Research Note

Response Surface Models for Effects of Temperature and Previous Growth Sodium Chloride on Growth Kinetics of *Salmonella* Typhimurium on Cooked Chicken Breast†

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ABSTRACT

Response surface models were developed and validated for effects of temperature (10 to 40°C) and previous growth NaCl (0.5 to 4.5%) on lag time (λ) and specific growth rate (μ) of *Salmonella* Typhimurium on cooked chicken breast. Growth curves for model development ($n = 55$) and model validation ($n = 16$) were fit to a two-phase linear growth model to obtain λ and μ of *Salmonella* Typhimurium on cooked chicken breast. Response surface models for natural logarithm transformations of λ and μ as a function of temperature and previous growth NaCl were obtained by regression analysis. Both λ and μ of *Salmonella* Typhimurium were affected ($P < 0.0001$) by temperature but not by previous growth NaCl. Models were validated against data not used in their development. Mean absolute relative error of predictions (model accuracy) was 26.6% for λ and 15.4% for μ. Median relative error of predictions (model bias) was 0.9% for λ and 5.2% for μ. Results indicated that the models developed provided reliable predictions of λ and μ of *Salmonella* Typhimurium on cooked chicken breast within the matrix of conditions modeled. In addition, results indicated that previous growth NaCl (0.5 to 4.5%) was not a major factor affecting subsequent growth kinetics of *Salmonella* Typhimurium on cooked chicken breast. Thus, inclusion of previous growth NaCl in predictive models may not significantly improve our ability to predict growth of *Salmonella* spp. on food subjected to temperature abuse.

Salmonella spp. are dangerous contaminants of food because they grow over a broad range of temperature (7 to 45°C), pH (4.5 to 9.5), and water activity (0.94 to 1.00) *(6, 15)*. Mathematical models that predict growth of *Salmonella* spp. as a function of temperature, pH, and water activity are available *(1, 10, 16)* and are a valuable tool for assessing the microbiological safety of temperature-abused foods *(2, 3)*. Most predictive models for growth of *Salmonella* spp. were developed with strains grown under optimal conditions of temperature, pH, and water activity *(1, 10, 16)*. However, *Salmonella* spp. that contaminate food may be derived from environments in which their previous growth occurred under nonoptimal conditions. Effects of previous nonoptimal growth conditions on subsequent growth kinetics of *Salmonella* spp. in temperature-abused food have been less extensively studied and modeled. Inclusion of previous growth conditions as variables in predictive models may enhance our ability to predict growth of *Salmonella* spp. in food subjected to temperature abuse.

Recently, our laboratory investigated and modeled effects of nonoptimal growth conditions on subsequent growth kinetics of *Salmonella* Typhimurium in brain heart infusion broth *(13)* and on cooked chicken breast *(14)*. These studies demonstrated that growth kinetics (i.e., lag time [λ] and specific growth rate [μ]) of *Salmonella* Typhimurium were not greatly affected by previous growth over a broad range of pH (5.5 to 8.5) *(13)* and temperature (16 to 34°C) *(14)*. Nonetheless, to further expand knowledge about effects of previous growth conditions on subsequent growth of *Salmonella* spp., the current study was undertaken to investigate and model effects of previous growth NaCl (0.5 to 4.5%) on subsequent growth kinetics of *Salmonella* Typhimurium on cooked chicken breast subjected to a range of abuse temperatures (10 to 40°C).

MATERIALS AND METHODS

Stock cultures. *Salmonella* Typhimurium (ATCC 14028) at a concentration of 9.0 to 9.4 $\log_{10}$ CFU/ml were maintained at $-20°C$ in brain heart infusion broth that contained 15% glycerol.

Starter cultures. Stock cultures of *Salmonella* Typhimurium were thawed at room temperature and diluted by 10^{-3}. Then 5 µl of the resuspended stock culture was added to 5 ml of brain heart infusion broth (pH 6.4), resulting in an initial concentration of 3.0 to 3.4 $\log_{10}$ CFU/ml. Brain heart infusion broth used in starter cultures contained 0.5, 1.5, 2.5, 3.5, or 4.5% NaCl. Starter cultures were incubated for 23 h at 34°C in 25-ml Erlenmeyer flasks sealed with foam plugs and shaken at 150 rpm. Regardless of NaCl level,

viable counts of starter cultures at the end of the 23-h incubation were between 10.0 and 10.4 $\log_{10}$ CFU/ml.

Experimental designs. The experiment for model development was a full 3 by 9 factorial arrangement of previous growth NaCl (0.5, 2.5, or 4.5%) and incubation temperature (10, 12, 14, 16, 20, 24, 28, 34, or 40°C) of cooked chicken breast. Twenty-four growth conditions were done twice, two growth conditions were done thrice, and one growth condition was done once, for a total of 55 growth curves. The experiment for model validation was a full 2 by 8 factorial arrangement of previous growth NaCl (1.5 or 3.5%) and incubation temperature (11, 13, 15, 18, 22, 26, 31, or 37°C) of cooked chicken breast, for a total of 16 growth curves.

Preparation of cooked chicken breast. Boneless chicken breast was obtained from a local supermarket and was ground twice through a 3/16-in. plate of a hand-powered meat grinder. Ten grams of ground chicken was formed into a circular patty by finger kneading followed by flattening with a 100-ml beaker. An indentation (1.2 cm^2) was made in the center of the chicken patty with a dilution tube cap to serve as an inoculation well. Eight to 12 patties were made for each growth curve. Background microflora were removed by autoclaving (18 min at 121°C). After cooling, cooked chicken breast was transferred under sterile conditions to Petri dishes and stored at 4°C in plastic bags until used within 4 days.

Inoculation of cooked chicken breast. Cooked chicken breasts were incubated for 16 h at the proper temperature (10 to 40°C) before inoculation. A sterile repeater pipette was used to surface inoculate cooked chicken breast with 100 μl of sterile distilled water that contained 5.2 $\log_{10}$ CFU of *Salmonella* Typhimurium from the appropriate starter culture. Incubations were conducted in plastic bags to prevent drying of cooked chicken breast.

Determination of viable counts. At selected times after inoculation (0 to 220 h), depending on the incubation temperature, cooked chicken breasts (6 g after autoclaving) were homogenized (model 400 stomacher, Seward, London, England) for 2 min in 94 ml of sterile distilled water. Fifty microliters of undiluted and diluted (10^{-1} to 10^{-5}) samples of homogenate was spiral plated (Whitley Automatic Sprial Plater, Don Whitley Scientific Limited, West Yorkshire, England) onto brain heart infusion agar (Difco Laboratories, Detroit, Mich.). Sampling times for each incubation temperature were based on predicted λ and μ from a response surface model for growth of *Salmonella* Typhimurium in brain heart infusion broth *(13)* and were selected to produce a growth curve that accurately defined the lag phase and exponential growth phase over two to three $\log_{10}$ cycles of growth.

Spiral plates were inverted and incubated for 18 to 24 h at 30°C, and colonies that formed on brain heart infusion agar were counted using an automated colony counter (Protos Colony Counter, Synoptics, Cambridge, England). Using this protocol, countable plates were obtained when the undiluted or diluted homogenate had a *Salmonella* Typhimurium concentration of 3.0 to 4.9 $\log_{10}$ CFU/ml.

Growth curve fitting. Growth curves of viable counts (*Y*, $\log_{10}$ CFU/ml) versus sample time (*X*, h) were iteratively fit using GraphPad PRIZM (GraphPad Software, San Diego, Calif.) to a two-phase linear growth model *(5, 12)*:

$$Y = \text{Baseline} + \text{Increase}$$
$$\text{Increase} = 0 \qquad \text{if } X \leq (\lambda)$$

$$\text{Increase} = (\mu) \times \triangle X \quad \text{if } X > (\lambda)$$
$$\triangle X = X - (\lambda)$$

where viable count *Y* was equal to baseline (initial viable count) plus increase (increase of viable count). In turn, increase was equal to 0 if sample time *X* was less than or equal to λ in hours; otherwise increase was equal to the μ; $\log_{10}$ CFU/h times $\triangle X$ (sample time minus λ).

Response surface modeling. A data set containing model variables (i.e., previous growth NaCl and incubation temperature of cooked chicken breast) and natural logarithm transformations (ln) of λ and μ from 55 growth curve fits was created. The data set was subjected to regression analysis (Statistical Analysis System, Cary, N.C.) using the following response surface model:

$$\ln \lambda \text{ or } \ln \mu = b_0 + b_1A + b_2B + b_3AB + b_4A^2 + b_5B^2 + \epsilon$$

where *A* was the NaCl level in starter cultures or previous growth NaCl, *B* was incubation temperature of cooked chicken breast, b_0 to b_5 were regression coefficients, and ϵ was random error.

Model validation. Models were validated against data not used in their development. Relative error (RE) of each prediction case was determined using the following equation *(7)*:

$$\text{RE} = (X_p - X_o)/X_o$$

where X_p was predicted λ or μ and X_o was observed λ or μ. Median relative error (MRE) of model predictions was used as the measure of prediction bias. Mean absolute relative error (MARE) of each model was used as the measure of prediction accuracy and was calculated using the following equation *(7)*:

$$\text{MARE} = \frac{1}{n} * \sum_{i=1}^{n} |\text{RE}|_i$$

where *n* was the number of prediction cases.

RESULTS AND DISCUSSION

Representative growth curve fits for data used in model development and validation are shown in Figure 1. The coefficient of determination (r^2) of growth curve fits ranged from 0.954 to 0.995, with a mean ± SEM of 0.978 ± 0.0015 for data used in model development. For data used in model validation, the r^2 of growth curve fits ranged from 0.926 to 0.995, with a mean ± SEM of 0.972 ± 0.0047. The range and mean r^2 for growth curve fits in this study agree with the range and mean r^2 for growth curve fits from similar modeling studies *(13, 14)*.

A data set containing model variables (i.e., previous growth NaCl in brain heart infusion broth and incubation temperature of cooked chicken breast) and ln λ and ln μ from 55 growth curve fits was created and subjected to regression analysis to yield quadratic polynomial response surface models (Table 1). The λ and μ underwent ln to stabilize model variance *(10)*. Both models had high r^2, indicating a high degree of goodness of fit to the data. In similar modeling studies using the same strain of *Salmonella* Typhimurium, the r^2 values for the λ model were 0.963 *(13)* and 0.925 *(14)*, and for the μ model, the r^2 values were 0.984 *(13)* and 0.979 *(14)*.

Regression analysis indicated that incubation temperature of cooked chicken breast had a large effect on λ and μ of *Salmonella* Typhimurium, whereas previous growth
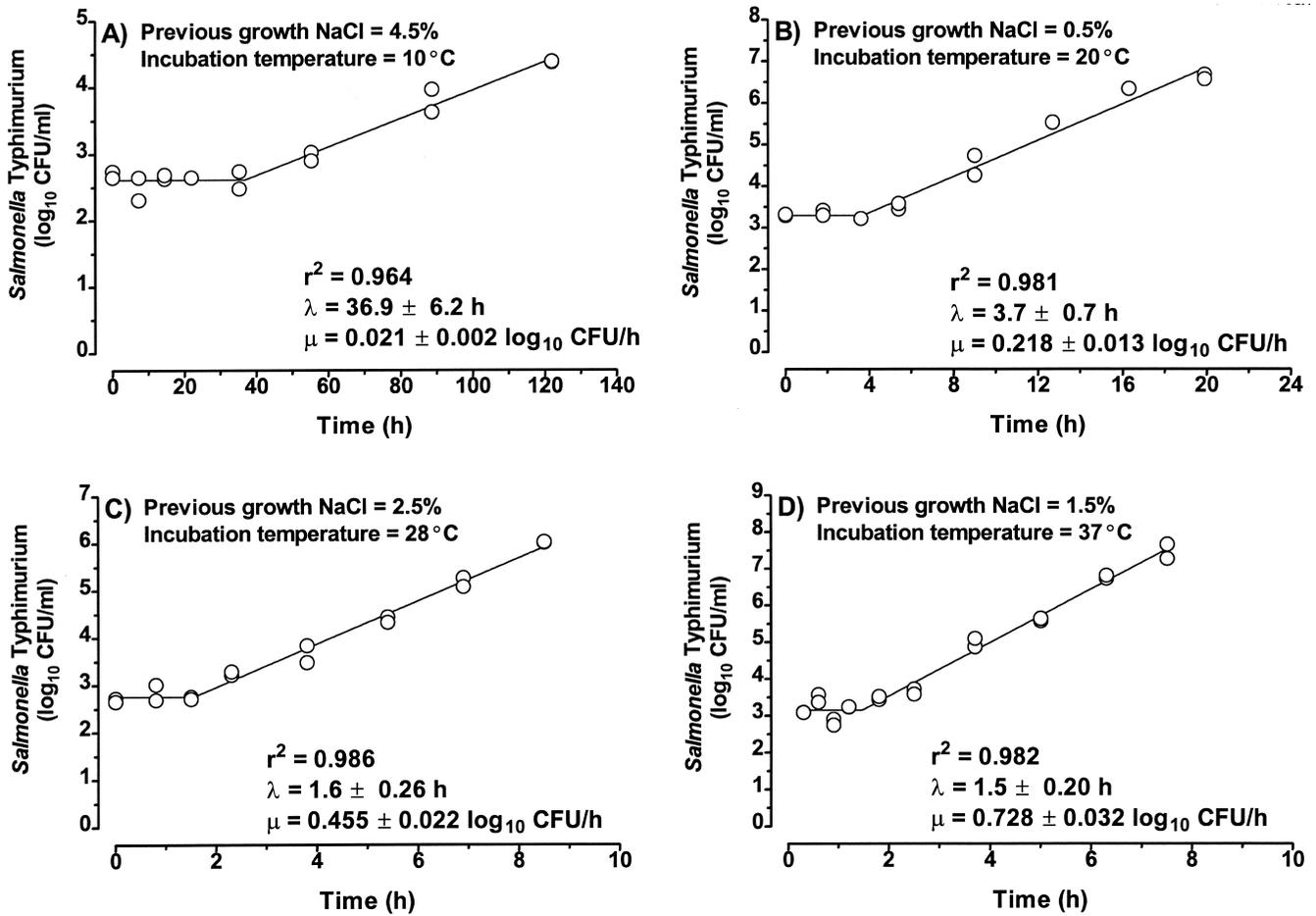
FIGURE 1. *Typical growth curve fits for* Salmonella *Typhimurium on cooked chicken breast for data used in model development (A through C) and data used in model validation (D). Viable counts are expressed per milliliter of homogenate.*

NaCl did not alter λ or μ of *Salmonella* Typhimurium on cooked chicken breast (Table 1). As expected, λ (Fig. 2a) decreased quadratically and μ (Fig. 2b) increased quadratically as incubation temperature increased from 10 to 40°C. In similar modeling studies using the same strain of *Salmonella* Typhimurium, previous growth pH (5.5 to 8.5) had a small effect on λ and no effect on μ *(13)*, whereas previous growth temperature (16 to 34°C) had no effect on λ or μ *(14)*. Together, these studies indicate that previous growth of *Salmonella* Typhimurium under a variety of nonoptimal growth conditions has minimal to no effect of subsequent growth kinetics. Thus, inclusion of previous growth

conditions in predictive models may not significantly improve our ability to predict growth of *Salmonella* spp. on food subjected to temperature abuse.

Similar to our studies, others have found that previous growth conditions do not alter subsequent growth rate of human bacterial pathogens *(4, 8, 11)*. In contrast, significant effects of previous growth temperature on subsequent λ have been observed *(4, 8, 9, 11)*. In general, temperature shifts from high (37 to 43°C) to low (5 to 14°C) result in extended λ. The extended λ for pathogens shifted from warm to cold environments may result from the need to synthesize new cell wall components before growth can be

TABLE 1. *Response surface models for effects of previous growth NaCl (pgNaCl) and temperature (T) on lag time (λ) and specific growth rate (μ) of* Salmonella *Typhimurium on cooked chicken breast*

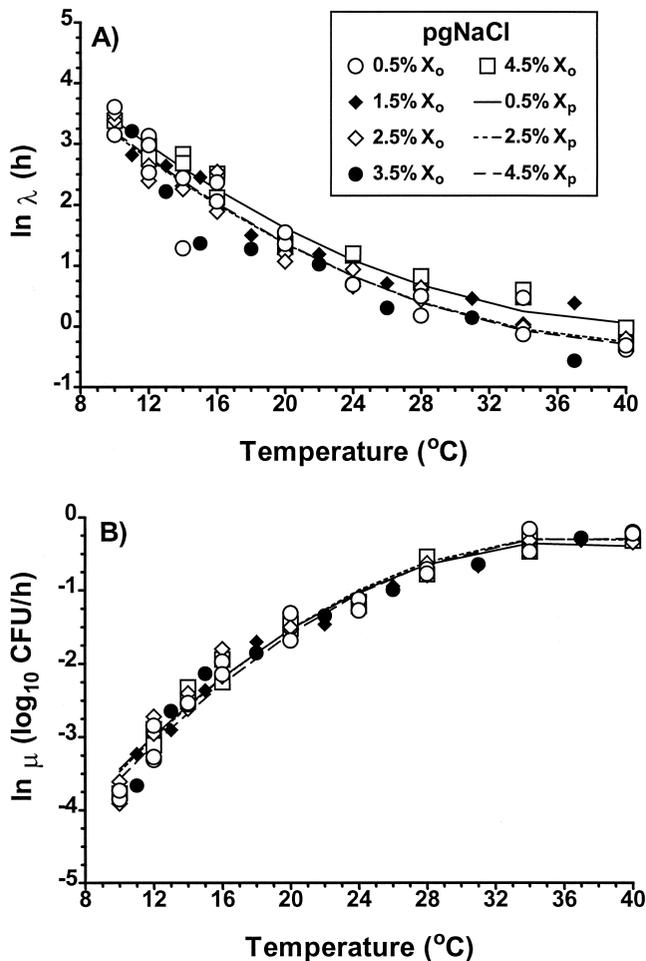| Parameter	ln λ, h			ln μ, $\log_{10}$ CFU/h		
	Estimate	*F* value	*P* value	Estimate	*F* value	*P* value
Intercept	5.9115			−6.2251		
pgNaCl	−0.2013	3.03	0.0882	−0.0114	0.02	0.8820
T	−0.2754	129.54	0.0001	0.3234	411.83	0.0001
pgNaCl × T	−0.0013	0.31	0.5790	0.0020	1.58	0.2154
pgNaCl × pgNaCl	0.0333	2.92	0.0941	−0.0085	0.44	0.5098
T × T	0.0033	51.36	0.0001	−0.0045	213.84	0.0001
r^2	0.952			0.978		

FIGURE 2. *Effect of previous growth NaCl (pgNaCl) in brain heart infusion broth and incubation temperature of cooked chicken breast on (A) ln λ and (B) ln μ of* Salmonella *Typhimurium on cooked chicken breast.*

quent growth kinetics of *Salmonella* Typhimurium. Nonetheless, results of our studies *(13, current study)* suggest that the physiological state of *Salmonella* Typhimurium is not greatly altered by previous growth under nonoptimal conditions of pH and NaCl.

The ability of our models to predict λ and μ of *Salmonella* Typhimurium was validated against data not used in their development. Data used in model validation was collected using the same strain and experimental protocol but with previous growth NaCl levels and incubation temperatures that were intermediate to those used to collect data for model development. The RE of each prediction case for each model and data set combination was calculated and used to calculate the MARE, a measure of prediction accuracy, and the MRE, a measure of prediction bias (Table 2). For both models, the MARE and MRE were similar for data used in model development and data used in model validation. Overall, prediction accuracy was better for the μ model than the λ model, whereas prediction bias was similar (Table 2).

A scatter plot of RE for λ predictions (Fig. 3a) indicated a random distribution of RE around 0% and thus a lack of systematic prediction bias. In contrast, a scatter plot of RE for μ predictions (Fig. 3b) showed systematic bias at low and intermediate μ. Nonetheless, the prediction bias was small and did not result in predictions that were grossly outside the range of observed μ (Fig. 2b).

In modeling studies using the same strain of *Salmonella* Typhimurium, MARE for λ predictions was 9.2% *(13)* and 13.4% *(14)*, whereas MARE for μ predictions was 9.1% *(13)* and 11.3% *(14)*. Prediction bias or MRE in similar modeling studies was −6.6% *(13)* and −3.0% *(14)* for λ and −7.6% *(13)* and 6.8% *(14)* for μ. Thus, MARE for λ and μ in the current study was higher and MRE was lower than our previous studies *(13, 14)*.

Delignette-Muller et al. *(7)* pooled 468 prediction cases from seven predictive modeling papers and calculated a MARE of 40.3% for λ and 36.2% for generation time. Oscar *(13)* calculated MARE for 16 predictive models representing 823 prediction cases in nine modeling papers for data used in model development and found that MARE ranged from 28.1 to 74.8% for λ and from 18.5 to 72.0% for generation time. In the current study, MARE was 26.6% for λ and 15.4% for μ. Thus, compared with models not developed in our laboratory, the current models had better prediction accuracy in all cases.

initiated at the lower temperature *(8)*. In our earlier study *(14)*, we used a narrower temperature range (16 to 34°C) than other investigators, which may explain why we did not observe an effect of previous growth temperature on subsequent λ of *Salmonella* Typhimurium.

Other investigators have not systematically investigated effects of previous growth under nonoptimal conditions of pH and NaCl on subsequent growth kinetics of human bacterial pathogens. Thus, we have no basis on which to evaluate our results for effects of previous growth pH *(13)* and previous growth NaCl (present study) on the subse-

TABLE 2. *Relative error of predictions for data used in development and data used in validation of predictive models for effects of previous growth NaCl and temperature on lag time (λ) and specific growth rate (μ) of* Salmonella *Typhimurium on cooked chicken breast*

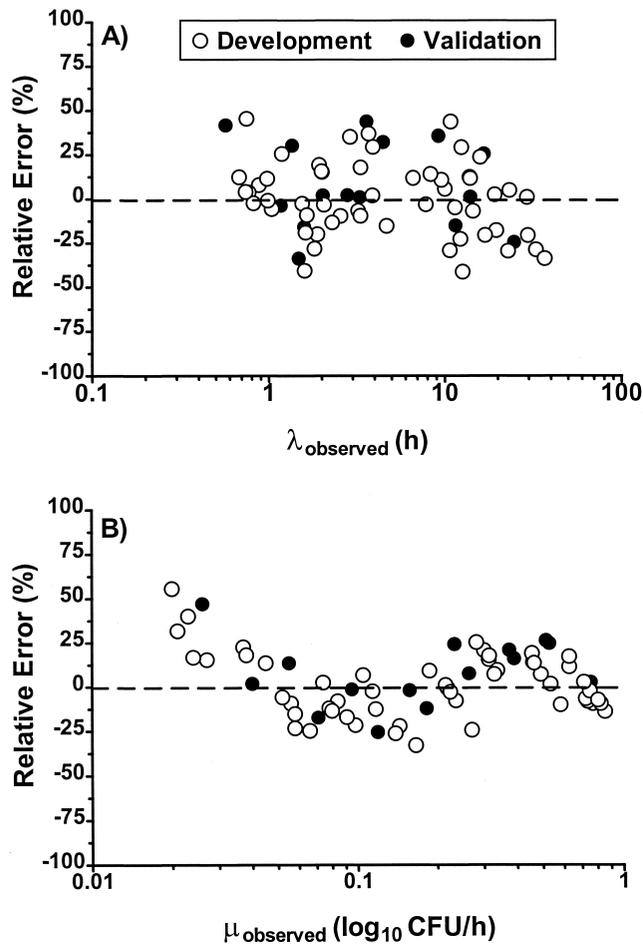| Model	Data	Cases	Mean absolute ± SEM	Relative error (%)		
				Median	Minimum	Maximum
λ	Development	55	20.2 ± 3.7	−1.0	−41.6	201.1
	Validation	16	26.6 ± 7.3	0.9	−36.3	119.6
μ	Development	55	14.1 ± 1.4	1.4	−33.0	55.4
	Validation	16	15.4 ± 3.2	5.2	−25.4	47.1

FIGURE 3. *Scatter plot of REs of model predictions for (A)* λ *and (B)* μ *for data used in model development (○) and model validation (●).*

In summary, predictive models for growth of *Salmonella* Typhimurium on cooked chicken breast as a function of previous growth NaCl and temperature were developed and validated against data not used in their development. Although validation results indicated that model predictions were less accurate than similar models developed in our laboratory, model predictions were more accurate than predictions of similar models published by other laboratories. Thus, the current models provide reliable predictions of λ and μ of *Salmonella* Typhimurium on cooked chicken breast within the range of previous growth NaCl (0.5 to 4.5%) and temperature (10 to 40°C) used to develop them. In addition, results of this study indicated that previous growth NaCl (0.5 to 4.5%) was not a major factor affecting subsequent growth kinetics of *Salmonella* Typhimurium on cooked chicken breast. Thus, inclusion of previous growth NaCl in predictive models may not significantly improve our ability to predict growth of *Salmonella* spp. on food subjected to temperature abuse.

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