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Research Paper

Neural Network Model for Thermal Inactivation of *Salmonella* Typhimurium to Elimination in Ground Chicken: Acquisition of Data by Whole Sample Enrichment, Miniature Most-Probable-Number Method

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ABSTRACT

Predictive models are valuable tools for assessing food safety. Existing thermal inactivation models for *Salmonella* and ground chicken do not provide predictions above 71°C, which is below the recommended final cooked temperature of 73.9°C for chicken. They also do not predict when all *Salmonella* are eliminated without extrapolating beyond the data used to develop them. Thus, a study was undertaken to develop a model for thermal inactivation of *Salmonella* to elimination in ground chicken at temperatures above those of existing models. Ground chicken thigh portions (0.76 cm^3) in microcentrifuge tubes were inoculated with 4.45 ± 0.25 log most probable number (MPN) of a single strain of *Salmonella* Typhimurium (chicken isolate). They were cooked at 50 to 100°C in 2 or 2.5°C increments in a heating block that simulated two-sided pan frying. A whole sample enrichment, miniature MPN (WSE-mMPN) method was used for enumeration. The lower limit of detection was one *Salmonella* cell per portion. MPN data were used to develop a multiple-layer feedforward neural network model. Model performance was evaluated using the acceptable prediction zone (APZ) method. The proportion of residuals in an APZ (pAPZ) from −1 log (fail-safe) to 0.5 log (fail-dangerous) was 0.911 (379 of 416) for dependent data and 0.910 (162 of 178) for independent data for interpolation. A pAPZ ≥0.7 indicated that model predictions had acceptable bias and accuracy. There were no local prediction problems because pAPZ for individual thermal inactivation curves ranged from 0.813 to 1.000. Independent data for interpolation satisfied the test data criteria of the APZ method. Thus, the model was successfully validated. Predicted times for a 1-log reduction ranged from 9.6 min at 56°C to 0.71 min at 100°C. Predicted times for elimination ranged from 8.6 min at 60°C to 1.4 min at 100°C. The model will be a valuable new tool for predicting and managing this important risk to public health.

Key words: Ground chicken; Neural network model; Predictive microbiology; *Salmonella* Typhimurium; Thermal inactivation; Whole sample enrichment, miniature most-probable-number method

Salmonella is a leading cause of foodborne illness *(19)*. Poultry meat and egg products are often linked to outbreaks of salmonellosis *(3)*. Most *Salmonella* pathogens are located on the surface of chicken *(20)* and are rapidly killed during cooking. However, *Salmonella* pathogens in ground chicken are located throughout the product *(1)*. Thus, if ground chicken is not thoroughly cooked, *Salmonella* can survive and cause salmonellosis *(8)*.

Predictive models are valuable tools for assessing food safety *(2)*. One important feature is the ability to predict pathogen behavior in food for conditions that were not investigated but that are within the ranges of variables used to develop the model *(22)*. In other words, the ability to interpolate. Thus, an important step in model development is validating the model for its ability to interpolate *(16)*. However, even after validation, a model should not be used to make predictions outside the ranges of independent variables used to develop and validate it because these predictions may not be reliable.

Models that predict thermal inactivation of *Salmonella* in ground chicken have been developed *(5, 7, 12, 13)*. However, the maximum temperature investigated and modeled is 71.1°C. This temperature is below the recommended final cooked temperature of 73.9°C or 165°F for chicken. One exception is the study of Murphy et al. *(10),* in which ground chicken was cooked to final temperatures of 75 and 80°C. However, the model was developed with a heat-resistant strain of *Salmonella* Senftenberg. Thus, it may not accurately predict thermal inactivation of other *Salmonella* strains. In fact, the model for *Salmonella* Senftenberg may be overly fail-safe, resulting in overcooking of the ground chicken to a point at which it would not be consumed. Therefore, existing models can be improved by including higher temperatures and by using *Salmonella*

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serotypes with more normal heat resistance than *Salmonella* Senftenberg to develop them.

Another limitation of existing models is that they do not predict when *Salmonella* pathogens are eliminated without extrapolating beyond the data used to develop them. This is because the lower limit of detection of the viable count method used to enumerate *Salmonella* is about 2.6 log/ml. Therefore, a study was undertaken to develop a model for thermal inactivation of *Salmonella* to elimination in ground chicken at temperatures above those of existing models. Data were acquired by a whole sample enrichment, miniature most-probable-number (WSE-mMPN) method. This enumeration and presence or absence method has a lower limit of detection of one *Salmonella* cell per portion. Thus, it was possible to investigate and model thermal inactivation of *Salmonella* to elimination in ground chicken.

MATERIALS AND METHODS

Experimental designs. For model development, an 8 × 13 full factorial of time (0, 0.5, 1, 2, 4, 6, 8, 10 min) and temperature (50, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 95, 100°C) was used. There were four replicate trials per temperature. For model validation, an 8 × 12 full factorial of time (0, 0.5, 1, 2, 4, 6, 8, 10 min) and temperature (52, 56, 60, 64, 68, 72, 76, 80, 84, 88, 92.5, 97.5°C) was used. There were two replicate trials per temperature. Temperature refers to that of the well surfaces in the heating block used to cook the ground chicken portions.

Ground chicken. Ground chicken portions with native microflora were prepared from thigh meat, as previously described *(18)*. Portions were cylindrical in shape, with a radius of 4.5 mm, a height of 12 mm, and a volume of 0.76 cm^3 ($V = \pi r^2 h$), which is equivalent to 0.76 g. They were housed in 1.5-ml polypropylene microcentrifuge tubes with 0.5-mm-thick walls. They were stored at –20°C until they were used in experiments.

Cooking trials and inoculation procedure. *Salmonella enterica* serotype Typhimurium was isolated from a chicken breast that was harvested from a whole broiler chicken obtained at retail, as described in a previous study *(17)*. Stock cultures were stored at –80°C in brain heart infusion broth (BD, Sparks, MD) that contained 15% glycerol (Sigma Aldrich, St. Louis, MO). Five microliters of stock culture was added to 9 ml of buffered peptone water (BPW; BD) in a glass dilution tube with cap. The tube was incubated for 72 h at 22°C without shaking to obtain stationary phase cells for inoculation of ground chicken portions. A fresh culture was prepared for each trial.

Chicken portions (nine per trial) were thawed at 22°C for 30 min. The 72-h culture was serially diluted (1:10) in BPW. Five microliters of the 10^{-2} dilution was inoculated into the center of the chicken portion. Inoculum size was 4.45 ± 0.25 (mean ± SD) log MPN per portion. Chicken portions were cooked for 0 to 10 min in a preheated heating block (Grant-bio PCH1, Grant Instruments, Cambridgeshire, UK). This model system simulated two-sided pan frying, but in the vertical rather than horizontal direction. However, it produced a similar meat temperature profile as two-sided pan frying of ground meat patties in the horizontal direction *(4)*. Thus, it provided a realistic and reliable model system for investigating and modeling the thermal inactivation of *Salmonella* in ground chicken during cooking.

An uninoculated portion was used to monitor meat temperature during cooking. A soldering iron was used to create a hole in the lid of the microcentrifuge tube. A thermometer (range = –50 to 150°C; Traceable Jumbo-Digital Display Thermometer, Control Company, Friendswood, TX) was inserted through the hole and into the center of the chicken portion. Initial temperature of chicken portions was 22.6 ± 1.3°C.

***Salmonella* enumeration by WSE-mMPN.** Before enumeration, chicken portions were cooled for 20 to 30 min at room temperature after cooking to simulate how consumers would handle ground chicken after cooking and to obtain results that reflected *Salmonella* number after a realistic cooking and cooling scenario. In contrast, in previous modeling studies *(7, 13, 14),* before enumeration, ground chicken was immediately cooled in an ice bath after cooking, which is not what is done in the real world. After cooling for 20 to 30 min, portions were transferred to plastic filter bags (207-ml; Whirl-Pak, Nasco, Fort Atkinson, WI) followed by addition of 9 ml of cold BPW. Samples were then pulsified (Pulsifier PUL 100, Microbiology International, Frederick, MD) for 15 s to recover *Salmonella* into BPW for enumeration by a 3 (replicate) × 8 (dilution) mMPN method, as previously described *(18)*.

In brief, the mMPN was performed in 2-ml, 96-well deep well plates. Serial dilutions (1:10) in BPW were performed by a robotic pipettor (SOLO Plus, Hudson Robotics, Springfield, NJ). The BPW plates were incubated for 24 h at 40°C. Next, 10 μl from each BPW well 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; BD) in each well. The RVB plates were incubated for 48 h at 42°C. *Salmonella*-positive (white) and -negative (blue) wells were then recorded and used to calculate the MPN, as previously described *(21)*.

The WSE was accomplished by incubating the sample remaining in the BPW bags for 24 h at 40°C. Next, 100 μl of the BPW incubate was transferred to 10 ml of RVB in a glass dilution tube with cap. The RVB tubes were incubated for 48 at 42°C. *Salmonella*-positive (white) and -negative (blue) tubes were recorded.

Portions with a single positive RVB well and a positive RVB tube were assigned an MPN value of 0.79 log. Portions with no positive RVB wells but a positive RVB tube were assigned an MPN value of 0.395 log. Portions with one positive RVB well and a negative RVB tube were assigned an MPN value of 0 log. Portions with no positive RVB wells and a negative RVB tube, or samples that tested negative for *Salmonella,* were assigned an MPN value of –1 log. This was done so that, when the model predicted survival and there was no survival or when the model predicted no survival and there was survival, the residual would be unacceptable according to the criteria of the acceptable prediction zone (APZ) method for evaluating model performance (see below). It should be stated that the WSE-mMPN method not only determines the number but also the presence or absence of *Salmonella* in the chicken portions. Thus, it is able to acquire data for modeling not only the reduction but also the elimination of *Salmonella* from ground chicken during cooking.

Model development. A data set was created in an Excel spreadsheet (version 2013, MicroSoft Corporation, Redmond, WA). It had four columns: (i) tag, (ii) temperature (independent numerical variable), (iii) time (independent numerical variable), and (iv) log MPN per portion (dependent numerical variable). The tag variable identified the dependent data for model development and the independent data for interpolation for model validation.

A spreadsheet add-in program (industrial version 6, NeuralTools, Palisade Corporation, Ithaca, NY) was used to develop a

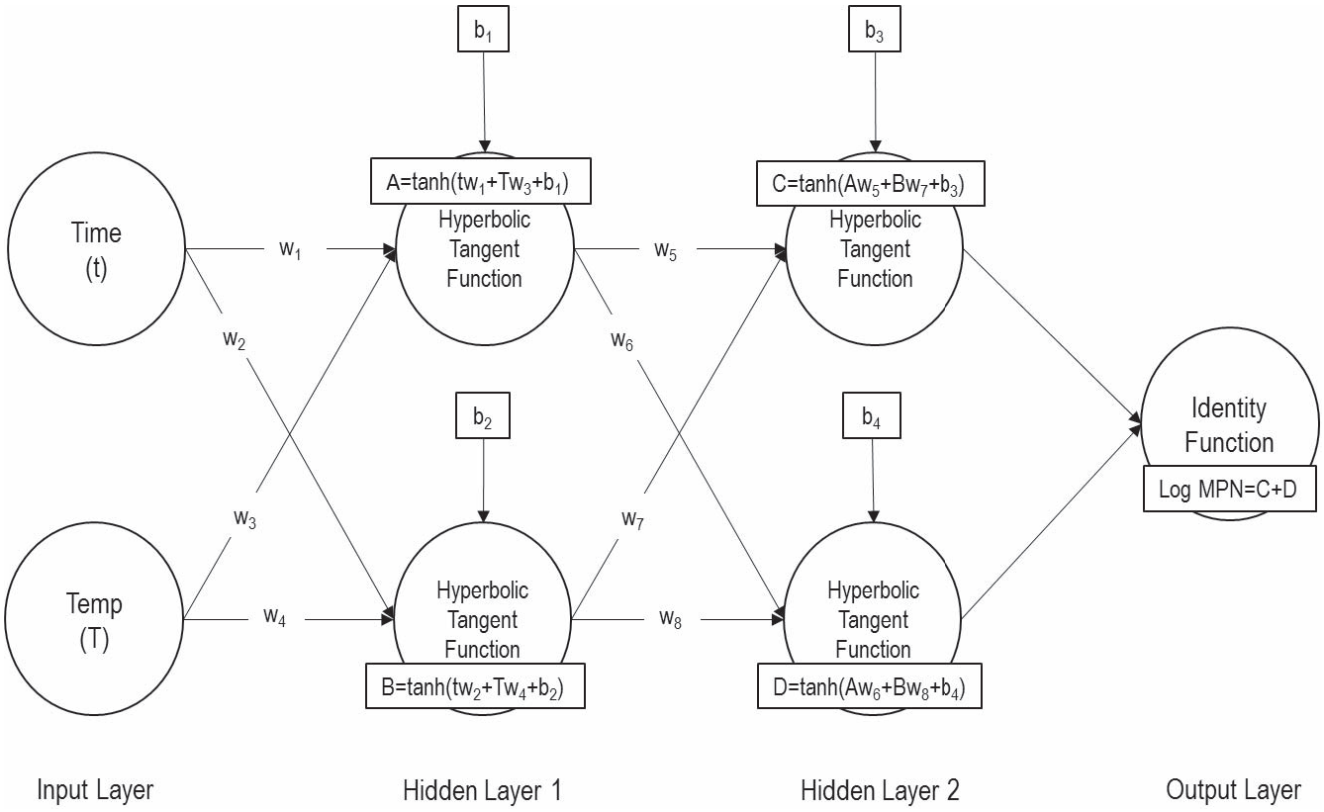


FIGURE 1. *Architecture of the multiple-layer feedforward neural network model for predicting the log MPN of* Salmonella *Typhimurium in ground chicken portions as a function of cooking time and temperature. w, weight; b, bias; tanh, hyperbolic tangent function.*

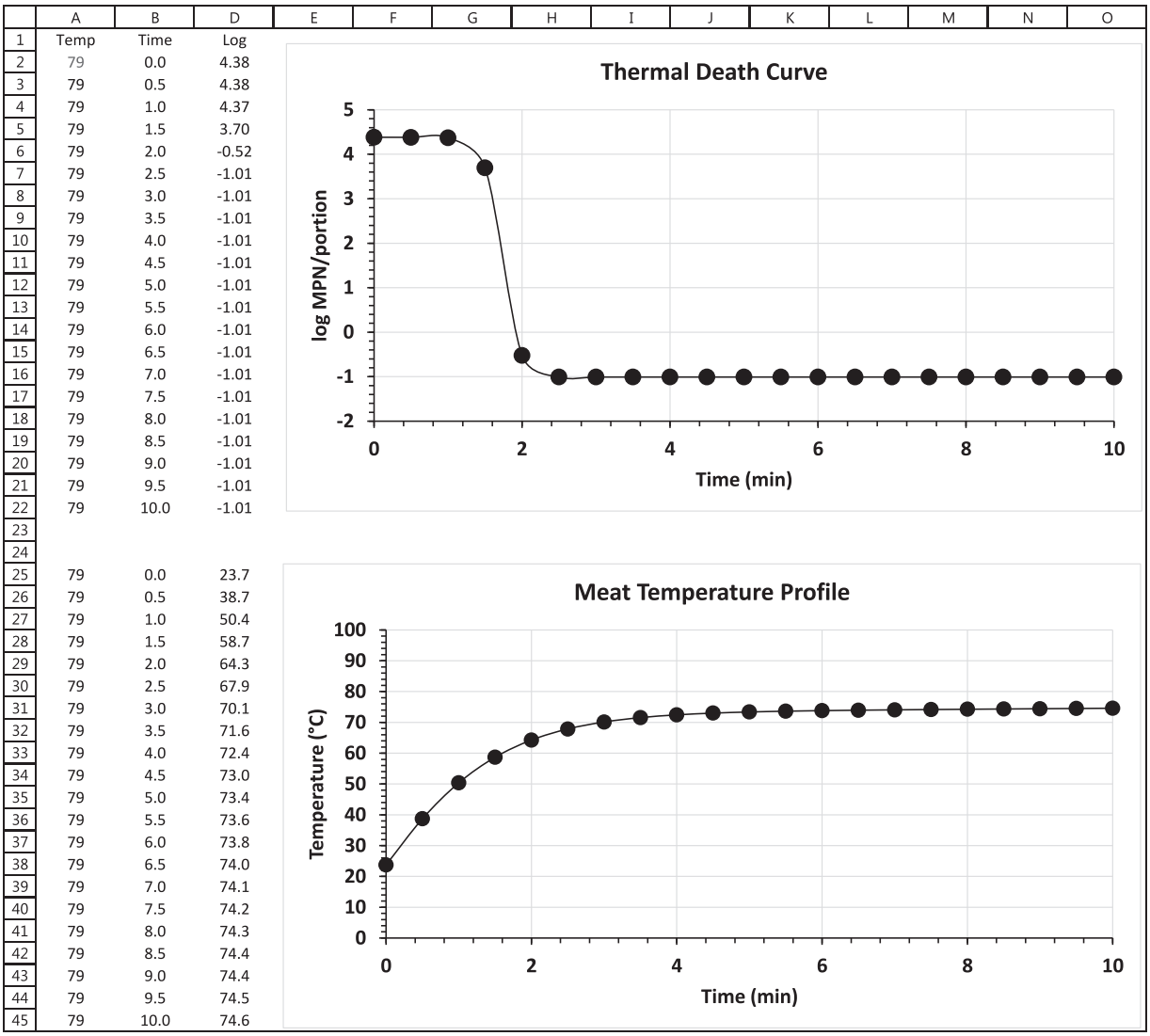
multiple-layer feedforward neural network model with two hidden layers of two nodes each for predicting log MPN per portion (Fig. 1). The activation functions were the hyperbolic tangent function for the hidden layers and the identity function for the output layer. In a similar manner, a multiple-layer feedforward neural network model with a single hidden layer of two nodes for predicting meat temperature as a function of cooking time and temperature was developed. The spreadsheet add-in program also calculated the root mean square error for dependent and independent data as the square root of the average square deviation of observed and predicted values.

NeuralTools does not provide weights and bias values for the neural networks it creates. However, it was possible to develop a stand-alone version. First, the PREDICT function of NeuralTools was used to create two arrays. Both arrays had four columns: (i) temperature; (ii) time; (iii) concatenate (temperature+time), and (iv) predicted log MPN or predicted meat temperature. Second, the CONCATENATE and VLOOKUP functions of Excel were used to return predicted values from the arrays. The stand-alone version of the model predicted the thermal inactivation curve and the meat temperature profile for cooking temperatures from 50 to 100°C in 1°C increments (Fig. 2).

Model performance. Performance of the thermal inactivation model was evaluated using the APZ method for models that predict log number *(15)*. A prediction was considered acceptable when the residual (observed − predicted) was in an APZ from −1 log (fail-safe) to 0.5 log (fail-dangerous). Model predictions had acceptable bias and accuracy when the proportion of residuals in the APZ (pAPZ) was $\geq$0.7 for whole sets of data (i.e., dependent data or independent data for interpolation) or individual thermal inactivation curves. The model was considered validated when the pAPZ values for dependent and independent data for interpolation were acceptable, there were no local prediction problems (i.e., two consecutive thermal inactivation curves with pAPZ $<$0.7), and the independent data for interpolation satisfied the test data criteria of the APZ method.

There are two test data criteria for independent data for interpolation *(16)*. First, independent data for interpolation must be collected using the same methods as dependent data. This ensures a valid comparison of observed and predicted values. Second, independent data for interpolation must provide uniform coverage of model predictions. This ensures an unbiased evaluation of model performance. The independent data for interpolation were collected with the same methods as the dependent data. In addition, they were collected at temperatures that were intermediate to those of the dependent data. Thus, they satisfied the test data criteria of the APZ method for independent data for interpolation.

Graphical analysis. Model predictions were graphed as a function of time and temperature (version 6, Prism, GraphPad Software, San Diego, CA). Predicted MPN at time zero was 4.38 log per portion for all temperatures. Horizontal lines were added at $Y = 3.38$ log MPN per portion to determine the time for a 1-log reduction and at $Y = 0$ log MPN per portion to determine the time for elimination. The cursor was placed at intersections of the horizontal lines and the thermal inactivation curve. Resulting coordinates from the x axis (time in min) were recorded. This method for obtaining the kinetic parameters is analogous to interpolation of values from a standard curve, which is a widely used scientific method. The nonlinear portion of the curve was included in the determination of kinetic parameters. Moreover, when the model predicts a log MPN value $<$0, it is predicting that *Salmonella* has been eliminated from the chicken portion.



	A	B	D
1	Temp	Time	Log
2	79	0.0	4.38
3	79	0.5	4.38
4	79	1.0	4.37
5	79	1.5	3.70
6	79	2.0	-0.52
7	79	2.5	-1.01
8	79	3.0	-1.01
9	79	3.5	-1.01
10	79	4.0	-1.01
11	79	4.5	-1.01
12	79	5.0	-1.01
13	79	5.5	-1.01
14	79	6.0	-1.01
15	79	6.5	-1.01
16	79	7.0	-1.01
17	79	7.5	-1.01
18	79	8.0	-1.01
19	79	8.5	-1.01
20	79	9.0	-1.01
21	79	9.5	-1.01
22	79	10.0	-1.01
23			
24			
25	79	0.0	23.7
26	79	0.5	38.7
27	79	1.0	50.4
28	79	1.5	58.7
29	79	2.0	64.3
30	79	2.5	67.9
31	79	3.0	70.1
32	79	3.5	71.6
33	79	4.0	72.4
34	79	4.5	73.0
35	79	5.0	73.4
36	79	5.5	73.6
37	79	6.0	73.8
38	79	6.5	74.0
39	79	7.0	74.1
40	79	7.5	74.2
41	79	8.0	74.3
42	79	8.5	74.4
43	79	9.0	74.4
44	79	9.5	74.5
45	79	10.0	74.6

FIGURE 2. *Neural network model for thermal inactivation of* Salmonella *Typhimurium to elimination in ground chicken. Users enter the cooking temperature in cell A2, and then the model predicts the thermal inactivation curve and meat temperature profile.*

RESULTS

A subset of data used in model development is shown in Figure 3. These representative data show that temperature of ground chicken rose rapidly during cooking and then plateaued. Final meat temperature was observed to be less than the cooking temperature (Table 1). Come-up time was about 4 min. Three patterns of thermal inactivation were observed: (i) none (Fig. 3A), (ii) concave downward (Fig. 3B), and (iii) sigmoidal (Fig. 3C to 3F).

The data set used to develop the model for thermal inactivation contained 416 log MPN values. The data set used to validate the model for interpolation contained 178 log MPN values. The root mean square error was 0.464 log for dependent data and 0.509 log for independent data for interpolation. Variable impacts were 54.1% for time and 45.9% for temperature.

Model performance was evaluated using the APZ method. The pAPZ for individual thermal inactivation curves ranged from 0.813 to 1.000 (Table 1). Thus, there were no local prediction problems. The pAPZ was 0.911 (379 of 416) for dependent data (Fig. 4A) and 0.910 (162 of 178) for independent data for interpolation (Fig. 4B). Because the independent data for interpolation satisfied the test data criteria of the APZ method, the pAPZ for dependent data and independent data were $\geq$0.7, and there were no local prediction problems, the model was successfully validated.

The validated model was used to determine the time for a 1-log reduction, as explained above. These values ranged from 9.6 min at 56°C to 0.71 min at 100°C (Table 1). The validated model was also used to determine the time for elimination, as explained above. These values ranged from 8.6 min at 60°C to 1.4 min at 100°C (Table 1).

A model for meat temperature profile was developed with 572 meat temperature readings. Independent data for interpolation consisted of 264 meat temperature readings. The root mean square error was 2.4°C for dependent data and 2.7°C for independent data for interpolation. Variable impacts were 57.5% for time and 42.5% for temperature.

DISCUSSION

The objective of the present study was accomplished. A model that predicts thermal inactivation of *Salmonella* to

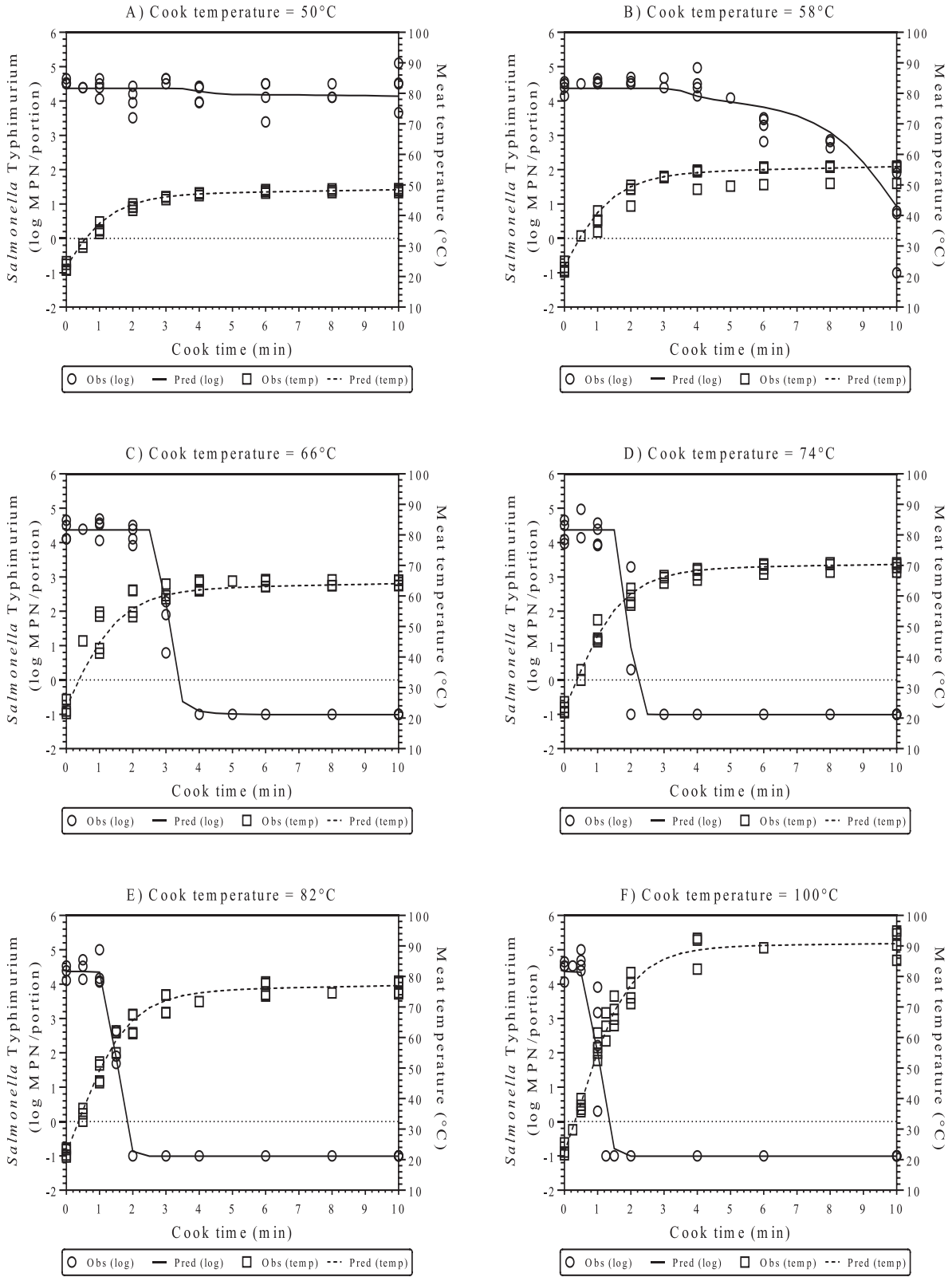


FIGURE 3. *Thermal inactivation curves for* Salmonella *Typhimurium in ground chicken and meat temperature profiles during cooking at (A) 50°C, (B) 58°C, (C) 66°C, (D) 74°C, (E) 82°C, and (F) 100°C. Symbols are observed data. Lines are predicted values.*

elimination in ground chicken at temperatures above existing models was developed and validated. The model simulates a scenario in which ground chicken is cooked by two-sided pan frying, cooled at room temperature for a short period, and then consumed. A small chicken portion was used to acquire data for model development and validation. To extrapolate model predictions to larger ground chicken portions, it can be assumed that the small chicken portion

TABLE 1. *Predictions and performance of a thermal inactivation model for* Salmonella *Typhimurium in ground chicken*[a]

Data set	CT (°C)	FT (°C)	*t*-log (min)	*t*-elim (min)	No. in APZ	Total	pAPZ
Dep	50.0	48.4	ND	ND	31	32	0.969
Ind	52.0	50.1	ND	ND	15	16	0.938
Dep	54.0	52.0	ND	ND	32	32	1.000
Ind	56.0	53.9	9.65	ND	13	16	0.813
Dep	58.0	56.0	7.45	ND	28	32	0.875
Ind	60.0	58.1	5.31	8.60	15	16	0.938
Dep	62.0	60.2	3.84	6.44	28	32	0.875
Ind	64.0	62.2	3.21	4.37	14	15	0.933
Dep	66.0	64.0	2.73	3.39	31	32	0.969
Ind	68.0	65.7	2.57	2.92	15	16	0.938
Dep	70.0	67.3	2.12	2.57	30	32	0.938
Ind	72.0	68.8	2.09	2.42	12	14	0.857
Dep	74.0	70.4	1.64	2.24	28	32	0.875
Ind	76.0	71.9	1.61	1.97	14	15	0.933
Dep	78.0	73.5	1.57	1.96	30	32	0.938
Ind	80.0	75.2	1.45	1.91	13	16	0.813
Dep	82.0	77.0	1.19	1.84	30	32	0.938
Ind	84.0	78.8	1.14	1.71	12	14	0.857
Dep	86.0	80.7	1.11	1.55	28	32	0.875
Ind	88.0	82.4	1.10	1.48	13	14	0.929
Dep	90.0	84.2	1.09	1.47	29	32	0.906
Ind	92.5	86.2	1.07	1.47	13	13	1.000
Dep	95.0	88.0	1.04	1.46	26	32	0.813
Ind	97.5	89.4	0.88	1.42	13	13	1.000
Dep	100.0	90.8	0.71	1.36	28	32	0.875

[a] CT, cooking temperature; FT, predicted final cooked temperature; *t*-log, predicted time for a 1-log reduction; *t*-elim, predicted time for elimination; no. in APZ, number of residuals in an acceptable prediction zone from −1 log (fail-safe) to 0.5 log (fail-dangerous); total, number of residuals; pAPZ, proportion of residuals in the acceptable prediction zone; Dep, dependent data; ND, not determined; Ind, independent data for interpolation.

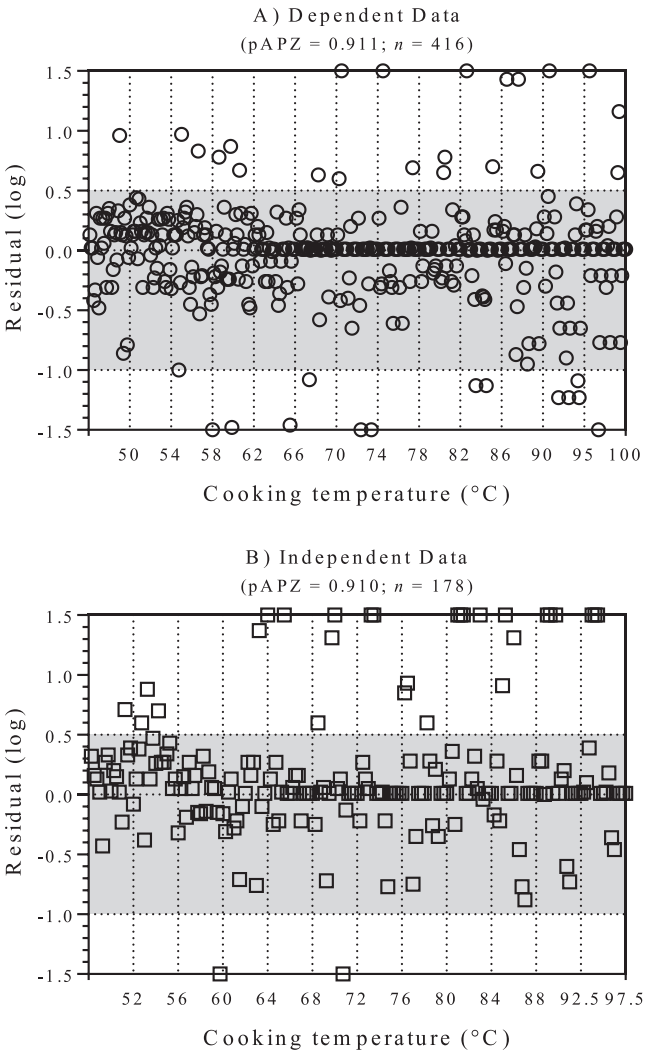


FIGURE 4. *Performance of the neural network model for thermal inactivation of* Salmonella *Typhimurium in ground chicken. (A) Dependent data and (B) independent data for interpolation. The acceptable prediction zone (gray box) was from −1 log (fail-safe) to 0.5 log (fail-dangerous). Residuals >1.5 log were graphed as 1.5 log for clarity of presentation.*

used for data acquisition contains the cold spot of the larger portion size and that all *Salmonella* are located in this cold spot. Thus, the model can be used to predict, independent of portion size, when all *Salmonella* are eliminated during cooking at a specific temperature. However, this is a conservative estimate because some or all of the *Salmonella* may actually be located in a spot closer to the heat source. Predictions of the model can be used to assess whether or not it is safe to consume ground chicken cooked by a specific time and temperature scenario. There are aspects of this study that are new. However, the model has limitations that require further research.

To my knowledge, this is the first study to use WSE-mMPN to acquire data for modeling thermal inactivation of *Salmonella* in ground chicken. Advantages of this method for this application are several. First, incubation in BPW allows injured cells of *Salmonella* to recover. Thus, an overestimation of the thermal inactivation of the pathogen is avoided. Second, the lower limit of detection was one *Salmonella* cell per portion. This made it possible to model thermal inactivation to elimination without extrapolating beyond the data. Third, incubation in RVB allowed enumeration of *Salmonella* in the presence of other microorganisms. Thus, there was no need to use marker strains *(12)* or remove background microflora *(5)* to measure thermal inactivation. The end result was more realistic data and predictions.

A second novel aspect was neural network modeling. Unlike regression models, neural networks are flexible. They can model data sets with different shaped thermal inactivation curves, such as those obtained in the present study (see Fig. 3). A second advantage is that neural network modeling involves one step instead of three. This reduces prediction error, simplifies modeling, and saves time. The study of Juneja et al. *(6)* is a good example of how complex regression modeling of thermal inactivation data can be. If the model had failed validation, one option would be to fit the data to a different model, perhaps a regression model.

A third novel aspect, in the sense that it is not often done by predictive microbiologists, was validation against independent data for interpolation. The validation data satisfied the test data criteria of the APZ method *(16)*.

Namely, they were collected with the same methods as dependent data. Thus, comparisons of observed and predicted values were not confounded. In addition, they were collected at temperatures intermediate to those of dependent data. Thus, they provided uniform coverage of model predictions. Consequently, evaluation of model performance was not biased. Proper validation is important because it provides users with confidence that model predictions are reliable.

A fourth novel aspect was that data were acquired under dynamic conditions that simulated two-sided pan frying of ground chicken. This was accomplished by cooking ground chicken in microcentrifuge tubes inserted into a heating block. This model system had an additional advantage in that it protected laboratory personnel from occupational exposure to *Salmonella*. In this simulation of pan frying, a period of survival before thermal inactivation was observed as ground chicken warmed to temperatures that caused thermal inactivation of the pathogen. Meat temperature profiles obtained were similar in pattern to those reported for pan frying of ground beef *(4)*. Thus, the model system provided a realistic simulation of a real cooking method.

In addition to predicting the number of *Salmonella* as a function of cooking time and temperature, the current model predicts the temperature profile of the cold spot of the ground chicken portion as a function of cooking time and temperature. Thus, the model predictions, which are based on cooking temperature rather than meat temperature at the cold spot, will not mislead consumers who use a meat thermometer to assess food safety. Rather, they will provide consumers the opportunity to compare their meat temperature readings with model predictions for meat temperature so they can judge for themselves how close the predicted meat temperature profile is to their cooking scenario and, thus, how reliable the model predictions of *Salmonella* inactivation and elimination may be for their cooking scenario.

There are several limitations of the current model that can be addressed with additional research. First, thermal resistance varies among strains and serotypes of *Salmonella (11)*. However, only one strain of *Salmonella* Typhimurium was used to develop and validate the present model. A single strain was used because it simplified inoculum preparation and provided specific information about the thermal inactivation of that strain. However, additional research is needed to see whether the model developed with this strain can be improved by including other strains and serotypes of *Salmonella*. This can be accomplished by collecting independent data for extrapolation and then using the APZ method to evaluate the ability of the current model to predict these data, as was done in a previous study for a growth model for *Salmonella* and ground chicken *(18)*. The model could then be expanded to include strains and serotypes for which it does not provide acceptable predictions.

A second limitation of the present model was that only one inoculum size (4.45 log) was investigated and modeled. This inoculum size does not cover all levels of *Salmonella* that may be present in ground chicken *(1)*. However, a study was recently completed in which inoculum sizes from 1.45 to 5.45 log in 1-log increments were investigated (T.P.O., unpublished data). Thus, a model with inoculum size as an independent variable will be available soon. This model will be especially valuable for quantitative microbial risk assessment.

A third limitation of the current model was that ground chicken was warmed to room temperature before cooking. In reality, consumers are more likely to cook chilled ground chicken. Thus, further research is needed to determine whether initial meat temperature affects thermal inactivation of *Salmonella* in ground chicken. If yes, the model can be expanded by additional research to include a range of initial meat temperatures as an independent variable.

A fourth limitation of the present model was that data were collected for only one distance from the heat source. The temperature profile of ground chicken during cooking varies as a function of distance from the heat source *(10)*. Moreover, Mackey et al. *(9)* showed that the distance from the heat source affects thermal inactivation of *Salmonella* Typhimurium in agar cylinders with, as expected, more rapid thermal inactivation of cells that are closer to the heat source. Thus, it is likely that the model can be improved by including other distances from the heat source as an independent variable.

Ultimately, the model will need to provide predictions of thermal inactivation at the "cold spot" or distance farthest from the heat source. This distance will vary as a function of patty thickness and cooking method, e.g., one-sided versus two-sided pan frying. Thus, having a model with distance from the heat source as an independent variable will likely provide model users with a more robust model that can predict thermal inactivation of *Salmonella* under a variety of cooking conditions and scenarios.

A fifth limitation of the current model was that maximum temperature of the heating block was 100°C. However, ground meat is usually pan fried at temperatures from 140 to 190°C *(4)*. Nonetheless, the manufacturer of the heating block used in this study also sells another model (temperature range up to 400°C; Grant BT5D, Grant Instruments) that can achieve these higher pan frying temperatures. Thus, it should be possible in the future to investigate and model cooking temperatures higher than 100°C. However, an important consideration is that the melting point of polypropylene is 160 to 170°C. This would necessitate a change in the model system from polypropylene microcentrifuge tubes to glass vials.

A sixth limitation of the present model was the use of a single formulation of ground chicken. It is known that the thermal inactivation of *Salmonella* differs among formulations of ground chicken *(7)*. In particular, level of fat affects thermal inactivation *(5)*. Thus, it may be advantageous to use the current model system to investigate thermal inactivation in ground chicken breast and mixtures of thigh, breast, and skin to develop and validate a model that includes type of chicken meat or meat formulation as an independent variable.

The approach to modeling thermal inactivation of *Salmonella* in the present study differs from previous studies. The approach used in previous studies involved placing ground chicken in plastic bags that were heat sealed and then submerged in preheated water baths held at constant temperatures. For example, Juneja et al. *(7)* used

this approach to investigate and model thermal inactivation of *Salmonella* in ground chicken cooked at 55 to 71°C. Irradiated meat samples (3 g) were inoculated with a mixture of eight serotypes of *Salmonella*. Inoculum size was 8 log/g. Meat samples were flattened to 1- to 2-mm thickness before cooking. Come-up time was immediate. *Salmonella* were enumerated by viable counts. Thermal inactivation curves were nonlinear. Data were modeled by regression methods. Time for a 1-log reduction was 0.29 min at 71°C.

Another example is the study of Murphy et al. *(13),* who investigated and modeled thermal inactivation of *Salmonella* in ground chicken cooked at 55 to 70°C. Meat samples (10 g) with native microflora were inoculated with a mixture of six antibiotic-resistant serotypes of *Salmonella*. Inoculum size was 7 log/g. Meat samples were flattened to 1-mm thickness and then were cooked in preheated water baths. Come-up time was instantaneous. *Salmonella* were enumerated by viable counts. Thermal inactivation curves were linear. Data were modeled by regression methods. Time for a 1-log reduction was 0.07 min at 70°C.

A final example is that of Osaili et al. *(14),* who investigated and modeled thermal inactivation of *Salmonella* in ground chicken cooked at 54 to 60°C. Meat samples (10 g) with native microflora were inoculated with a single strain of *Salmonella* Typhimurium. Inoculum size was 7.5 log/g. Meat samples were flattened to 3 mm. They were cooked in preheated water baths. Come-up time was immediate. *Salmonella* Typhimurium was enumerated by viable counts. Thermal inactivation curves were linear. Data were modeled by regression methods. Time for a 1-log reduction was 0.49 min at 60°C.

In the present study, thermal inactivation of *Salmonella* in ground chicken cooked at 50 to 100°C was investigated and modeled. Meat samples (0.76 cm^3) with native microflora were inoculated with a single strain of *Salmonella* Typhimurium. Inoculum size was 4.45 log MPN per portion. Meat samples in microcentrifuge tubes were 9 mm thick. They were cooked in a preheated heating block. Come-up time was 4 min. *Salmonella* were enumerated by WSE-mMPN. Thermal inactivation curves were nonlinear. Data were modeled by neural network methods. Time for a 1-log reduction was 2.12 min at 70°C.

Thermal inactivation of *Salmonella* was slower in the present study than in the aforementioned studies. Although there were many differences in experimental methods and conditions, distance of *Salmonella* from the heat source may be the critical factor. It was 1 to 3 mm in the other studies and 5 mm in the current study. Consequently, come-up time was immediate in the other studies but was about 4 min in the present study. There was a short period of time when the *Salmonella* in the present study did not experience temperatures high enough to kill them. This resulted in a shoulder in the inactivation curve even at 100°C.

In conclusion, when using models to predict thermal inactivation of a pathogen, one may find that existing models are not perfect for the scenario of interest. Although the current model is not perfect or applicable to all cooking scenarios, it does address some limitations of existing models. First, it can predict thermal inactivation of *Salmonella* at the recommended final cooked temperature of 73.9°C (165°F) for chicken. Second, it can predict when all *Salmonella* are eliminated from ground chicken during cooking without extrapolating beyond the data used in model development. This is because the WSE-mMPN method used to enumerate *Salmonella* also determined the presence or absence of the pathogen in the chicken portions after cooking. Moreover, not extrapolating beyond the data used in model development is important because, when a model is used to make predictions beyond the data used to develop it, the predictions may not be reliable. Third, the current model was properly validated using the test data and model performance criteria of the APZ method. This was important; it ensured that the comparisons of observed and predicted values were not confounded because the independent data were collected with the same methods as the dependent data and ensured that the validation was not biased because the independent data for interpolation provided uniform coverage of model predictions. Fourth, the cooking and cooling scenario simulated and modeled in the present study more closely represented how chicken is cooked and cooled in the real world than the cooking and cooling scenario used in previous studies to develop thermal inactivation models for *Salmonella* in ground chicken. Thus, the current model is expected to provide more realistic and reliable predictions than previous models for the thermal inactivation of *Salmonella* in ground chicken. After publication, the data and model will be made available for free at www.ars.usda.gov/nea/errc/PoultryFARM.

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REFERENCES

1. Anonymous. 1996. Nationwide raw ground chicken microbiological survey. U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, DC.
2. Buchanan, R. L. 1993. Predictive food microbiology. *Trends Food Sci. Technol.* 4:6–11.
3. Foley, S. L., R. Nayak, I. B. Hanning, T. J. Johnson, J. Han, and S. C. Ricke. 2011. Population dynamics of *Salmonella enterica* serotypes in commercial egg and poultry production. *Appl. Environ. Microbiol.* 77:4273–4279.
4. Ikediala, J. N., L. R. Correia, G. A. Fenton, and N. Ben-Abdallah. 1996. Finite element modeling of heat transfer in meat patties during single-sided pan-frying. *J. Food Sci.* 61:796–802.
5. Juneja, V. K., B. S. Eblen, and H. M. Marks. 2001. Modeling non-linear survival curves to calculate thermal inactivation of *Salmonella* in poultry of different fat levels. *Int. J. Food Microbiol.* 70:37–51.
6. Juneja, V. K., U. Gonzales-Barron, F. Butler, A. S. Yadav, and M. Friedman. 2013. Predictive thermal inactivation model for the combined effect of temperature, cinnamaldehyde and carvacrol on starvation-stressed multiple *Salmonella* serotypes in ground chicken. *Int. J. Food Microbiol.* 165:184–199.
7. Juneja, V. K., A. S. Yadav, C. A. Hwang, S. Sheen, S. Mukhopadhyay, and M. Friedman. 2012. Kinetics of thermal destruction of *Salmonella* in ground chicken containing trans-cinnamaldehyde and carvacrol. *J. Food Prot.* 75:289–296.

8. Kosa, K. M., S. C. Cates, S. Bradley, E. Chambers IV, and S. Godwin. 2015. Consumer-reported handling of raw poultry products at home: results from a national survey. *J. Food Prot.* 78:180–186.
9. Mackey, B. M., A. F. Kelly, J. A. Colvin, P. T. Robbins, and P. J. Fryer. 2006. Predicting the thermal inactivation of bacteria in a solid matrix: simulation studies on the relative effects of microbial thermal resistance parameters and process conditions. *Int. J. Food Microbiol.* 107:295–303.
10. Murphy, R. Y., E. R. Johnson, B. P. Marks, M. G. Johnson, and J. A. Marcy. 2001. Thermal inactivation of *Salmonella senftenberg* and *Listeria innocua* in ground chicken breast patties processed in an air convection oven. *Poult. Sci.* 80:515–521.
11. Murphy, R. Y., B. P. Marks, E. R. Johnson, and M. G. Johnson. 1999. Inactivation of *Salmonella* and *Listeria* in ground chicken breast meat during thermal processing. *J. Food Prot.* 62:980–985.
12. Murphy, R. Y., T. Osaili, L. K. Duncan, and J. A. Marcy. 2004. Effect of sodium lactate on thermal inactivation of *Listeria monocytogenes* and *Salmonella* in ground chicken thigh and leg meat. *J. Food Prot.* 67:1403–1407.
13. Murphy, R. Y., T. Osaili, L. K. Duncan, and J. A. Marcy. 2004. Thermal inactivation of *Salmonella* and *Listeria monocytogenes* in ground chicken thigh/leg meat and skin. *Poult. Sci.* 83:1218–1225.
14. Osaili, T. M., A. A. Al-Nabulsi, R. R. Shaker, A. N. Olaimat, Z. W. Jaradat, and R. A. Holley. 2013. Thermal inactivation of *Salmonella* Typhimurium in chicken shawirma (gyro). *Int. J. Food Microbiol.* 166:15–20.
15. Oscar, T. P. 2005. Development and validation of primary, secondary and tertiary models for predicting growth of *Salmonella* Typhimurium on sterile chicken. *J. Food Prot.* 68:2606–2613.
16. Oscar, T. P. 2005. Validation of lag time and growth rate models for *Salmonella* Typhimurium: acceptable prediction zone method. *J. Food Sci.* 70:M129–M137.
17. Oscar, T. P. 2014. Use of enrichment real-time PCR to enumerate *Salmonella* on chicken parts. *J. Food Prot.* 77:1086–1092.
18. Oscar, T. P. 2015. Neural network model for survival and growth of *Salmonella enterica* serotype 8,20:–:z_6 in ground chicken thigh meat during cold storage: extrapolation to other serotypes. *J. Food Prot.* 78:1819–1827.
19. Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M.-A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States—major pathogens. *Emerg. Infect. Dis.* 17:7–15.
20. Thomas, C. J., and T. A. McMeekin. 1981. Attachment of *Salmonella* spp. to chicken muscle surfaces. *Appl. Environ. Microbiol.* 42:130–134.
21. Thomas, H. A. 1942. Bacterial densities from fermentation tube tests. *J. Am. Water Works Assoc.* 34:572–576.
22. Whiting, R. C., and R. L. Buchanan. 1994. Microbial modeling. *Food Technol.* 48:113–120.