General Regression Neural Network Model for Behavior of *Salmonella* on Chicken Meat during Cold Storage

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Abstract: A study was undertaken to investigate and model behavior of *Salmonella* on chicken meat during cold storage at constant temperatures. Chicken meat (white, dark, or skin) portions (0.75 cm^3) were inoculated with a single strain of *Salmonella* Typhimurium DT104 (2.8 log) followed by storage for 0 to 8 d at –8, 0, 8, 12, 14, or 16 °C for model development and at –4, 4, 10, or 14 °C for model validation. A general regression neural network model was developed with commercial software. Performance of the model was considered acceptable when the proportion of residuals (observed – predicted) in an acceptable prediction zone (pAPZ) from –1 log (fail-safe) to 0.5 logs (fail-dangerous) was ≥0.7. Growth of *Salmonella* Typhimurium DT104 on chicken meat was observed at 12, 14, and 16 °C and was highest on dark meat, intermediate on skin, and lowest on white meat. At lower temperatures (–8 to 10 °C) *Salmonella* Typhimurium DT104 remained at initial levels throughout 8 d of storage except at 4 °C where there was a small (0.4 log) but significant decline. The model had acceptable performance (pAPZ = 0.929) for dependent data (n = 482) and acceptable performance (pAPZ = 0.923) for independent data (n = 235). Results indicated that it is important to include type of meat as an independent variable in the model and that the model provided valid predictions of the behavior of *Salmonella* Typhimurium DT104 on chicken skin, white, and dark meat during storage for 0 to 8 d at constant temperatures from –8 to 16 °C.

Keywords: chicken, predictive modeling, *Salmonella*

Practical Application: A model for predicting behavior of *Salmonella* on chicken meat during cold storage was developed and validated. The model will help the chicken industry to better predict and manage this risk to public health.

Introduction

During cold storage, the number of *Salmonella* on chicken meat may stay the same, increase, or decrease depending on time and temperature of storage and type of meat. For example, when *Salmonella* are inoculated into white and dark meat of chicken and stored for 100 d at –20, –5, –2, or 1 °C, there is a smaller decrease in number in white meat than in dark meat and at –20 °C than at –5, –2, or 1 °C (Foster and Mead 1976). The effect of frozen storage on behavior of *Salmonella* on chicken skin was not investigated. Thus, there is a need to further investigate behavior of *Salmonella* on chicken meat during frozen storage as a function of time, temperature, and type of meat.

Several studies have investigated behavior of *Salmonella* on chicken meat stored at refrigeration temperatures. Zaher and Fujikawa (2011) investigated behavior of *Salmonella* Enteritidis in ground chicken stored at 8 to 16 °C and Pradhan and others (2012) investigated behavior of *Salmonella* Typhimurium on chicken white meat stored for 21 d at 0, 4, or 8 °C. Oscar (2011a) studied survival and growth of *Salmonella* Typhimurium DT104 on chicken skin stored for 0 to 10 d at 4 to 12 °C. However, none of these studies investigated the effect of all types of chicken meat on behavior of *Salmonella* during refrigerated storage.

Models that predict changes in number of *Salmonella* over time, temperature, and other independent variables are routinely used in the chicken industry to predict and manage this risk to public health. The chicken industry is most interested in models that predict behavior of *Salmonella* in chicken meat rather than in laboratory broth. The study of Foster and Mead (1976) suggests that type of chicken meat might affect behavior of *Salmonella* during cold storage but thus far there are no predictive models that include this variable. Therefore, the current study was undertaken to investigate and model behavior of *Salmonella* on chicken meat during cold storage as a function of time, temperature, and type of meat. Data from a previous study (Oscar 2011a) were not used in model development and validation because they were collected with different methods (that is, different inoculum size, different previous history of inoculum, and different chicken skin 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., U.S.A.). Stock cultures of the organism were maintained at –80 °C in brain heart infusion broth (BBL™, Becton, Dickinson and Co., Sparks, Md., U.S.A.) that contained 15% (volume/volume) glycerol

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(Sigma-Aldrich Corp., St. Louis, Mo., U.S.A.). This organism was used for model development and validation because it has a phenotype that allows it to be enumerated in the presence of other microorganisms and because it has been isolated from chicken (Parveen and others 2007).

Preparation of chicken portions

Chicken breasts (white meat) and thighs (dark meat) with skin were purchased from local retail stores. Skin was removed and placed on a plastic cutting board followed by freezing for 15 min at –20 °C. This was done to facilitate cutting of circular portions (0.5 cm^2) with a cork borer (#5). Chicken breasts and thighs were deboned and then white and dark meats were ground separately through the coarse plate and then fine plate of an electric tabletop meat grinder (Model 586.8 Zelmer, The Sausage Maker, Buffalo, N.Y., U.S.A.). Ground white meat and ground dark meat were packed into separate plastic petri dishes (100 × 15 mm) and frozen at –20 °C to facilitate cutting into equal-sized cylindrical portions (0.75 cm^3) with a cork borer (#5). White meat and dark meat portions were transferred to 1.5-mL polypropylene microcentrifuge tubes. Skin portions were placed on top of some white meat or dark meat portions. All chicken meat portions were stored at –20 °C until used in experiments.

Measurement of pH

The pH of randomly selected chicken meat portions ($n = 4$ to 6 per type of meat) was measured using a handheld instrument whose probe could be directly inserted into the meat portion (pH Spear, Oakton Instruments, Vernon Hills, Ill., U.S.A.).

Enumeration of native microflora

The number of native microflora of randomly selected chicken meat portions ($n = 4$ to 6 per type of meat) was determined by the most probable number (MPN) method. Individual portions of chicken meat were placed in 207-mL plastic bags with filter screens (Whirl-Pak®, Nasco, Fort Atkinson, Wis., U.S.A.). After adding 9 mL of buffered peptone water (BPW; Difco™, Becton, Dickinson and Co.), the sample was pulsified (model PUL 1, Microbiology Intl., Frederick, Md., U.S.A.) for 1 min to recover native microflora into BPW. The pulsifate was used to set up a 3 (replicate) by 6 (dilution [1:10]) MPN assay in BPW. The MPN assay was incubated for 24 h at 30 °C and then 5 μL from each MPN tube was drop plated onto brain heart infusion agar (BBL™, Becton, Dickinson and Co.). Drop plates were incubated for 24 h at 30 °C to confirm the pattern of positive and negative tubes. The MPN result was calculated as described below.

Inoculation and incubation of chicken meat portions

Stock culture of *Salmonella* Typhimurium DT104 was thawed, resuspended by gentle shaking, and then 5 μL was inoculated into 9 mL of BPW in a glass dilution tube (16 × 125 mm) with plastic cap. The inoculated BPW tube was incubated for 72 h at 22 °C without shaking to obtain stationary phase cells for inoculation of chicken meat portions. Immediately before inoculation of chicken meat portions, the 72-h culture was serially diluted (1:10) in BPW to 10^{-6}. Concentration of *Salmonella* Typhimurium DT104 in the 72-h culture was determined by spiral plating (Whitley Automated Spiral Plater, Microbiology Intl.) 50 μL of the 10^{-5} and 10^{-6} dilutions in duplicate onto XLT4 agar base medium (Difco™, Becton, Dickinson and Co.) supplemented with 25 mM HEPES (Sigma-Aldrich Corp.) and 25 μg per mL of the following antibiotics (Sigma-Aldrich Corp.): chloramphenicol (C), ampicillin (A), tetracycline (T), and streptomycin (S); hereafter, referred to as XLH-CATS. Spiral plates were incubated for 24 h at 40 °C and then typical black colonies of *Salmonella* Typhimurium DT104 that formed were counted using an automated colony counter (ProtoCOL, Microbiology Intl.).

Chicken meat portions were spot inoculated on their surface with 5 μL of the 10^{-4} dilution of the 72-h culture of *Salmonella* Typhimurium DT104 for an initial inoculum level of 2.8 ± 0.1 (mean ± standard deviation) log per portion. The inoculated chicken meat portions in 1.5-mL microcentrifuge tubes were inserted into individual wells of a heating and cooling dry block (Peltier PCH-1, Grant Instruments, Cambridge, UK or Eppendorf ThermoStat Plus, Hamburg, Germany) and incubated for 0, 1, 2, 4, 6, or 8 d at –8, 0, 8, 12, 14, or 16 °C for model development or at –4, 4, 10, or 14 °C for model validation. The heating and cooling dry block was located in a refrigerator for storage temperatures from –8 to 4 °C and at room temperature for storage temperatures from 8 to 16 °C. Two to four replicate trials with duplicate meat samples per time were conducted per storage temperature.

Enumeration of *Salmonella* Typhimurium DT104

Duplicate chicken meat portions were enumerated separately for *Salmonella* Typhimurium DT104 at each sampling time. Individual portions were considered as independent observations because it was assumed that the microniche of the inoculated cells differed among portions. The cap of the 1.5 mL microcentrifuge tube was opened and then a microtube cutter was used to cut off the bottom of the microcentrifuge tube. The chicken meat portion was then pushed out of the tube and into a 207-mL sample bag with filter screen. Nine milliliter of BPW was added to the chicken meat portion in the bag and then the sample was pulsified for 1 min to recover *Salmonella* Typhimurium DT104 into BPW for enumeration by MPN and spiral plating methods.

A 3 (replicate) by 4 (dilution) MPN assay in BPW was used when the number of *Salmonella* Typhimurium DT104 was between 0 and 3.26 log per portion. After setting up the MPN assay, 9 mL of BPW was added to the residual pulsifate and chicken meat portion in the bag. The MPN assay tubes and bag contents were incubated for 24 h at 40 °C and then 5 μL from each MPN tube and the filter bag was spot inoculated onto XLH-CATS. After 24 h of incubation at 40 °C, MPN tubes and bags that were positive for *Salmonella* Typhimurium DT104 produced a black spot on XLH-CATS whereas MPN tubes and bags that were negative for *Salmonella* Typhimurium DT104 produced no spot on XLH-CATS.

The MPN result was calculated as follows (Thomas 1942):

$$\frac{MPN}{portion} = \log\{[(P/\sqrt{N \times T})] \times V\} \quad (1)$$

where P was the number of positive tubes, N was the total amount of pulsifate (mL) in all negative tubes, T was the total amount of pulsifate (mL) in all tubes, and V was the total volume of BPW (that is, 9 mL) in the original sample. It was assumed that the solid meat portion (0.75 cm^3) did not contribute significantly to the total sample volume because it remained behind the filter screen when samples were pulled for the MPN assay.

The number of *Salmonella* Typhimurium DT104 per portion was also determined by spiral plating undiluted or serially diluted (1:10 in BPW) samples (50 μL) of pulsifate onto XLH-CATS

followed by incubation for 24 h at 40 °C and automated counting of black colonies that formed on the spiral plate during incubation.

Model development

A general regression neural network (GRNN) model was developed rather than a regression model based on primary, secondary, and tertiary modeling because GRNN models are more flexible, require less data, are easier to develop and validate, only require one modeling step, and they outperform regression models in predictive microbiology applications (Hajmeer and others 1997; Jeyamkondan and others 2001; Garcia-Gimeno and others 2003; Palanichamy and others 2008).

To develop the GRNN model, a data set was created in a computer spreadsheet (Excel 2007, Microsoft Corp., Redmond, Wash., U.S.A.) with separate columns for tag (to designate dependent data for model development and to designate independent data for model validation), type of meat (independent categorical variable), temperature (independent numerical variable), time (independent numerical variable), and log number per portion (dependent numerical variable). The GRNN model (Figure 1) was developed by the method of Specht (1991) using a spreadsheet add-in program (industrial version 5.7, NeuralTools, Palisade Corp., Ithaca, N.Y., U.S.A.) and equations as previously described (Oscar 2009a). The model parameters (that is, smoothing factors) are not provided by the software for proprietary reasons. However, after publication the model will be made available through the Poultry Food Assess Risk Models website (www.ars.usda.gov/naa/errc/PoultryFARM).

Model validation

A concern when using a GRNN model to make predictions is overtraining that would result in predictions closely mimicking data used in model development. Thus, it was important to validate the GRNN model against independent data. To accomplish this, the data set was divided into dependent data ($n = 482$) and independent data ($n = 235$) sets. The independent data were collected with the same methods as dependent data but at intermediate temperatures with the exception of data collected at 14 °C. This was done to see how well the model could interpolate over its entire prediction region.

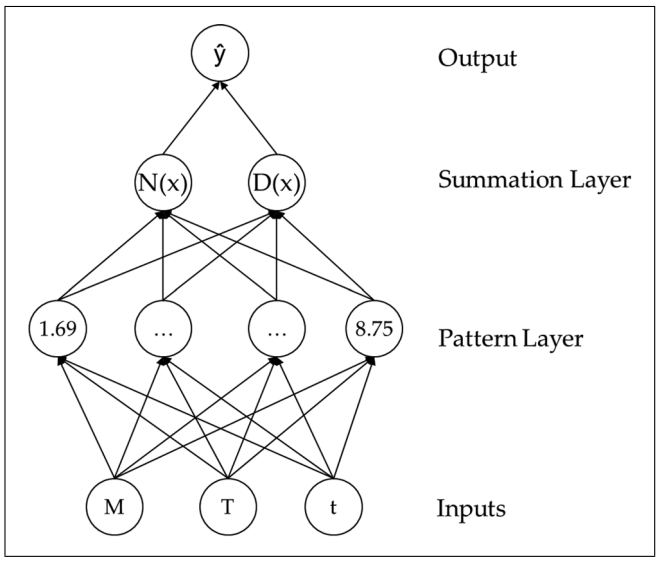


Figure 1–Diagram of the GRNN model for survival, death, and growth of *Salmonella* Typhimurium DT104 on chicken meat as a function of time (t), temperature (T), and type of meat (M). The pattern layer shows the range of log number data.

Model performance was evaluated using the acceptable prediction zone (APZ) method (Oscar 2005a). This method has criteria for test data and model performance that must be satisfied for a model to be classified as validated. It involves 3 sequential steps: (1) goodness-of-fit; (2) interpolation; and (3) extrapolation.

A model is considered validated in the APZ method when it meets test data and model performance criteria for the 1st 2 steps. The 3rd step is optional and is performed to see how broadly the model can be applied to independent variables (for example, other strains) not included in model development. Although optional, the 3rd step is important because it identifies independent variables for which new models are not needed, which saves time and money.

The APZ method can be used to evaluate and validate all types of predictive models (Oscar 2005b). The APZ boundaries used for each type of model are based on an evaluation of experimental error associated with determining dependent variables (for example, lag time). For models that predict log number, it was found that the absolute relative error among replicate samples was about 0.5 log (Oscar 2005a). Thus, a prediction is considered acceptable in the APZ method when the difference between observed and predicted values is <0.5 log. This metric is the same as that used by Thurette and others (1998) to evaluate performance of a predictive model for *Listeria monocytogenes* and smoked fish products.

When using a model to predict food safety, it is desirable to allow the model to err more in the fail-safe direction to provide an extra level of assurance that model predictions will protect public health. The precedent in predictive microbiology is to allow a model to err twice as much in the fail-safe direction (Ross and others 2000). Consequently, a prediction is considered acceptable in the APZ method when the residual (observed – predicted) is in an APZ from –1 log (fail-safe) to 0.5 logs (fail-dangerous).

There is not a precedent in predictive microbiology for what proportion of residuals must be acceptable for classification of a model as providing predictions with acceptable accuracy and bias. However, in the U.S.A. education system an established performance criterion is that a test score of 70% correct answers is the minimum for a classification of acceptable performance. This established criterion is used in the APZ method. Thus, when the proportion of residuals in the APZ (pAPZ) is ≥0.7, the model is classified as providing acceptable predictions.

In addition to criteria for model performance, criteria for test data must be met for a model to be classified as validated. Table 1 shows the progression of questions that must be answered in the affirmative for a model to be validated by the APZ method. Thus, in the APZ method, it is not possible to validate a model for extrapolation if it was not validated for interpolation and it is not possible to validate a model for interpolation unless it provided acceptable predictions of dependent data.

An important component of the APZ method is a plot of residuals as a function of independent variables or a plot of the dependent variable as a function of the independent variables with the APZ indicated on the plot. These types of plots are used to check for local prediction problems.

Statistical analysis

To provide an objective way to conclude whether *Salmonella* Typhimurium DT104 survived, died, or grew on chicken meat during cold storage and to objectively assess whether type of meat

Table 1–Progression of questions that must be answered in the affirmative for a model to be classified as validated in the acceptable prediction zone (APZ) method.

Q1	Were predictions for dependent data acceptable (pAPZ ≥ 0.7)?
Q2	Were validation data for interpolation independent?
Q3	Were validation data for interpolation collected with dependent data methods?
Q4	Did validation data for interpolation provide complete coverage of model predictions?
Q5	Were predictions for validation data for interpolation acceptable (pAPZ ≥ 0.7)?
Q6	Was validation for interpolation successful (yes to Q1 to Q5)?
Q7	Were validation data for extrapolation independent?
Q8	Were validation data for extrapolation collected with dependent data methods except for the new independent variable?
Q9	Did validation data for extrapolation provide complete coverage of model predictions?
Q10	Were predictions for validation data for extrapolation acceptable (pAPZ ≥ 0.7)?
Q11	Was validation for extrapolation successful (yes to Q1 to Q10)?

affected behavior of *Salmonella* Typhimurium DT104 on chicken during cold storage, two-way analysis of variance (version 6.03, Prism, GraphPad Software, San Diego, Calif., U.S.A.) was used within storage temperature to determine effects of storage time, type of meat, and their interaction on log number of *Salmonella* Typhimurium DT104 per portion. Data used in model development and validation were combined into a single data set for this analysis. When a significant ($P < 0.05$) effect of type of meat or interaction of type of meat and storage time was observed, means among types of meat within a storage time and temperature were compared using Tukey's multiple comparison tests with a significance level of $P < 0.05$.

One-way analysis of variance (Prism, GraphPad Software) was used to determine effect of type of meat on pH and log number of native microflora per portion. When a significant ($P < 0.05$) effect of type of meat was observed, means were compared using Tukey's multiple comparison tests with a significance level of $P < 0.05$.

Results and Discussion

Behavior of *Salmonella* Typhimurium DT104 on chicken meat during cold storage

Based on a study by Foster and Mead (1976) it was expected that depending on type of meat and time and temperature of cold storage that the log number of *Salmonella* Typhimurium DT104 inoculated onto chicken meat would stay the same, decrease, or increase. To provide an objective assessment of whether *Salmonella* Typhimurium DT104 survived, died, or grew on chicken meat during cold storage, results within a storage temperature were analyzed by two-way analysis of variance. A significant ($P < 0.05$) main effect of time or a significant ($P < 0.05$) interaction of time and type of meat indicated that *Salmonella* Typhimurium DT104 either died or grew on chicken meat. On the other hand, when the main effect of time and interaction of time and type of meat on the log number of *Salmonella* Typhimurium DT104 per portion were not significant ($P > 0.05$) this indicated survival and not death or growth.

The main effect of time and interaction of time and type of meat on log number of *Salmonella* Typhimurium DT104 per portion were not significant ($P > 0.05$) for storage temperatures of –8, –4, 0, 8, and 10 °C (results not shown). Thus, *Salmonella* Typhimurium DT104 survived and did not die or grow on chicken meat during 8 d of cold storage at these temperatures. However, at a storage temperature of 4 °C, the main effect of time and the main effect of type of meat were significant ($P < 0.05$). Here (Figure 2), log number of *Salmonella* Typhimurium DT104 was lower ($P < 0.05$) on skin than white meat at 2 d of storage, lower ($P < 0.05$) on dark meat than white meat at 4 d of storage, and lower ($P < 0.05$) on skin than on dark meat at 8 d of storage. Although there was not a consistent pattern of results among types of meat, results indicated that log number of *Salmonella* Typhimurium DT104 per portion decreased slightly (0.4 log) during storage for 0 to 8 d at 4 °C.

At higher cold storage temperatures (12, 14, and 16 °C), log number of *Salmonella* Typhimurium DT104 was affected by a significant ($P < 0.05$) time by type of meat interaction. At 12 °C (Figure 3), log number of *Salmonella* Typhimurium DT104 was higher ($P < 0.05$) on dark meat than white meat and skin at 4, 6, and 8 d of storage whereas at 8 d of storage it was higher ($P < 0.05$) on skin than on white meat. At 14 °C (Figure 4), log number of *Salmonella* Typhimurium DT104 was higher ($P < 0.05$) on dark meat than on white meat and skin at 2, 4, 6, and 8 d of storage whereas it was higher ($P < 0.05$) on skin than on white meat

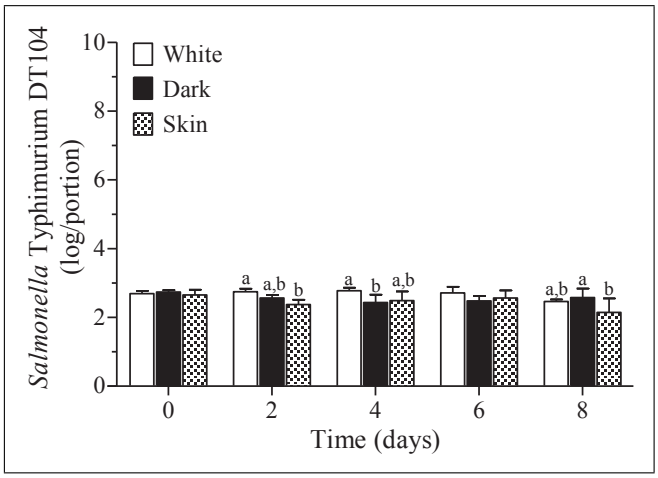


Figure 2–Effect of time and type of meat on log number of *Salmonella* Typhimurium DT104 on chicken meat stored for 0 to 8 d at 4 °C. Bars are means ± standard deviations. Bars within a cluster with different superscripts differ ($P < 0.05$).

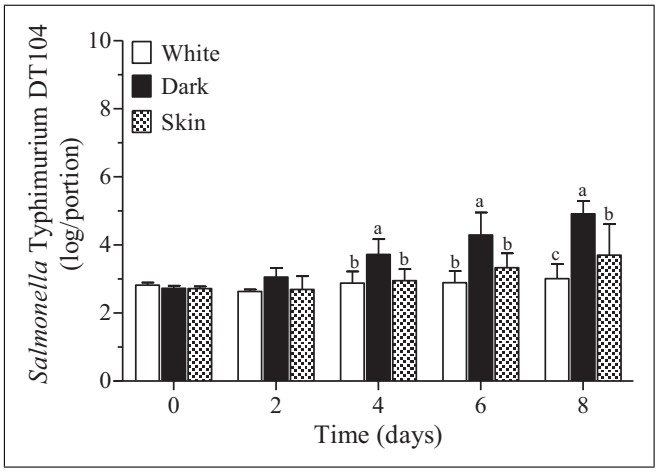


Figure 3–Effect of time and type of meat on log number of *Salmonella* Typhimurium DT104 on chicken meat stored for 0 to 8 d at 12 °C. Bars are means ± standard deviations. Bars within a cluster with different superscripts differ ($P < 0.05$).

at 4, 6, and 8 d of storage. At 16 °C (Figure 5), log number of *Salmonella* Typhimurium DT104 was higher ($P < 0.05$) on dark meat and skin than on white meat at 2, 4, 6, and 8 d of storage and was higher ($P < 0.05$) on dark meat than on skin at 2 and 4 d of storage. Thus, *Salmonella* Typhimurium DT104 grew on chicken meat stored for 8 d at 12, 14, or 16 °C and growth was highest on dark meat, intermediate on skin, and lowest on white meat.

Differences in pH and native microflora could help explain differences in growth of *Salmonella* Typhimurium DT104 on skin, white, and dark meat of chicken during cold storage at 12, 14, or 16 °C. One-way analysis of variance indicated that pH differed ($P < 0.05$) among types of chicken meat; it was higher ($P < 0.05$) for skin (6.41 ± 0.12) and dark meat (6.46 ± 0.03) than for white meat (5.92 ± 0.11). However, initial log number of native microflora was similar ($P > 0.05$) for skin (2.82 ± 0.41 log/portion), dark meat (2.59 ± 0.18 log/portion), and white meat (2.70 ± 0.24 log/portion). Thus, differences in initial level of native microflora did not explain differences in growth of *Salmonella* Typhimurium DT104 among different types of chicken meat during cold storage at 12, 14, or 16 °C. However, the lower pH of white meat may have been a contributing factor to lower growth of *Salmonella* Typhimurium DT104 on white meat compared to dark meat and skin, which had higher but similar pH.

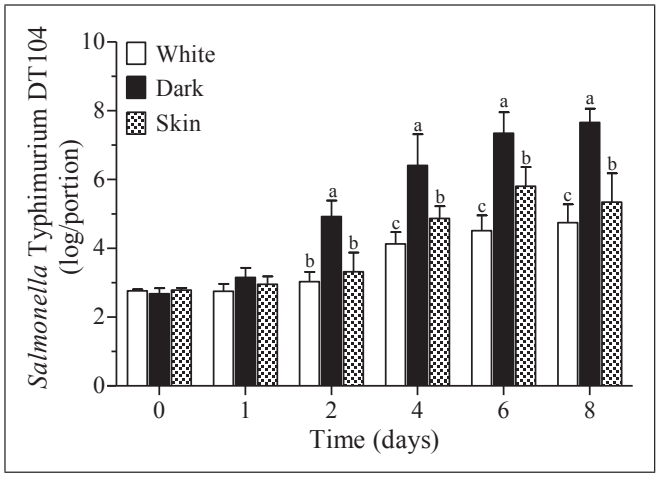


Figure 4–Effect of time and type of meat on log number of *Salmonella* Typhimurium DT104 on chicken meat stored for 0 to 8 d at 14 °C. Bars are means ± standard deviations. Bars within a cluster with different superscripts differ ($P < 0.05$).

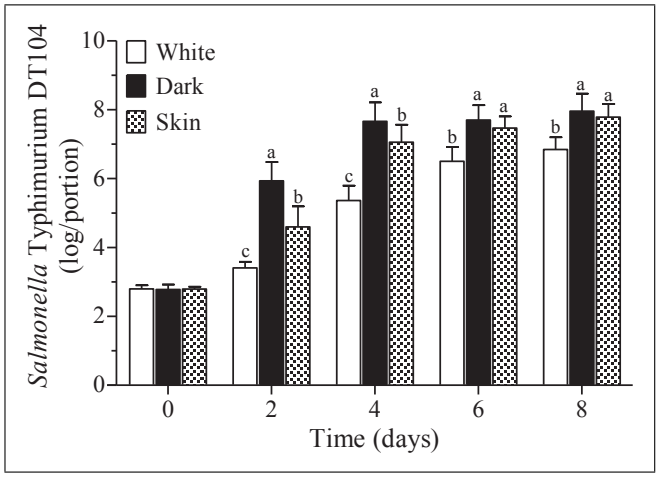


Figure 5–Effect of time and type of meat on log number of *Salmonella* Typhimurium DT104 on chicken meat stored for 0 to 8 d at 16 °C. Bars are means ± standard deviations. Bars within a cluster with different superscripts differ ($P < 0.05$).

Based on a previous study (Foster and Mead 1976) it was expected that the log number of *Salmonella* Typhimurium DT104 on chicken meat would decrease during storage at – 8 to 0 °C and that the decrease would be greater in dark meat than in white meat. Instead, log number of *Salmonella* Typhimurium DT104 on chicken meat during storage at –8 to 0 °C remained the same throughout 8 d of storage and was not affected by type of meat. The pH of white meat was 5.80 and pH of dark meat was 6.40 in the previous study (Foster and Mead 1976) as compared to pH of 5.92 for white meat and 6.46 for dark meat in the present study. Thus, pH of chicken meat was similar among studies and therefore does not explain the difference in results. However, other experimental conditions, such as strain of *Salmonella* Typhimurium, previous history of the inoculum, inoculum size, and native microflora, differed among these studies and could account for the difference in results.

Behavior of *Salmonella* on chicken meat stored at refrigeration temperatures (0 to 6 °C) is variable among studies. Shin and others (2010) report that *Salmonella* Typhimurium grow from 3.2 to 6 logs per gram on chicken breast meat during 10 d of storage at 4 °C. In contrast, Cosansu and Ayhan (2010) found that the number of *Salmonella* Enteritidis decreased on chicken breast meat from 5.3 log per cm^2 on day 0 to 4.1 log per cm^2 on day 10 of storage at 4 °C. Sharma and others (2012) observed that the number of *Salmonella* Typhimurium on chicken breast fillets decreased from 6.33 to 5.01 logs per gram during 7 d of storage at 4 °C. In the current study, the number of *Salmonella* Typhimurium DT104 on chicken meat declined slightly (0.4 log) during storage for 8 d at 4 °C. Other studies (Szczawinska and others 1991; Nychas and Tassou 1996; Oscar 2011a; Pradhan and others 2012) report that the level of *Salmonella* on chicken meat stays the same during refrigerated storage. Together these studies indicate that behavior of *Salmonella* on chicken meat during refrigerated storage is complex and may depend on multiple factors whose interactions are not well understood at the present time.

Similar to behavior of *Salmonella* on chicken meat during proper refrigeration (0 to 6 °C), behavior of *Salmonella* on chicken meat during improper refrigeration (7 to 16 °C) is different among studies indicating a potential interaction among multiple factors. In the present study, *Salmonella* Typhimurium DT104 did not grow on chicken meat stored for 8 d at 8 or 10 °C. In contrast, Baker and others (1986) report that the number of *Salmonella* Typhimurium on minced chicken meat (breast and leg) increases from 4.2 to 6.1 log after 5 d of storage at 7 °C. Nissen and others (2001) found that the number of *Salmonella* Enteritidis on chicken breast meat with skin increases from 3.5 to 7 log per cm^2 after 4 d of storage at 10 °C.

Based on a previous study (Oscar 2012) it was expected that growth of *Salmonella* Typhimurium DT104 would be the same on skin and white and dark meat of chicken. Instead, growth of *Salmonella* Typhimurium DT104 was highest on dark meat, intermediate on skin, and lowest on white meat in the present study. One important difference between studies was the storage temperature, which was 30 °C in the previous study (Oscar 2012) and 12, 14, or 16 °C in the current study. Thus, the effect of type of meat on growth of *Salmonella* Typhimurium DT104 on chicken meat may depend on storage temperature; additional studies are needed to clarify this issue.

Model development and validation

Results of two-way analysis of variance indicated that time, temperature, and type of meat were all important variables affecting behavior of *Salmonella* Typhimurium DT104 on chicken meat during cold storage. Thus, a GRNN model was developed to predict log number of *Salmonella* Typhimurium DT104 on chicken meat during cold storage as a function of these variables. Data collected at storage temperatures of –8, 0, 8, 12, 14 (1st 2 storage trials), and 16 °C were used to develop the GRNN model whereas data collected at storage temperatures of –4, 4, 10, and 14 °C (last 1 or 2 storage trials) were used to validate the GRNN model for its ability to interpolate. After the GRNN model was developed, residuals (observed log number – predicted log number) were evaluated using the APZ method.

A pAPZ of 0.929 was obtained for dependent data (Figure 6) and a pAPZ of 0.923 was obtained for independent data (Figure 7). Because the pAPZ for dependent data was ≥ 0.7, the GRNN model had acceptable goodness-of-fit (yes to Q1 in Table 1). In addition, the GRNN model was validated for interpolation (yes to Q6 in Table 1) because the pAPZ for independent data for interpolation was ≥ 0.7 (yes to Q5 in Table 1) and the validation data were independent (yes to Q2 in Table 1), collected with the same methods as dependent data (yes to Q3 in Table 1), and provided complete coverage of model predictions (yes to Q4 in Table 1).

To further evaluate and validate performance of the GRNN model, plots of the dependent variable (log number per portion) as a function of the independent variables (time, temperature, and

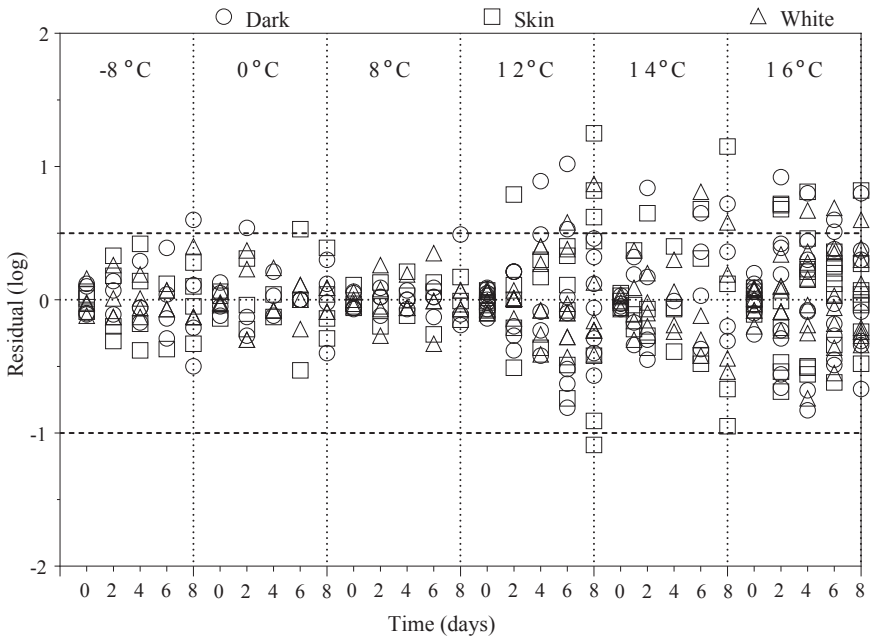


Figure 6–Acceptable prediction zone (APZ) analysis of a GRNN model for behavior of *Salmonella* Typhimurium DT104 on chicken meat (dependent data) as a function of time, temperature, and type of meat. Dashed lines are APZ boundaries.

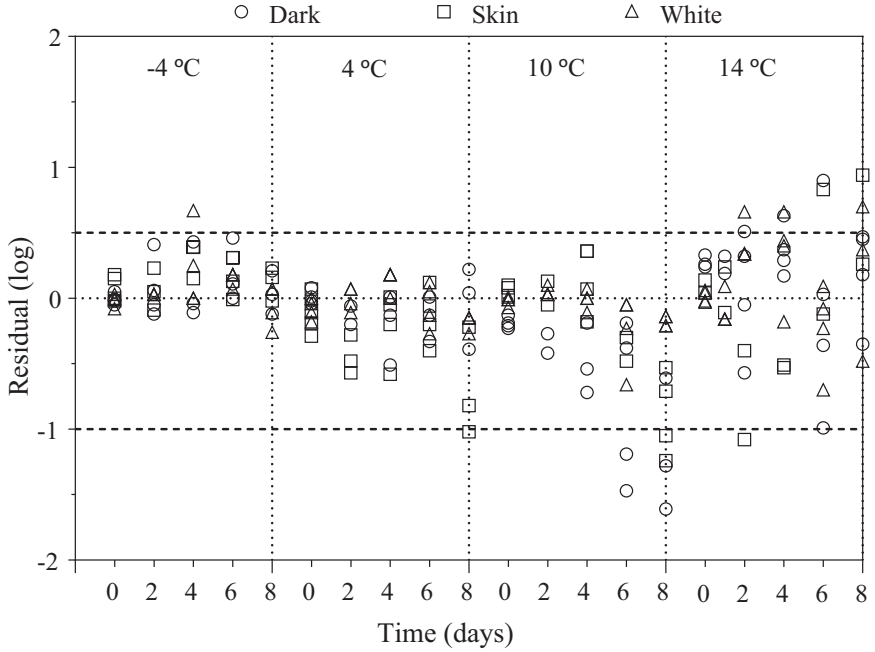


Figure 7–APZ analysis of a GRNN model for behavior of *Salmonella* Typhimurium DT104 on chicken meat (independent data) as a function of time, temperature, and type of meat. Dashed lines are APZ boundaries.

type of meat) were made and examined. These plots contained observed data (symbols), predicted log number (solid line), and APZ boundaries (dotted lines). The purpose of the plots was to look for local prediction problems. Only plots with local prediction problems are shown.

For dependent data, all combinations of time, temperature, and type of meat had pAPZ ≥ 0.7 except for skin stored for 8 d at 12 °C (Figure 8). Here, failure of the GRNN model to provide an acceptable prediction was due to variation of log number data. This could be due to experimental error or it could indicate that an important independent variable was missing from the model.

For independent data, all combinations of time, temperature, and type of meat had pAPZ ≥ 0.7 except for 5. They were storage on skin for 8 d at 10 °C (Figure 9), storage on skin for 6 or 8 d at 14 °C (Figure 10), and storage on dark meat for 6 or 8 d at 10 °C (Figure 11). Here, local prediction problems resulted from the GRNN model predicting growth at 10 °C when survival occurred and in the case of skin at 14 °C, limited data. Nonetheless, in all cases, the unacceptable predictions were close to the APZ boundaries and thus, not a major concern.

The final step was to construct a user-friendly version of the GRNN model for use by the chicken industry to assess impact of process deviations on *Salmonella* behavior (Figure 12). The final model was designed like the those in the U.S. Dept. of Agriculture, Pathogen Modeling Program; it predicts log number of *Salmonella* Typhimurium DT104 on chicken as a function of time (0 to 8 d), temperature (–8 to 16 °C), and type of meat (white, dark, or skin). As with all models of this type, it predicts pathogen behavior for conditions used in model development and for conditions not used in model development but that fall within ranges of independent variables used to develop the model. For example, in Figure 12,

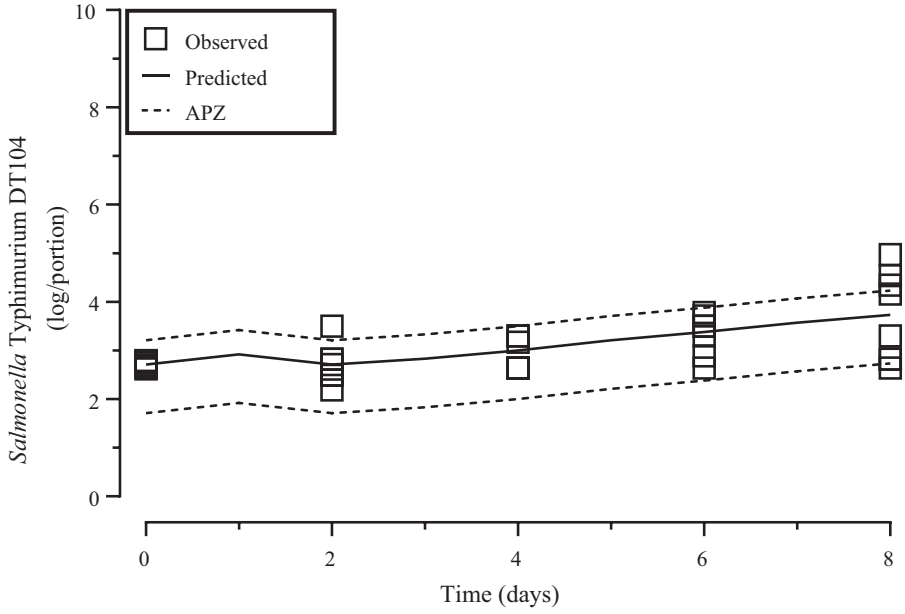


Figure 8–APZ analysis of a GRNN model for behavior of *Salmonella* Typhimurium DT104 on chicken skin stored at 12 °C. Symbols are observed log number (dependent data), solid line is predicted log number, and dashed lines are APZ boundaries.

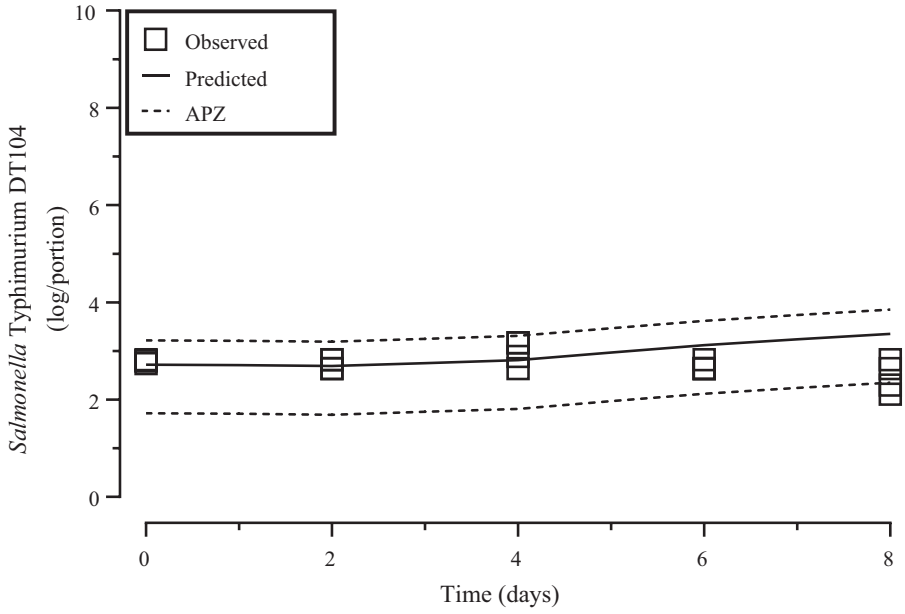


Figure 9–APZ analysis of a GRNN model for behavior of *Salmonella* Typhimurium DT104 on chicken skin stored at 10 °C. Symbols are observed log number (independent data), solid line is predicted log number, and dashed lines are APZ boundaries.

the model predicts log number of *Salmonella* Typhimurium DT104 on chicken skin for 8 d of storage at a temperature (15 °C) not used in model development.

The original models for prediction of food safety were developed in laboratory broth as a function of time, temperature, pH, and water activity (McClure and others 1994; Whiting 1995). The guiding philosophy was that these models would provide fail-safe predictions of pathogen behavior in food. However, broth models do not consider microbial competition and as a result provide overly fail-safe predictions. As shown in a previous study for *Salmonella* and chicken meat (Oscar 2007), broth models can overpredict growth of *Salmonella* on chicken by as much as 7 logs. Consequently, the chicken industry prefers models developed with chicken meat rather than laboratory broth.

Investigating and modeling behavior of *Salmonella* on chicken meat is different from investigating and modeling behavior of *Salmonella* in laboratory broth. In fact, the independent variables that have the most influence on behavior of *Salmonella* in laboratory broth and in chicken meat differ. Of note, pH and water activity vary little within a single type of chicken meat and thus, are not highly influential independent variables in predictive models developed with chicken meat. Identifying which independent variables are important to include in a predictive model for *Salmonella* and chicken meat is important. Thus far, 3 independent variables, in addition to time and temperature, have been identified that are important to include in predictive models for *Salmonella* and chicken meat, they are: (1) serotype (Oscar 2009a); (2) inoculum size (Oscar 2007, 2011b); and (3) type of meat (present study).

An important consideration when developing and validating a predictive model for food safety is that it can err more in the fail-safe direction than in the fail-dangerous direction (Ross and others 2000). However, it should not be allowed to err too much in the fail-safe direction because models that provide overly

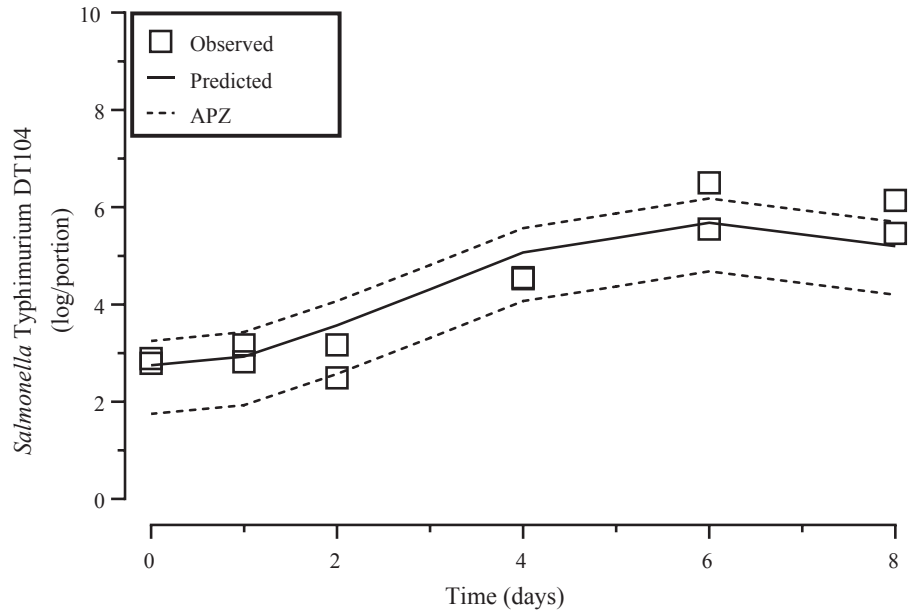


Figure 10–APZ analysis of a GRNN model for behavior of *Salmonella* Typhimurium DT104 on chicken skin stored at 14 °C. Symbols are observed log number (independent data), solid line is predicted log number, and dashed lines are APZ boundaries.

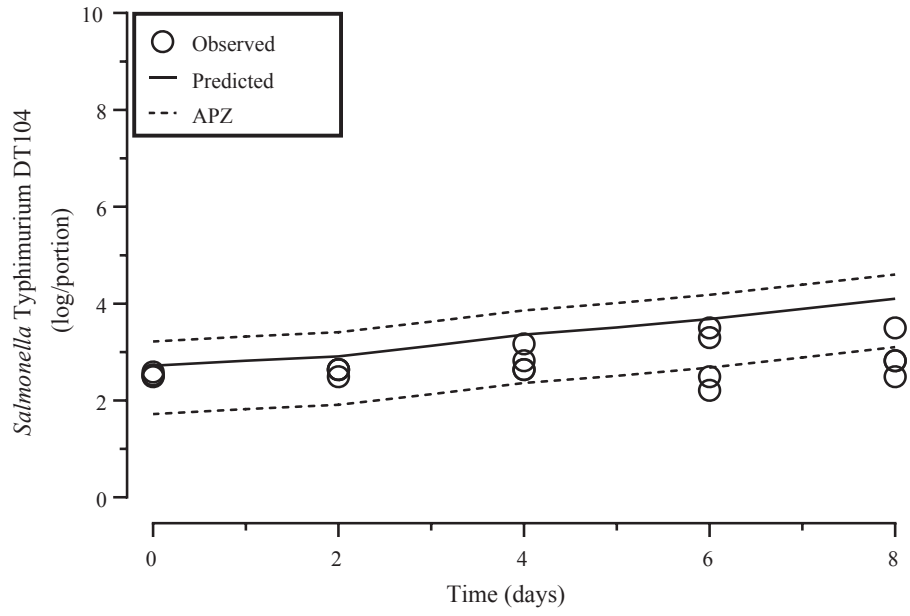


Figure 11–APZ analysis of a GRNN model for behavior of *Salmonella* Typhimurium DT104 on chicken dark meat stored at 10 °C. Symbols are observed log number (independent data), solid line is predicted log number, and dashed lines are APZ boundaries.

fail-safe predictions prevent consumption of safe food that could have benefited public health. On the other hand, a model that provides overly fail-dangerous predictions harms public health by allowing distribution and consumption of food that presents a significant risk.

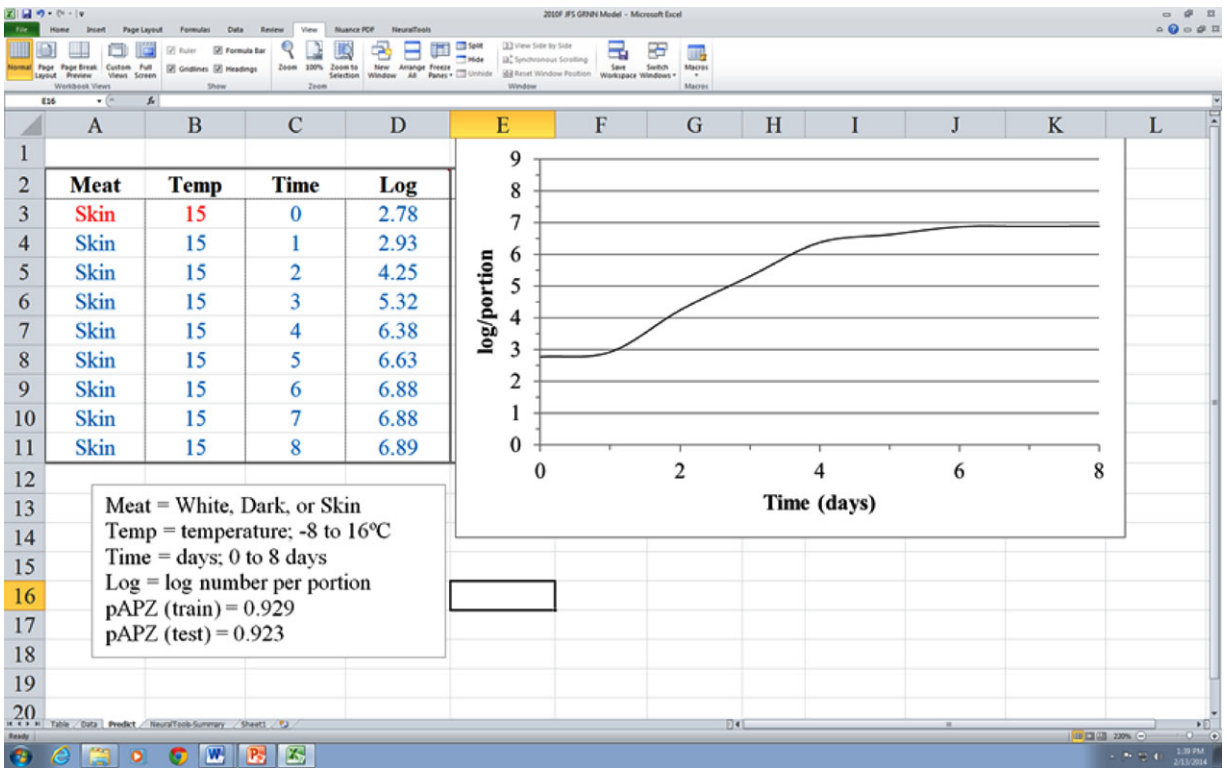
The precedent in predictive microbiology is to allow models to err twice as much in the fail-safe direction (Ross and others 2000). Consequently, predictions of the current GRNN model were considered acceptable when they were in an APZ from –1 log (fail-safe) to 0.5 logs (fail-dangerous). Moreover, the GRNN model was considered to provide predictions with acceptable accuracy and bias when the proportion of residuals in the APZ (pAPZ) was ≥ 0.7. In the current study, pAPZ was 0.929 for dependent data and 0.923 for independent data. Because the independent data for validation met the test data criteria for the APZ method (Table 1), the GRNN model was classified as validated meaning that it provided predictions with acceptable accuracy and bias or in other words, predictions that were neither overly fail-safe nor overly fail-dangerous.

In previous studies (Abou-Zeid and others 2009; Oscar 2009b), the APZ method was applied to complete sets of data and more recently (Oscar 2011b, 2013) to individual survival and growth curves. In the current study, the APZ method was also applied to individual combinations of independent variables. This was done to better detect local prediction problems. Although some local prediction problems were detected, as discussed above, they were not of sufficient concern to warrant a classification of nonvalidation. However, they do indicate that the model can be improved by collection of additional data.

Most models that are used to predict food safety have been developed using regression methods and 3 steps: (1) primary modeling, (2) secondary modeling, and (3) tertiary modeling (Whiting and Buchanan 1994). Primary modeling involves fitting log number data obtained under one combination of independent variables to a mathematical model that describes growth, survival, or death as a function of time. Secondary modeling involves modeling fitted parameters of the primary model as a function of independent variables. Substituting secondary models for parameters in the primary model forms a tertiary model that predicts log number over time as a function of independent variables. The value of the tertiary model is that it can generate predicted growth, survival, or death curves for combinations of independent variables that were used in model development or were not used in model development but that fall within ranges of independent variable combinations used in model development.

In the current study, a GRNN model was developed for behavior of *Salmonella* Typhimurium DT104 on chicken meat during cold storage. The GRNN model was developed in one step using commercial software and functions like a tertiary model in that it provides predictions of survival, death, and growth curves for combinations of independent variables that were and were not used in model development. Development of tertiary or tertiary-like models in one step, as was done in the present study, saves time and money, is simpler than a 3-step modeling process, and as shown in a previous study (Martino and Marks 2007) reduces prediction error of the final model.

Collection of data for development of predictive models for *Salmonella* and chicken meat is time consuming and expensive. Small chicken meat portions (0.75 cm^3) in 1.5 mL microcentrifuge tubes that were incubated in tabletop heating and cooling dry blocks were used in the present study to make it less time



Meat	Temp	Time	Log
Skin	15	0	2.78
Skin	15	1	2.93
Skin	15	2	4.25
Skin	15	3	5.32
Skin	15	4	6.38
Skin	15	5	6.63
Skin	15	6	6.88
Skin	15	7	6.88
Skin	15	8	6.89

Figure 12–GRNN model for predicting survival, death, and growth of *Salmonella* Typhimurium DT104 on chicken meat as a function of time (0 to 8 d), constant temperature (–8 to 16 °C), and type of meat (white, dark, or skin).

consuming and expensive to collect data for model development and validation. In addition, use of small meat portions facilitated enumeration of low levels of *Salmonella*.

One final issue to address is why the prediction bias (B_f) and accuracy factor (A_f) method (Ross 1996; Ross and others 2000) was not used to evaluate and validate the present GRNN model. The B_f and A_f method was originally used to evaluate performance of secondary models for generation time (GT):

$$B_f = 10^{\left(\sum log\left(GT_{predicted}/GT_{observed}\right)/n\right)} \quad (2)$$

$$A_f = 10^{\left(\sum |log\left(GT_{predicted}/GT_{observed}\right)|/n\right)} \quad (3)$$

The performance indices are mean values of normalized predicted values. However, when the B_f and A_f method are applied to GRNN models that predict log number of *Salmonella* on chicken meat (Oscar 2009a), for the same log difference between observed and predicted values (for example, 1 log), normalized predicted values are large (for example, $1.1/0.1 = 11$) when observed values are small and they are small ($9.5/8.5 = 1.1$) when observed values are large. Thus, B_f and A_f were not used to evaluate and validate the current GRNN model because of a systematic prediction bias error in their computation when applied to models that predict log number.

Conclusions

A GRNN model for survival, death, and growth of *Salmonella* Typhimurium DT104 on chicken meat during cold storage was successfully developed and validated against independent data. Results indicated that during improper refrigeration (12 to 16 °C) growth of *Salmonella* Typhimurium DT104 was highest on dark meat, intermediate on skin, and lowest on breast meat. Thus, it was important to include type of meat as an independent variable in the model to obtain reliable predictions of *Salmonella* behavior on chicken meat during cold storage. How broadly the model can be applied to other strains of *Salmonella*, other inoculum sizes, other previous histories, and fluctuating temperatures remains to be determined in future validation studies for extrapolation.

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