Journal of Food Protection, Vol. 68, No. 12, 2005, Pages 2606–2613

Development and Validation of Primary, Secondary, and Tertiary Models for Growth of *Salmonella* Typhimurium on Sterile Chicken†

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MS 05-154: Received 31 March 2005/Accepted 9 July 2005

ABSTRACT

Models are used in the food industry to predict pathogen growth and to help assess food safety. However, criteria are needed to determine whether models provide acceptable predictions. In the current study, primary, secondary, and tertiary models for growth of *Salmonella* Typhimurium ($10^{4.8}$ CFU/g) on sterile chicken were developed and validated. Kinetic data obtained at 10 to 40°C were fit to a primary model to determine initial density (N_0), lag time (λ), maximum specific growth rate (μ_{max}), and maximum population density (N_{max}). Secondary models for N_0, λ, μ_{max}, and N_{max} as a function of temperature were developed and combined with the primary model to create a tertiary model that predicted pathogen density (N) at times and temperatures used and not used in model development. Performance of models was evaluated using the acceptable prediction zone method in which experimental error associated with growth parameter determinations was used to set criteria for acceptable model performance. Models were evaluated against dependent and independent (validation) data. Models with 70% prediction or relative errors (RE) in an acceptable prediction zone from −0.3 to 0.15 for μ_{max}, −0.6 to 0.3 for λ, and −0.8 to 0.4 for N, N_0, and N_{max} were classified as acceptable. All secondary models had acceptable goodness of fit and were validated against independent (interpolation) data. Percent RE in the acceptable prediction zone for the tertiary model was 90.7 for dependent data and 97.5 for independent (interpolation) data. Although the tertiary model was validated for interpolation, an unacceptable %RE of 2.5 was obtained for independent (extrapolation) data obtained with a lower N_0 ($10^{0.8}$ CFU/g). The tertiary model provided overly fail-dangerous predictions of N from a lower N_0. Because *Salmonella* concentrations on chicken are closer to $10^{0.8}$ than $10^{4.8}$ CFU/g, the tertiary model should not be used to help assess chicken safety.

Kinetic data for development of models that predict growth of pathogens on food are usually obtained in challenge studies with sterile broth and then modeled in three stages: primary, secondary, and tertiary (Fig. 1) *(21)*. Primary models predict changes in pathogen density as a function of time, whereas secondary models predict changes in primary model parameters (e.g., lag time and growth rate) as a function of independent variables (e.g., temperature, pH, and water activity). Primary and secondary models are combined in a computer software application to create a tertiary model *(3)*. The tertiary model uses predicted values of growth parameters from secondary models in the primary model to predict changes in pathogen density at times and levels of independent variables used and not used in model development (Fig. 1).

Similar to model development, evaluation of model performance involves three stages: goodness of fit and verification, interpolation, and extrapolation *(11)*. Central to the evaluation process is the calculation of performance factors that assess the bias and accuracy of model predictions *(6, 15)* and the definition of criteria that permit a decision as to whether a model provides acceptable predictions *(18)*. Although tertiary model predictions of pathogen density have been compared in previous studies *(8, 12, 23)*, performance factors were not calculated and criteria were not defined to assess whether predictions were acceptable.

In the current study, primary, secondary, and tertiary models for growth of *Salmonella* Typhimurium ($10^{4.8}$ CFU/g) on sterile chicken as a function of temperature (10 to 40°C) were developed. Ability of the models to predict pathogen growth for data used in model development (dependent data) and data not used in model development (independent data for interpolation or extrapolation) was quantified using the acceptable prediction zone method *(11)*. Criteria were established to assess whether model predictions were acceptable based on an assessment of experimental error associated with determination of growth parameters.

Because *Salmonella* numbers on chicken are usually less than 30 CFU per carcass *(17, 19, 20)*, tertiary model predictions of pathogen density from a lower initial density ($10^{0.8}$ CFU/g) were evaluated to determine whether the tertiary model should be used to help assess chicken safety. Growth from a high density was investigated and modeled because the limit of enumeration by viable counts was 10^3 CFU/g.

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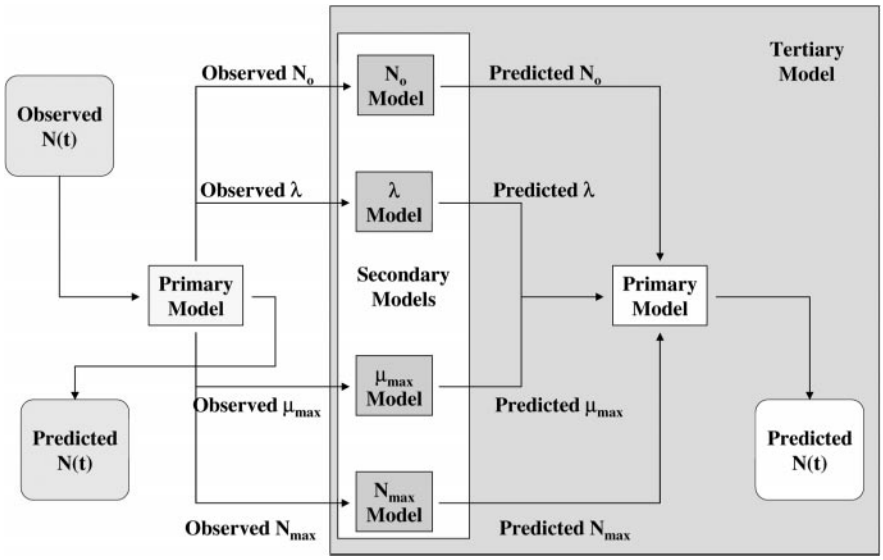

FIGURE 1. *Relationship among the three stages of predictive model development: primary, secondary, and tertiary.* N(t)*, pathogen density at time* t*;* N_0*, initial pathogen density;* λ*, lag time;* μ_{max}*, maximum specific growth rate;* N_{max}*, maximum population density.*

MATERIALS AND METHODS

Salmonella. *Salmonella* Typhimurium (ATCC 14028, American Type Culture Collection, Manassas, Va.) was maintained in stock culture at −70°C in brain heart infusion broth (Becton Dickinson, Sparks, Md.) that contained 15% glycerol (Sigma, St. Louis, Mo.).

Sterile chicken. Ten-gram portions of ground chicken breast meat were formed into uniform patties and then sterilized in an autoclave at 121°C for 18 min. A 1.5-cm-diameter cork borer was used to cut plugs from the patties. Plugs were cut in half and trimmed to yield 1-g portions. Portions were transferred to individual wells of a 12-well tissue culture dish for subsequent inoculation.

Inoculum culture. Stock culture (5 μl) was added to 5 ml of brain heart infusion broth in a 25-ml Erlenmeyer flask sealed with a foam plug. Inoculum cultures were incubated at 30°C and 150 orbits per min for 23 h before serial dilution in buffered peptone water (BPW; Becton Dickinson).

Challenge study. Diluted inoculum culture (5 μl) was pipetted onto the surface of the chicken portions for an initial density of $10^{4.8}$ CFU/g, and inoculated portions were incubated at 10 to 40°C. At selected incubation times, a 1-g portion was homogenized (model 80 stomacher blender, Seward, London, UK) in 9 ml of BPW. Samples of stomachate (50 μl), either undiluted or serially diluted in BPW, were spiral plated (Whitley Automatic Spiral Plater, Microbiology International, Frederick, Md.) onto brain heart infusion agar (Becton Dickinson). Plates were incubated at 30°C for 24 h, and then colonies were counted with an automated counter (ProtoCol, Microbiology International).

Experimental design. Kinetic data used in model development were collected at 10, 12, 14, 16, 20, 24, 28, 32, 36, 38, and 40°C. Twelve pathogen density (*N*) determinations were made per temperature and were fit to a primary model to determine initial density (N_0), lag time (λ), maximum specific growth rate (μ_{max}), and maximum population density (N_{max}). Determinations of *N* were targeted for lag ($n = 3$), exponential ($n = 6$), and stationary ($n = 3$) phases of growth.

Independent *N* data for evaluating performance of secondary and tertiary models for the ability to interpolate within the response surface were collected at 11, 13, 15, 18, 22, 26, 30, 34, 37, and 39°C with the same experimental methods as used for model development. These *N* data could not be used to evaluate the primary model for interpolation as a function of time because they were collected at different temperatures than the *N* data used in primary modeling.

For secondary models, 12 *N* determinations were made per temperature and were fit to the primary model to generate N_0, λ, μ_{max}, and N_{max} data for performance evaluation for interpolation. For the tertiary model, four of 12 *N* determinations per temperature were randomly selected for performance evaluation for interpolation. These four *N* determinations for tertiary model evaluation were targeted for lag, early exponential, late exponential, and stationary phases to satisfy the test data criteria for interpolation in the acceptable prediction zone method, which requires that test data be uniformly distributed within the response surface *(11).*

Independent *N* data for evaluating the ability of the tertiary model to extrapolate were collected at 10, 12, 14, 16, 20, 24, 28, 32, 36, and 40°C using the same experimental methods as used for model development except that a lower N_0 ($10^{0.8}$ CFU/g) was used. The N_0 used in these experiments ($10^{0.8}$ CFU/g) was calculated from the viable count determination of the inoculum culture. Thus, the tertiary model was evaluated for the ability to predict *N* from a lower N_0, which was outside the level of independent variables (i.e., N_0) used to develop the model.

To evaluate performance of the tertiary model for extrapolation from a lower N_0, four *N* determinations were made per temperature targeted for mid-exponential, late exponential, early stationary, and stationary phases. Determinations of *N* in lag and early exponential phases were below the detection limit (10^3 CFU/g) of viable counts and thus could not be included.

Because complete growth curves could not be generated from the lower N_0, it was not possible to perform primary modeling and directly evaluate secondary models for extrapolation. However, the ability of primary and secondary models to extrapolate was indirectly assessed when the tertiary model was evaluated for extrapolation because the primary and secondary models were combined in a computer software application to form the tertiary model (Fig. 1).

Primary modeling. Viable count data were graphed as a function of time and fit (Prism version 4.0, GraphPad Software, San Diego, Calif.) to the logistic-with-delay primary model *(1)*:

$$N(t) = \begin{cases} N_0 & \text{if } t \le \lambda \\ \dfrac{N_{max}}{1 + [(N_{max}/N_0) - 1]\exp[-\mu_{max}(t - \lambda)]} & \\ & \text{if } t > \lambda \end{cases} \quad (1)$$

where $N(t)$ is pathogen density (log CFU per gram) at time t (hours), N_0 is initial pathogen density (log CFU per gram), λ is lag time (hours), N_{max} is maximum pathogen density (log CFU per gram), and μ_{max} is maximum specific growth rate (hours^{-1}).

In addition to the logistic-with-delay primary model, N data were fit to the three-phase linear model *(4)*, the logistic model *(1)*, and the modified Gompertz model *(24)*. However, the logistic-with-delay model provided the highest goodness-of-fit value, as assessed by Akaike's information criteria (results not shown). To simplify secondary and tertiary modeling, only growth parameters obtained with the logistic-with-delay primary model were used in the next two steps of model development. Thus, use of the logistic-with-delay primary model was justified by comparison with other primary models.

Secondary modeling. For technical reasons, not all growth conditions could be evaluated on the same day. Consequently, multiple inoculum cultures were used. As expected, N_0 did not change as a function of temperature (T, °C); therefore, mean N_0 was used as the secondary model.

Although N_0 is not expected to change as a function of temperature, inconsistent preparation and pipetting of the inoculum could result in significant variation of N_0 among growth curves and temperatures. If N_0 affects pathogen growth, as occurred in the current study, inconsistent preparation and delivery of inoculum among growth curves and temperatures would introduce experimental error into the secondary models for other growth parameters and into the tertiary model. Consequently, mean N_0 was used as a secondary model, mainly for quality control purposes, to assess whether preparation and pipetting of the inoculum was consistent among growth curves and temperatures.

Secondary models for λ *(10)*, μ_{max} (developed in this study), and N_{max} *(22)* as a function of temperature were as follows:

$$\lambda = [p/(T - T_{min})]^m \quad (2)$$

$$\mu_{max} = \begin{cases} \mu_{max-min} & \text{if } T \le T_0 \\ \mu_{max-min} + [b(T - T_0)]^m & \text{if } T > T_0 \end{cases} \quad (3)$$

$$N_{max} = \exp\left\{\frac{a[(T - T_{min})(T - T_{max})]}{(T - T_{submin})(T - T_{supmax})}\right\} \quad (4)$$

where p is a regression coefficient, T_{min} is the minimal growth temperature, m is a shape factor, $\mu_{max-min}$ is the minimal predicted μ_{max}, b is a regression coefficient, T_0 is the temperature at which μ_{max} increases from $\mu_{max-min}$, a is a regression coefficient, T_{submin} is a temperature just below T_{min}, and T_{supmax} is a temperature just above T_{max}, the maximal growth temperature. Secondary models were fit to growth parameter data from primary modeling using the Prism software program.

In a previous study *(10)*, the λ model was compared with four other λ models and provided the highest goodness-of-fit value for *Salmonella* Typhimurium and sterile chicken. The comparison of λ models in the previous study *(10)* accounted for differences in the number of parameters among models. The shape parameter m was included in the λ model to remove prediction bias at higher temperatures *(10)*.

In the current study, a modified version of the square root model for μ_{max} was developed because a significant prediction bias at low temperatures (<14°C) was observed when the square root model *(13)* was used. Although the assumption of a constant μ_{max} below T_0 in the modified square root model was not realistic, this assumption fit the observed data better than the assumption of the square root model of a decreasing μ_{max} to T_{min}. The modified version of the square root model for μ_{max} removed a prediction bias at low temperatures. Overparameterization of the modified square root model was evaluated by comparing its predictions to data not used in model development. The modified square root model had acceptable goodness-of-fit values and was validated for interpolation and thus provided acceptable predictions of μ_{max} between 10 and 40°C.

The N_{max} model was compared with the assumption that N_{max} did not change as a function of temperature. Results of the comparison (not shown) indicated that use of mean N_{max} to predict N_{max} as a function of temperature introduced significant prediction bias at the lower and upper ends of the temperature range used in model development, i.e., regions where N_{max} decreased significantly. Consequently, the aforementioned secondary model was used to predict N_{max} as a function of temperature.

Use of these secondary models for λ, μ_{max}, and N_{max} was justified by comparison with other secondary models. To simplify tertiary modeling, only one secondary model was used per growth parameter.

Tertiary modeling. The primary model and secondary models were combined in a computer spreadsheet (Excel 2000, Microsoft Corporation, Redmond, Wash.) to create a tertiary model (Fig. 1). The tertiary model used predicted values from the secondary models in the primary model to predict N at times and temperatures that were used and not used in model development.

Performance evaluation. Performance of primary, secondary, and tertiary models was evaluated using the acceptable prediction zone method *(11)*. Prediction errors or relative errors (REs) for individual prediction cases were calculated:

$$\text{RE for } \lambda = (\text{predicted} - \text{observed})/\text{predicted} \quad (6)$$

$$\text{RE for } N, N_0, \mu_{max}, \text{ and } N_{max} = (\text{observed} - \text{predicted})/\text{predicted} \quad (7)$$

such that an RE of less than zero represented fail-safe predictions and an RE greater than zero represented fail-dangerous predictions. Relative errors for N, N_0, and N_{max} were calculated using CFU per gram rather than log CFU per gram. Calculation of RE with log-transformed values would have overestimated model performance and therefore was not done.

Percent RE in an acceptable prediction zone from −0.3 (fail safe) to 0.15 (fail dangerous) quantified performance of the μ_{max} model *(11)*. Performance of the secondary model for μ_{max} was classified as acceptable when %RE was ≥70 *(11)*, i.e., 70% of the predictions of μ_{max} could not deviate from observed values by more than 30% in the fail-safe direction or by more than 15% in the fail-dangerous direction for the model to be classified as acceptable.

Different acceptable prediction zones were used for evaluating performance of λ and N models because experimental error associated with determining these growth parameters differs from experimental error associated with determining μ_{max}. For example, λ of *Salmonella* on sterile chicken is twice as variable among replicate growth curves as is μ_{max} *(9)*. Consequently, an acceptable prediction zone that was twice as wide (−0.6 to 0.3) as the acceptable prediction zone for μ_{max} was used to evaluate performance of the λ model.

Performance of models that predict N (i.e., primary, secondary for N_0, secondary for N_{max}, and tertiary) is limited by the

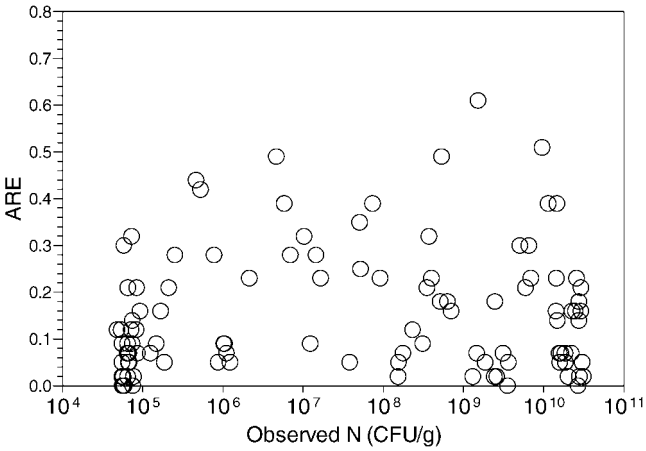

FIGURE 2. *Absolute relative error (ARE) of pathogen density (*N*) determinations among serial dilutions of the same sample of inoculated sterile chicken. Samples were inoculated with* Salmonella *Typhimurium (10$^{4.8}$ CFU/g) and incubated for selected times at 11 temperatures from 10 to 40°C.*

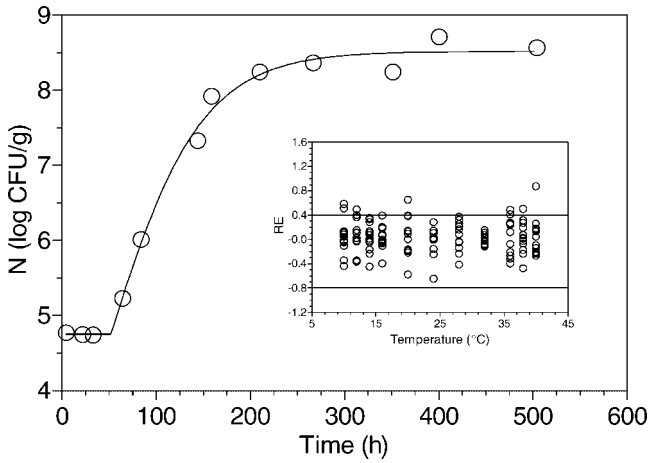

FIGURE 3. *Primary model fit to kinetic data for growth of* Salmonella *Typhimurium on sterile chicken. Data were obtained at 10°C. Inset: acceptable prediction zone analysis of the goodness of fit of the primary model to* N *(pathogen density) data used in model development and obtained at 11 temperatures from 10 to 40°C. RE, relative error.*

precision of the enumeration method. To establish performance criteria for this class of models, the absolute relative error (ARE) among serial dilutions of the same sample was quantified:

$$\text{ARE} = |(A - B)/[(A + B)/2]|$$

where dilutions *A* and *B* were observed *N* in CFU per gram. The distribution of ARE was then evaluated and the 95th percentile of ARE was used to establish the boundaries of the acceptable prediction zone, which were −0.8 (fail safe) and 0.4 (fail dangerous). Thus, width of the acceptable prediction zone was most narrow for the μ_{max} model (−0.3 to 0.15), intermediate for the λ model (−0.6 to 0.3), and widest for the *N* models (−0.8 to 0.4).

The acceptable prediction zone for *N* models is based on a conservative estimate of experimental error because it does not account for other sources of experimental error for *N* such as variation of *N* among duplicate samples. For technical reasons, duplicate samples were not processed at each sampling time. Thus, experimental error associated with among-sample variability (e.g., pipetting error for N_0, sample processing deviations) was not quantified and included in the acceptable prediction zone for *N* models.

Statistical analysis. Because the performance factor of the acceptable prediction zone method, %RE, is a proportion, comparison among performance evaluations can be made using a 2 × 2 contingency table and Fisher's exact test. In the current study, Fisher's exact test in the Prism software was used to compare performance of the primary model and tertiary model for predicting the same *N* data (i.e., *N* data used in model development).

RESULTS

The ARE values among serial dilutions of the same sample had a mean of 0.155, a median of 0.115, a range of 0.000 to 0.608, and a 95th percentile of 0.400. ARE values were randomly distributed as a function of observed *N* (Fig. 2). The 95th percentile of AREs, which was a one-tailed distribution, was used to establish the fail-dangerous boundary of the acceptable prediction zone for *N* models. The 95th percentile was used because in a one-tailed or two-tailed statistical test, such as the *t* test, the 95th percentile (i.e., two standard deviations) is used as the criterion for significance. The fail-safe boundary was set at twice the fail-dangerous boundary as per the acceptable prediction zone method *(11)* where predictions can err twice as much in the fail-safe direction. Thus, the acceptable prediction zone for evaluation of models that predicted *N* was established as −0.8 (fail safe) to 0.4 (fail dangerous).

When the acceptable prediction zone (−0.8 to 0.4) for *N* was used to evaluate goodness of fit of the primary model to kinetic data obtained at 11 temperatures from 10 to 40°C (Fig. 3), 121 of 129 REs were in the acceptable prediction zone (Fig. 3, inset) for an acceptable %RE of 93.8.

Use of the mean value of 4.821 log CFU/g or 66,222 CFU/g to model N_0 as a function of temperature (Fig. 4) was evaluated against dependent data and independent (in-

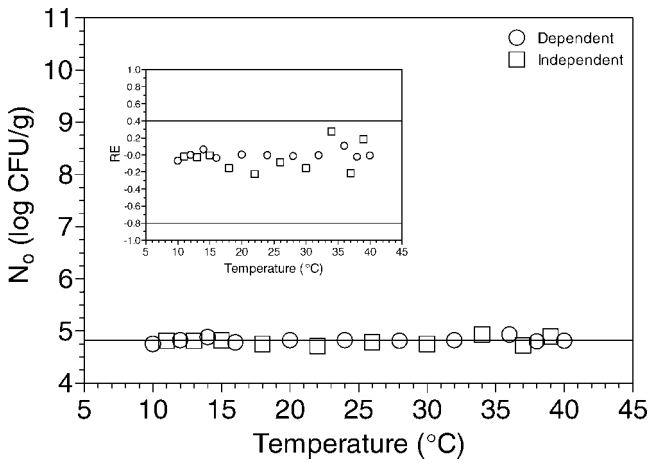

FIGURE 4. *Secondary model fit to data for initial density (*N_0*)* of Salmonella *Typhimurium on sterile chicken as a function of temperature (10 to 40°C). Inset: acceptable prediction zone analysis of the goodness of fit of the secondary model for* N_0*. RE, relative error; dependent data,* N_0 *data used in model development; independent data,* N_0 *data not used in model development.*

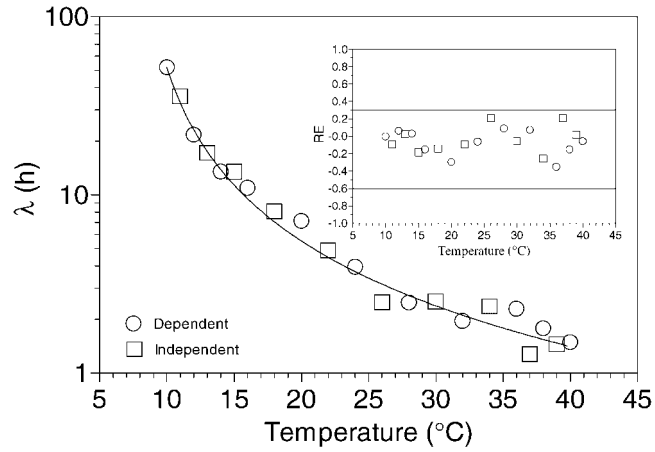

FIGURE 5. *Secondary model fit to data for lag time (λ) of* Salmonella *Typhimurium on sterile chicken as a function of temperature (10 to 40°C). Inset: acceptable prediction zone analysis of the goodness of fit of the secondary model for λ. RE, relative error; dependent data, λ data used in model development; independent data, λ data not used in model development.*

terpolation) data. For both dependent and independent (interpolation) data, all RE (100%) for N_0 were inside the acceptable prediction zone (−0.8 to 0.4; Fig. 4, inset). Thus, the secondary model for N_0 had acceptable goodness of fit and was validated for interpolation. These results also indicated that preparation and delivery of inoculum among growth curves and temperatures was acceptable.

The secondary model for λ as a function of temperature (Fig. 5) was

$$\lambda = [41.47/(T - 7.325)]^{1.44}$$

and the 95% confidence intervals and constraints for fitting this and the other secondary models are presented in Table 1. For both dependent and independent (interpolation) data, all REs (100%) for λ were inside the acceptable prediction zone (−0.6 to 0.3; Fig. 5, inset). Thus, the secondary model for λ had acceptable goodness of fit and was validated for interpolation.

The secondary model for μ_{max} as a function of temperature (Fig. 6) was

$$\mu_{max} = \begin{cases} 0.01885 & \text{if } T \leq 11.43 \\ 0.01885 + [0.004325(T - 11.43)]^{1.306} & \text{if } T > 11.43 \end{cases}$$

For both dependent and independent (interpolation) data, all REs (100%) for μ_{max} were inside the acceptable prediction zone (−0.3 to 0.15; Fig. 6, inset). Thus, the secondary model for μ_{max} had acceptable goodness of fit and was validated for interpolation.

The secondary model for N_{max} as a function of temperature (Fig. 7) was

$$N_{max} = \exp\left\{2.348\left[\frac{(T - 9.64)(T - 40.74)}{(T - 9.606)(T - 40.76)}\right]\right\}$$

For both dependent and independent (interpolation) data, all REs (100%) for N_{max} were inside the acceptable prediction zone (−0.8 to 0.40; Fig. 7, inset). Thus, the secondary model for N_{max} had acceptable goodness of fit and was validated for interpolation.

The tertiary model was evaluated for its ability to predict *N* data used in model development and *N* data not used in model development but inside (interpolation) or outside (extrapolation) its response surface. For dependent data, 117 of 129 REs (%RE = 90.7) were inside the acceptable prediction zone (−0.8 to 0.4; Fig. 8). Thus, the tertiary model had acceptable performance for predicting *N* data used in model development.

Because the same *N* data (i.e., *N* data used in model development) were used to evaluate performance of the tertiary model and goodness of fit of the primary model (Fig.

TABLE 1. *Best-fit value, 95% confidence interval (CI), and constraints for secondary model parameters*

Secondary model	Parameter	Best-fit value	95% CI	Constraints
N_0	Mean N_0	4.822	4.790–4.854	
λ	*p*	41.47	37.38–45.55	
	T_{min}	7.325	7.033–7.617	
	m	1.440		Fixed
μ_{max}	$\mu_{max\text{-}min}$	0.01885	0–0.06124	>0
	T_0	11.43	1.417–21.43	
	b	0.004325	0–0.01543	>0
	m	1.306	0.6283–1.984	
N_{max}	*a*	2.348	2.326–2.369	
	T_{min}	9.640	9.225–10.06	
	T_{max}	40.74	38.96–42.52	
	T_{submin}	9.606	9.149–10.06	
	T_{supmax}	40.76	38.93–42.60	

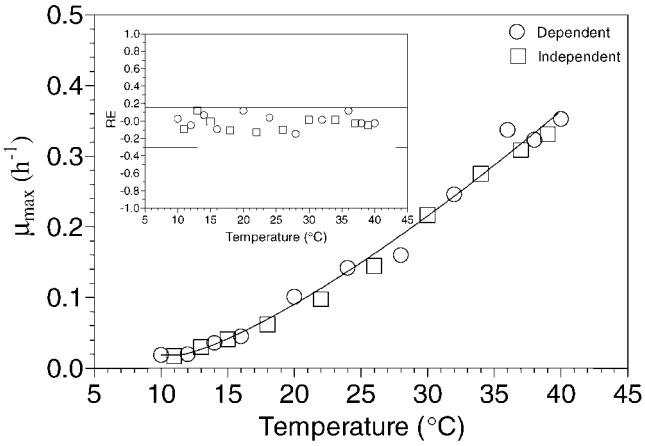

FIGURE 6. *Secondary model fit to data for maximum specific growth rate (μ_{max}) of* Salmonella *Typhimurium on sterile chicken as a function of temperature (10 to 40°C). Inset: acceptable prediction zone analysis of the goodness of fit of the secondary model for μ_{max}. RE, relative error; dependent data, μ_{max} data used in model development; independent data, μ_{max} data not used in model development.*

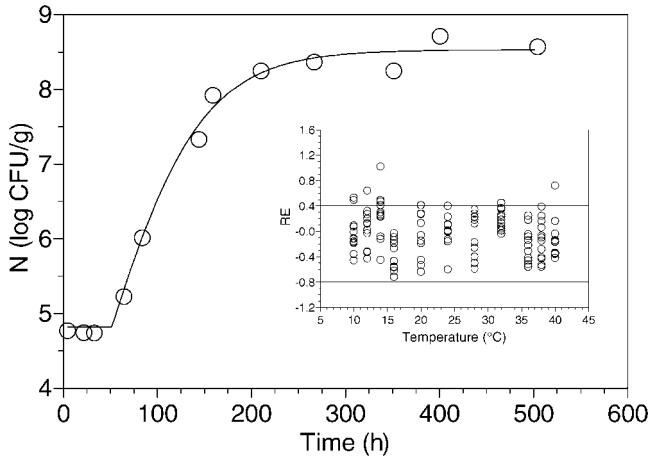

FIGURE 8. *Tertiary model prediction (solid line) of pathogen density (*N*) data (circles) obtained at a single temperature (10°C) for* Salmonella *Typhimurium on sterile chicken. Inset: acceptable prediction zone analysis of tertiary model predictions of* N *data used in model development and obtained at temperatures from 10 to 40°C. RE, relative error.*

3), Fisher's exact test was used to compare performance among these models. Although %RE was lower for the tertiary model (90.7) than for the primary model (93.8) for predicting the same *N* data, the reduction in performance from the primary to the tertiary modeling step was not significant ($P = 0.48$). Thus, model error from combining the primary model with four secondary models to create the tertiary model was small.

For independent *N* data that were inside the response surface, 39 of 40 REs (%RE = 97.5) for the tertiary model were inside the acceptable prediction zone (−0.8 to 0.4; Fig. 9). Thus, the tertiary model was validated for interpolation.

For independent *N* data that were outside the response surface because they were from challenge studies conducted with a lower N_0 ($10^{0.8}$ CFU/g), only 1 of 40 REs (%RE = 2.5) for the tertiary model were in the acceptable prediction zone (−0.8 to 0.4; Fig. 10). All REs were positive, which indicated that the tertiary model provided overly fail-dangerous predictions of *N* from a lower N_0. These results indicated that the tertiary model developed for high N_0 was not validated for extrapolation of *N* from a lower N_0.

DISCUSSION

In primary and secondary modeling, models are iteratively fit to data, and therefore comparison of model pre-

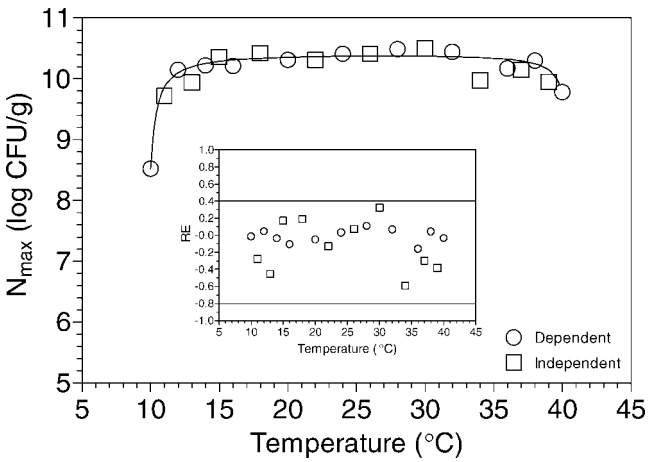

FIGURE 7. *Secondary model fit to data for maximum pathogen density (N_{max}) of* Salmonella *Typhimurium on sterile chicken as a function of temperature (10 to 40°C). Inset: acceptable prediction zone analysis of the goodness of fit of the secondary model for N_{max}. RE, relative error; dependent data, N_{max} data used in model development; independent data, N_{max} data not used in model development.*

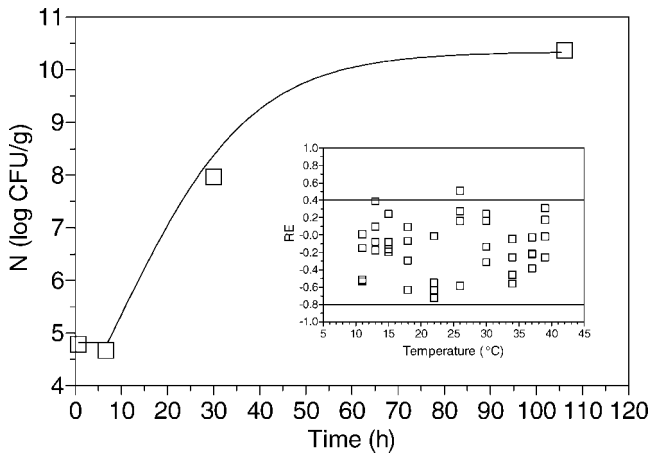

FIGURE 9. *Tertiary model prediction (solid line) of pathogen density (*N*) data (squares) obtained at a single temperature (18°C) for evaluation of interpolation of* Salmonella *Typhimurium growth on sterile chicken. Inset: acceptable prediction zone analysis of tertiary model predictions of* N *data for evaluation of interpolation at temperatures from 11 to 39°C. RE, relative error.*

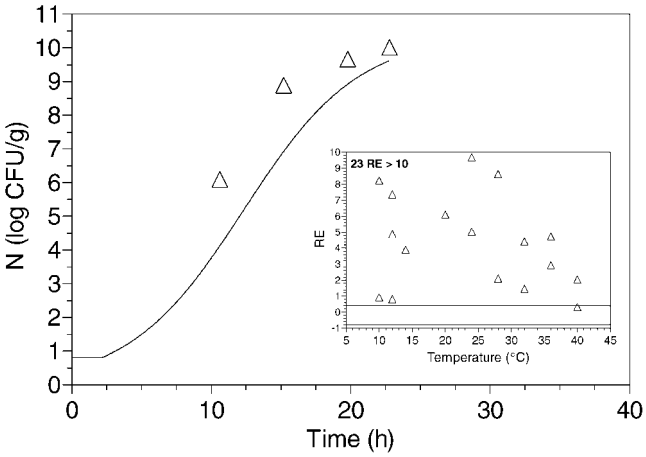

FIGURE 10. *Tertiary model prediction (solid line) of pathogen density (*N*) data (triangles) obtained at a single temperature (32°C) for evaluation of extrapolation of* Salmonella *Typhimurium growth on sterile chicken from a lower initial density ($10^{0.8}$ CFU/g). Inset: acceptable prediction zone analysis of tertiary model predictions of* N *data for evaluation of extrapolation of growth from a lower initial density ($10^{0.8}$ CFU/g) at temperatures from 10 to 40°C. For clarity of presentation, relative errors (REs) greater than 10 (*n = 23*) were not included.*

dictions to dependent data assesses goodness of fit. In contrast, combining primary and secondary models creates tertiary models. Therefore, comparison of tertiary model predictions to dependent data does not assess goodness of fit but rather verifies that the tertiary model was properly constructed.

Some researchers have proposed that predictive models for food pathogens can err more in the fail-safe direction *(16).* With the acceptable prediction zone method *(11),* evaluation of model performance is based on this assumption. The reasons for using the acceptable prediction zone method rather than other methods, such as the bias and accuracy factors of Ross *(15),* to evaluate predictive model performance have been fully addressed previously *(11).*

Use of predictions of growth parameters from secondary models outside the primary model from which they were derived can result in inaccurate predictions of *N* because growth parameter values derived from the same *N* data differ among primary models. For example, Buchanan et al. *(4)* found that the three-phase linear model predicted shorter λ and longer generation times (by 22 to 32%) than did the Gompertz and Baranyi primary models and that the Gompertz model predicted lower N_{max} than did the three-phase linear and Baranyi primary models. Augustin and Carlier *(1)* found differences among primary models for estimates of λ and growth rate. The extent of these differences depends on the quality of kinetic data, and these differences do not follow a pattern *(2).* Therefore, use of correction factors to normalize estimates of growth parameters among primary models, an approach used by some, is not recommended *(2).* Thus, to avoid inaccurate predictions of *N,* it is important to use predicted growth parameters from secondary models in the primary model from which they were derived, such as was done in the tertiary modeling step of the current study (Fig. 1).

Creation of a tertiary model allows prediction of *N* for conditions not used in model development. A potential disadvantage of using tertiary rather than primary models to predict *N* is that prediction errors of secondary models are combined, resulting in poorer model performance. For example, %RE decreased from 93.8 to 90.7 between the primary and tertiary modeling steps in the current study. However, this reduction in model performance was not significant.

Although it is only necessary to evaluate performance of the tertiary model, when prediction problems occur it is also important to evaluate performance of primary and secondary models to find the source of the problems. Prediction problems can result from experimental error, model error, and biological variation *(7).* It may be necessary to repair a tertiary model by collecting additional data and by fitting existing and new data to other primary and secondary models *(11).* In the current study, no prediction problems occurred in the tertiary model or in the primary and secondary models from which it was created. All secondary models had acceptable goodness of fit and were validated for interpolation.

Ross et al. *(16)* proposed criteria for validating predictive models for growth rate using the bias factor (B_f). They proposed that growth rate models with B_f of 0.9 to 1.05 are good, models with B_f of 0.7 to 0.9 or 1.06 to 1.15 are acceptable, and models with B_f of <0.7 or >1.15 are unacceptable. These criteria are the basis for the acceptable prediction zone used to evaluate the performance of secondary models for μ_{max} *(11).* These criteria also have been applied to other growth rate models *(7)* and λ models *(11)* but not to models that predict *N.*

In the current study, criteria for models that predict *N* (i.e., primary, secondary for N_0, secondary for N_{max}, and tertiary) were developed based on an evaluation of experimental error associated with determination of *N* by viable counts. This approach provided a conservative estimate of experimental error because it considered variation of *N* only among serial dilutions of the same sample and not among duplicate samples; that variation comes from other sources of experimental error (e.g., variation of N_0 due to pipetting error) encountered in challenge studies. The 95th percentile for the ARE of *N* among serial dilutions was 0.4. This value was used to establish a wide acceptable prediction zone for RE from −0.8 (fail safe) to 0.4 (fail dangerous) for evaluation of model performance for *N.* The wide acceptable prediction zone for *N* reflects the imprecision of the viable count method.

In addition to criteria for *N* models, new criteria for λ models were established in this study based on an examination of experimental error associated with determining this growth parameter in challenge studies. In a previous study *(9),* variation of λ among replicate growth curves for *Salmonella* on sterile chicken was twice as large as μ_{max}. Thus, the acceptable prediction zone for evaluation of the λ model in the current study was set at twice the width (−0.6 to 0.3 versus −0.3 to 0.15) of the acceptable prediction zone for evaluation of the μ_{max} model.

Coleman et al. *(5)* compared growth from low N_0 (1

to 10 CFU/ml) and high N_0 (10^3 CFU/ml) for *Escherichia coli* O157:H7 in sterile broth at 10, 19, and 37°C and found few differences in growth parameters. Likewise, under optimal conditions in sterile broth, λ of *Listeria monocytogenes* is not different from low N_0 ($10^{0.1}$ CFU/ml) to high N_0 (10^7 CFU/ml) *(14).* In contrast, growth of *Salmonella* Typhimurium on sterile chicken from a low N_0 ($10^{0.8}$ CFU/g) in this study was much more rapid than growth from a high N_0 ($10^{4.8}$ CFU/g). All REs for *N* were positive, indicating that the high N_0 model underpredicted growth from a lower N_0. Because *Salmonella* concentrations on chicken are closer to $10^{0.8}$ than $10^{4.8}$ CFU/g *(17, 19, 20),* the tertiary model was not considered appropriate for assessing chicken safety.

Although the tertiary model developed here was not validated for use in the chicken industry, this study is important because it is the first in which performance of a tertiary model that predicts *N* has been quantified. This study is also the first in which criteria were developed and applied to allow a decision as to whether a tertiary model and its component models (i.e., primary and secondary models) provided acceptable predictions of pathogen growth. Experimental error associated with imprecision of the enumeration assay was a more important source of prediction error than was modeling error; very little loss in predictive performance was observed between the primary and tertiary modeling steps.

ACKNOWLEDGMENTS

The author appreciates the excellent technical and administrative assistance of J. Ludwig and P. Shannon (Agricultural Research Service, U.S. Department of Agriculture) and D. Blakeney (University of Maryland Eastern Shore).

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