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Extrapolation of a Predictive Model for Growth of a Low Inoculum Size of *Salmonella* Typhimurium DT104 on Chicken Skin to Higher Inoculum Sizes†

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ABSTRACT

Validation of model predictions for independent variables not included during model development can save time and money by identifying conditions for which new models are not needed. A single strain of *Salmonella* Typhimurium DT104 was used to develop a general regression neural network (GRNN) model for growth of a low inoculum size (0.9 log) on chicken skin with native microflora as a function of time (0 to 8 h) and temperature (20 to 45°C). The ability of the GRNN model to predict growth of higher inoculum sizes (2, 3, or 4.1 log) was evaluated. When the proportion of residuals in an acceptable prediction zone (pAPZ) from −1 log (fail-safe) to 0.5 log (fail-dangerous) was ≥0.7, the GRNN model was classified as providing acceptable predictions of the test data. The pAPZ for dependent data was 0.93 and for independent data for interpolation was 0.88. The pAPZs for extrapolation to higher inoculum sizes of 2, 3, or 4.1 log were 0.92, 0.73, and 0.77, respectively. However, residual plots indicated local prediction problems with pAPZs of <0.7 for an inoculum size of 3 log at 30, 35, and 40°C and for an inoculum size of 4.1 log at 35 and 40°C where predictions were fail-dangerous, indicating faster growth at higher inoculum sizes. The model provided valid predictions of *Salmonella* Typhimurium DT104 growth on chicken skin from inoculum sizes of 0.9 and 2 log at all temperatures investigated and from inoculum sizes of 3 and 4.1 log at some but not all temperatures investigated. Thus, the model can be improved by including inoculum size as an independent variable.

Predictive microbiology has an important role to play in efforts to improve food safety. Predictive models can be used to help assess the impact of food handling and storage conditions on pathogen levels in food and the risk to public health. An important step in model development is validation that predictions have acceptable accuracy and bias *(20)*. Evaluation of predictive models in the author's laboratory consists of three stages *(12)*: (i) demonstration of acceptable goodness of fit to dependent data; (ii) demonstration of acceptable predictions of independent data for interpolation; and (iii) demonstration of acceptable predictions of independent data for extrapolation. Validation for extrapolation to independent variables not included during model development can save time and money by identifying conditions for which new models are not needed.

Salmonella organisms are a leading cause of foodborne illness, and poultry foods are often implicated as sources of salmonellosis in humans *(1)*. Some studies *(9, 10)* indicate that *Salmonella* growth in food is not affected by inoculum size, whereas other studies *(2, 5, 11, 14)* indicate the opposite. Predictive models are usually developed with one inoculum size because of the cost associated with including it as an independent variable. In the present study, a predictive model for growth of a low inoculum size of *Salmonella* Typhimurium DT104 on chicken skin with native microflora was developed and then evaluated for its predictions of growth from higher inoculum sizes. The purpose of this study was to determine whether or not inoculum size should be included as an independent variable in the model.

MATERIALS AND METHODS

Organism. A multiple-antibiotic-resistant strain (ATCC 700408) of *Salmonella enterica* serotype Typhimurium definitive phage type 104 (DT104) was obtained from a commercial source (American Type Culture Collection, Manassas, VA). This serotype was selected because it is a predominant serotype isolated from chickens in the author's geographical region *(17, 19)*. In addition, this strain was used because it has a phenotype that can be quantified in the presence of native microflora by plating on agar media with multiple antibiotics *(13)*. A stock culture of this strain was maintained at −70°C in brain heart infusion broth (Difco, BD, Sparks, MD) that contained 15% (vol/vol) glycerol (Sigma, St. Louis, MO).

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TABLE 1. *Experimental designs for model development and evaluation*

Data	No. of replicates	Temp (°C)	Time (h)	Inoculum (log)[a]
Development	4	20, 25, 30, 35, 40, 45	0, 2, 4, 6, 8	0.9 ± 0.05
Interpolation	2	22.5, 27.5, 32.5, 37.5, 42.5	0, 2, 4, 6, 8	0.9 ± 0.07
Extrapolation	2	20, 25, 30, 35, 40, 45	0, 2, 4, 6, 8	2 ± 0.05
Extrapolation	2	20, 25, 30, 35, 40, 45	0, 2, 4, 6, 8	3 ± 0.07
Extrapolation	2	20, 25, 30, 35, 40, 45	0, 2, 4, 6, 8	4.1 ± 0.06

[a] Mean ± standard deviation.

Plating media. XLT4 base agar medium (BD) supplemented with 25 mM HEPES and 25 μg of antibiotics chloramphenicol (C), ampicillin (A), tetracycline (T), and streptomycin (S) per ml (hereinafter referred to as CATS) was used for drop plating and spiral plating. All CATS supplements were from Sigma.

Chicken skin preparation. Chicken thighs with native microflora were purchased weekly at local retail stores. The skin was removed, placed on a plastic cutting board, held at −70°C for 15 min, and then cut into circular portions (2.14 cm^2) with a no. 10 cork-borer. A different batch of chicken skin portions was prepared and used for each replicate storage trial.

Inoculum. Stationary-phase cells of *Salmonella* Typhimurium DT104 for inoculation of chicken skin portions were obtained by adding 5 μl of stock culture to 5 ml of brain heart infusion broth in a 25-ml Erlenmeyer flask sealed with a foam plug followed by incubation at 30°C and 150 rpm for 23 h.

Experimental designs. Full factorial designs were used for model development and evaluation (Table 1). Independent data for model evaluation for interpolation were collected at temperatures intermediate to those used during model development but at the same storage times. Independent data for model evaluation for extrapolation were obtained by inoculating chicken skin portions with higher inoculum sizes of *Salmonella* Typhimurium DT104. Four (20, 30, 35, 40, 45°C) or five (25°C) replicate storage trials were conducted per temperature for model development, whereas two replicate storage trials were conducted per temperature for model evaluation.

Chicken skin inoculation. Chicken skin portions with native microflora were spot inoculated with 5 μl of a 10^{-7}, 10^{-6}, 10^{-5}, or 10^{-4} dilution of the 23-h inoculum culture to obtain four inoculum sizes (Table 1). Serial dilutions (1:10) were prepared in sterile buffered peptone water (BPW; BD).

Sample processing. Individual chicken skin portions were placed in 9 ml of BPW in a 207-ml capacity bag with filter screen (Whirl-Pak, Nasco, Fort Atkinson, WI). Samples were pulsified (Pulsifier model PUL 100, Microbiology International, Frederick, MD) for 1 min to recover *Salmonella* Typhimurium DT104 into BPW, which was then used for pathogen enumeration. Duplicate samples were processed at each sampling time, and results were averaged to obtain the log number of *Salmonella* Typhimurium DT104 at sampling time t.

MPN assay. A three-tube most-probable-number (MPN) assay in BPW (three replicates with 4 dilutions each) was used to enumerate levels of *Salmonella* Typhimurium DT104 from 0 to 4 log per chicken skin portion *(13)*. Because the entire sample including the chicken skin portion was included in the MPN assay, the lower limit of detection was 0 log per chicken skin portion. MPN tubes were incubated for 24 h at 38°C, and then 5 μl from each MPN tube and sample bag with chicken skin portion were drop plated onto CATS. Positive MPN tubes produced a black colony on CATS after 24 h of incubation at 38°C.

Viable counts. Higher levels (>4 log per chicken skin portion) of *Salmonella* Typhimurium DT104 were enumerated by spiral plating because this method was less labor-intensive than the MPN method. Pulsified samples were serially diluted (1:10) in BPW, and then 50-μl volumes of appropriate dilutions were spiral plated (WASP, Microbiology International) onto CATS. Spiral plates were incubated for 24 h at 38°C before automated counting of black colonies (ProtoCol, Microbiology International).

Data analysis. MPN was determined by use of an MPN table, and these values and CFU data were expressed as log number change (Δ) per skin portion:

$$\Delta = N(t) - N_0$$

where $N(t)$ was the log number of *Salmonella* Typhimurium DT104 cells per chicken skin portion at sampling time t and N_0 was the initial log number of *Salmonella* Typhimurium DT104 cells per chicken skin portion.

Model development. A general regression neural network (GRNN) model *(21)* was developed with MPN and CFU data for growth of a low inoculum size (0.9 log) of *Salmonella* Typhimurium DT104 on chicken skin stored at 20 to 45°C for 0 to 8 h (Table 1). A data set was created in a computer spreadsheet (Excel 2007, Microsoft, Redmond, WA) with separate columns for temperature (independent numerical variable in degrees Celsius), time (independent numerical variable in hours), and Δ (dependent numerical variable in log change). The model was trained by using a spreadsheet add-in program (Neural Tools version 5.5, Palisade, Newfield, NY).

The model had an input layer with two nodes, one per independent variable, and a pattern layer with one node per observed value (Fig. 1). Each node in the pattern layer computed its distance from the presented observed value:

$$D(T,t) = \sum_{j=1}^{p} \left(\frac{x_j - x_{ij}}{\sigma_j} \right)^2$$

where $D(T,t)$ was the distance function for independent variables (x) of temperature (T), and time (t), j is the index for independent variables that ran from 1 to p, i is the index for observed values that ran from 1 to n, and σ is the smoothing factor. Each calculated distance was passed to the summation layer where the summed values from the numerator node, $N(x)$, were divided by the summed values from the denominator node, $D(x)$, to obtain the predicted value in the output layer:

$$\hat{y}(x) = \frac{\sum_{i=1}^{n} y_i \exp(-D(T,t))}{\sum_{i=1}^{n} \exp(-D(T,t))} = \frac{N(x)}{D(x)}$$

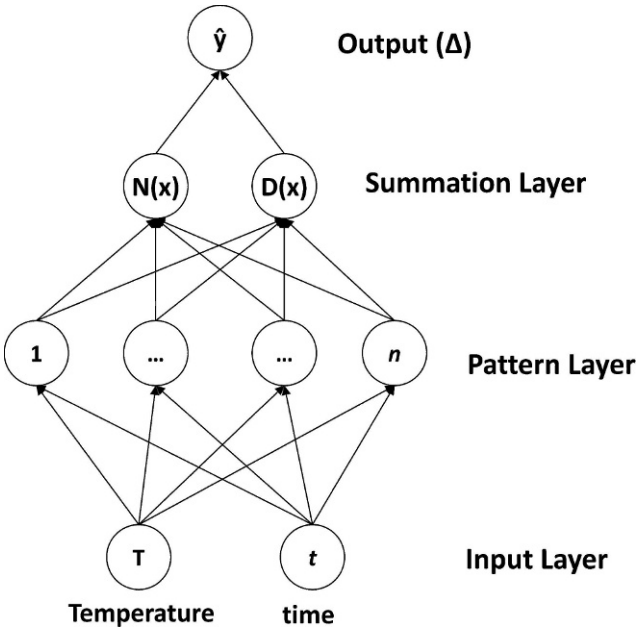

FIGURE 1. *Diagram showing the structure of the general regression neural network used in model development and evaluation.*

where y was the observed value and $\hat{y}$ was the predicted value. Training of the model involved optimizing smoothing factors to minimize mean squared error (e):

$$e = (\hat{y} - y)^2$$

Smoothing factors of the GRNN model could not be extracted from the commercial software application for proprietary reasons. However, the GRNN model will be incorporated into the U.S. Department of Agriculture Pathogen Modeling Program, and the MPN and CFU data used in model development and evaluation will be archived in ComBase. The Pathogen Modeling Program and ComBase can be accessed at http://portal.arserrc.gov/. Alternatively, the GRNN model can be obtained from the author and then run with the commercial software applications mentioned above.

Model performance. Data obtained at 2, 4, 6, and 8 h of storage were used to evaluate model performance. Data obtained at 0 h of storage were not used because residuals were zero due to calculation of Δ. Model performance was evaluated by the acceptable prediction zone method *(11, 12)*. In brief, the proportion of residuals (observed Δ − predicted Δ) in an acceptable prediction zone (pAPZ) from −1 log (fail-safe) to 0.5 log (fail-dangerous) was determined. When pAPZ was ≥0.7, the model was classified as providing acceptable predictions of the test data. The model was evaluated for goodness of fit, interpolation, and extrapolation. Residuals were graphed as a function of independent variables, and pAPZs were determined for individual growth curves or storage temperatures to evaluate the GRNN model for local prediction problems.

RESULTS

Data used in development of the GRNN model were previously used to develop a regression (REG) model using the primary, secondary, and tertiary modeling approach *(16)*. The primary model used in the REG model was the Baranyi model. Predictions of the REG model were compared with predictions of the GRNN model (Table 2). For four of the five sets of data, the GRNN model provided better (i.e., higher-pAPZ) predictions than the REG model. Consequently, only results for the GRNN model are presented for evaluating the ability of a predictive model developed with a low inoculum size to extrapolate to higher inoculum sizes.

TABLE 2. *Evaluation of model performance: comparison of regression (REG) and general regression neural network (GRNN) models*

Data	Inoculum (log)	n_{trials}	$n_{evaluate}$	pAPZ[a]	
				REG	GRNN
Development	0.9	25	100	0.84	0.93
Interpolation	0.9	10	40	0.92	0.88
Extrapolation	2	12	48	0.83	0.92
Extrapolation	3	12	48	0.58	0.73
Extrapolation	4.1	12	48	0.67	0.77

[a] Proportion of residuals in an acceptable prediction zone from −1 log (fail-safe) to 0.5 log (fail-dangerous).

Figures 2 and 3 provide a visual summary of the growth responses and quality of kinetic data used to develop and evaluate model performance in the current study. It should be noted that growth of *Salmonella* Typhimurium DT104 was observed at all temperatures investigated and that the optimum temperature for growth was 40°C.

Figure 4 shows results of the acceptable prediction zone analysis for each of the test data sets. Table 2 reports the overall pAPZs for the GRNN model predictions of the test data sets, whereas Figure 4 shows the pAPZs for individual growth curves or storage temperatures. The latter pAPZs were used to identify local prediction problems (i.e., pAPZs for individual growth curves or storage temperatures that were <0.7).

The overall pAPZ for dependent data was 0.93 (Table 2), and the residual plot (Fig. 4A) did not show signs of local prediction problems, as all pAPZs for individual growth curves or storage temperatures were >0.7. Thus, the GRNN model had acceptable goodness of fit.

The overall pAPZ for independent data collected at intermediate temperatures was 0.88 (Table 2), and the residual plot (Fig. 4B) indicated no local prediction problems, as all pAPZs for individual growth curves or storage temperatures were >0.7. Thus, the GRNN model was validated for interpolation.

The overall pAPZ for independent data collected with a higher inoculum size of 2 log was 0.92 (Table 2), and the residual plot (Fig. 4C) did not show signs of local prediction problems, as all pAPZs for individual growth curves or storage temperatures were >0.7. Thus, the GRNN model was validated for extrapolation to an inoculum size of 2 log.

The overall pAPZ for independent data collected with an inoculum size of 3 log was 0.73 (Table 2). However, the residual plot (Fig. 4D) indicated local prediction problems with fail-dangerous predictions (i.e., residuals of >0.5 log) at 30, 35, and 40°C where pAPZs were <0.7. Thus, the

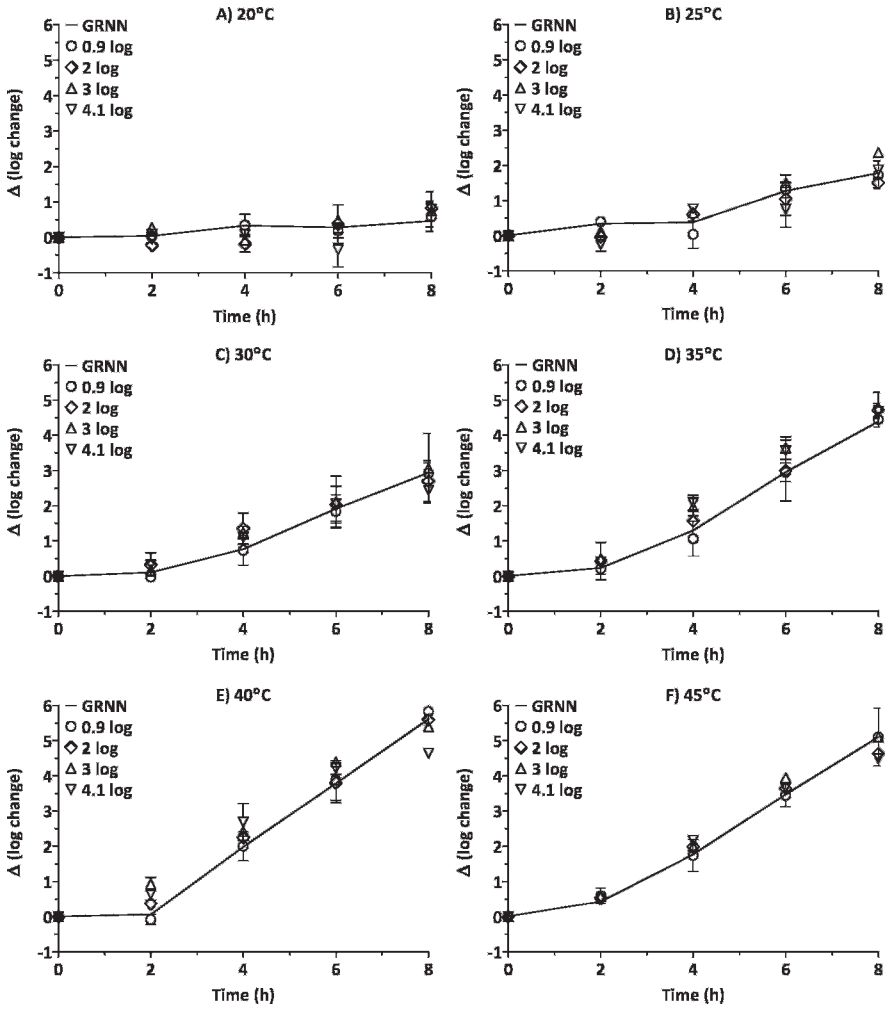

FIGURE 2. *Growth of different inoculum sizes of* Salmonella *Typhimurium DT104 on chicken skin stored at (A) 20°C, (B) 25°C, (C) 30°C, (D) 35°C, (E) 40°C, or (F) 45°C. Symbols represent mean values ± standard deviations.*

GRNN model was only validated for extrapolation to an inoculum size of 3 log at 20, 25, and 45°C.

The overall pAPZ for independent data collected with an inoculum size of 4.1 log was 0.77 (Table 2). Again, the residual plot (Fig. 4E) indicated local prediction problems with fail-dangerous predictions (i.e., residuals of >0.5 log) at 35 and 40°C where pAPZs were <0.7. Thus, the GRNN model only provided valid predictions for extrapolation to an inoculum size of 4.1 log at 20, 25, 30, and 45°C.

Figure 5 shows the final spreadsheet form of the GRNN model. Based on results of the acceptable prediction zone analysis (Fig. 4), the GRNN model can be used with confidence to predict growth of low inoculum sizes (≤2 log) of *Salmonella* Typhimurium DT104 on chicken skin with native microflora that are within the ranges of time (0 to 8 h) and temperature (20 to 45°C) used in model development.

DISCUSSION

In a previous study *(14),* the author compared growth of a small (1.1 log) and a large (3.7 log) inoculum size of *Salmonella* Typhimurium DT104 on ground chicken breast meat with native microflora that was stored for >40 h at 10 to 40°C. He found that inoculum size affected pathogen growth with the largest effect on maximum population density followed by lag time and then growth rate. In the present study, a small effect of inoculum size on growth of the same strain of *Salmonella* Typhimurium DT104 on chicken skin with native microflora was observed during storage at 20 to 45°C for 8 h, which was not long enough for growth to reach maximum levels. Had longer storage times been studied, a larger effect of inoculum size on pathogen growth might have been observed. However, it is unlikely that chicken producers or consumers would store chicken at 20 to 45°C for greater than 8 h and still sell or consume the product, respectively. Thus, it seemed reasonable to limit the storage trials to 8 h, which is the normal time for a production run in a chicken processing plant. Lower growth temperatures (5 to <20°C) were not included in this study because the lag time of *Salmonella* Typhimurium DT104 on chicken skin is greater than 8 h at these storage temperatures *(16).*

Mackey and Kerridge *(10)* conducted a similar study in which they inoculated minced beef with a cocktail of antibiotic-resistant serotypes of *Salmonella* and then studied the effect of inoculum size on growth at 10 to 35°C. They found that inoculum size (1.6 versus 4 log) did not alter lag time or growth rate. In contrast, the author found that growth of *Salmonella* Typhimurium DT104 on chicken skin with native microflora at 35°C was slightly faster from inoculum sizes of 3 and 4.1 log than from 0.9 log and at 30°C was slightly faster from an inoculum size of 3 log.

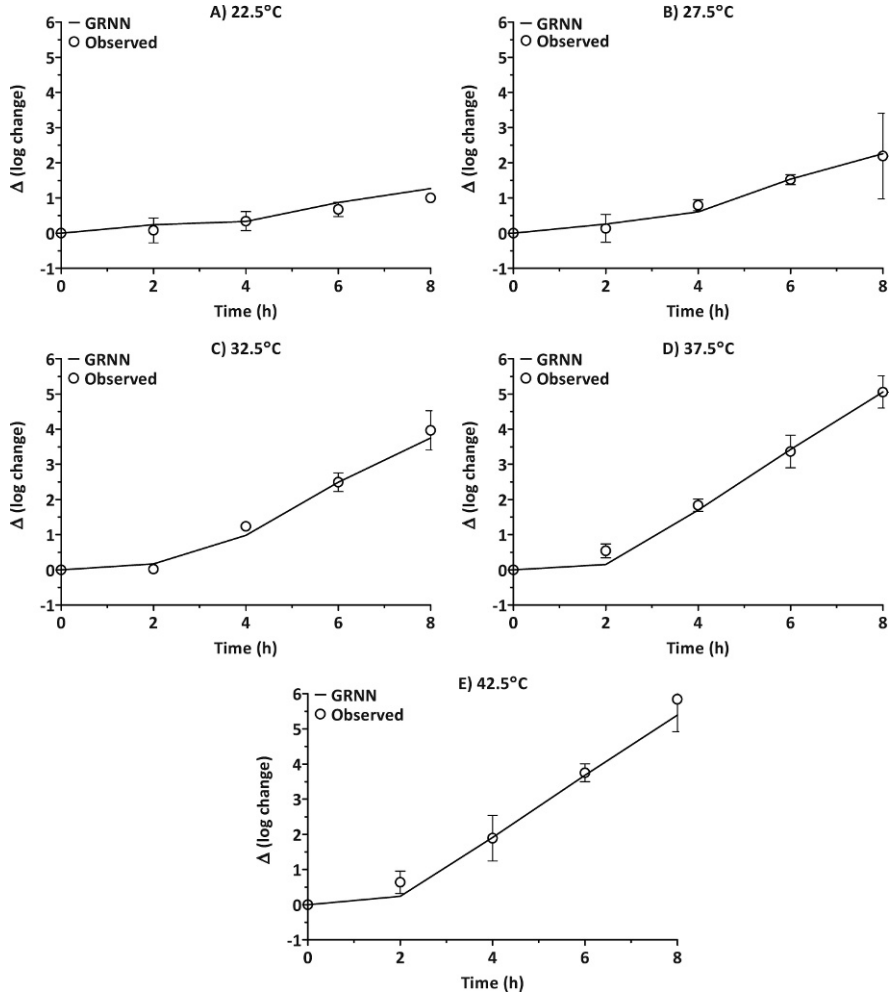

FIGURE 3. *Growth of a low inoculum size (0.9 log) of* Salmonella *Typhimurium DT104 on chicken skin stored at (A) 22.5°C, (B) 27.5°C, (C) 32.5°C, (D) 37.55°C, or (E) 42.5°C. Symbols represent mean values ± standard deviations.*

Interestingly, in an earlier study with sterile chicken breast meat *(11),* the author observed faster growth from a low inoculum size (0.8 log) than from a higher inoculum size (4.8 log) at all storage temperatures from 10 to 40°C, which could indicate that native microflora interacts with inoculum size to affect growth of *Salmonella* Typhimurium DT104 in the chicken meat matrix.

Liao *(9)* reported that the growth rate of a cocktail of antibiotic-resistant serotypes of *Salmonella* on sprouting alfalfa seeds at 20°C was not affected by inoculum sizes from <1 to 3 log. In agreement with Mackey and Kerridge and with Liao, the author found that inoculum size did not affect growth of *Salmonella* Typhimurium DT104 on chicken skin at 20°C in the present study. Together these results indicate that the effect of inoculum size on growth of *Salmonella* in food is complex and as with other pathogens may differ depending on the interaction of several factors, such as pathogen strain, food structure, storage temperature, gas atmosphere, and native microflora *(3, 6).* Thus, inclusion of inoculum size as an independent variable for *Salmonella* in predictive models seems justified, as previously suggested *(14).*

Performance of predictive models is usually evaluated by calculation of bias (B_f) and accuracy (A_f) factors *(20).* When B_f and A_f meet the criteria for performance, the model is classified as providing acceptable or valid predictions of the test data. Because models used to predict food safety can err more in the fail-safe direction than in the fail-dangerous direction, criteria for acceptable performance are wider in the fail-safe direction. For example, the criterion for acceptable model performance for B_f is twice as wide in the fail-safe direction as in the fail-dangerous direction *(20).*

The B_f and A_f method was developed for secondary models that predict generation time as a function of independent variables *(20).* Although this method has been successfully applied to secondary models for lag time, it yields inaccurate results when applied to models that predict log numbers of microbes, such as primary models, secondary models for maximum population density, and tertiary models *(15).* In contrast, the acceptable prediction zone method provides an accurate assessment of the performance of these types of models and therefore was used in the present study rather than the B_f and A_f method *(12, 15).*

The acceptable prediction zone method was applied for the first time to individual growth curves or storage temperatures in the present study. This assisted the author in identifying local prediction problems. Had the method only been applied to entire data sets or all storage temperatures at once, a different conclusion would have been obtained. Namely, the GRNN model would have been validated for extrapolation to higher inoculum sizes when in

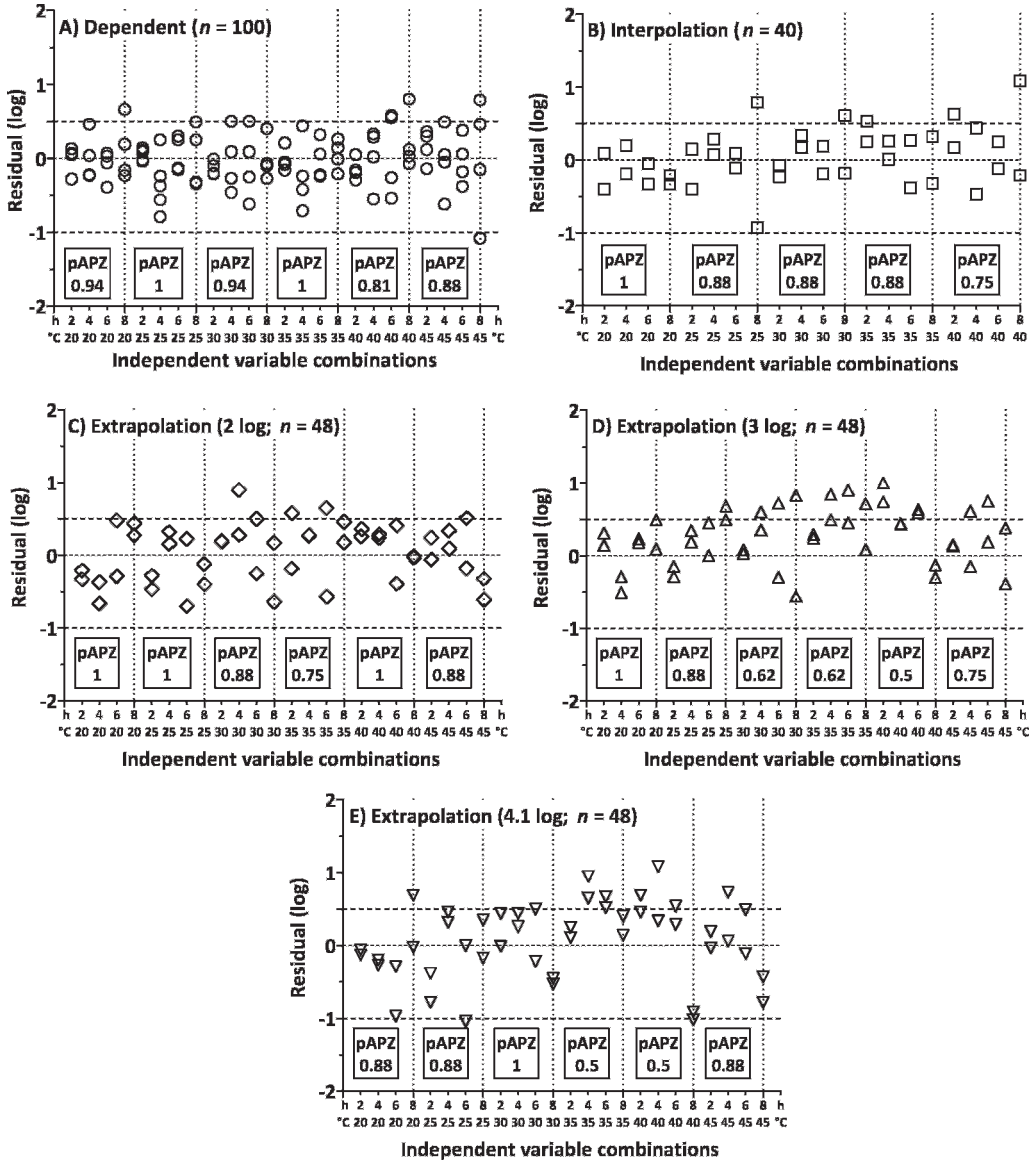

FIGURE 4. *Residual plots and acceptable prediction zone analysis of model performance for (A) dependent data collected with an inoculum size of 0.9 log, (B) independent data for interpolation collected with an inoculum size of 0.9 log, (C) independent data for extrapolation to a higher inoculum size of 2 log, (D) independent data for extrapolation to a higher inoculum size of 3 log, and (E) independent data for extrapolation to a higher inoculum size of 4.1 log. The proportion of residuals in an acceptable prediction zone (pAPZ) from −1 log (fail-safe) to 0.5 log (fail-dangerous) was calculated for individual growth curves.*

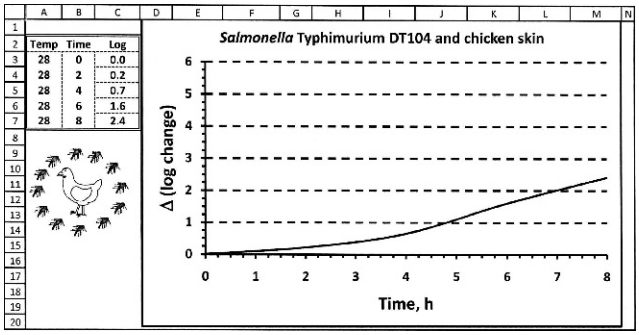

FIGURE 5. *General regression neural network model for predicting growth of low inoculum sizes (≤2 log) of* Salmonella *Typhimurium DT104 on chicken skin stored for 0 to 8 h at 20 to 45°C. The model requires off-the-shelf software (Excel and Neural Tools) to run.*

fact it did not provide acceptable predictions under all storage temperatures and inoculum sizes investigated. Consequently, results of this study indicated that inclusion of inoculum size as an independent variable would improve model performance.

There is general consensus in predictive microbiology that models should be evaluated against independent data. However, there is not general consensus on how those independent data should be collected. In most cases, independent data used to ''validate'' predictive models result in confounded comparisons and invalid conclusions about model performance. For example, when the performance of a model developed with strain A in broth culture is compared with data collected with strain B in food, it is not possible to conclude whether or not the model provides

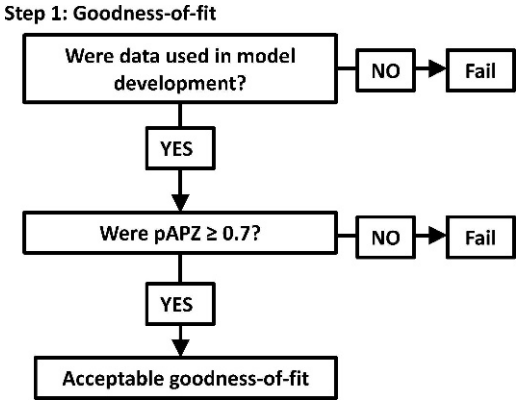

FIGURE 6. *Flow diagram of the first step in the process used to develop, evaluate, and potentially validate predictive models (see the text for more details).*

acceptable predictions of the other strain or the food because comparisons are confounded. Moreover, if independent data only correspond to some conditions used in model development, only partial validation of the model is possible. In fact, if independent data are not uniformly distributed within the matrix of independent variables used in model development, then the resulting model evaluation will be biased. Thus, design of experiments for collecting data to properly evaluate model performance is important. In other words, criteria for test data as well as model performance are needed if a model evaluation is going to result in a model validation.

In a previous study *(12),* the author described a systematic process for model evaluation and validation that involves criteria for both test data and model performance. This process is important because it provides users of the author's models with confidence that they provide predictions with acceptable accuracy and bias. Here, the author revisits and updates this process because it is yet to be widely adopted by the larger predictive microbiology community.

Figures 6 to 8 show flow diagrams of the process for evaluating and validating predictive models. This process is most appropriately applied to tertiary models (e.g., current model) that predict changes in log numbers of microbes as a function of independent variables, which should be the targeted end point of predictive microbiology modeling studies. It can also be applied to primary and secondary models to find potential sources of fatal prediction errors in tertiary models if a regression approach rather than a neural network modeling approach is used *(11).* In this process, calculation of pAPZs and residual plots compose model evaluation, whereas meeting of criteria for test data and model performance compose model validation. In other words, model evaluation involves comparing observed and predicted values, and model validation involves meeting criteria for test data and model performance.

The first step of this process (Fig. 6) is to evaluate the model for goodness of fit by comparing predicted values to observed data used in model development (i.e., dependent data). The only criterion for test data is that they were used in model development. The criterion for model performance is the same for all three steps; pAPZs for individual growth curves or combinations of independent variables must be ≥0.7.

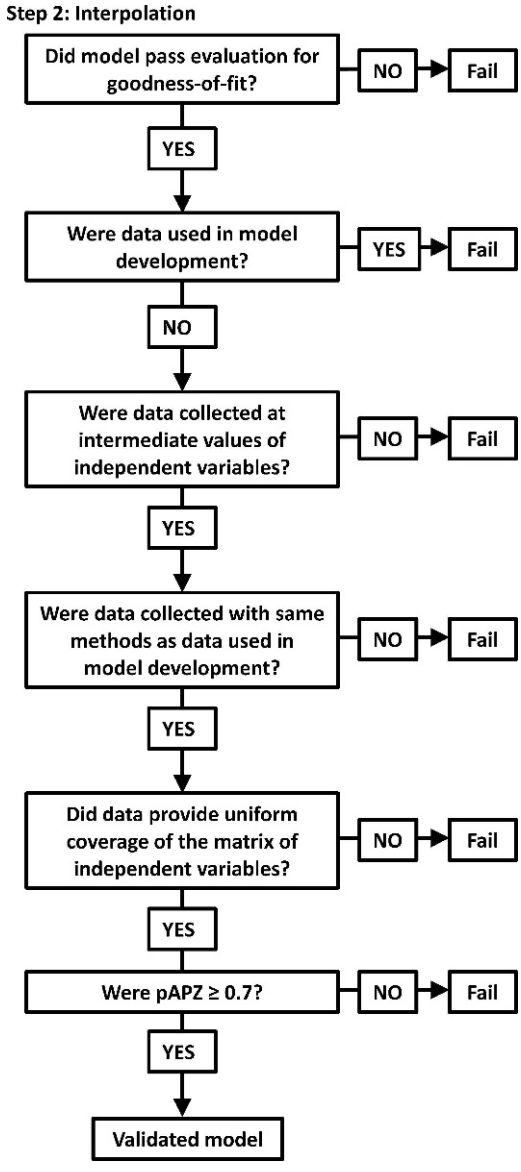

FIGURE 7. *Flow diagram of the second step in the process used to develop, evaluate, and potentially validate predictive models (see the text for more details).*

The second step of this process (Fig. 7) is to evaluate the model for interpolation by comparing predicted values to observed data collected with the same experimental methods but at intermediate values of independent variables. The criteria for test data are (i) that they were not used in model development (i.e., independent); (ii) that they be collected with the same experimental methods (e.g., same strain, same previous history of inoculum, same food matrix, same enumeration methods, etc.); (iii) that they be collected for intermediate combinations of independent variables; and (iv) that a uniform distribution of data throughout the matrix of independent variables was used in model development so as to provide an unbiased and complete assessment of model performance. In addition to the aforementioned criterion for model performance (pAPZ

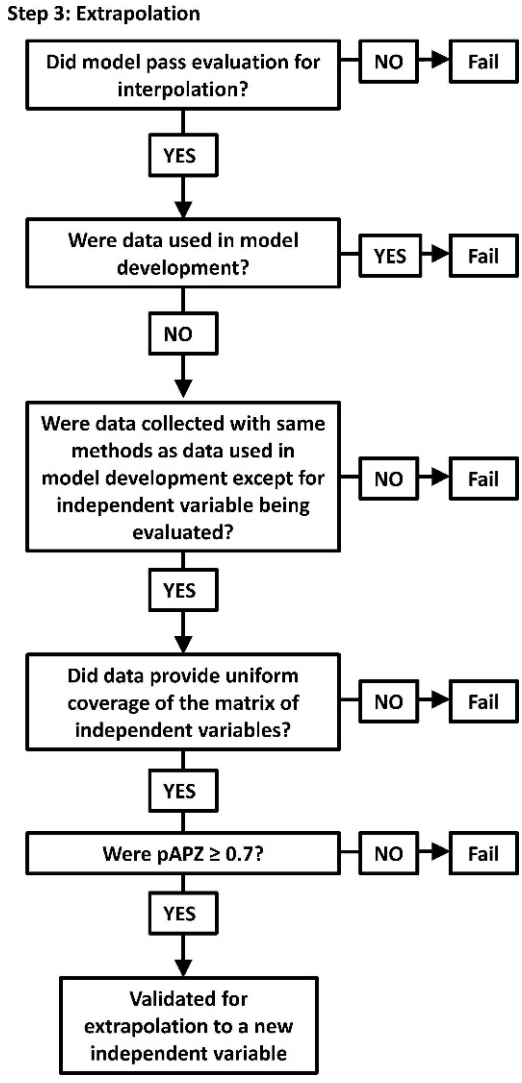

FIGURE 8. *Flow diagram of the third step in the process used to develop, evaluate, and potentially validate predictive models (see the text for more details).*

≥ 0.7), the model must pass the goodness of fit test before it can be validated for interpolation.

The third step in the process (Fig. 8) is to evaluate the model for extrapolation to independent variables not included during model development but collected with the same experimental methods except the independent variable being evaluated. Here, it is best to use the same experimental design as used in model development so that the new data can be added to the model in the event that it is determined that the independent variable being evaluated should be added to the model. The criteria for test data are (i) that they were not used in model development (i.e., independent); (ii) that they be collected with the same experimental methods except the one independent variable being evaluated; and (iii) that uniform coverage of the matrix of independent variables be used in model development to provide an unbiased and complete evaluation of model extrapolation. In addition to the aforementioned criterion for model performance (i.e., pAPZ ≥ 0.7), the model must pass tests for goodness of fit and validation for interpolation before it can be validated for extrapolation.

If a model fails any step in the model validation process, it can be repaired by collection of additional data or by fitting the data to better primary and secondary models in the case of a regression-based tertiary model. In the present study, the GRNN model met the criteria for test data for all model evaluations and met the model performance criterion for goodness of fit, interpolation, and extrapolation to an inoculum size of 2 log. However, it only partially met the performance criterion for extrapolation to inoculum sizes of 3 and 4.1 log. The data for higher inoculum sizes can be added to the model because the experimental design used to collect these data was the same as used in model development. However, an additional two replications of storage trials at the three higher inoculum sizes will likely be needed to generate a new model with acceptable goodness of fit. In addition, two additional replications at intermediate temperatures for the three higher inoculum sizes will be needed to evaluate the expanded model for interpolation. A less costly and time-consuming approach may be to develop future models with a higher inoculum size, such as 3 log. Data from this study indicate that such a model would provide fail-safe predictions of *Salmonella* growth from lower inoculum levels and would likely yield pAPZs of ≥ 0.7 for all four inoculum sizes and combinations of times and temperatures investigated.

In the present study, a simple one-step modeling method, GRNN, that uses off-the-shelf software to predict pathogen growth as a function of independent variables was used. The author found that this method was easier than the traditional regression approach of primary, secondary, and tertiary modeling and resulted in better model performance (Table 2), which is in agreement with other studies *(4, 7, 8, 18)*. In addition, this approach required fewer data for model development and has the potential to simultaneously model growth, survival, and inactivation data, which is a difficult task for regression-based methods. Thus, the author recommends it as a way of reducing the technical difficulty and cost of developing predictive models in food.

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