

Predictive Model for Survival and Growth of *Salmonella* Typhimurium DT104 on Chicken Skin during Temperature Abuse[†]

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ABSTRACT

To better predict risk of *Salmonella* infection from chicken subjected to temperature abuse, a study was undertaken to develop a predictive model for survival and growth of *Salmonella* Typhimurium DT104 on chicken skin with native flora. For model development, chicken skin portions (2.14 cm²) were inoculated with 0.85 log of *Salmonella* Typhimurium DT104 (ATCC 700408) and then stored at 5 to 50°C for 8 h. Kinetic data from the storage trials were fit to a primary model to determine lag time (λ), specific growth rate (μ), and the 95% prediction interval (PI). Secondary models for λ , μ , and PI as a function of storage temperature were developed and then combined with the primary model to create a tertiary model. Performance of the tertiary model was evaluated against dependent data, independent data for interpolation, and independent data for extrapolation to kosher chicken skin by using an acceptable prediction zone from -1 (fail-safe) to 0.5 (fail-dangerous) log per skin portion. Survival of *Salmonella* Typhimurium DT104 on chicken skin was observed during 8 h of storage at 5 to 20°C and at 50°C, whereas growth was observed from 25 to 45°C and was optimal at 40°C with a λ of 2.5 h and a μ of 1.1 log/h. Variation of pathogen growth, as assessed by PI, increased in a nonlinear manner as a function of temperature and was greater for growth conditions than no-growth conditions. The percentage of acceptable prediction errors was 82.6% for dependent data, 83.7% for independent data for interpolation, and 81.6% for independent data for extrapolation to kosher skin, which all exceeded the performance criterion of 70% acceptable predictions. Thus, it was concluded that the tertiary model provided valid predictions for survival and growth of *Salmonella* Typhimurium DT104 from a low initial dose on both nonkosher and kosher chicken skin with native flora.

Salmonella is a leading cause of illness from food with an estimated 1.4 million cases and 500 deaths per year in the United States (20). Poultry are often implicated as a vehicle of *Salmonella* transmission to humans (7). Microbiological surveys indicate that most chickens are not contaminated with *Salmonella* and those that are contaminated usually contain low numbers of the organism (i.e., <10 cells per carcass) (9, 36, 39, 40). However, *Salmonella* can grow to high numbers on chicken that is subjected to temperature abuse (15, 18), and thus, even low numbers at processing or at retail can pose significant risk to human health when the product is not properly handled after processing or purchase.

Models that predict survival and growth of *Salmonella* are valuable tools for helping assess and manage risk of human illness from food because they can predict pathogen behavior under conditions where no data exist. However, existing models for *Salmonella* were developed in sterile food systems without background flora and using a high initial dose (>3 log) of *Salmonella* (13–15, 37). Since na-

tive flora and the initial dose of the pathogen can affect and interact to affect growth of *Salmonella* (6, 22), existing models might not accurately estimate risk from *Salmonella* for food that contains competitive flora and a low initial dose of the pathogen and that has been subjected to temperature abuse. Thus, there is a need to develop models that better predict behavior of *Salmonella* during temperature abuse in food with background flora and that is contaminated with a low initial dose of *Salmonella* (28–30).

Marketing of chickens in the United States has changed from predominantly whole chicken in 1965 to predominantly further-processed chicken today. Nonetheless, over 50% of chickens are still sold whole (11%) or as cut-up parts (43%) with skin (3). Although skin is a major component of retail chicken and a likely source of *Salmonella* contamination and transmission, there are no published studies regarding survival and growth of *Salmonella* on this important tissue of chicken. Therefore, the current study was undertaken to investigate and model survival and growth of *Salmonella* from a low initial dose on chicken skin with native flora and during short-term (<8-h) temperature abuse that might be encountered during the processing of chickens or during meal preparation. A separate study is currently in progress to model the survival and growth of *Salmonella* from a low initial dose on chicken

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skin subjected to longer-term temperature abuse (0 to 13 days) at low temperatures (4 to 12°C).

MATERIALS AND METHODS

Organism. A multiple-antibiotic-resistant strain (ATCC 700408, American Type Culture Collection, Manassas, VA) of *Salmonella* Typhimurium definitive phage type 104 (DT104) was used in all experiments. Stock cultures were maintained at -70°C in brain heart infusion (BHI) broth (Difco, Becton Dickinson, Sparks, MD) containing 15% (vol/vol) glycerol (Sigma, St. Louis, MO).

Preparation of chicken skin portions. Nonkosher chicken thighs were purchased fresh at local retail outlets, whereas kosher chicken thighs were purchased frozen via the Internet. Skin was removed, spread on a plastic cutting board, frozen at -20°C for 15 min, cut into circular 2.14 cm² portions, and placed on top of skinless, deboned thighs in a 500-ml polycarbonate jar with a screw-cap lid (Fisher Scientific, Hampton, NH). Chicken skin portions, prepared in this manner, were stored at 4°C for 1 day before use in storage trials.

Inoculation of chicken skin portions. Five microliters of stock culture (i.e., *Salmonella* Typhimurium DT104) was combined with 5 ml of BHI broth, pH 7.4, in a 25-ml Erlenmeyer flask that was sealed with a foam plug and then incubated at 30°C and 150 orbits per min for 23 h to obtain stationary-phase cells for inoculation. Immediately before inoculation of chicken skin portions, the 23-h culture of Typhimurium DT104 was serially diluted in buffered peptone water (BPW; Difco, Becton Dickinson), and then 5 µl of the 10⁻⁷ dilution (i.e., 3.15 log/ml) was spot inoculated onto the surface of individual skin portions for an initial median dose of 0.85 log.

The typical *Salmonella* cell is about 2 µm in length, and the median number of cells inoculated in this study was 7/2.14 cm². A typical chicken carcass has a surface area of about 2,000 cm², and the typical number of *Salmonella* organisms on a contaminated carcass is about 10 cells or fewer (36). Thus, the model system used in the current study is fairly representative of what occurs in nature if one assumes that each skin portion inoculated in this study represents a *Salmonella*-contaminated chicken carcass in nature.

The skin portions and underlying thigh meat were cold (i.e., 4°C) at the time of inoculation. The time course for the skin portion to reach the abuse temperature was not determined. Rather, a single short-term temperature abuse scenario was used for model development and validation. The robustness of the model for predicting other temperature abuse scenarios was not assessed in this study but will be assessed in future studies. Nonetheless, the tertiary model developed in this study with storage temperature as the independent variable was successfully validated against independent data.

Experimental designs. Nonkosher chicken skin portions were used for model development. The experimental design for model development was a 10 × 5 factorial arrangement of temperature (5, 10, 15, 20, 25, 30, 35, 40, 45, and 50°C) and time (0, 2, 4, 6, and 8 h). Two, four, or five storage trials were conducted per temperature, and a different batch of chicken skin portions was used in each storage trial. Longer storage times were not included at low temperatures because a separate study is in progress to develop and validate a predictive model for the survival and growth of *Salmonella* from a low initial dose on chicken skin with native flora and stored at 4 to 12°C for 0 to 13 days.

To validate the model for interpolation, independent data

were collected at intermediate temperatures. Data were collected using the same methods as used in model development. The experimental design for interpolation was a 9 × 5 factorial arrangement of temperature (7.5, 12.5, 17.5, 22.5, 27.5, 32.5, 37.5, 42.5, and 47.5°C) and time (0, 2, 4, 6, and 8 h). Two storage trials were conducted per temperature, and a different batch of nonkosher chicken skin portions was used in each storage trial.

To validate the model for extrapolation, independent data were collected using the same experimental methods except that kosher chicken skin was used instead of nonkosher skin. The experimental design for extrapolation was a 10 × 5 factorial arrangement of temperature (5, 10, 15, 20, 25, 30, 35, 40, 45, and 50°C) and time (0, 2, 4, 6, and 8 h). Two trials were conducted per temperature, and a different batch of kosher chicken skin portions was used in each storage trial.

Bacterial enumeration. Most-probable-number (MPN) and viable count (CFU) methods were used to enumerate background flora and Typhimurium DT104 on chicken skin portions before or during the storage trials, respectively. Duplicate skin portions were processed for enumeration at each sampling time by combining an individual skin portion (2.14 cm²) with 9 ml of BPW in a filter bag followed by pulsifying (Pulsifier, Microbiology International, Frederick, MD) for 1 min.

A 3 (replicate samples) × 4 (dilutions) MPN assay in BPW was used when the background flora or Typhimurium DT104 was present at 0 to 3.26 log per skin portion (28). After setting up the 3 × 4 MPN assay, 40 ml of BPW was added to the residual pulsifate and skin portion in the filter bag. The MPN assay was incubated for 24 h at 30°C for background flora and at 38°C for Typhimurium DT104, and then 5 µl from each MPN tube and the filter bag was spot plated onto BHI agar (Difco, Becton Dickinson) for background flora or, for Typhimurium DT104, on xylose-lysine (XL) agar base medium (Difco, Becton Dickinson) that contained 25 mM HEPES (H) and 25 µg/ml of the following antibiotics: chloramphenicol (C), ampicillin (A), tetracycline (T), and streptomycin (S) (this is hereafter referred to as XLH-CATS). All media supplements were from Sigma. After 24 h of incubation at 30°C for background flora or 38°C for Typhimurium DT104, results of the MPN assay were read (a white spot for background flora or black spot for Typhimurium DT104 meant a positive tube; no spot meant a negative tube), and MPN results were calculated by the method of Thomas (38).

When background flora or Typhimurium DT104 were present at >3 log per skin portion, 50 µl of undiluted and diluted pulsifate in BPW was spiral plated onto BHI agar for background flora or XLH-CATS for Typhimurium DT104. After 24 h of incubation at 30°C for background flora and 38°C for Typhimurium DT104, colonies that formed were counted using an automated colony counter (Protocol, Microbiology International). Thus, the MPN method was used to enumerate low levels of background flora or Typhimurium DT104 (i.e., 0 to 3.26 log per skin portion), whereas the CFU method was used to enumerate higher levels (i.e., >3 log per skin portion) of background flora or Typhimurium DT104.

A spreadsheet model based on survival and growth kinetics of Typhimurium DT104 on chicken breast meat with native flora (28) was used to generate the sampling schedule, which included a determination of whether the MPN, CFU, or both methods should be applied to the enumeration of a particular sample.

The CFU and MPN per milliliter of pulsifate were determined and used to calculate the total number of cells on the skin portion at time *t*. Results were not expressed on a square centimeter basis because the author did not want to assume a uniform distribution of cells on the skin surface. Rather, it was assumed

that the cells of Typhimurium DT104 were distributed in clusters on the skin surface.

Chicken skin portions placed on the top of thigh meat were used as the model system rather than whole chicken parts because the combination MPN and CFU enumeration method was designed for small food portions rather than large food portions. Use of large food portions would have resulted in a redesign of the enumeration method to include a 3×6 or greater MPN assay to create an enumeration overlap between the MPN and CFU methods, which is needed to produce uninterrupted growth curves. This would have increased the cost and technical difficulty of the data collection step for model development and validation and thus was not done.

Primary modeling. MPN and CFU data within a temperature were combined among storage trials, graphed as a function of time, and fit by least-squares regression (GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, CA) to the modified (i.e., the parameter for y_{\max} was removed) Baranyi model (4):

$$\log N(t) = \log N_0 \pm \text{PI}/2 + \ln[1 - \exp(-\mu \times \lambda)] + \exp[\mu \times (t - \lambda)] \quad (1)$$

where $N(t)$ is pathogen number at time t (hours), N_0 is initial pathogen number, μ is specific growth rate (log per hour), λ is lag time (hours), and PI is the 95% prediction interval. When no growth was observed, lag time was fixed at 8 h and specific growth rate was fixed at 0 log/h during model fitting to obtain PI.

The 95% prediction interval was calculated as follows (21):

$$\text{PI} = 2 \times (S_{y \cdot x} \times K) \quad (2)$$

where $S_{y \cdot x}$ is the standard deviation of the residuals (log) and K is the number of standard deviations from the mean that are needed to calculate PI and where K is dependent on the number of data points analyzed (n) and K is equal to 1.96 when n is infinity.

The growth curves obtained in this study did not contain an upper asymptote because the storage trials were of short duration. Therefore, the Baranyi model was modified by removing the term for maximum population density (i.e., y_{\max}) because it caused problems in curve fitting (i.e., inappropriate model).

Secondary modeling. In the second stage of model development, growth parameters from equation 1 were graphed as a function of temperature (T in degrees Celsius) and were fitted by least-squares regression (GraphPad Prism) to the equations described below. Lag time (λ) data were fitted to a modified (i.e., parameter names were changed) version of the two-phase exponential model found in GraphPad Prism:

$$\lambda = \begin{cases} \lambda_{\max} & \text{if } T \leq T_{\min} \\ \lambda_{\min} + (\lambda_{\max} - \lambda_{\min}) \times \exp[-\lambda_{\text{rate}} \times (T - T_{\min})] & \text{if } T > T_{\min} \end{cases} \quad (3)$$

where λ_{\max} is maximal lag time (hours), T_{\min} is the minimal growth temperature (in degrees Celsius), λ_{\min} is the minimal lag time (in hours), and λ_{rate} is the rate of change of lag time as a function of temperature ($1/^\circ\text{C}$).

Specific growth rate (μ) data were fitted to the cardinal temperature model (34):

$$\mu = \begin{cases} 0 & \text{if } T \leq T_{\min} \text{ or } T \geq T_{\max} \\ \mu_{\text{opt}} \times (A/B) & \text{if } T_{\min} < T < T_{\max} \end{cases}$$

$$A = (T - T_{\max}) \times (T - T_{\min})^2$$

$$B = (T_{\text{opt}} - T_{\min}) \times \{(T_{\text{opt}} - T_{\min}) \times (T - T_{\text{opt}}) - (T_{\text{opt}} - T_{\max}) \times [T_{\text{opt}} + T_{\min} - (2 \times T)]\} \quad (4)$$

where μ is specific growth rate (in log per hour), μ_{opt} is optimal specific growth rate (log per hour), T_{\min} is minimal growth temperature (in degrees Celsius), T_{opt} is optimal growth temperature (in degrees Celsius), and T_{\max} is maximal growth temperature (in degrees Celsius).

Prediction interval (PI) data were fit to a modified (i.e., parameter names were changed) version of the two-phase linear model (10):

$$\text{PI} = \begin{cases} \text{PI}_{\min} & \text{if } T \leq T_1 \\ \text{PI}_{\min} + \text{PI}_{\text{rate}}(T - T_1) & \text{if } T > T_1 \end{cases} \quad (5)$$

where PI_{\min} (log) is the 95% prediction interval below the temperature (T_1) at which PI increases as a result of pathogen growth and PI_{rate} is the linear rate (log/degrees Celsius) of increase of PI as a function of temperature.

Tertiary modeling. In the third stage of model development, secondary models for λ (equation 3), μ (equation 4), and PI (equation 5) were combined with the modified Baranyi primary model (equation 1) in a computer spreadsheet (Microsoft Office Excel 2003, Professional Edition, Microsoft Corporation, Redmond, WA) to create a tertiary model for predicting survival and growth of Typhimurium DT104 from a low initial dose (0.85 log) on nonkosher chicken skin as a function of time and temperature:

$$\log N(t) = \log N_0 \pm \text{PI}^{\text{(eq. 5)}/2} + \ln\{1 - \exp[-\mu^{\text{(eq. 4)}} \times \lambda^{\text{(eq. 3)}}]\} + \exp\{\mu^{\text{(eq. 4)}} \times [t - \lambda^{\text{(eq. 3)}}]\} \quad (6)$$

Outputs of the tertiary model were the predicted growth curve and its 95% PI as well as predicted values for μ , and PI. As illustrated in Figure 1, the tertiary model is capable of predicting the behavior of Typhimurium DT104 from a low initial dose (0.85 log) on chicken skin for times and temperatures that were not investigated (e.g., 38°C).

To validate performance of the tertiary model (equation 6), prediction errors (PE; in log) or residuals for individual prediction cases were calculated:

$$\text{PE} = O - P \quad (7)$$

where O is the observed value (in log), P is the predicted value (in log), PE values of <0 log are fail-safe predictions, and PE values of >0 log are fail-dangerous predictions. Median PE was used as a measure of prediction bias, whereas median absolute PE was used as a measure of prediction accuracy.

To determine whether individual prediction errors were acceptable, an acceptable prediction zone (APZ) was used (26, 27): $-1.0 \text{ log} < \text{acceptable PE} < 0.5 \text{ log}$, where the APZ was twice as wide in the fail-safe direction because greater error can be tolerated in the fail-safe direction when a model is used to predict food safety (33). The percentage of PE (%PE) in the APZ was calculated and used as an overall measure of model performance:

$$\% \text{PE} = \left(\frac{\text{PE}_{\text{in}}}{\text{PE}_{\text{total}}} \right) \times 100$$

Input	Unit	Range	Value
Number	log	Fixed	0.85
Temperature	°C	5 to 50	38
Output	Unit	Value	
λ	h	2.73	
μ	log/h	1.07	
PI	log	1.78	

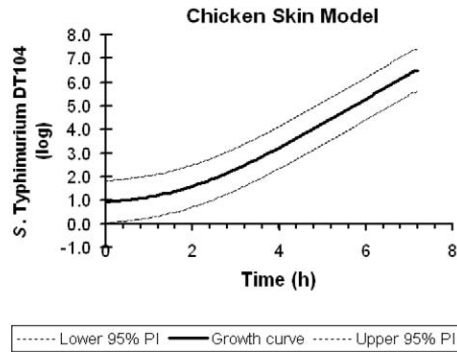


FIGURE 1. Tertiary model for survival and growth of *Salmonella Typhimurium DT104* from a low initial dose (0.85 log) on chicken skin as a function of time (0 to 8 h) and temperature (5 to 50°C). Abbreviations: λ , lag time; μ , specific growth rate; PI, 95% prediction interval.

where PE_{in} is the number of PE in the APZ and PE_{total} is the total number of PE in the evaluation. A %PE of $\geq 70\%$ indicated a tertiary model that provided acceptable or valid predictions for the test data set (26).

Performance of the model was not evaluated against existing models and data in the scientific literature because the comparisons would have been confounded by more than one difference in the independent variables (e.g., strain, food matrix, previous history, and initial dose), experimental methods, and modeling methods. Rather, the robustness of the tertiary model was evaluated in the present study and will be further evaluated in future studies by comparison with MPN and CFU data collected using the same experimental and modeling methods and only one different independent variable at a time.

The ability of an existing model (28) for the growth of *Typhimurium DT104* from a low initial dose (0.6 log) on ground chicken breast meat with native flora to predict the MPN and CFU data collected in this study was evaluated using the acceptable prediction zone method.

RESULTS AND DISCUSSION

Survival of *Typhimurium DT104* was observed on nonkosher chicken skin stored at 5 to 20°C or at 50°C for 8 h (Table 1). Growth at 5°C was not expected because the minimum growth temperature for *Salmonella* is usually above 5°C (17) and growth at 50°C was not expected because the maximum growth temperature for *Salmonella* is typically below 50°C (11). Growth of *Salmonella* at 10, 15, and 20°C was also not expected because lag time at these

temperatures is normally longer than 8 h (13), which was the total time of the storage trials in this study. In contrast, growth of *Typhimurium DT104* was observed within 8 h on chicken skin stored at 25 to 45°C (Table 1). Optimal growth was observed at 40°C with a lag time of 2.5 h and a specific growth rate of 1.1 log per h. Figure 2 illustrates primary modeling results obtained at 10, 20, 30 and 40°C.

Growth of *Typhimurium DT104* on chicken skin exhibited significant variation among storage trials with 95% PI that ranged from 1.55 log at 25°C to 2.03 log at 45°C (Table 1). This variation of *Typhimurium DT104* growth among storage trials is similar to growth variation observed in previous studies with this strain and ground chicken breast meat (28, 29) and chicken frankfurters (30). Inoculation of chicken skin with a low number (i.e., 0.85 log or 7 CFU) of *Typhimurium DT104* could have contributed to this growth variation as McKellar and Lu (19) reported that variation of lag time increases as the initial number of microbial cells decreases from 500 to 5 CFU. This occurs because differences in lag times among individual cells in the population are better expressed when a small number rather than a large number of cells are present initially (19).

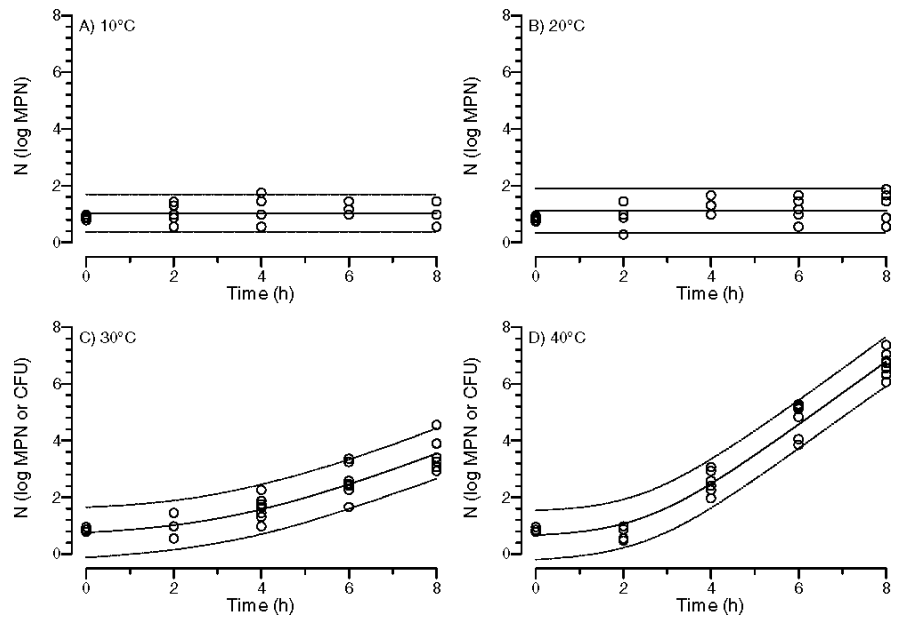
Differences in number and types of background flora (5, 23, 31, 35) among batches of chicken skin may have also contributed to the observed variation of *Typhimurium DT104* growth among storage trials. A simple and valid method for quantifying the variation of pathogen growth

TABLE 1. Survival and growth of *Salmonella Typhimurium DT104* on chicken skin: primary modeling results^a

Temp (°C)	Lag time (h)		Specific growth rate (log/h)		95% PI (log)			
	BFV	SE	BFV	SE	PI	S_{y-x}	<i>n</i>	<i>K</i>
5	8.00	0.00	0.000	0.000	1.41	0.327	19	2.16
10	8.00	0.00	0.000	0.000	1.32	0.321	38	2.06
15	8.00	0.00	0.000	0.000	1.34	0.325	40	2.05
20	8.00	0.00	0.000	0.000	1.58	0.384	40	2.05
25	5.34	0.79	0.469	0.115	1.55	0.382	50	2.03
30	3.46	0.65	0.600	0.081	1.71	0.418	40	2.05
35	3.13	0.32	0.928	0.062	1.61	0.393	40	2.05
40	2.53	0.26	1.118	0.054	1.69	0.409	39	2.06
45	2.55	0.41	0.904	0.070	2.03	0.492	39	2.06
50	8.00	0.00	0.000	0.000	2.00	0.490	40	2.05

^a BFV, best-fit value; SE, standard error; PI, 95% prediction interval; S_{y-x} , standard deviation of the residuals; *n*, number of data points; *K*, number of deviations from the mean to calculate PI.

FIGURE 2. Primary model fits for survival and growth of a low initial dose (0.85 log) of *Salmonella Typhimurium* DT104 on chicken skin stored at (A) 10°C, (B) 20°C, (C) 30°C, or (D) 40°C.



among storage trials is to use a 95% PI to quantify the uncertainty of the curve fit as well as the scatter of MPN and CFU data around the best-fit curve due to unaccounted for independent variables, such as background flora and pH (12, 28, 30).

After primary modeling, secondary models for primary model parameters as a function of storage temperature were developed. To model lag time as a function of storage temperature (Fig. 3A), a lag time of 8 h was assigned to those temperatures where no growth was observed. Lag time data were fit to a two-phase exponential model, which resulted in a curve fit with high goodness of fit (Table 2) when the lag time value at 50°C was excluded. Exclusion of the lag time value at 50°C did not have a negative impact on tertiary model performance because although the tertiary model predicted a lag time of 2.5 h at 50°C, it predicted a specific growth rate of 0 log/h at 50°C, and thus, the predicted growth curve was for no growth as was observed.

Specific growth rate was modeled as a function of storage temperature by using a cardinal temperature model (Fig. 3B), which displayed high goodness of fit to the dependent data (Table 2). In a previous study (24), the cardinal temperature model was found to provide a slightly better goodness of fit to dependent data for specific growth rate of *Salmonella Typhimurium* on autoclaved and sterile chicken breast and thigh meat than two versions of the Ratkowsky square root model. The cardinal temperature values for growth of *Typhimurium* DT104 on chicken skin (Table 2) are in agreement with other studies (24, 25) for growth of *Salmonella Typhimurium* with the exception of T_{\min} , which was higher in the present study because of the short duration (i.e., only 8 h) of the storage trials.

Similar to previous studies for survival and growth of *Typhimurium* DT104 on chicken breast meat (28) and chicken frankfurters (30), the 95% PI was observed to increase in a nonlinear manner as a function of storage temperature (Fig. 3C) and was wider for growth than for no-growth conditions. This occurs because under no-growth

conditions the 95% PI is due solely to experimental error, whereas under growth conditions the 95% PI is due to experimental error plus variation of microbial growth (30). Parameters obtained for the two-phase linear model for PI are summarized in Table 2.

Performance of secondary models is usually evaluated by calculating prediction bias and accuracy factors that are either mean or median relative errors (8) or mean log ratios of observed and predicted values (32). However, prediction cases involving no growth cannot be included in calculations of such performance factors because it is not possible to divide by infinity for lag time or to divide by zero for specific growth rate or to take the log of infinity for lag time or the log of zero for specific growth rate. Five of 10 storage temperatures used in model development in the present study resulted in no growth (Table 1), and thus, it was not possible to accurately assess prediction bias and accuracy of secondary models by using established methods. However, it was possible to indirectly evaluate performance of the secondary models by evaluating performance of the tertiary model by using a published acceptable prediction zone method based on absolute differences between observed and predicted values for $N(t)$ (26, 27).

In stage one of tertiary model validation, a plot of prediction errors for dependent data was generated and found to have a random distribution of prediction errors around zero, indicating a lack of systematic prediction bias (Fig. 4A). In addition, prediction bias and accuracy factors for dependent data indicated low median prediction bias (i.e., a value close to zero) and high median prediction accuracy (i.e., a value close to zero) (Table 3). Median values rather than mean values were used in calculation of performance factors to avoid potential bias due to large prediction errors that could inflate the mean. Overall, 82.6% of prediction errors were in the acceptable prediction zone (%PE) from -1 to 0.5 log, where a %PE value of $\geq 70\%$ is considered acceptable (26, 27), and thus, it was concluded that the tertiary model provided valid predictions of dependent data.

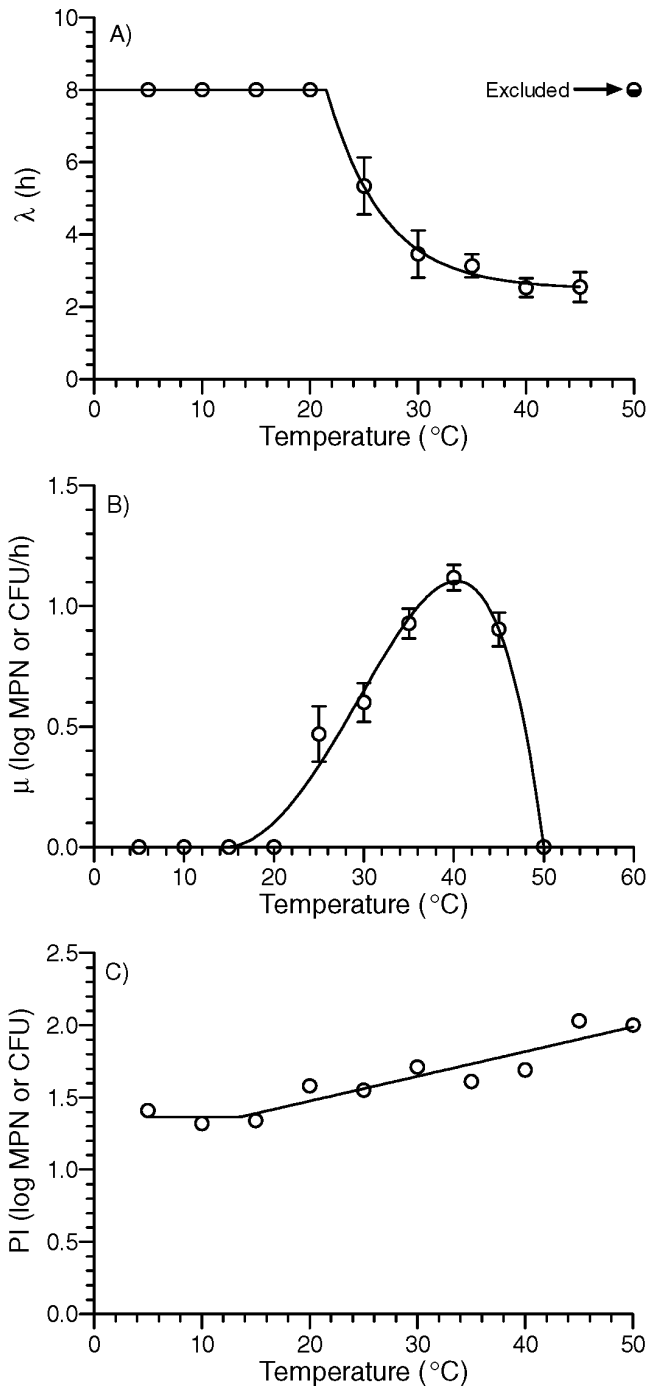


FIGURE 3. Secondary models for (A) lag time (λ), (B) specific growth rate (μ), and (C) 95% prediction interval (PI) of *Salmonella Typhimurium* DT104 on chicken skin as a function of temperature.

Once a model is found to provide valid predictions for dependent data, it can be evaluated for its ability to interpolate within its response surface (27). However, for an unbiased evaluation of model performance, independent data for interpolation should be collected using the same methods and the test data should provide uniform coverage of the response surface. In the present study, independent data for interpolation met the aforementioned criteria for test data and a prediction error plot of these data indicated a lack of systematic prediction bias (Fig. 4B). Prediction

TABLE 2. Survival and growth of *Salmonella Typhimurium* DT104 on chicken skin: secondary modeling results^a

Model	Parameter	Units	BFV	SE	R ²
λ	T_{\min}	°C	21.5	0.59	0.999
	λ_{\max}	h	8.00	0.06	
	λ_{\min}	h	2.49	0.13	
	λ_{rate}	1/°C	0.192	0.031	
μ	T_{\min}	°C	14.5	2.3	0.984
	T_{opt}	°C	40.3	0.75	
	T_{\max}	°C	50.0	0.25	
	μ_{opt}	log/h	1.10	0.05	
	PI	PI _{min}	log	1.37	
T_1	°C	13.6	5.7		
	PI _{rate}	log/°C	0.017	0.003	

^a BFV, best-fit value; SE, standard error; R², coefficient of determination; λ , lag time; μ , specific growth rate; PI, 95% prediction interval; T, temperature; min, minimum; max, maximum; opt, optimum.

bias and accuracy factors for interpolation data indicated low median prediction bias and high median prediction accuracy (Table 3). Overall, 83.7% of prediction errors were in the acceptable prediction zone, indicating that the tertiary model was successfully validated for interpolation.

Once a model is found to provide valid predictions for interpolation, it can be evaluated for its ability to extrapolate (27). However, for an unbiased evaluation of model performance, independent data for extrapolation should be collected using the same methods except for the factor under test and the data should provide uniform coverage of the response surface. In the present study, independent data for extrapolation to kosher chicken skin met the aforementioned criteria, and although a prediction error plot suggested some possible systematic prediction bias (Fig. 4C), prediction bias and accuracy factors indicated low median prediction bias and high median prediction accuracy (Table 3). Overall, 81.6% of prediction errors were in the acceptable prediction zone, indicating that the tertiary model was successfully validated for extrapolation to kosher chicken skin.

The storage conditions associated with large prediction errors (i.e., >1 log) are summarized in Table 4. In general, large prediction errors were evenly distributed among data sets. However, 75% of large prediction errors occurred at temperatures at which growth was observed. For dependent data, large prediction errors were mainly observed at 50°C and were positive, indicating growth might have occurred at this temperature in some storage trials even though the tertiary model predicted no growth. For interpolation data, most large prediction errors were negative, indicating that the tertiary model predicted more growth than was observed. All large prediction errors for interpolation occurred at 6 or 8 h of storage, and most were from two storage trials and two temperatures. These data indicate that variation of *Typhimurium* DT104 growth on chicken skin increases as a function of time of temperature abuse. This could be due to increased interactions of *Typhimurium* DT104 with the background flora (28–30).

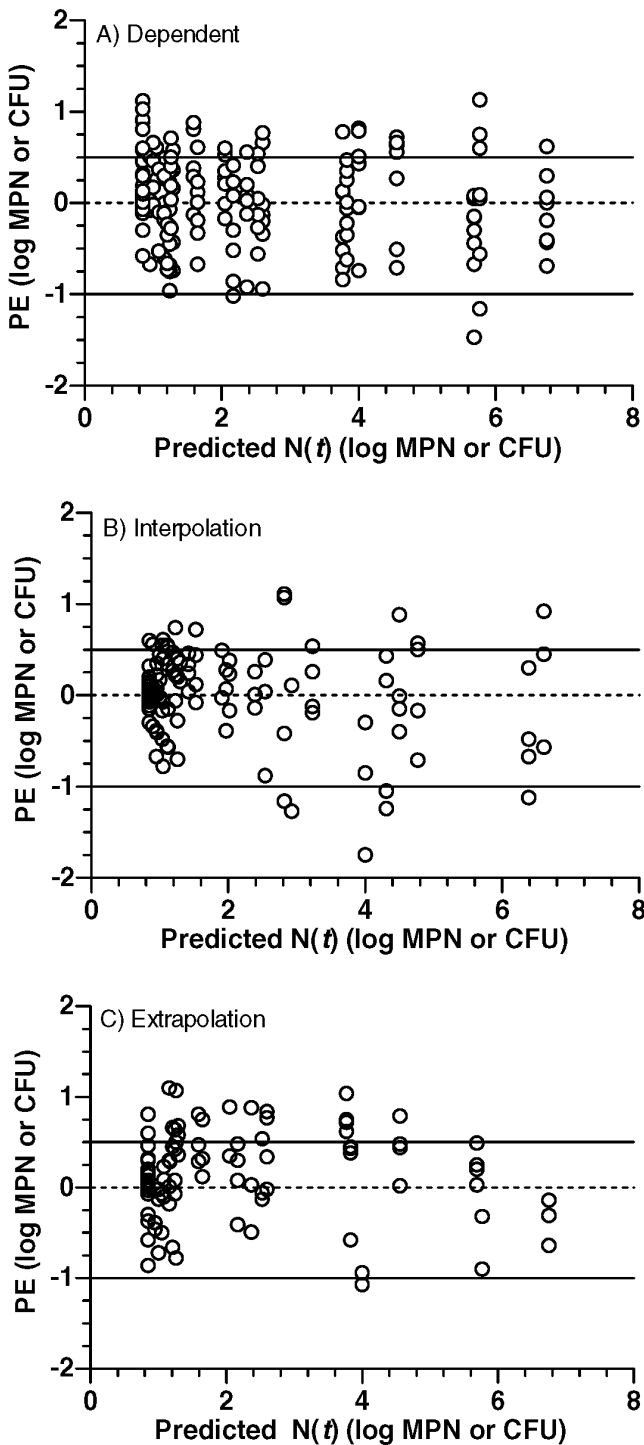


FIGURE 4. Prediction error (PE) plots for tertiary model predictions of (A) dependent data, (B) independent data for interpolation, and (C) independent data for extrapolation to kosher chicken skin. Solid lines are the upper and lower boundaries of the acceptable prediction zone.

For extrapolation data, most large prediction errors occurred at 45°C and were from a single storage trial at 45°C, where little growth was observed. In addition, there were five kosher chicken skin samples (results not shown) for which after 4, 6, or 8 h of storage at 50°C no Typhimurium DT104 organisms were detected, which indicated total death of the pathogen on these samples. Negative samples

were not observed under any other storage conditions. Thus, there was some evidence that survival and growth of Typhimurium DT104 on kosher chicken skin was less at storage temperatures near the upper growth boundary. Lower water activity associated with kosher salt retention by chicken skin during kosher processing (1, 2) could explain the latter observation, as salt (i.e., lower water activity) has been shown to cause a downward shift of the growth/no growth boundary for *Salmonella* (16).

The initial distribution of Typhimurium DT104 cells among chicken skin portions was estimated by spot plating 5 μ l of the 23-h culture used for inoculation onto XLH-CATS. Results were similar across all experiments and thus were combined to obtain the distribution shown in Figure 5. These results indicated that the initial number of Typhimurium DT104 organisms inoculated onto chicken skin portions had a median value of 7 CFU or 0.85 log with a range from 0 to 14 (1.15 log) CFU. When these data were evaluated as discrete data by using @Risk (version 5.0, Professional Edition, Palisade Corporation, Newfield, NY), the top three best-fitting distributions per the chi-square test were the binomial ($\chi^2 = 14.11$), HyperGeo ($\chi^2 = 14.45$), and Poisson ($\chi^2 = 22.1$) distributions. These results indicate that the distribution of Typhimurium DT104 in the inocula was not uniform and thus was a significant source of variation of $N(t)$ among chicken skin portions in this study, especially under no-growth conditions.

Aerobic plate count (APC) of skin portions was measured the day before initiation of the storage trials. These results indicated that the level of background flora was initially higher on nonkosher skin portions used to develop the tertiary model and validate it for interpolation than on the kosher skin portions used to validate the tertiary model for extrapolation (Fig. 6). Kosher skin portions were obtained from chicken thighs that were frozen immediately after processing, whereas nonkosher skin portions were obtained from chicken thighs that were refrigerated but not frozen after processing. This difference in handling of the kosher and nonkosher chicken thighs after processing likely explains the difference in APC.

On average, the skin portions had initial levels of background flora that were well below the value of 7 log per cm^2 that is often associated with spoilage. In fact, there were no signs of spoilage of the chicken skin portions during the short-term storage trials conducted in the present study.

Although the initial APC of kosher skin portions used in this study were about 2 log lower than the initial APC of nonkosher skin portions, the survival and growth levels of Typhimurium DT104 were similar on the two types of chicken skin. These results suggest that APC of the skin portion was not correlated with survival and growth of Typhimurium DT104 on chicken skin, and consequently, it was not included as an independent variable in the present model. It is more likely that the number and types of background flora in close proximity to the inoculated cells of Typhimurium DT104 are highly correlated with growth of the pathogen. However, it is technically not possible to

TABLE 3. Survival and growth of *Salmonella Typhimurium DT104* on chicken skin: tertiary model performance results

Type of chicken skin	Data set	<i>n</i>	5th percentile ^a	25th percentile ^a	Median ^a	75th percentile ^a	95th percentile ^a	APZ ^b
Bias								
Nonkosher	Dependent	384	-0.70	-0.15	0.03	0.29	0.80	82.6
Nonkosher	Interpolation	178	-0.90	-0.11	0.04	0.31	0.59	83.7
Kosher	Extrapolation	196	-0.87	-0.18	0.06	0.32	0.80	81.6
Accuracy								
Nonkosher	Dependent	384	0.01	0.08	0.20	0.52	0.84	NA ^c
Nonkosher	Interpolation	178	0.01	0.08	0.23	0.47	1.06	NA
Kosher	Extrapolation	196	0.01	0.08	0.31	0.59	0.92	NA

^a Values are prediction errors (bias) and absolute prediction errors (accuracy) [observed $N(t)$ - predicted $N(t)$], in log.

^b Values are the percentages of prediction errors that were within an acceptable prediction zone (APZ) from -1 log (fail-safe) to 0.5 log (fail-dangerous).

^c NA, not applicable.

measure and characterize the background flora in the niche occupied by the inoculated cells of *Typhimurium DT104*.

Predictions of a model that was developed and validated using the same strain and similar initial dose (i.e., 0.6 log) of *Typhimurium DT104* and with the same history but using ground chicken breast meat with native flora (28) were compared to the MPN and CFU data obtained in the present study with nonkosher and kosher chicken skin. The results of this evaluation (Table 5 and Fig. 7) indicated that survival and growth of *Typhimurium DT104* from a low initial dose (0.6 log) on ground chicken breast meat and

survival and growth of *Typhimurium DT104* from a low initial dose (0.85 log) on chicken skin were similar at temperatures from 10 to 40°C. There was a small but acceptable prediction bias in the fail-dangerous direction that was representative of the 0.25-log-lower initial dose used to develop the predictive model for ground chicken breast meat. The only indication of systematic prediction bias occurred in the data set for extrapolation to kosher chicken skin, where the predictive model for ground chicken breast meat underpredicted $N(t)$ at values of >4 log per skin portion (Fig. 7C).

TABLE 4. Survival and growth of *Salmonella Typhimurium DT104* on chicken skin: storage conditions resulting in large prediction errors (PE)

Case no.	Trial code	Type of chicken skin	Data set	Temp (°C)	Time (h)	PE (log)
1	D10c	Nonkosher	Dependent	15	2	1.02
2	D17d	Nonkosher	Dependent	35	4	-1.02
3	D17d	Nonkosher	Dependent	35	8	-1.48
4	D8b	Nonkosher	Dependent	45	8	1.12
5	D4a	Nonkosher	Dependent	50	4	1.11
6	D4a	Nonkosher	Dependent	50	6	1.02
7	D4a	Nonkosher	Dependent	50	8	1.02
8	D7b	Nonkosher	Dependent	50	4	1.02
9	D14c	Nonkosher	Dependent	50	8	1.02
10	I4a	Nonkosher	Interpolation	27.5	8	-1.17
11	I20b	Nonkosher	Interpolation	27.5	8	1.10
12	I20b	Nonkosher	Interpolation	27.5	8	1.06
13	I2a	Nonkosher	Interpolation	37.5	6	-1.06
14	I2a	Nonkosher	Interpolation	37.5	6	-1.25
15	I2a	Nonkosher	Interpolation	37.5	8	-1.13
16	I3a	Nonkosher	Interpolation	47.5	6	-1.28
17	I3a	Nonkosher	Interpolation	47.5	6	-1.28
18	I3a	Nonkosher	Interpolation	47.5	8	-1.76
19	I3a	Nonkosher	Interpolation	47.5	8	-2.84
20	E8b	Kosher	Extrapolation	30	8	-1.04
21	E4a	Kosher	Extrapolation	35	2	1.09
22	E2a	Kosher	Extrapolation	45	6	-2.14
23	E2a	Kosher	Extrapolation	45	6	-2.36
24	E2a	Kosher	Extrapolation	45	8	-3.53
25	E2a	Kosher	Extrapolation	45	8	-4.03
26	E6b	Kosher	Extrapolation	45	2	1.06
27	E6b	Kosher	Extrapolation	45	6	-1.08

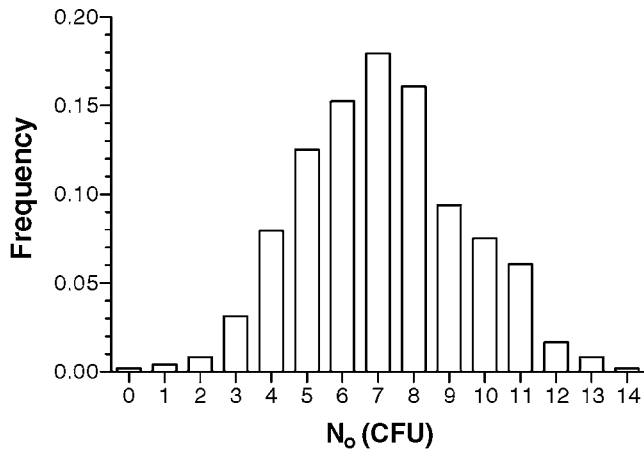


FIGURE 5. Distribution of the initial number (N_0) of *Salmonella Typhimurium* DT104 inoculated onto chicken skin portions.

A predictive model (Fig. 1) for survival and growth of *Typhimurium* DT104 from a low initial number (0.85 log per portion) on chicken skin with native flora was developed. The model was found to provide valid predictions within its response surface, and the model was found to provide valid predictions when extrapolated to kosher chicken skin. Predictive models such as the one developed and validated in this study are valuable tools for food safety because of their ability to interpolate or predict responses of pathogens to conditions that were not investigated, for example, growth of *Typhimurium* DT104 on chicken skin stored at 38°C (Fig. 1).

Although the model that was developed and validated in this study provides predictions of *Typhimurium* DT104 survival and growth during 8 h of temperature abuse that are similar to those of a previously published model for survival and growth of *Typhimurium* DT104 from a low initial dose on ground chicken breast meat with native flora (28), the current model is a valuable addition because it provides predictions over a wider range of temperature (i.e., 5 to 50°C) than the previous model (i.e., only 10 to 40°C).

The main potential limitation of the current model is

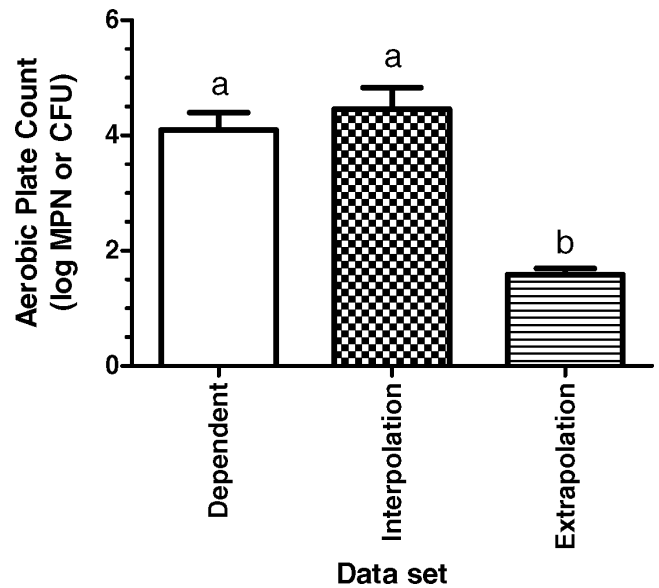


FIGURE 6. Aerobic plate counts for skin portions used in model development (i.e., dependent data) and in model validation for interpolation and for extrapolation to kosher chicken skin. Values are means \pm SEM. Bars with different superscripts differ at P values of <0.05 per one-way analysis of variance followed by means comparison using Tukey's multiple comparison test in GraphPad Prism (version 5.0, GraphPad Software, Inc., San Diego, CA).

its robustness. However, future studies will further evaluate the ability of the model to extrapolate to other strains of *Salmonella* (e.g., Kentucky, Hadar, *Typhimurium*), other initial doses (e.g., 1.85, 2.85, and 3.85 log) of the pathogen, other poultry products (e.g., turkey skin and meat, chicken nuggets, deli meats made from turkey or chicken, and poultry sausages, such as frankfurters), and other histories (e.g., freezing, cooking, refrigeration, pH, and water activity) of the pathogen and chicken products. These studies will further characterize the robustness of the model and identify conditions for which new model development is indicated

TABLE 5. Performance evaluation for a tertiary model for survival and growth of *Salmonella Typhimurium* DT104 from a low initial dose (0.6 log) on ground chicken breast meat with native flora (28): comparison with MPN and CFU data collected with chicken skin stored at 10 to 40°C in the present study

Type of chicken skin	Data set	n	5th percentile ^a	25th percentile ^a	Median ^a	75th percentile ^a	95th percentile ^a	APZ ^b
Bias								
Nonkosher	Dependent	286	-0.70	-0.09	0.24	0.38	0.80	79.4
Nonkosher	Interpolation	120	-0.57	-0.03	0.22	0.41	0.77	80.0
Kosher	Extrapolation	138	-0.32	0.11	0.29	0.56	0.95	71.7
Accuracy								
Nonkosher	Dependent	286	0.07	0.19	0.28	0.57	0.98	NA ^c
Nonkosher	Interpolation	120	0.04	0.17	0.30	0.52	0.80	NA
Kosher	Extrapolation	138	0.04	0.18	0.31	0.60	0.95	NA

^a Values are prediction errors (bias) and absolute prediction errors (accuracy) [observed $N(t)$ - predicted $N(t)$], in log.

^b Values are the percentages of prediction errors that are within an acceptable prediction zone (APZ) from -1 log (fail-safe) to 0.5 log (fail-dangerous).

^c NA, not applicable.

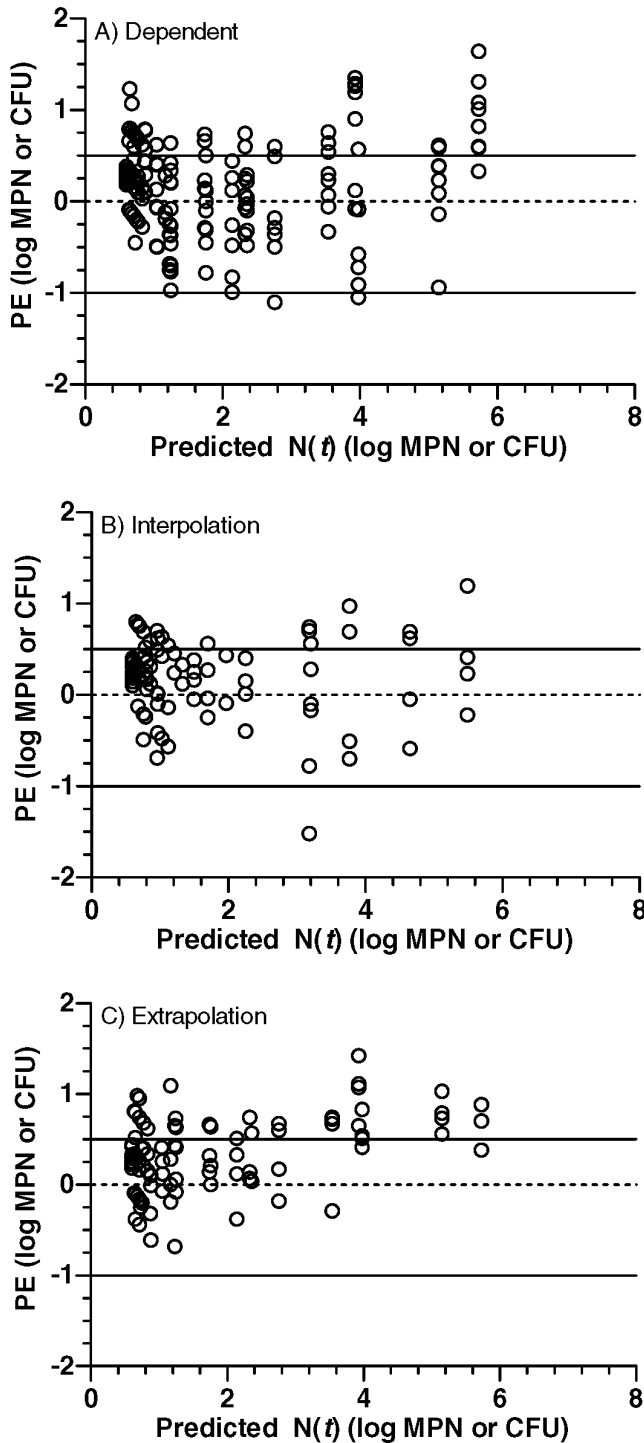


FIGURE 7. Prediction error (PE) plots for tertiary model predictions of (A) dependent data, (B) independent data for interpolation, and (C) independent data for extrapolation to kosher chicken skin. Solid lines are the upper and lower boundaries of the acceptable prediction zone. The model used in this evaluation was for survival and growth of *Salmonella Typhimurium* DT104 from a low initial dose (0.6 log) on ground chicken breast meat with native flora as a function of time and temperature from 10 to 40°C (28).

and conditions for which new model development is not needed.

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