Research Note

Growth of *Salmonella* Typhimurium DT104 at 30°C Is Not Affected by Anatomical Location on the Chicken Carcass[†]

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ABSTRACT

Development of models for growth of *Salmonella* in the chicken food matrix is time-consuming and expensive. The current study was undertaken to examine growth of *Salmonella* on different anatomical locations of the chicken carcass. The purpose was to determine whether anatomical location should be included as an independent variable in predictive models for chicken. Eleven anatomical locations were studied: skin (wing, breast, drumstick, and thigh), meat surface (wing, breast, drumstick, and thigh), and meat interior (breast, drumstick, and thigh). Background microflora, pH, and growth (lag time, λ; growth rate, μ; and time for a 3-log increase, t_3) at 30°C for a small inoculum size (0.92 ± 0.30 log per portion) of *Salmonella* Typhimurium DT104 were examined. Four or six replicate storage trials were conducted per anatomical location ($n = 46$ growth curves). Portion sizes were 1.12 ± 0.17 g (mean $\pm$ standard deviation) for meat and 0.25 ± 0.08 g for skin. A two-phase linear model was used to determine λ and μ. The effect of anatomical location on dependent variables was assessed by one-way analysis of variance. pH values differed ($P < 0.001$) among anatomical locations, with skin (6.86 ± 0.20) > dark meat (6.39 ± 0.20) > white meat (5.97 ± 0.20). Background microflora (4.32 ± 1.66 log per portion) was variable and not affected ($P > 0.05$) by anatomical location. Likewise, λ (1.90 ± 0.75 h), μ (0.648 ± 0.120 log/h), and t_3 (6.71 ± 0.82 h) at 30°C were not affected ($P > 0.05$) by anatomical location. Although there were differences in pH among anatomical locations, these differences were not sufficient to affect growth of *Salmonella* Typhimurium DT104 at 30°C. If this observation holds for other storage conditions and strains, then anatomical location does not need to be included as an independent variable in predictive models for chicken. This would save significant time and money for the predictive microbiologist.

Predictive microbiology is a valuable approach for helping food producers and consumers assess when food storage and handling conditions support growth of *Salmonella* to levels that could result in foodborne illness. Several models for growth of *Salmonella* under temperature abuse conditions have been developed for assessing food safety *(3, 8, 11)*. One of the aforementioned models *(8)* was developed for chicken skin with native microflora, using a single strain of *Salmonella enterica* serotype Typhimurium DT104 (ATCC 700408). This strain was used for model development because it has a multiple-drug resistance phenotype that allows it to be quantified in the presence of other microorganisms.

Development of predictive models for growth of *Salmonella* Typhimurium DT104 on chicken meat is time-consuming and expensive. One way to reduce the cost of model development is to identify conditions for which new models are not needed. In other words, a model developed

for one chicken meat might provide acceptable predictions of *Salmonella* growth on a similar chicken meat. For example, growth of *S. enterica* serotype Typhimurium (ATCC 14028) on sterile cooked chicken breast meat is indistinguishable from its growth on sterile cooked chicken thigh meat *(7)*. Whether a similar relationship exists for growth of *Salmonella* Typhimurium DT104 on chicken meats with native microflora has not been determined.

The chicken carcass is composed of three general types of meat: skin, white, and dark. There are differences in the chemical composition (i.e., pH, myoglobin levels, and fat content) of these meats that are related to their physiological function and location on the carcass. These chemical and anatomical differences could affect growth of *Salmonella* during temperature abuse of chicken meat but are yet to be investigated.

Electron microscopy studies reveal that most microorganisms are located on the surface of chicken meat and that few microorganisms are located in the meat interior *(5, 12)*. Moreover, differences in feathering patterns (i.e., spacing and size of feather follicles) result in differences in skin microenvironments on different anatomical regions of the carcass. Cutting of chicken meat during processing transfers bacteria from the meat surface to the meat interior and at the

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same time releases soluble nutrients from muscle fibers *(6)*. All of these chemical, microbiological, and physical factors may affect growth of *Salmonella* during temperature abuse of chicken meat but are yet to be examined in a systematic manner. Consequently, the present study was undertaken to determine whether or not anatomical location affects growth of *Salmonella* Typhimurium DT104 on the chicken carcass during temperature abuse at 30°C, which is an outdoor temperature commonly encountered during the late spring to early fall in temperate climate zones.

MATERIALS AND METHODS

Test strain. A single strain (ATCC 700408) of *S. enterica* serotype Typhimurium DT104 was obtained from a commercial source (American Type Culture Collection, Manassas, VA). The strain was stored at −70°C in brain heart infusion (BHI) broth that contained 15% (vol/vol) glycerol (Sigma Chemical Co., St. Louis, MO). Before use in storage trials, the strain was revived by incubation for 23 h at 30°C and 150 rpm in 5 ml of BHI broth in a 25-ml Erlenmeyer flask sealed with a foam plug. Serial dilutions (1:10) of the 23-h culture, which had a final pathogen level of 10.28 ± 0.06 log/ml (mean ± standard deviation [SD]), were prepared in buffered peptone water (BPW; BD, Franklin Lakes, NJ). The 10^{-7} dilution was used to inoculate chicken portions.

Chicken preparation. Chicken parts were purchased from local retail stores. Skin was removed from wings, breasts, thighs, and drumsticks, placed on a plastic cutting board, frozen for 15 min at −70°C, and then cut into 2.14-cm^2 portions with a no. 10 cork borer. White meat was obtained from wings and breasts, whereas dark meat was obtained from thighs and drumsticks. White and dark meats were cut into 1-g portions.

Anatomical locations. The anatomical locations studied were as follows: (i) wing skin, ws; (ii) wing meat surface, wm(s); (iii) breast skin, bs; (iv) breast meat surface, bm(s); (v) breast meat interior, bm(i); (vi) drumstick skin, ds; (vii) drumstick meat surface, dm(s); (viii) drumstick meat interior, dm(i); (ix) thigh skin, ts; (x) thigh meat surface, tm(s); and (xi) thigh meat interior, tm(i).

Native microflora. The number of native microflora organisms was determined by most-probable-number (MPN) and viable count (CFU) methods *(8)*. Individual portions of chicken preparations were placed into a 207-ml-capacity plastic bag with filter screen (Whirl-Pak, Nasco, Fort Atkinson, WI). After adding 9 ml of BPW, the sample was pulsified (model PUL 1, Microbiology International, Frederick, MD) for 1 min to recover native microflora into the BPW. The pulsifate was used to set up a three (replicate) by four (dilution [1:10]) MPN assay in BPW. In addition, serial dilutions (1:10) of pulsifate were prepared in BPW, and appropriate dilutions were spiral plated (WASP, Microbiology International) onto BHI agar (BD). The MPN tubes and spiral plates were incubated at 30°C for 24 h. Five microliters from each MPN tube was drop plated onto BHI agar. Drop plates were incubated at 30°C for 24 h to confirm the pattern of positive and negative tubes. The MPN was determined from an MPN table. Colonies that formed on spiral plates were counted by an automated colony counter (Protocol Automated Colony Counter, Microbiology International).

pH. The pH of meat samples was determined by a pH probe that could be directly inserted into the meat sample (pH Spear, Oakton Instruments, Vernon Hills, IL).

Storage trials. After preparation, meat samples were stored overnight at 4°C in 12-well tissue culture flasks (Falcon Multiwell, BD) encased in a plastic bag (Ziploc, S. C. Johnson and Sons, Inc., Racine, WI). Just before the start of a storage trial, meat samples were removed from cold storage and 5 µl of the 10^{-7} dilution of the 23-h culture of *Salmonella* Typhimurium DT104 was spot inoculated onto the outside surface of skin portions or onto the outside surface (i.e., intact epimysium) or onto the interior (i.e., cut surface) of white and dark meat portions. The inoculated meat samples were stored at 30°C for 0 to 8 h. The log number of *Salmonella* Typhimurium DT104 cells on meat samples was determined hourly. Four replicate storage trials were conducted per anatomical location investigated except for bm(i), for which six replicate storage trials were completed for a total of 46 storage trials.

Pathogen enumeration. The MPN and CFU methods for determining the number of *Salmonella* Typhimurium DT104 cells on a chicken meat portion were similar to the MPN and CFU methods described above for native microflora *(8)*. There were two main differences. First, the agar medium used for drop plating and spiral plating was CATS rather than BHI. CATS was composed of xylose lysine Tergitol (XLT4) base agar medium (BD) that contained 25 mM HEPES (Sigma) and 25-µg/ml concentrations of each of the following four antibiotics (Sigma): chloramphenicol, ampicillin, tetracycline, and streptomycin. Second, the incubation temperature for the CATS plates was 38°C instead of 30°C.

Growth curve fitting. Data for the log number of *Salmonella* Typhimurium DT104 cells on chicken meat portions were graphed as a function of time. The growth data were then fitted to the two-phase linear model *(2)* by using a commercial software application (Prism 5, GraphPad Software, Inc., San Diego, CA):

$$N(t) = N_0 \text{ if } t \leq \lambda$$

$$N(t) = N_0 + \mu(t - \lambda) \text{ if } t > \lambda$$

where $N(t)$ was the log number of *Salmonella* Typhimurium DT104 cells per chicken meat portion at sampling time t (in hours), N_0 was the initial log number of *Salmonella* Typhimurium DT104 cells per chicken meat portion, λ was lag time (in hours), and μ was growth rate (log per hour). The time (in hours) for a 3-log increase (t_3) in the number of *Salmonella* Typhimurium DT104 cells on chicken meat portions was calculated using the following equation:

$$t_3 = \left(\frac{3}{\mu}\right) + \lambda$$

Statistical analysis. One-way analysis of variance (ANOVA) was used to determine whether anatomical location affected ($P < 0.05$) the chemical (i.e., pH) or microbiological (i.e., native microflora) composition of chicken meat portions or growth (i.e., λ, μ, or t_3) of *Salmonella* Typhimurium DT104 on the chicken meat portions at 30°C. ANOVA was performed using a commercial software application (Prism 5, GraphPad). When ANOVA results were significant ($P < 0.05$), differences in means of dependent variables among anatomical locations were compared using Tukey's posttest.

RESULTS AND DISCUSSION

A challenge facing predictive microbiologists as they attempt to develop models in food is the additional time and cost associated with collecting the required kinetic data with complex food matrices and from small inoculum sizes. In
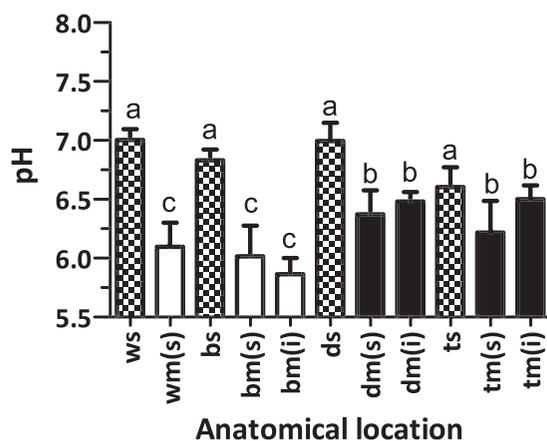
FIGURE 1. *Effect of anatomical location on the chicken carcass on pH of meat portions. See ''Materials and Methods'' for explanations of the abbreviations for anatomical locations. Bars are means ± SD. Bars with different letters differ (P < 0.05).*

the current study, the portion sizes were 1.12 ± 0.17 g (mean $\pm$ SD; $n = 30$ storage trials) for white and dark meat and 0.25 ± 0.08 g ($n = 16$ storage trials) for skin. The initial inoculum size for *Salmonella* Typhimurium DT104 in the present study was 0.92 ± 0.30 log per portion, as determined from the curve-fit-to-growth data from 46 storage trials. The small portion sizes used allowed the enumeration of low to high numbers of *Salmonella* Typhimurium DT104, using a combination of MPN and CFU methods at reduced cost because of the smaller volumes of media required *(8)*. The latter approach makes it possible and cost-effective to investigate and model microbial behavior in food with native microflora and from a low inoculum size.

The main goal of the present study was to determine whether anatomical location on the chicken carcass affects growth of *Salmonella* Typhimurium DT104 on chicken meat subjected to temperature abuse at 30°C for up to 8 h. It was believed that differences in the chemical, microbiological, and physical properties of different anatomical locations on the chicken carcass would result in differences in growth of the pathogen under the aforementioned temperature abuse scenario *(6)*. It was observed, as expected, that the pH of chicken meat samples (Fig. 1) was affected ($P < 0.001$) by anatomical location, with skin (6.86 ± 0.20) having a higher pH than dark meat (6.39 ± 0.20), which in turn had a higher pH than white meat (5.97 ± 0.20). No other measures of chemical composition were made, but it is well established that there are differences in nutrient composition among different anatomical locations and meat types of the chicken carcass.

The growth of *Salmonella* Typhimurium DT104 on chicken meat is also likely to be affected by both the numbers and types of native microflora encountered at the inoculation site. It was not possible to measure the numbers and types of native microflora encountered at the inoculation site, which would correspond to the immediate vicinity surrounding the approximately 10 cells of *Salmonella* Typhimurium DT104 inoculated onto each chicken meat portion. Nonetheless, the total number of native microflora
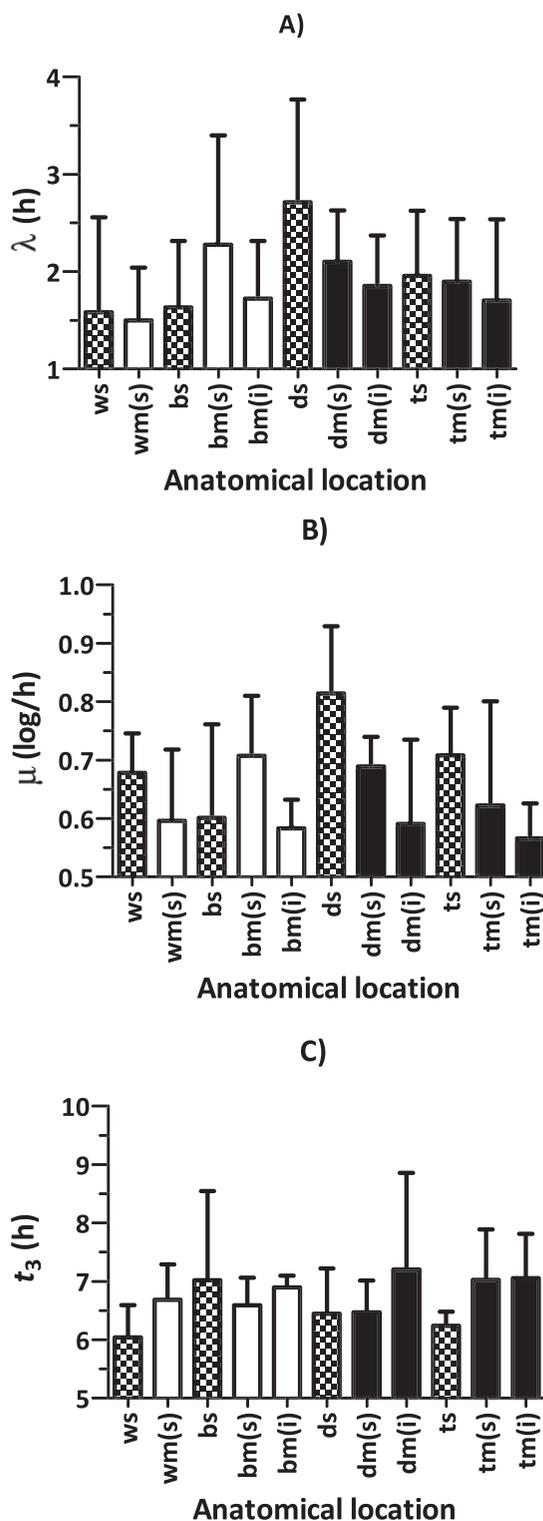

FIGURE 2. *Effect of anatomical location on the chicken carcass on (A) lag time (λ), (B) growth rate (μ), and (C) time for a 3-log increase (t₃) of* Salmonella *Typhimurium DT104 at 30°C. See ''Materials and Methods'' for explanations of the abbreviations for anatomical locations. Bars are means ± SD of four replicate storage trials except for bm(i), which represents six replicate storage trials.*

organisms recovered and subsequently able to grow on BHI agar incubated at 30°C was determined for random samples of the chicken meat portions used in the storage trials. It was observed that the initial levels of native microflora
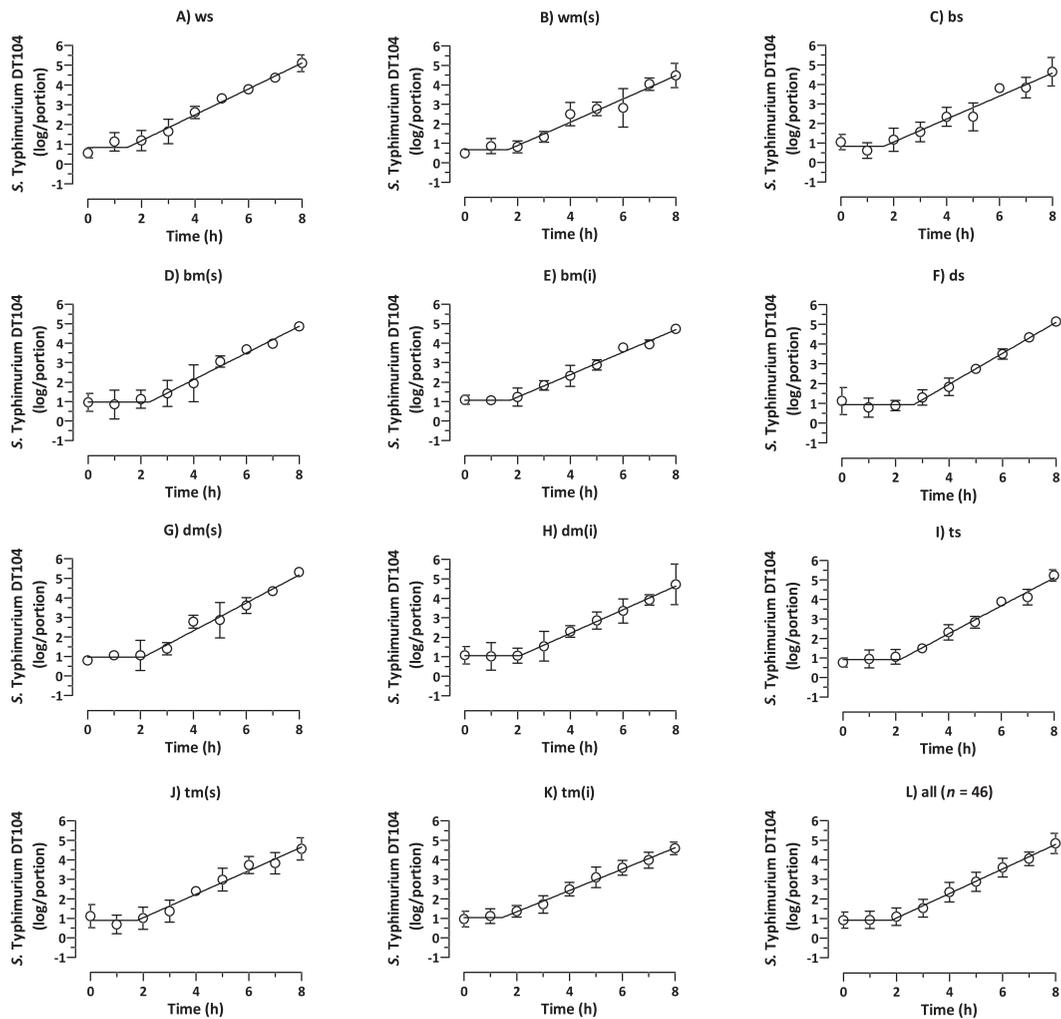
FIGURE 3. *Summary graphs of the growth of* Salmonella *Typhimurium DT104 on chicken meat portions from different anatomical locations of the chicken carcass during temperature abuse at 30°C for 8 h. Symbols are the observed values for log number (mean $\pm$ SD) from four (A to D and F to K) or six (E) replicate storage trials. The lines represent the best-fit lines from the two-phase linear model. The data and curve fit in panel L are for all of the data combined, which represent 408 data points from 46 growth curves. The model parameters from panel L are as follows: $N_0 = 0.92$ log; $\lambda = 1.84$ h; $\mu = 0.626$ h; $t_3 = 6.63$ h; and $R^2 = 0.997$ (when the model was fitted using the mean values) and 0.901 (when the model was fitted using the individual values).*

recovered were variable (4.32 ± 1.66 log per portion; $n = 45$) and did not differ ($P > 0.05$) among anatomical locations (results not shown). In 4 of 45 samples, the initial level of native microflora was at or around the spoilage level (i.e., 7 log/g); however, the growth of *Salmonella* was similar to that of meat samples with lower initial levels of native microflora (results not shown).

A physical characteristic considered in this study was the difference in skin microstructure due to differing feathering patterns on the chicken carcass. This was assessed by investigating the growth kinetics of *Salmonella* Typhimurium DT104 on skin harvested from different locations of the carcass, which included the wings, breasts, thighs, and drumsticks. It was believed that these differences in skin microstructure could alter growth of *Salmonella* Typhimurium DT104 as a result of differences in microbial competition for and availability of essentials nutrients for pathogen growth.

A second physical characteristic examined was the difference in microstructure of white and dark meat. This

was assessed by comparing growth kinetics of *Salmonella* Typhimurium DT104 inoculated onto the intact epimysium covering the surface of white or dark meat with growth kinetics of *Salmonella* Typhimurium DT104 inoculated onto the cut surface or interior of white or dark meat. It was thought that these differences in meat microstructure would alter growth of the pathogen as previously demonstrated for *Listeria innocua (6)*. More specifically, it was thought that growth of *Salmonella* Typhimurium DT104 on the meat surface would be slower due to higher microbial competition and lower availability of essential nutrients. Here it was believed that cutting the meat would release soluble nutrients from muscle fibers and thus make it easier for *Salmonella* Typhimurium DT104 to grow on the meat interior. Also, based on previous electron microscopy studies *(5, 12)*, it was believed that the inoculated cells of the pathogen would have less competition for the released nutrients from members of the native microflora, which predominate on the meat surface and are observed less often in the meat interior.

Despite expectations that anatomical location might affect growth kinetics of *Salmonella* Typhimurium DT104 on chicken meat for the aforementioned reasons, one-way ANOVA indicated that anatomical location on the chicken carcass did not alter ($P > 0.05$) lag time (1.90 ± 0.75 h), growth rate (0.648 ± 0.120 log/h), or time for a 3-log increase (6.71 ± 0.82 h) of *Salmonella* Typhimurium DT104 on chicken meat stored at $30°$C for 8 h (Fig. 2). However, there was considerable variation in growth kinetics of *Salmonella* Typhimurium DT104 among replicate storage trials in this study. Consequently, the two-phase linear model was used to fit the growth data because it is a very robust model that is capable of providing successful curve fits for data sets with considerable variation. In fact, kinetic data for growth of *Salmonella* Typhimurium DT104 on chicken meat incubated at $30°$C for 8 h fit well to a two-phase linear model with an overall coefficient of determination (R^2) of 0.9407 ± 0.0457, which was the mean $\pm$ SD among all 46 curve fits (Fig. 3).

The mean coefficients of variation among anatomical locations were 39.3% for lag time and 16.1% for growth rate. Two factors may have contributed to these high values. First, chicken samples were obtained from local retail outlets in which there was considerable variation in native microflora among batches of chicken portions, which could reflect chicken at different stages of shelf life. Second, a small inoculum size (i.e., 10 cells) of *Salmonella* Typhimurium DT104 was used in this study to simulate conditions encountered in nature, where a low level of *Salmonella* is usually observed on chicken meat contaminated with this pathogen (1, 10). A consequence of this small inoculum size could be increased variation in growth kinetics among replicate storage trials. In fact, it has been shown that variation of lag time increases as inoculum size decreases (4, 9).

Nonetheless, by calculating t_3 for the curve fits, it was possible to better assess the impact of anatomical location on growth of *Salmonella* Typhimurium DT104, as the mean coefficient of variation among anatomical locations for t_3 was only 11.0%. The results for t_3 clearly indicated that anatomical location did not affect growth of *Salmonella* Typhimurium DT104 at $30°$C (Fig. 2C). In future surveys of this type, better control of the initial level of native microflora and use of a larger inoculum size could help reduce variation of pathogen growth among replicate storage trials and result in an even better test of the study objective.

Although there were differences in pH among anatomical locations, these differences were not sufficient to affect growth of *Salmonella* Typhimurium DT104 at $30°$C for 8 h. If this observation holds for other storage conditions (e.g., other temperatures) and strains of *Salmonella,* then anatomical location does not need to be included as an independent variable in predictive models for chicken. This would save significant time and money for the predictive microbiologist.

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