Effect of Native Microflora on the Growth Kinetics of *Salmonella* Enteritidis Strain 04-137 in Raw Ground Chicken

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ABSTRACT

Effects of native microflora (NM) on growth kinetics of *Salmonella* Enteritidis strain 04-137 were studied in raw ground chicken. First, samples of ground chicken with high and low levels of NM (10^7.1 and 10^4.9 CFU/g, respectively) were spiked with *Salmonella* at doses ranging from 10^1 to 10^4 CFU/g. The growth kinetics, including the rate constant of growth, \( r \), and the lag period, were similar, but the maximum cell level, \( N_{\text{max}} \), was higher at higher initial *Salmonella* doses for both NM levels. Second, samples of ground chicken with high and low NM levels (10^6.8 and 10^4.7 CFU/g, respectively) were spiked with *Salmonella* and then stored at various constant temperatures ranging from 8 to 32°C. Both \( N_{\text{max}} \) and \( r \) for *Salmonella* were higher at higher temperatures for both NM levels. Although \( r \) for total bacteria, which consisted of NM and *Salmonella*, was also higher at higher temperatures, \( N_{\text{max}} \) was constant at all temperatures for both NM levels. Further, *Salmonella* growth was compared among samples of ground chicken with high and low NM levels and samples of sterilized chicken. *Salmonella* growth, characterized by both \( N_{\text{max}} \) and \( r \), was highest in sterilized chicken, followed by chicken with the low NM level. Our growth model successfully described and analyzed the growth of *Salmonella* and total bacteria in chicken at constant temperatures; using the data obtained, the model also successfully predicted the growth of *Salmonella* and total bacteria in chicken stored at dynamic temperatures. Our study clarified the effects that different doses of NM in ground chicken had on the growth kinetics of the *Salmonella* strain and demonstrated the usability of the growth model for foods with NM.

One of the most important environmental factors influencing the microbiological safety of food is temperature. When a food is exposed to high temperature from the production to the distribution processes, native microflora (NM) can grow. Once NM grow in the food, they will not decrease in number even when the food is then cooled in a refrigerator. If the NM include pathogens, food poisoning outbreaks can occur. Also, even if there are no pathogens, an increased number of NM can lead to food spoilage. Thus, prediction of microbial growth in food at various temperatures is important for food safety and quality.

Over the last two decades, a number of mathematical models have been developed to quantitatively describe microbial growth in culture media and food (22). Among the growth models proposed so far, the modified Gompertz model, the Baranyi model, and a three-phase linear model are well known worldwide (2, 3, 13). We also have developed a growth model, which is an extended form of the logistic model (7, 8, 10, 11). The model, which we call a new logistic model, has successfully predicted bacterial growth in broth, milk, and pouched food, and also on the surface of an agar plate at a variety of temperatures, very similar to the Baranyi model (8, 12).

Many of the articles published on predictive food microbiology have dealt with the growth of pathogens in sterile foods and media. We also have successfully described growth kinetics of *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus* in sterile foods and media with our model (7, 8, 10, 11). However, most retail foods and food materials are not sterilized, and competition between a pathogen and NM in a food would greatly influence the growth of the pathogen. But our model has not been validated for such a food yet.

A few investigators have studied the growth kinetics of a pathogen as well as NM in a food (14, 15, 21). Studies on microbial growth kinetics in a food with NM are becoming important for the next stage of predictive food microbiology. Therefore, we need to analyze and predict growth of pathogens and NM in many kinds of food and apply the obtained data to microbial food safety.

*Salmonella enterica* serovar Enteritidis often contaminates chicken and eggs and has caused serious food poisoning outbreaks worldwide. In this study, we therefore conducted a systematic study of the growth kinetics of *Salmonella* Enteritidis and NM in raw ground chicken. We focused on growth parameters such as the rate constant of growth, the lag period, and especially the maximum cell population for both *Salmonella* and NM. Then we used these data to predict growth of both *Salmonella* and NM in
ground chicken at dynamic temperatures with our model. Further, we studied the *Salmonella* growth kinetics in sterilized ground chicken to evaluate the effect of NM on *Salmonella* growth in ground chicken. Here we selected a test strain that showed the average growth characteristics among four *Salmonella* Enteritidis strains.

**MATERIALS AND METHODS**

*Salmonella* cell preparation. *Salmonella* Enteritidis strain 04-137, an isolate from a food poisoning outbreak in Tokyo, Japan, was activated on a nutrient agar plate (Nissui Pharmaceuticals, Tokyo, Japan) at 35°C for 24 h. Cells of several well-grown colonies on the plate were incubated in Trypticase soy broth (Oxoid, Basingstoke, UK) at 35°C with shaking (MK161, Yamato Co., Tokyo, Japan) at 80 rpm for 24 h. Cultured cells (1 ml) were washed with saline (0.85% [wt/vol] sodium chloride solution) by centrifugation at 13,000 × g at 4°C for 15 min. Cells were thoroughly suspended in saline (1 ml) and then diluted to 1:10^4 with saline, yielding a cell suspension of about 10^5 CFU/mL. In the experiment at different initial cell doses, the cell suspension was diluted to corresponding ratios with saline.

**Chicken.** All ground chicken samples, which were made from the breast meat of young, domestic chicken and then wrapped on plastic trays, were purchased at a retail store in Tokyo and examined for contamination with *Salmonella* spp. by the conventional method (1) before use. Ground chicken (6 kg) was purchased, and then two ground chicken samples with low and high levels of NM were prepared for experiments under various constant and dynamic temperature conditions. The low-NM sample was composed of 3 kg of untreated ground chicken purchased at the store, and the high-NM sample was prepared by incubating 3 kg of purchased ground chicken at 30°C for 10 h to increase the level of NM. After thorough mixing, about 250 g of each ground chicken sample was placed in sterile glass bottles. The bottles were frozen at −20°C until use and then thawed at <10°C overnight for use. Retail ground chicken was also sterilized at 121°C for 15 min for experiments with sterile chicken.

*Salmonella* spiking and storage. Ground chicken samples were spiked with the *Salmonella* cell suspension prepared above (2 ml/100 g of chicken). After thorough mixing, 10-g portions were placed in sterile glass bottles (vacant volume, 110 ml) with tight caps.

The glass bottles were stored either in a water bath unit (SM-05R, Taitech, Koshigaya, Japan) for trials at a constant temperature above room temperature or in a programmable incubator (SU-221, Espec Co., Osaka, Japan) for trials at constant temperature below room temperature. The time for the sample in the bottle to reach the designated temperature (i.e., 30 min) in each incubator was measured with a digital thermometer (AM-7002, Anritsu Meter Co., Tokyo, Japan) and was taken into consideration during the experiment (8, 10, 11). Immediately after incubation, each sample (one bottle per data point) was taken from the incubator and cooled in ice water. At least two trials were performed at each constant temperature.

For a dynamic temperature experiment, the glass bottles were placed in the programmable incubator, and the sample temperature was measured in duplicate every 30 s throughout the experiment with the digital thermometer (7, 8, 10). Immediately after each incubation period, the sample in duplicate was taken from the incubator and cooled in ice water. One trial was performed for each dynamic temperature pattern.

**Bacterial cell counts.** The ground chicken samples in the bottles were mixed with buffered sodium chloride peptone solution (90 ml) (Nissui Pharmaceuticals) to make 10% food homogenate and then transferred to a filtered plastic bag. The homogenate was thoroughly mixed in a stomacher (SH-IIM, Elmex, Tokyo, Japan) for 1 min. Each sample was then serially 10-fold diluted with saline (0.85% sodium chloride solution) without peptone, to suppress microbial growth in the diluted samples (1). Total (aerobic) bacteria counts of the sample were enumerated in duplicate with the surface-plating method using standard method agar plates (Nissui Pharmaceuticals) after incubation at 35°C for 48 h (1). *Salmonella* counts of the sample were enumerated in duplicate with the surface-plating method using deoxycholate-hydrogen sulfide-lactose (DHL) agar plates (Nissui Pharmaceuticals) or xylose lysine deoxycholate agar (XLD) plates (Oxoid). For *Salmonella* counts, agar plates were incubated at 42°C for 24 h to suppress the growth of microorganisms other than the test strain. Suspected colonies were examined for identification with a serological test using antiserum to *Salmonella* O antigens (Denka Seiken, Tokyo, Japan) on a glass slide or a real-time PCR method (17). The average bacterial count with two plates for each data point was calculated for *Salmonella* and total bacteria.

*Salmonella* cells at a very low dose were enumerated with the five-tube most-probable-number method (1). Namely, three dilutions consisting of 10, 1, and 0.1 ml of a 10% food homogenate of a sample were cultured in each of five tubes containing *Enterobacteriaceae* enrichment mannitol broth (Merck, Darmstadt, Germany) and then isolated on a DHL or XLD plate. Suspected colonies were then tested as described above.

**Growth model and statistical analysis.** Averages of bacterial counts for the two trials of the constant temperature experiments or the two samples of the dynamic temperature experiments were then analyzed with the new logistic model, which is expressed as follows (10):

\[
\frac{dN}{dt} = r N \left\{ 1 - \left( \frac{N}{N_{\text{max}}} \right)^{m} \right\} \left\{ 1 - \left( \frac{N_{\text{ini}}}{N} \right)^{n} \right\}
\]  

(1)

Here \(N\) is the population of a microorganism (CFU per gram) at time \(t\) (hours), \(r\) is the rate constant of growth (1/h), \(N_{\text{max}}\) is the maximum population (CFU per gram), and \(N_{\text{ini}}\) is the initial population (CFU per gram). Parameters \(m\) and \(n\) (\(>0\)) are related to the curvature of the deceleration phase and the period of the lag phase, respectively. The equation was solved numerically with the fourth-order Runge-Kutta method. Numerical data of microbial counts were analyzed with a computer program to fit to the growth model, which was developed using a spreadsheet software program, Microsoft Excel (9). Microbial populations estimated with the model (CFU per gram) were then log-transformed to make a growth curve (9).

Growth at a dynamic temperature was predicted using the values of parameters in equation 1 studied at constant temperatures (7, 8, 10, 11). The value of \(r\) at the measured temperature of the time interval during an experiment was obtained from the square root model (22).

The mean of the square error between log-transformed cell concentrations predicted with the model and observed at the observation points was calculated. Statistical analysis of data, including regression analyses, was performed with Microsoft Excel. The lag period in a growth curve, \(lag\), defined as the period between the initial point and the point where the regression line for the exponential phase intersected the horizontal line passing through the initial point on the semilogarithmic plot (3, 5, 10), was estimated with our computer program (9).
RESULTS

Growth kinetics at various initial doses. Growth kinetics of Salmonella Enteritidis strain 04-137 in raw ground chicken at various initial doses of Salmonella were studied. Ground chicken with a high level of NM (10^{7.1} CFU/g) was spiked with Salmonella at various initial doses ranging from 10^4 to 10^1 CFU/g and then stored at a given temperature. Salmonella growth curves were similar during the storage period (Fig. 1A). The values of the rate constant of growth, or $r$, were similar among them, being 0.53, 0.51, 0.55, and 0.46 (1/h) at the initial doses of 10^{4.2}, 10^{3.2}, 10^{2.0}, and 10^{1.1} CFU/g, respectively. Also, values of lag in the growth curves were all very short (<1 h). However, the maximum level in the stationary phase, $N_{\text{max}}$, was higher at the higher initial doses (Fig. 1A). Values for $N_{\text{max}}$ were 10^{8.5}, 10^{8.0}, 10^{7.6}, and 10^{7.0} CFU/g at the initial doses of 10^{4.2}, 10^{3.2}, 10^{2.0}, and 10^{1.1} CFU/g, respectively. No Salmonella cells were detected in the original ground chicken.

Growth of total bacteria in the ground chicken spiked at 10^{2.0} CFU/g of Salmonella was measured for comparison (Fig. 1A). Since the ground chicken used in this experiment was originally contaminated with a high level of NM (10^{7.1} CFU/g), growth of NM was much less than that of Salmonella.

Similar phenomena on the growth kinetics were observed in ground chicken with a low level of NM (10^{0.9} CFU/g) (Fig. 1B). $N_{\text{max}}$ for Salmonella was higher at the higher initial doses; values for $N_{\text{max}}$ were 10^{8.6}, 10^{8.0}, 10^{7.4}, and 10^{6.5} CFU/g at the initial doses of 10^{4.3}, 10^{3.3}, 10^{2.3}, and 10^{1.4} CFU/g, respectively. Also, values for $r$ were similar and values of lag were all short for those growth curves (data not shown). No Salmonella cells were detected in the original ground chicken. It is interesting that $N_{\text{max}}$ for Salmonella was dependent on the initial dose in the ground chicken with the high and low NM levels (Fig. 1A and 1B).

Growth kinetics at constant temperatures. Since the initial dose of Salmonella Enteritidis strain 04-137 did not remarkably affect its growth characteristics except for $N_{\text{max}}$, growth of the pathogen at various constant temperatures was then studied at a given initial dose. Ground chicken with low and high levels of NM (10^{4.7} and 10^{6.8} CFU/g, respectively) were spiked with Salmonella at about 10^{1.2} CFU/g and then stored at temperatures ranging from 8 to 32°C to study the effect of NM on the growth of the pathogen. No Salmonella cells were detected in the original ground chicken.

Growth curves of Salmonella and total bacteria in the ground chicken with the low and high levels of NM stored at the constant temperatures were all sigmoidal and were described well by our growth models (data not shown). At 8°C, no Salmonella growth was observed in the chickens with the low and high levels of NM. Growth parameter values obtained for Salmonella and total bacteria in the two ground chicken samples are shown in Table 1. Values for the parameters in the table were then studied as follows.

Value of $N_{\text{max}}$ for Salmonella increased with the temperature in both low- and high-NM chicken samples, and the value in low-NM chicken was higher than that in high-NM chicken at <32°C (Fig. 2). The values of $N_{\text{max}}$ for both low and high levels of NM were precisely described with the polynomial equations 2 and 3, with high values for coefficient of determination of 0.976 and 0.945, respectively (Fig. 2).

$$N_{\text{max}} = -0.0073T^2 + 0.4756T + 1.592$$ (2)

$$N_{\text{max}} = -0.0047T^2 + 0.4343T + 0.208$$ (3)

Values of $N_{\text{max}}$ for total bacteria in the two ground chicken samples at these temperatures were both constant with the same averages of 10^{9.4} CFU/g (Table 1 and Fig. 2).

The value of $r$ for Salmonella also increased with the temperature at both NM levels, and the value was higher in low-NM chicken than in high-NM chicken (Fig. 3A). Values of $r$ for Salmonella at the storage temperatures for both NM levels were successfully described with the square root model (Fig. 3A). Linear regression lines for $r$ in both
low- and high-NM chicken were described by equations 4 and 5, respectively.

\[
\sqrt{r} = 0.0399(T - 5.11) \tag{4}
\]

\[
\sqrt{r} = 0.0285(T - 4.18) \tag{5}
\]

The coefficients of determination for the low- and high-NM chicken were 0.976 and 0.986, respectively. The steeper slope of \( r \) for low-NM chicken indicated that it was more sensitive to temperature. Values for \( r \) in both NM levels at 8°C, which were measured to be almost zero, obviously deviated from the regression lines (Fig. 3A).

Values of \( r \) for total bacteria were also well described with the square root model (Fig. 3B). The regression lines

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### TABLE 1. Growth characteristics of Salmonella Enteritidis strain 04-137 and total bacteria in ground chicken with high and low native microflora levels at various constant temperatures

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>( r ) (1/h)</th>
<th>lag (h)</th>
<th>( N_{\text{max}} ) (log CFU/g)</th>
<th>( r ) (1/h)</th>
<th>lag (h)</th>
<th>( N_{\text{max}} ) (log CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Salmonella</td>
<td>Total bacteria</td>
<td></td>
<td>Ground chicken with high level of native microflora</td>
<td></td>
<td>Ground chicken with low level of native microflora</td>
</tr>
<tr>
<td>32</td>
<td>0.59</td>
<td>&lt;1</td>
<td>9.4</td>
<td>0.60</td>
<td>&lt;1</td>
<td>9.6</td>
</tr>
<tr>
<td>28</td>
<td>0.44</td>
<td>&lt;1</td>
<td>8.5</td>
<td>0.53</td>
<td>&lt;1</td>
<td>9.3</td>
</tr>
<tr>
<td>24</td>
<td>0.37</td>
<td>&lt;1</td>
<td>8.0</td>
<td>0.34</td>
<td>&lt;1</td>
<td>9.8</td>
</tr>
<tr>
<td>20</td>
<td>0.20</td>
<td>1.3</td>
<td>6.4</td>
<td>0.28</td>
<td>&lt;1</td>
<td>9.4</td>
</tr>
<tr>
<td>16</td>
<td>0.10</td>
<td>2.8</td>
<td>6.5</td>
<td>0.17</td>
<td>&lt;1</td>
<td>9.2</td>
</tr>
<tr>
<td>12</td>
<td>0.038</td>
<td>5.5</td>
<td>4.3</td>
<td>0.088</td>
<td>&lt;1</td>
<td>9.2</td>
</tr>
</tbody>
</table>

| 32        | 1.0          | <1       | 9.4                           | 0.99         | 1.2      | 9.6                           |
| 28        | 0.96         | <1       | 9.1                           | 0.84         | 1.0      | 9.7                           |
| 24        | 0.59         | <1       | 8.6                           | 0.65         | 1.4      | 9.3                           |
| 20        | 0.36         | 2.0      | 8.1                           | 0.38         | 2.2      | 9.3                           |
| 16        | 0.16         | <1       | 7.6                           | 0.34         | 5.3      | 9.5                           |
| 12        | 0.055        | 8.0      | 5.9                           | 0.19         | 5.8      | 9.3                           |

\( a \) Parameters \( r \), lag, and \( N_{\text{max}} \) are the rate constant of growth, the lag period, and the maximum cell population, respectively. Values are the averages of two trials. No Salmonella growth was observed in either ground chicken sample at 8°C.

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FIGURE 2. The maximum population for Salmonella Enteritidis strain 04-137 and total bacteria in ground chicken with the low and high levels of native microflora at various constant temperatures. Total and Sal indicate total bacteria and Salmonella, respectively. Curves are depicted with a polynomial equation.

FIGURE 3. The rate constant of growth for Salmonella Enteritidis strain 04-137 (A) and total bacteria (B) in ground chicken with the low and high levels of native microflora at various constant temperatures. Values are analyzed with the square root model. Straight lines are linear regression lines.
for low- and high-NM chicken were described with equations 6 and 7, respectively.

\[ \sqrt{r} = 0.0277(T + 4.40) \] (6)

\[ \sqrt{r} = 0.0238(T - 1.45) \] (7)

The coefficients of determination for low- and high-NM chicken samples were 0.989 and 0.986, respectively. Interestingly, the value for \( r \) was higher in low-NM chicken than in high-NM chicken in the temperature range of this study, similar to Salmonella (Fig. 3B).

The value of \( lag \) for Salmonella tended to be longer at lower temperatures for both NM levels of ground chicken (Table 1). This was also seen for total bacteria in low-NM chicken, while values for \( lag \) were all small (i.e., \(<1 \) h) for total bacteria in high-NM chicken (Table 1).

When total bacteria counts of a chicken sample reached 10^9 CFU/g, the chicken was found to spoil with an offensive smell, but the storage experiments were continued until the Salmonella population reached the maximum level.

**Growth prediction at dynamic temperatures.** Based on the above results, our model predicted the growth of Salmonella Enteritidis strain 04-137 and total bacteria at various dynamic temperatures in ground chicken at the two NM levels. Namely, equations 2 and 3 for Salmonella and the average of \( N_{\text{max}} \) for total bacteria were introduced for \( N_{\text{max}} \) in the growth model. Equations 4 to 7 were also introduced for \( r \) in the growth model. For \( m \) and \( n \) in equation 1, the averages obtained at constant temperatures for both NM levels of ground chicken shown in Table 2 were used for prediction.

The model’s predictions of the growth of Salmonella and total bacteria in both NM levels of chicken stored at various dynamic temperatures are shown in Figures 4 and 5; here, the dynamic temperatures included high (A) and low (B) temperature ranges. Similar results were obtained in ground chicken exposed at a wider temperature range between 11.4 and 30.5°C (data not shown). Values of the mean of the square error for those predictions were very small. The averages of the mean of the square error for Salmonella and total bacteria were 0.049 \( \pm \) 0.021 and 0.075 \( \pm \) 0.070 log units in low-NM chicken and 0.18 \( \pm \) 0.059 and 0.10 \( \pm \) 0.055 log units in high-NM chicken, respectively, for three dynamic patterns.

**Growth kinetics in sterile chicken.** Growth of Salmonella Enteritidis strain 04-137 in sterilized ground chicken at various constant temperatures between 8 and 32°C was studied for comparison with that in ground chicken with low and high NM levels. An example of the growth curve at 24°C is shown in Fig. 6A. Salmonella growth, characterized by both \( N_{\text{max}} \) and \( r \), was highest in sterilized chicken, followed by chicken with the low NM level; the values of \( N_{\text{max}} \) for sterilized chicken, low-NM chicken, and high-NM chicken were 10^{10.3}, 10^{8.6}, and 10^{8.0} CFU/g, and values of \( r \) were 0.85, 0.59, and 0.37 h^{-1}, respectively. Values of \( lag \) were all small, \(<1 \) h. Similar results were observed at other temperatures. These results showed that the level of NM in ground chicken influenced Salmonella growth kinetics. While the ground chicken sterilized by heating might have developed better nutrients for bacterial growth, the results showed potential growth of the bacteria under optimal conditions without any competitors.

The slope in the log phase, or \( r \), was greater at higher temperatures in sterilized ground chicken, similar to that in

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**TABLE 2. Values for parameters m and n of the new logistic model for Salmonella Enteritidis strain 04-137 and total bacteria in ground chicken with low and high native microflora levels**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Salmonella</th>
<th>Total bacteria</th>
<th>Salmonella</th>
<th>Total bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>( m )</td>
<td>0.28</td>
<td>0.45</td>
<td>0.67</td>
<td>0.68</td>
</tr>
<tr>
<td>( n )</td>
<td>62</td>
<td>14</td>
<td>67</td>
<td>100</td>
</tr>
</tbody>
</table>

\( a \) Values are the averages for \( m \) and \( n \) of the model (equation 1) in ground chicken samples with low and high native microflora levels that were stored at constant temperatures ranging from 8 to 32°C and then used for growth prediction at dynamic temperatures.

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**FIGURE 4. Predictions of Salmonella Enteritidis strain 04-137 and total bacteria in ground chicken with the low level of native microflora at dynamic temperature patterns A and B.**

- **A** Salmonella; •, total bacteria. Growth curves are predicted with our growth model. Regularly changing curves during the storage period are the measured temperature of the chicken.

- **B** Low High

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the low- and high-NM ground chicken (Fig. 6B). However, strikingly, the values of $N_{\text{max}}$ between 12 and 32 °C were almost constant, at about $10^{10}$ CFU/g (Fig. 6B), and were distinctly different from those in the ground chicken with NM (Table 1 and Fig. 2). At 12 °C, Salmonella finally grew to $10^{10}$ CFU/g after 240 h of storage (not shown in Fig. 6B).

Nongrowth of Salmonella in the sterilized chicken was also observed at 8 °C for about 7 days, similar to results in the low- and high-NM chicken. This means that nongrowth at 8 °C would be due to a physiological characteristic of the organism, not to microbial competition in chicken.

**DISCUSSION**

NM in raw ground chicken are thought to consist of many kinds of microorganisms such as bacteria, yeasts, and molds. In this study the level of NM was evaluated with standard method agar plates before spiking with Salmonella cells. On the other hand, total bacteria of ground chicken samples spiked with Salmonella cells consisted of NM and Salmonella. Therefore, total bacteria and NM were used with these corresponding meanings in this study.

NM was always dominant in the ground chicken samples during storage in our study, as compared with spiked Salmonella cells. At constant temperatures, ratios of Salmonella counts to total bacteria counts in most samples were very low, 2 to 3% at maximum even in ground chicken with the low NM level. At higher temperatures, the ratio increased as the storage period continued; the maximum ratio reached 40% at the stationary phase at 32 °C for ground chicken with both NM levels (Table 1).

This study clarified three parameters of microbial growth kinetics for Salmonella Enteritidis strain 04-137 in ground chicken with NM: the period of lag, the rate constant of growth, and $N_{\text{max}}$. Especially, the value of $N_{\text{max}}$ for a microbe of concern is necessary for a mathematical model that describes and predicts microbial growth over a given time; but, so far, few researchers’ models of microbial growth have focused on values of $N_{\text{max}}$ for pathogens in food with NM (19, 27, 28). In the present study, we determined values of $N_{\text{max}}$ for the Salmonella strain in raw ground chicken according to temperature (Fig. 2), initial Salmonella level (Fig. 1), and level of NM (Figs. 2 and 6A). Our results in Figure 2 are similar to those of Koseki and Isobe (19), who studied the growth of Listeria monocytogenes, Escherichia coli, and Salmonella serovars Enteritidis and Typhimurium on lettuce leaves; they found that $N_{\text{max}}$ for pathogens increased at higher temperatures. Oscar (27,
also reported that \( N_{\text{max}} \) for Salmonella Typhimurium differed with the initial level of the strain and temperature. However, these studies (19, 27, 28) used foods with a single NM level and did not study the growth kinetics of NM in the foods. In the present study, we found that a higher level of NM yielded a smaller value of \( N_{\text{max}} \) for Salmonella Enteritidis strain 04-137 (Figs. 2 and 6A). As shown in Figure 1A and 1B, as total bacteria in ground chicken increased to the maximum population, Salmonella growth was suppressed, resulting in a lowered maximum population. We infer that this is due to microbial competition between Salmonella and NM in the ground chicken. This phenomenon is known as the Jameson effect and has been observed for several microorganisms (6, 15, 18, 29).

In our study we also found that values of \( N_{\text{max}} \) for Salmonella Enteritidis strain 04-137 in sterile ground chicken at various constant temperatures were almost constant (Fig. 6B), in contrast to the values in raw ground chicken with NM (Fig. 2). The result in sterile ground chicken is similar to reported results for \( E. \) coli grown in broth and Salmonella Typhimurium grown on sterile chicken (8, 26). Values of \( N_{\text{max}} \) for \( E. \) coli grown at various initial cell doses in broth are also constant (8). In our preliminary study, values of \( N_{\text{max}} \) for Salmonella Enteritidis strain 04-137 at various initial doses in sterile chicken were also constant at a given temperature, in contrast to raw chicken with NM (Fig. 1A and 1B). These results suggest that this difference in \( N_{\text{max}} \) was also due to the Jameson effect by NM in ground chicken.

No remarkable differences in values of \( r \) and \( lag \) in raw ground chicken were observed at the various initial Salmonella doses in our study (Fig. 1A and 1B). Mackey and Kerridge (21) inoculated Salmonella cells at 10^4 and 40 CFU/g in minced beef and reported that the maximum growth rate and lag period of Salmonella spp. in minced beef are unaffected by the inoculum size. Oscar (28) also reported no remarkable differences in the maximum specific growth rate and lag period for Salmonella at 10^{1-12} CFU/g and 10^{3.7} CFU/g in ground chicken, and Gimenez and Dalgaard (14) reported similar results for \( L. \) monocytogenes in cold-smoked salmon. While the contamination level of Salmonella in retail chicken is generally very low, with <1 most probable number per g in most positive samples (27, 30, 31), the abovementioned articles suggest that the initial dose would not affect the growth kinetics of the organism. Thus, for parameters other than the value of \( N_{\text{max}} \), we believe that the analysis and prediction methods used in this study can be extended to Salmonella growth in foods with very low initial levels of Salmonella.

The present study also showed the effect of NM levels on the growth parameter \( r \) for Salmonella Enteritidis strain 04-137. The value of \( r \) at a given temperature was smaller at the higher level of NM (Figs. 3A and 6A), which suggests that NM suppress Salmonella growth during the log phase. On the other hand, no obvious effect on the value for \( lag \) for Salmonella was observed (Table 1). We hypothesize that the population of NM in the ground chicken was not enough to suppress Salmonella growth at the beginning of storage in this study.

Our results were obtained from two trials for each experiment. Although an increased number of trials could result in changes in the values for the growth parameters in our model, we believe that the results from the present study such as the Jameson effect and others would not be unique, but common.

There are various methods for studying microbial growth of multiple species. We studied microbial growth in raw food with NM, as did Oscar (27, 28) and Koseki and Isobe (19). Guillier et al. (15) studied microbial competition by inoculating sterilized food with both a pathogen (\( L. \) monocytogenes) and natural biofilm microflora. Other investigators studied microbial growth for coculture in broth with specific microorganisms (4, 23, 25). Each method has advantages and disadvantages. Our method, using real foods, can obtain applicable microbial data for the tested food, but it is difficult to adjust the populations of NM to the desired levels; by trial and error we were able to prepare ground chicken with an NM population higher by two orders of magnitude by incubating the original sample at 30°C for 10 h.

Strain 04-137 used in this study was selected from a survey of four Salmonella Enteritidis strains with different origins in Trypticase soy broth (Oxoid). Because the growth curves of the four strains were very similar to each other (results not shown), the results obtained in this study are thought to be applicable to other Salmonella Enteritidis strains.

For enumeration of Salmonella cells in foods with NM, some investigators have used selective agar media specific to the target organism (20, 24); however, it is difficult to select Salmonella colonies on agar plates on which a number of bacterial colonies are growing. Suspected colonies need to be examined for further microbiological identification. Other investigators spiked antibiotic-resistant target cells or target cells artificially transformed with fluorescent proteins (16, 21, 27, 28). In this study, we incubated food samples inoculated on DHL or XLD agar plates at a high temperature of 42°C for Salmonella enumeration. This high temperature suppressed the growth of most NM in chicken on selective agar plates; thus, it became much easier to count the Salmonella colonies. In preliminary experiments, we confirmed that the Salmonella counts of samples incubated at 42°C were the same as those at 35 or 37°C. This method is based on the physiological characteristics of Salmonella for temperature and did not require any special Salmonella cells described above.

For Salmonella enumeration, we used DHL as well as XLD agar plates in this study. The DHL agar plate is an official selective medium for Salmonella in Japan (1). In a preliminary study, we confirmed that the DHL agar plate was equivalent to the XLD agar plate; Salmonella counts of samples on DHL agar plates were the same as those on XLD agar plates.

The predominant species of microorganism in ground chicken might change with the storage period and temperature, but we believe that our growth model can describe and predict Salmonella Enteritidis 04-137 as well as total bacteria in ground chicken at various temperature
patterns. To our knowledge, no researchers have yet successfully reported growth prediction for both pathogen and total bacteria in food under various temperature conditions. Delignett-Muller et al. (6) showed first-order simulations of growth of \textit{L. monocytogenes} and food flora in cold-smoked salmon at 4°C, but they did not confirm their results with microbial experiments. On the other hand, competition among microbial species within a food has not been introduced in our model. We would like to improve our model to describe microbial competition mathematically, as in the Lotka-Volterra model (14), in the near future.

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**REFERENCES**