA; $n = 22$) and without (Control; $n = 22$) a 0.03% PAA additive; mean and 95% confidence intervals. PAA, peracetic acid.

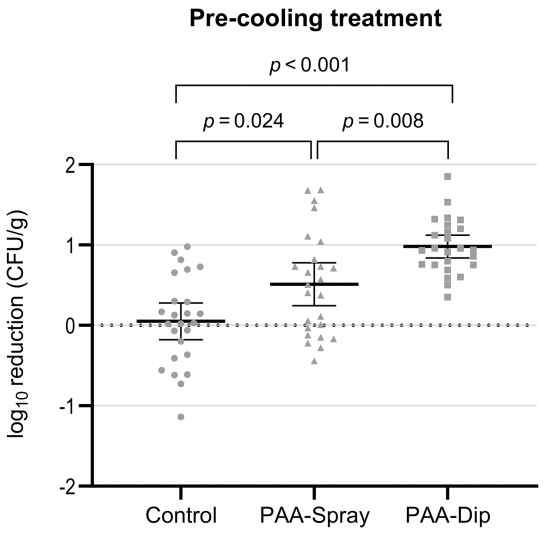

FIGURE 4 Results of the pre-cooling treatment in the experimental slaughtering facility showing the $\log_{10}$ reductions (CFU/g) of the total colony counts on the carcass neck skin between before and after the dipping and spraying treatments with 0.1% PAA (PAA-Dip, $n = 25$; PAA-Spray, $n = 25$) and dipping treatment without PAA (Control, $n = 25$); mean and 95% confidence intervals. PAA, peracetic acid.

($n = 25$) with the same PAA concentration resulted in a 0.98 ± 0.07 $\log_{10}$ CFU/g reduction ($p < 0.001$). Moreover, the $\log_{10}$ level reduction was significantly higher for the dip treatment in comparison to the spray treatment ($p = 0.008$).

4 | DISCUSSION

The aim of the present study was to investigate the efficacy of two organic acids, FA and LA, and PAA as an oxidizing agent for the hygienization in the chicken slaughter process. The results indicate variations in the susceptibility of different pathogens and the impact of organic matter on the antimicrobial performance of these acids. Overall, all tested disinfectants, but especially PAA, show promising results in the decontamination of process water and chicken carcasses.

4.1 | Decontamination of scalding water

Scalding is a critical process in the poultry slaughter chain known for its potential to cause cross-contamination (McBride et al., 1980; Mulder et al., 1978). Zeng et al. (Zeng et al., 2021) reported the presence of *Salmonella* in scalding water, even during the slaughter of the first flock in a chicken processing plant, highlighting the risk of contaminating subsequent carcasses through the scalding process. Various methods have been evaluated to enhance the hygiene of scalding water, including adjustments to temperature and pH values (Humphrey et al., 1981; McKee et al., 2008; Yang et al., 2001).

The biocidal action of organic acids relies on pH reduction, leading to an increase in undissociated acid concentration within cells. This influx of undissociated acid results in the accumulation of toxic anions and protons within the cell, disrupting the structure of the cytoplasmic membrane and inhibiting cellular transport processes (Eklund, 1985; Ricke, 2003). PAA, as an oxidizing agent, reacts with proteins and lipids, including those in the cell membranes of microorganisms, by oxidizing and denaturing them (Kitis, 2004; Maris, 1995). Similar to our study, Okrend et al. (Okrend et al., 1986) used acetic acid for scalding water decontamination, achieving substantial reduction of the time necessary for a 1-$\log_{10}$ decrease in *Salmonella* and *Campylobacter* numbers at 52°C, even at low concentrations such as 0.1% acetic acid. However, the majority of other studies primarily focused on evaluating the impact of scalding water decontamination on the bacterial counts on scalded chicken carcasses. Sakhare et al. (Sakhare et al., 1999) added 0.5% acetic acid and 0.25% LA into poultry scalding water. The addition of 0.25% LA to the scalding water resulted in the most substantial reduction in total mesophilic plate counts on broiler carcasses, demonstrating a 1-$\log_{10}$ level reduction, akin to our findings when incorporating PAA into scalding water within the experimental slaughtering facility.

Our study revealed that the type and concentration of organic matter significantly affected the required disinfectant concentration for a 5-$\log_{10}$ pathogen reduction in the scalding water. Higher organic matter concentrations reduced decontamination efficacy. This finding aligns with a study conducted by Thomas et al. (Thomas et al., 2020), which investigated the efficacy of PAA and LA on steel surfaces in a chicken slaughterhouse, both with and without organic residue. Their research revealed that the presence of an artificial organic residue substance reduced the reduction of *E. coli* and *P. aeruginosa* from more than 4 $\log_{10}$ CFU/mL to less than 1.4 $\log_{10}$ CFU/mL for both LA and PAA. However, it is noteworthy that both disinfectants still

achieved substantial reductions even in the presence of the artificial organic residue, which parallels the results of our study. These findings are consistent with other studies where the presence of organic matter increased the decimal reduction time of various *Salmonella* species and elevated the free chlorine residual required to control biofilms, respectively (Ndiongue et al., 2005; Paul et al., 2017). Similarly, the addition of bovine serum albumin to ozonated water reduced the inactivation rates of tested bacterial strains (Restaino et al., 1995).

These combined results suggest that especially oxidizing agents, such as PAA, can decrease their effectiveness in the presence of organic matter. This phenomenon can be attributed to the mechanism of action of PAA. In addition to oxidizing cell membrane components, PAA also reacts with other proteins and lipids present, such as the organic matter, potentially limiting its reactivity against microorganisms.

PAA is known to degrade at higher temperatures (Wang et al., 2020). In our suspension tests, we evaluated the efficacy of PAA at 52°C, and the results demonstrated its robust efficacy even at this elevated temperature. A study by Ramirez-Hernandez et al. (Ramirez-Hernandez et al., 2018) investigated the effectiveness of various disinfectants, including PAA, in reducing *Salmonella* contamination by spraying them on chicken parts at three different temperatures: 21, 38, and 54°C. For PAA, they tested concentrations of 200 and 400 ppm, which were similar to our observed effective concentration in the suspension tests. Interestingly, they reported no significant differences in PAA's effectiveness between high and low temperatures. However, it is worth noting that they also did not observe a significant reduction in *Salmonella* contamination on the tested chicken parts for either of the tested PAA concentrations (200 and 400 ppm).

4.2 | Decontamination using a pre-cooling dip

In the evisceration process, the risk of carcass contamination with intestinal contents is significant. Beterams et al. (Beterams et al., 2024) showed an increase in *Campylobacter* spp. load from 2.0 to 2.6 $\log_{10}$ CFU/mL after scalding to 2.9–3.4 $\log_{10}$ CFU/mL after evisceration. Various approaches for decontaminating chicken carcasses post-evisceration have been explored, including chemical and physical methods before, during, and after chilling (Dogan et al., 2022). Chlorine is a commonly used chemical for this purpose, with studies demonstrating its efficacy (Northcutt et al., 2005; Tsai et al., 1992). Yang et al. (Yang et al., 2001) showed that chlorine concentrations as low as 10 ppm reduced *Salmonella Typhimurium* and *Campylobacter jejuni* in chiller water. However, when looking at the bacterial survival on chicken skin, no reduction was observed on the skin when dipped in water containing 50 ppm of chlorine for up to 50 min.

It is known that PAA possesses a stronger oxidizing potential compared to chlorine (De Luca et al., 2008). In line with this understanding, our study has demonstrated that PAA exhibits excellent efficacy in reducing bacterial contamination on the carcass skin, even when used at low concentrations. To support this observation, a study by Bauermeister et al. (Bauermeister et al., 2008) investigated the impact of an 85 ppm PAA mixture additive in chiller water within a commercial chicken slaughtering facility, comparing it to a 30 ppm chlorine additive. Their results strongly indicated that the PAA mixture additive outperformed the chlorine additive in reducing the prevalence of *Salmonella* and *Campylobacter* on chicken carcasses. Chen et al. (Chen et al., 2014) also reported significant reductions in *Salmonella* and *Campylobacter* counts on poultry parts following a post-chill treatment with 0.1% PAA.

In our laboratory-based investigation, both FA and LA demonstrated modest reductions of *E. hirae* (1-$\log_{10}$) and *Salmonella* (2.2-$\log_{10}$ for FA and 1.6-$\log_{10}$ for LA) on the chicken skin carriers when used at a 10% concentration in a pre-cooling treatment. In contrary, Laury et al. (Laury et al., 2009) achieved a 2.3-$\log_{10}$ reduction in *Salmonella* by dipping chicken carcasses in an acid blend containing a concentration of only 2.5% LA and citric acid, potentially benefiting from the inclusion of citric acid in their antimicrobial product.

Another relevant study assessed the impact of dipping and spraying chicken carcasses inoculated with *C. jejuni* for a duration of 15 s using two concentrations of LA: 10% at 10°C, which closely resembles our surface tests, and 15% at 30°C (Ellebroek et al., 2007). Their findings indicated that 10% LA at 10°C had minimal effectiveness in reducing *Campylobacter* levels on the chicken carcasses. However, when the concentration was increased to 15% and the temperature increased to 30°C, a substantial reduction in *Campylobacter* counts of 1.5 $\log_{10}$ levels for dipping and 0.8 $\log_{10}$ levels for spraying was observed. This suggests that LA may have reduced antimicrobial efficacy at lower temperatures, which aligns with the limited effectiveness of LA and FA in our surface tests, which were conducted at 4°C. Additionally, a study by Izat et al. (Izat et al., 1989) affirmed this temperature sensitivity, demonstrating that 2% lactic acid had no significant effect on reducing bacteria on chicken carcasses when the suspension temperature was reduced from 37 to 4°C. This temperature-dependent response underlines the importance of considering environmental conditions when evaluating the effectiveness of antimicrobial agents like LA, particularly in scenarios where lower temperatures are encountered.

In our pre-cooling treatment at the experimental slaughtering facility, we assessed the effectiveness of 0.1% PAA on chicken carcasses through dipping and spraying. Dipping treatment yielded significantly higher reductions in bacterial load on chicken skin compared with spraying, although the latter still achieved a 0.5-$\log_{10}$ reduction compared with the control group.

Kumar et al. (Kumar et al., 2020) conducted a comparative analysis involving the dipping and spraying of chicken breasts inoculated with *Salmonella Typhimurium* and *Campylobacter coli* using a 500 ppm PAA solution. Their results demonstrated a significant reduction of approximately 1 $\log_{10}$ level on chicken breasts for both dipping and spraying treatments. In their spraying treatment, chicken breasts were sprayed for 10 s at a rate of 15 mL per second, resulting in a total volume of 150 mL per chicken sample. In contrast, our study used a spraying treatment with a total volume of 24 mL per carcass. This

difference in treatment volume may account for the differences observed in the efficacy of the spraying treatment between our study and Kumar et al.'s study.

However, Meredith et al. (Meredith et al., 2013) explored the impact of various chemical treatments, including dipping and spraying with PAA and LA, on *Campylobacter* levels in chicken skin samples. In line with our results concerning the pre-cooling treatment of chicken carcasses, they reported that spraying skin samples with a 5% LA solution led to a reduction of less than 0.5 $\log_{10}$ levels compared to the water control, whereas dipping the skin samples in 5% LA resulted in a significant reduction of 1.12 $\log_{10}$ levels in *Campylobacter* counts. Regarding PAA, both dipping and spraying treatments using 200 ppm PAA displayed no significant effect on *Campylobacter* levels on the skin. This differs from the results of our investigation, potentially attributable to methodological differences, such as variations in exposure time and the total volume of spraying treatment.

For both methods, decontamination of chicken carcasses through scalding water and a pre-cooling treatment using LA, FA or PAA, the question of environmental and consumer protection needs to be addressed.

PAA and FA are included in the disinfectant list of the German Veterinary Association for the food sector and are widely used. The choice to incorporate LA in this study was informed by its authorization for use in cattle slaughtering to reduce microbial contamination on beef carcasses, as stipulated in the Commission Regulation (EU) No 101/2013 of 4 February 2013.

The EFSA has conducted comprehensive safety evaluations for LA and PAA. In 2018, they assessed the safety of applying 2–5% LA to reduce microbiological contamination on pork carcasses (EFSA, 2018). Likewise, in 2014, the EFSA evaluated the safety of using PAA solutions of up to 2000 ppm on poultry carcasses (EFSA, 2014). Their evaluations concluded that the use of LA or PAA in these concentrations poses neither risks to human health nor the environment. Furthermore, LA and PAA are registered as a food additives in the EU at "quantum satis", and the levels utilized for decontamination purposes are well below those that would exceed typical dietary exposure according to the EFSA reports from 2014 and 2018. It is also important to note that wastewater from slaughterhouses is typically subject to treatment before being released. Naturally, PAA degrades into harmless byproducts, such as water, oxygen, and acetic acid, all of which have negligible environmental toxicity, especially at the low concentrations used in our study.

In conclusion, our findings suggest that all disinfectants tested, with a particular highlight on PAA, showed promising potential for the effective decontamination of process water like scalding water, as well as chicken carcasses. Nevertheless, it is crucial to acknowledge that environmental factors, such as temperature and the presence of organic matter, play a vital role in selecting the most suitable disinfectant. Therefore, when making disinfection choices, these factors must be taken into account. Overall, our results present a straightforward and cost-effective method for reducing microbial contaminants on chicken carcasses, applicable across various operational scales, from large-scale production facilities to smaller meat processors, and under all kinds of pre-harvest conditions. Nevertheless, further research on possible sensory changes of the final product and the practical implementation are essential to optimize these strategies.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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