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Development and validation of a tertiary simulation model for predicting the potential growth of *Salmonella typhimurium* on cooked chicken☆

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Abstract

The growth of *Salmonella typhimurium* (ATCC 14028) on the surface of autoclaved ground chicken breast and thigh burgers incubated at constant temperatures from 8 to 48 °C in 2 °C increments was investigated and modeled. Growth curves at each temperature were fit to a two-phase linear primary model to determine lag time (λ) and specific growth rate (μ). Growth of *S. typhimurium* on breast and thigh meat was not different. Consequently, secondary models that predicted lag time and specific growth rate as a function of temperature were developed with the combined data for breast and thigh meat. Five secondary models for lag time and three secondary models for specific growth rate were compared. A new version of the hyperbola model and a cardinal temperature model were selected as the best secondary models for lag time and specific growth rate, respectively. The secondary models were combined in a computer spreadsheet to create a tertiary simulation model that predicted the potential growth ($\log_{10}$ increase) of *S. typhimurium* on cooked chicken as a function of time and temperature. Probability distributions and simulation were used in the tertiary model to model the secondary model parameters and the times and temperatures of abuse. The outputs of the tertiary model were validated (prediction bias of -4% for λ and 1% for μ and prediction accuracy of 10% for λ and 8% for μ) and integrated with a previously developed risk assessment model for *Salmonella*. Published by Elsevier Science B.V.

Keywords: Cooked chicken; *Salmonella typhimurium*; Predictive model; Growth; Simulation; Validation

1. Introduction

Mathematical models that predict the growth of *Salmonella* as a function of food and environmental factors have been developed (Smith, 1985; Thayer et al., 1987; Gibson et al., 1988; Oscar, 1999c) and promoted as a means of assessing the microbiological safety of food (Buchanan, 1993; Skinner et al., 1994; McClure et al., 1994; Schaffner and Labuza, 1997; Whiting and Buchanan, 1997b; Soboleva et al., 2000). However, growth models are limited in the ability to predict food safety because they do not consider whether or not the pathogen is present. In addition, they do not consider other pathogen events, such as contamination, physical removal, nonthermal inactivation, thermal inactivation and dose–response, which

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determine the exposure and response of consumers to pathogens of food origin. Consequently, growth models only predict the potential growth of the pathogen and not the absolute growth of the pathogen on or in food that has been improperly handled.

One way of improving the effectiveness of predictive models as food safety tools is to integrate them with risk assessment models that predict the absolute change in the pathogen load of a food as it moves from the farm to the table. The most common approach for integrating predictive models with risk assessment models is to place them inside the framework of the risk assessment model (Whiting and Buchanan, 1997a; Cassin et al., 1998; McNab, 1998). A potential problem with this approach is that it creates complex models that are difficult to use. Reducing the complexity of risk assessment models is a major hurdle for their routine application in the food industry.

As an alternative, predictive models can be used outside the framework of the risk assessment models in an attempt to keep the risk assessment models simple and easy to use (Oscar, 1998b). The USDA, ARS, Poultry Food Assess Risk Model or Poultry FARM (www.arserrc.gov/mfs/) is a computer software application that contains four predictive models for growth of *Salmonella* and risk assessment models for *Salmonella* and *Campylobacter* (Oscar, 1999d). Poultry FARM provides chicken processors with a computer-modeling tool that they can use to assess the microbiological safety of chicken destined for specific distribution channels and consumer populations (Oscar, 1999d).

A limitation of the growth models in Poultry FARM is that their output cannot easily serve as input in the risk assessment models. In addition, the growth models in Poultry FARM (Oscar, 1999a,b,c) do not predict growth on dark chicken meat or growth at temperatures below 10 °C or above 40 °C. Therefore, the current study was undertaken to investigate and model the growth kinetics of *Salmonella typhimurium* ATCC 14028 on sterile ground chicken breast and thigh burgers incubated at constant temperatures from 8 to 48 °C.

S. typhimurium ATCC 14028 has been used extensively in our laboratory for investigating and modeling the growth of *Salmonella* in laboratory medium (Oscar, 1998a, 1999a) and on sterile ground chicken breast burgers (Oscar, 1999b,c, 2000). In general, it exhibits the same growth kinetics as other strains of *Salmonella* that are commonly found on chicken in the United States (Oscar, 1998a, 2000) and thus, it is a good strain to use for developing predictive growth models for *Salmonella* and chicken.

During the secondary modeling stage of model development, the data for breast and thigh meat were analyzed separately and then ultimately combined because growth of *S. typhimurium* was not different on the two types of meat. However, during the secondary modeling step, which model best-described lag time and specific growth rate, as a function of temperature became an issue. Thus, an objective of the current study was to compare secondary models for the ability to predict lag time and specific growth rate as a function of temperature.

In the final step of model development, the best-fitting secondary models for lag time and specific growth rate were combined in a computer spreadsheet to create a tertiary model that predicted the potential growth (i.e., $\log_{10}$ increase) of *Salmonella* as a function of time and temperature. An objective of the tertiary modeling step was to create a predictive model whose output could serve as input in the previously developed risk assessment model for *Salmonella* in Poultry FARM.

2. Materials and methods

2.1. Organism

Kinetic data for development of the model were collected using a single strain of *S. typhimurium* (ATCC 14028, American Type Culture Collection, Rockville, MD, USA). Stationary phase cells of the organism (10^9 CFU/ml) were maintained at −70 °C in brain heart infusion broth (Difco Laboratories, Detroit, MI, USA) that contained 15% (v/v) glycerol.

2.2. Experimental design

Autoclaved (121 °C for 18 min) ground chicken breast and thigh burgers were inoculated on their surface with 10^6 *S. typhimurium* in a 1.2-cm^2 inoculation well (Oscar, 1999c) and then incubated at constant temperatures from 8 to 48 °C in 2 °C increments for a total of 42 growth curves, 21 with breast meat and 21

with thigh meat. Surface growth was measured rather than growth throughout the burger because most bacteria are located on the surface of intact chicken (Cunningham, 1982). Ground chicken burgers were used rather than intact chicken pieces to create a more homogeneous model system and thus, reduce experimental error.

2.3. Microbiological methods

Details of the microbiological methods have been published (Oscar, 1999a) and therefore, only a general description will be provided here. *S. typhimurium* for inoculation were grown in brain heart infusion broth (pH 6.4) under aerobic conditions at 37 °C for 23 h. After dilution in sterile buffered peptone water (Difco), 100 μl was inoculated onto the surface of the burgers for an initial concentration of 10^6 *S. typhimurium* per 1.2 cm^2. A high initial concentration was used to facilitate viable cell counting (Oscar, 1999c).

Burgers were inoculated at 4 °C and then were incubated at constant temperatures from 8 to 48 °C in 2 °C increments. Each growth curve involved eight burgers or sampling times. At each sampling time, an inoculated burger (6 g) was homogenized (model 400 stomacher blender, Seward, London, UK) in 94 ml of sterile buffered peptone water. After centrifugation, undiluted and diluted (10^{-1} to 10^{-3}) samples were spiral plated (WASP spiral plater, Don Whitley Scientific, West Yorkshire, UK) onto brain heart infusion agar (Difco). Spiral plates were inverted and incubated at 30 °C for 24 h before counting of colonies using an automated colony counter (Protocol, Microbiology International, Frederick, MD, USA).

2.4. Primary modeling

Viable cell counts in the homogenate (N; $\log_{10}$ CFU/ml) were graphed as a function of sampling time (t; h). Lag time (λ; h) and specific growth rate (μ; $\log_{10}$ CFU/h) were determined by nonlinear regression (version 3.0, Prism®, GraphPad Software, San Diego, CA, USA) using a two-phase linear model (Einarsson, 1994; Buchanan et al., 1997):

$$\begin{aligned} N &= N_0 && \text{if } t \le \lambda \\ N &= N_0 + \mu(t-\lambda) && \text{if } t > \lambda, \end{aligned} \tag{1}$$

where N_0 was the initial viable cell count in the homogenate ($\log_{10}$ CFU/ml). In three cases, the curve-fit predicted a lag time that was much shorter than observed. To obtain a curve-fit that was in agreement with the observed data, lag time was fixed at the last sampling time where the growth curve was observed to be in lag phase.

2.5. Secondary modeling

2.5.1. Lag time models

The following secondary models were evaluated for predicting lag time as a function of temperature (T; °C).

$$\lambda = \exp[p/(T-q)] \tag{2}$$

$$\lambda = [p/(T-q)]^2 \tag{3}$$

$$\lambda = [p/(T-q)]^m \tag{4}$$

$$\lambda = A + (B/T) + (C/T^2) \tag{5}$$

$$\lambda = 1/[b(T-T_{\min})^2] \tag{6}$$

Models (2)–(4) are different forms of the hyperbola model (Zwietering et al., 1991, 1994; Duh and Schaffner, 1993) where p (h) is the rate of change of lag time as a function of temperature, q is the temperature at which lag time is infinite and m is an exponent. Model (5) is the nonlinear Arrhenius model of Davey (Davey, 1989, 1991; Daughtry et al., 1997) where A, B and C are regression coefficients without biological meaning. Model (6) is the inverse square root model of Ratkowsky (Adair et al., 1989; Duh and Schaffner, 1993; Wijtzes et al., 1995) where b is the rate of change of lag time as a function of temperature and $T_{\min}$ is the minimum temperature for growth. Transformations (logarithm and square root) to stabilize model variance (Gibson et al., 1988) were applied to the model rather than the data to facilitate curve-fitting operations.

2.5.2. Specific growth rate models

The following secondary models were evaluated for predicting the specific growth rate as a function of temperature (T; °C).

$$\mu = (b(T - T_{\min})\{1 - \exp[c(T - T_{\max})]\})^2 \quad (7)$$

$$\mu = [b(T - T_{\min})]^2\{1 - \exp[c(T - T_{\max})]\} \quad (8)$$

$$\begin{aligned} \mu &= 0 && \text{if } T \leq T_{\min} \text{ or } \geq T_{\max} \\ \mu &= \mu_{\text{opt}}(D/E) && \text{if } T_{\min} < T < T_{\max} \end{aligned} \quad (9)$$

$$D = (T - T_{\max})(T - T_{\min})^2$$

$$E = (T_{\text{opt}} - T_{\min})[(T_{\text{opt}} - T_{\min})(T - T_{\text{opt}}) - (T_{\text{opt}} - T_{\max})(T_{\text{opt}} + T_{\min} - 2T)]$$

Eqs. (7) and (8) are variations of the square root model of Ratkowsky and are referred to in the scientific literature as the Ratkowsky 2 and 3 models, respectively (Ratkowsky et al., 1983; Zwietering et al., 1991, 1994), where b is the rate of change of the specific growth rate between $T_{\min}$ and T_{opt}, c is the rate of change of the specific growth rate between T_{opt} and $T_{\max}$, $T_{\min}$ is the minimum growth temperature, T_{opt} is the optimum growth temperature, and $T_{\max}$ is the maximum growth temperature. Eq. (9) is the cardinal temperature model with inflection (Rosso et al., 1993, 1995) where $T_{\min}$, T_{opt}, and $T_{\max}$ are the cardinal temperatures and μ_{opt} is the specific growth rate at T_{opt} or the optimum specific growth rate.

2.5.3. Evaluation of the secondary models

The secondary models were fit to the combined data for breast and thigh meat using Prism®. The goodness-of-fit of the data to each model was evaluated using the coefficient of determination (R^2) and the standard deviation of the residuals (S_{yx}):

$$S_{yx} = \sqrt{\Sigma(Y_o - Y_p)^2/(n - s)},$$

where Y_o was the observed lag time or specific growth rate, Y_p was the predicted lag time or specific growth rate, n was the number of lag time or specific growth rate determinations ($n = 42$) and s was the number of fitted parameters ($s = 2$, 3 or 4) in the model.

The bias of the model predictions was evaluated by calculating the relative error (RE) of each prediction case (Delignette-Muller et al., 1995; Rosso et al., 1995):

$$\text{RE} = [(Y_o - Y_p)/Y_p]100.$$

The median relative error (MRE) was used to quantify the prediction bias of the model, whereas the mean absolute relative error (MARE) was used to quantify the prediction accuracy of the model. The prediction bias and accuracy of the model predictions were also quantified by calculating the bias factor (BF) and accuracy factor (AF) of Ross (1996):

$$\text{BF} = 10^{(\Sigma \log(Y_p/Y_o)/n)},$$

$$\text{AF} = 10^{(\Sigma |\log(Y_p/Y_o)|/n)}.$$

In addition, prediction bias was evaluated by calculating the number of runs (Prism®), where a run was a set of consecutive residuals that were either above or below zero on the residual plot. The higher the number of runs, the more random the distribution of residuals was around zero and the more desirable was the model.

The confidence intervals for the best-fit values (BFV) of the model parameters were evaluated by calculating the absolute relative standard error (ARSE):

$$\text{ARSE} = |(\text{SE}/\text{BFV})|100,$$

where SE was the standard error of the best-fit value as provided by Prism®. Model parameters with ARSE exceeding 20% were considered to have wide confidence intervals. Based on R^2, S_{yx}, MRE, MARE, BF, AF, runs and ARSE, one lag time and one specific growth rate model were selected for the development of the tertiary simulation model.

2.6. Tertiary modeling

The lag time and specific growth rate models were incorporated into a computer spreadsheet (Excel 2000, Microsoft, Redmond, WA, USA) to create a tertiary simulation model that predicted the potential growth

($\log_{10}$ increase) of *S. typhimurium* on cooked chicken as a function of time and temperature. Pert distributions, which were defined by minimum, most likely and maximum values, were used to model the parameters of the lag time and specific growth rate models and the times and temperatures of abuse. The model was simulated using @Risk (version 4.0, Palisade, Newfield, NY), a spreadsheet add-in program.

3. Results

3.1. Evaluation of the primary modeling step

Comparison of the best-fit values for lag time and specific growth rate indicated that growth was similar on breast and thigh meat at all the temperatures investigated (Table 1). The confidence intervals of the best-fit values for lag time and specific growth rate, as assessed by the absolute relative standard error (ARSE), were low (< 20%) in most cases. Only at the extremes of the growth temperature range were high (> 20%) ARSE values observed. The mean ARSE for lag time (15%) was two-fold higher than the mean ARSE for specific growth rate (5%). Overall, the ARSE values for the lag time and specific growth rate parameters were acceptable and similar for breast and thigh meat. The high R^2 values (mean > 0.97) and low S_{yx} values (mean < 0.14) indicated that the two-phase linear model fit the data well (Fig. 1). As expected from the ARSE results, the lowest R^2 and highest S_{yx} were seen at the extremes of the growth temperature range (Table 1). Overall, the quality of the kinetic

Table 1
Best-fit values and statistical summary of the primary modeling step[a]

Temperature (°C)	Lag time				Specific growth rate				Goodness-of-fit			
	Best-fit (h)		ARSE (%)		Best-fit ($\log_{10}$/h)		ARSE (%)		R^2		S_{yx}	
	Breast	Thigh	Breast	Thigh	Breast	Thigh	Breast	Thigh	Breast	Thigh	Breast	Thigh
8	43.8	46.8	33	18	0.005	0.012	20	8	0.930	0.980	0.064	0.105
10	19.6	21.6	15	Fixed[b]	0.025	0.025	4	5	0.991	0.966	0.099	0.245
12	14.9	10.3	15	32	0.045	0.048	4	6	0.989	0.974	0.129	0.209
14	11.3	9.1	12	13	0.079	0.078	4	3	0.987	0.991	0.152	0.131
16	6.5	5.7	15	14	0.103	0.107	4	3	0.989	0.992	0.131	0.113
18	5.3	4.5	3	22	0.145	0.155	3	5	0.992	0.985	0.109	0.169
20	3.7	3.9	15	10	0.192	0.186	5	3	0.985	0.995	0.136	0.081
22	3.8	3.2	6	8	0.247	0.269	3	3	0.995	0.994	0.073	0.098
24	3.3	2.8	12	16	0.343	0.311	7	6	0.984	0.983	0.148	0.161
26	2.5	2.4	5	8	0.365	0.382	3	3	0.997	0.995	0.056	0.087
28	2.2	2.2	5	14	0.438	0.411	2	6	0.997	0.983	0.055	0.149
30	2.1	2.3	8	5	0.527	0.540	5	3	0.986	0.996	0.114	0.073
32	1.6	1.5	6	7	0.603	0.554	3	2	0.995	0.996	0.077	0.082
34	1.4	1.6	7	10	0.607	0.664	3	4	0.995	0.992	0.071	0.120
36	1.4	1.4	5	8	0.613	0.679	2	3	0.997	0.992	0.058	0.111
38	1.3	1.4	7	7	0.699	0.776	3	3	0.995	0.996	0.080	0.088
40	1.3	1.2	9	10	0.751	0.757	4	3	0.993	0.991	0.111	0.131
42	1.0	1.5	25	8	0.609	0.784	7	5	0.977	0.990	0.186	0.126
44	1.1	1.1	17	Fixed	0.648	0.655	6	4	0.988	0.982	0.129	0.184
46	0.9	1.0	15	Fixed	0.514	0.490	4	6	0.992	0.954	0.084	0.283
48	1.6	1.0	74	62	0.260	0.249	22	10	0.795	0.972	0.389	0.151
Mean	6.22	6.03	14.7	15.2	0.372	0.387	5.5	4.6	0.978	0.986	0.117	0.138
SEM	2.17	2.29	3.3	3.2	0.054	0.058	1.1	0.4	0.010	0.002	0.016	0.012

[a] ARSE = absolute relative standard error; R^2 = coefficient of determination; S_{yx} = standard error of the residuals; and SEM = standard error of the mean.

[b] Lag time was fixed during primary modeling and thus, it was not possible to calculate ARSE.

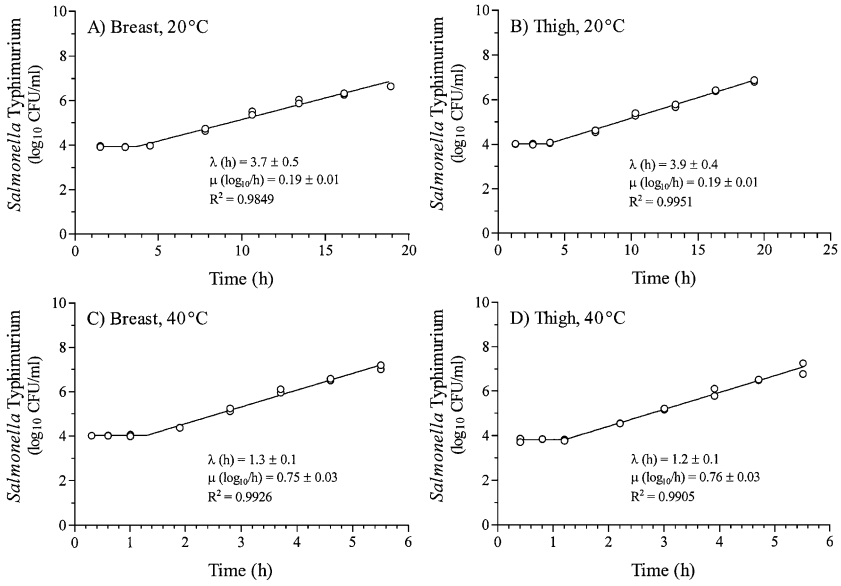

Fig. 1. Examples of primary model fits to kinetic data collected on cooked chicken breast (A and C) and thigh (B and D) meat burgers. Lag time (λ) and specific growth rate (μ) values are the best-fit values ± their standard errors from the primary model fit, whereas R^2 is the coefficient of determination of the primary model fit.

data was high and not different for breast and thigh meat.

3.2. Comparison of the secondary models

3.2.1. Evaluation of the lag time models

Five models (models (2)–(6)) were evaluated for the ability to predict lag time as a function of temperature (Table 2). The coefficients of determination, R^2, were high (>0.98) for all models indicating that they all fit the data well (Fig. 2A to E). However, the hyperbola (^m) model had the highest R^2 or best fit to the data (Table 2). Greater differences were noted among the models for S_{yx}, the goodness-of-fit criterion that adjusted for differences in the number of fitted parameters. Nonetheless, the best fit was once again obtained with the hyperbola (^m) model, which had the lowest S_{yx} (Table 2).

Table 2
Statistical summary of the secondary modeling step for lag time (models (2)–(6)) and specific growth rate (models (7)–(9))[a]

Model	Goodness-of-fit		Runs test			Prediction bias		Prediction accuracy	
	R^2	S_{yx}	Runs	Above	Below	MRE	BF	MARE	AF
(2) Hyperbola (exp)	0.9896	1.042	11	8	34	− 20.4	1.29	23.9	1.34
(3) Hyperbola (^2)	0.9903	1.009	10	36	6	30.1	0.75	40.6	1.36
(4) Hyperbola (^m)	0.9937	0.825	20	18	24	− 3.9	1.00	10.1	1.10
(5) Davey (exp)	0.9927	0.884	19	13	29	− 5.6	1.07	13.1	1.15
(6) Inverse Ratkowsky	0.9903	1.009	10	36	6	30.1	0.75	40.5	1.36
(7) Ratkowsky 2	0.9868	0.0305	15	18	24	− 1.7	1.04	7.0	1.08
(8) Ratkowsky 3	0.9877	0.0294	21	22	20	1.0	1.01	5.7	1.06
(9) Cardinal temperature	0.9877	0.0294	19	23	19	0.9	0.97	8.6	1.08

[a] R^2 = coefficient of determination; S_{yx} = standard error of the residuals; MRE = median relative error; BF = bias factor; MARE = mean absolute relative error; and AF = accuracy factor.

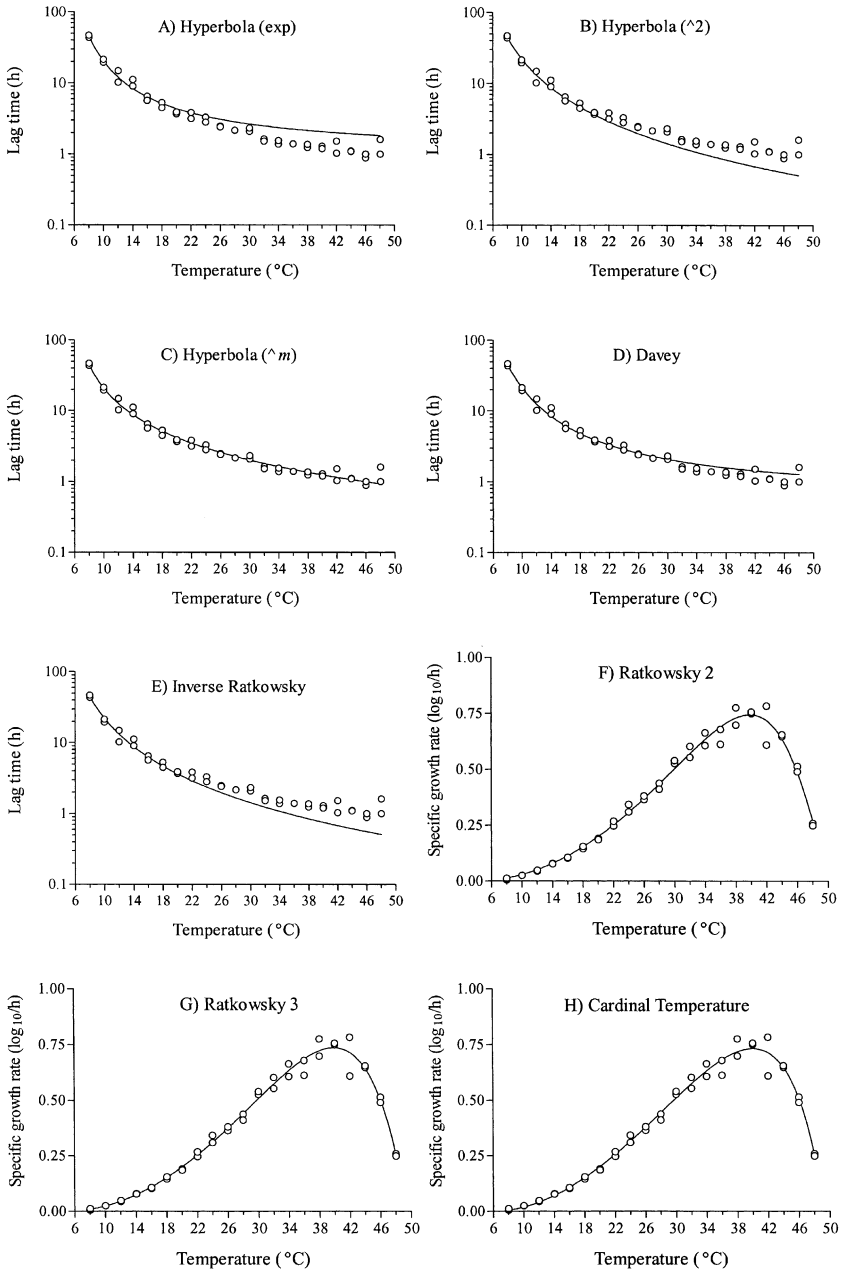

Fig. 2. Secondary model fits of the lag time (A to E) and specific growth rate (F to H) data as a function of temperature.

The runs test, which quantifies the distribution of the residuals around zero, was one of the three statistics used to evaluate the bias of the model predictions. The model with the most number of runs and thus, the most random distribution of its residuals around zero, was the hyperbola (^*m*) model (Table 2 and Fig. 3C). Two other indices of prediction bias were the median relative error of the predictions (MRE) and the bias factor (BF). Again, the model with the MRE closest to zero, no bias, and

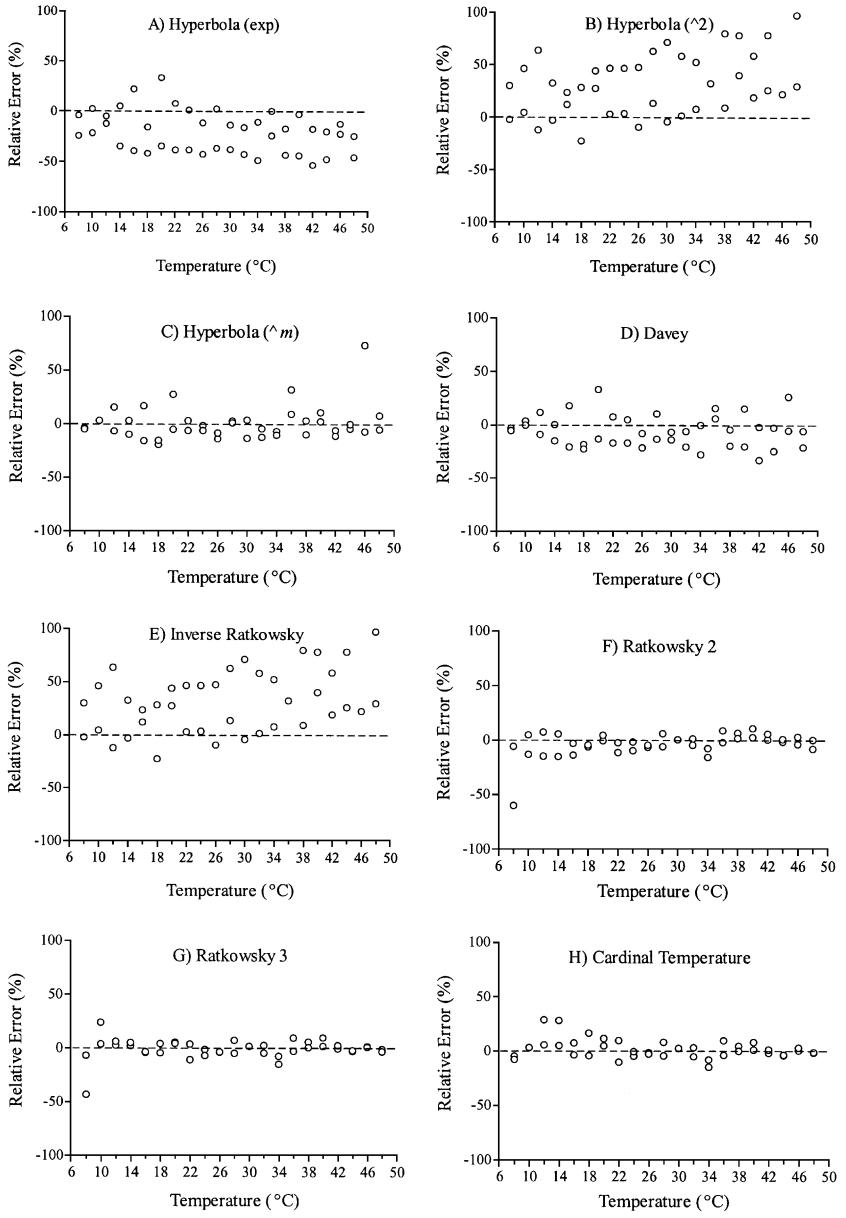

Fig. 3. Scatter plots of the relative errors of prediction of the secondary models for lag time (A to E) and specific growth rate (F to H) as a function of temperature. Positive values on the lag time plots are fail-safe, whereas positive values on the specific growth rate plots are fail-dangerous.

the BF closest to one, no bias, was the hyperbola (^*m*) (Table 2).

The hyperbola (^*m*) represents a new version of this model. Earlier versions of the hyperbola model produced predictions with considerable systematic bias (Fig. 3A and B). In contrast, the hyperbola (^*m*) (Fig. 3C) model showed little systematic prediction bias. The Davey model showed some systematic prediction bias at optimal to high temperatures (Fig. 3D), whereas the inverse Ratkowsky exhibited

considerable systematic bias throughout the entire temperature range for growth (Fig. 3E).

Accuracy of the model predictions was assessed using the mean absolute relative error (MARE) of the predictions and the accuracy factor (AF). Again, the most accurate model or the model with the lowest MARE and the AF closest to one was the hyperbola (^*m*) (Table 2).

The final criterion used to evaluate the secondary models for lag time was the ARSE value, a measure of the width of the confidence intervals for the best-fit values of the model parameters. The only models with high ARSE values (>20%) were the hyperbola (exp) and Davey models (Table 3). The hyperbola (^*m*) model had low ARSE (< 10%) for all its parameters. Thus, the best secondary model for lag time was the hyperbola (^*m*), which had the best goodness-of-fit (R^2 and S_{yx}), the lowest prediction bias (runs, MRE and BF), the highest prediction accuracy (MARE and AF) and the tightest confidence intervals for its fitted parameters.

3.2.2. Evaluation of the specific growth rate models

Three models (models (7)–(9)) were evaluated for their ability to predict specific growth rate as a function of temperature. All three models fit the data well (Fig. 2F to H) as indicated by high (> 0.985) and similar R^2 values and as indicated by low (< 0.31) and similar S_{yx} values (Table 2).

All three models for specific growth rate showed very little (< 5%) prediction bias (Table 2). The model with the highest number of runs (Table 2) or most random distribution of its residuals (Fig. 3F to H) was the Ratkowsky 3. The cardinal temperature model had the MRE closest to zero, whereas the Ratkowsky 3 had the closest BF value to one. Thus, the Ratkowsky 3 model showed the lowest prediction bias. In addition, the Ratkowsky 3 model had the lowest MARE and the closest AF value to one and thus, was the most accurate of the three models (Table 2).

Most of the best-fit values for the parameters of the secondary models for specific growth rate had

Table 3
Best-fit values and 95% confidence intervals for the parameters of the secondary models for lag time (models (2)–(6)) and specific growth rate (models (7)–(9))

Model	Parameter	Best-fit value	ARSE[a] (%)	95% Confidence interval
(2) Hyperbola (exp)	p	28.86	4	26.80 to 30.93
	q	0.4372	63	− 0.1232 to 0.9977
(3) Hyperbola (^2)	p	32.00	3	30.04 to 33.96
	q	3.217	5	2.893 to 3.541
(4) Hyperbola (^m)	p	40.67	7	34.95 to 46.39
	q	5.251	6	4.601 to 5.901
	m	1.415	7	1.228 to 1.602
(5) Davey (exp)	A	− 0.6199	28	− 0.9718 to − 0.2679
	B	42.64	9	34.77 to 50.51
	C	− 57.63	36	− 99.50 to − 15.77
(6) Inverse Ratkowsky	b	0.0009765	6	0.0008571 to 0.001096
	T_{min}	3.217	5	2.893 to 3.541
(7) Ratkowsky 2	b	0.02771	4	0.02520 to 0.03023
	T_{min}	3.797	24	1.917 to 5.676
	c	0.1769	10	0.1429 to 0.2110
	T_{max}	51.12	1	50.46 to 51.77
(8) Ratkowsky 3	b	0.02973	5	0.02644 to 0.03301
	T_{min}	4.703	20	2.820 to 6.587
	c	0.1172	13	0.08655 to 0.1478
	T_{max}	49.44	0	49.07 to 49.81
(9) Cardinal temperature	T_{max}	49.26	0	48.89 to 49.64
	T_{min}	5.699	14	4.090 to 7.308
	T_{opt}	40.01	1	39.51 to 40.51
	μ_{opt}	0.7320	1	0.7143 to 0.7498

[a] Absolute relative standard error.

tight confidence intervals or low ARSE values (Table 3). The one exception was the T_{min} parameter of the Ratkowsky 2 and 3 models, which had an ARSE value in excess of 20%. Overall, the ARSE values were best for the cardinal temperature model.

Although the Ratkowsky 3 model exhibited slightly less prediction bias and had slightly better prediction accuracy than the cardinal temperature model, the cardinal temperature model was selected as the best secondary model for specific growth rate because it had tighter confidence intervals for all its fitted parameters, which (as described below) was an advantage for the development of the tertiary model.

3.3. Tertiary modeling

The hyperbola (^*m*) model for lag time and the cardinal temperature model for specific growth rate were combined in a computer spreadsheet to create a tertiary simulation model that predicted the potential growth ($\log_{10}$ increase) of *S. typhimurium* on cooked chicken as a function of time and temperature (Fig. 4). Pert distributions were used to model the best-fit values and their 95% confidence intervals for the parameters of the secondary models (Table 3). This was done to account for the uncertainty associated with estimating these parameters. Consequently, having parameters with low ARSE was a desirable characteristic of the selected models because high ARSE would have increased the uncertainty of the tertiary model predictions. The uncertainty of the times and temperatures of abuse were also modeled using pert distributions.

3.3.1. Validation of the tertiary model predictions

To validate the predictions of the tertiary model, the temperature of abuse was held constant at values from 8 to 48 °C in 2 °C increments, the lag time and specific growth rate cells in the model were temporarily designated as output cells, and then the model was simulated for 10,000 iterations per temperature. The mean lag time and specific growth rate values predicted by the tertiary model were then compared to the 42 observed lag time and specific growth rate values by calculating prediction bias and accuracy values. Results of this analysis indicated that the prediction bias and accuracy of the tertiary model predictions for both lag time and specific growth rate were low (< 10%; Table 4). In addition, the 95% confidence intervals of the predictions (mean ± 2SD) for lag time and specific growth rate were tight, with coefficients of variation (SD/mean) less than 20%, at all temperatures, except for 8 °C, where the coefficient of variation was 46%. Thus, using pert distributions to model the uncertainty of the secondary model parameters rather than just using the best-fit values was a valid approach for predicting the potential growth of *S. typhimurium* on cooked chicken.

	A	B	C	D	E	F
1	**Abuse Scenario**	**Pert**	**Minimum**	**Median**	**Maximum**	**Formula in Cells of Column B**
2	Time (h)	3.8	0	2	6	=RiskPert(C2, D2, E2)
3	Temperature (8-48°C)	22.1	20	22	25	=RiskPert(C3, D3, E3)
4						
5	**Growth Parameters**					
6	Lag time (h)	3.10				=(B11/(B3-B12))^B13
7	Specific growth rate ($\log_{10}$/h)	0.285				=B17*(B18/B19)
8	Potential growth ($\log_{10}$)	0.101				=RiskOutput() + IF(B2<B6,0,B7*(B2-B6))
9						
10	**Model Parameters**	**Pert**	**Minimum**	**Median**	**Maximum**	**Pert**
11	*p*	39.14	34.95	40.67	46.39	=RiskPert(C11,D11,E11)
12	*q*	5.18	4.601	5.251	5.901	=RiskPert(C12,D12,E12)
13	*m*	1.37	1.228	1.415	1.602	=RiskPert(C13,D13,E13)
14	T_{max}	49.40	48.89	49.26	49.64	=RiskPert(C14,D14,E14)
15	T_{min}	4.56	4.090	5.699	7.308	=RiskPert(C15,D15,E15)
16	T_{opt}	39.62	39.51	40.01	40.51	=RiskPert(C16,D16,E16)
17	μ_{opt}	0.73	0.7143	0.7320	0.7498	=RiskPert(C17,D17,E17)
18	D	-8,413				=(B3-B14)*((B3-B15)^2)
19	E	-21,546				=(B16-B15)*((B16-B15)*(B3-B16)-(B16-B14)*(B16+B15-(2*B3)))

Fig. 4. Tertiary simulation model for predicting the potential growth ($\log_{10}$ increase) of *S. typhimurium* on cooked chicken as a function of time and temperature.

Table 4
Predicted lag time and specific growth rate values from 10,000 iterations of the tertiary simulation model[a]

Temperature (°C)	Lag time (h)		Specific growth rate (log_{10}/h)	
	Mean	SD	Mean	SD
8	46.79	11.67	0.0059	0.0027
10	21.24	4.00	0.0197	0.0046
12	12.84	2.04	0.0416	0.0061
14	8.87	1.24	0.0712	0.0073
16	6.61	0.84	0.1080	0.0082
18	5.19	0.60	0.1516	0.0088
20	4.22	0.46	0.2014	0.0092
22	3.52	0.36	0.2566	0.0094
24	3.00	0.29	0.3163	0.0096
26	2.60	0.24	0.3795	0.0096
28	2.28	0.20	0.4446	0.0096
30	2.02	0.17	0.5101	0.0094
32	1.81	0.15	0.5736	0.0091
34	1.64	0.13	0.6322	0.0086
36	1.49	0.12	0.6820	0.0078
38	1.36	0.11	0.7177	0.0071
40	1.25	0.10	0.7319	0.0067
42	1.16	0.09	0.7138	0.0074
44	1.07	0.08	0.6473	0.0093
46	1.00	0.08	0.5072	0.0125
48	0.93	0.07	0.2514	0.0205
MRE	−4.38		0.88	
BF	1.01		0.97	
MARE	10.07		8.42	
AF	1.10		1.08	

[a] SD = standard deviation; MRE = median relative error; BF = bias factor; MARE = mean absolute relative error; and AF = accuracy factor.

3.3.2. *Use of the tertiary model for providing risk assessment model inputs*

A single scenario (Fig. 4) was simulated to demonstrate how the tertiary model could be used to provide input settings for a previously developed risk assessment model. Simulation of the scenario (10,000 iterations) resulted in the output distribution shown in Fig. 5. This output distribution was filtered to remove the iterations where no growth occurred. The number of no growth events was 8226 (cell B36) for an incidence of potential growth events of 17.7%, the first input setting needed for the risk assessment model in Poultry FARM. The other three input settings needed for the risk assessment model in Poultry FARM were the minimum, median and maximum extent of potential growth, which were obtained from cells B5, B23 and B6, respectively, of the filtered output distribution (Fig. 5).

	A	B
1		
2	Name	Potential growth
3	Description	Output
4	Cell	B8
5	Minimum	0.000
6	Maximum	1.030
7	Mean	0.180
8	Std Deviation	0.144
9	Variance	0.021
10	Skewness	1.090
11	Kurtosis	4.260
12	Errors Calculated	0.000
13	Mode	0.011
14	5% Perc	0.011
15	10% Perc	0.023
16	15% Perc	0.038
17	20% Perc	0.052
18	25% Perc	0.068
19	30% Perc	0.082
20	35% Perc	0.098
21	40% Perc	0.114
22	45% Perc	0.130
23	50% Perc	0.146
24	55% Perc	0.164
25	60% Perc	0.186
26	65% Perc	0.207
27	70% Perc	0.232
28	75% Perc	0.263
29	80% Perc	0.299
30	85% Perc	0.336
31	90% Perc	0.381
32	95% Perc	0.461
33	Filter Minimum	1E-20
34	Filter Maximum	
35	Type (1 or 2)	1
36	# Values Filtered	8226
37		
38		
39		

Fig. 5. Filtered results of the output distribution for the scenario simulated using the input settings and tertiary model shown in Fig. 4.

4. Discussion

A predictive model for growth of *Salmonella* on cooked chicken was developed that covered a broader range of temperature than previously developed models (Smith, 1985; Thayer et al., 1987; Gibson et al., 1988; Oscar, 1999a,b,c). In addition, the model developed was designed to provide the input settings (incidence, minimum, median and maximum extent of potential growth) for growth events in a previously developed risk assessment model for *Salmonella* (Oscar, 1999d). The predictive model was developed by following a six-step approach: (1) kinetic data collection; (2) database creation; (3) primary modeling; (4) secondary modeling; (5) tertiary modeling; and (6) model validation (Buchanan, 1993; Whiting and Buchanan, 1997b). A major focus of the predictive modeling process was the identification of the best-fitting secondary models for lag time and specific growth rate.

Comparison of the performance of secondary models for predicting lag time as a function of temperature has been done before. Zwietering et al. (1991) used an *F*-test to compare the hyperbola (exp), the inverse Ratkowsky, the inverse Ratkowsky 2 and the inverse Ratkowsky 3 models and found that all of the tested models had acceptable *f*-values but that the hyperbola (exp) had the lowest *f*-value or best fit to the data. Duh and Schaffner (1993) compared the hyperbola (exp) and the inverse Ratkowsky models and found that both made fail-dangerous predictions of lag time at high temperatures. In contrast, in the present study, the inverse Ratkowsky made mostly fail-safe predictions, whereas the hyperbola (exp) made mostly fail-dangerous predictions. A general observation of secondary models for lag time is that they do not predict well at high temperatures (Duh and Schaffner, 1993; Schaffner, 1995; this study); the exceptions in the current study were the hyperbola (^*m*) and Davey models.

The performance of secondary models for predicting specific growth rate as a function of temperature has also been compared. Zwietering et al. (1991) compared the Ratkowsky 2 and 3 models for growth rate using an *F*-test and found that the Ratkowsky 3 model had slightly better goodness-of-fit, which is in agreement with the current study. In contrast, Duh and Schaffner (1993) found that the Ratkowsky 2 model had slightly better goodness-of-fit (lower *f*-value and higher R^2) than the Ratkowsky 3 model. However, similar to the current study, Duh and Schaffner (1993) reported little systematic bias in the predictions of the Ratkowsky 2 and 3 models. Finally, Rosso et al. (1993) compared the cardinal temperature model with the Ratkowsky 2 and 3 models and found similar but slightly better goodness-of-fit for the cardinal temperature model. In addition, they observed significant correlations among the parameters of the Ratkowsky 2 and 3 models but not the cardinal temperature model. These parameter correlations were identified as the cause of difficulties encountered in obtaining accurate parameter estimates. Likewise, in the present study, the cardinal temperature model, although similar in goodness-of-fit to the Ratkowsky 2 and 3 models, was easier to fit and yielded more accurate parameter estimates than the Ratkowsky 2 and 3 models.

Deciding which secondary model provided the best fit was not easy because there was no single statistical measurement that proved entirely satisfactory. Rather, the final decision was based on considering a number of statistical measures. The hyperbola (^*m*) model was identified as the best-fitting model for lag time and represents a new version of this model that was discovered during the early stages of secondary modeling when the untransformed hyperbola (^1) and square root transformed hyperbola (^2) models were being evaluated. When the lag time data were fit to the untransformed hyperbola (^1) model (results not shown), the model predicted well at low temperatures but over predicted at temperatures corresponding to the bottom plateau of lag time. In contrast, when the lag time data were fit to the square root transformed hyperbola (^2) model, the model predicted well at low temperatures but under predicted at temperatures corresponding to the bottom plateau of lag time. Thus, a transformation of the model using an exponent between one and two was needed to eliminate the prediction bias. In fact, when the exponent (*m*) was introduced as a fitted parameter in the model, the resulting secondary model fit, with an *m* parameter value of 1.4, showed little prediction bias for lag time throughout the entire temperature range for growth.

Probability distributions for secondary model parameters and simulation have been used inside risk assessment models (Whiting and Buchanan, 1997a; Cassin et al., 1998; McNab, 1998) and in tertiary

predictive models (this study) to predict the growth of pathogens on food. However, the validity of this approach for predicting pathogen growth has not been tested. One potential concern of this modeling approach is the generation of nonsensical predictions due to the random sampling of the probability distributions. To test for such predictions, the current model was simulated and the mean predicted values for lag time and specific growth rate at each temperature were compared to the observed values by calculating prediction bias and accuracy statistics. In addition, the 95% confidence intervals for the tertiary model predictions at each temperature were evaluated. Results of this analysis did not suggest any problems regarding nonsensical predictions of the growth parameters. Thus, use of probability distributions for secondary model parameters and simulation to predict microbial growth is a valid approach. However, it may require that the modeler accurately estimate the secondary model parameters (i.e., have tight confidence intervals for each parameter estimate), as nonsensical predictions may be more common for tertiary models with secondary model parameter distributions with large uncertainty.

The simulation results in the present study demonstrated that not all temperature abuse events resulted in the potential growth of *Salmonella* on cooked chicken because not all times of abuse exceeded the lag time for growth. However, to predict the absolute growth of *Salmonella* on chicken that has been improperly handled, the results of the tertiary model simulation need to be entered into the risk assessment model for *Salmonella* in Poultry FARM and simulated along with the other pathogen events that determine the change in the number of *Salmonella* on chicken as it moves from packaging at the processing plant to the consumer's table (Oscar, 1998b, 1999d). Results of such simulations, which are beyond the scope of this study, would demonstrate even further that not all temperature abuse events result in the growth of *Salmonella* because not all portions of chicken are contaminated with *Salmonella*; especially after proper cooking when the presence of *Salmonella* is likely to be a very rare event.

The predictive model developed in the current study is not a complete model for predicting the growth of *Salmonella* on chicken. Some of the important factors that were not considered in the development of this model are strain variation, physiological state of the pathogen, initial pathogen density, fluctuating temperatures and competing microorganisms. Current research in the author's laboratory is directed at expanding the current model to include the aforementioned factors.

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