Reduction of *Salmonella* in skinless chicken breast fillets by lauric arginate surface application\(^1,2\)

C. S. Sharma,*\(^3\) A. Ates,* P. Joseph,† R. Nannapaneni,† and A. Kiess*

*Poultry Science Department, and †Department of Food Science, Nutrition and Health Promotion, Mississippi State University, Mississippi State 39762

**ABSTRACT** Lauric arginate (LAE) has been found to be effective against various foodborne pathogens. In this study, the antimicrobial efficacy of LAE against *Salmonella* and mesophilic organisms was evaluated in fresh, skinless, boneless, uncooked chicken breast fillets. The effect of LAE treatments on pH and color of breast fillets was also assessed. Chicken breast fillets were inoculated with a 4-strain *Salmonella* cocktail (*Salmonella* Enteritidis ATCC 4931, *Salmonella* Heidelberg ATCC 8326, *Salmonella* Kentucky ATCC 9263, and *Salmonella* Typhimurium ATCC 14028) and then treated with sterile dionized water (positive control) and 200 ppm and 400 ppm of LAE. The chicken breast fillets were stored at 4 ± 1°C and analyzed on d 0, 1, 3, 5, and 7 for *Salmonella*, total aerobes, color, and pH. The fillets destined for color analysis were not inoculated with *Salmonella* cocktail and stored under conditions simulating the retail display. The fillets treated with 400 ppm LAE had lower (*P < 0.05*) *Salmonella* counts compared with the positive control from d 0 through d 7 of storage except on d 3, when no effect of LAE was observed. Treating fillets with 200 ppm of LAE caused a significant reduction in *Salmonella* counts (*P < 0.1*) on d 0, 1, and 7. Reductions in *Salmonella* spp. were 0.7 log cfu/g and 0.7 to 1.0 log cfu/g for 200 and 400 ppm treatments, respectively. Lauric arginate did not exhibit any treatment effect on the growth of mesophilic microorganisms, pH, and color of chicken breast fillets (*P > 0.05*) when applied at 200 and 400 ppm concentrations. These results indicate that surface application of LAE in chicken breast fillets significantly reduces *Salmonella* during refrigerated aerobic storage without negatively affecting the color of chicken breast fillets.

**Key words:** lauric arginate, *Salmonella*, chicken breast fillet, color, total aerobes

1419

**INTRODUCTION**

Salmonellosis caused by nontyphoidal *Salmonella* spp. is still the leading cause of bacterial foodborne illnesses in the United States with an estimated 1 million cases occurring each year (Scallan et al., 2011). Consumption of undercooked poultry products contaminated with *Salmonella* has been implicated in foodborne illnesses (CDC, 2010, 2011). Poultry meat was cited to be the most common cause of illness among different outbreaks attributed to a single food commodity (CDC, 2010). Live birds harbor these pathogenic bacteria in their intestines, feathers, and skin, which leads to contamination of carcasses during slaughtering and processing. The USDA’s Food Safety and Inspection Services (FSIS) has also introduced new performance standards for poultry processing plants to further reduce *Salmonella* contamination in poultry carcasses and their parts (USDA FSIS, 2010). Many postslaughter interventions are being employed at commercial poultry processing facilities in the form of prechill and postchill sprays and dips with a wide variety of USDA-approved antimicrobials (Kemp et al., 2001; Skandamis et al., 2002; Bourassa et al., 2004; Bauermeister et al., 2008; Over et al., 2009; Sharma et al., 2012; USDA FSIS, 2012) to restrict the occurrence of *Salmonella* in fresh poultry carcasses and their parts, but none has resulted in total elimination of *Salmonella*.

Lauric arginate (LAE) is among the various antimicrobials approved by the USDA FSIS as a processing aid for use on poultry carcasses and their parts (USDA FSIS, 2012). Lauric arginate is a cationic preservative derived from lauric acid and arginine. The antimicrobial properties of LAE against *Salmonella* are due to its action on the cytoplasmic membrane. Rodríguez et al. (2004) examined the cellular effects of LAE on *Salmonella* Typhimurium by electron microscopy, flow cytom-
etry, ion leakage, and disruption of membrane potential. Treating Salmonella with LAE caused loss of cell viability and structural changes along with disturbance in membrane potential without causing lysis of treated cells. Laurie arginate has been found to be effective in reducing Salmonella and Listeria monocytogenes in different food products (Stopforth et al., 2010; Soni et al., 2012; Theinsathid et al., 2012). In a previous study, the combined spray applications of LAE along with polylysine and acidic calcium sulfate resulted in significant reduction of Salmonella on chicken carcasses (Benli et al., 2011). The LAE can be applied as an antimicrobial to the surface of fresh cuts of poultry such as chicken breasts at levels not exceeding 200 ppm by weight of the finished product. The objective of the present study was to determine whether surface application of LAE on skinless chicken breast fillets had any inhibitory effect on the growth of Salmonella and total aerobic bacteria under aerobic refrigeration conditions. The effect of LAE treatments on the pH of breast meat and color of chicken breast fillets stored under refrigeration was also determined.

MATERIALS AND METHODS

Bacterial Strains and Inoculum Preparation

A 4-strain cocktail of Salmonella consisting of serotypes that are commonly associated with poultry carcasses and their products (Salmonella Enteritidis ATCC 4931, Salmonella Heidelberg ATCC 8326, Salmonella Kentucky ATCC 9263, and Salmonella Typhimurium ATCC 14028) were selected in this study to evaluate the antimicrobial efficacy of LAE against Salmonella. These strains were maintained on tryptic soy agar (TSA; MP Biomedicals LLC, Solon, OH) slants and cultured at 37°C for 20 h in 10 mL of tryptic soy broth (MP Biomedicals LLC) to obtain cell concentrations of approximately 10^9 cfu/mL. Cells were pelleted by centrifugation at 10,000 x g for 10 min at 4°C, harvested, washed twice, and resuspended in 10 mL of sterile 0.1% peptone water (CM009, Oxoid Ltd., Basingstoke, UK). A 4-strain cocktail of Salmonella was prepared by mixing equal volumes of the washed cell suspensions containing approximately 10^9 cfu/mL, and appropriate serial dilutions were made to get the final concentration of 10^8 cfu/mL in 0.1% sterile peptone water.

Chicken Breast Fillet Sample Preparation, Inoculation, and Treatment

Skinless, boneless, uncooked chicken breast fillets with sell-by date of at least 1 wk were procured from a local supermarket, transported on ice, and processed on the same day or kept at 4°C for use within 24 h. Chicken breast fillets were aseptically cut and weighed into 25-g samples, transferred to sterile Whirlpak bags, and inoculated with 100 μL of approximately 10^8 cfu/mL of the Salmonella cocktail. The samples were stored under a biosafety cabinet for 20 min to allow attachment of bacteria to breast meat surface before LAE treatments. The LAE was obtained from Vedeqsa Inc. (New York, NY), which contained 10% active LAE dissolved in propylene glycol (solvent) and polysorbate 20 (emulsifier) and used for treatment of Salmonella-inoculated chicken breast fillets. The LAE was applied on the surface of chicken breast fillets samples and the delivery volume for all treatments was 1 mL, which consisted of untreated control [1 mL of sterile dionized (DI) water], LAE 200 ppm (50 μL of 10% LAE added to 950 μL of DI water), and LAE 400 ppm (100 μL of 10% LAE added to 900 μL of DI water). Following the treatments, samples were gently massaged manually to ensure proper distribution of LAE over surface of chicken breast meat and stored at 4 ± 1°C for 7 d. Duplicate samples for each treatment were analyzed after 0, 1, 3, 5, and 7 d of storage for Salmonella recovery, total aerobic counts, and pH measurements. Three separate trials were conducted for this study.

Microbiological and pH Analyses

Microbiological and pH analysis was done as described in earlier study (Sharma et al., 2012). Briefly, on each day of sampling, chicken breast meat samples (25 g each) were homogenized with 225 mL of sterile 0.1% peptone water in a stomacher (Seward model 400C, Seward Laboratory Services Inc., Port St. Lucie, FL) for 2 min at 100 rpm to loosen and suspend the bacteria into the solution. Serial dilutions were prepared by transferring 1.0 mL of the sample homogenate to 9 mL of 0.1% sterile peptone water. A volume of 0.1 mL from each dilution was pipetted onto duplicate prepoured XLT-4 agar plates (Becton Dickinson and Company, Sparks, MD) for Salmonella recovery and TSA plates for total aerobic counts. The XLT-4 and the TSA plates were incubated aerobically for 24 h at 35°C. Black colonies or colonies that were black-centered with a yellow periphery on XLT-4 agar were considered to be presumptively positive for Salmonella. Uninoculated chicken breast samples were tested for the presence of Salmonella at the beginning of each experiment and were found to be negative for Salmonella. After incubation, cfu from each plate were counted, averaged, and reported as log cfu/g of the sample for both Salmonella and total aerobes. The pH for each sample homogenate was measured by placing the pH probe into the sample homogenate after the microbiological analyses were completed. All pH measurements were recorded in duplicate using an Accumet pH meter (AB15 Accumet Basic, Fisher Scientific, Pittsburgh, PA).

Retail Display for Color Analysis

Chicken breast fillets were subjected to respective treatments (control, 200 ppm of LAE, 400 ppm of LAE) as surface application. Sterile deionized water was added to control fillets to maintain the constant delivery.
volume as done in LAE treatments. Fillets were kept in Styrofoam trays, overwrapped with polyvinyl chloride film (15,500 to 16,275 cm$^2$/m$^2$ per 24 h oxygen transmission rate at 23°C), and assigned for refrigerated retail display (1,000 lx lighting; 2°C) for 0, 1, 3, 5, and 7 d. The instrumental color was evaluated on the breast fillets assigned for respective storage days.

**Color Measurement**

Instrumental color was measured on the light-exposed surface of each breast fillet using a chroma meter (Chroma meter Model CR-400, Minolta Camera Co. Ltd., Osaka, Japan Serial No. C8202489) with an 8 mm aperture, illuminant D65, and calibrated using a standard white calibration plate (model no. 20933026, Minolta Camera Co. Ltd., Osaka, Japan). Three measurements were taken at 3 identical locations for each breast fillet. Color for each sample was expressed in terms of Commission Internationale d’Eclairage values for lightness ($L^*$), redness ($a^*$), and yellowness ($b^*$). On a given day, 4 breast fillets were analyzed per treatment within a replication.

**Statistical Analysis**

The experiment was replicated 3 times with a factorial design within a randomized complete block design (replications as blocks). Data were analyzed by GLIMMIX procedures of SAS (SAS Institute, 2009) to determine differences between trials, treatments and storage days, and treatment × day interactions. Tukey-Kramer test was used to separate treatment means when significant differences occurred ($P < 0.1$).

**RESULTS AND DISCUSSION**

**Effect of LAE on Growth of Salmonella in Chicken Breasts**

The efficacy of LAE on *Salmonella* survivability in chicken breast fillets was determined under aerobic refrigerated storage. Counts of *Salmonella* in positive control ranged from 4.3 to 5.1 log cfu/g throughout 7 d of storage (Table 1). Except on d 3, treating chicken breast fillets with 400 ppm of LAE caused a significant reduction ($P < 0.05$) in *Salmonella* populations on all days of analysis compared with positive control with the only exception on d 5 with a $P$-value of ($P \leq 0.0953$). The reductions in *Salmonella* after treatment with 400 ppm ranged from 0.7 to 1.0 log cfu/g with 1.0 log reduction on d 0 and 1 of storage. The reduction of *Salmonella* 400 ppm LAE treatment was maintained till d 7 of storage with 0.9 log reduction observed on d 7 compared with the control. Treatment of chicken breast fillets with 200 ppm also caused significant reductions ($P \leq 0.0953$) of *Salmonella* populations on d 0, 1, and 7 of storage with level of reduction of 0.7 log cfu/g as compared with control on each day. There was no difference ($P > 0.05$) between the 2 LAE treatments (200 and 400 ppm LAE) on all days of analysis, although treating chicken breast fillets with 400 ppm LAE exhibited greater reduction of *Salmonella* up to 1.0 log compared with 0.7 log reduction with 200 ppm LAE treatment. The LAE has been reported to be effective against *Salmonella* and other foodborne pathogens in different food products (Martin et al., 2009; Stopforth et al., 2010; Soni et al., 2012; Theinsathid et al., 2012). Benli et al. (2011) reported a significant reduction of *Salmonella* (2.2 logs) after a spray treatment of 200 ppm LAE solution followed by 30% acidic calcium sulfate on chicken carcasses. In a previous study conducted in our laboratory (unpublished data), LAE was unable to exert any inhibitory effect on growth of *Salmonella* in ground chicken compared with this study, under similar conditions (i.e., the same level of LAE treatments). However, in this study there was 1.0 log reduction of *Salmonella* on d 0 and 1. The possible reason for this difference in antimicrobial effects of LAE could be due to a higher fat percentage of ground chicken versus low fat levels in chicken breast fillets because in this study skinless chicken breast fillets were used and in poultry most of the fat is associated with chicken skin. The other reason could be the substrate effect because in this study LAE was applied as a surface treatment on chicken breast fillets, which are intact cuts of poultry meat, whereas ground chicken is a totally different product with a different environment for survival of *Salmonella*. Fat has a protective effect for *Salmonella* against the antimicrobial potency of LAE in ground poultry products such as ground turkey; increasing the level of fat in ground turkey resulted in decreased efficacy of LAE against *Salmonella* (Oladunjoye et al., 2013). The findings from this study indicate the potential of LAE as an antimicrobial for restricting the growth of *Salmonella* in fresh cuts of poultry such as chicken breast fillets. Moreover, further studies are needed to determine the synergistic effect of LAE with

Table 1. Presumptive *Salmonella* counts (log cfu/g) for chicken breast fillets inoculated with *Salmonella*, treated with lauric arginate (LAE), and stored at 4 ± 1°C for 7 d

<table>
<thead>
<tr>
<th>Treatment</th>
<th>d 0</th>
<th>d 1</th>
<th>d 3</th>
<th>d 5</th>
<th>d 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>4.3b,x</td>
<td>4.5b,x</td>
<td>4.9b,x</td>
<td>4.3b,x</td>
<td>4.5b,x</td>
</tr>
<tr>
<td>200 ppm of LAE</td>
<td>4.1b,x</td>
<td>4.3b,x</td>
<td>4.1b,x</td>
<td>3.9b,x</td>
<td>3.9b,x</td>
</tr>
<tr>
<td>400 ppm of LAE</td>
<td>4.1b,x</td>
<td>3.9b,x</td>
<td>3.9b,x</td>
<td>3.9b,x</td>
<td>3.9b,x</td>
</tr>
</tbody>
</table>

*a,b*Means within a column lacking a common superscript differ ($P < 0.05$). *b*Means ($P < 0.1$). *Means within a row lacking a common superscript differ ($P < 0.05$).
other food-grade antimicrobials without affecting the organoleptic properties of poultry products. Encouraging results in this regard were reported by Oladunjoye et al. (2013), where greater inhibition/reduction of Salmonella was seen when LAE was used in combination with carvacrol, a food-grade essential oil.

**Effect of LAE on Total Aerobic Counts and pH Analysis**

No significant differences \((P > 0.05)\) were observed for mesophilic counts in chicken breast treated with LAE and stored for 7 d, although lower counts of total mesophilic organisms were seen compared with the control on each day of sampling. Treatment of chicken breast fillets with 400 ppm produced 0.6 to 0.9 log cfu/g reductions \((P > 0.05)\) in total aerobe counts from d 0 through d 7 (Table 2). In addition, LAE had no effect on the pH of chicken breast fillets. The pH values were similar \((P > 0.05)\) for the untreated control and for both 200 and 400 ppm LAE treatments from d 0 through d 7 (Table 3). The pH is very important for functional properties of muscle proteins such as myosin and actin and manufacturing a variety of further processed poultry products. Because LAE had no significant effect on pH, the breasts treated with LAE would behave similarly compared with untreated meat during manufacturing of processed poultry products.

**Effect of LAE Treatments on Color of Chicken Breast Fillets**

No significant treatment \(\times\) day interactions \((P > 0.05)\) were observed for instrumental meat color attributes \((L^*, a^*, b^*)\) of chicken breast fillets. Table 4 represents the effects of treatments on meat color of chicken breast during refrigerated retail display, numerically expressed as CIE \(L^*\) (lightness), \(a^*\) (redness), and \(b^*\) (yellowness) values. With respect to \(L^*\) and \(a^*\) values, the treatments exhibited no significant differences \((P > 0.05)\). The positive control had a greater \((P < 0.05)\) \(b^*\) value than 200 ppm LAE treatment initially. During storage days, \(L^*\) value demonstrated an inconsistent trend \((P > 0.05)\) in all the treatments. The display days also were not significantly different \((P > 0.05)\) for \(a^*\) and \(b^*\) values.

The data obtained for chicken breast color in the present experiment correspond to the range of \(L^*, a^*, \text{and} b^*\) values reported in the scientific literature (Fletcher et al., 2000; Liu et al., 2004; Battula et al., 2008). The \(L^*\) value is indicative of meat lightness/darkness, and Van Laack et al. (2000) reported that a greater \(L^*\) value (above 60) combined with lower ultimate meat pH would lead to the occurrence of pale chicken breast meat. In meat and poultry, \(a^*\) value indicates redness and typically is used as an indicator for meat color stability (Mancini and Hunt, 2005), whereas an increase in \(b^*\) value denotes an increase in the yellowness in meat.

Meat color and appearance influence the purchasing decisions of consumers. Results of the present study revealed that surface application of LAE does not influence meat color/color stability of chicken breast fillets during refrigerated storage. Our previous experiment (unpublished data) on the effect of LAE on color attributes of ground chicken documented similar results. In their investigations to determine the antimicrobial effects of sodium metasilicate in chicken breast fillets, Huang et al. (2011) reported no adverse effects of the treatment on meat color during retail storage for 9 d. On the other hand, application of certain antimicrobial applications could negatively influence meat color; chlorine dioxide sachets in combination with modified atmosphere packaging resulted in greenish brownish discoloration of fresh chicken breast fillets during refrigerated storage (Ellis et al., 2006). In summary, our results concluded that surface application of LAE at 200 and 400 ppm levels in chicken breast fillets did not

---

**Table 2.** Total aerobic counts (log cfu/g) for chicken breast fillets inoculated with Salmonella, treated with lauric arginate (LAE), and stored at 4 ± 1°C for 7 d

<table>
<thead>
<tr>
<th>Treatment</th>
<th>d 0</th>
<th>d 1</th>
<th>d 3</th>
<th>d 5</th>
<th>d 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>5.5a,x</td>
<td>5.6a,x</td>
<td>5.5a,x</td>
<td>5.5a,x</td>
<td>6.4a,x</td>
</tr>
<tr>
<td>200 ppm of LAE</td>
<td>5.1a,x</td>
<td>5.0a,x</td>
<td>4.9a,x</td>
<td>5.0a,x</td>
<td>5.7a,x</td>
</tr>
<tr>
<td>400 ppm of LAE</td>
<td>4.9a,x</td>
<td>4.7a,x</td>
<td>4.7a,x</td>
<td>4.8a,x</td>
<td>5.5a,x</td>
</tr>
</tbody>
</table>

*Means within a column lacking a common superscript differ \((P < 0.05)\).

---

**Table 3.** The pH measurements for chicken breast fillets inoculated with Salmonella, treated with lauric arginate (LAE), and stored at 4 ± 1°C for 7 d

<table>
<thead>
<tr>
<th>Treatment</th>
<th>d 0</th>
<th>d 1</th>
<th>d 3</th>
<th>d 5</th>
<th>d 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>6.0a,x</td>
<td>6.0a,x</td>
<td>6.1a,x</td>
<td>6.1a,x</td>
<td>6.3a,x</td>
</tr>
<tr>
<td>200 ppm of LAE</td>
<td>6.0a,x</td>
<td>6.0a,x</td>
<td>6.1a,x</td>
<td>6.1a,x</td>
<td>6.2a,x</td>
</tr>
<tr>
<td>400 ppm of LAE</td>
<td>6.0a,x</td>
<td>6.0a,x</td>
<td>6.1a,x</td>
<td>6.1a,x</td>
<td>6.2a,x</td>
</tr>
</tbody>
</table>

*Means within a column lacking a common superscript differ \((P < 0.05)\).

*Means within a row lacking a common superscript differ \((P < 0.05)\).
negatively influence meat color during refrigerated retail display under aerobic packaging for 7 d.

In conclusion, this study revealed that the surface application of LAE reduced the Salmonella populations in fresh, skinless chicken breast fillets without affecting the color of chicken breast fillets. The antimicrobial efficacy of LAE against Salmonella increased with an increase in concentration from 200 to 400 ppm. This study corroborates the findings from previous studies in which LAE was able to reduce Salmonella on chicken carcasses and other food products. However, LAE had little effect on the growth of total aerobes in chicken breast fillets inoculated with Salmonella. Also, LAE did not exhibit any treatment effect on the pH of chicken breast fillets. These findings revealed the need for further research to determine the validity of using higher levels of LAE (up to 400 ppm) in fresh poultry products.

ACKNOWLEDGMENTS

The funding for this research was supported by the Special Research Initiative award by the Mississippi Agricultural and Forestry Experiment Station (MAFES). The authors thank Oluseye Oderinlo, Vikram Kurve, and Monil Desai at Mississippi State University (Mississippi State) for their assistance.

REFERENCES


