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Original article

# Neural network models for growth of *Salmonella* serotypes in ground chicken subjected to temperature abuse during cold storage for application in HACCP and risk assessment

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**Summary** Predictive microbiology models are valuable tools for helping to assess and manage the risk of illness from food contaminated with human pathogens, such as *Salmonella*. However, multiple versions of a model may be needed for different food safety applications, such as hazard analysis and critical control point (HACCP) programs and risk assessment. A neural network model for growth of *Salmonella* in ground chicken as a function of time (0 to 8 days) at 16 °C and serotype ($n$ = 8) was developed. The proportion of residuals in an acceptable prediction zone (pAPZ) from −1 log (fail-safe) to 0.5 log (fail-dangerous) was 0.948 for training data ($n$ = 192) and 0.988 for testing data ($n$ = 84). A pAPZ ≥0.7 indicated that the model provided predictions with acceptable bias and accuracy. Thus, the model was successfully validated. Different versions of the model were developed for application in HACCP and risk assessment.

**Keywords** Ground chicken, hazard analysis and critical control point, neural network models, risk assessment, *Salmonella*, temperature abuse.

## Introduction

Growth of pathogens in food is affected by internal and external factors of the food, as well as pathogen characteristics. Internal factors of food include composition, water activity and pH. External factors of food include time and temperature. Pathogen characteristics include physiological state and strain variation. There are limited data in the scientific literature regarding variation of growth among serotypes of *Salmonella* in chicken meat with native microflora. Existing studies (Oscar, 2009, 2015) show that growth of *Salmonella* is affected by serotype but only a limited number of serotypes have been investigated. Consequently, in this study, data for growth of five *Salmonella* serotypes (Thompson, Typhimurium, Typhimurium var 5-, Kentucky and 8,20:-:$z_6$) in ground chicken thigh meat with native microflora from a previous study (Oscar, 2015) were combined with new data for three additional serotypes (Enteritidis, 4,5,12:Nonmotile and 4,12:Nonmotile) to further evaluate the effect of serotype on growth and to develop and validate a model for growth of *Salmonella* in ground chicken as a function of serotype and time of cold storage at 16 °C. The temperature of 16 °C was selected because it is a temperature at which *Salmonella* grow and differences among serotypes can be detected (Oscar, 2015).

Models that predict growth of foodborne human pathogens, such as *Salmonella*, in unit operations of the food production chain are valuable tools for food safety. These types of models can predict whether a process deviation (e.g. temperature rise) at a critical control point (e.g. cold storage) results in growth of the pathogen by a critical amount (e.g. one log increase). In addition, these models can provide output distributions of pathogen growth that serve as input distributions in risk assessment models that predict changes in pathogen number within a unit operation (e.g. cold storage) of a food production chain. Thus, predictive models have application in both hazard analysis and critical control point (HACCP) programs and risk assessment. However, different versions of the model are needed for these different food safety

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applications. Thus, a second objective of the current study was to demonstrate how to develop different versions of the model for use in HACCP and risk assessment.

## Materials and methods

### Experimental design

A 6 × 8 full factorial design of time (0, 1, 2, 4, 6, or 8 days) and serotype (8,20:-:$z_6$, Kentucky, Typhimurium, Typhimurium var 5-, Thompson, Enteritidis, 4,5,12:Nonmotile or 4,12:Nonmotile) was used to investigate and model growth of *Salmonella* serotypes in ground chicken subjected to temperature abuse during cold storage at 16 °C. Six replicate storage trials were conducted per serotype except for Enteritidis and 4,12: Nonmotile where five replicate storage trials were conducted. Six replicates were supposed to be conducted for all serotypes but the last replicates for serotypes Enteritidis and 4,12:Nonmotile were accidently missed.

### Storage trials

*Salmonella* were isolated in previous studies (Oscar, 2013, 2014b) from chicken parts harvested from whole broilers chickens sold in flow pack wrappers and obtained at retail. The isolates were maintained in stock culture as previously described (Oscar, 2015). Stock cultures were thawed at room temperature, and then, 5 μL was added to 9 mL of buffered peptone water (BPW, Difco™, Becton, Dickinson and Co., Sparks, MD, USA) in glass dilution tubes. Precultures were incubated for 72 h at 22 °C without shaking to obtain stationary-phase cells for inoculation of ground chicken thigh meat portions (0.75 $cm^3$ in 1.5 mL microcentrifuge tubes), which were prepared as previously described (Oscar, 2014a). Precultures were serially diluted (1:10) in BPW, and then, 5 μL of the $10^{-5}$ dilution of the appropriate serotype was inoculated into the centre of ground chicken portions at room temperature. Inoculated portions (six per trial) were incubated at 16 °C in a heating and cooling block (ThermoStat Plus, Eppendorf, Hamburg, Germany).

### Most probable number

At each sampling time, a ground chicken portion was transferred to a plastic bag (207-mL) that had a filter screen (Whirl-Pak®, Nasco, Fort Atkinson, WI, USA) and 9 mL of BPW. The sample was pulsified (model PUL 100, Microbiology International, Frederick, MD, USA) for 15 s to recover *Salmonella* into BPW for enumeration by a three (replicate) by eight (dilution) most probable number (MPN) method that was performed in 2 mL, 96-well deep-well plates (Axygen Scientific, Union City, CA, USA), as previously described (Oscar, 2015).

In brief, a 1 mL sample of a $10^0$, $10^{-1}$ or $10^{-2}$ serial dilution (1:10) of pulsified sample in BPW was added to an empty cell in the first row of the deep-well plate that contained 0.9 mL of BPW in all other wells. After the first row was filled with samples, serial dilutions (1:10) were performed by a robotic pipettor (SOLO Plus, Hudson Robotics, Springfield, NJ, USA). After incubation for 24 h at 40 °C, 10 μL from each well of the BPW plate was transferred by the robotic pipettor to corresponding wells of a second deep-well plate that contained 1 mL of Rappaport Vassiliadis R10 broth (RVB; Difco™, Becton, Dickinson and Co.) in each well. After incubation of the RVB plate for 48 h at 42 °C, *Salmonella*-positive (white) and *Salmonella*-negative (blue) wells were recorded and used to calculate the log MPN per portion by the method of Thomas (1942) as described by Oscar (2014a). This automated mini-MPN method was validated in a previous study (Oscar, 2015) using an AOAC-approved lateral flow assay that showed complete concordance between white and blue wells in RVB and positive and negative results in the lateral flow assay, respectively.

### Model development

A data set was created in a computer spreadsheet (Excel 2013, MicroSoft Corp., Redmond, WA, USA) with four columns: (i) tag (tag), (ii) serotype (independent categorical variable), (iii) time (independent numerical variable) and (iv) log MPN per portion (dependent numerical variable). Within data for a serotype and time, the highest four of six (66.7%) log MPN values for serotypes Typhimurium, Typhimurium var 5-, Thompson, 4,5,12:Nonmotile, Kentucky or 8,20:-:$z_6$ or highest four of five (80%) log MPN values for serotypes Enteritidis or 4,12:Nonmotile were tagged as training data ($n = 192$) and the lowest 33.3% or 20% of the log MPN values, respectively, were tagged as testing data ($n = 84$). This was done to create a slightly fail-safe model, to provide a unique solution for the neural network model so that predictions are consistent and to provide independent data to test the model for the ability to generalise. A spreadsheet add-in program (industrial version 6, NeuralTools, Palisade Corp., Ithaca, NY, USA) was used to develop a multiple-layer feedforward neural network model with a single hidden layer of two nodes (Fig. 1). The activation function was the hyperbolic tangent function in the hidden layer and the identity function in the output layer.

The first model used an add-in program (NeuralTools) that for proprietary reasons did not provide the parameters (weights and bias) of the neural network model. This is a problem because not all

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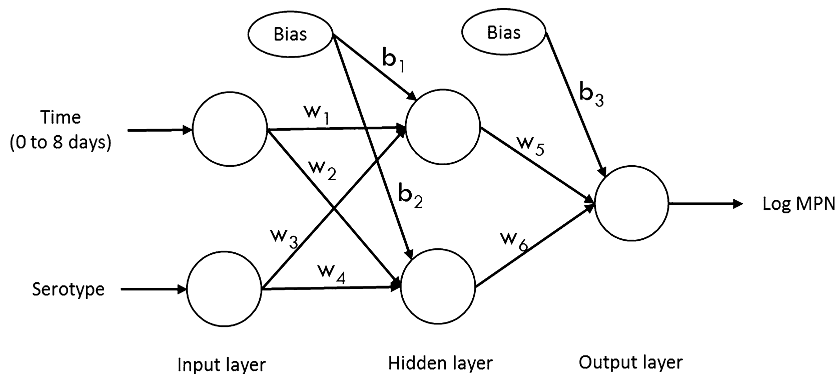
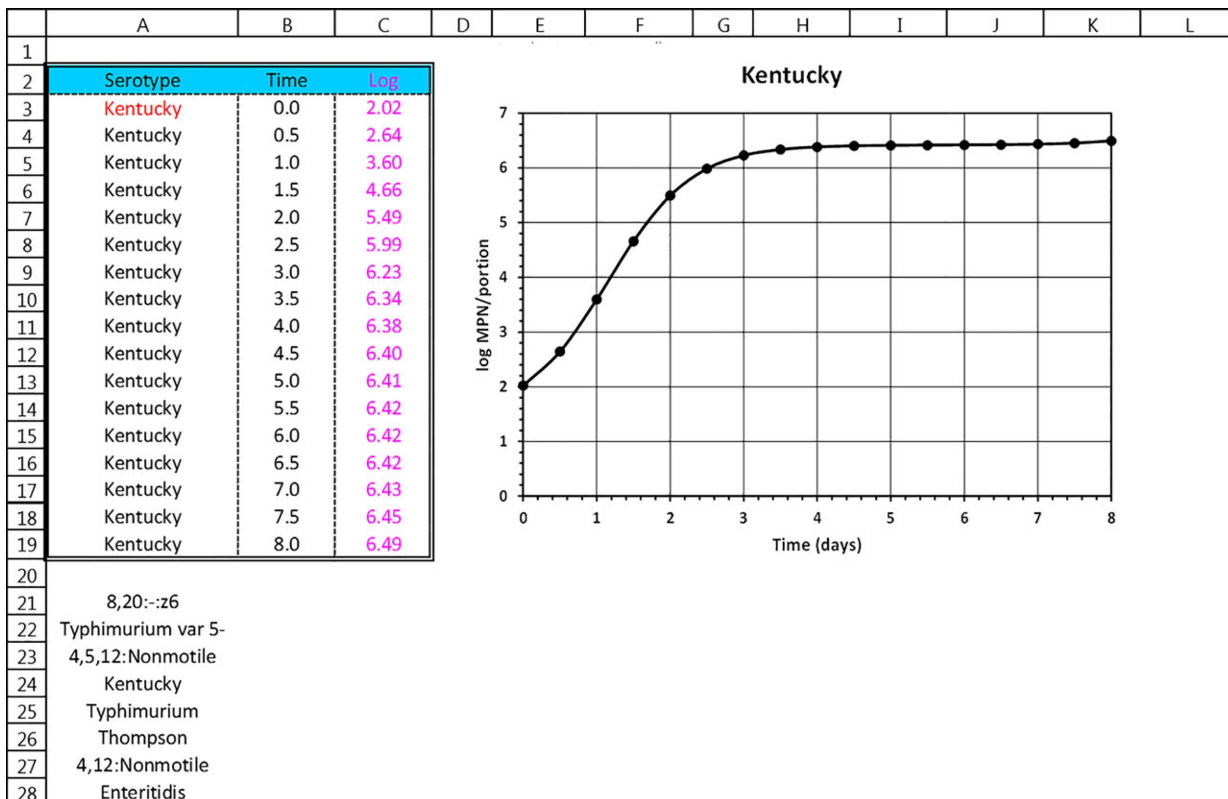



**Figure 1** Structure of the neural network model for predicting growth of *Salmonella* serotypes in ground chicken stored at 16 °C. Abbreviations: w = weight; b = bias.

potential users of the model are willing or able to purchase and maintain the spreadsheet add-in program that is needed to run the model. Thus, to reduce the cost, a second version of the model was developed that did not require the add-in program. This version was developed by creating an array of predicted log MPN values as a function of the independent variables (i.e. time and serotype) using the PREDICT function of NeuralTools. The VLOOKUP function of Excel was then used to return the predicted log MPN value from the array for the selected serotype and times from 0 to 8 days of cold storage at 16 °C. This version of the model, which is not shown, is similar in appearance to the original version (Fig. 2).

The limitation of the second model is that it cannot be used for risk assessment. Thus, a third version of the model (Fig. 3) was developed for risk assessment. Risk assessors desire models that predict pathogen behaviour as a function of the variability of independent variables. Consequently, independent variables




| | A | B | C |
|---|---|---|---|
| 1 | | | |
| 2 | Serotype | Time | Log |
| 3 | Kentucky | 0.0 | 2.02 |
| 4 | Kentucky | 0.5 | 2.64 |
| 5 | Kentucky | 1.0 | 3.60 |
| 6 | Kentucky | 1.5 | 4.66 |
| 7 | Kentucky | 2.0 | 5.49 |
| 8 | Kentucky | 2.5 | 5.99 |
| 9 | Kentucky | 3.0 | 6.23 |
| 10 | Kentucky | 3.5 | 6.34 |
| 11 | Kentucky | 4.0 | 6.38 |
| 12 | Kentucky | 4.5 | 6.40 |
| 13 | Kentucky | 5.0 | 6.41 |
| 14 | Kentucky | 5.5 | 6.42 |
| 15 | Kentucky | 6.0 | 6.42 |
| 16 | Kentucky | 6.5 | 6.42 |
| 17 | Kentucky | 7.0 | 6.43 |
| 18 | Kentucky | 7.5 | 6.45 |
| 19 | Kentucky | 8.0 | 6.49 |
| 20 | | | |
| 21 | 8,20:-:z6 | | |
| 22 | Typhimurium var 5- | | |
| 23 | 4,5,12:Nonmotile | | |
| 24 | Kentucky | | |
| 25 | Typhimurium | | |
| 26 | Thompson | | |
| 27 | 4,12:Nonmotile | | |
| 28 | Enteritidis | | |

**Figure 2** Neural network model for growth of *Salmonella* serotypes in ground chicken stored at 16 °C. This version requires Excel and NeuralTools to run.

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| | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| 1 | | | | | | | |
| 2 | Serotype | Time | Log | | Prevalence | Code | Serotype |
| 3 | Enteritidis | 0.0 | 1.93 | | 2 | 1 | Thompson |
| 4 | Enteritidis | 1.3 | 3.90 | | 24 | 2 | Typhimurium var 5- |
| 5 | Output | Δ | 1.97 | | 8 | 3 | Typhimurium |
| 6 | | | | | 2 | 4 | 4,5,12:Nonmotile |
| 7 | | | | | 2 | 5 | 4,12:Nonmotile |
| 8 | | | | | 5 | 6 | 8,20:-:z6 |
| 9 | | | | | 4 | 7 | Kentucky |
| 10 | | | | | 2 | 8 | Enteritidis |
| 11 | | | | | | | |

**Figure 3** Stochastic version of a neural network model for growth of *Salmonella* serotypes in ground chicken stored at 16 °C. This version requires Excel, NeuralTools, and @Risk to run.

must be expressed as probability distributions rather than single values and the model must use a random sampling method like Monte Carlo simulation to incorporate this variability into model predictions. To accomplish this, another spreadsheet add-in program (version 6.0, @Risk, Palisade Corp.) was used that allowed introduction of probability distributions for the independent variables, that had an algorithm for Monte Carlo simulation and that was compatible with the first spreadsheet add-in program (i.e. NeuralTools).

The third version of the model contained an array (E3:G10) for prevalence of *Salmonella* serotypes in ground chicken portions. Results from previous studies with chicken parts harvested from whole broiler chicken in flow pack wrappers at retail (Oscar, 2013, 2014b) were used to define the prevalence of individual serotypes in the array (Fig. 3). The array was in turn used to define a discrete distribution for *Salmonella* serotype prevalence:

A3 = VLOOKUP(RiskDiscrete(F3:F10,E3:E10),F3:G10,2,FALSE)

where the output of the distribution during each calculation of the model was the name of one of the eight serotypes of *Salmonella*. The name of the serotype was then used in the neural network model to predict the log MPN value at 0 days of storage (cell C3 in Fig. 3).

The model also contained a lognormal distribution for time of cold storage:

B4 = RiskLognorm(1,1,RiskTruncate(0,8))

with a mean of 1 day, a standard deviation of 1 day, and was truncated at 0 and 8 days to prevent predictions outside the range of time used to develop the model. What needs to be mentioned is that this distribution is for demonstration purposes and is not intended to represent the situation in any particular chicken production chain. The actual distribution will depend on how chicken is stored in the production chain being simulated.

During simulation of the model with the second spreadsheet add-in program (version 6, @Risk, Palisade Corp.), the randomly sampled time of cold storage at 16 °C from the lognormal distribution was used with the randomly selected serotype of *Salmonella* from the discrete distribution in the neural network model to predict the log MPN value at that time. Next, the log increase of *Salmonella* for the simulated ground chicken portion was calculated as follows:

C5 = RiskOutput()+C4-C3

where C4 was the predicted log MPN value at time $t$ (days) and C3 was the predicted log MPN value at $t = 0$ days. Cell C5 (Fig. 3) was designated as the output cell using the @Risk command ‘RiskOutput’.

The model was simulated with settings of Latin Hypercube sampling, Mersenne Twister, a random number generator seed of 1, and 10 000 iterations. The simulation result was a cumulative distribution of log increase values (Fig. 4) that can be used as an input distribution in a risk assessment model. Output distributions generated with this model in this study are for illustration purposes and are not intended to represent what occurs in a particular chicken production chain.

## Model performance

Prediction bias and accuracy of the original version of the model (Fig. 2) were evaluated using the test data

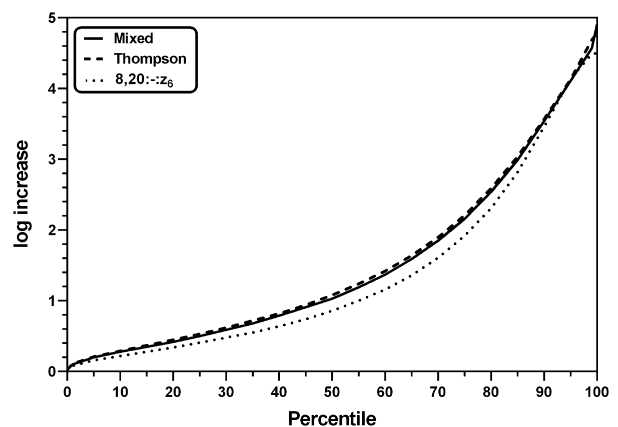


**Figure 4** Simulation results for the stochastic version of the model shown in Fig. 3. Mixed is prevalence of serotypes shown in Fig. 3, whereas Thompson and 8,20:-:$z_6$ indicate a prevalence of 100% for these serotypes.

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and model performance criteria of the acceptable prediction zone (APZ) method, which were established as previously described (Oscar, 2005, 2015). The test data evaluated in this study met the test data criteria of the APZ method; namely, they were not used to develop the model (i.e. they were independent data) and they were collected using the same methods as the data used to develop the model. Thus, the comparisons of observed and predicted values were not confounded.

In the APZ method, a prediction is considered acceptable when the residual (observed – predicted) is in an acceptable prediction zone from −1 log (fail-safe) to 0.5 log (fail-dangerous). The model is considered to provide predictions with acceptable bias and accuracy when the proportion of residuals in the acceptable prediction zone (pAPZ) is ≥0.7. A single metric (i.e. pAPZ) is used to simultaneously assess prediction bias and accuracy.

The analytical units for this model performance analysis were the complete data set ($n$ = 276), the training data set ($n$ = 192), the testing data set ($n$ = 84), growth curves for individual serotypes ($n$ = 30 or 36) and individual combinations of serotype and time ($n$ = 5 or 6). A local prediction problem was considered to exist when three consecutive times of cold storage within a growth curve for an individual serotype had pAPZ <0.7. The model was considered validated when it provided acceptable predictions for both the training and testing data, and there were no local prediction problems.

### Statistical analysis

To determine the effect of time, serotype and their interaction on growth of *Salmonella* in ground chicken, data were analysed by two-way analysis of variance (version 6.03, Prism, GraphPad Software, San Diego, CA, USA). When a significant ($P$ < 0.05) effect occurred, means were compared using Tukey's multiple comparison test at $P$ < 0.05.

## Results and discussion

### Growth of *Salmonella* serotypes in ground chicken

Figure 5 shows results for growth of all eight *Salmonella* serotypes in ground chicken including the three newly investigated serotypes Enteritidis, 4,5,12:Nonmotile and 4,12:Nonmotile. Two-way analysis of variance (Table 1) indicated that growth of *Salmonella* was affected ($P$ < 0.05) by time and serotype but not their interaction. Regardless of the serotype, *Salmonella* growth increased from 0 to 1 to 2 to 4 days and then plateaued from 4 to 6 to 8 days of cold storage at 16 °C (Table 2).

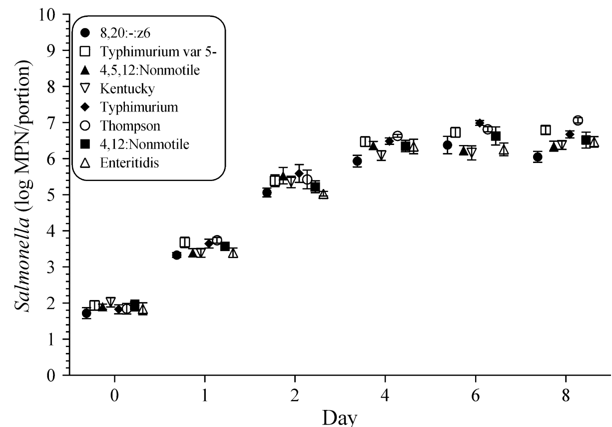


**Figure 5** Growth of *Salmonella* serotypes in ground chicken stored at 16 °C. Symbols are means and error bars are standard errors of the mean. This is a grouped plot from the two-way analysis of variance. Symbols were used instead of bars to increase clarity of presentation.

**Table 1** Results of two-way analysis of variance for growth of *Salmonella* serotypes in ground chicken thigh meat stored for 0 to 8 days at 16 °C

| Source of Variation | % of total variation | $P$ value | $P$ value summary | Significant? |
|---|---|---|---|---|
| Interaction | 0.585 | 0.2167 | ns | No |
| Time | 94.68 | <0.0001 | **** | Yes |
| Serotype | 0.8763 | <0.0001 | **** | Yes |

There were no differences in growth among serotypes after 0, 1 or 2 days of storage at 16 °C (Fig. 5); however, after 4, 6 or 8 days of storage at 16 °C, there were differences ($P$ < 0.05) among serotypes (Table 3). In general, serotypes 8,20:-:$z_6$, Kentucky and 4,5,12:Nonmotile grew at 0.63 to 1.01 log less ($P$ < 0.05) than serotypes Thompson, Typhimurium and Typhimurium var 5- at 4, 6, or 8 days of storage at 16 °C. Although lag time and growth rate could not be determined from these data because there were too few sampling times, these results showed that maximum population density differed among serotypes.

A storage temperature of 16 °C was investigated because this is a temperature that occurs in domestic refrigerators, although on rare occasion (Kennedy *et al.*, 2005; Pouillot *et al.*, 2010). In addition, it is a temperature at which *Salmonella* grow well on chicken meat with native microflora and where differences in growth among serotypes can be detected (Oscar, 2015). Although the normal shelf-life of ground chicken is 1 to 2 days, some consumers may consume chicken that has been stored for 5 or more days


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**Table 2** Comparison of time (day) on growth (mean log difference) of *Salmonella* serotypes in ground chicken thigh meat stored at 16 °C: Tukey's multiple comparison test ($P < 0.05$)

| Comparison | 8,20:-:$z_6$ | Typhimurium var 5- | 4,5,12:Nonmotile | Kentucky | Typhimurium | Thompson | 4,12:Nonmotile | Enteritidis | Significant? |
|---|---|---|---|---|---|---|---|---|---|
| 0 vs. 1 | −1.61 | −1.75 | −1.49 | −1.37 | −1.82 | −1.90 | −1.64 | −1.55 | Yes |
| 0 vs. 2 | −3.34 | −3.45 | −3.63 | −3.33 | −3.76 | −3.58 | −3.29 | −3.19 | Yes |
| 0 vs. 4 | −4.21 | −4.53 | −4.46 | −4.07 | −4.66 | −4.79 | −4.42 | −4.50 | Yes |
| 0 vs. 6 | −4.66 | −4.79 | −4.33 | −4.14 | −5.16 | −4.97 | −4.70 | −4.42 | Yes |
| 0 vs. 8 | −4.33 | −4.86 | −4.45 | −4.35 | −4.85 | −5.22 | −4.58 | −4.62 | Yes |
| 1 vs. 2 | −1.73 | −1.71 | −2.14 | −1.97 | −1.94 | −1.68 | −1.65 | −1.63 | Yes |
| 1 vs. 4 | −2.60 | −2.79 | −2.97 | −2.70 | −2.84 | −2.89 | −2.78 | −2.94 | Yes |
| 1 vs. 6 | −3.05 | −3.05 | −2.84 | −2.77 | −3.34 | −3.07 | −3.06 | −2.86 | Yes |
| 1 vs. 8 | −2.72 | −3.11 | −2.95 | −2.99 | −3.03 | −3.32 | −2.94 | −3.07 | Yes |
| 2 vs. 4 | −0.87 | −1.08 | −0.84 | −0.73 | −0.90 | −1.21 | −1.13 | −1.31 | Yes |
| 2 vs. 6 | −1.32 | −1.34 | −0.70 | −0.81 | −1.40 | −1.39 | −1.41 | −1.23 | Yes |
| 2 vs. 8 | −0.99 | −1.40 | −0.82 | −1.02 | −1.08 | −1.64 | −1.29 | −1.44 | Yes |
| 4 vs. 6 | −0.45 | −0.26 | 0.13 | −0.07 | −0.50 | −0.19 | −0.28 | 0.08 | No |
| 4 vs. 8 | −0.12 | −0.32 | 0.02 | −0.29 | −0.19 | −0.43 | −0.17 | −0.13 | No |
| 6 vs. 8 | 0.33 | −0.07 | −0.12 | −0.22 | 0.32 | −0.25 | 0.11 | −0.21 | No |

**Table 3** Effect of serotype on growth of *Salmonella* in ground chicken thigh meat stored at 16 °C: Tukey's multiple comparison test ($P < 0.05$)

| Day | Comparison | Mean log difference | Significant? |
|---|---|---|---|
| 4 | 8,20:-:z6 vs. Thompson | −0.70 | Yes |
| 6 | 4,5,12:Nonmotile vs. Typhimurium | −0.76 | Yes |
| 6 | Kentucky vs. Typhimurium | −0.83 | Yes |
| 6 | Kentucky vs. Thompson | −0.66 | Yes |
| 6 | Typhimurium vs. Enteritidis | 0.73 | Yes |
| 8 | 8,20:-:z6 vs. Typhimurium var 5- | −0.74 | Yes |
| 8 | 8,20:-:z6 vs. Typhimurium | −0.63 | Yes |
| 8 | 8,20:-:z6 vs. Thompson | −1.01 | Yes |
| 8 | 4,5,12:Nonmotile vs. Thompson | −0.71 | Yes |
| 8 | Kentucky vs. Thompson | −0.69 | Yes |

(Evans, 1992; Straver *et al.*, 2007). Thus, data were collected over 8 days of cold storage to cover a period that included the normal shelf-life as well as potential extremes of the storage life of chicken before its consumption. Extremes of distributions are important rare events in risk assessments because they often result in cases of foodborne illness. Thus, it is important to investigate and model these events.

Serotypes in this study were selected based on the following criteria: (i) isolated from chicken (all serotypes), (ii) top human clinical isolate (Typhimurium var 5-, Typhimurium, Enteritidis, Thompson), (iii) top chicken isolate (Typhimurium var 5-, Typhimurium, Kentucky, Enteritidis), (iv) grow slower on chicken meat (Kentucky, Enteritidis, 8,20:-:$z_6$) (Oscar, 2009, 2015) and (v) unknown growth on chicken meat (4,5,12:Nonmotile, 4,12:Nonmotile). This provided a diverse set of *Salmonella* for investigating and modelling variation of growth among serotypes under an important condition found in the chicken production chain.

In agreement with previous studies (Oscar, 2009, 2015), results indicated that there were significant differences in growth among serotypes of *Salmonella* in chicken meat with native microflora. In general, serotypes Typhimurium var 5-, Typhimurium and Thompson grew better (0.6 to 1 log) than serotypes 8,20:-:$z_6$, Kentucky and 4,5,12:Nonmotile with the other serotypes falling in between. Thus, including serotype as an independent variable in the model was justified.

Differences in growth among serotypes of *Salmonella* is important because it indicates that under the same scenario of temperature abuse and initial level of the pathogen, consumer exposure would be higher for faster growing serotypes. This may explain, in part, why serotypes Typhimurium var 5-, Typhimurium and Thompson are top human clinical isolates whereas serotypes 8,20:-:$z_6$, 4,5,12:Nonmotile and Kentucky are not and why most human clinical cases with serotype Enteritidis are attributed to eggs rather than chicken. In other words, in addition to higher virulence, higher consumer exposure due to better survival and growth in the food of attribution could be an important reason why a serotype is a top human clinical isolate whereas another serotype is not.

### Development and validation of the model

The complete data set for model development and validation contained 276 log MPN values. Of these, 192 were used to train the neural network and 84 were used to test it for the ability to generalise. As described above in 'Materials and methods' subsection 'Model

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development', a tagging method that biased predictions in the fail-safe direction was used to develop the model. Consequently, the mean residual or average prediction bias was 0.00 log, as expected, for the training data and −0.49 log (fail-safe) for the testing data.

Performance of the model was further evaluated using the acceptable prediction zone method. This method allows predictions to err twice as much in the fail-safe direction. This was done to provide an added level of safety when using the model to predict food safety. In the acceptable prediction zone method, if greater than 70% of the residuals are in an acceptable prediction zone from −1 log (fail-safe) to 0.5 log (fail-dangerous), then the model is classified as providing predictions with acceptable bias and accuracy.

Although model predictions were biased in the fail-safe direction, the model provided predictions with acceptable bias and accuracy for all eight serotypes of *Salmonella* (Table 4). The pAPZ ranged from 0.917 for serotype Kentucky to 1.000 for serotypes Enteritidis and Typhimurium var 5-. When data for all serotypes were combined, pAPZ was 0.948 (182/192) for training data, 0.988 (83/84) for testing data and 0.960 (265/276) for the complete data set. There were no signs of local prediction problems (Fig. 6). Thus, the model was successfully validated.

Predictive models are often used by the chicken industry to verify the critical control points in HACCP programs. For example, a chicken company may use a model to evaluate the effect of a process deviation on growth of *Salmonella* on chicken meat. If the process deviation results in less than a one log increase in *Salmonella,* then the process is considered to be in control; otherwise, corrective action is needed.

Chicken companies are more likely to use a model for HACCP when it is user-friendly and free. Although the model developed (Fig. 2) is user-friendly, it is not free because to operate the model users have to purchase a spreadsheet add-in program and renew its license annually. Therefore, to reduce the cost for the chicken industry, a standalone version of the model was developed, as described above, to meet the needs of this important group of stakeholders.

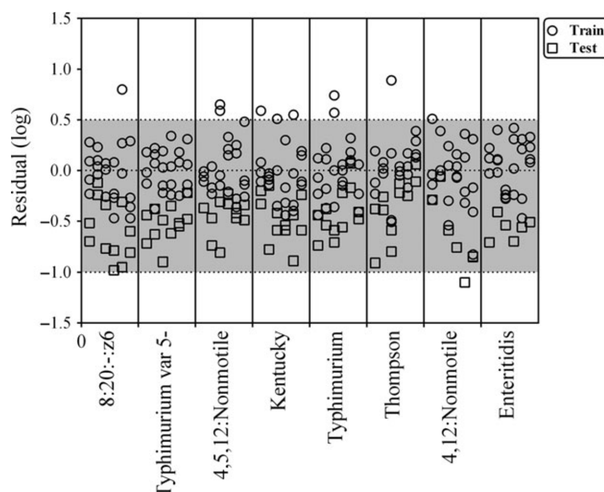


**Figure 6** Acceptable prediction zone analysis of a neural network model (Fig. 2) for growth of *Salmonella* serotypes in ground chicken stored at 16 °C for 0 to 8 days. The grey box is the acceptable prediction zone, which is from −1 log (fail-safe) to 0.5 log (fail-dangerous).

### Stochastic version of the model for risk assessment

Another important group of stakeholders are risk assessors in regulatory agencies that use models to inform policy decisions aimed at protecting public health. To meet the needs of this group, a third version of the model (Fig. 3) was developed. To demonstrate this version, a temperature abuse scenario was simulated in which ground chicken was contaminated

**Table 4** Performance of the neural network model for growth of *Salmonella* serotypes in ground chicken stored at 16 °C for 0 to 8 days: Acceptable prediction zone (APZ) method

| | Train | | | Test | | | Train + Test | | |
|---|---|---|---|---|---|---|---|---|---|
| Serotype | In | Total | pAPZ[a] | In | Total | pAPZ | In | Total | pAPZ |
| 8,20:-:z6 | 23 | 24 | 0.958 | 12 | 12 | 1.000 | 35 | 36 | 0.972 |
| Typhimurium var 5- | 24 | 24 | 1.000 | 12 | 12 | 1.000 | 36 | 36 | 1.000 |
| 4,5,12:Nonmotile | 22 | 24 | 0.917 | 12 | 12 | 1.000 | 34 | 36 | 0.944 |
| Kentucky | 21 | 24 | 0.875 | 12 | 12 | 1.000 | 33 | 36 | 0.917 |
| Typhimurium | 22 | 24 | 0.917 | 12 | 12 | 1.000 | 34 | 36 | 0.944 |
| Thompson | 23 | 24 | 0.958 | 12 | 12 | 1.000 | 35 | 36 | 0.972 |
| 4,12:Nonmotile | 23 | 24 | 0.958 | 5 | 6 | 0.833 | 28 | 30 | 0.933 |
| Enteritidis | 24 | 24 | 1.000 | 6 | 6 | 1.000 | 30 | 30 | 1.000 |
| | 182 | 192 | 0.948 | 83 | 84 | 0.988 | 265 | 276 | 0.960 |

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


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with all eight serotypes of *Salmonella*. The output was a probability distribution for log increase of *Salmonella* among 10 000 portions of ground chicken (Fig. 4). The log increase values for this scenario ranged from 0.029 to 4.91 with a median of 1.03. This distribution can be used in a risk assessment model to predict the change in *Salmonella* number among chicken portions subjected to temperature abuse during refrigerated storage.

For comparison, the model was simulated for two additional scenarios: first, where all ground chicken portions were contaminated with only the fastest growing serotype (Thompson) and second, where all ground chicken portions were contaminated with only the slowest growing serotype (8,20:-:$z_6$). The log increase for serotype Thompson ranged from 0.034 to 4.78 with a median of 1.08, whereas the log increase for serotype 8,20:-:$z_6$ ranged from 0.025 to 4.50 with a median of 0.86 (Fig. 6). These scenarios further demonstrated that serotype was an important independent variable to include in the model.

## Conclusion

In the present study, growth of *Salmonella* serotypes in ground chicken thigh meat stored at 16 °C was investigated using an automated mini-MPN method that allowed enumeration of low to high levels of the pathogen in the presence of native microflora. The MPN data were then used to develop and validate a neural network model that predicts growth of *Salmonella* in ground chicken as a function of time and serotype. The model was validated against independent data using the acceptable prediction zone method. Additional versions of the model for use in HACCP and risk assessment were developed and demonstrated. Once this work is published, all the models will be available on our Poultry FARM website: www.ars.usda.gov/naa/errc/PoultryFARM, where they can be used with confidence to help make important food safety decisions aimed at protecting public health.

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