Research Note

Development and Validation of a Predictive Microbiology Model for Survival and Growth of *Salmonella* on Chicken Stored at 4 to 12°C[†]

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ABSTRACT

Salmonella spp. are a leading cause of foodborne illness. Mathematical models that predict *Salmonella* survival and growth on food from a low initial dose, in response to storage and handling conditions, are valuable tools for helping assess and manage this public health risk. The objective of this study was to develop and to validate the first predictive microbiology model for survival and growth of a low initial dose of *Salmonella* on chicken during refrigerated storage. Chicken skin was inoculated with a low initial dose (0.9 log) of a multiple antibiotic-resistant strain of *Salmonella* Typhimurium DT104 (ATCC 700408) and then stored at 4 to 12°C for 0 to 10 days. A general regression neural network (GRNN) model that predicted log change of *Salmonella* Typhimurium DT104 as a function of time and temperature was developed. Percentage of residuals in an acceptable prediction zone, from −1 (fail-safe) to 0.5 (fail-dangerous) log, was used to validate the GRNN model by using a criterion of 70% acceptable predictions. Survival but not growth of *Salmonella* Typhimurium DT104 was observed at 4 to 8°C. Maximum growth of *Salmonella* Typhimurium DT104 during 10 days of storage was 0.7 log at 9°C, 1.1 log at 10°C, 1.8 log at 11°C, and 2.9 log at 12°C. Performance of the GRNN model for predicting dependent data ($n = 163$) was 85% acceptable predictions, for predicting independent data for interpolation ($n = 77$) was 84% acceptable predictions, and for predicting independent data for extrapolation ($n = 70$) to *Salmonella* Kentucky was 87% acceptable predictions. Thus, the GRNN model provided valid predictions for survival and growth of *Salmonella* on chicken during refrigerated storage, and therefore the model can be used with confidence to help assess and manage this public health risk.

Salmonella is estimated to cause 1.3 million cases of foodborne illness per year in the United States, which results in 15,608 hospitalizations and 553 deaths *(20)*. Eggs, chicken, and turkey are leading sources of salmonellosis *(2)*. However, freshly processed chickens are contaminated with low levels of *Salmonella,* typically fewer than 30 cells per carcass *(5, 30–32)*. Results of human feeding trials indicate that the dose of *Salmonella* that causes illness in 50% of healthy humans ranges from 10^5 to 10^9 cells and depends on the serotype and strain of *Salmonella* ingested *(17–19)*. Thus, in order for *Salmonella* to cause foodborne illness, it must multiply from a low initial dose on chicken to a higher dose.

Mathematical models that predict survival and growth of *Salmonella* from a low initial dose, in response to storage and handling conditions, are valuable tools for helping assess and manage this public health risk. Oscar *(24, 25)* recently developed predictive models for survival and growth of *Salmonella* Typhimurium DT104 from a low initial dose on chicken skin with native microflora, stored at 10 to 50°C for 0

to 8 h. Other studies *(1, 13, 15, 16)* that investigated the effect of refrigerated storage (4 to 10°C) on survival and growth of *Salmonella* were conducted in the absence of microbial competition (i.e., in laboratory broth), with substrates other than chicken in some cases, and from high initial doses (i.e., >3 log) of *Salmonella,* and as a result did not optimally simulate scenarios found in commercial practice. Thus, there is a need to develop a model that predicts survival and growth of a low initial dose of *Salmonella* on chicken skin during cold storage for more than 8 h and at temperatures lower than 10°C. Therefore, the current study was undertaken to investigate and model survival and growth of a low initial dose (0.9 log) of *Salmonella* on chicken skin with native microflora, stored at 4 to 12°C for the normal shelf life (i.e., <10 days) of the product.

MATERIALS AND METHODS

Materials. A multiple antibiotic-resistant strain of *Salmonella enterica* serotype Typhimurium DT104 (ATCC 700408) was obtained from the American Type Culture Collection (Manassas, VA), whereas a multiple antibiotic-resistant strain of *S. enterica* serotype Kentucky was obtained from a poultry company. Brain heart infusion (BHI) broth, buffered peptone water (BPW), and xylose lysine agar base medium were obtained from BD (Franklin Lakes, NJ). Chloramphenicol, ampicillin, tetracycline, streptomycin, novobiocin, and HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-

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ethanesulfonic acid) were from Sigma (St. Louis, MO). Chicken thighs were obtained from local retail outlets.

Experimental designs. A full 5 × 5 factorial arrangement of temperature (4, 6, 8, 10, and 12°C) and time (0, 1, 3, 6, and 10 days) was used for model development with *Salmonella* Typhimurium DT104. A full 4 × 5 factorial arrangement of temperature (5, 7, 9, and 11°C) and time (0, 1, 3, 6, and 10 days) was used for model validation for interpolation with *Salmonella* Typhimurium DT104 and for model validation for extrapolation with *Salmonella* Kentucky. The refrigerated bench top incubator shaker (model Innova 4230, New Brunswick Scientific, Edison, NJ) used maintained the storage temperature within ±0.5°C. Storage conditions were replicated from two to eight times.

Preparation and inoculation of chicken. Skin was removed from chicken thighs, placed on a cutting board, frozen at −70°C for 15 min, and then cut into portions 2.14 cm² by using a no. 10 cork borer. Skin portions were placed on top of thigh meat (~100 g) in 500-ml plastic jars with screw-cap lids and then stored for 24 to 48 h at 4°C before use in storage trials.

Stock cultures of *Salmonella* Typhimurium DT104 and *Salmonella* Kentucky were maintained at −70°C in BHI broth with 15% glycerol. Five microliters of the appropriate stock culture was added to 5 ml of BHI broth in a 25-ml Erlenmeyer flask. The flask was sealed with a foam plug and then incubated at 30°C and 150 rpm for 23 h in the Innova 4230. After 23 h of incubation, cultures were serially diluted in BPW to a final concentration of 3.2 log/ml for *Salmonella* Typhimurium DT104 and 3.1 log/ml for *Salmonella* Kentucky. Five microliters of the appropriate cell suspension was spot inoculated onto individual skin portions, at an initial level of 0.9 log per skin portion for *Salmonella* Typhimurium DT104 and 0.8 log per skin portion for *Salmonella* Kentucky.

Enumeration of *Salmonella*. At the appropriate sampling time, a single skin portion was placed in a 207-ml capacity Whirl-Pak bag with filter screen (Nasco, Fort Atkinson, WI) that contained 9 ml of BPW. The sample was pulsified (model PUL1, Microbiology International, Frederick, MD) for 1 min to recover *Salmonella* into BPW, and then the pulsifate was used for enumeration.

A three-tube most-probable-number (MPN) approach and a viable count (CFU) method were used to enumerate *Salmonella,* as previously described *(23, 25)*. Viable counts were determined by spiral plating (Whitely Automated Spiral Plater, Microbiology International) undiluted and diluted pulsifate in BPW onto xylose lysine agar base medium, HEPES, chloramphenicol, ampicillin, tetracycline, and streptomycin for *Salmonella* Typhimurium DT104, and onto xylose lysine agar base medium, HEPES, novobiocin, ampicillin, tetracycline, and streptomycin for *Salmonella* Kentucky. MPN tubes, which were prepared in BPW, were incubated for 24 h at 38°C before drop plating 5 µl from each MPN tube and the skin portion enrichment onto xylose lysine agar base medium, HEPES, chloramphenicol, ampicillin, tetracycline, and streptomycin for *Salmonella* Typhimurium DT104, and onto xylose lysine agar base medium, HEPES, novobiocin, ampicillin, tetracycline, and streptomycin for *Salmonella* Kentucky. Drop plates (MPN) and spiral plates (CFU) were incubated for 24 h at 38°C. Colonies that formed on spiral plates were counted with an automated colony counter (Protocol, Microbiology International). An MPN table was used to interpret results of the drop plates.

Model development. Data sets for *Salmonella* Typhimurium DT104 and *Salmonella* Kentucky were created in computer spreadsheets (Excel 2003, Microsoft Corp., Redmond, WA) with separate columns for temperature (independent numerical variable), time (independent numerical variable) and log change (Δ; dependent numerical variable), which was calculated as follows:

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

where $N(t)$ was the log at sampling time $t,$ and N_0 was the initial log. A general regression neural network (GRNN) model was developed with a spreadsheet add-in program (version 1.0, Neural Tools, Palisade Corp., Newfield, NY), as described previously *(24, 25)*.

Model validation. The GRNN model was validated for goodness of fit by using the dependent data, for interpolation utilizing independent data for *Salmonella* Typhimurium DT104, and for extrapolation by using independent data for *Salmonella* Kentucky. Performance of the model (i.e., prediction bias and accuracy) was determined with the acceptable prediction zone method, as previously described *(23, 24)*. In brief, model performance was classified as acceptable when 70% of residuals were in an acceptable prediction zone, from −1 (fail-safe) to 0.5 (fail-dangerous) log *(23, 24)*. The performance index for model performance was the percentage of residuals in the acceptable prediction zone. This performance index assesses prediction bias and prediction accuracy at the same time. Prediction bias and accuracy factors *(29)* were not used, because as shown in previous studies *(22, 24),* they do not provide as accurate an assessment of model performance as does the acceptable prediction zone method.

Data analysis and interpretation. Within a storage time, the mean log change (Δ) of *Salmonella* number was calculated. Considering precision of the enumeration methods used and for the sake of facilitating discussion of the data, death was defined as a mean Δ < −0.5 log per skin portion, survival was defined as a mean Δ = −0.5 to 0.5 log per skin portion, and growth was defined as mean Δ > 0.5 log per skin portion.

RESULTS

During storage trials for model development (Fig. 1), *Salmonella* Typhimurium DT104 was observed to grow on chicken skin incubated at 10°C for 3 or 6 days (Fig. 1D) and on chicken skin stored at 12°C for 1, 3, 6, or 10 days (Fig. 1E). At all other storage times and temperatures, *Salmonella* Typhimurium DT104 survived, and death was not observed.

During the storage trials for model validation for interpolation (Fig. 2), *Salmonella* Typhimurium DT104 was observed to grow at 6 and 10 days of storage at 9°C and 3, 6, and 10 days of storage at 11°C. At all other storage times and temperatures, *Salmonella* Typhimurium DT104 survived, and death was not observed.

During storage trials for model validation for extrapolation (Fig. 3), *Salmonella* Kentucky was observed to grow after 10 days of storage at 11°C (Fig. 3D). At all other storage times and temperatures, *Salmonella* Kentucky survived, except for 6 days of storage at 7°C, during which time death was observed.

Performance of the GRNN model for predicting data (n =163) used in model development was 85% acceptable predictions, and there were no signs of local prediction problems as a function of the independent variables (Fig. 4A). Performance of the GRNN model for interpola-
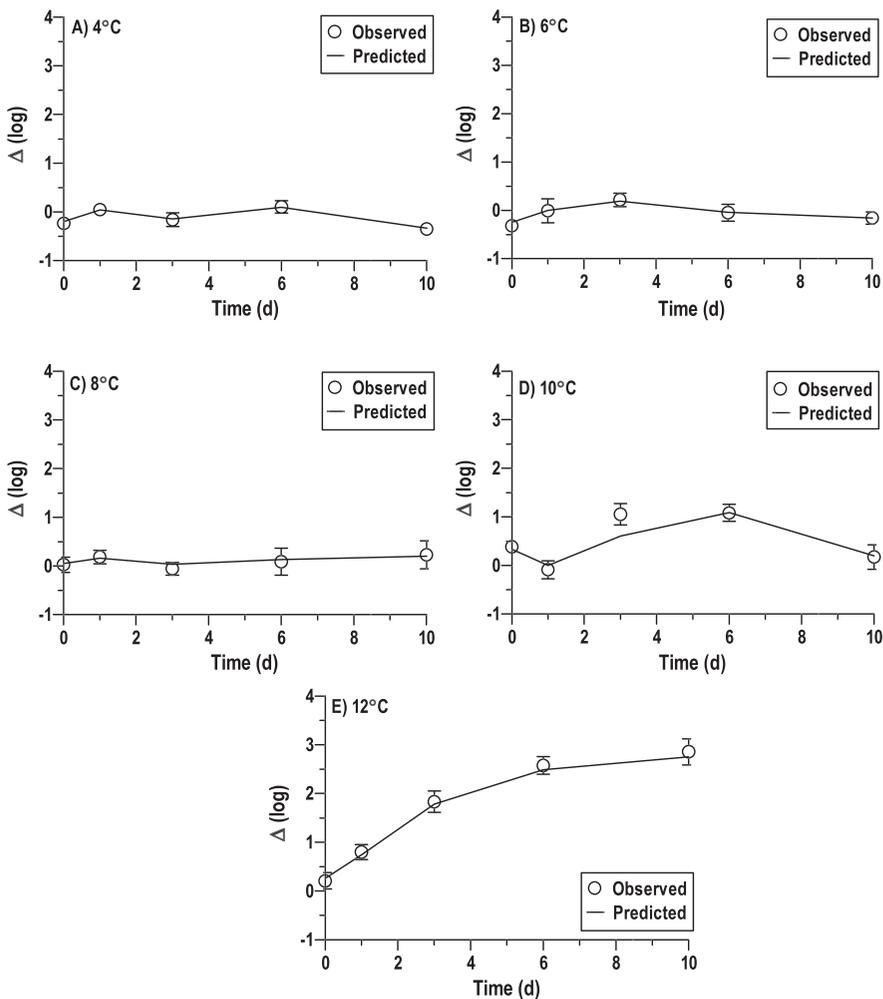
FIGURE 1. *Survival and growth of a low initial dose (0.9 log) of* Salmonella Typhimurium DT104 *on chicken skin stored at (A) 4°C, (B) 6°C, (C) 8°C, (D) 10°C, or (E) 12°C, for 0, 1, 3, 6, or 10 days. Symbols are the observed data (mean log changes [Δ] ± standard errors of the mean) that were used in model development, whereas the lines are the responses predicted by the general regression neural network model.*

tion was evaluated using independent data ($n = 77$) for *Salmonella* Typhimurium DT104 that was collected at intermediate temperatures (5, 7, 9, and 11°C), but at the same storage times (0, 1, 3, 6, and 10 days) as data used in model development. The percentage of acceptable predictions for interpolation was 84%, and there were no signs of local prediction problems as a function of the independent variables (Fig. 4B).

Finally, ability of the GRNN model to extrapolate to another serotype of *Salmonella* was evaluated. Independent data ($n = 70$) for survival and growth of a low initial dose (0.8 log) of *Salmonella* Kentucky on chicken skin were obtained with the identical experimental design and methods as those used in model validation for interpolation, except that *Salmonella* Kentucky rather than *Salmonella* Typhimurium DT104 was used as the test strain. The percentage of acceptable predictions for extrapolation of the GRNN model to *Salmonella* Kentucky was 87% (Fig. 4C). Although most residuals were below 0, indicating less survival and growth of *Salmonella* Kentucky on chicken skin than growth of *Salmonella* Typhimurium DT104, most of these residuals were within the acceptable prediction zone. However, at 11°C and storage times of 3 and 6 days, the GRNN model provided overly fail-safe predictions of *Salmonella* Kentucky growth (Fig. 3D), indicating existence of a local prediction problem.

DISCUSSION

There have been a number of previous studies that have investigated survival and growth of *Salmonella* on chicken incubated at refrigeration temperatures. Burnette and Yoon *(3)* investigated survival and growth of a high initial dose (i.e., 6 log) of *Salmonella* Typhimurium (ATCC 14028) on cooked chicken breast meat without native microflora. At 4°C, *Salmonella* Typhimurium numbers declined by about 0.3 log after 10 days, which is similar to results of the current study. At 8°C, Burnette and Yoon *(3)* observed that *Salmonella* Typhimurium numbers increased slightly (ca. 0.2 log) over 8 days of storage, which agrees with results of the present study. In contrast, at 10°C, *Salmonella* Typhimurium counts increased about 2 log after 11 days of storage in the study of Burnette and Yoon *(3),* whereas in the present study, *Salmonella* Typhimurium DT104 only increased about 1.1 log after 6 days of storage at 10°C and 0.2 log after 10 days of storage at 10°C.

Jung and others *(14)* investigated growth of *Salmonella* Typhimurium (ATCC 13311) from an initial density of 3 log/ml in BHI broth incubated at 10°C for 20 days. They reported a 5-log increase of *Salmonella* Typhimurium after 20 days, which is much greater than that of *Salmonella* Typhimurium DT104 at 10°C in the current study.

Dominguez and Schaffner *(4)* investigated growth of a cocktail of antibiotic- and non–antibiotic-resistant strains of

FIGURE 2. *Survival and growth of a low initial dose (0.9 log) of* Salmonella *Typhimurium DT104 on chicken skin stored at (A) 5°C, (B) 7°C, (C) 9°C, or (D) 11°C, for 0, 1, 3, 6, or 10 days. Symbols are the observed data (mean log changes [Δ] ± standard errors of the mean) that were used to validate the model for interpolation, whereas the lines are the responses predicted by the general regression neural network model.*
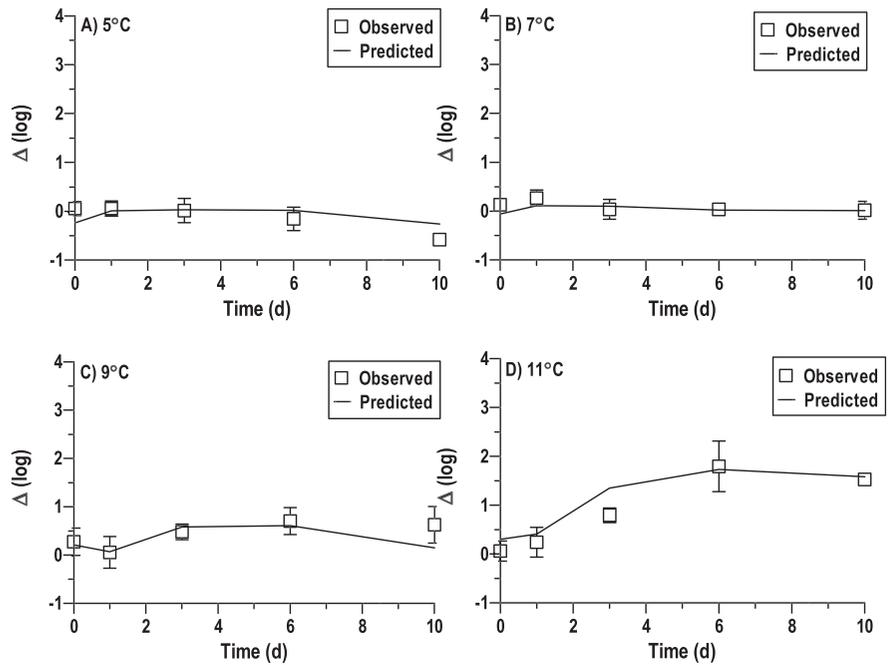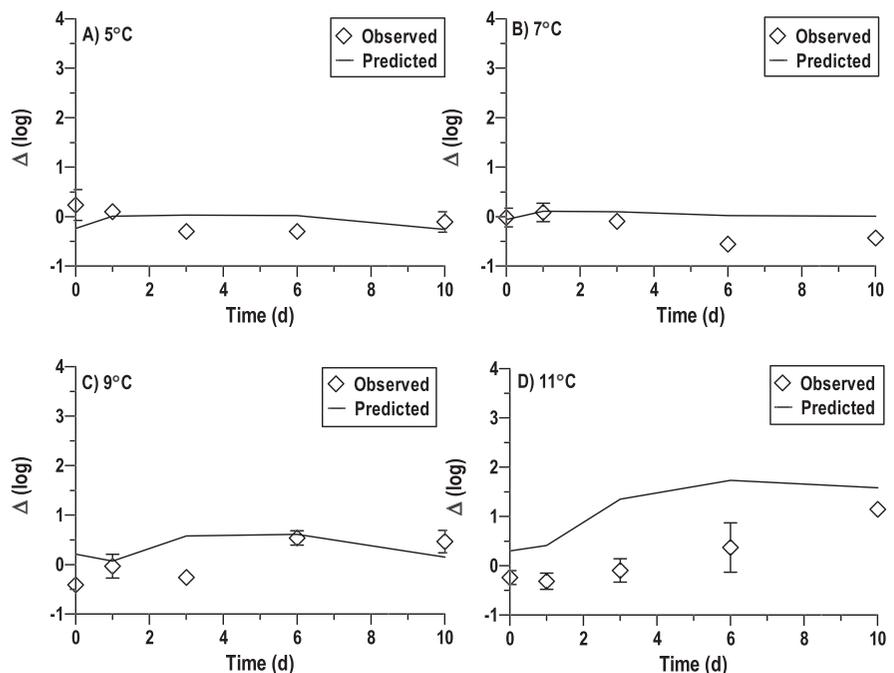

Salmonella Kentucky and Typhimurium inoculated onto chicken breast tenderloins at an initial dose of 3.3 log/cm^2 and then stored at 10 and 12°C. Observed mean growth rates were 0.24 log/day at 10°C and 0.66 log/day at 12°C. Complete growth data were not reported and thus, it was not possible to directly compare their results with ours. Nonetheless, the study of Dominguez and Schaffner *(4)* indicates that *Salmonella* grows on chicken with native microflora at 10 and 12°C, which is in general agreement with the results of the current study.

Oscar *(21)* investigated the growth of a high initial dose (6 log) of *Salmonella* Typhimurium (ATCC 14028) on cooked chicken breast meat without native microflora. Total growth of *Salmonella* Typhimurium (ATCC 14028) was 0.5 log after 5.7 days at 8°C, 2.5 log after 5.1 days at 10°C, and 2.7 log after 3.3 days at 12°C. In comparison, predicted growth of *Salmonella* Typhimurium DT104 in the present study was 0 log after 5.7 days at 8°C, 1 log after 5.1 days at 10°C, and 1.4 log after 3.3 days at 12°C. Absence of microbial competition or differences in other experimental conditions could explain the higher observed growth of *Salmonella* Typhimurium (ATCC 14028) in the study of Oscar *(21)*. Pathogen strain is not a likely explanation, as *Salmonella* Typhimurium (ATCC 14028) and *Salmonella* Typhimurium DT104 (ATCC 700408) have similar growth kinetics on cooked chicken breast meat without native microflora *(26)*.

Oscar *(23)* also investigated the growth of a low initial dose (0.6 log) of *Salmonella* Typhimurium DT104 (ATCC

FIGURE 3. *Survival and growth of a low initial dose (0.8 log) of* Salmonella *Kentucky on chicken skin stored at (A) 5°C, (B) 7°C, (C) 9°C, or (D) 11°C, for 0, 1, 3, 6, or 10 days. Symbols are the observed data (mean log changes [Δ] ± standard errors of the mean) that were used to validate the model for extrapolation, whereas the lines are the responses predicted by the general regression neural network model.*
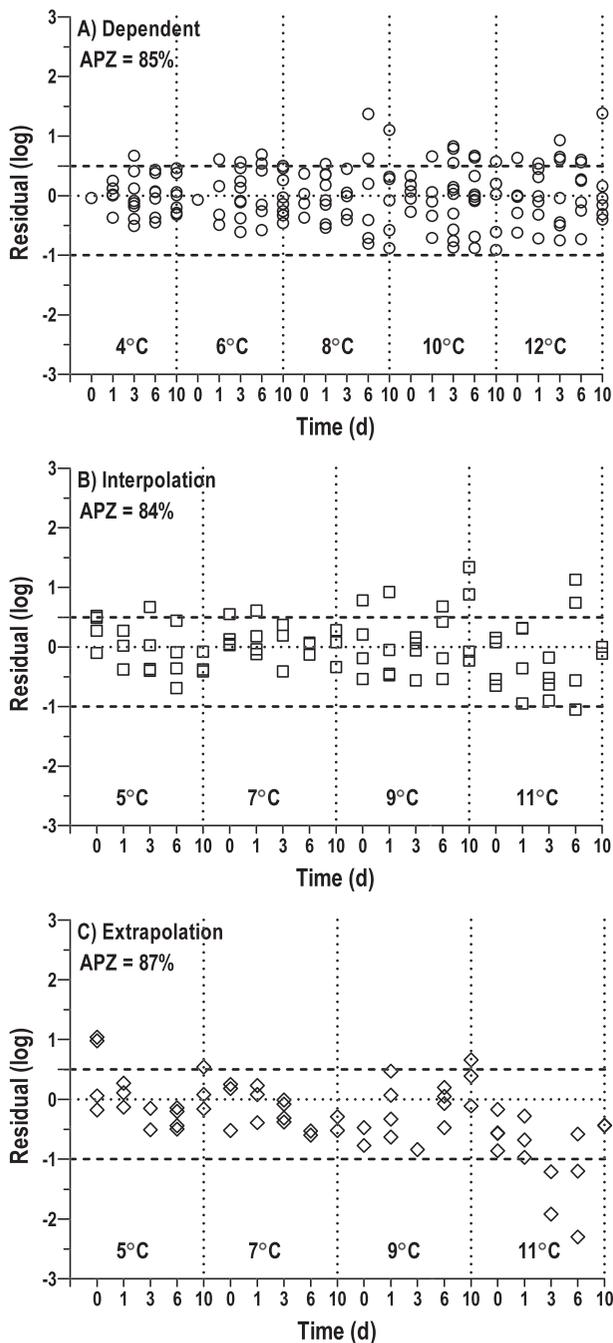
FIGURE 4. *Residual plots for (A) dependent data for* Salmonella *Typhimurium DT104, (B) independent data for interpolation with* Salmonella *Typhimurium DT104, and (C) independent data for extrapolation to* Salmonella *Kentucky. Residuals were sorted by the independent variables of temperature and time. Horizontal dashed lines indicate upper and lower bounds of the acceptable prediction zone, which were from −1 (fail-safe) to 0.5 (fail-dangerous) log.*

700408) on chicken breast meat with native microflora. Total growth of *Salmonella* Typhimurium DT104 (ATCC 700408) was 1 log after 8.8 days at 10°C, 1.7 log after 7.5 days at 11°C, and 2.1 log after 7.7 days at 12°C *(23)*. In comparison, predicted growth of the same strain of *Salmonella* Typhimurium DT104 (ATCC 700408) in the present study was 0.5 log after 8.8 days at 10°C, 1.4 log after 7.5 days at 11°C, and 2.2 log after 7.7 days at 12°C. These results indicate that growth

of *Salmonella* Typhimurium DT104 (ATCC 700408) is similar on the skin and the breast meat of chicken.

Previous studies demonstrate that GRNN models outperform other types of neural network models and regression models in predictive microbiology applications *(6–12, 27)*. However, overtraining of neural networks, such as the GRNN model developed in the present study, can result in prediction errors when the model is applied to independent data. Thus, it is important to test the GRNN model for its ability to predict data not used in model development but that fall within the ranges of independent variables used to develop the model. Ideally, the test data should cover the prediction region of the model in a uniform manner to provide a complete and nonbiased assessment of model performance. In the current study, an independent set of data was collected to test the ability of the GRNN model to interpolate within its prediction region from 4 to 12°C and from 0 to 10 days of refrigerated storage. Independent data for interpolation testing were collected with the same experimental procedures but with intermediate temperatures (5, 7, 9, and 11°C). To evaluate model performance, the percentage of residuals that fell within an acceptable prediction zone, from −1 (fail-safe) to 0.5 (fail-dangerous) log, was determined for data used in model development (i.e., dependent data) and not used in model development (i.e., independent data for interpolation). When the percentage of residuals in the acceptable prediction zone exceeded 70%, the GRNN model was classified as providing acceptable predictions of the test data. Residuals were graphed as a function of independent variables to look for regional prediction problems. The percentage of acceptable predictions was 85% for dependent data and 84% for independent data for interpolation. There were no signs of regional prediction problems for either set of data and thus, the model was classified as providing acceptable and valid predictions for survival and growth of a low initial dose (0.9 log) of *Salmonella* Typhimurium DT104 on chicken thigh skin stored at 4 to 12°C for 0 to 10 days. The GRNN model was not validated for extrapolation to higher initial levels of *Salmonella* Typhimurium DT104 and thus, should not be used for such predictions until such a validation is completed.

Testing ability of the GRNN model to extrapolate to other conditions (i.e., independent variables) not used in model development can save time and money by identifying conditions for which new models are not needed. In this study, ability of the GRNN model to extrapolate to another serotype of *Salmonella* (i.e., Kentucky) was evaluated. Parveen et al. *(28)* reported that the predominant serotypes of *Salmonella* on postchill chickens in a commercial processing plant in the Mid-Atlantic region (i.e., the region of this study) of the United States were Kentucky and Typhimurium. Consequently, it was of interest to see how well the GRNN model developed in this study with *Salmonella* Typhimurium DT104 could predict survival and growth of *Salmonella* Kentucky on chicken during refrigerated storage.

In general, the GRNN model provided acceptable predictions of the survival and growth of a low initial dose (i.e., 0.8 log) of *Salmonella* Kentucky on chicken skin, with

an acceptable prediction rate of 87%. However, more than 30% of residuals for survival and growth of *Salmonella* Kentucky on chicken skin at 11°C in the present study were outside the acceptable prediction zone and negative, indicating that the model developed with *Salmonella* Typhimurium DT104 overpredicted survival and growth of *Salmonella* Kentucky at this cold-storage temperature. These results are in agreement with the observation of Oscar *(24),* that *Salmonella* Kentucky grows slower than *Salmonella* Typhimurium DT104 on chicken skin subjected to temperature abuse at 25 to 45°C for 0 to 8 h.

ACKNOWLEDGMENTS

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