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Development and Validation of a Stochastic Model for Predicting the Growth of *Salmonella* Typhimurium DT104 from a Low Initial Density on Chicken Frankfurters with Native Microflora†

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ABSTRACT

The presence of native microflora is associated with increased variation of *Salmonella* growth among batches and portions of chicken meat and as a function of temperature. However, variation of *Salmonella* growth can be modeled using a 95% prediction interval (PI). Because there are no reports of predictive models for growth of *Salmonella* on ready-to-eat poultry meat products with native microflora and because *Salmonella* is usually present at low levels on poultry meat, the current study was conducted to develop and validate a stochastic model for predicting the growth of *Salmonella* from a low initial density on chicken frankfurters with native microflora. One-gram portions of chicken frankfurters were inoculated with 0.5 log CFU of a single strain (ATCC 700408) of *Salmonella* Typhimurium DT104. Changes in pathogen numbers over time, $N(t)$, were fit to a two-phase linear primary model to determine lag time (λ), growth rate (μ), and the 95% PI, which characterized the variation of pathogen growth. Secondary quadratic polynomial models for natural log transformations of λ, μ, and PI as a function of temperature (10 to 40°C) were obtained by nonlinear regression. The primary and secondary models were combined in a computer spreadsheet to create a tertiary model that predicted the growth curve and PI. The pathogen did not grow on chicken frankfurters incubated at 10 to 12°C, but μ ranged from 0.003 log CFU/g/h at 14°C to 0.176 log CFU/g/h at 30°C to 0.1 log CFU/g/h at 40°C. Variation of $N(t)$ increased as a function of time (i.e., PI was lower during lag phase than during growth phase) and temperature (i.e., PI was higher at 18 to 40°C than at 10 to 14°C). For dependent data (n = 338), 90.5% of observed $N(t)$ values were in the PI predicted by the tertiary model, whereas for independent data (n = 86), 89.5% of observed $N(t)$ values were in the PI predicted by the tertiary model. Based on this performance evaluation, the tertiary model was considered acceptable and valid for stochastic predictions of *Salmonella* Typhimurium DT104 growth from a low initial density on chicken frankfurters with native microflora.

Salmonella is frequently isolated from raw poultry and red meat but is infrequently isolated from ready-to-eat products such as frankfurters, which are heat processed. Palumbo et al. *(11)* reported that the normal thermal process used to manufacture frankfurters completely inactivates gram-negative bacteria such as *Salmonella,* but more heat-resistant gram-positive bacteria can survive and eventually cause spoilage. Heat processing reduces the total microflora of frankfurters on average from 10^5 to 10^2 CFU/g *(11),* indicating that although frankfurters are thermally processed and ready to eat, they are not sterile products.

Although *Salmonella* are not expected to survive thermal processing, they can still be found on the finished product, usually as a result of cross-contamination during peeling and packaging. In September 2000 during routine microbiological testing, the U.S. Department of Agriculture Food Safety and Inspection Service detected *Salmonella* in frankfurter samples obtained at a commercial plant, prompting a product recall *(1).*

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† Mention of trade names or commercial products in this publication is solely for providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Although Palumbo et al. *(11)* did not observe growth of *Salmonella* Senftenberg 775W or *Salmonella* Dublin on frankfurters held at 5 or 37°C, growth of *Salmonella* on frankfurters held at other temperatures has been reported. Bayne and Michener *(3)* found *Salmonella* Enteritidis growing on frankfurters at 20°C but not at 7°C. Rice and Pierson *(12)* observed growth of *Salmonella* Infantis and *Salmonella* Enteritidis on frankfurters stored at 15 and 27°C, and Whichard et al. *(15)* reported growth of *Salmonella* Typhimurium DT104 on chicken frankfurters held at 22°C for 24 h. These studies indicate that *Salmonella* is capable of growing at temperatures encountered during storage and handling of the product after manufacture. However, the data available on growth of *Salmonella* on frankfurters are not sufficient to allow development and validation of a model for predicting food safety.

A technical hurdle for modeling pathogen growth in food with native microflora has been the lack of a naturally occurring strain with a phenotype that can be followed and enumerated in the presence of other microorganisms. Recent studies have revealed that a multiple-antibiotic–resistant strain of *Salmonella* Typhimurium DT104 (ATCC 700408) that occurs in nature could be used to investigate and model growth on ground chicken breast meat with na-

tive microflora; this strain has a phenotype that can be followed and enumerated in the presence of other microorganisms *(9).*

The level of *Salmonella* on chicken frankfurters, although not reported, is likely to be low (<2 log CFU/g), as suggested by the results of Palumbo et al. *(11).* Previous studies with sterile *(7)* and nonsterile *(10)* chicken indicate that growth of *Salmonella* Typhimurium from low initial densities (<1 log CFU/g) is often faster than growth from higher initial densities (>3 log CFU/g). These results suggest that it might be best to develop a model for chicken frankfurters using a low initial density of *Salmonella.* Recently, a most-probable-number (MPN) drop-plate method was used to successfully model growth of *Salmonella* Typhimurium DT104 from a low initial density (0.6 log CFU/g) on ground chicken breast meat contaminated with native microflora *(9).*

Another important factor to consider when developing a model for predicting growth of *Salmonella* on food with native microflora is the heterogeneity of the food matrix. In a previous study *(9),* growth of *Salmonella* Typhimurium DT104 differed among batches and portions of ground chicken breast meat and as a function of temperature. This growth variation was attributed to variation of the number and types of native microflora among batches and portions of chicken meat, the nonuniform distribution of the native microflora in the meat, and changes in the types and numbers of native microflora as storage temperature increased *(9, 10).* In a more processed product, such as frankfurter, that contains additional ingredients such as antimicrobial chemicals, the heterogeneity of the food matrix in terms of both abiotic (i.e., chemical) and biotic (i.e., microbiological) factors at the microniche or microscopic level is likely greater than that in fresh meat. To model the variation of *Salmonella* growth in a heterogeneous food matrix, a 95% prediction interval (PI) that captures experimental error, the uncertainty of the curve fit, and the scatter of the growth data around the curve has been used successfully *(9).*

The objective of the present study was to develop and validate a stochastic model for predicting the growth of *Salmonella* Typhimurium DT104 from a low initial density on chicken frankfurters with native microflora. The ability of the model to predict growth of *Salmonella* on other frankfurter formulations, to predict growth of other strains of *Salmonella* on frankfurters, and to predict growth from other initial densities of *Salmonella* were not addressed in this study but will be addressed in future studies.

MATERIALS AND METHODS

Organism. A multidrug-resistant strain of *Salmonella* Typhimurium definitive phage type 104 (DT104; ATCC 700408, American Type Culture Collection, Manassas, Va.) was used for model development and validation because this strain occurs in nature, has a phenotype that can be followed, can be enumerated in the presence of other microorganisms, and has growth patterns similar to those of other strains of *Salmonella (9).* The stock culture was maintained at −70°C in brain heart infusion (BHI) broth (Becton Dickinson, Sparks, Md.) that contained 15% (vol/vol) glycerol (Sigma, St. Louis, Mo.).

Preparation of chicken frankfurter portions. A single brand of chicken frankfurters (Gwaltney, Smithfield, Va.) was purchased weekly from local retail outlets. Listed ingredients were mechanically separated chicken, water, corn syrup, modified food starch, salt, potassium lactate, sodium phosphate, sodium diacetate, flavorings, sodium erythorbate, and sodium nitrite. Composition of frankfurters was reported on the label as 9 g of total fat, 2.5 g of saturated fat, 50 mg of cholesterol, 760 mg of sodium, 5 g of total carbohydrates, 0 g of dietary fiber, 1 g of sugars, and 5 g of protein per frankfurter or per 56-g serving. The listed formulation and listed composition did not change during this study.

On a weekly basis, frankfurters from a newly purchased package were sliced and trimmed to yield circular 1-g portions that were transferred to individual wells of a 12-well tissue culture dish (Falcon Multiwell 12-well polystyrene, Becton Dickinson) for subsequent inoculation. In this study, the term ''batch'' refers to the weekly preparation of frankfurter portions from a single package.

Challenge trials. On the day before a challenge trial, 2 μl of the thawed stock culture was added to 5 ml of BHI broth in a 25-ml Erlenmeyer flask. The flask was then sealed with a foam plug and incubated at 30°C and 150 rpm for 23 h to obtain stationary phase cells for inoculation. Just before inoculation of the chicken frankfurter portions, the 23-h culture (10.2 log CFU/ml) was serially diluted in buffered peptone water (BPW; Becton Dickinson), and 2 μl of the 10^{-7} dilution was spot inoculated onto the surface of each chicken frankfurter portion for an average initial density of 0.5 log CFU/g.

Inoculated chicken frankfurters were incubated at 10, 11, 12, 14, 18, 22, 26, 30, 34, or 40°C. Two (10, 11, and 12°C) or four (14, 18, 22, 26, 30, 34, and 40°C) trials were conducted per temperature, with a different batch of frankfurters and a different inoculation culture in each trial.

Pathogen enumeration. At selected incubation times, a 1-g frankfurter portion was homogenized (model 80 Stomacher blender, Seward, London, UK) for 1 min in 9 ml of BPW. For samples with a low density (0 to 3.28 log CFU/g) of *Salmonella* Typhimurium DT104, the sample homogenate was used in a 3 × 4 MPN assay *(9)* for pathogen enumeration. The MPN assay samples were prepared and incubated in BPW for 24 h at 38°C before pathogen detection was performed by drop plating onto XLH-CATS, which is xylose lysine agar medium (Becton Dickinson) supplemented with 25 mM HEPES (N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid]) and 25 μg/ml chloramphenicol, ampicillin, tetracycline, and streptomycin (Sigma). The MPN tubes that were positive for *Salmonella* Typhimurium DT104 formed a black drop on XLH-CATS after 24 h of incubation at 38°C. The log MPN per gram was calculated using the method of Thomas *(14)* as described by Oscar *(9).*

For samples with a higher density (>3 log CFU/g) of *Salmonella* Typhimurium DT104, the sample homogenate was serially diluted in BPW, and 50 μl was spiral plated (Whitley Automatic Spiral Plater, Microbiology International, Frederick, Md.) onto XLH-CATS plates, which were incubated at 38°C for 24 h. Colonies were counted with an automated counter (Protocol, Microbiology International).

Primary modeling. Growth of *Salmonella* Typhimurium DT104 on chicken frankfurters was not observed at 10 to 12°C, but growth was observed at 14 to 40°C. When growth was observed, pathogen enumeration data for all trials within a temperature were combined and graphed as a function of time and were fit by least squares regression (version 5.0, Prism, GraphPad Soft-

ware, Inc., San Diego, Calif.) to a two-phase linear primary model *(5)*:

$$N(t) = \begin{cases} N_0 & \text{if } t \leq \lambda \\ N_0 + \mu\cdot(t - \lambda) \pm \text{PI}_{\text{growth}} & \text{if } t > \lambda \end{cases}$$

where $N(t)$ is pathogen density (log MPN or CFU per gram) at time t (hours), N_0 is the initial pathogen density (log MPN or CFU per gram), λ is lag time (hours), μ is growth rate (log MPN or CFU per gram per hour) and $\text{PI}_{\text{growth}}$ is the 95% PI (log MPN or CFU per gram) for the growth phase. $N(t)$ data from replicate challenge trials within a temperature were combined so the PI for the primary model fit would include the variation of pathogen growth among portions and batches of chicken frankfurters. Fitting the $N(t)$ data for individual challenge trials to the primary model would have resulted in a PI that included only the variation of pathogen growth among portions of chicken frankfurters, thus underestimating the variation of pathogen growth and complicating secondary modeling by requiring the development of stochastic secondary models for λ and μ.

No-growth and lag phase data were fit to a linear regression model where the slope was fixed at zero to obtain a 95% PI for characterizing the variation of $N(t)$ during the lag phase (PI_{lag}; log MPN per gram). Both PI_{lag} and $\text{PI}_{\text{growth}}$ were determined by interpolation from the graph of the primary model fit.

Secondary modeling. The natural logarithm (ln) transformations of λ, μ, PI_{lag}, and $\text{PI}_{\text{growth}}$ from primary modeling and the maximum sampling time (Ω) were graphed as a function of temperature (T, °C) and were fit by least squares regression to a quadratic polynomial secondary model (Prism version 5.0):

$$\ln Y = b_0 + (b_1 T) + (b_2 T^2)$$

where Y is the primary model parameter and b_0, b_1, and b_2 are regression coefficients. For secondary modeling of the no-growth data at 10, 11, and 12°C, λ was fixed at Ω. A secondary model was developed for Ω to limit extrapolation of tertiary model predictions beyond the sampling times investigated and modeled. This limitation is especially important for tertiary models that employ a primary model, such as the two-phase linear model, which does not have an upper asymptote. The square root model of Ratkowsky ($\mu = b\cdot[T - T_{\text{min}}]^2$) was not used to model growth rate as a function of temperature because growth rate was optimal at 30°C and decreased at 34 and 40°C and thus the square root model did not fit the data well because it describes linear increases as a function of temperature above T_{min}, whereas the growth rate data in this study increased in a nonlinear manner above T_{min}.

Tertiary modeling. The quadratic polynomial secondary models for λ, μ, PI_{lag}, $\text{PI}_{\text{growth}}$, and Ω were combined with the two-phase linear primary model in a computer spreadsheet (Excel 2000, Microsoft Corporation, Redmond, Wash.) to create a tertiary model for predicting the variation of *Salmonella* Typhimurium DT104 growth on chicken frankfurters as a function of time and temperature (10 to 40°C) and from a low N_0 (0.5 log MPN or CFU/g). Outputs of the tertiary model are a predicted growth curve and 95% PI and the predicted primary model parameters. Complete description of the tertiary model is beyond the scope of this article. However, the tertiary model can be obtained from the author upon request.

Verification of tertiary model performance. To verify that the tertiary model provided acceptable stochastic predictions of $N(t)$ data used in model development, the proportion of dependent $N(t)$ data ($n = 338$) in the PI of the primary model (pPM) fits and the proportion of dependent $N(t)$ data in the PI predicted by the tertiary model (pTM) were determined. The concordance index for verification (CI_v) was then calculated as follows:

$$\text{CI}_v = \frac{\text{pTM}}{\text{pPM}}$$

where a CI_v of 1.0 indicated that the variation of *Salmonella* Typhimurium DT104 growth among batches and portions of chicken frankfurters in the model development trials was well predicted by the tertiary model. A $\text{CI}_v < 1.0$ indicated that the tertiary model predicted less variation of *Salmonella* Typhimurium DT104 growth among batches and portions of chicken frankfurters than was observed in the model development trials and/or that the tertiary model provided inaccurate and/or biased predictions of pathogen growth. A $\text{CI}_v > 1.0$ indicated that the tertiary model predicted more variation of *Salmonella* Typhimurium DT104 growth among batches and portions of chicken frankfurters than was observed in the model development trials.

Validation of tertiary model performance. To validate the tertiary model for making acceptable predictions of *Salmonella* Typhimurium DT104 growth among batches and portions of chicken frankfurters, an independent set of $N(t)$ data ($n = 86$) for validation for interpolation within the response surface of the model was collected using the same experimental methods and experimental design. Within a temperature, sampling times were randomly selected to provide $N(t)$ data throughout both the lag phase and the growth phase for the purpose of providing uniform and complete coverage of the response surface for both time and temperature. The proportion of dependent $N(t)$ data (pD) and the proportion of independent $N(t)$ data (pI) in the PI predicted by the tertiary model were determined. The concordance index for validation (CI_V) was then calculated as follows:

$$\text{CI}_V = \frac{\text{pI}}{\text{pD}}$$

where a CI_V of 1.0 indicated that the future variation of *Salmonella* Typhimurium DT104 growth among batches and portions of chicken frankfurters was well predicted by the tertiary model. A $\text{CI}_V < 1.0$ indicated that the tertiary model predicted less variation of *Salmonella* Typhimurium DT104 growth among batches of chicken frankfurters than was observed in independent trials and/or that the tertiary model provided inaccurate and/or biased predictions of pathogen growth. A $\text{CI}_V > 1.0$ indicated that the tertiary model predicted more variation of *Salmonella* Typhimurium DT104 growth among batches of chicken frankfurters than was observed in independent trials.

RESULTS

Growth of *Salmonella* Typhimurium DT104 on chicken frankfurters incubated at 10, 11, or 12°C was not observed in the time frames investigated, and only minor and slow growth was observed at 14°C (Fig. 1). In contrast, significant growth (i.e., >1 log MPN or CFU) was observed when chicken frankfurters were incubated at 18 to 40°C (Fig. 2). Variation of *Salmonella* Typhimurium DT104 growth among batches and portions of chicken frankfurters was considerable, as indicated by the $\text{PI}_{\text{growth}}$, which ranged from 1.4 to 4.6 log MPN or CFU/g (Table 1). These PIs indicated that 95% of $N(t)$ data were within $\pm$0.7 to 2.3 log MPN or CFU of the best-fit line.

The two-phase linear model was selected as the primary model because it is robust and easy to fit to data with considerable scatter. This model facilitated generation of

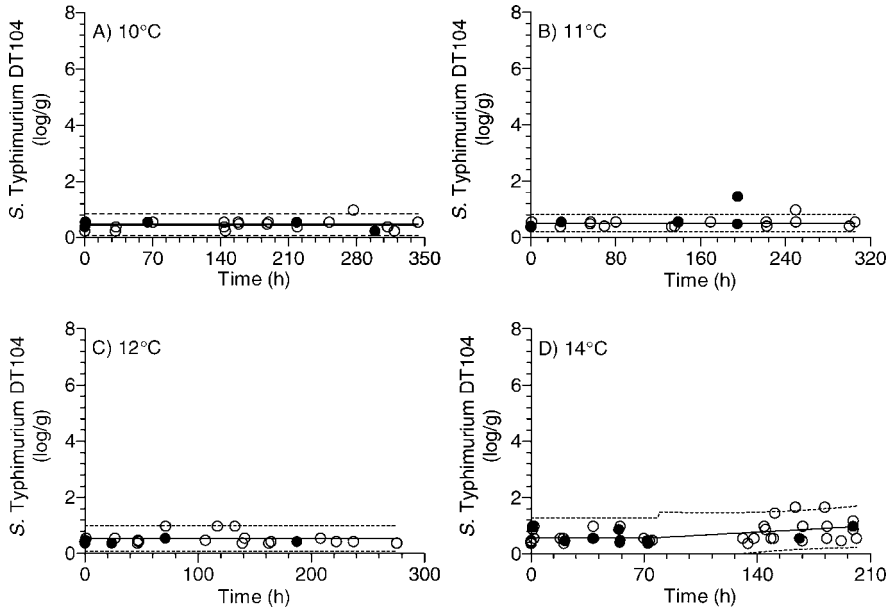

FIGURE 1. *Primary model fits and 95% prediction intervals for growth of* Salmonella *Typhimurium DT104 on chicken frankfurters incubated at (A) 10°C, (B) 11°C, (C) 12°C, and (D) 14°C.* ○, *dependent* N(t) *data;* ●, *independent* N(t) *data.*

separate PIs for the lag phase and the growth phase, which allowed development of a more precise tertiary model. The low R^2 values for the primary model fits were due to the observed variation of *Salmonella* Typhimurium DT104 growth among batches and portions of chicken frankfurters rather than to use of an inappropriate primary model; other primary models did not provide improved R^2 values and were more difficult to fit to the data (results not shown).

The goodness of fit of secondary models to primary model parameters as a function of temperature (Fig. 3) ranged from R^2 of 0.78 to 0.95 (Table 2). The lag phase for no-growth conditions was set at Ω, the maximum time of sampling, to facilitate construction of a tertiary model that provided reasonable predictions of λ for no-growth data; the resulting secondary model for λ had high goodness-of-fit values, indicating the success of this approach (Table 2).

Variation of $N(t)$ within a temperature included exper-

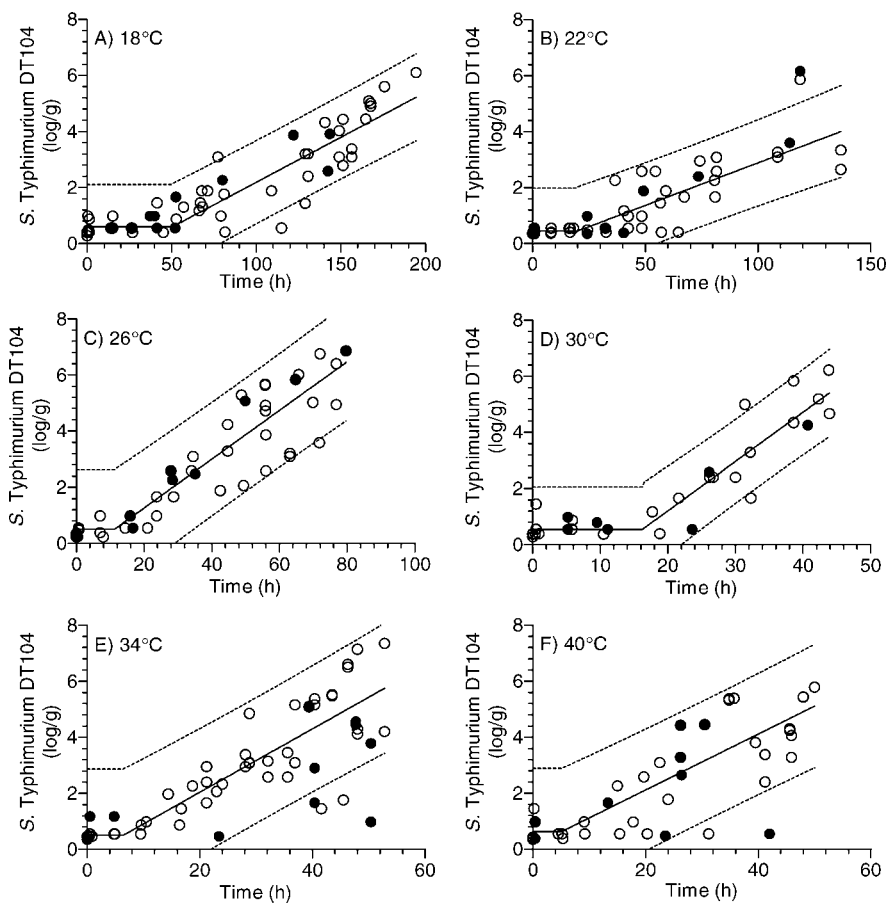

FIGURE 2. *Primary model fits and 95% prediction intervals for growth of* Salmonella *Typhimurium DT104 on chicken frankfurters incubated at (A) 18°C, (B) 22°C, (C) 26°C, (D) 30°C, (E) 34°C, and (F) 40°C.* ○, *dependent* N(t) *data;* ●, *independent* N(t) *data.*

TABLE 1. *Results of primary model fits for growth of* Salmonella *Typhimurium DT104 on chicken frankfurters*[a]

Temp (°C)	λ (h)	μ (log MPN or CFU/g/h)	PI_{lag} (log MPN or CFU/g)	PI_{growth} (log MPN or CFU/g)	Ω (h)	R^2
10	343.2	0	0.76	No growth	343.2	0
11	305.5	0	0.61	No growth	305.5	0
12	275.4	0	0.91	No growth	275.4	0
14	79.1	0.003	0.98	1.41	201.3	0.173
18	50.6	0.032	1.37	3.09	194.5	0.827
22	19.7	0.030	0.40	3.30	136.8	0.685
26	11.4	0.087	1.36	4.18	76.8	0.809
30	16.2	0.176	1.60	3.12	43.9	0.887
34	6.4	0.113	0.23	4.56	52.8	0.720
40	5.1	0.100	0.97	4.39	50.0	0.706

[a] λ, lag time; μ, growth rate; PI_{lag}, 95% prediction interval for lag phase; PI_{growth}, 95% prediction interval for growth rate; Ω, maximum sampling time; R^2, coefficient of determination.

imental error and pathogen growth variation among batches and portions of the frankfurters and was less (i.e., smaller PI) during the lag phase than during the growth phase (Fig. 3c) because during the lag phase the variation of $N(t)$ is primarily experimental error, with only a minor contribution from pathogen growth variation, which results from variation of the duration of the lag phase among replicate challenge trials (e.g., Fig. 2a). The contribution of pathogen growth variation to PI during the lag and growth phases increased in a nonlinear manner as a function of temperature, and after natural log transformation to homogenize model variance, ln PI fit well to a secondary quadratic polynomial model (Fig. 3c). However, to obtain a secondary model fit for ln PI during lag phase, ln PI data at 22 and 34°C had to be excluded. The justification for excluding these data was the observation that when the independent data (closed symbols in Fig. 2b and 2e) for $N(t)$ at 22 and 34°C were included in the primary model fit, the PI at these temperatures was larger and more in line with the rest of the PI data for lag phase. Thus, we felt justified in excluding these PI data from the secondary model fit; however, we did not feel justified in excluding the $N(t)$ data from 22 and 34°C from the evaluation of tertiary model performance because that exclusion would have introduced a bias into the verification and validation of this modeling approach (i.e., use of a quadratic polynomial model for ln PI_{lag} as a function of temperature and exclusion of ln PI_{lag} data at 22 and 34°C during secondary model development).

The tertiary model developed is shown in Figure 4, and the tertiary model predictions of the data used and not used in model development are shown in Figure 5 for temperatures from 10 to 14°C and in Figure 6 for temperatures from 18 to 40°C. The tertiary model developed predicts the *Salmonella* Typhimurium DT104 growth curve on chicken frankfurters and its 95% PI and the primary model parameters and maximum sampling time for temperatures used and not used in model development but between 10 and 40°C. Predictions beyond the times used in model devel-

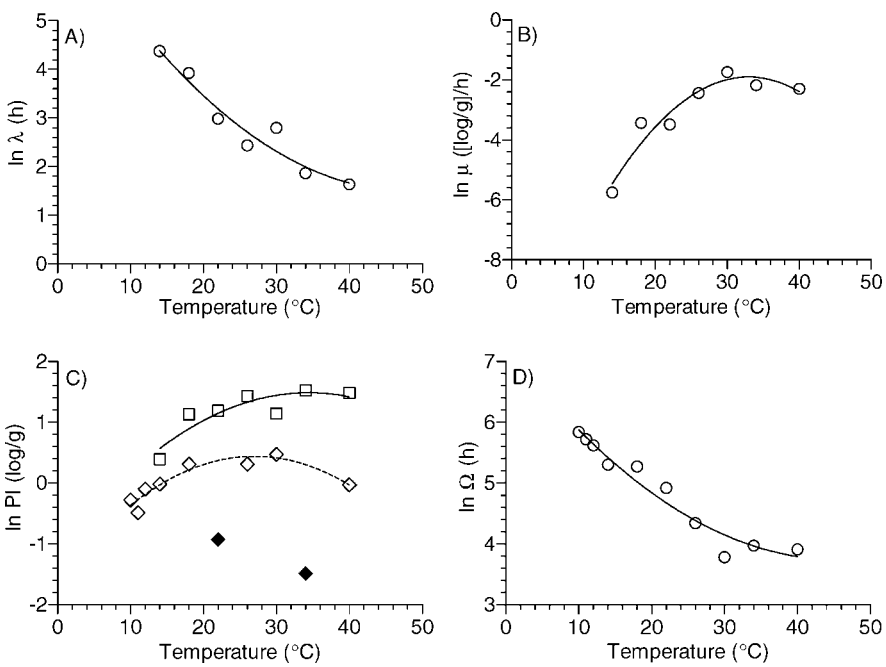

FIGURE 3. *Secondary quadratic polynomial model fits for ln transformations of (A) lag time (λ), (B) growth rate (μ), (C) 95% prediction interval for lag phase (PI_{lag}: ◇, included; ◆, excluded) and growth phase (PI_{growth}; □), and (D) maximum time of sampling (Ω) for growth of* Salmonella *Typhimurium DT104 on chicken frankfurters as a function of temperature.*

TABLE 2. *Results for secondary quadratic polynomial modeling of* Salmonella *Typhimurium DT104 growth on chicken frankfurters*

Dependent variable[a]	Regression coefficients	Best-fit value	SE	R^2
ln λ	b_0	7.231	1.239	0.9347
	b_1	−0.2383	0.09815	
	b_2	0.002475	0.001807	
ln μ	b_0	−12.67	1.809	0.9231
	b_1	0.6535	0.1434	
	b_2	−0.009919	0.002639	
ln PI_{lag}	b_0	−1.591	0.2839	0.8778
	b_1	0.1505	0.02792	
	b_2	−0.002789	0.000572	
ln PI_{growth}	b_0	−1.121	0.8693	0.7803
	b_1	0.1514	0.06888	
	b_2	−0.002199	0.001268	
ln Ω	b_0	7.245	0.3999	0.9498
	b_1	−0.1541	0.03827	
	b_2	0.001696	0.000789	

[a] λ, lag time; μ, growth rate; PI_{lag}, 95% prediction interval for lag phase; PI_{growth}, 95% prediction interval for growth rate; Ω, maximum sampling time; R^2, coefficient of determination.

Input	Unit	Range	Value
Number	log/g	0 to 1	0.5
Temperature	°C	10 to 40	24

Output	Unit	Value
λ	h	18.9
μ	[log/g]/h	0.067
PI_{lag}	log/g	1.51
PI_{growth}	log/g	3.48
Ω	h	92.2
	days	3.8

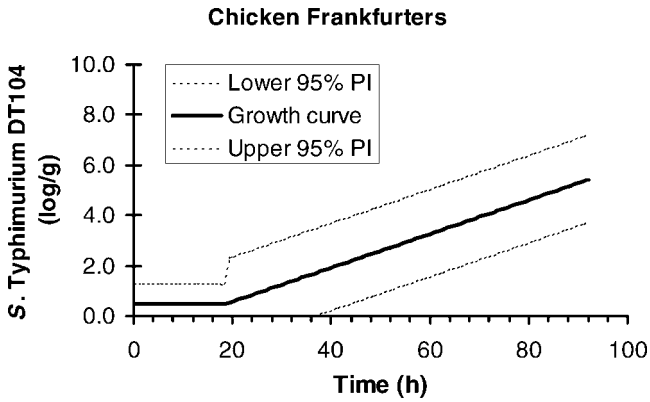

FIGURE 4. *Tertiary model for stochastic prediction of* Salmonella *Typhimurium DT104 growth on chicken frankfurters as a function of time and temperature. λ, lag time; μ, growth rate; PI_{lag}, 95% prediction interval for lag phase; PI_{growth}, 95% prediction interval for growth rate; Ω, maximum sampling time.*

opment are limited by inclusion of the secondary model for maximum sampling time (Ω). The latter model was important to include in the tertiary model because the primary model (i.e., two-phase linear model) used in the tertiary model did not predict an upper asymptote to growth. Thus, to limit extrapolation of tertiary model predictions beyond the sampling times used in model development, the secondary model for Ω was developed and included in the tertiary model.

Performance of the tertiary model was evaluated by calculating the CI_v and the CI_V. As expected, the proportion of dependent $N(t)$ data in the PI of the primary model fits was close to 0.95 (Table 3). The CI_v, which is the proportion of dependent $N(t)$ data in the PI predicted by the tertiary model divided by the proportion of dependent $N(t)$ data in the PI of the primary model fits, was 0.948 (i.e., 0.908/0.959). A CI_v of <1.0 indicated that overall the tertiary model predicted slightly less variation of *Salmonella* Typhimurium DT104 growth among batches and portions of chicken frankfurters than was observed in the model development trials.

A CI_v <1.0 could also be obtained if predictions of the tertiary model were inaccurate and/or biased, resulting in dependent $N(t)$ data falling outside the PI predicted by the tertiary model. The only evidence of this inaccuracy or bias occurred at 18°C (Table 3), where the highest number of dependent $N(t)$ data that were outside the PI predicted by the tertiary model were observed (Fig. 6a). Examination of the secondary model fits for μ (Fig. 3b) and PI_{growth} (Fig. 3c) indicated that the observed μ and PI_{growth} at 18°C were above the best-fits lines, and therefore the secondary models predicted slower growth and less variation of $N(t)$ at 18°C than was observed in the model development trials; these prediction errors explain the higher proportion of $N(t)$ that were outside the PI predicted by the tertiary model. Nonetheless, the overall ability of the tertiary model to predict the dependent $N(t)$ data was acceptable because the CI_v was close to 1.0.

The CI_V, which is the proportion of independent $N(t)$ data in the PI predicted by the tertiary model divided by the proportion of dependent $N(t)$ data in the PI predicted by the tertiary model, was 0.986 (i.e., 0.895/0.908). A CI_V of <1.0 indicated that the variation of *Salmonella* Typhimurium DT104 growth among batches of chicken frankfurters with native microflora was slightly higher during the validation trials than during the model development trials. There was little evidence (i.e., only at 34°C, Fig. 6e) of inaccurate and/or biased predictions of the independent $N(t)$ data by the tertiary model. Nonetheless, the CI_V, like the CI_v, was close to 1.0, indicating that overall the tertiary model provided acceptable stochastic predictions of *Salmonella* Typhimurium DT104 growth from a low initial density on chicken frankfurters stored at temperatures of 10 to 40°C.

To more precisely model the variation of *Salmonella* Typhimurium DT104 growth from a low initial density on chicken frankfurters with native microflora, separate secondary models were developed for the PIs during the lag and growth phases because less variation of $N(t)$ was ob-

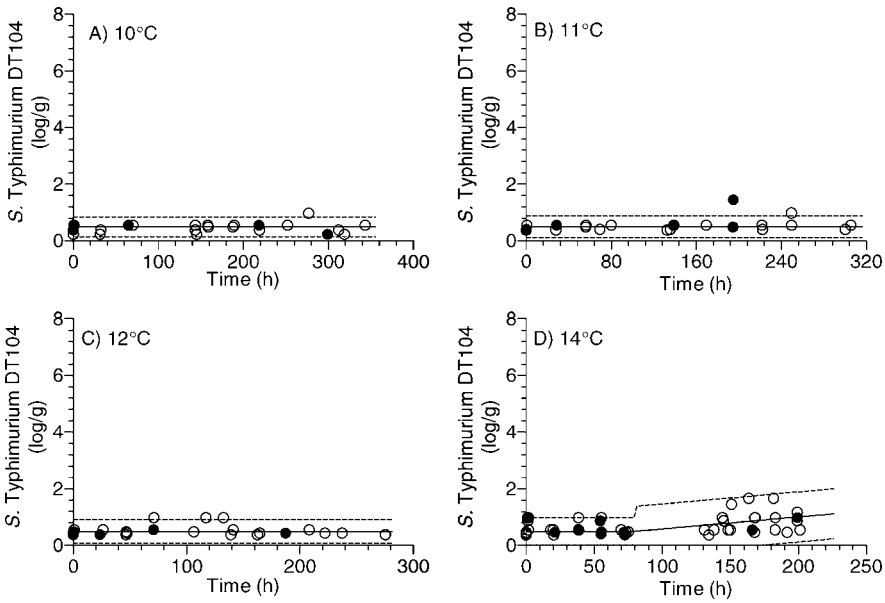

FIGURE 5. *Tertiary model prediction of the growth curve and its 95% prediction interval for* Salmonella *Typhimurium DT104 on chicken frankfurters incubated at (A) 10°C, (B) 11°C, (C) 12°C, and (D) 14°C.* ○, *dependent* N(t) *data;* ●, *independent* N(t) *data.*

served during the lag phase than during the growth phase. The significance of this modeling approach can be seen by comparing the PI in Figure 2 with the PI in Figure 6 for the same $N(t)$ data. The PI in Figure 2 overestimates the variation of $N(t)$ during the lag phase, whereas the PI in Figure 6 provides a much better estimate of the variation of $N(t)$ during the lag phase. This difference occurs because the observed PIs for the growth phase are used by the primary model fits in Figure 2, whereas the predicted PIs for the lag phase are used for the tertiary model predictions in Figure 6. Thus, by using two secondary models for the PI, one for the lag phase and one for the growth phase, more precise predictions of the variation of *Salmonella* Typhimurium DT104 growth on chicken frankfurters with native microflora were obtained.

DISCUSSION

Cross-contamination of frankfurters with pathogens after thermal processing occurs on the exterior surface, whereas contamination of the interior is the likely scenario for pathogens in the frankfurter emulsions that survive because of underprocessing. Frankfurters in the current study were inoculated on the interior cut surface with a low initial density (0.5 log CFU/g) and then incubated under aerobic conditions at temperatures from 10 to 40°C to obtain kinetic data for development and validation of a predictive growth model. Although the initial density was realistic, a potential criticism of this approach is that it does not exactly simulate the growth conditions of these scenarios for pathogen contamination of frankfurters. However, Bayne and Michener *(3)* reported that growth of *Salmonella* was similar for cells inoculated into the interior or applied on the exterior of frankfurters. Nonetheless, future studies are needed to determine how broadly the current model can be applied. For example, how well does the model predict growth of other strains and growth for other formulations? To examine these questions, reduced data sets (i.e., less than needed for full model development) will be collected and used to evaluate the model for extrapolation to these other conditions. If the model does not provide acceptable predictions of *Salmonella* growth under a new condition (e.g., another formulation of frankfurters), then a new model will be developed.

A potential limitation of the current study was that *Salmonella* Typhimurium DT104 growth was investigated and modeled only for the lag phase and the linear phase of growth. Although maximum population density (N_{max}) was not determined, the data and model are of value for assessing food safety because the storage times investigated and modeled were extensive. For example, the model predicts no growth or growth of the pathogen up to 14 days at 10°C, 5 days at 22°C, and 3 days at 30°C. The refrigerated storage life of frankfurters is 7 days for an opened package and 14 days for an unopened package *(2),* indicating that the model provides predictions of *Salmonella* growth that cover the typical shelf life of the product.

In the present study, the variation of *Salmonella* Typhimurium DT104 growth (i.e., 95% PI of the primary model fit) among batches and portions of chicken frankfurters increased in a nonlinear manner as the storage temperature increased and was well predicted by a secondary quadratic polynomial model for the ln PI as a function temperature. This finding is similar to that of a previous study *(9),* in which the variation of growth of the same strain of *Salmonella* Typhimurium DT104 was investigated and modeled as a function of temperature in ground chicken breast meat with native microflora. However, this finding differs from results with sterilized cooked chicken breast meat, in which much less variation of *Salmonella* Typhimurium growth was observed *(6).* Beckers et al. *(4)* reported variable growth of *Salmonella* in food sample homogenates, with N_{max} ranging from 3 to 7 log CFU/ml. These authors concluded that the type of native microflora rather than initial numbers was a more important predictor of *Salmonella* growth. Thus, variation of numbers and types of native microflora among batches and portions and changes in the number and types of native microflora as storage

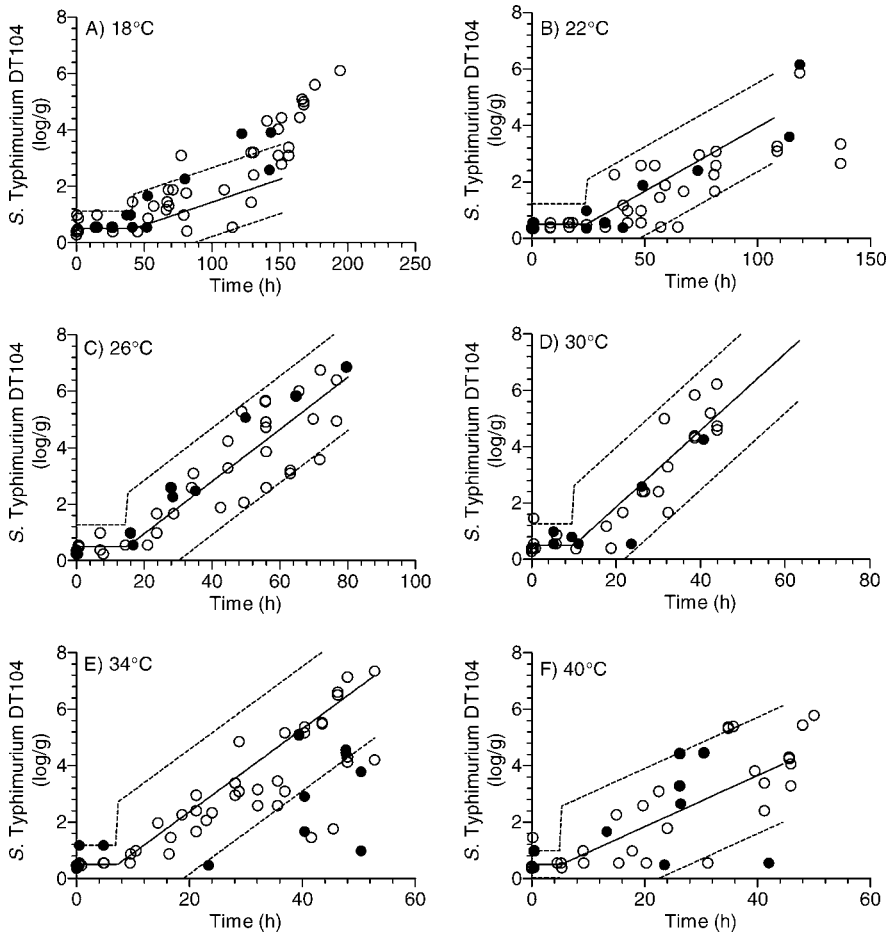

FIGURE 6. *Tertiary model prediction of the growth curve and its 95% prediction interval for* Salmonella *Typhimurium DT104 on chicken frankfurters incubated at (A) 18°C, (B) 22°C, (C) 26°C, (D) 30°C, (E) 34°C, and (F) 40°C.* ○, *dependent* N(t) *data;* ●, *independent* N(t) *data.*

temperature increases could explain in part the nonlinear increase in the variation of *Salmonella* Typhimurium DT104 growth observed in this and a previous study *(9)* as storage temperature increased.

The nonlinear increase in the variation of *Salmonella* Typhimurium DT104 growth among batches and portions of chicken frankfurters as the storage temperature increased could also result in part from variations in the concentration and activity of antimicrobial compounds, such as potassium lactate or sodium diacetate, in frankfurter formulations. The concentrations of antimicrobials among packages of chicken frankfurters used in this study were not measured in this study or reported by the manufacturer. However, a single brand of chicken frankfurters from the same manufacturer was used throughout this study, and the formulation and composition of frankfurters reported on the label did not

TABLE 3. *Evaluation of primary and tertiary model performance: concordance method*

	Total		Primary model[a]		Tertiary model[b]	
Temp (°C)	Dependent	Independent	Dependent	Independent	Dependent	Independent
10	19	5	1	0	1	0
11	18	5	1	1	1	1
12	18	5	0	0	3	0
14	36	10	2	0	0	0
18	46	11	3	0	12	2
22	39	10	1	1	3	0
26	45	11	1	0	1	0
30	27	7	2	0	1	0
34	43	13	2	2	4	5
40	47	9	1	1	5	1
Total	338	86	14	5	31	9
Concordance[c]			0.959	0.942	0.908	0.895

[a] Values are the number of $N(t)$ data points that are outside of the 95% prediction interval of the primary model fit.
[b] Values are the number of $N(t)$ data points that are outside of the 95% prediction interval predicted by the tertiary model.
[c] The proportion of $N(t)$ data points in the 95% prediction intervals of the primary model fit or predicted by the tertiary model.

change. Thus, large differences in concentrations of antimicrobials among packages of chicken frankfurters probably did not occur. Nonetheless, additional research is needed to assess how much of the variation of *Salmonella* Typhimurium DT104 growth among batches and portions of chicken frankfurters and as a function of temperature can be explained by variations in the concentration and activity of antimicrobial compounds.

Rather than include numbers and types of native microflora and concentration and activity of antimicrobials as independent variables in the model, the variation of *Salmonella* Typhimurium DT104 growth among portions and batches of chicken frankfurters as a function of time was modeled by combining $N(t)$ data from replicate challenge trials within temperatures and then using the primary model fit to predict growth variation in the form of a 95% PI. The PI included experimental error, the uncertainty of the curve fit, and pathogen growth variation among batches and portions of frankfurters. The PI for chicken frankfurters was affected in a nonlinear manner by temperature and was wider for the growth phase than for the lag phase. For the growth phase, the PI ranged from 1.4 to 4.6 log MPN or CFU/g at 14 to 40°C, whereas for the lag phase the PI ranged from 0.23 to 1.6 log MPN or CFU/g at 10 to 40°C. In comparison, in a previous study with the same strain of *Salmonella* Typhimurium DT104 grown on ground chicken breast meat with native microflora, the PI for the growth phase ranged from 1.3 to 2.6 log MPN or CFU/g at 10 to 40°C, indicating less variation in pathogen growth on freshly ground meat than on frankfurters *(9).*

In the present study, no growth of *Salmonella* Typhimurium DT104 was observed on chicken frankfurters for up to 345 h of incubation at 10°C, whereas at 11 and 12°C no growth of *Salmonella* Typhimurium DT104 was observed for up to 310 and 275 h of incubation, respectively. These results are in contrast to growth of this same strain of *Salmonella* Typhimurium DT104 on ground chicken breast meat with native microflora *(9).* From a low initial density (i.e., 0.6 log CFU/g) on ground chicken breast meat, this same strain of *Salmonella* Typhimurium DT104 grew 1.06 log cycles after 185 h at 10°C, 1.79 log cycles after 175 h at 11°C, and 2.47 log cycles after 164 h at 12°C *(9).* The presence of antimicrobial compounds (e.g., spices, potassium lactate, and sodium diacetate) and differences in numbers and types of native microflora may help to explain the reduced growth rate and growth temperature range of *Salmonella* Typhimurium DT104 on chicken frankfurters as compared with ground chicken breast meat in a previous study *(9).* Thus, use of the tertiary model developed and validated in the present study rather than the existing model for growth of *Salmonella* Typhimurium DT104 on chicken breast meat with native microflora will significantly reduce overprediction of the risk of consumer exposure to *Salmonella* on chicken frankfurters subjected to temperature abuse.

Sodium nitrite is included in frankfurter emulsions to add color, flavor, and preservation. However, Bayne and Michener *(3)* reported that nitrite (156 ppm before processing and 39 ppm after processing) had little to no effect on growth of *Salmonella* inoculated in or on frankfurters and then incubated at 20°C. Likewise, Palumbo et al. *(11)* observed no effect of nitrite on levels of *Salmonella* inoculated onto frankfurters and then stored for 24 h at 37°C, and Rice and Pierson *(12)* found no inhibitory effect of 50 or 156 ppm nitrite on growth of nalidixic acid–resistant strains of *Salmonella* on frankfurters incubated at 15 and 27°C. Thus, sodium nitrite did not likely contribute to the reduced growth rate and growth temperature range observed for *Salmonella* Typhimurium DT104 on chicken frankfurters in the present study as compared with growth of this same strain on ground chicken breast meat in a previous study *(9).*

Predictive models for growth of pathogens usually are developed based on pure broth cultures growing in higher numbers than would be found in the natural environment. Performance of such models is often evaluated by calculation of prediction bias and accuracy factors that quantify the average prediction error and bias of the model *(13).* However, when predictive models for growth of pathogens are developed with a low, more biologically relevant level of the pathogen growing on food with native microflora and considerable biological variation of growth among replicate challenge trials, evaluation of model performance shifts from a focus on calculating the average prediction error of the model to a focus on evaluating how well the model predicts the observed variation of pathogen growth. If the model predicts substantially more or less variation of pathogen growth than what is observed, then the model does not provide acceptable predictions of pathogen growth and is in need of repair, which could involve collection of additional data to better define the response surface and variation of pathogen growth.

In the present study, *Salmonella* Typhimurium DT104 growth from a low, naturally occurring level was investigated and modeled on chicken frankfurters with native microflora, and considerable biological variation of pathogen growth was observed among replicate challenge trials and as a function of temperature. This variation was modeled using a 95% PI, and the ability of the resulting tertiary model to predict the variation of *Salmonella* Typhimurium DT104 growth among batches and portions of chicken frankfurters and as a function of time and temperature was evaluated using a CI method for model verification against dependent $N(t)$ data and a CI method for model validation against independent $N(t)$ data. The CI_v was 0.948 for dependent $N(t)$ data, indicating that the tertiary model predicted slightly less variation of *Salmonella* Typhimurium DT104 growth among batches of chicken frankfurters than was observed in the model development trials. The use of a separate secondary model for PI during the lag phase could explain this observation because the variation of $N(t)$ during the lag phase was less than the variation of $N(t)$ during the growth phase. In primary modeling, the wider PI for the growth phase was used for the lag phase, thus accounting for a CI_v of less than 1.0 for dependent $N(t)$ data.

The CI_V against independent $N(t)$ data in the present study was very close to 1.0 (0.986), indicating that the ter-

tiary model provided similar and thus acceptable predictions of the variation of *Salmonella* Typhimurium DT104 growth on chicken frankfurters with native microflora as was observed in the model development trials. The independent $N(t)$ data used to evaluate model performance were collected using the same experimental methods and an experimental design that provided fairly uniform coverage of the entire response surface of the model in both time and temperature. Independent data must be collected in this manner because evaluation of model performance using independent data that only cover part of the response surface, are more heavily concentrated in one area of the response surface, or are collected using different experimental methods could lead to confounded, biased, incomplete, and/or inaccurate assessments of model performance *(8).*

A tertiary model for predicting the variation of growth of a single strain of *Salmonella* Typhimurium DT104 on a single formulation of chicken frankfurters with native microflora was successfully developed and validated against independent data. The model provides stochastic predictions of *Salmonella* growth on chicken frankfurters with native microflora over a wide range of temperatures and from a low, naturally occurring initial density of the pathogen, which should make the model highly relevant for use in microbial risk assessments and in the food industry. Further studies are needed to evaluate how broadly the model can be applied (i.e., robustness) to other strains of *Salmonella,* to other frankfurter formulations, and to other storage and handling conditions.

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