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Effects of various chemical decontaminants on *Salmonella* Typhimurium survival in chicken carcasses

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Abstract

Salmonella Typhimurium is one of the most common foodborne pathogens isolated from poultry meat. The goal of this study was to investigate *S.* Typhimurium survival in broiler carcasses exposed to ozone, lactic acid, sodium hypochlorite and levulinic acid. *S.* Typhimurium was inoculated into broiler carcasses, which were divided into eight treatment groups, including a positive and negative control group. After standardized bacterial culture methods, microbiological analysis revealed a statistically significant relationship between the number of bacteria detected and the concentration and application time in all the treatment groups ($p < 0.05$). As compared with the control group, all the treatments were effective in terms of inactivation of *S.* Typhimurium. There was a significant decrease in the 1.5 ppm ozone treatment group (5 min and 15 min) as compared with that in the other chemical decontaminant groups ($p < 0.05$). However, there was no significant difference in reduction of *S.* Typhimurium among the other treatment groups, irrespective of the treatment time ($p > 0.05$). Among the decontaminants, the most effective chemical solution was 3% LEV. The ozone treatment caused a lower logarithmic decrease in *S.* Typhimurium numbers at all treatment times as compared with that in the other treatment groups.

Keywords: lactic acid; levulinic acid; ozone, *Salmonella* Typhimurium; sodium hypochlorite.

Practical Application: Determining effective decontaminants and appropriate concentrations with application times on *Salmonella* Typhimurium in chicken carcasses.

1 Introduction

Meat derived from animals is an important source of nutrients in the human diet (Pereira & Vicente, 2013). Poultry meat is an important source of protein, essential polyunsaturated fatty acids, and other dietary components, such as vitamins and minerals (Givens et al., 2006; Barroeta, 2007; Farrell, 2013). Poultry meat consumption is increasing in many developed countries, with chicken meat the most commonly consumed products, followed by turkey meat (Rouger et al., 2017; Wang et al., 2018). Contamination of chicken carcasses with *Salmonella* spp., *Listeria monocytogenes,* and *Campylobacter* spp. may occur at various stages of processing in poultry processing plants (Goncuoglu et al., 2016; Zhu et al., 2017; Oliveira et al., 2018). These foodborne pathogens can cause life-threatening infections in humans (van Nierop et al., 2005; Cetin et al., 2019).

Foodborne pathogens are a major concern and represent a public health problem worldwide (Manoj et al., 2015; Sánchez-Gamboa et al., 2018; Chavez-Martinez et al., 2019; Cruz et al., 2019; Yang et al., 2020). *Salmonella* spp. are one of the most commonly isolated pathogens from foods of animal origin (Heredia & García, 2018; Mendonça et al., 2019; Cunha-Neto et al., 2019). An earlier study estimated that 93.8 million foodborne illnesses occurred annually worldwide and that 155,000 humans worldwide died annually from salmonellosis (Eng et al., 2015). *Salmonella* serotypes are the most important pathogens in chicken meat (Antunes et al., 2016; Zwe et al., 2018). Previous studies reported a high prevalence of *Salmonella* spp. and *Salmonella* Typhimurium (*S.* Typhimurium) in chicken meat (Yang et al., 2010; Freitas et al., 2010; El-Aziz, 2013; Abd-Elghany et al., 2015; Thung et al., 2016; Ren et al., 2017; Trongjit et al., 2017; Xu et al., 2018; Sharma et al., 2019; Jia et al., 2020). Mani-López et al. (2012) reported that *S.* Typhimurium was the most widespread serotype associated with foodborne infections.

Chicken carcasses and their components are often contaminated with spoilage bacteria because of excessive carcass manipulation, including stunning, scalding in a water bath, feather removal, and chilling, in processing plants (Rouger et al., 2017). Various methods are used to reduce microorganism contamination in chicken carcasses and meats during processing (Del Río et al., 2007). These include chemical methods (organic acids, phosphates, acid-phosphate mixtures), physical methods (ionizing radiation, pulsed X-rays, steam, or hot water dips/sprays), electromagnetic waves (ultraviolet light and microwaves), high-intensity pulsed electric fields, oscillating magnetic fields, and natural antimicrobials (Pothakamury et al., 1993; Lillard,1994; Zhang et al., 1994; Dorsa et al., 1996; Bautista et al., 1997; Farkas, 1998; Sofos & Smith, 1998; Barbosa-Canovas et al., 2000; Sastry et al., 2000; Datta & Davidson, 2000; Huffman, 2002; Portela et al., 2019). The objective of the present study was to determine the survival of *S.* Typhimurium in broiler carcasses treated with different concentrations of ozone, lactic acid (LA), sodium hypochlorite (NaClO), and levulinic acid (LEV) applied for different times.

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2 Material and methods

2.1 Bacterial strain

S. Typhimurium ATCC 14028 was used for contamination of chicken carcasses. Cultures were grown overnight in tryptic soy broth broth (Oxoid, England) culture at 37°C. The culture was adjusted to a 0.5 McFarland turbidity standard (approximately 10^8 cfu/mL^{-1}). Chicken carcasses were then contaminated with standardized bacterial culture for the experiments (Durak et al., 2012).

2.2 Application of the decontaminants

The chicken carcasses (n = 144) were obtained from local producers in Burdur Province, Turkey. All the chicken carcasses (1.2–1.4 kg) were quickly taken to the laboratory under cold-chain conditions. There were eight treatment groups, including a negative and positive control group. The treatment groups were as follows: LA (90%) (Merck, Germany), 30 ppm NaClO (Tekkim, Turkey), and 2%, 2.5%, and 3% LEV (99%) (Sigma-Aldrich, USA), with each treatment applied for 5, 10, and 15 min. Post-treatment, the samples were stored at 4° C. The groups were examined 0, 3, and 7 d post-treatment to determine the effects of the storage conditions on chicken carcasses. The experimental design of the study is shown in Figure 1. An ozone generator (Genozon, GN-Q1005S, Turkey) was used to standardize the ozone level (1.5 ppm) in the decontaminant solution.

2.3 Microbiological analysis

Microbiological analysis of *S.* Typhimurium in the decontaminated chicken carcasses at the different application times was analyzed using conventional culture methods.

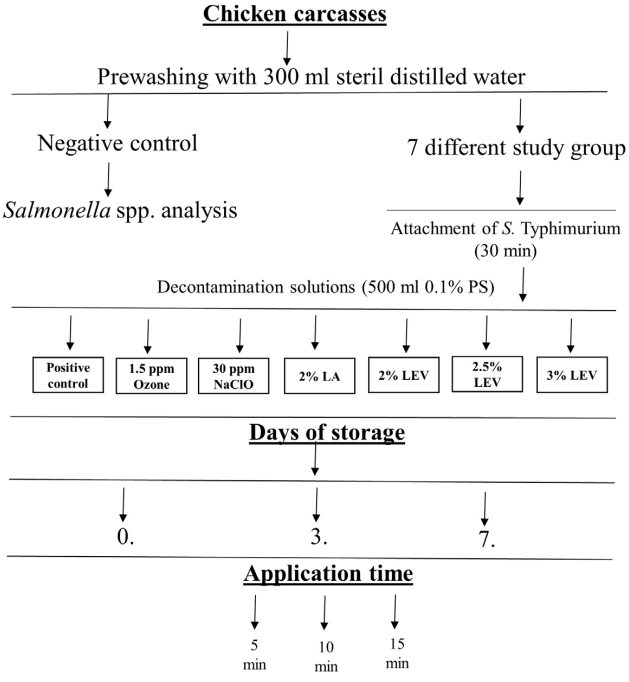

Figure 1. Decontamination of chicken carcasses with the chemical decontaminants. NaClO: sodium hypochlorite, LA: lactic acid, LEV: levulinic acid.

For this purpose, freshly processed broiler carcasses were rinsed, and the rinses were serially diluted 10-fold with 0.1% peptone water (Oxoid, England). The samples were then spread on xylose lysine deoxycholate agar (Merck) and brilliant-green phenol-red lactose sucrose agar (Merck). Colonies were inoculated into triple sugar iron (Merck) agar and lysine iron agar (Merck). The isolates were then tested with *Salmonella* antiserum (Difco 2264-47-2) (Mulder et al., 1987; Andrews et al., 2019).

2.4 Statistical analysis

The SPSS software package (version 25.0 for Windows) was used for statistical analysis. The appropriateness of the data to an analysis of variance in the factorial order was evaluated by a multivariate normal distribution and homogeneity of Box-M variance test. A factorial analysis of variance was used to compare the means. If the parametric (variance analysis in factorial order) did not meet the prerequisites, the data were recovered by Box–Cox data transformation, and the variance analysis in the factorial order was used with the transformed data obtained. Multiple comparisons were averaged according to Fisher's least significant difference method. The significance level was accepted as $p < 0.01$ and $p < 0.05$.

3 Results

3.1 Control group

In the positive control group, 6.60 log cfu/mL was detected on d 0, 6.81 log cfu/mL was detected on d 3, and 6.88 log cfu/mL was detected on d 7.

3.2 Inactivation of S. Typhimurium by ozone

In this study, as compared with the control group, the application of ozone for 5 min reduced the levels of *S.* Typhimurium, with 1.21, 1.51, and 1.57 log cfu/mL recorded on d 0, 3, and 7, respectively. In the 10-min treatment group, the *S.* Typhimurium counts on d 0, 3, and 7 were reduced to 1.43, 1.77, and 1.88 log cfu/mL, respectively. The application of ozone for 15 min decreased the levels of *S.* Typhimurium to 1.13 log cfu/mL, 1.21 log cfu/mL, and 1.47 log cfu/mL on d 0, 3, and 7, respectively. When the effect of the ozone application on *S.* Typhimurium survival was examined, the most effective treatment time was 10 min (Figure 2). There was also a statistically significant difference in the 5-min treatment group after 0, 3, and 7 d as compared with that in the 15-min treatment group on the same days. ($p < 0.05$).

3.3 Inactivation of S. Typhimurium by LA

Table 1 and Figure 3 show the reduction in the levels of *S.* Typhimurium following the application of 2% LA for 5, 10, and 15 min as compared with those in the positive control.

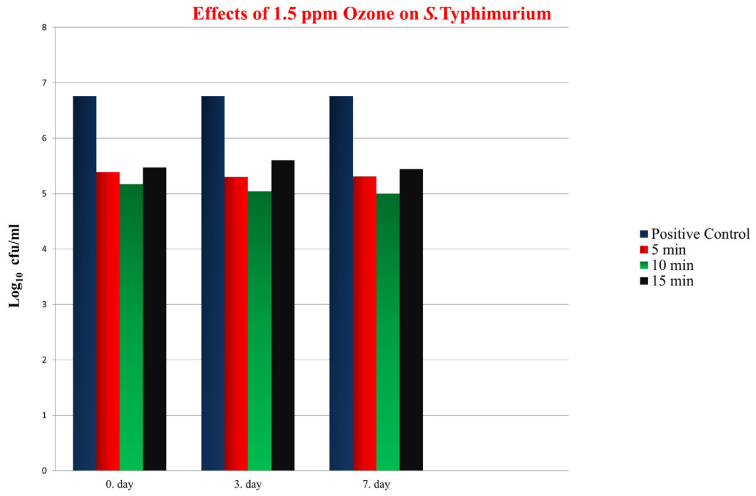

Figure 2. Effects of the 1.5 ppm ozone treatment on *S.* Typhimurium survival.

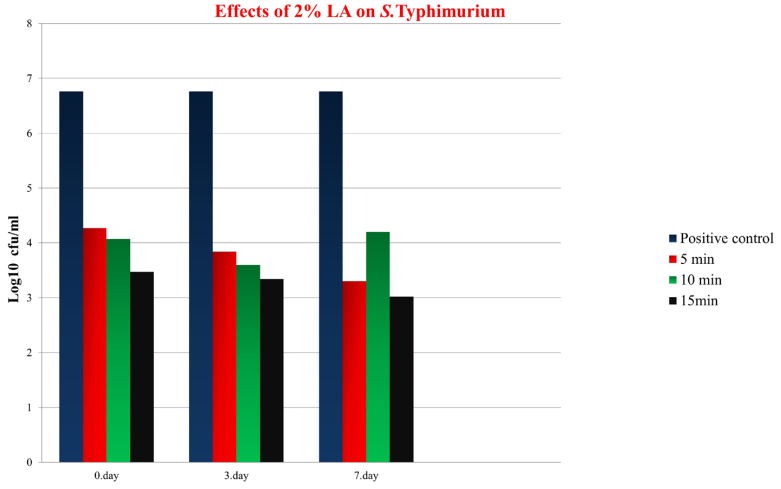

Figure 3. Effects of 2% LA on *S.* Typhimurium survival. LA: Lactic acid.

Table 1. Reduction in the levels of *S.* Typhimurium in the group treated with 2% LA.

	Reduction Level (log cfu/mL)								
	5 min			10 min			15 min		
2% LA	**0. day**	**3. day**	**7. day**	**0. day**	**3. day**	**7. day**	**0. day**	**3. day**	**7. day**
	2.33	2.97	3.58	2.53	3.21	2.68	3.13	3.47	3.86

Table 2. Reduction in the levels of *S.* Typhimurium in the group treated with 2%, 2.5%, and 3% LEV.

Concentration of LEV	Reduction Level (log cfu/mL)								
	5 min			10 min			15 min		
	0. day	3. day	7. day	0. day	3. day	7. day	0. day	3. day	7. day
2%	2.43	2.79	2.86	(-)	3.21	2.82	(-)	(-)	(-)
2.5%	2.79	2.77	2.58	3.43	3.42	3.41	(-)	(-)	(-)
3%	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)

(-): All the *S.* Typhimurium population was inactivated by LEV. LEV: levulinic acid.

3.4 Inactivation of S. Typhimurium by 2%, 2.5%, and 3% LEV

Table 2 and Figure 4 show the reduction in the levels of *S.* Typhimurium after the 2%, 2.5%, 3% LEV applications for 5 min, 10 min, and 15 min, respectively, as compared with those in the positive control.

3.5 Inactivation of S. Typhimurium by NaClO

Table 3 and Figure 5 provide information on the reduction in the levels of *S.* Typhimurium in the 30 ppm NaClO application group after 5, 10, and 15 min as compared with those in the positive control.

Table 3. Reduction in the level of *S.* Typhimurium in the 30 ppm NaClO group.

	Reduction Level (log cfu/mL)								
	5 min			10 min			15 min		
30 ppm NaClO	**0. day**	**3. day**	**7. day**	**0. day**	**3. day**	**7. day**	**0. day**	**3. day**	**7. day**
	2.58	3.81	4.18	1.21	2.51	3.88	3.21	(-)	(-)

(-): All the *S.* Typhimurium population was inactivated by the NaClO treatment. NaClO: sodium hypochlorite.

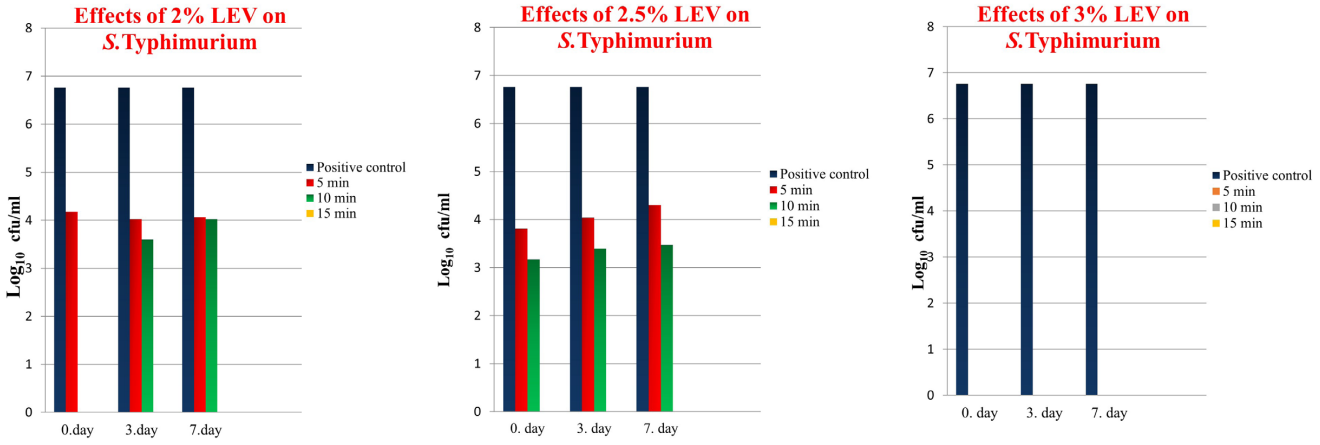

Figure 4. Effects of 2%, 2.5%, and 3% LEV on *S.* Typhimurium counts. LEV: levulinic acid.

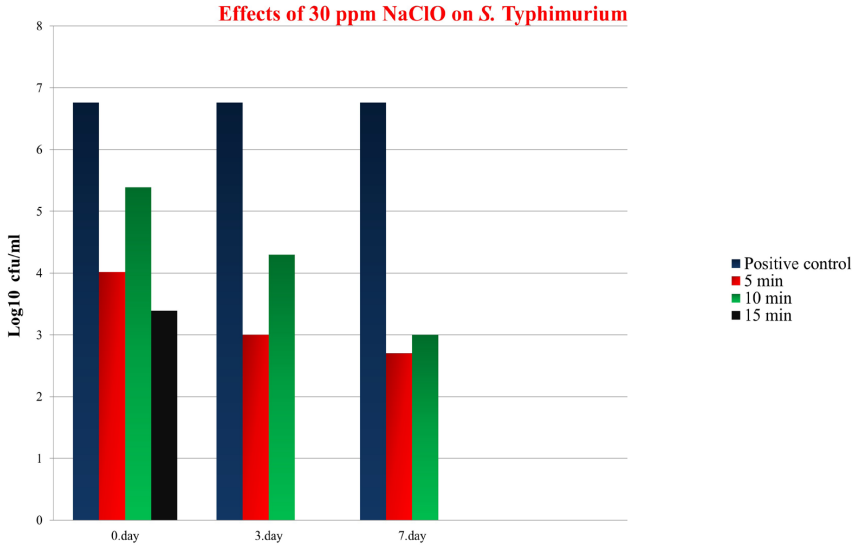

Figure 5. Effects of 30 ppm NaClO on *S.* Typhimurium.

4 Discussion

Salmonella spp. are an important foodborne pathogen in the poultry industry. The occurrence of *Salmonella* spp. in animal products is indicative of flaws in poultry handling or mishandling of chicken carcasses during processing (Freitas et al., 2010). Abbassi-Ghozzi et al. (2012) reported the highest occurrence of *Salmonella* spp. (48.3%) in chickens among different meat samples (beef, lamb meat, and minced meat). High levels *Salmonella* infection (salmonellosis) associated with chicken products have been reported in Brazil, Egypt, China, Malaysia, Thailand, and India (Freitas et al., 2010; Yang et al., 2010; El-Aziz, 2013; Abd-Elghany et al., 2015; Thung et al., 2016; Ren et al., 2017; Trongjit et al., 2017; Sharma et al., 2019). The findings indicate that contamination of chicken and broiler products with *Salmonella* spp. are common worldwide. For this reason, effective methods need to be developed to prevent contamination of chicken products with *Salmonella* spp. in poultry processing plants.

Various chemical agents and methods can be used for decontamination of chicken carcasses. LA is a harmless, cheap, and safe compound used in the decontamination of foods. LA is generally recognized as safe by the U.S. Food and Drug Administration

for meat products (Mani-López et al., 2012). The effectiveness of organic acids, such as LA, against microorganisms depends on the concentration, exposure time, and temperature (Dickson & Anderson, 1992). The effectiveness and antimicrobial effects of LA in poultry has been studied extensively. In one study, researchers observed a 2.2 log cfu/mL reduction in *S.* Typhimurium following the application of 1% and 2% LA to chickens for 30 s at 20 °C (Xiong et al., 1998). Mohamed & Abdel-Naeem (2018) found a 1 log cfu/cm^2 and 3.3 log cfu/cm^2 reduction in *S.* Typhimurium in chicken carcasses following the application of 1% and 2% LA, respectively. Other studies also reported that 1% LA was effective against *S.* Typhimurium (Slavik et al., 1997; Madushanka et al., 2018). The present study determined the effect of 2% LA against *S.* Typhimurium in chicken carcasses. The results pointed to similarities with the findings of the aforementioned studies, despite differences in concentrations and application times.

LEV is a permeable substance that allows the penetration of cells (Helander et al., 1997; Alakomi et al., 2000). There have been only a few studies on the effect of LEV on *Salmonella* spp. inactivation in broiler carcasses. Zhao et al. (2011) reported that LEV was effective in decontamination of *Salmonella* spp., reporting a 5 log cfu/cm^2 reduction in treated chicken carcasses. LEV was reported to be effective, even at low concentrations (Zhao et al., 2009). In our study, we found similar findings in terms of the decontamination effectiveness of LEV against *S.* Typhimurium in chicken carcasses. In the present study, the most effective concentration of LEV (2%, 2.5%, and 3%) and application time (5, 10, and 15 min) was 3% and 15 min, respectively (Figure 4). Differences in the application times and decontaminant concentration are likely the main reason for differences observed between our findings and the results of other studies.

The effect of ozone varies according to the pH, temperature, humidity, presence of additives, and organic substances in the environment (Kim et al., 1999). The half-life of ozone in water is only 20–30 min. In the present study, the ozone treatment was applied at room temperature (20 °C) because previous research demonstrated that it was most effective at this temperature (Guzel-Seydim et al., 2004). Cho et al. (2015) treated chicken breasts with ozone gas during cold storage for 3 d. They reported a decrease of 0.79 log cfu/g in the number of *S.* Typhimurium. In another study on chicken carcasses contaminated with *S.* Typhimurium and treated with ozone (10 ppm) carcasses for 45 min, the authors observed a 0.7 log cfu/mL decrease (Fabrizio et al., 2002). Based on our results and those of other studies, the oxidation capacity of ozone in terms of decontamination appears to decrease with time.

Chlorine is a broad-spectrum decontamination agent. Hypochlorites have been shown to have antimicrobial activity against bacteria, bacterial spores, viruses, fungi, algae, and protozoa (Park et al., 2001). NaClO is a disinfectant widely used in the food industry due to its ease of use and low cost (Meireles et al., 2016; Park et al., 2016). Chlorine at a concentration 20–50 ppm is recommended because it causes an undesirable odor at higher concentrations in decontamination of food (Sofos & Smith, 1998). Thus, in the present study, the NaClO concentration used was 30 ppm. Yang et al. (2001) reported a reduction of 1 log unit in chicken skin inoculated with *S.* Typhimurium and treated with 50 ppm chlorinated water for 50 min. In other research, spraying chicken carcasses with NaClO (20–40 ppm) for 3.5 s reduced the number of *S.* Typhimurium from 90% to 96% (Bailey et al., 1986). Despite differences in decontamination methods and application times among the reported studies, the effectiveness of NaClO is similar to those in the literature.

5 Conclusion

Despite technological advances in poultry meat cutting lines, developments in logistics infrastructure, innovations in packaging technology, application of preservation methods, and consumer awareness, microorganism contamination of chicken products remains a problem. Decontamination processes in poultry slaughterhouses require more economical and shorter decontamination processes. In the current study, among the various decontamination solutions, the most effective chemical solution was 3% LEV. The effect of the ozone solution application on *S.* Typhimurium was detected a lower logarithmic reduction compared to the other groups.

In conclusion, chemical decontamination agents used for decontamination of broiler carcasses should not cause residues, color and taste changes, or unwanted odor formation. To prevent such adverse effects, appropriate concentrations and application times should be used. In comparison with ozone, LEV has advantages, such as ease of use and no need for expensive instrument equipment. Considering the observed decontamination effect of LEV on chicken carcasses in the present study. LEV could be used as an alternative to ozone for decontamination of broiler carcasses.

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