

# Effects of Sodium Lactate on the Survival of *Listeria monocytogenes*, *Escherichia Coli* O157:H7, and *Salmonella* spp. in Cooked Ham at Refrigerated and Abuse Temperatures\*

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## ABSTRACT

The objective of this study was to determine the effect of sodium lactate on the survival of *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella* spp. in cooked ham during storage at refrigerated and abuse temperatures. Cooked ham was added with 0% - 3% lactate, inoculated with a multiple-strain mixture of *L. monocytogenes*, *E. coli* O157:H7, or *Salmonella* spp. and stored at 4°C - 15°C for up to 35 day. The growth of the three pathogens was inhibited in ham containing 3% lactate, and no growth of *E. coli* O157:H7 and *Salmonella* spp. occurred at the lowest storage temperatures of 6 and 8°C, respectively. In ham containing no lactate, the average growth rates were 0.256 - 0.380 log CFU/day for *L. monocytogenes* at 4°C - 8°C, 0.242 - 0.315 log CFU/day for *E. coli* O157:H7 at 8°C - 15°C, and 0.249 - 0.328 log CFU/day for *Salmonella* spp. at 10°C - 15°C. The addition of 1% or 2% lactate significantly ( $P < 0.05$ ) reduced the growth rates of the three pathogens, and the effect was more profound at lower temperatures. *Salmonella* spp. were more sensitive to the effect of lactate than *L. monocytogenes* and *E. coli* O157:H7. Polynomial models were developed to describe the growth rates of the three pathogens as affected by the lactate concentration and storage temperature. Results from this study demonstrate the effect of lactate on the growth of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp. in cooked ham and indicate the effective lactate concentrations and storage temperatures that can be used to enhance the microbiological safety of ready-to-eat ham products.

**Keywords:** Ham, Lactate, *Listeria Monocytogenes*, *Escherichia Coli* O157:H7, *Salmonella* spp.

## 1. Introduction

Refrigerated ready-to-eat (RTE) meat products are processed with a heat treatment to kill vegetative microorganisms; therefore, the products are generally free of vegetative pathogens. However, reports have indicated that the products are susceptible to recontamination with *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella* spp. after heat processing. This is evidenced by several outbreaks of foodborne illness that were linked to the consumption of RTE meats [1-4]. *L. monocytogenes* causes listeriosis with a high mortality

rate. *E. coli* O157:H7 causes serious illnesses that include hemorrhagic colitis and hemolytic uremic syndromes, while *Salmonella* spp. cause severe gastroenteritis in human [5,6]. Post-thermal processing contamination of RTE meats mainly occurs during product handling, slicing, or packaging in the manufacturing facilities. RTE meats are consumed without prior cooking, therefore controlling the growth of the pathogens that may contaminate RTE meats is particularly important for ensuring the microbial safety of the products. Since *L. monocytogenes* is capable of growing at refrigerated temperatures, it is a particular concern among pathogens that may contaminate RTE meats. RTE meats contaminated with *L. monocytogenes* were implicated in several listeriosis outbreaks [1,2]. Although not as prominent as

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*L. monocytogenes* in RTE meats, *E. coli* O157:H7 and *Salmonella* spp. have been increasingly linked to RTE meats as frequent contaminants in raw meats used as ingredients for RTE meat products. A survey reported that *Salmonella* spp. were found in 1.9% retail beef samples [7], and, in 2007, 9.9 million kg of fresh and frozen ground beef were recalled due to *E. coli* O157:H7 contamination [8]. This implicates that both pathogens are likely to be introduced into RTE meat processing environment through the raw meat ingredient.

Salts of lactate have been increasingly used in meat, poultry, and smoked seafood products to control the growth of foodborne pathogens [9,10]. They are used in RTE meats to enhance microbial food safety and shelf life and as flavor agents. Lactate is affirmed as generally recognized as safe (GRAS) and can be used as a direct food ingredient in meat and poultry products at levels not to exceed 4.8% by weight of total formulation [11,12]. The antimicrobial effect of lactate, alone or in combination with other food additives, have been examined and reported. Ukuku *et al.* [13] used a solution of 1% sodium lactate, 1% hydrogen peroxide, 25 µg/ml nisin, and 0.5% citric acid as a washing solution to treat *E. coli* O157:H7-inoculated whole cantaloupes and honey dew melons. The levels of the pathogen on the surface of melons were reduced by 3 - 4 log CFU/cm<sup>2</sup>. Raybaudi-Massilia *et al.* [14] used a dipping solution containing 1% N-acetyl-L-cysteine, 1% glutathione, and 1% calcium lactate to treat fresh-cut Fuji apple and reported reductions of 2.0 log CFU/g for *L. monocytogenes*, 1.5 log CFU/g for *S. Enteritidis*, and 3.0 log CFU/g for *E. coli* O157:H7 in apple pieces after a 30-d storage at 5°C. Quilo *et al.* [15] examined a treatment of 200 ppm peroxyacetic acid followed by 3% lactate for beef trimmings inoculated with *E. coli* and *S. Typhimurium*. They reported a significant reduction of *S. Typhimurium*, but not *E. coli*, on the trimmings during a 7-d refrigerated storage. Maks *et al.* [16] reported that lactate and pediocin together rendered *L. monocytogenes* more sensitive to heat in bologna. Studies have also examined the combination of lactate with UV light (254 nm) in inactivating *L. monocytogenes* on frankfurters [17], lactate in bioactive alginate coatings to control *L. monocytogenes* on cold-smoked salmon slices and fillets [18], the combination of lactate and oregano oil in ground beef to control *Salmonella* spp. [19], and the combination of lactate and lauric arginate to control *L. monocytogenes* on frankfurters [20,21]. These and many other studies [3,22-25] show that studies examining the antimicrobial effect of lactate in RTE meats have been mainly focused on *L. monocytogenes*; few have examined the effect of lactate on *E. coli* O157:H7 and *Salmonella* spp. The objective of this

study was to examine the effects of lactate on *L. monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella* spp. in RTE cooked ham under refrigerated and temperature-abuse conditions. *L. monocytogenes* was included in this study to serve as a comparison to *E. coli* O157:H7 and *Salmonella* spp.

## 2. Materials and Methods

### 2.1. *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp.

Five strains of *L. monocytogenes*, MFS 2 (pork processing plant isolate), MFS 102 (frankfurters isolate), Scott A (clinical isolate), 101M (beef and pork sausage isolate), and F6854 (turkey frankfurters isolate), four strains of *E. coli* O157:H7, EC505B (beef isolate), EC204P (pork isolate), C7927 (apple cider isolate), and C9490 (hamburger isolate), and four species of *Salmonella* spp., *S. Typhimurium* (beef isolate), *S. Kentucky*, *S. Enteritidis* (clinic isolate), and *S. Saintpaul* (stuffed ham isolate) were used in this study. All bacterial strains were obtained from the culture collection of the Microbial Food Safety Research Unit, Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture. Each bacterial strain was maintained at -80°C in Brain Heart Infusion (BHI; Difco Laboratories Inc., Detroit, MI., U.S.A.) broth containing 10% glycerol (Sigma Chemical Co., St. Louis, MO., U.S.A.). A loopful of cell suspension of each strain was subcultured twice in 10 ml BHI broth and incubated at 37°C for 24 h. Cell suspension from each strain was mixed together, and the mixture was diluted with sterile 0.1% (w/v) peptone water (PW) to achieve a population of approximately 10<sup>5</sup> CFU/ml for use as inoculum. The cell count of each inoculum was determined by spread-plating 0.1 ml diluted cell mixture onto PALCAM medium base formulated with antimicrobial supplement (Becton, Dickinson and Company, Sparks, MD., U.S.A.) for *L. monocytogenes*, Sorbitol MacConkey agar (SMAC; Becton, Dickinson and Company) for *E. coli* O157:H7, and Xylose-Lysine-Tergitol agar (XLT; Becton, Dickinson and Company) for *Salmonella* spp. Plates were incubated at 37°C for 24 - 48 h before typical colonies were counted.

### 2.2. Ham Preparation

A restructured cooked ham product (76% moisture, 2.9% fat, 16.5% protein, 1.9% salt, and <0.1 µg/g nitrite) purchased from a local supplier was used in this study. The ham, in ground form, was added with sodium lactate to ensure that the selected levels of lactate were presented in the ham. To prepare the ham samples, ham was cut

into 1 cm × 1 cm × 1 cm pieces, and the ham pieces, in stomacher bags, were heated in a water bath to 63°C and held for 30 min to inactivate the native microflora. After the heat treatment, the ham pieces were cooled under running water and kept at -20°C for overnight. The frozen, heat-treated ham pieces were ground in a Waring blender (Waring Commercial, Torrington, CT., U.S.A.). One hundred grams of ground ham were placed into stomacher bags (Spiral Biotech Inc., Norwood, MA., U.S.A.) and added with sodium lactate (65% syrup, Sigma Chemical Co.). The amount of lactate syrup added was adjusted for its concentration to achieve a final lactate concentration of 0, 1%, 2%, or 3% (w/w) in ground ham. The bags were hand-massaged for 30 s and then pummeled for 2 min in a BagMixer 400 Stomacher (Interscience, St. Nom, France). The bags were stored at 4°C overnight for equilibration. The pH of ham after equilibration was measured using a SevenMulti pH meter fitted with an InLab RoutinPro pH electrode (Mettler-Toledo, Schwerzenbach, Switzerland). The pHs of samples were pH 5.7 - 6.1. After storage, 4 g of ground ham were placed into 100-ml stomacher bags (Spiral Biotech Inc.) for inoculation.

### 2.3. Sample Inoculation and Storage

One-tenth of one milliliter of the *L. monocytogenes*, *E. coli* O157:H7, or *Salmonella* spp. inoculum was added into bags and mixed thoroughly by hands. The bags were vacuum-sealed using a Multivac A300 vacuum sealer (Multivac Inc., Kansas, MO., U.S.A.). The inoculum level of *L. monocytogenes*, *E. coli* O157:H7, or *Salmonella* spp. in ham was approximately 10<sup>2</sup> CFU/g. The samples were stored at 4, 8, and 10°C for *L. monocytogenes*, 6, 8, 10, 12, and 15°C for *E. coli* O157:H7, and 8, 10, 12, and 15°C for *Salmonella* spp. for up to 35 d. The temperatures were refrigerated and abuse temperatures or the growth/no growth boundary temperatures for each pathogen.

### 2.4. Microbial Analysis

During storage, samples were enumerated for counts of *L. monocytogenes*, *E. coli* O157:H7, or *Salmonella* spp. at 6 different times. At each sampling time, two samples were added with 8 ml PW, and the mixture was pummeled for 2 min. Additional dilutions were prepared in serial PW, and duplicated 0.1 ml of appropriate dilution were surface-plated on PALCAM, SMAC, and XLT to enumerate cell counts of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp., respectively. All plates were incubated at 35°C for 24 - 48 h before typical colonies were counted. The experiment was performed twice.

### 2.5. Average Growth Rate and Effect Analysis

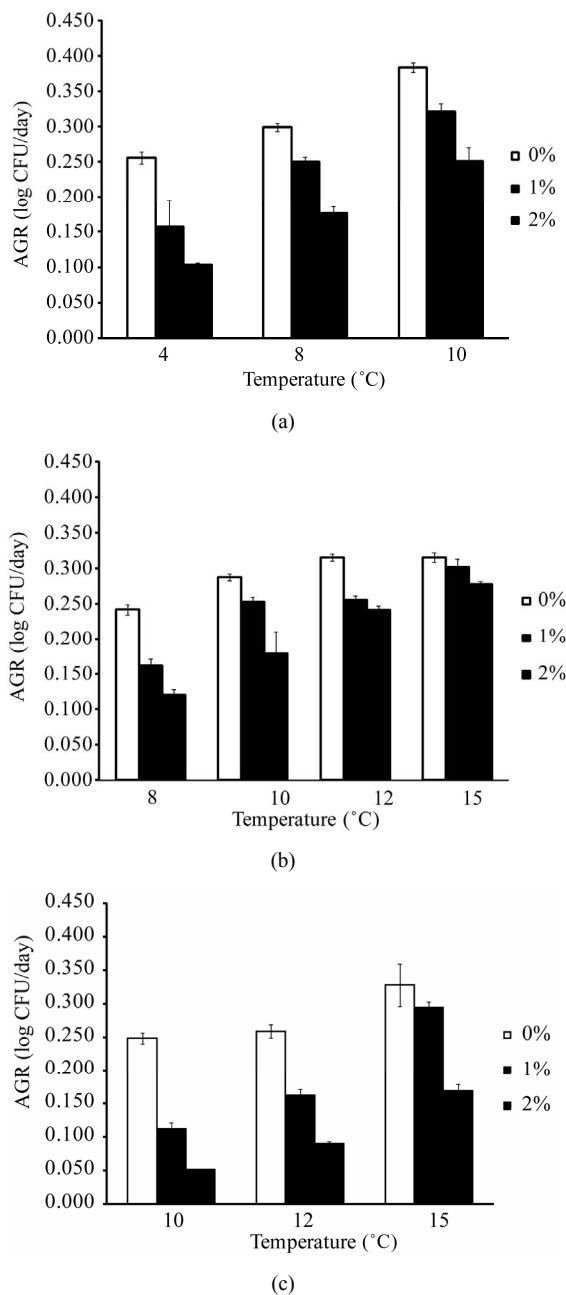
Means of cell counts (log CFU/g) of each pathogen in ham during storage were plotted against sampling times to obtain growth curves. From the curves the average growth rates (AGR, log CFU/d) for each pathogen were estimated by dividing the numbers of net population increase (log CFU/g) by the length of time of storage (d). Since most of the treatments resulted in growth curves without lag phases, the AGR were a good indication of the effect of lactate and storage temperature on the pathogens. To analyze the individual and combined effect of lactate and storage temperature, the AGR were transformed into square root. The GR were transformed into square root to stabilize the variance [26] and analyzed by the General Linear Model (GLM) of Statistical Analysis System 9.1 (SAS Institute Inc., Cary, NC., U.S.A.) as a function of lactate concentration, storage temperature, and their interaction with the following quadratic equation:

$$\sqrt{AGR} = \alpha + \beta_1(\text{lactate}\%) + \beta_2(\text{temperature}) + \beta_3(\text{lactate}\% * \text{temperature}) + \beta_4(\text{lactate}\%)^2 + \beta_5(\text{temperature})^2$$

where  $\alpha$  is the intercept, and  $\beta_1$ -  $\beta_5$  are estimated coefficients.

### 3. Results and Discussion

*E. coli* O157:H7 and *Salmonella* spp. were not able to grow in ham samples stored at 6°C and 8°C, respectively, regardless of the concentration of lactate in ham. The temperatures observed in this study were similar to the minimal growth temperatures (approximately 7°C - 8°C) reported for pathogenic *E. coli* and *Salmonella* spp. [19,27]. *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp. did not grow (less than 0.5 log CFU/g increase during storage) in ham containing 3% lactate at all storage temperatures. The addition of lactate in ham at 1% or 2% reduced the growth rates of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp. when compared to ham containing no lactate. The results indicate that lactate at 3% or higher concentrations may prevent the growth of these pathogens in ham at refrigerated and mild abuse temperatures for up to 35 d, while lactate at lower levels slow the growth of these pathogens. The AGR of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp. in ham containing 0% - 2% lactate are shown in **Figure 1**. In ham containing no lactate, the AGR were 0.256 - 0.380 log CFU/d for *L. monocytogenes* at 4°C - 10°C, 0.242 - 0.315 log CFU/d for *E. coli* O157:H7 at 8°C - 15°C, and 0.249 - 0.328 log CFU/d for *Salmonella* spp. at 10°C - 15°C. The addition of 1% or 2% lactate in ham significantly ( $P < 0.05$ ) reduced the AGR of the



**Figure 1.** Average growth rates of *L. monocytogenes* (a), *E. coli* O157:H7 (b), and *Salmonella* spp. (c) in cooked ham formulated with 0% - 2% lactate and stored at refrigerated and abuse temperatures ( $n = 4$ ).

three pathogens, and the effect was more profound at lower storage temperatures. The AGR of *L. monocytogenes* in ham containing no lactate at 4°C was 0.256 log CFU/d, and it was reduced to 0.158 and 0.104 log CFU/d with the addition of 1% and 2% lactate, respectively. Lactate at 1% and 2% in ham reduced the AGR of *L. monocytogenes* by 35% and 60% at 4°C, respectively,

whereas the reductions were 16% and 41% at 8°C, and 16% and 34% at 10°C (**Figure 1(a)**). At the lowest storage temperatures that supported the growth of *E. coli* O157:H7 and *Salmonella* spp., the AGR reductions caused by 1% and 2% lactate for *E. coli* O157:H7 were 33% and 50% at 8°C (**Figure 1(b)**), and were 55% and 79% for *Salmonella* spp. at 10°C (**Figure 1(c)**). The lactate-induced AGR reduction decreased as the storage temperature for *E. coli* O157:H7 and *Salmonella* spp. increased. The reductions were 4% - 19% and 12% - 38% for *E. coli* O157:H7 at 12 and 15°C, and were 10% - 37% and 49% - 65% for *Salmonella* spp. at 12 and 15°C, respectively. The results indicated that *Salmonella* spp. were more sensitive to the growth-rate reducing effect of lactate than *L. monocytogenes* and *E. coli* O157:H7.

The findings regarding the antimicrobial effect of lactate on *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp. in RTE ham is in agreement with results reported by other studies using different foods or growth media. Thompson *et al.* [23] reported that *L. monocytogenes* levels in turkey roll containing 2% lactate increased only 2 log CFU/cm<sup>2</sup>, which was significant lower than those in samples containing no lactate, after a 6-week refrigerated storage. The growth-delaying effect of lactate on *L. monocytogenes* in food products or microbiological growth media were also reported by Glass *et al.* [24], Stekelenburg [28], Legan *et al.* [29], and Abou-Zeid *et al.* [30]. McWilliam Leitch and Steward [31, 32] reported a reduced viability of *E. coli* O157:H7 and non-O157 *E. coli* isolates in a lactate solution (100 mM) at 37°C. Neetoo *et al.* [18] reported that lactate (1.2 or 2.4%) suppressed the growth of spoilage aerobic and anaerobic microorganisms at 4°C. In this study, *Salmonella* spp. were observed to grow in ham containing 1 and 2% lactate stored at 10°C - 15°C, albeit the growth was slower comparing to those in samples containing no lactate. Similar observations have also been reported. Jung *et al.* [33] examined the growth kinetics of lactic acid-stressed and unstressed *S. Typhimurium* in broth and chicken patties containing a mixture of 2.2% potassium lactate and 0.16% sodium diacetate and reported that the growth of lactic acid-stressed *S. Typhimurium* was inhibited by the mixture at 10°C, whereas the growth of unstressed *S. Typhimurium* was not inhibited. Juneja *et al.* [5] also reported that *Salmonella* spp. was not able to grow at 15°C in heat-treated ground beef containing 1.5% lactate.

The individual and combined effects of lactate and storage temperature on the  $\sqrt{AGR}$  of the three pathogens were analyzed using a quadratic regression. The estimated coefficients for each parameter and their significance levels are shown in **Table 1**. The resulted

**Table 1. Coefficients and significance of fitted parameters.**

Parameter <sup>1</sup>	<i>L. monocytogenes</i>		<i>E. coli</i> O157:H7		<i>Salmonella</i> spp.	
	Estimate	Pr >  t  <sup>2</sup>	Estimate	Pr >  t	Estimate	Pr >  t
Intercept	0.476	<0.0001	0.027	0.6182	0.092	0.6935
Lactate	-0.048	0.0005	-0.082	<0001	-0.183	<0001
Temperature	-0.007	0.048	0.081	<0001	0.047	0.0181
Lactate*temperature	0.001	0.046	0.006	<0001	0.004	0.0177
Lactate*lactate	-0.017	<0.0001	-0.020	<0001	0.013	0.004
Temperature*temperature	0.002	0.014	-0.003	<0001	-0.001	0.0459

regression equations that describe the  $\sqrt{AGR}$  for each of the three pathogens in ham containing 0% - 2% lactate and at storage temperatures that supported their growth are:

*L. monocytogenes* (4°C - 10°C)

$$\begin{aligned} \sqrt{AGR} = & 0.476 - 0.048 * (\text{lactate}\%) \\ & - 0.007 * (\text{temperature}) \\ & + 0.001 * (\text{lactate}\% * \text{temperature}) \\ & - 0.017 * (\text{lactate}\%)^2 + 0.002 * (\text{temperature})^2 \end{aligned}$$

$$(R^2 = 0.97, P < 0.0001)$$

*E. coli* O157:H7 (8°C - 15°C)

$$\begin{aligned} \sqrt{AGR} = & 0.027 - 0.082 * (\text{lactate}\%) \\ & + 0.081 * (\text{temperature}) \\ & + 0.006 * (\text{lactate}\% * \text{temperature}) \\ & - 0.020 * (\text{lactate}\%)^2 - 0.003 * (\text{temperature})^2 \end{aligned}$$

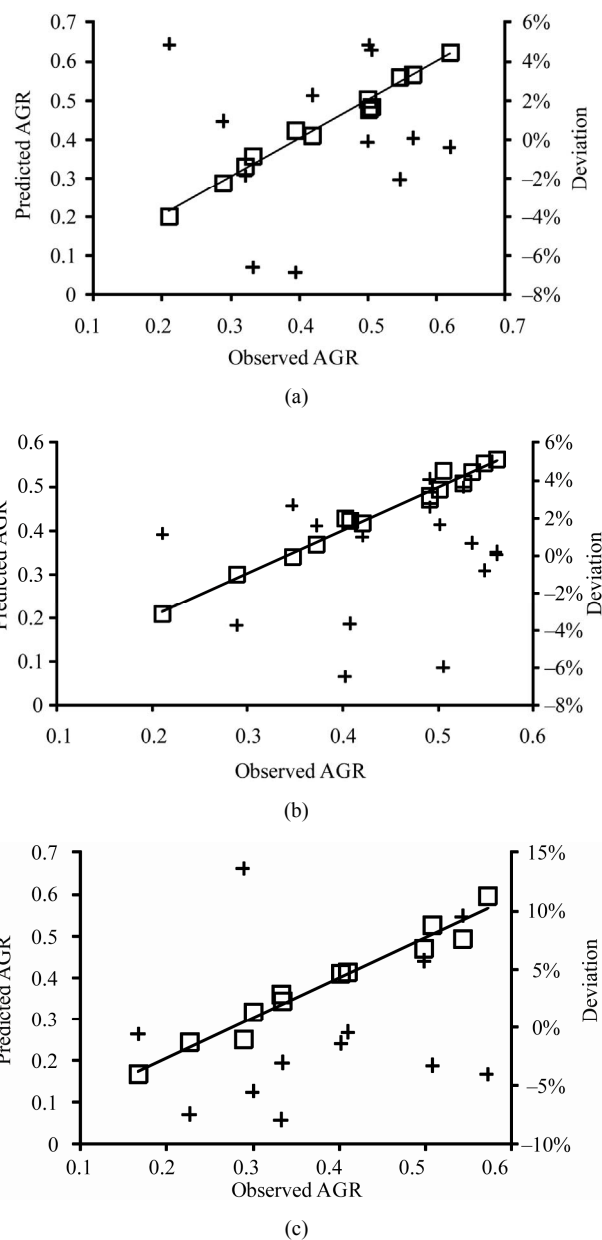
$$(R^2 = 0.97, P < 0.0001)$$

*Salmonella* spp. (10°C - 15°C)

$$\begin{aligned} \sqrt{AGR} = & 0.092 - 0.183 * (\text{lactate}\%) \\ & + 0.047 * (\text{temperature}) \\ & + 0.004 * (\text{lactate}\% * \text{temperature}) \\ & + 0.013 * (\text{lactate}\%)^2 - 0.001 * (\text{temperature})^2 \end{aligned}$$

$$(R^2 = 0.95, P < 0.0001)$$

In general lactate concentration, storage temperature, and their interaction significantly ( $P < 0.05$ ) affect the  $\sqrt{AGR}$  of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp. To determine the goodness-of-fit of the equations, the predicted  $\sqrt{AGR}$ , obtained from the equations, were plotted against the observed  $\sqrt{AGR}$  (Figure 2). The linear relationship between the predicted and observed  $\sqrt{AGR}$  is indicated by the correlation coefficients ( $r^2$ ) of 0.98 for *L. monocytogenes*, 0.96 for *E. coli* O157:H7, and 0.98 for *Salmonella* spp., indicating a consistent closeness of the predicted and observed values within the ranges of lactate concentration and temperature examined. The predicted  $\sqrt{AGR}$  are mostly within  $\pm 8\%$  of the observed for *L. monocytogenes* and *E. coli*



**Figure 2. Predicted versus observed  $\sqrt{AGR}$  ( $\square$ ) for *L. monocytogenes* (a), *E. coli* O157:H7 (b), and *Salmonella* spp. (c) and the deviations of predicted values to the observed values (+).**

O157:H7, and are within  $\pm 10\%$  of the observed for *Salmonella* spp., indicating that the equations closely describe the observed  $\sqrt{AGR}$  of the three pathogens in ham containing 0% - 2% lactate at the storage temperatures that supported their growth.

#### 4. Conclusions

The present study showed that lactate had a broad antimicrobial effect in ham as it delayed the growth of *L. monocytogenes* as well as *E. coli* O157:H7 and *Salmonella* spp. at refrigerated and abuse temperatures. The results indicated the individual and combined effect of lactate and storage temperature on delaying the growth of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp. in ham. The mathematical models that describe the average growth rates of the three pathogens in ham will be beneficial to the manufacturers of RTE meats in formulating lactate concentrations in ham for food-safety and sensory quality purposes.

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