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Research Paper

# Development and Validation of a Predictive Model for Growth of *Salmonella* Infantis in Ground Turkey

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ABSTRACT

Most retail samples (25 g) of ground turkey contain no or low levels of *Salmonella*. However, temperature abuse after retail can lead to spread and growth of *Salmonella* in the package. In addition, it can lead to levels that pose a significant risk of salmonellosis. This is especially true when the serotype is a top human clinical isolate, like Infantis. Therefore, the current study was undertaken to develop and validate a predictive model for the growth of *Salmonella* Infantis in ground turkey subjected to temperature abuse. The purpose was to fill a gap for serotype-specific data and models in risk assessments for this pathogen and food combination. Storage trials with a low initial inoculum (0.85 $\log_{10}$) of *Salmonella* Infantis in commercial ground turkey samples (0.2 g) with native microflora were conducted at 16–40 °C for 0–28 h. *Salmonella* was enumerated in ground turkey samples using an automated, whole sample enrichment, miniature, most probable number (MPN) assay. The MPN data were fitted to a three-phase linear primary model. Secondary models for primary model parameters were developed and used in the primary model to create a tertiary model that predicted the growth of *Salmonella* Infantis in ground turkey as a function of time and temperature. Data and tertiary model predictions were evaluated using the test data, model performance, and model validation criteria of the Acceptable Prediction Zones method in the Validation Software Tool. The tertiary model predictions were considered to have acceptable bias and accuracy when the proportion of residuals (observed − predicted) in the partly and fully acceptable prediction zones (pAPZ) was ≥0.7. The overall pAPZ of the tertiary model was 0.866 for dependent data ($n$ = 406) and 0.853 for independent data for interpolation ($n$ = 177). However, there were local prediction problems that limited the validated prediction range to a region from 0 to 8 h at 16–40 °C. Nonetheless, this validation range was sufficient to simulate temperature abuse of ground turkey during meal preparation in the consumers' home. Thus, the model fills an important data and modeling gap in risk assessments for *Salmonella* and ground turkey. Additional data are needed to repair and fully validate the model.

Most retail samples (25 g) of ground turkey have no or low levels of *Salmonella* (Erickson et al., 2018). However, temperature abuse after retail can lead to spread and growth of *Salmonella* in the package and to levels that pose a significant risk of salmonellosis (Oscar, 2017), especially when the serotype is a leading human clinical isolate like Infantis (Powell & Williams, 2024). In 2011, a multiple antibiotic resistance strain of *Salmonella* Heidelberg from ground turkey caused an outbreak of salmonellosis involving 134 people in 34 U. S. states (Routh et al., 2015). This demonstrates the potential risk of this pathogen and food combination to public health.

Quantitative microbial risk assessment is a valuable tool for assessing and managing the risk of salmonellosis from turkey products (Bemrah et al., 2003) and other foods (Collineau et al., 2020; Lambertini et al., 2017). A gap in risk assessments for foodborne salmonellosis is serotype-specific data and models for growth of *Salmonella* in food with native microflora (Kim et al., 2024). Therefore, the current study was undertaken to develop and validate a serotype-specific model for the growth of *Salmonella* Infantis in ground turkey with native microflora. This serotype was selected because its isolation from humans and chickens is on the rise (Powell & Williams, 2024). Also, it was isolated (Oscar, 2020a) from the commercial source of ground turkey used in the present study.

The serotype-specific model developed in this study is not the first model for growth of *Salmonella* in ground turkey. Ingham et al. (2007)

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developed a model for the growth of *Salmonella* in ground turkey using a mixture of serotypes that included Infantis. Consequently, its predictions were compared to those of the model developed in this study.

## Materials and methods

**Stock culture.** *Salmonella* Infantis was isolated from a commercial package of ground turkey in a previous study (Oscar, 2020a). It was maintained at −80 °C in tryptic soy broth with 15% glycerol (Hardy Diagnostics).

**Experimental designs.** A replicated ($n = 6$), full 7 by 10 factorial design of temperature (16, 20, 24, 28, 32, 36, 40 °C) and time (0, 2, 4, 6, 8, 12, 16, 20, 24, 28 h) was used for model development, whereas a replicated ($n = 3$), full 6 by 10 factorial design of temperature (18, 22, 26, 30, 34, 38 °C) and time (0, 1, 3, 5, 7, 10, 14, 18, 22, 26 h) was used for model validation.

**Inoculum culture.** Five microliters of the stock culture were added to 0.7 mL of buffered peptone water (Microbiology International) in a 1.5 mL microcentrifuge tube. The culture was incubated for 96 h at 22 °C in a ThermoStat Plus programmable incubator (Eppendorf). A fresh inoculum culture was prepared weekly.

**Ground turkey samples.** Ground turkey was purchased weekly from a local retail store and transported to the laboratory within 30 min at ambient temperature before storage at 4 °C for 4–6 h and preparation of 0.2 g samples. The samples were placed in 1.5 mL microcentrifuge tubes that were stored at 4 °C for 16–18 h before use in the storage trials.

**Inoculation procedure.** Five microliters of the $10^{-6}$ dilution of the inoculum culture of *Salmonella* Infantis were spot inoculated onto the surface of the cold (4 °C) ground turkey samples (0.2 g) for an initial inoculum level of about 0.85 $\log_{10}$ most probable number.

**Storage trials.** After inoculation with *Salmonella* Infantis, ground turkey samples in 1.5 mL microcentrifuge tubes were inserted into a heating and cooling block (ThermoStat Plus) at the test temperature. Two storage trials were conducted per week.

**Sample processing**. At the designated time, a sample was removed and 0.7 mL of cold (4 °C) buffered peptone water was added. Samples were vortexed in a Digital Disruptor Genie (Scientific Industries) for one min at 3,000 rpm to recover *Salmonella* into the medium for enumeration.

**Most probable number.** A miniature most probable number assay was used to enumerate *Salmonella* in the recovery medium (Oscar, 2018). The assay was conducted in 96-well, deep-well plates, had a 6 replicate by 16 dilution design, and involved two steps. First, there was nonselective enrichment in buffered peptone water for 24 h at 40 °C. Second, there was selective enrichment in Rappaport Vassiliadis broth (Becton Dickinson) for 48 h at 42 °C. The serial dilution and transfer steps were performed by a robotic pipettor (Solo Plus, Hudson Robotics). The Jarvis et al. (2010) calculator (version 6) was used to calculate the most probable number.

**Primary modeling.** The most probable number data ($\log_{10}$ MPN/0.2 g) from replicate storage trials within a temperature were graphed as a function of time and fitted (Prism, version 10, GraphPad Software) to the three-phase linear model (Buchanan et al., 1997):

$$\begin{aligned} Y &= Y_1 && \text{IF } X \leqslant X_1 \\ Y &= Y_1 + (\{(Y_2 - Y_1)/(X_2 - X_1)\}(X - X_1)) && \text{IF } X_1 < X < X_2 \\ Y &= Y_2 && \text{IF } X \geqslant X_2 \end{aligned} \quad (1)$$

where Y was the $\log_{10}$ most probable number per 0.2 g at time X (h), $X_1$ was lag time (h), $X_2$ was time (h) to $Y_2$ or final Y ($\log_{10}$ MPN/0.2 g), and $Y_1$ was initial Y ($\log_{10}$ MPN/0.2 g). The 99% prediction interval was used to identify and remove outliers.

**Secondary Modeling.** The best-fit values of the primary model parameters were graphed as a function of temperature (T, °C). The dependent data were then fitted to secondary models:

$$\begin{aligned} X_1 &= B_0 + B_1 T && \text{IF } T < T_o \\ X_1 &= (B_0 + B_1 T_o) + B_2 (T - T_o) && \text{IF } T \geqslant T_o \end{aligned} \quad (2)$$

where $X_1$ was lag time (h), $B_0$, $B_1$, and $B_2$ were regression coefficients, T was temperature (°C), and $T_o$ was the temperature (°C) where the two linear lines met.

$$\begin{aligned} X_2 &= B_0 && \text{If } T \leqslant T_1 \\ X_2 &= B_0 + B_1 (T - T_1) + B_2 (T - T_1)^2 && \text{If } T > T_1 \end{aligned} \quad (3)$$

where $X_2$ was the time to $Y_2$ (h), $B_0$, $B_1$, and $B_2$ were regression coefficients, T was temperature (°C), and $T_1$ was the temperature (°C) where $X_2$ began to decline.

$$Y_1 = B_0 + B_1 T \quad (4)$$

where $Y_1$ was the initial most probable number ($\log_{10}$ /0.2 g), $B_0$ and $B_1$ were regression coefficients, and T was temperature (°C).

$$Y_2 = \exp\left(\{a(T - T_{min})(T - T_{max})\}/\{(T - T_{submin})(T - T_{supmax})\}\right) \quad (5)$$

where $Y_2$ was final $\log_{10}$ most probable number per 0.2 g, $a$ was a regression coefficient, T was temperature (°C), $T_{min}$ was the minimum growth temperature (°C), $T_{max}$ was the maximum growth temperature (°C), $T_{submin}$ was a temperature (°C) just below $T_{min}$, and $T_{supmax}$ was a temperature (°C) just above $T_{max}$.

**Tertiary modeling.** The tertiary model was created in an Excel spreadsheet (MicroSoft Office 365) by inserting the secondary models into the primary model:

$$\begin{aligned} Y &= {}^{(4)}Y_1 && \text{IF } X \leqslant X_1 \\ Y &= {}^{(4)}Y_1 + \left(\{({}^{(5)}Y_2 - {}^{(4)}Y_1)/({}^{(3)}X_2 - {}^{(2)}X_1)\}(X - {}^{(2)}X_1)\right) && \text{IF } X_1 < X < X_2 \\ Y &= {}^{(5)}Y_2 && \text{IF } X \geqslant X_2 \end{aligned} \quad (6)$$

where Y was the $\log_{10}$ most probable number per 0.2 g at time X (h), ${}^{(2)}X_1$ was the secondary model for lag time, ${}^{(3)}X_2$ was the secondary model for time to $Y_2$, ${}^{(4)}Y_1$ was the secondary model for initial Y, and ${}^{(5)}Y_2$ was the secondary model for final Y.

The predicted primary model parameters were used to calculate the growth rate ({$\log_{10}$ MPN/0.2 g}/h):

$$= (Y_2 - Y_1)/(X_2 - X_1) \quad (7)$$

where $Y_2$, $Y_1$, $X_2$, and $X_1$, were as described above.

**Model validation.** Data and predictions of the tertiary model ($\log_{10}$ MPN/0.2 g) were evaluated using the test data, model performance, and model validation criteria of the Acceptable Prediction Zones (APZ) method in the Validation Software Tool (Oscar, 2020b). The model provided predictions with acceptable bias and accuracy when the proportion of residuals in the partly and fully acceptable prediction zones (pAPZ) was ≥0.7. The model was validated when it met all criteria for test data and model performance for the dependent data and the independent data for interpolation.

## Results and discussion

The results of the three-phase linear primary modeling step for the dependent data are summarized in Table 1, and representative curve fits are presented in Figure 1. The growth curves at 16 and 20 °C had only two phases (lag and exponential). Therefore, the time to final most probable number was fixed to 28 h to obtain a curve fit.

The coefficient of determination for the primary model fits ranged from 0.8921 at 16 °C to 0.9838 at 28 °C (Table 1). The 99% prediction interval identified 14 outliers in the dependent data. When fitted to the independent data for interpolation (results not shown), 3 outliers were identified by the 99% prediction interval method. Thus, the overall proportion of outliers was 0.028 (17/600).

The most probable number assay was not serotype-specific. Consequently, outliers from indigenous *Salmonella* were possible. However, only 2 of 17 outliers were above the growth curves. Thus, inflation of

**Table 1**
Results of the primary modeling step for the dependent data for growth of *Salmonella* Infantis in ground turkey[a]

| Best-fit values | Units | 16 °C | 20 °C | 24 °C | 28 °C | 32 °C | 36 °C | 40 °C |
|---|---|---|---|---|---|---|---|---|
| $X_1$ | h | 13.9 | 10.09 | 4.004 | 1.934 | 1.227 | 1.657 | 1.694 |
| $Y_1$ | $\log_{10}$ MPN/0.2 g | 0.8247 | 0.9961 | 0.8648 | 0.9395 | 0.7662 | 0.9358 | 0.8082 |
| $X_2$ | h | 28.00 | 28.00 | 21.17 | 17.46 | 13.11 | 9.706 | 8.554 |
| $Y_2$ | $\log_{10}$ MPN/0.2 g | 3.723 | 7.987 | 10.16 | 11.20 | 11.02 | 11.08 | 10.21 |
| 95% CI (profile likelihood) | | | | | | | | |
| $X_1$ | h | 12.17–15.24 | 8.673–11.29 | 2.750–4.931 | 1.098–2.655 | 0.3818–2.000 | 1.059–2.216 | 1.055–2.220 |
| $Y_1$ | $\log_{10}$ MPN/0.2 g | 0.7150–0.9343 | 0.7729–1.219 | 0.4170–1.161 | 0.4907–1.267 | 0.2284–1.304 | 0.4063–1.453 | 0.1966–1.386 |
| $X_2$ | h | 28.00–28.00 | 28.00–28.00 | 20.14–22.40 | 16.74–18.23 | 12.44–13.89 | 9.210–10.29 | 8.098–9.090 |
| $Y_2$ | $\log_{10}$ MPN/0.2 g | 3.459–3.987 | 7.579–8.394 | 9.784–10.54 | 10.94–11.47 | 10.75–11.29 | 10.83–11.32 | 9.923–10.49 |
| Goodness of Fit | | | | | | | | |
| Degrees of Freedom | | 53 | 56 | 55 | 55 | 55 | 53 | 53 |
| $R^2$ | | 0.8921 | 0.9429 | 0.9748 | 0.9838 | 0.9761 | 0.9769 | 0.9644 |
| Adjusted $R^2$ | | 0.8880 | 0.9409 | 0.9735 | 0.9829 | 0.9748 | 0.9756 | 0.9624 |
| Sy.x | $\log_{10}$ MPN/0.2 g | 0.3280 | 0.6104 | 0.6266 | 0.5485 | 0.6572 | 0.6467 | 0.7469 |
| RMSE | $\log_{10}$ MPN/0.2 g | 0.3220 | 0.5997 | 0.6102 | 0.5341 | 0.6400 | 0.6292 | 0.7266 |
| Constraints | | | | | | | | |
| $X_2$ | h | = 28 | = 28 | None | None | None | None | None |
| Number of points | | | | | | | | |
| # of X values | | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| # Y values analyzed | | 56 | 59 | 59 | 59 | 59 | 57 | 57 |
| Outliers | h | 20,24,28,28 | 28 | 24 | 24 | 12 | 6,20,24 | 8,16,16 |

[a] Abbreviations: CI = confidence interval; $X_1$ = lag time; h = hours; $Y_1$ = initial most probable number (MPN); $X_2$ = time to $Y_2$ (final MPN); $R^2$ = coefficient of determination; Sy.x = standard deviation of the residuals; and RMSE = root mean squared error.

most probable numbers by indigenous *Salmonella* did not occur often, if at all. The use of small samples (0.2 g) of ground turkey may have mitigated this potential problem.

A low inoculum size of *Salmonella* Infantis (0.85 $\log_{10}$ MPN/0.2 g) was used. Lag time is more variable when low inoculum sizes are used (Koutsoumanis & Lianou, 2013; Pin & Baranyi, 2006). This occurs because variation of physiological states among individual cells is more apparent. Thus, some of the variation of *Salmonella* growth among replicate storage trials was likely from this phenomenon.

Results of the secondary modeling step are shown in Figure 2. Dependent data for lag time ($X_1$) fitted well to a two-segmented linear model with a coefficient of determination of 0.9926 (Fig. 2A). The two linear lines in the model met at 26.18 °C. Lag time decreased as the temperature went from 16 to 26.18 °C, whereas it did not change much from 26.18 to 40 °C where the slope of the second line was close to zero. Independent data for lag time at 18 and 20 °C deviated from the fitted curve, whereas closer agreement was observed for lag times from 22 to 38 °C (Fig. 2A). Some local prediction problems in the tertiary model (see below) can be traced back to this secondary model.

The secondary model fit for time to final most probable number ($X_2$) is shown in Figure 2B. The time to the final most probable number decreased as the temperature increased from 19.88 to 40 °C. The decrease was nonlinear and was predicted well by a two-phase, quadratic polynomial model with a coefficient of determination of 0.9973. The independent data for time to final most probable number ($X_2$) were close to the fitted curve. Thus, the local prediction problems observed in the tertiary model (see below) could not be traced back to this secondary model.

The secondary model fit for initial most probable number ($Y_1$) is shown in Figure 2C. The slope of this linear model was close to zero and the Y-intercept was 0.94 $\log_{10}$ most probable number per 0.2 g, which was a little higher than the average most probable number at time zero, which was 0.83 $\log_{10}$ per 0.2 g for dependent data and 0.80 $\log_{10}$ per 0.2 g for independent data. Both the dependent data and the independent data for interpolation were close to the fitted line (Fig. 2C). Thus, the local prediction problems in the tertiary model (see below) could not be traced back to this secondary model.

The secondary model fit for the final most probable number ($Y_2$) is shown in Figure 2D. The final most probable number changed in a nonlinear manner as a function of temperature. The minimum (13.99 °C) and maximum (71.85 °C) temperatures for growth of *Salmonella* were nonsensical. This was because the range of temperatures investigated was not close enough to the growth/no growth boundaries. Yet, within the range of temperatures investigated, the model fitted the dependent data well with a coefficient of determination of 0.9979. On the other hand, the final most probable number values for the independent data for interpolation were mostly below those predicted by the secondary model. Notably, the data point at 26 °C deviated the most from the fitted curve, which may help explain some of the local prediction problems observed in the tertiary model at this temperature (see below).

The secondary models for the primary model parameters (Fig. 2) were incorporated back into the primary model to create a tertiary model (Fig. 3) that predicted the change in $\log_{10}$ most probable number of *Salmonella* Infantis in 0.2 g of ground turkey as a function of time (0–28 h) and temperature (16–40 °C). The temperature (25.2 °C) simulated in Figure 3 was not investigated but was within the range of temperatures modeled. This ability to interpolate is a valuable feature of predictive models.

The performance of the tertiary model for predicting the dependent data was evaluated using the criteria of the Acceptable Prediction Zones method in the Validation Software Tool (Fig. 4). The decision tree contained yes or no questions for test data (questions 1–3), model performance (questions 4–6), and model validation (question 7) criteria.

The first pivot table counted the prediction cases per combination of time and temperature and was used to answer questions 1–3 for test data in the decision tree for dependent data (Fig. 4). The test data were used to develop the model. Therefore, the answer to question 1 was yes. The times and temperatures used to collect the test data were evenly spaced. Thus, the answer to question 2 was yes. The minimum number of test data or prediction cases per combination of time and temperature was four. Consequently, the answer to question 3 was yes. These results indicated that the data met all the criteria for test data for dependent data.

The second pivot table calculated the proportion of residuals in the partly and fully acceptable prediction zones (pAPZ) and was used to answer questions 4–6 for model performance in the decision tree for

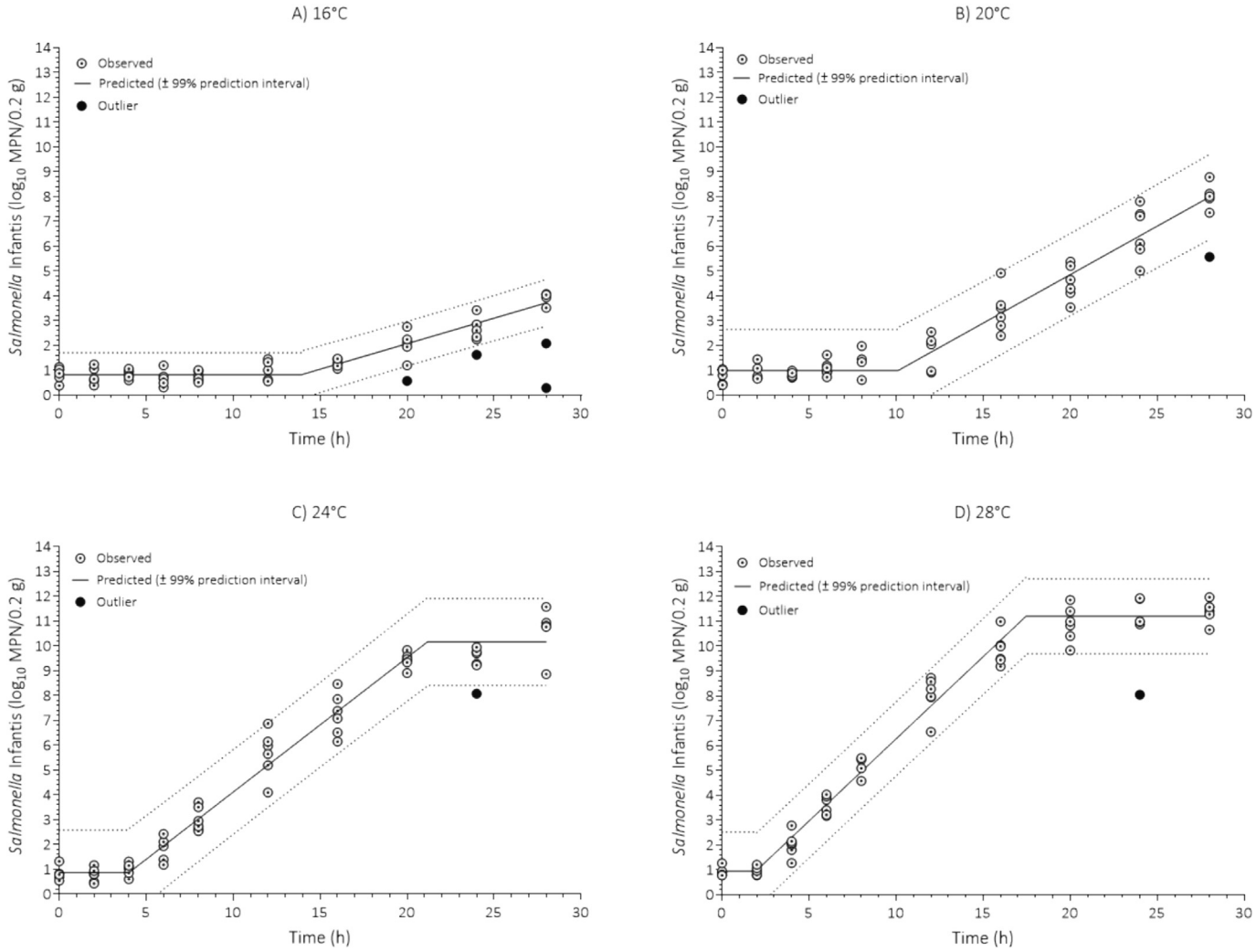


**Figure 1.** Three-phase linear primary model fits to the dependent data for the growth of *Salmonella* Infantis in ground turkey stored at (A) 16 °C; (B) 20 °C; (C) 24 °C; and (D) 28 °C.

dependent data (Fig. 4). The overall pAPZ for the tertiary model predictions of the dependent data ($n$ = 406) was 0.866. Thus, the answer to question 4 was yes. The minimum pAPZ for time was 0.793 at 12 h, and the minimum pAPZ for temperature was 0.769 at 36 °C. Consequently, the answer to question 5 was yes. The maximum number of consecutive pAPZ that were less than 0.7 for combinations of time and temperature was two. Therefore, the answer to question 6 was yes. These results indicated that the tertiary model satisfied all criteria for model performance in the decision tree for dependent data.

The tertiary model satisfied the criteria for test data and model performance as indicated by answers of yes to questions 1–6 in the decision tree for the dependent data (Fig. 4). Consequently, the answer to question 7 for model validation was yes. Successful validation of the tertiary model for the dependent data indicated that it was now eligible to be validated for interpolation.

The performance of the tertiary model for predicting an independent set of test data is shown in Figure 5. Here, questions 2–5 in the decision tree were for test data criteria, questions 6–8 were for model performance criteria, and questions 1 and 9 were for model validation criteria. The answer to question 1 automatically populated from the answer to question 7 in the decision tree for dependent data (Fig. 4). This was done to ensure that users do not validate a model for interpolation that failed the validation for dependent data.

Again, the first pivot table counted the number of test data or prediction cases per combination of time and temperature and was used to answer questions 2–5 for test data criteria in the decision tree for interpolation (Fig. 5). The test data were not used to develop the model. Thus, the answer to question 2 was yes. The test data were collected using the same methods as those used to collect the dependent data. Therefore, the answer to question 3 was yes. The test data were collected at times and temperatures that were intermediate to those used in model development. Consequently, the answer to question 4 was yes. Lastly, there was a minimum of two test data or prediction cases per combination of time and temperature. Thus, the answer to question 5 was yes. These results indicated that the data met all criteria for test data in the decision tree for independent data for interpolation.

The overall pAPZ for the tertiary model predictions of the independent data for interpolation ($n$ = 177) was 0.853 (Fig. 5). Therefore, the answer to question 6 was yes. The minimum pAPZ for time was 0.687 at 14 h, and the minimum pAPZ for temperature was 0.713 at 22 °C. Thus, the answer to question 7 was no. The maximum number of consecutive pAPZ that were less than 0.7 for individual combinations of time and temperature was seven. Consequently, the answer to question 8 was no. These results indicated that the tertiary model did not meet all the criteria for model performance because it had local

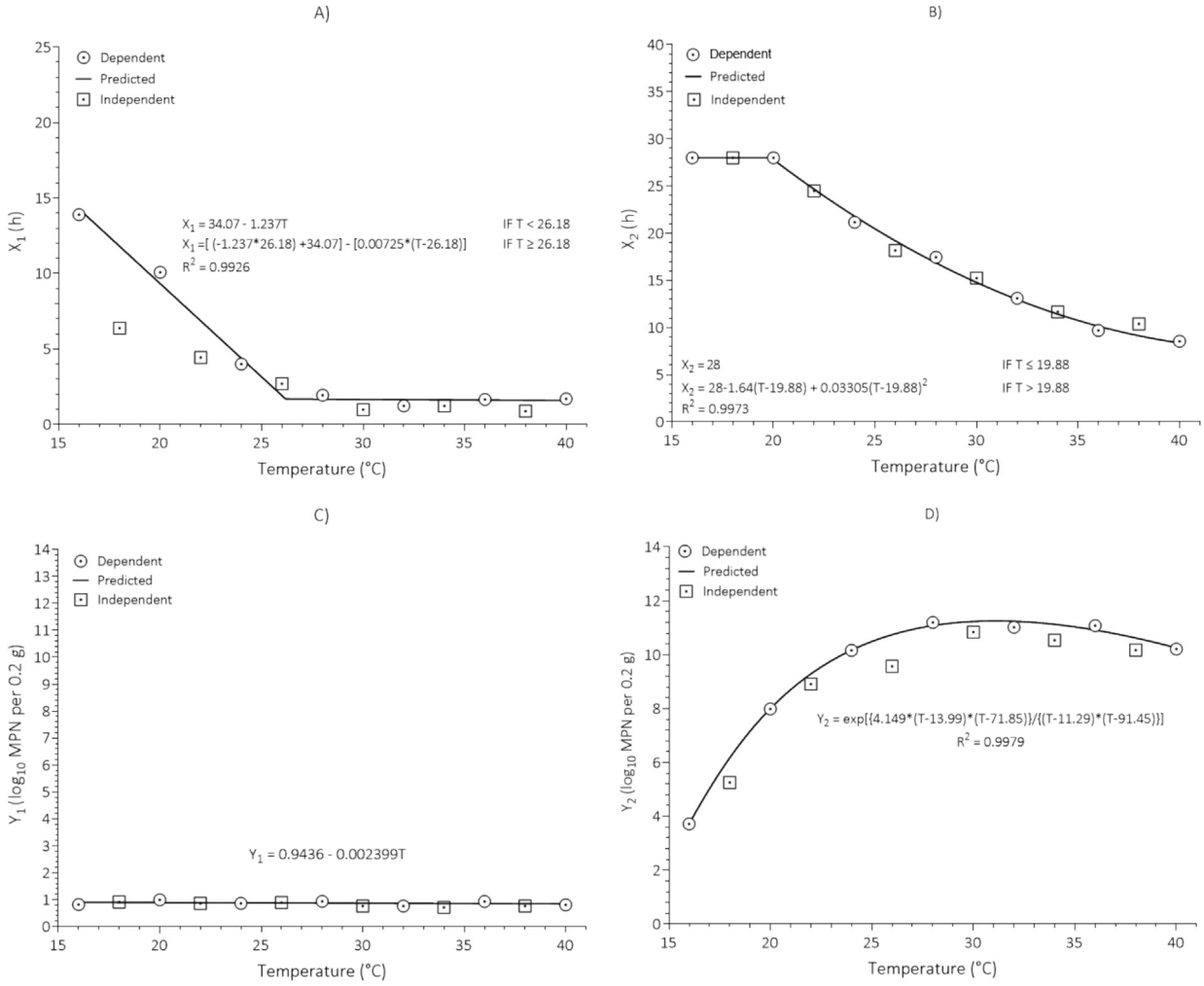


**Figure 2.** Secondary model fits to the three-phase linear primary model parameters of (A) lag time ($X_1$); (B) time to final most probable number ($X_2$); (C) initial most probable number ($Y_1$); and (D) final most probable number ($Y_2$) of *Salmonella* Infantis in ground turkey as a function of temperature. $R^2$ is the coefficient of determination.

prediction problems. Specifically, it did not provide acceptable predictions from 10 to 18 h at 18 °C, from 5 to 26 h at 22 °C, and from 10 to 18 h at 26 °C.

The answer to question 9 in the decision tree for interpolation was no (Fig. 5). This indicated that the tertiary model was not validated for interpolation because it had local prediction problems. Thus, per the model validation criteria of the Acceptable Prediction Zones method, it was not eligible for validation for extrapolation to a new independent variable like another serotype of *Salmonella* or another source of ground turkey.

Graphs of the observed most probable number data for interpolation and tertiary model predictions over time were examined to further evaluate the local prediction problems (Fig. 6). These graphs showed most of the deviations of the observed data from predicted growth curves were just enough to result in pAPZs that were just below the threshold of 0.7. In fact, 8 of the 14 unacceptable pAPZ in the unacceptable prediction region were between 0.639 and 0.681 (Fig. 5). Thus, the collection of additional data in these unacceptable regions could raise the pAPZ for enough combinations of time and temperature that the tertiary model would be validated for interpolation. However, the region from 10 to 18 h at 18 °C where pAPZ ranged from 0.199 to 0.521 may present the greatest challenge. Here, the local prediction problem can be traced back to the secondary model where the lag time at 18 °C deviated greatly from the secondary model curve fit (Fig. 2A).

The purpose of the Acceptable Prediction Zones method is not to reject models (Oscar, 2020b). Rather, it is to develop better models. This is done by identifying problems in models and repairing them before they are released to end-users. In this case, an attempt was made to repair the model by using the most probable number data to train and test a neural network model (results not shown). However, this was unsuccessful because the local prediction problems observed in the tertiary model persisted in the neural network model. Thus, they appeared to be due to inconsistencies in the data and not to the choice of the model.

The latter conclusion was supported by results of the secondary modeling step (Fig. 2). Here, all secondary models had high goodness-of-fit ($R^2 > 0.99$) for the dependent data. However, the local prediction problems in the tertiary model appeared to be associated with deviations of the independent data for interpolation from the secondary model predictions, specifically, the lag time data at 18 and 22° (Fig. 2A) and the final most probable number data at 26 °C (Fig. 2D).

A second approach to model repair was to see if the tertiary model could be partially validated. Here, values for time in the pivot tables for the independent data for interpolation were removed one by one starting at 26 h until the tertiary model met all criteria for test data,

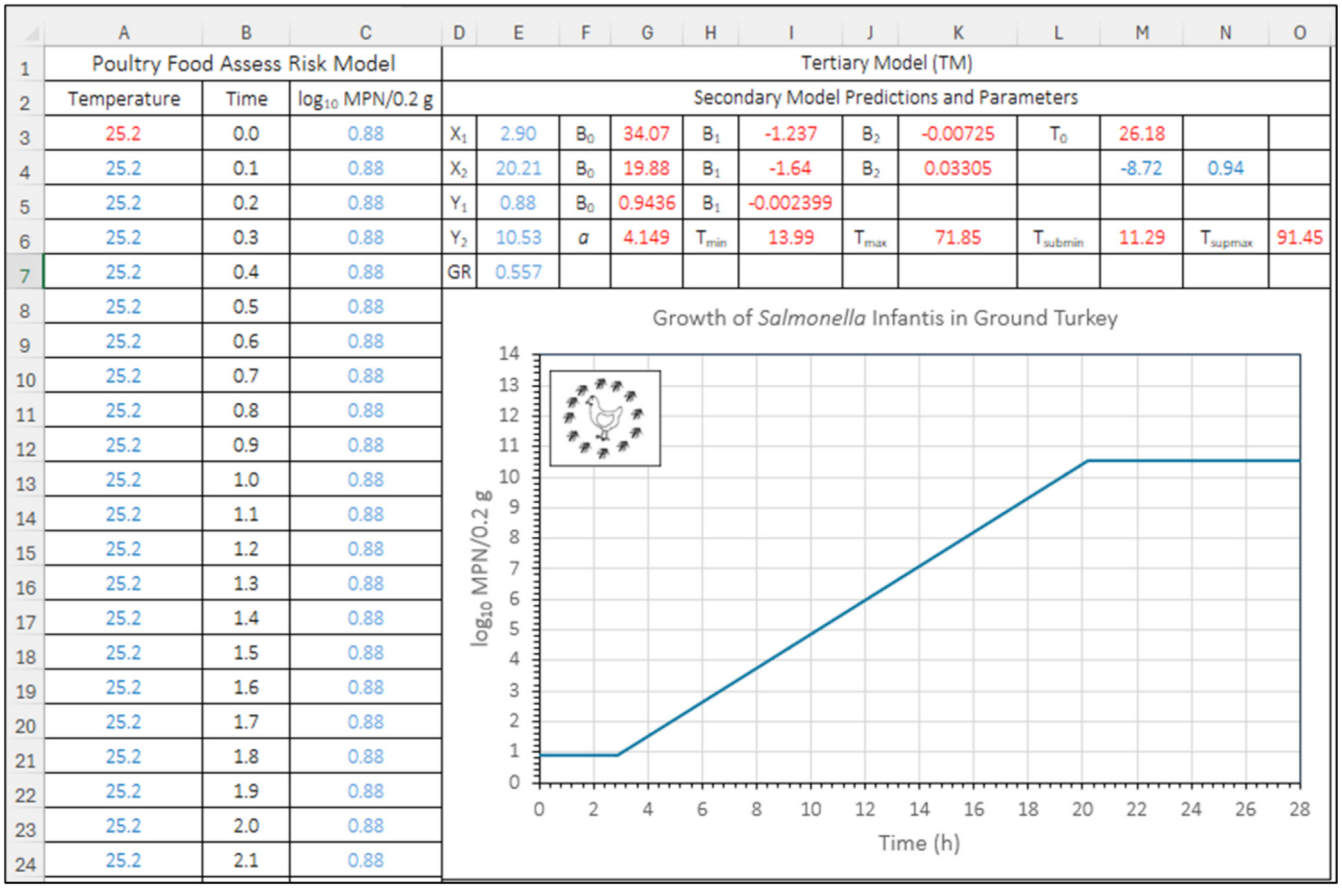


**Figure 3.** Screenshot of the tertiary model for predicting growth of *Salmonella* Infantis in ground turkey as a function of time (0–28 h) and temperature (16–40 °C).

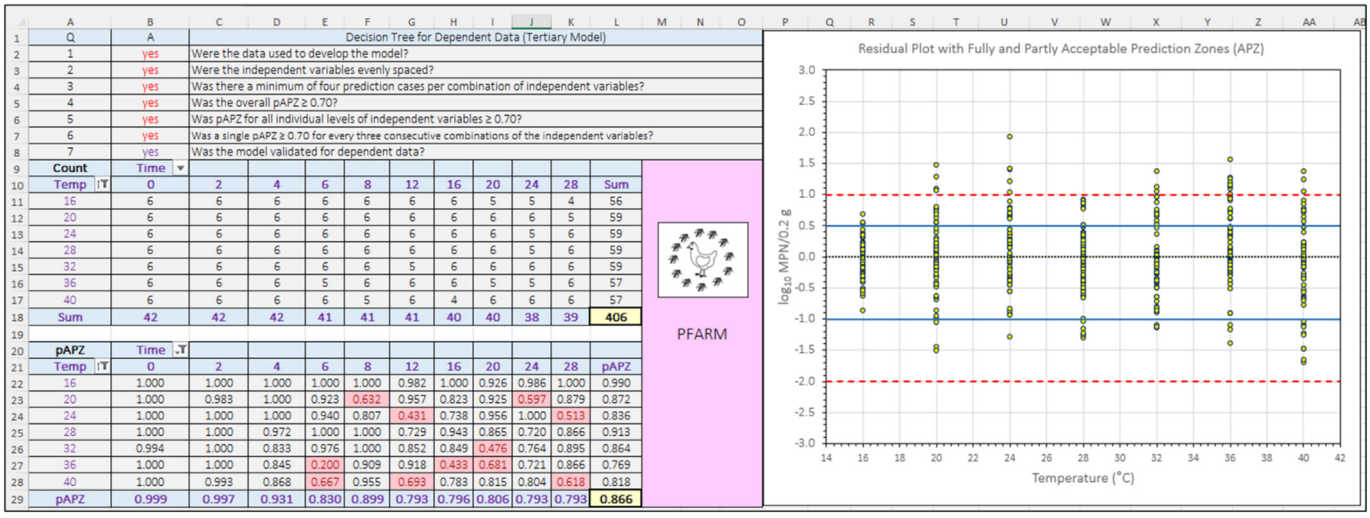


**Figure 4.** Screenshot of the Validation Software Tool for the Acceptable Prediction Zones analysis of the tertiary model predictions of the dependent data for growth of *Salmonella* Infantis in ground turkey. pAPZ is the proportion of residuals in the partly and fully acceptable prediction zones.

model performance, and model validation. This occurred when the only remaining values for time were from 0 to 7 h at 18–38 °C. Here, the overall pAPZ was 0.946 and there were no local prediction problems (results not shown).

Next, the values for time from 10 to 28 h in the pivot tables for the tertiary model predictions of the dependent data were removed. This was done to confirm that the tertiary model provided acceptable predictions of the dependent data in the region that immediately bordered the validated region for the independent data for interpolation. Here, the overall pAPZ was 0.932 and there were no local prediction problems or violations of the criteria for test data (results not shown). Thus, the partial validation of the tertiary model for interpolation was successful for a prediction region from 0 to 8 h at 16–40 °C. This validated range covers typical temperature abuse sce-

| Q | A | Decision Tree for Interpolation (Tertiary Model) |
|---|---|---|
| 1 | yes | Was the model validated for dependent data? |
| 2 | yes | Were the data independent? |
| 3 | yes | Were the data collected using the same methods as dependent data? |
| 4 | yes | Were the independent variables at values intermediate to those used in model development? |
| 5 | yes | Was there a minimum of two prediction cases per combination of independent variables? |
| 6 | yes | Was the overall pAPZ ≥ 0.70? |
| 7 | no | Was pAPZ for all individual levels of independent variables ≥ 0.70? |
| 8 | no | Was a single pAPZ ≥ 0.70 for every three consecutive combinations of the independent variables? |
| 9 | no | Was the model validated for interpolation? |

| Count | Time | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Temp | 0 | 1 | 3 | 5 | 7 | 10 | 14 | 18 | 22 | 26 | Sum |
| 18 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 30 |
| 22 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 30 |
| 26 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 29 |
| 30 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 29 |
| 34 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 3 | 3 | 29 |
| 38 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 30 |
| Sum | 18 | 18 | 18 | 17 | 18 | 18 | 17 | 18 | 18 | 17 | 177 |

| pAPZ | Time | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Temp | 0 | 1 | 3 | 5 | 7 | 10 | 14 | 18 | 22 | 26 | pAPZ |
| 18 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.521 | 0.199 | 0.421 | 0.843 | 0.855 | 0.784 |
| 22 | 1.000 | 1.000 | 1.000 | 0.565 | 0.639 | 0.667 | 0.528 | 0.667 | 0.674 | 0.387 | 0.713 |
| 26 | 1.000 | 1.000 | 1.000 | 1.000 | 0.859 | 0.667 | 0.667 | 0.681 | 0.796 | 0.642 | 0.838 |
| 30 | 1.000 | 1.000 | 1.000 | 1.000 | 0.667 | 0.926 | 0.869 | 0.874 | 0.886 | 0.795 | 0.898 |
| 34 | 1.000 | 1.000 | 1.000 | 1.000 | 0.692 | 0.929 | 0.948 | 0.958 | 1.000 | 1.000 | 0.953 |
| 38 | 1.000 | 1.000 | 1.000 | 1.000 | 0.978 | 0.631 | 1.000 | 1.000 | 0.831 | 0.952 | 0.939 |
| pAPZ | 1.000 | 1.000 | 1.000 | 0.923 | 0.806 | 0.723 | 0.687 | 0.767 | 0.838 | 0.780 | 0.853 |

PFARM

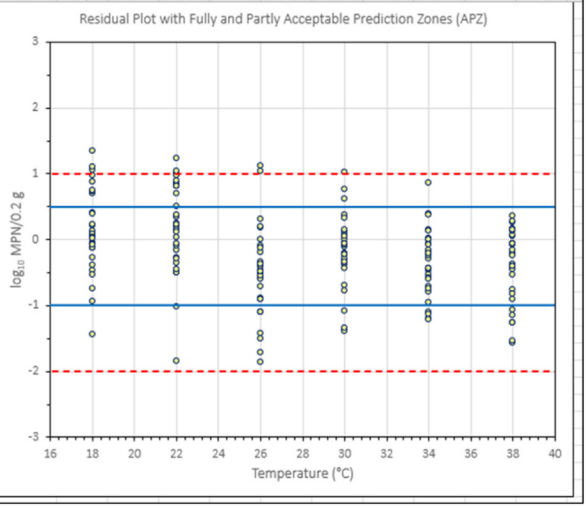


**Figure 5.** Screenshot of the Validation Software Tool for Acceptable Prediction Zones analysis of the tertiary model prediction of the independent data for interpolation for growth of *Salmonella* Infantis in ground turkey. pAPZ is the proportion of residuals in the partly and fully acceptable prediction zones.

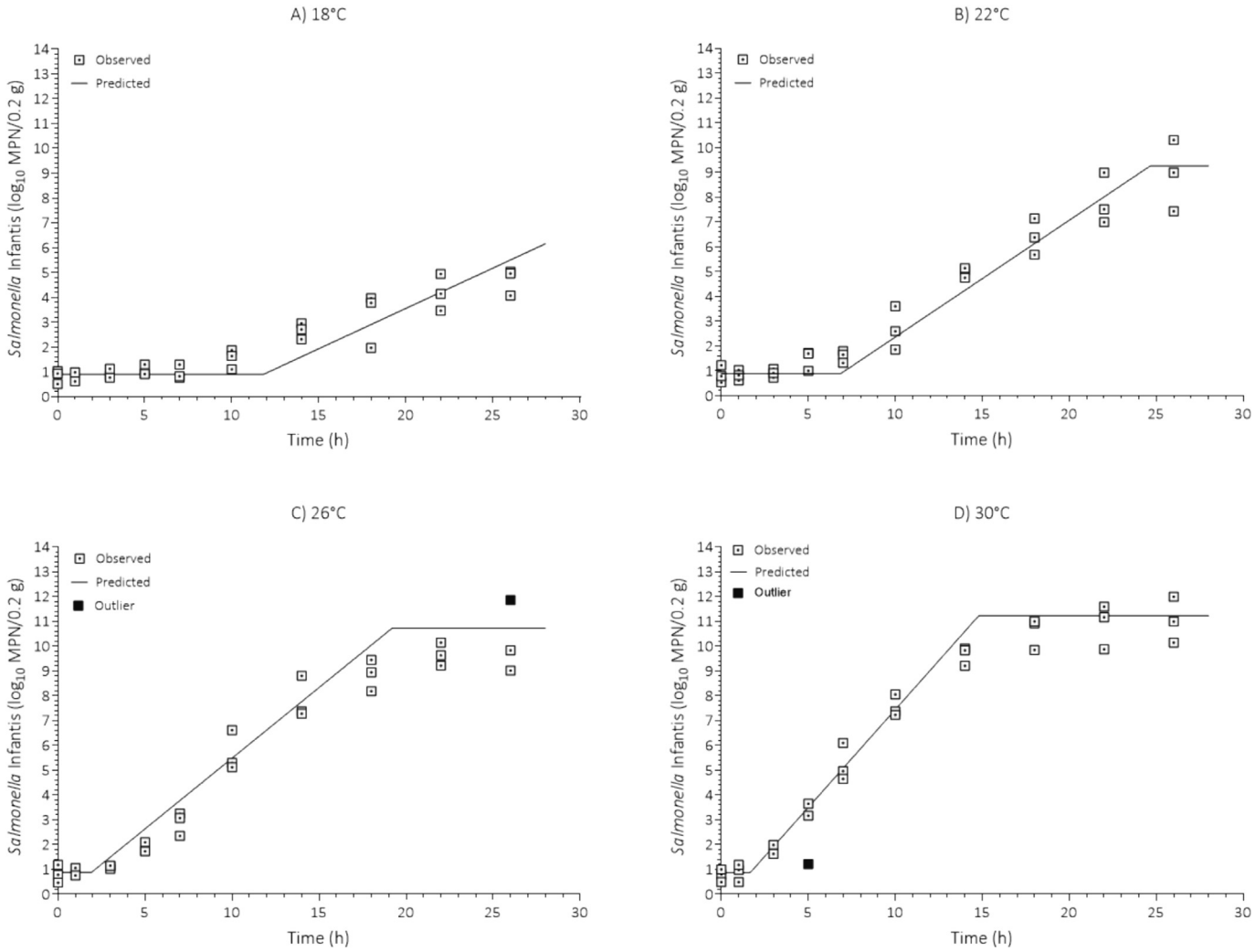


**Figure 6.** Tertiary model predictions of the independent data for growth of *Salmonella* Infantis in ground turkey at (A) 18 °C; (B) 22 °C; (C) 26 °C; and (D) 30 °C.

narios encountered during the preparation of ground turkey for consumption. Thus, it fills an important data and modeling gap in risk assessments for *Salmonella* and ground turkey (Oscar, 2020a), namely, the acquisition of serotype-specific data and the development of a serotype-specific model for an important unit operation (i.e. meal preparation) and pathogen event (i.e. growth) in the risk pathway.

In a recent study, Noviyanti et al. (2024) developed growth models for *Salmonella* spp. in chicken juice, sliced chicken, ground chicken, and chicken patty with native microflora. The dependent data were collected from 0 to 39 h at 3-h intervals at 10, 15, 20, and 25 °C. Three replicate storage trials were conducted per temperature. The dependent data were fitted to the Baranyi primary model. A secondary model for growth rate was developed and used in a tertiary model to predict the log count data from six scenarios. The proportion of residuals in the fully Acceptable Prediction Zone (pAPZ) was used to compare observed and predicted log counts at the end of the six scenarios. A pAPZ of 1.00 was obtained, and it was concluded that the model provided valid predictions of *Salmonella* growth. However, the models for growth of *Salmonella* in chicken products developed by Noviyanti et al. (2024) were not fully developed and validated per the test data, model performance, and model validation criteria of the Acceptable Prediction Zones method in the Validation Software Tool (Oscar, 2020b). Nonetheless, they can be repaired by the collection of additional data, development of secondary models for all primary model parameters, and development and validation of the tertiary models for interpolation.

Haque et al. (2024) developed a model for the growth of *Salmonella* in raw ground pork with native microflora. They collected dependent data ($n = 3$) at 10, 15, 20, 25, 30, and 40 °C. They used the Acceptable Prediction Zones method in the Validation Software Tool to evaluate the primary model for dependent data, the secondary model for growth rate for dependent data, and the tertiary model for extrapolation to a new independent variable (i.e., changing temperature). The overall pAPZ of the primary model for the dependent data ($n = 660$) was 0.96, which was acceptable, and there were no local prediction problems. However, the criteria for test data were not used and were not completely met because of insufficient replication at all temperatures and no data at 35 °C.

Haque et al. (2024) did not use the proper version of the Acceptable Prediction Zones method in the Validation Software Tool to evaluate performance of the secondary model for growth rate (Oscar, 2023). They report the wrong acceptable prediction zone boundaries and units. In addition, they did not evaluate the dependent data for growth rate using the criteria for test data and like the dependent data used in the evaluation of the primary model they were insufficient replication at all temperatures and no data at 35 °C.

Finally, Haque et al. (2024) reported an overall pAPZ of 0.92, which was acceptable, and no local prediction problems for the tertiary model prediction of independent data ($n = 63$) for extrapolation to a new independent variable (i.e., changing temperature). However, the model was not eligible for this evaluation because it was not validated for interpolation. In fact, no independent data for interpolation were collected.

Regardless, the model of Haque et al. (2024) can be repaired by collecting additional dependent data to fill the gaps needed to satisfy the test data criteria for dependent data and by collecting data at intermediate values of time and temperatures used to collect the dependent data so that the model can be evaluated and validated for interpolation. Also, a secondary model for maximum population density would need to be developed and validated.

Mishra et al. (2017) used the Acceptable Prediction Zone method to evaluate the performance of a growth model for *Salmonella enterica* in leafy greens. Data for model development were obtained from eight published studies involving six different leafy green products. The total number of growth curves was 35, and the temperature range was 7–37 °C. The number of replicated growth curves per study was uneven and was 1 ($n = 4$), 2 ($n = 2$), or 3 ($n = 2$). The dependent data did not satisfy the test data criteria of the Acceptable Prediction Zones method.

Mishra et al. (2017) fitted the log count data from the 35 growth curves to the three-phase linear primary model like was done in the present study. They developed a secondary model for growth rate as a function of temperature. The coefficient of determination was 0.41, which indicated that an important independent variable was missing from the model.

Mishra et al. (2017) did not validate the secondary model for growth rate for dependent data or for independent data for interpolation. Also, they did not develop secondary models for the other primary model parameters. This was because some of the growth curves analyzed had too few log count data to properly estimate them.

Mishra et al. (2017) incorporated the secondary model for growth rate into the primary model to create a tertiary model for predicting the growth of *Salmonella enterica* in leafy greens under changing temperature conditions in the supply chain. They obtained three sets of test data from the scientific literature. The observed log counts were ten in scenarios 1 and 2 and eight in scenario 3. The time courses were 70 h in scenarios 1 and 2 and 5 h in scenario 3. The span of log count values was 0.4 in scenario 1, 1.2 in scenario 2, and 0.5 in scenario 3. They used the model performance criteria in the Acceptable Prediction Zones method and reported a pAPZ 1.00. However, the tertiary model of Mishra et al. (2017) did not meet the test data and model validation criteria for extrapolation in the Acceptable Prediction Zones method. Moreover, the model cannot be repaired because data do not exist in the scientific literature to fill the data gaps in the model. Also, the span of log count values was not sufficient to provide a rigorous test of model performance.

These (Haque et al., 2024; Mishra et al., 2017; Noviyanti et al., 2024) and other studies (Jayeola et al., 2019; Li et al., 2011; Luo et al., 2015; Min & Yoon, 2010) show that the Acceptable Prediction Zones method is being used in predictive modeling studies but that it is not being used to its full potential. Note that, the test data, local model performance, and model validation criteria are not being used. These unused criteria are important because they ensure that the model validation process is complete, accurate, unbiased, objective, rigorous, and objective. The quality of predictive models could be improved by fully utilizing the test data, model performance, and model validation criteria of the Acceptable Prediction Zones method in the Validation Software Tool (Oscar, 2020b, 2023).

The proper validation of predictive models is important because it provides users with confidence that model predictions are reliable, and it allows modelers to identify and repair problems leading to the development and distribution of better models. In the present study, the full potential of the criteria of the Acceptable Prediction Zones method was realized when they were used to successfully identify minor but important local prediction problems in the tertiary model that can be repaired by additional data collection and result in a better and more reliable model for end-users.

The final objective of the current study was to compare the tertiary model developed in this study (Fig. 3) with the THERM model developed by Ingham et al. (2007) for the growth of *Salmonella* in ground turkey. This was done using the time for a 3-$\log_{10}$ increase ($t_{1000}$) in *Salmonella* number as a function of temperature (Fig. 7). The $t_{1000}$ was calculated using secondary model predictions of lag time and tertiary model calculations of growth rates (Fig. 3) from the current study (PFARM) and reported lag times and growth rates for *Salmonella* in the THERM study (Table 2). Thus, the PFARM and THERM model comparisons were equivalent to a two-phase linear tertiary model.

The regression line was forced to go through the axis-origin ($X = 0$, $Y = 0$). The hypothesis tested was that the model predictions of the time for a 3-$\log_{10}$ increase of *Salmonella* number were the same. If true, the slope of the line should not be significantly different from one or the line of unity where there is perfect agreement between predictions

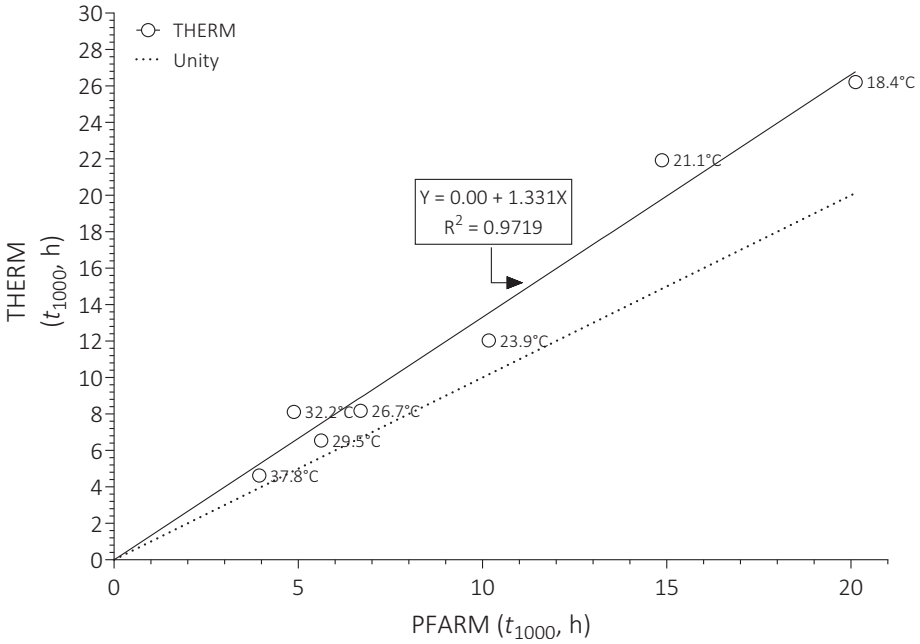


**Figure 7.** Comparison of the predictions of the time for a 3-$\log_{10}$ increase in growth of Salmonella in ground turkey from the THERM study of Ingham et al. (2007) and the current study (PFARM). $R^2$ is the coefficient of determination. Unity represents perfect agreement between THERM and PFARM predictions.

**Table 2**
Comparison of the THERM and PFARM tertiary model predictions for growth of *Salmonella* in ground turkey[a]

| | THERM | | | PFARM | | |
|---|---|---|---|---|---|---|
| Temperature (°C) | Lag time (h) | Growth rate ({$\log_{10}$ CFU/g}/h) | Time to a 3-$\log_{10}$ increase (h) | Lag time (h) | Growth rate ({$\log_{10}$ MPN/0.2 g}/h) | Time to a 3-$\log_{10}$ increase (h) |
| 18.4 | 8.35 | 0.168 | 26.21 | 11.31 | 0.340 | 20.13 |
| 21.1 | 4.68 | 0.174 | 21.92 | 7.97 | 0.435 | 14.87 |
| 23.9 | 3.10 | 0.336 | 12.03 | 4.51 | 0.530 | 10.17 |
| 26.7 | 3.45 | 0.636 | 8.17 | 1.68 | 0.599 | 6.69 |
| 29.5 | 2.27 | 0.702 | 6.54 | 1.66 | 0.758 | 5.62 |
| 32.2 | 2.23 | 0.510 | 8.12 | 1.64 | 0.927 | 4.88 |
| 37.8 | 1.53 | 0.972 | 4.62 | 1.60 | 1.284 | 3.94 |

[a] THERM = Ingham et al. (2007); PFARM = present study; CFU = colony forming unit; MPN = most probable number.

made by PFARM and THERM. However, the observed slope was 1.331, which differed ($P$ = 0.0005) from one. Thus, the hypothesis was rejected, and it was concluded that the THERM model (Ingham et al., 2007) predicted slower growth of *Salmonella* in ground turkey than the PFARM model (Fig. 3).

Differences in data collection and modeling methods could explain the difference in results. Ingham et al. (2007) used a mixture of *Salmonella* serotypes Typhimurium, Heidelberg, Infantis, Hadar, and Enteritidis to model growth in ground turkey, whereas a single isolate of *Salmonella* Infantis was used to model growth in the current study. The inoculum size used by Ingham et al. (2007) was 3.9 $\log_{10}$ colony-forming units per 0.2 g of ground turkey, whereas it was 0.85 $\log_{10}$ most probable number per 0.2 g of ground turkey in the present study. The laboratory ground turkey used by Ingham et al. (2007) contained less fat and salt than commercial ground turkey, which was used in this study. The growth data in the study of Ingham et al. (2007) were fitted to the Baranyi primary model to obtain values for lag time and growth rate as a function of temperature, whereas, in the current study, they were fitted to the three-phase linear primary model, which has been shown to yield different lag time and growth rate values than the Baranyi model (Buchanan et al., 1997). Clearly, additional research is needed to better understand how these differences affect the modeling of *Salmonella* growth in ground turkey.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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