ABSTRACT Contamination and penetration of salmonellae into hatching eggs may comprise an important link in the transmission of these bacteria to growing birds, processed carcasses, and eventually to the consumer. In this study, a predictive model for Salmonella typhimurium as a function of initial cell number and storage or incubation time at a nearly constant temperature and humidity was developed and evaluated to compute the bacterial load after 1 d (holding), 10 d (candling), 17 d (incubation), and 21 d (chick processing). Experiments were conducted for S. typhimurium with both high initial bacterial load (HIBL) and low initial bacterial load (LIBL) of 6.0 and 3.5 log cfu/egg, respectively. Eggs with HIBL experienced 2.0 log reduction in the bacterial load after holding at 4°C for 24 h and 3.0 log increase in the bacterial load during incubation and hatch at approximately 37°C between 17 d and 21 d. Experimental data showed that bacterial load of S. typhimurium from holding to chick processing changed from 3.7 to 6.6 log cfu/egg and from 3.7 to 2.7 log cfu/egg in HIBL and LIBL eggs, respectively. The developed model was able to predict bacterial load of S. typhimurium from 3.6 to 6.6 log cfu/egg in HIBL eggs and from 3.4 to 2.7 log cfu/egg in LIBL eggs from holding to chick processing. Root mean square errors and plot of predicted compared with observed bacterial load of S. typhimurium in contaminated eggs yielded a good fit and prediction. The predicted and experimental results indicated that incubated broiler eggs have an increase in internal bacterial loads between incubation and hatch. This model can be used as a tool to predict bacterial load of S. typhimurium in contaminated eggs as well as help predict the behavior of S. typhimurium during hatch.

(Key words: predictive model, Salmonella typhimurium, hatchery, incubation, chick processing)


INTRODUCTION

Foodborne diseases have become a serious issue with significant health and economic consequences worldwide. In the past few decades, many cases of food poisoning have been associated with the foodborne pathogen Salmonella. Major sources of this pathogen have been linked to poultry products including eggs and meat (Stern et al., 1991; Takase et al., 1999; Latimer et al., 2002). Egg products are deemed the main mode of Salmonella contamination in food (Berrang et al., 1991; Schoeni et al., 1995; Guertler and Fehlhaber, 2004). Salmonella is a common cause of gastrointestinal (GI) infection in both humans and animals. The Centers for Disease Control and Prevention estimates that each year 1,341,873 illnesses and 553 deaths are associated with Salmonella in the US (McEntire, 2004). Broiler hatcheries have been shown to be reservoirs for Salmonella and can be the source of dissemination of these pathogenic bacteria from breeder feed to the nest box, the hatchery onto the grow-out farm, and to dressed carcasses (Cox et al., 1990; Berrang et al., 1999a,b). In a previous study of commercial broiler hatcheries, 75% of 175 samples were salmonellae-positive, and 38 of 40 randomly selected samples contained greater than 10³ salmonellae cells per sample (Cox et al., 1990). Two pathways are the main mode of salmonellae entry into hatching eggs: 1) vertical transmission (salmonellae from an infected hen contaminate her egg) and 2) horizontal integration (salmonellae contaminate the egg through the shell after the egg is laid) (Miyamoto et al., 1998; Cox et al., 2000). Although the possibility exists that vertical transmission of bacteria results in the internal contamination of intact shell eggs, horizontal bacterial contamination is likely to be the major culprit, having serious negative food safety implications (Wang and Slavik, 1998). Several studies indicate that the percentage of fertile hatching eggs contaminated with Salmonella is very low (0.01 to 0.05%), but...
these eggs can spread the bacteria posthatch to chicks in the same hatchery, resulting in further cross-contamination during grow-out and processing (Bailey et al., 1992, 1994, 1998; Berrang et al., 1999b; Russell, 2003).

Several studies have demonstrated the rapid and deep penetration of Salmonella in eggs with no way of preventing further infection to the developing embryo (Pardon, 1990; Berrang et al., 1998; Miyamoto et al., 1998; Cox et al., 2000; Almonacid et al., 2002). Microbial detection using routine standard methods are limited by lengthy test time and high cost. An alternative methodology called predictive microbiology provides benefits to the food industry by yielding a faster and more efficient approach to poultry. It provides a basis for comparing data from diverse sources on the behavior of microorganisms in foods, resulting in increased productivity by reducing the need for the time-consuming and invasive microbial testing procedures (Ross and McMee-kin, 1994).

However, no previous reports assessed the prediction and enumeration of Salmonella typhimurium at each stage in a broiler hatchery from storage to chick processing. No predictive model exists in the literature for the prediction of behavior (growth, survival, and death) of Salmonella as it penetrates and moves through fertile hatching eggs at different stages in a poultry hatchery. The hatchery is the first link in the production and processing chain in the poultry industry; therefore, the current study will attempt to elucidate the behavior and level of S. typhimurium in hatching eggs, which could provide a valuable tool in planning intervention strategies to prevent the introduction of this and other pathogens into commercial hatcheries, grow-out operations, and processing facilities. The objectives of the study are to collect microbial data regarding the level of S. typhimurium at each stage in a broiler hatchery from storage to chick processing and to develop and evaluate a predictive model for the behavior of S. typhimurium in the broiler hatchery.

**MATERIALS AND METHODS**

**Microorganism and Culture**

In this study, a nalidixic acid (NA)-resistant mutant of S. typhimurium (USDA/ARS, Poultry Production and Product Safety Research Unit, Fayetteville, AR) maintained at −80°C in a cryogenic state was used. Before each experiment, a fresh overnight culture of NA-resistant marker strain of S. typhimurium was grown for 18 to 20 h at 37°C in brain heart infusion broth (Difco Laboratories, Detroit, MI). The cell count was determined by serially diluting (1:10) and spread plating the culture onto tryptic soy agar (TSA)-NA agar plates, which contained TSA (Difco Laboratories) with 200 ppm of NA. Through this plate count method, the original culture was enumerated to approximately 8 log cfu/mL after incubating for 48 h at 37°C. This marker strain of Salmonella was used in the experiments to eliminate the natural occurring strains of bacteria when allowed to grow on TSA-NA plates.

**Eggs and Incubators**

A total of 120 freshly laid eggs (Cobb 700 × Ross 508) were obtained from a local commercial hatchery (George’s Hatchery, Springdale, AR) on the first day of each test. Eggs were divided equally into 2 groups identified as control and treatment. Five eggs were set aside as negative controls, and the remaining control eggs were covered in foil, identified, and placed into a refrigerated storage room for 24 h at 4°C to simulate real commercial hatchery conditions. Eggs were allowed to warm to room temperature following refrigerated storage prior to placing eggs in the incubator. This procedure of prewarming prior to setting reduces sweating or condensation of water on the eggshell surface, which reduces bacterial multiplication and penetration and improves hatchability and chick quality (Dozier et al., 2002). Control and treatment eggs were incubated and hatched independently in 2 different Sportsman Incubators (model 1502, GQF Manufacturing Co., Savannah, GA), which have the complete combination of incubator-hatcher units with 3 automatically turned trays and a fourth stationary hatching tray in the bottom of the incubator. Each incubator was equipped with an electronic thermostat, a hygrometer, and humidity control and could accommodate 180 eggs in a single batch of hatch- ing. Incubators were sanitized with formaldehyde prior to the placement of eggs for incubation and hatching.

**Inoculation of Eggs and Incubation**

Two levels of inoculum, a low and a high, were used to simulate 2 types of bacterial loads. The low level culture was serially diluted (1:10) and added to PBS solution for a final inoculum level of 4.0 log cfu/mL. For the high level culture, 10 mL of bacterial culture were suspended into 990 mL of PBS solution to obtain an inoculum level of 6.3 log cfu/mL. These 2 inoculum levels were chosen based on the preliminary results of bacterial detection and enumeration with different bacterial inoculation levels for the time course studies of hatching eggs. Any inoculation level below the low level showed no detectable growth in the samples of eggs after refrigerated storage for 24 h at 4°C and similarly, inoculation levels greater than the high level had tremendous bacterial growth at different stages, which showed no time course changes in the bacterial count. Treatment eggs were placed into an inoculum bath for 15 min and then removed and air-dried for 30 min according to the procedures by Cason et al. (1993). Five treatment eggs inoculated with S. typhimurium were separated to test for the positive control bacterial load. Finally, the remaining treatment eggs were placed into cartons, covered, identified, moved into a refrigerated storage room for 24 h at 4°C, and then prewarmed to room temperature prior to setting, which was a process...
similar to the control eggs. After holding, treatment eggs were transferred to one incubator, and control eggs were transferred to another incubator of the same environmental conditions. At the end of each stage [1 d (holding), 10 d (candling), 17 d (incubation), 21 d (chick processing)], 5 control and 10 treatment eggs were randomly selected and taken out for sampling and enumerating the bacterial load. The major stages of a poultry hatchery considered during these experiments are described in Figure 1. Candling is a process of discarding eggs that will not hatch, including infertile and dead eggs.

**Sample Collection**

Eggshell samples after 1 d (holding); eggshell and yolk samples after 10 d (candling); eggshell, shell membrane, yolk, and GI tract samples after 17 d (incubation); and eggshell, beak, and cloacal samples after 21 d (chick processing) were collected from both control and treatment eggs. For treatment eggs, eggshell samples were taken from one set of 5 eggs, and egg content samples were taken from another set of 5 eggs. The eggshell samples were taken from 5 treatment eggs for microbial enumeration, and the inner egg contents of these 5 treatment eggs were discarded aseptically. Another 5 treatment eggs were aseptically washed with 70% alcohol 3 times to remove bacteria from the outer shells, and the eggshells were aseptically cut around the equatorial center using sterile disposable scalpel blades for obtaining the inner egg contents. Egg contents of these eggs were then placed into sterile Petri dishes. This procedure was followed to avoid the cross-contamination of bacteria from eggshell to the inner egg contents. Eggshell samples of 5 treatment eggs were also taken after bacterial inoculation and drying for the enumeration of bacteria to obtain the initial bacterial load in eggs, known as the positive control. Eggshells were placed into Stomacher bags (7.5 in. × 12 in., Nasco Whirlpak, Fort Atkinson, WI) with buffered peptone water (BPW) and were hand-shaken for 1 min to obtain homogenized samples for high recovery of bacteria. Egg yolks were sampled by aseptically separating the vitelline membrane and then swabbing the yolk. The yolk sample was then placed into BPW and vortexed for 10 s. Eggshell membranes were sampled by removing the whole membrane, placing it into BPW, and vortexing for 10 s. The GI tracts of developing fetus were obtained by aseptic removal in Petri dishes. They were then placed into BPW and vortexed for 10 s. Samples from cloaca and beak were taken with the help of sterile swabs. They were placed into BPW and vortexed for 10 s.

**Microbial Enumeration**

For the eggs inoculated with high initial bacterial load (HIBL), samples were auto-plated in triplicate using a WASP spiral plater (DW Scientific, W. Yorkshire, UK) in which 0.5 mL of each wash sample was placed onto 150-mm TSA-NA plates and subsequent enumeration was done using the ProtoCol hardware/software system (Synoptics, Frederick, MD). Samples were enumerated using the 3-tube most probable number (MPN) method for the eggs inoculated with low initial bacterial load (LIBL). For the LIBL test, egg samples were serially diluted (1:10) up to a fourth dilution in BPW and incubated for 24 h at 37°C for pre-enrichment. Pre-enriched cultures were transferred to tetraionate broth (Difco Laboratories) and again incubated for 48 h at 37°C. All pre-enriched and incubated samples were then streak-plated in triplicate into TSA-NA plates and incubated for 48 h at 37°C for subsequent enumeration of S. typhimurium.
The TSA-NA plates were placed in incubators at each hatchery stage, and there was no growth of NA-resistant marker strain of *S. typhimurium* observed. Samples from all control eggs did not show any growth of this marker strain of *S. typhimurium* at any stage in the hatchery process.

**Model Development and Evaluation**

Most of the efforts on predictive microbiology have focused on the development of kinetic models in which the effect of various factors (e.g., temperature, pH, water activity, and preservatives) on microbial death or growth is expressed quantitatively by mathematical equations (Koutsoumanis et al., 2004). The approach to kinetic modeling has been greatly enhanced by coupling polynomial or response surface analysis, which employs nonlinear regression techniques, to model equations such as logistic and Gompertz functions, which can be used to depict death or growth curves mathematically.

The ability of bacteria to contaminate an egg and further increase in number is dependent on several factors such as *Salmonella* penetration ability, survivability on the shell surface, the albumen environment, storage temperature, humidity, shell contact with the liquids, position of the yolk relative to the site of the infection, shell quality, shell porosity, number of eggshell pores, shell membrane thickness, and concentration of natural antimicrobials (Berrang et al., 1999b; Cox et al., 2000). Depending on the amount of bacteria present on the shell at the time of infection, the majority of organisms are believed to remain dormant until favorable growth conditions such as temperature, humidity, and availability of nutrients from yolk allow bacterial growth. Incubated broiler eggs have a sharp increase in internal bacterial loads between 17 d and hatch (Cason et al., 1993). Extended incubation to 17 d allows growth of the chick fetus; by that time, the fetus replaces the allantoic fluid, providing protection to bacteria from chemical barriers of allantoic fluid and causing exponential growth thereafter. Various theories suggest that biological barriers of the egg and their strength consistency with age of the egg inhibit the movement of bacteria and reduce the bacterial concentration of a contaminated egg by up to 17 d (Cason et al., 1993; Berrang et al., 1999b; Cox et al., 2000). However, bacterial loads increase dramatically between 17 d and hatch. Based on these theories and assumptions, a predictive model for *S. typhimurium* as a function of initial cell number and storage or incubation time at a nearly constant temperature and humidity was developed and evaluated with necessary modifications to the Gompertz function. The model is described as

\[
N_t = N_0 - C_1 \left\{ \exp\left(\exp\left(-B_1(17 - t)\right)\right) \right\} \quad \text{if } d \leq 17 \tag{1}
\]

\[
N_t = N_0 + C_2 \left\{ \exp\left(\exp\left(-B_2(17 - t)\right)\right) \right\} \quad \text{if } d > 17 \tag{2}
\]

where

\[
N_t = \log \text{ count of } S. \text{ typhimurium} \text{ after day } t,
\]

\[
N_0 = \text{ initial number of } S. \text{ typhimurium} \text{ in eggs in log scale},
\]

\[
C_1 = \text{ asymptotic amount of inactivation that occurs as } t \text{ increases indefinitely (i.e., number of log cycles of survival and/or death) on or before } 17 \text{ d},
\]

\[
C_2 = \text{ asymptotic amount of growth that occurs as } t \text{ increases indefinitely (i.e., number of log cycles of survival and or growth) after } 17 \text{ d},
\]

\[
B_1 = \text{ maximum relative survival/death rate on or before } 17 \text{ d},
\]

\[
B_2 = \text{ maximum relative survival/growth rate after } 17 \text{ d}.
\]

Parameters \( B_1, B_2, C_1, \) and \( C_2 \) were determined by nonlinear curve fittings of bacterial load of *S. typhimurium* at all different hatchery stages in HIBL and LIBL eggs. The model was evaluated for HIBL and LIBL of *S. typhimurium* in contaminated eggs by comparing the root mean square errors (RMSE) and plotting the predicted in contrast to the observed bacterial load.

**Statistical Analyses**

All data were subjected to log transformation and expressed in \( \log_{10} \) units before analyses were conducted. Data were presented as the means \( \pm \) SE. The mean of bacterial loads of all samples from different locations of hatching eggs at each stage were computed to express the concentration of *S. typhimurium* per egg basis. Significant differences between bacterial loads at different stages were determined by Tukey’s multiple mean comparison test at a significance level of \( \alpha = 0.05 \). The parameter estimation and model evaluation for the Gompertz
PREDICTIVE MODEL OF SALMONELLA IN HATCHERY

Table 1. Bacterial load of Salmonella typhimurium at different locations in contaminated hatching eggs

<table>
<thead>
<tr>
<th>Hatchery stage</th>
<th>Sample</th>
<th>HIBL eggs&lt;sup&gt;2&lt;/sup&gt;</th>
<th>LIBL eggs&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>Eggshell</td>
<td>5.97 ± 0.03</td>
<td>3.48 ± 0.12</td>
</tr>
<tr>
<td>Holding</td>
<td>Eggshell</td>
<td>3.70 ± 0.04</td>
<td>3.67 ± 0.19</td>
</tr>
<tr>
<td>10 d, candling</td>
<td>Eggshell</td>
<td>3.24 ± 0.23</td>
<td>2.41 ± 0.56</td>
</tr>
<tr>
<td>Yolk</td>
<td>2.91 ± 0.55</td>
<td>2.79 ± 0.81</td>
<td></td>
</tr>
<tr>
<td>17 d, incubation</td>
<td>Eggshell</td>
<td>2.83 ± 0.03</td>
<td>1.13 ± 0.17</td>
</tr>
<tr>
<td>Shell membrane</td>
<td>2.68 ± 0.21</td>
<td>1.48 ± 0.00</td>
<td></td>
</tr>
<tr>
<td>Yolk</td>
<td>2.87 ± 0.05</td>
<td>1.27 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>6.68 ± 0.05</td>
<td>1.10 ± 0.50</td>
<td></td>
</tr>
<tr>
<td>Chick processing</td>
<td>Eggshell</td>
<td>6.64 ± 0.02</td>
<td>3.28 ± 0.25</td>
</tr>
<tr>
<td>Beak</td>
<td>6.58 ± 0.04</td>
<td>2.07 ± 0.49</td>
<td></td>
</tr>
<tr>
<td>Cloaca</td>
<td>6.46 ± 0.10</td>
<td>2.82 ± 0.21</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Salmonella typhimurium (log cfu/egg) is expressed as mean ± 1 SE. Mean and SE were calculated from samples of 5 different eggs (n = 5).

<sup>2</sup>High initial bacterial load hatching eggs; enumeration for S. typhimurium was done by the standard plating method with a WASP spiral plater (DW Scientific, W. Yorkshire, UK) and ProtoCol hardware/software system (Synoptics, Frederick, MD).

<sup>3</sup>Low initial bacterial load hatching eggs; enumeration for S. typhimurium was done by the 3-tube most probable number method.

nonlinear model was performed using the nonlinear platform of the JMP statistical analysis software (SAS Inst., Inc., Cary, NC) (the equivalent of PROC NLIN of SAS software). All data were analyzed using JMP software. Experiments for LIBL and HIBL eggs were conducted at different periods in the same incubator; therefore, incubator and hatcher effect did not present any confounding factors.

RESULTS AND DISCUSSION

**Eggs with HIBL**

**Bacterial Load on Different Parts of Hatching Eggs.** Bacterial cells were found in eggshells as shown in the micrograph of a treatment egg (Figure 2) using scanning electron microscopy. Bacterial load of S. typhimurium on different parts of the HIBL hatching eggs at different hatchery stages is shown in Table 1. The mean initial bacterial load of S. typhimurium was approximately 6.0 log cfu/egg in positive control eggshell samples, which implied that the bacterial cells penetrated into the eggshells after inoculation. This result is consistent with the observation of previous researchers who demonstrated that penetration of salmonellae into the cuticle and shell occurred almost immediately in some eggs; in fact, penetration below both membranes in the egg was detected as early as 6 min following shell exposure (Cox et al., 2000). Reduction of bacterial load in treatment eggs after holding was approximately 2.3 log cfu/egg. This refrigeration environment might be hostile to bacteria for their survival and growth. Some bacterial cells might be protected within the eggshells and membranes and remain dormant until favorable conditions allow their growth. Earlier studies demonstrated that rapid growth of Salmonella was observed in artificially inoculated eggs stored at ≥12°C, where as little or no growth was evident at 4 to 8°C (Gast and Beard, 1991).

The bacterial load further decreased up to 10 d (candling) because of the migration of bacteria from eggshell and membrane to the allantoic fluid that has antimicrobial properties, including the alkaline pH, which is unfavorable for bacterial growth. Samples from egg yolks and membranes were not tested until 10 d because preliminary tests and results showed that there was no detectable growth in these egg samples until 10 d. The bacterial load in eggshell, shell membrane, and yolk samples after 17 d (incubation) decreased compared with the bacterial load after candling because of the migration and colonization of the bacteria in the developing chick fetus. After 17 d, the samples of GI tracts of the chick fetuses showed a mean bacterial load of

Table 2. Values of parameters of the model for the behavior of Salmonella typhimurium in HIBL (high initial bacterial load) and LIBL (low initial bacterial load) eggs with RMSE (root mean square error) of estimations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HIBL</th>
<th>LIBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;1&lt;/sub&gt;</td>
<td>8.7</td>
<td>6.1</td>
</tr>
<tr>
<td>B&lt;sub&gt;1&lt;/sub&gt; (d&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>-0.017</td>
<td>-0.098</td>
</tr>
<tr>
<td>RMSE</td>
<td>0.152</td>
<td>0.212</td>
</tr>
<tr>
<td>C&lt;sub&gt;2&lt;/sub&gt;</td>
<td>1.9</td>
<td>-2.2</td>
</tr>
<tr>
<td>B&lt;sub&gt;2&lt;/sub&gt; (d&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.041</td>
<td>0.014</td>
</tr>
<tr>
<td>RMSE</td>
<td>0.093</td>
<td>0.864</td>
</tr>
</tbody>
</table>

<sup>1</sup>C<sub>1</sub> = asymptotic amount of inactivation that occurs as t increases indefinitely (i.e., number of log cycles or survival or death) on or before d 17; C<sub>2</sub> = asymptotic amount of growth that occurs as t increases indefinitely (i.e., number of log cycles of survival or growth) after 17 d; B<sub>1</sub> = maximum relative survival or death rate on or before 17 d; B<sub>2</sub> = maximum relative survival/growth rate after 17 d.
Upon rapid growth in the GI tract, subsequent bacterial cells migrated into the beak and other parts of the developing chick and proliferated. Mean bacterial load values of *S. typhimurium* in eggshell, beak, and cloacal samples after 21 d (chick processing) were in the range of 6.5 to 6.6 log cfu/egg. Although a decrease in bacterial load levels was observed in eggshell samples up to 17 d of incubation, these levels increased from 17 d to hatch because of deposition of excreta.

**Bacterial Load on Hatching Eggs per Egg Basis.**

Bacterial count of all eggshells and yolk samples were averaged to obtain the bacterial load per egg at candling. Similarly, means of the eggshell, shell membrane, yolk, and GI tract samples after 17 d and means of the eggshell, beak, and cloacal samples after 21 d were computed for bacterial load per egg after incubation and chick processing, respectively. Figure 3a shows the bacterial load of *S. typhimurium* in hatching eggs per egg basis for HIBL at each hatchery stage. As shown in Figure 3a, there was a reduction in the bacterial load of approximately 2.3 log cfu/egg after holding eggs at 4°C for 24 h. As described earlier, this refrigeration temperature was not suitable for bacterial growth, and bacteria that migrated to the allantoic fluid were killed. Bacterial loads of *S. typhimurium* at 1 d (holding), 10 d (candling), and 17 d (incubation) were not significantly different (P > 0.05) from each other; however, bacterial loads at 17 d (incubation) and 21 d (chick processing) were significantly different (P < 0.05; Figure 3a). Bacterial load increased dramatically after 17 d, and at this point, bacteria cells migrated and colonized and increased in number in the GI tracts of the developing chicks. The existing conditions during incubation, with the warm temperature inside the incubator, supported the proliferation and multiplication of *S. typhimurium* in a hatching egg.

**Eggs with LIBL**

**Bacterial Load on Different Parts of Hatching Eggs.**

Table 1 shows the bacterial load of *S. typhimurium* on different parts of LIBL hatching eggs. Bacterial cells penetrated into eggshells during the process of inoculation, resulting in a mean initial bacterial load of 3.5 log cfu/egg in positive control eggshell samples. Samples from positive control eggshells were not pre-enriched before plating, as growth occurred without pre-enrichment for these samples. All remaining samples from holding to chick processing were pre-enriched, incubated, and plated for microbial enumeration because without pre-enrichment, bacteria were not detectable from these samples. Bacterial load in egg samples decreased from holding to 17 d (incubation) and increased from 17 d to chick processing (Table 1). Bacterial load decreased from holding to candling because of the migration of bacteria from eggshells and membranes to the allantoic fluid, which has antimicrobial properties unfavorable for bacterial growth. A portion of bacteria might actually be protected within the shell and membranes, providing a safe niche and allowing bacterial cells to remain dormant until favorable growth conditions are present. At hatch, these bacteria within the shells and membranes are ingested by the chick, thus causing cross-contamination to other chicks (Bailey et al., 1994; Berrang et al., 1998, 1999a). Messens et al. (2004) reported that salmonellae
were unable to grow in albumen at temperatures <10°C, and growth becomes negligible <7°C (Gast and Holt, 2000, 2001). Previous research indicates that maximum bactericidal effect of allantoic fluid occurs at normal hatching temperatures and is the highest for the first several days after incubation begins (Cason et al., 1993).

On eggshell samples, bacterial load gradually decreased from holding to 17 d (incubation) and increased at 21 d (chick processing) because of the deposition of excreta on shells at hatch. Hammack et al. (1993) demonstrated that eggshell fecal contamination is the most frequent mode of contamination, which was exacerbated in cracked compared with intact eggs. Cason et al. (1993), who studied the location of *Salmonella* in inoculated eggs during the last few days of incubation and hatching, found that 100% of shell and membrane samples were *Salmonella*-positive 30 min after inoculation and that 38% of shell and membrane samples tested positive after 17 to 21 d of incubation.

**Bacterial Load on Hatching Eggs Per Egg Basis.**

As mentioned earlier, microbial enumeration for LIBL tests were performed through the MPN method, which gave the estimation of the bacterial load. Figure 3b shows the bacterial load of *S. typhimurium* in hatching eggs per egg basis for LIBL at each hatchery stage. A significant difference was observed at only 17 d of incubation. The range of bacterial load of *S. typhimurium* in LIBL hatching eggs estimated by the MPN method in eggshell and yolk samples after candling was 1.5 to 5.0 log cfu/egg and in eggshell, beak, and cloacal samples after chick processing was 0.6 to 4.0 log cfu/egg, which in turn increased the variability in the estimation. The bacterial load of *S. typhimurium* in the chick processing samples of LIBL eggs increased significantly (*P* < 0.05) compared with the 17-d (incubation) samples (Figure 3b).

**Predicted Bacterial Load from the Model**

Bacterial loads were computed from the prediction model at each hatchery stage from holding to chick processing and plotted simultaneously for both HIBL and LIBL eggs (Figure 4). Experimental results showed that bacterial load of *S. typhimurium* from holding to chick processing changed from 3.7 to 6.6 log cfu/egg and from 3.7 to 2.7 log cfu/egg in HIBL and LIBL eggs, respectively. The developed model was able to predict bacterial load of *S. typhimurium* from holding to chick processing changed from 3.6 to 6.6 log cfu/egg in HIBL eggs and from 3.4 to 2.7 log cfu/egg in LIBL eggs from holding to chick processing. All data at different hatchery stages for both HIBL and LIBL eggs were analyzed using the nonlinear platform of the JMP software, which gave the parameter estimation by fitting the Gompertz nonlinear model with incorporation of initial bacterial load and time at different hatchery stages in equations 1 and 2. This analysis also predicted bacterial loads at different hatchery stages, which were saved to JMP data sheets and compared with the observed bacterial loads (Figure 4). Predicted and experimental results indicated that incubated broiler eggs have an increase in internal bacterial loads between 17 d (incubation) and hatch. The parameters of the model as well as RMSE to evaluate the fit of the nonlinear model for HIBL and LIBL eggs were determined and listed in Table 2.
The plot of predicted compared with the observed bacterial load of \textit{S. typhimurium} (Figure 4) and RMSE (Table 2) showed a good fit and prediction. Values of RMSE for LIBL eggs were higher compared with HIBL eggs (0.21 > 0.15 and 0.86 > 0.09), indicating the model’s better performance for HIBL eggs compared with LIBL eggs. This is evident because the MPN method used for enumeration of bacterial loads yielded estimations based on statistics for the LIBL group. Comparing the observed and predicted results for HIBL and LIBL eggs, it was observed that this proposed model can predict the bacterial load more accurately when initial bacterial load is high; thus, this model can be used as a predictive tool to measure the behavior and bacterial load of \textit{S. typhimurium} in a commercial hatchery from storage to hatch.

**ACKNOWLEDGMENTS**

This research was supported in part by the USDA, grant number 2000-51110-9740, and the Food Safety Consortium. The authors gratefully express their appreciation for the extensive support from George’s Hatchery (Springdale, AR), which supplied the freshly laid hatching eggs for this study.

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