Final Report

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Project Title: Determine the location and influence of physical characteristics on *Salmonella* in poultry fat intended for pet food use.

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Industry Summary

1. Introduction

Recent estimates suggest that over 25 million tons of raw animal materials are rendered in the U.S. and Canada each year (Informa Economics, 2011). The utility of the rendering practice towards the efficiency and sustainability of the meat industry is tremendous (Meeker and Meisenger, 2015). Complementary to this, assuring the safety of products generated from rendering operations is of paramount importance to the industry. In this regard, many rendering companies have taken a proactive stance in assuring the safety of their products, as is evident by the industry-wide programs (Rendering Code of Practice) and testing/monitoring practices (National Renderers Association, 2014). These programs have noted the industry's success in eliminating the threat of foodborne pathogens found in raw materials (Troutt et al., 2001).

Without question, the rendering industry has a history of concerted efforts towards the mitigation of pathogens in the rendering process; however, the implementation of the Food Safety Modernization Act (FSMA) is set to revolutionize the management of microbiological hazards in rendered red meat and poultry products. The new regulatory policies provided by FSMA will serve to transition the industry to a preventative based approach towards the management of food safety hazards. Preventative based approaches will require validation of the efficacy of the rendering process on pathogen reduction. In this regard, although previous data certainly depicts tremendous reductions in microbial populations during rendering, others (including a study conducted in our laboratory; Murphy et al., 2015) have noted the persistence of pathogens during and following processing. To date, the mechanisms by which pathogens are introduced into and persist within a complex oil matrix aren't well understood. Thus, efforts towards understanding the routes of introduction into, distribution within, and influence of physical and environmental parameters on

pathogens within rendered animal fats is an imperative component in the development of targeted interventions to assure a safe finished product.

2. Objectives

(i) Utilize fluorescently-tagged *Salmonella* to assess the distribution of *Salmonella* in a rendered fat matrix.

(ii) Assess the influence of post-inoculation time and moisture content on the distribution of fluorescently-tagged *Salmonella* in rendered poultry fat.

(iii) Assess the influence of post-inoculation time and physical parameters (i.e., impurity level and moisture content) on the survival of three *Salmonella* serotype strains in rendered poultry fat stored at 25˚C or 45˚C.

3. Summary

The purpose of this project was to enhance understanding of the distribution within and influence of physical and environmental parameters on populations of *Salmonella* within rendered poultry fat. Generation of this information can be utilized in the development of targeted interventions. Results from this study indicate that, overall, greater storage/incubation temperatures result in more lethality of *Salmonella* spp. within a poultry fat matrix. However, the rate of *Salmonella* death is influenced by strain and physical parameters (moisture content and impurity level). Equally, this study revealed that at a lower storage/incubation temperature, *Salmonella* spp. can persist in a poultry fat matrix. Overall, however, these data reiterate the importance of temperature/heat as a viable strategy for the elimination of *Salmonella* spp. in poultry fat.

Abstract

The aims of this study were to: (i) utilize fluorescently-tagged *Salmonella* to assess the distribution of *Salmonella* in a rendered fat matrix; (ii) assess the influence of post-inoculation time and moisture content on the distribution of fluorescently-tagged *Salmonella* in rendered poultry fat; and, (iii) assess the influence of post-inoculation time and physical parameters (i.e., impurity level and moisture content) on the survival of three *Salmonella* serotype strains in rendered poultry fat stored at 25˚C or 45˚C. Three studies, designated as Study I(a), I(b) and II were conducted to address the objectives outlined above. In Study I(a), a green fluorescent protein (GFP)-expressing strain of *Salmonella* Typhimurium was used to visually and microbiologically map the organism within warmed (45˚C) poultry fat formulations comprised of a low impurity level (0.2%) and three moisture contents (low: 0.5%; medium: 2.2%; high: 4.5%). In Study I(b), using the same fat formulations as in Study I(a), the survivability of the GFP-expressing *Salmonella* strain was compared in samples that were either stored at 25˚C or 45˚C. In Study II, the survivability of three *Salmonella* serotype (Enteritidis, Senftenberg, Typhimurium) strains was compared in fat formulations of two impurity levels (0.5%, 1.0%), three moisture contents (low: 0.5-0.7%; medium: 2.1-3.0%; high: 3.9-4.8%) and two temperatures (25˚C, 45˚C). Two replications were performed for Study I(a) and I(b), and three replications were performed for Study II. Surviving population of *Salmonella* Typhimurium and their location in a rendered fat matrix were achieved for each treatment combination (Study I). For Study I(b) and II, death/survival/growth curves were obtained and comparisons between time, temperature and moisture contents were made. Therefore, a better understanding of how certain physical (moisture, impurity) and environmental (temperature) characteristics on survival of *Salmonella* spp. in rendered poultry fat survival were achieved.

Key Words: poultry fat, rendering, impurity, moisture, *Salmonella*, survival

Introduction

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Without question, the rendering industry has a history of concerted efforts towards the mitigation of pathogens in the rendering process; however, the implementation of the Food Safety Modernization Act (FSMA) is set to revolutionize the management of microbiological hazards in rendered red meat and poultry products. The new regulatory policies provided by FSMA will serve to transition the industry to a preventative based approach towards the management of food safety hazards. Preventative based approaches will require validation of the efficacy of the rendering process on pathogen reduction. In this regard, although previous data certainly depicts tremendous reductions in microbial populations during rendering, others (including a study conducted in our laboratory; Murphy et al., 2015) have noted the persistence of pathogens during and following processing. To date, the mechanisms by which pathogens are introduced into and persist within a complex oil matrix aren't well understood. Thus, efforts towards understanding the routes of introduction into, distribution within, and influence of physical and environmental parameters on pathogens within rendered animal fats is an imperative component in the development of targeted interventions to assure a safe finished product.

Materials and Methods

A. Experimental Design

Three studies, designated as Study I(a), I(b) and II were conducted to address the objectives outlined above. In Study I(a), a green fluorescent protein (GFP)-expressing strain of *Salmonella* Typhimurium was used to visually and microbiologically map the organism within warmed (45˚C) poultry fat formulations comprised of a low impurity level (0.2%) and three moisture contents (low: 0.5%; medium: 2.2%; high: 4.5%). In Study I(b), using the same fat formulations as in Study I(a), the survivability of the GFP-expressing *Salmonella* strain was compared in samples that were either stored at 25˚C or 45˚C. In Study II, the survivability of three *Salmonella* serotype (Enteritidis, Senftenberg, Typhimurium) strains was compared in fat formulations of two impurity levels (0.5%, 1.0%), three moisture contents (low: 0.5-0.7%; medium: 2.1-3.0%; high: 3.9-4.8%) and two temperatures (25˚C, 45˚C).

B. Procurement and Preparation of Poultry Fat Formulations

Poultry fat comprised of “low”, “medium” and “high” impurity levels was procured with the assistance of Dr. Ansen Pond (Darling Ingredients Inc., Irving, TX). One container per impurity level was received. From each of the batches of poultry fat, three 5-g samples were removed for moisture content analysis. The poultry fat in each container was warmed to 35˚C and thoroughly mixed by vigorous stirring before removal of these aliquots. Moisture determination was performed by weighing out 1 ± 0.1 g aliquots, in duplicate, from each 5-g sample and drying the samples in a laboratory convention oven set at 60˚C, for 72 h.

Once the initial moisture content of each of the impurity levels of poultry fat were known, a Pearson Square calculator was used to determine the proportions of poultry fat and added water needed to achieve the proposed target moisture levels. Regardless of impurity level,

the target moisture levels were 0.5% ("low" moisture content), 2.5% ("medium" moisture content) and 4.5% ("high" moisture content). All poultry fat formulations were thoroughly mixed after addition of any added moisture, following which, three 5-g samples were collected for moisture analysis as described above. Additionally, samples were collected for impurity level analysis, kindly performed by Darling Analytical Labs (Butler, KY), and water activity analysis (AquaLab model series 3, Decagon Devices, Pullman, WA).

C. Salmonella *Strains*

As indicated, a GFP-expressing strain, specifically *Salmonella* Typhimurium DT104 ATCC 700408/ISSAGFP (Noah et al., 2005), was used in Study I(a) and I(b). Use of this strain allowed for visualization of the location of fluorescing cells, with a UV light source, within the inoculated poultry fat. This strain was also used in Study II, as were *Salmonella* Enteritidis (isolated from the gastrointestinal tract of live broilers) and *Salmonella* Senftenberg 775W ATCC 43845 (a well-documented heat resistant strain which was also used in a previously FPRF (Fats and Proteins Research Foundation, Inc.)-funded study (Murphy et al., 2015) (Table 1).

The strains were available as frozen cultures in the culture collection of the Food Safety/Microbiology laboratory of the Center for Meat Safety & Quality (Department of Animal Sciences, Colorado State University, Fort Collins, CO). The strains were activated by transferring an aliquot of the frozen culture into 10 ml tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) and incubating at 35˚C (20-24 h). Working cultures were maintained on xylose lysine deoxycholate agar (XLD; Acumedia-Neogen, Lansing, MI).

D. Inoculum Preparation

Two days prior to each experiment replication, an individual colony of each strain was picked off of each respective XLD agar plate and separately cultured and subcultured (35˚C, 22

± 0.5 h) in 10 ml of TSB. After incubation, broth cultures were transferred to separate sterile centrifuge tubes and cells were harvested by centrifugation (5,590 × *g*, 15 min, 4˚C; J2-MC, Beckman Coulter). Cell pellets were washed twice with 10 ml of phosphate-buffered saline (PBS, pH 7.4; PBS; Sigma-Aldrich, St. Louis, MO). After the final wash with PBS and decanting of the supernatant, cell pellets were vortexed to generate a thin layer of cells over the bottom of the centrifuge tube. The tubes, with the lids removed, were then placed under a biological safety cabinet for 2 h in an attempt to allow evaporation of some of the remaining moisture associated with the cell pellets. Following the 2-h drying period, cells were resuspended in 10 ml of pre-warmed (35˚C) autoclave-sterilized poultry fat. As shown in our previous work (Murphy et al., 2015), use of pre-warmed poultry fat prevented clumping of cells. The formulation of the poultry fat (i.e., impurity level and moisture content) used for resuspension of cells corresponded with the fat formulation to be inoculated. Inocula were vortexed and visually inspected for homogeneity before use for inoculation of poultry fat samples. The concentration of each of the prepared inocula was evaluated by serial tenfold dilution and plating of appropriate dilutions onto XLD agar and tryptic soy agar (TSA; Acumedia-Neogen). Cell concentrations of the inocula ranged from 8 to 9 log CFU/ml (data not shown).

E. Study I(a): Visual and Microbiological Mapping of GFP-expressing Salmonella *Typhimurium in Poultry Fat*

This study was initiated with the low, medium and high moisture content formulations of the lowest impurity level of poultry fat received (i.e., 0.2%; Table 2). For the first replication of this study, 15 burettes (50 ml, Eisco, India) constituting one burette per moisture level (low, medium and high) and per sampling time (0, 2, 6, 12 and 24 h) were filled with 50 ml of poultry fat and held in 50˚C incubators overnight to allow the fat to equilibrate to the test temperature.

The equilibration step was necessary since the viscosity of fat varies with temperature and a consistent temperature exposure for the duration of the trial was imperative. The aliquoted fat within the burettes was inoculated the morning following temperature equilibration, by gently depositing 50 µl of one of the prepared *Salmonella* Typhimurium inocula to the top of the fat sample. Three different *Salmonella* Typhimurium inocula were used to inoculate each of the three moisture content (i.e., low, medium and high) fat formulations; for example, burettes filled with the low moisture content poultry fat were inoculated with *Salmonella* Typhimurium cells that had been resuspended in fat of the same composition, etc.

At each of the five sampling intervals (0, 2, 6, 12 and 24 h post-inoculation), one burette per moisture content was removed from the incubator and visually inspected for fluorescence using a handheld UV light (365 nm, UVGL-58 Handheld, UVP, Upland, CA). The presence or absence of fluorescence was captured by taking photographs (Figure 1). Following visual assessment, five sequential 10 ml samples (designated as samples A through E) were obtained per burette as shown in Figure 2. The sampling time for each 10 ml aliquot was approximately 4 min. Immediately following collection, samples were microbiologically analyzed as outlined below (section H) to determine numbers of surviving bacteria over the incubation period, in addition to their location within the poultry fat matrix.

The results of the first replication of this trial indicated that there were no surviving *Salmonella* beyond the 0-h sampling time (data not shown). Based on this result, a reduced incubation temperature (45˚C) was utilized for the subsequent replications. Two replications were performed, as outlined above, at the 45˚C temperature. Regrettably there was insufficient sample material of the 0.2% impurity level to conduct a third replication.

Following completion of the portion of the study utilizing a 0.2% impurity poultry fat, a medium impurity level (0.5%) was prepared for the same evaluation as described above. However, physical impurities within the poultry fat matrix repeatedly clogged the burettes, making it impossible to aseptically remove five sequential aliquots for analysis.

F. Study I(b): Survival of Salmonella *Typhimurium in Poultry Fat Stored at 25˚C or 45˚C*

In addition to visual and microbiological mapping of the GFP-expressing *Salmonella* strain within the low, medium and high moisture content formulations of the low impurity level of fat, it was thought to be of interest to also compare the survivability of this strain within these fat formulations when stored at two incubation temperatures, specifically 25˚C and 45˚C. This study was run concurrently with the mapping study (Study I[a]). In brief, 20 ml of the three moisture content and fat formulations were separately distributed into glass bottles, in duplicate. One set was equilibrated overnight at 25˚C and the second set at 45˚C. The next morning, the 20-ml aliquots were inoculated with 20 µl of the *Salmonella* Typhimurium inoculum that corresponded with the moisture content of the fat sample. The target inoculation level was 6-7 log CFU/ml. Inoculated samples were vortexed and sampled at 0, 2, 6, 12 and 24 h post-inoculation by removing a 1-ml aliquot and analyzing it for surviving populations (section H).

G. Study 2: Comparison of Survival of Three Salmonella *Serotype Strains in Poultry Fat with Impurity Levels of 0.5% and 1.0%*

As indicated above, visualizing and microbial mapping was not capable at higher impurity levels (0.5 and 1.0%). Instead, the survivability of three *Salmonella* serotype strains (Enteritidis, Senftenberg, Typhimurium; Table 1) was evaluated in poultry fat containing either 0.5% or 1.0% impurities and formulated to attain three moisture contents (low, medium, high).

Salmonella survivability at two temperatures (25˚C, 45˚C) over a 48 h post-inoculation period was evaluated (intervals: 0, 2, 5, 8, 12, 24, 48 h). Three replications (n=3) were performed on separate days for each impurity level.

The experimental setup was similar to that described for Study I(b). Briefly, on the morning of each replication, 20-ml aliquots of the various fat formulations were separately distributed into 50 ml conical centrifuge tubes. One set of nine tubes (one per strain and moisture content; i.e., 3 strains × 3 moisture contents) was equilibrated for 2.0 ± 0.5 h in an incubator set at 25˚C and a second set of nine tubes in a 45˚C incubator (Figure 3). Following temperature equilibration, each 20-ml sample was inoculated with 20 µl of one of the prepared inocula. There were a total of nine *Salmonella* inocula; one for each *Salmonella* serotype strain, and for each strain, one for each moisture level of fat. Immediately after inoculation, samples were vortexed and 1-ml aliquots were removed and analyzed for 0-h bacterial counts (section H). Inoculated tubes were placed into their respective incubators and sampled again at 2, 5, 8, 12, 24, and 48 h post-inoculation.

H. Microbiological Analysis

The methodology developed by Murphy et al. (2015) was used for microbial analysis of the poultry fat samples. This included using warmed (35˚C; in a water bath) 0.1% buffered peptone water (Difco, Becton Dickinson) supplemented with 1% Tween 80 (VWR International, West Chester, PA) (BPW-Tween) for tenfold serial dilution of samples. Furthermore, the first dilution was always performed in a 50 ml conical centrifuge tube and this tube was vortexed for 30 s before removing an aliquot for plating or further dilution. The warm BPW-Tween, larger surface area of the conical tube compared to that of a regular glass test tube (16×150 mm), and extended vortexing time aided in emulsification of the poultry fat, and as such, homogeneous

distribution of bacterial cells within the sample. As reported by Murphy et al. (2015), these procedures mitigated phase separation of the fat samples and ultimately led to an expected tenfold serial dilution of microbial populations.

Appropriate dilutions of the fat samples were surface-plated, in duplicate, onto a selective agar for *Salmonella* (i.e., XLD agar) as well as a non-selective recovery culture medium (i.e., TSA with 1% sodium pyruvate; TSAp). Bacterial counts obtained from the XLD agar plates were those of the inoculum while those recovered with TSAp included any sub-lethally injured inoculum cells that were able to recover, as well as any background microflora associated with the fat samples. Colonies were manually counted after incubation of XLD agar plates at 35˚C for 48 h and TSAp plates at 25˚C for 72 h. The detection limit of the microbiological analysis was 1 log CFU/ml (10 CFU/ml). Uninoculated poultry fat was also analyzed for counts of any natural microflora associated with the product received, as well as for the presence or absence of any naturally-present *Salmonella* populations.

I. Statistical Analysis

Study I(a) and I(b) were replicated two times and analyzed using the Mixed Procedure of SAS version 9.4 and expressed as least squares means. No statistical comparisons were conducted for the data of Study I(a). Study I(b) was designed as a paired comparison over sampling time per treatment (Moisture Content × Sampling Time × Temperature). Data were presented as least squares means with differences reported using a significance level of $\alpha = 0.05$ and no comparisons were made between culture media. Study II was designed as a paired comparison over sampling time per treatment (Moisture Content × Strain × Sampling Time × Temperature) and was replicated three times. No comparisons were made between impurity levels and culture media. Data were analyzed using the Mixed Procedure of SAS version 9.4 and

expressed as least squares means. Data are presented as least squares means with differences reported using an α of 0.05.

Results and Discussion

A. Physical Properties of Poultry Fat Formulations

The initial moisture content of the three impurity levels (low: 0.2%; medium: 0.5%; high: 1.0%) of poultry fat, as well as the moisture content of the three moisture-content formulations (low, medium, and high) are shown in Table 2. The range in moisture content, across all impurity levels, following formulation was: Low Moisture Content- 0.5 to 0.7%; Medium Moisture Content- 2.1 to 3.0%; High Moisture Content- 3.9 to 4.8%. The water activity of each moisture content formulation within each of the three impurity levels is shown in Table 3.

B. Study I(a): Visual and Microbiological Mapping of GFP-expressing Salmonella Typhimurium in Poultry Fat

Microbial populations ranging from <1.00 to 1.70 log CFU/ml were observed in uninoculated poultry fat samples. Furthermore, no naturally-present *Salmonella* were detected in these samples.

Table 4 shows mean (log CFU/ml) surviving bacterial populations and their location (A-E; Figure 2) within the burette model. At the time of inoculation, sample aliquot A was the furthest from the point of inoculation while sample aliquot E was at the point of inoculation (Figure 2). At the 0 h sampling time, which occurred within 2 min following inoculation, *Salmonella* Typhimurium was recovered from aliquots C, D and E, irrespective of moisture content, indicating rapid migration of the cells through the fat matrix. Differences were, however, noted in concentration levels of the organism at each of these locations among the fat

formulations (Table 4). For the low moisture-content formulation, no surviving *Salmonella* Typhimurium (<1.00 log CFU/ml) were detected after the 0 h sampling time. For medium and high moisture content samples, *Salmonella* were recovered at the 2 h sampling interval for all aliquots (A through E) plated on the non-selective agar (TSAp). In comparison, corresponding *Salmonella* counts recovered with the selective agar (XLD) were 1.93- >2.67 log CFU/ml (medium moisture-content formulation), and 1.18 to 1.78 log CFU/ml (high moisture-content formulation) lower than those recovered with TSAp (Table 4). Differences in *Salmonella* Typhimurium populations recovered with the non-selective and selective agars suggests the presence of sub-lethally injured cells within the fat matrix; these cells were not able to recover and grow on the selective agar but were able to do so on the non-selective agar. Regardless, no surviving *Salmonella* Typhimurium were recovered, with either culture medium (TSAp or XLD), from any aliquots of all fat formulations sampled at 6, 12 and 24 h post-inoculation. The only exception was aliquot C of the high moisture-content product sampled at 6 h and plated on TSAp. Photos of the burettes were taken after inoculation (Figure 1) and after sampling was completed (Figure 4).

C. *Study I(b): Survival of* Salmonella *Typhimurium in Poultry Fat Stored at 25˚C or 45˚C*

Figures 5 and 6 show the death/survival curves for *Salmonella* Typhimurium in fat formulations comprised of the low impurity level (0.2%) and three moisture contents (low: 0.5%; medium: 2.2%; high: 4.5%), stored at 25˚C (Figure 5) and 45˚C (Figure 6) for up to 24 h. These data are also presented in table form (Tables 5 and 6).

As shown, inoculated populations of *Salmonella* Typhimurium (5.49 to 5.57 log CFU/ml) in the low moisture fat formulation were reduced to non-detectable levels (<1.00 log CFU/ml) by

6 h (XLD agar counts) and 12 h (TSAp counts) of incubation at 25˚C (Table 5, Figure 5). On the other hand, high numbers of surviving *Salmonella* Typhimurium (4 to 6 log CFU/ml) were still culturable in medium and high moisture-content fat samples at the end (24 h) of the 25˚C incubation period. Increasing the incubation temperature from 25˚C to 45˚C resulted in quicker lethality of *Salmonella* in low moisture poultry fat and no recoverable *Salmonella*, at or beyond 6 h incubation, were obtained in medium and high moisture-content fat samples (Table 6, Figure 6). Regardless of incubation temperature, no recoverable *Salmonella* were obtained from the low moisture poultry fat beyond 6 h of incubation. In contrast, *Salmonella* was recoverable from medium and low moisture samples incubated at 25˚C for 6 h and longer (Table 6, Figure 6).

D. Study 2: Survival of Three Salmonella *Serotype Strains in Poultry Fat with a 0.5% Impurity Level*

No *Salmonella* were recovered from the uninoculated poultry fat samples. On TSAp, generic microflora recovered from the uninoculated samples ranged from <1.00 to 1.00 log CFU/ml. Initial (0 h) populations of *Salmonella*, for each formulation, immediately following inoculation are presented in Table 7.

Death/survival curves for the three *Salmonella* serotype strains in formulations representing the medium (0.5%) impurity level and three moisture contents (low: 0.5%; medium: 2.1%; high: 3.9%), stored at 25˚C or 45˚C (48 h) are shown in Figures 7 and 8, respectively. These data are also presented in table form (Tables 8-19).

Incubation temperature had a notable effect on survival of all three strains of *Salmonella*; higher numbers of survivors were obtained at 25˚C than at 45˚C (Figures 7 and 8). Furthermore, as seen previously for the low impurity (0.2%) fat products, moisture content also had an effect on *Salmonella* survival. Populations of all three strains in the low moisture poultry fat incubated

at 25˚C steadily declined over the 48 h period (Figure 7). In contrast, *Salmonella* populations of >6 log CFU/ml were obtained even after 48 h at 25˚C in the corresponding medium and high moisture-content formulations (Figure 7). Within each incubation temperature, sporadic differences ($P < 0.05$) in survival between the three *Salmonella* strains were noted (Tables 11-13 and 17-19).

E. Study 2: Survival of Three Salmonella *Serotype Strains in Poultry Fat with a 1.0% Impurity Level*

No *Salmonella* were recovered from any of the uninoculated 1.0% impurity fat samples. Generic microflora recovered with TSAp ranged from <1.00 to 3.61 log CFU/ml. Inoculated levels of the three *Salmonella* serotypes, for each tested fat formulation, are presented in Table 7.

Figures 9 and 10 show the death/survival/growth curves of the three *Salmonella* serotype strains in poultry fat representing the high (1.0%) impurity level and three moisture contents (low: 0.7%; medium: 3.0%; high: 4.8%), stored at 25˚C (Figure 9) and 45˚C (Figure 10) for up to 48 h. These data are also presented in table form (Tables 20-31).

When fat formulations were incubated at 25˚C, *Salmonella* levels in the low moisture fat steadily declined over time whereas those in the medium moisture content product remained relatively unchanged throughout the incubation period (Figure 9). In contrast, growth ($P < 0.05$) of *Salmonella* was obtained in the high moisture fat formulation incubated at 25˚C (Figure 9). Specifically, *Salmonella* populations, regardless of strain, increased by up to 1 log CFU/ml (TSAp: 0.73-1.04 log CFU/ml; XLD: 0.97-1.07 log CFU/ml) (Figure 9, Table 25). Likely reasons for the observed growth are a combination of the relatively high water activity (0.918 ± 0.023; Table 3), high moisture content (4.8 ± 0.31%; Table 2) and high impurity level (1.0%) of

the formulation. As noted for the 0.5% impurity level, temperature had an effect on the survival of the *Salmonella* strains in the 1.0% impurity formulations.

Conclusions

These data suggest the control of moisture content, temperature, impurity level and water activity is very important for controlling survival of *Salmonella* spp. in poultry fat. Based on our experimental design, statistical comparison of data could not be performed among the three impurity levels; however, trends indicated that lower impurity levels of fat were not necessarily better at controlling survival of *Salmonella*. Storage of poultry fat with medium or high moisture content at 25˚C allowed survival of large populations of *Salmonella*, and even permitted growth of the pathogen when a high impurity level, high moisture content and high water activity were present.

Based on the observations of this work, it can be concluded that low impurity poultry fat with low moisture content that is stored at a high temperature (45˚C and above) for a period of time would effectively control *Salmonella* contamination in poultry fat. The findings of the study provided a better understanding on how certain physical characteristics (moisture, impurity, water activity) and temperature influence the survival of *Salmonella* spp. in poultry fat.

While this study yielded valuable information related to the influence of physical parameters on *Salmonella* survivability in poultry fat, more research is necessary to better understand why *Salmonella* spp. survivability in poultry fat differs among impurity levels. Additionally, although limited in its scope, the burette portion of this study resulted in the visual observation of fluorescently-tagged *Salmonella* Typhimurium on the sides of the burette (Figure 4). This suggests the potential for biofilm formation, or persisting *Salmonella* in storage

vessels—which may result in cross-contamination or reintroduction of pathogens into a sample matrix. Additional research with industry-representative vessels would be useful in determining this possibility.

Table 1. Strains of *Salmonella* spp. used in the study.

***Salmonella* Serotype**	**Strain ID**	**Source**
Salmonella Enteritidis	FFSRU SE NN	Dr. Thomas Edrington (USDA-ARS-SPA, Food and Feed Safety Research Unit, College Station, TX)
Salmonella Senftenberg 775W	ATCC 43845	--
Salmonella Typhimurium DT104	ATCC 700408/ISSAGFP	Noah et al. (2005)

Table 2. Initial moisture content (%; mean ± SD) and ending moisture content (%; mean ± SD) for each impurity level of poultry fat.

Impurity level (%)	**Initial Moisture Content**	**Moisture Content of Fat Formulations After Addition of Moisture**		
		Low Moisture	**Medium Moisture**	**High Moisture**
0.2 ± 0.0	0.4 ± 0.1	0.5 ± 0.2	2.2 ± 0.9	4.5 ± 0.1
0.5 ± 0.1	0.1 ± 0.1	0.5 ± 0.1	2.1 ± 0.5	3.9 ± 0.5
1.0 ± 0.0	0.0 ± 0.1	0.7 ± 0.2	3.0 ± 0.2	4.8 ± 0.3

SD: standard deviation.

Table 3. Water activity values of each poultry fat formulation.

Impurity level (%)	Water Activity (Mean ± SD)		
	Low Moisture[1]	Medium Moisture[1]	High Moisture[1]
0.2 ± 0.0	0.461 ± 0.010	0.905 ± 0.003	0.923 ± 0.003
0.5 ± 0.1	0.571 ± 0.012	0.842 ± 0.010	0.910 ± 0.005
1.0 ± 0.0	0.554 ± 0.005	0.837 ± 0.025	0.918 ± 0.023

SD: standard deviation.
[1]Refer to Table 2 for moisture content details.

Table 4. Effect of time and moisture content (low: 0.5%; medium: 2.2%; high: 4.5%) on *Salmonella* Typhimurium populations (log CFU/ml) recovered from poultry fat with a 0.2% impurity content, incubated at 45˚C for up to 24 h.

Medium	Time (h)	Sample	Low moisture		Medium moisture		High moisture	
			LSMean	SEM	LSMean	SEM	LSMean	SEM
TSAp	0	A	<1.00	0.46	<1.00	0.46	<1.00	0.46
		B	<1.00	0.46	<1.00	0.46	<1.00	0.46
		C	2.75	0.46	4.74	0.46	5.34	0.46
		D	4.86	0.46	4.69	0.46	6.70	0.46
		E	5.96	0.46	6.63	0.46	7.11	0.46
	2	A	<1.00	0.46	3.97	0.46	5.18	0.46
		B	<1.00	0.46	1.35	0.46	4.18	0.46
		C	<1.00	0.46	3.67	0.46	4.19	0.46
		D	<1.00	0.46	2.83	0.46	3.82	0.46
		E	<1.00	0.46	2.66	0.46	4.29	0.46
	6	A	<1.00	0.46	<1.00	0.46	<1.00	0.46
		B	<1.00	0.46	<1.00	0.46	<1.00	0.46
		C	<1.00	0.46	<1.00	0.46	1.00	0.46
		D	<1.00	0.46	<1.00	0.46	<1.00	0.46
		E	<1.00	0.46	<1.00	0.46	<1.00	0.46
	12	A	<1.00	0.46	<1.00	0.46	<1.00	0.46
		B	<1.00	0.46	<1.00	0.46	<1.00	0.46
		C	<1.00	0.46	<1.00	0.46	<1.00	0.46
		D	<1.00	0.46	<1.00	0.46	<1.00	0.46
		E	<1.00	0.46	<1.00	0.46	<1.00	0.46
	24	A	<1.00	0.46	<1.00	0.46	<1.00	0.46
		B	<1.00	0.46	<1.00	0.46	<1.00	0.46
		C	<1.00	0.46	<1.00	0.46	<1.00	0.46
		D	<1.00	0.46	<1.00	0.46	<1.00	0.46
		E	<1.00	0.46	<1.00	0.46	<1.00	0.46
XLD	0	A	<1.00	0.40	<1.00	0.40	<1.00	0.40
		B	<1.00	0.40	<1.00	0.40	<1.00	0.40
		C	2.16	0.40	4.59	0.40	5.24	0.40

	D	4.61	0.40	5.40	0.40	6.57	0.40
	E	5.12	0.40	6.77	0.40	7.04	0.40
2	A	<1.00	0.40	2.04	0.40	4.00	0.40
	B	<1.00	0.40	<1.00	0.40	2.75	0.40
	C	<1.00	0.40	<1.00	0.40	2.46	0.40
	D	<1.00	0.40	<1.00	0.40	2.45	0.40
	E	<1.00	0.40	<1.00	0.40	2.51	0.40
6	A	<1.00	0.40	<1.00	0.40	<1.00	0.40
	B	<1.00	0.40	<1.00	0.40	<1.00	0.40
	C	<1.00	0.40	<1.00	0.40	<1.00	0.40
	D	<1.00	0.40	<1.00	0.40	<1.00	0.40
	E	<1.00	0.40	<1.00	0.40	<1.00	0.40
12	A	<1.00	0.40	<1.00	0.40	<1.00	0.40
	B	<1.00	0.40	<1.00	0.40	<1.00	0.40
	C	<1.00	0.40	<1.00	0.40	<1.00	0.40
	D	<1.00	0.40	<1.00	0.40	<1.00	0.40
	E	<1.00	0.40	<1.00	0.40	<1.00	0.40
24	A	<1.00	0.40	<1.00	0.40	<1.00	0.40
	B	<1.00	0.40	<1.00	0.40	<1.00	0.40
	C	<1.00	0.40	<1.00	0.40	<1.00	0.40
	D	<1.00	0.40	<1.00	0.40	<1.00	0.40
	E	<1.00	0.40	<1.00	0.40	<1.00	0.40

Sample A-E: Sample aliquots were collected as shown in Figure 2.
SEM: standard error of the mean.
LSMean: Least squares means.
Detection limit = 1.00 log CFU/ml

Table 5. Effect of time and moisture content (low: 0.5%; medium: 2.2%; high: 4.5%) on *Salmonella* Typhimurium populations (log CFU/ml) recovered from poultry fat with a 0.2% impurity content, incubated at 25˚C for up to 24 h.

Medium	Time (h)	Low moisture		Medium moisture		High moisture		*P-value*
		LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	0	5.57^{b}	0.54	6.91^{ab}	0.54	7.04^{a}	0.54	0.0234
	2	2.35^{b*}	0.54	6.61^{a}	0.54	6.65^{a}	0.54	<0.0001
	6	1.00^{b*}	0.54	6.50^{a}	0.54	6.47^{a}	0.54	<0.0001
	12	$<1.00^{b*}$	0.54	5.97^{a}	0.54	6.35^{a}	0.54	<0.0001
	24	$<1.00^{b*}$	0.54	4.84^{a*}	0.54	6.02^{a}	0.54	<0.0001
XLD	0	5.49^{b}	0.54	6.93^{a}	0.54	7.01^{a}	0.54	0.0160
	2	3.28^{b*}	0.54	6.40^{a}	0.54	6.53^{a}	0.54	<0.0001
	6	$<1.00^{b*}$	0.54	6.25^{a}	0.54	6.39^{a}	0.54	<0.0001
	12	$<1.00^{b*}$	0.54	5.67^{a}	0.54	6.04^{a}	0.54	<0.0001
	24	$<1.00^{b*}$	0.54	4.18^{a*}	0.54	5.17^{a*}	0.54	<0.0001

SEM: standard error of the mean.
$^{a\text{-}c}$: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$).
Detection limit = 1 log CFU/ml
*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column and within each culture medium.

Table 6. Effect of time and moisture content (low: 0.5%; medium: 2.2%; high: 4.5%) on *Salmonella* Typhimurium populations (log CFU/ml) recovered from poultry fat with a 0.2% impurity content, incubated at 45˚C for up to 24 h.

Medium	Time (h)	Low moisture		Medium moisture		High moisture		*P-value*
		LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	0	5.48^{b}	0.18	7.09^{a}	0.18	7.01^{a}	0.18	<0.0001
	2	$<1.00^{b*}$	0.18	5.82^{a*}	0.18	5.98^{a*}	0.18	<0.0001
	6	$<1.00^{a*}$	0.18	$<1.00^{a*}$	0.18	$<1.00^{a*}$	0.18	1.0000
	12	$<1.00^{a*}$	0.18	$<1.00^{a*}$	0.18	$<1.00^{a*}$	0.18	1.0000
	24	$<1.00^{a*}$	0.18	$<1.00^{a*}$	0.18	$<1.00^{a*}$	0.18	1.0000
XLD	0	5.47^{b}	0.18	6.90^{a}	0.18	6.93^{a}	0.18	<0.0001
	2	$<1.00^{c*}$	0.18	4.73^{b*}	0.18	5.72^{a*}	0.18	<0.0001
	6	$<1.00^{a*}$	0.18	$<1.00^{a*}$	0.18	$<1.00^{a*}$	0.18	1.0000
	12	$<1.00^{a*}$	0.18	$<1.00^{a*}$	0.18	$<1.00^{a*}$	0.18	1.0000
	24	$<1.00^{a*}$	0.18	$<1.00^{a*}$	0.18	$<1.00^{a*}$	0.18	1.0000

SEM: standard error of the mean.

$^{a-c}$: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$).

Detection limit = 1 log CFU/ml

*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column and within each culture medium.

Table 7. Initial (inoculated) populations (log CFU/ml; mean ± SD) of *Salmonella* spp. strains immediately following inoculation (0 h) as recovered on XLD agar.

			Moisture Content		
Impurity (%)	**Temperature (˚C)**	***Salmonella* Serotype**	**Low**	**Medium**	**High**
0.5	25	*Salmonella* Enteritidis	6.37 ± 0.30	6.63 ± 0.18	6.94 ± 0.09
		Salmonella Senftenberg	5.73 ± 0.31	6.64 ± 0.07	6.59 ± 0.12
		Salmonella Typhimurium	6.74 ± 0.25	6.97 ± 0.09	6.98 ± 0.07
0.5	45	*Salmonella* Enteritidis	5.68 ± 0.47	6.75 ± 0.05	6.82 ± 0.20
		Salmonella Senftenberg	5.08 ± 0.60	6.52 ± 0.08	6.78 ± 0.08
		Salmonella Typhimurium	6.24 ± 0.24	7.03 ± 0.07	7.00 ± 0.05
1.0	25	*Salmonella* Enteritidis	5.72 ± 0.26	6.89 ± 0.07	6.88 ± 0.06
		Salmonella Senftenberg	5.50 ± 0.27	6.66 ± 0.16	6.67 ± 0.11
		Salmonella Typhimurium	6.23 ± 0.18	7.03 ± 0.19	6.98 ± 0.24
1.0	45	*Salmonella* Enteritidis	4.59 ± 0.46	6.75 ± 0.11	6.86 ± 0.16
		Salmonella Senftenberg	4.28 ± 1.00	6.58 ± 0.16	6.64 ± 0.25
		Salmonella Typhimurium	5.48 ± 0.29	7.02 ± 0.21	7.17 ± 0.15

SD: standard deviation.

Table 8. Effect of time and moisture content (low: 0.5%; medium: 2.1%; high: 3.9%) on *Salmonella* Enteritidis populations (log CFU/ml) recovered from poultry fat with a 0.5% impurity content, incubated at 25˚C for up to 48 h.

Medium	Time (h)	Low moisture		Medium moisture		High moisture		
		LSMean	SEM	LSMean	SEM	LSMean	SEM	*P-value*
TSAp	0	6.53[a]	0.14	6.66[a]	0.14	6.92[a]	0.14	0.1212
	2	4.95[b*]	0.14	6.88[a]	0.14	6.88[a]	0.14	<0.0001
	5	3.77[b*]	0.14	6.85[a]	0.14	6.89[a]	0.14	<0.0001
	8	3.32[b*]	0.14	6.83[a]	0.14	6.92[a]	0.14	<0.0001
	12	2.47[b*]	0.14	6.79[a]	0.14	6.96[a]	0.14	<0.0001
	24	1.20[b*]	0.14	6.77[a]	0.14	6.84[a]	0.14	<0.0001
	48	<1.00[b*]	0.14	6.56[a]	0.14	6.74[a]	0.14	<0.0001
XLD	0	6.37[b]	0.14	6.63[b]	0.14	6.94[a]	0.14	0.0126
	2	4.85[b*]	0.14	6.70[a]	0.14	6.92[a]	0.14	<0.0001
	5	3.51[b*]	0.14	6.81[a]	0.14	6.82[a]	0.14	<0.0001
	8	3.19[b*]	0.14	6.72[a]	0.14	6.84[a]	0.14	<0.0001
	12	2.35[b*]	0.14	6.75[a]	0.14	6.81[a]	0.14	<0.0001
	24	<1.00[b*]	0.14	6.44[a]	0.14	6.71[a]	0.14	<0.0001
	48	<1.00[b*]	0.14	6.19[a]	0.14	6.50[a]	0.14	<0.0001

SEM: standard error of the mean.
[a-c]: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$).
Detection limit = 1 log CFU/ml
*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column and within each culture medium.

Table 9. Effect of time and moisture content (low: 0.5%; medium: 2.1%; high: 3.9%) on *Salmonella* Senftenberg populations (log CFU/ml) recovered from poultry fat with a 0.5% impurity content, incubated at 25˚C for up to 48 h.

Medium	Time (h)	Low moisture		Medium moisture		High moisture		*P-value*
		LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	0	5.98[b]	0.14	6.81[a]	0.14	6.76[a]	0.14	<0.0001
	2	4.89[b*]	0.14	6.68[a]	0.14	6.80[a]	0.14	<0.0001
	5	4.16[b*]	0.14	6.70[a]	0.14	6.82[a]	0.14	<0.0001
	8	3.89[b*]	0.14	6.61[a]	0.14	6.80[a]	0.14	<0.0001
	12	3.58[b*]	0.14	6.64[a]	0.14	6.77[a]	0.14	<0.0001
	24	3.18[b*]	0.14	6.61[a]	0.14	6.86[a]	0.14	<0.0001
	48	2.71[b*]	0.14	6.49[a]	0.14	6.77[a]	0.14	<0.0001
XLD	0	5.73[b]	0.14	6.64[a]	0.14	6.59[a]	0.14	<0.0001
	2	4.58[b*]	0.14	6.44[a]	0.14	6.67[a]	0.14	<0.0001
	5	3.95[b*]	0.14	6.42[a]	0.14	6.66[a]	0.14	<0.0001
	8	3.77[b*]	0.14	6.47[a]	0.14	6.72[a]	0.14	<0.0001
	12	3.25[b*]	0.14	6.41[a]	0.14	6.60[a]	0.14	<0.0001
	24	2.95[b*]	0.14	6.27[a]	0.14	6.64[a]	0.14	<0.0001
	48	2.35[b*]	0.14	6.08[a*]	0.14	6.40[a]	0.14	<0.0001

SEM: standard error of the mean.
[a-c]: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$).
Detection limit = 1 log CFU/ml
*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column and within each culture medium.

Table 10. Effect of time and moisture content (low: 0.5%; medium: 2.1%; high: 3.9%) on *Salmonella* Typhimurium populations (log CFU/ml) recovered from poultry fat with a 0.5% impurity content, incubated at 25˚C for up to 48 h.

Medium	Time (h)	Low moisture		Medium moisture		High moisture		*P-value*
		LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	0	6.65[a]	0.14	6.96[a]	0.14	7.08[a]	0.14	0.0664
	2	5.45[b*]	0.14	7.08[a]	0.14	7.08[a]	0.14	<0.0001
	5	4.75[b*]	0.14	7.03[a]	0.14	7.03[a]	0.14	<0.0001
	8	4.45[b*]	0.14	6.98[a]	0.14	7.00[a]	0.14	<0.0001
	12	3.67[b*]	0.17	7.09[a]	0.14	7.00[a]	0.17	<0.0001
	24	1.62[b*]	0.14	6.83[a]	0.14	6.95[a]	0.14	<0.0001
	48	1.10[b*]	0.14	6.70[a]	0.14	6.91[a]	0.14	<0.0001
XLD	0	6.74[a]	0.14	6.97[a]	0.14	6.98[a]	0.14	0.3541
	2	5.25[b*]	0.14	6.92[a]	0.14	7.06[a]	0.14	<0.0001
	5	4.07[b*]	0.14	6.94[a]	0.14	6.90[a]	0.14	<0.0001
	8	4.09[b*]	0.14	6.85[a]	0.14	6.95[a]	0.14	<0.0001
	12	2.61[b*]	0.17	6.80[a]	0.14	6.94[a]	0.17	<0.0001
	24	<1.00[b*]	0.14	6.56[a]	0.14	6.76[a]	0.14	<0.0001
	48	<1.00[b*]	0.14	6.09[a*]	0.14	6.52[a*]	0.14	<0.0001

SEM: standard error of the mean.
[a-c]: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$).
Detection limit = 1 log CFU/ml
*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column and within each culture medium.

Table 11. *Salmonella* spp. populations (log CFU/ml) recovered from poultry fat with a 0.5% impurity level and low moisture content (0.5%), incubated at 25˚C for up to 48 h.

Medium	Time (h)	*Salmonella* Enteritidis		*Salmonella* Senftenberg		*Salmonella* Typhimurium		
		LSMean	SEM	LSMean	SEM	LSMean	SEM	*P-value*
TSAp	0	6.53[a]	0.14	5.98[b]	0.14	6.65[a]	0.14	0.0010
	2	4.95[b*]	0.14	4.89[b*]	0.14	5.45[a*]	0.14	0.0058
	5	3.77[b*]	0.14	4.16[b*]	0.14	4.75[a*]	0.14	<0.0001
	8	3.32[c*]	0.14	3.89[b*]	0.14	4.45[a*]	0.14	<0.0001
	12	2.47[b*]	0.14	3.58[a*]	0.14	3.67[a*]	0.17	<0.0001
	24	1.20[b*]	0.14	3.18[a*]	0.14	1.62[b*]	0.14	<0.0001
	48	<1.00[b*]	0.14	2.71[a*]	0.14	1.10[b*]	0.14	<0.0001
XLD	0	6.37[a]	0.14	5.73[b]	0.14	6.74[a]	0.14	<0.0001
	2	4.85[b*]	0.14	4.58[b*]	0.14	5.25[a*]	0.14	0.0023
	5	3.51[b*]	0.14	3.95[ab*]	0.14	4.07[a*]	0.14	0.0097
	8	3.19[b*]	0.14	3.77[a*]	0.14	4.09[a*]	0.14	<0.0001
	12	2.35[b*]	0.14	3.25[a*]	0.14	2.61[b*]	0.17	<0.0001
	24	<1.00[b*]	0.14	2.95[a*]	0.14	<1.00[b*]	0.14	<0.0001
	48	<1.00[b*]	0.14	2.35[a*]	0.14	<1.00[b*]	0.14	<0.0001

SEM: standard error of the mean.

[a-c]: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$). Detection limit = 1 log CFU/ml

*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column and within each culture medium.

Table 12. *Salmonella* spp. populations (log CFU/ml) recovered from poultry fat with a 0.5% impurity level and medium moisture content (2.1%), incubated at 25˚C for up to 48 h.

Medium	Time (h)	*Salmonella* Enteritidis		*Salmonella* Senftenberg		*Salmonella* Typhimurium		
		LSMean	SEM	LSMean	SEM	LSMean	SEM	*P-value*
TSAp	0	6.66[a]	0.14	6.81[a]	0.14	6.96[a]	0.14	0.2889
	2	6.88[a]	0.14	6.68[a]	0.14	7.08[a]	0.14	0.1072
	5	6.85[a]	0.14	6.70[a]	0.14	7.03[a]	0.14	0.2354
	8	6.83[a]	0.14	6.61[a]	0.14	6.98[a]	0.14	0.1484
	12	6.79[ab]	0.14	6.64[b]	0.14	7.09[a]	0.14	0.0564
	24	6.77[a]	0.14	6.61[a]	0.14	6.83[a]	0.14	0.4954
	48	6.56[a]	0.14	6.49[a]	0.14	6.70[a]	0.14	0.5119
XLD	0	6.63[a]	0.14	6.64[a]	0.14	6.97[a]	0.14	0.1273
	2	6.70[ab]	0.14	6.44[b]	0.14	6.92[a]	0.14	0.0412
	5	6.81[ab]	0.14	6.42[b]	0.14	6.94[a]	0.14	0.0190
	8	6.72[a]	0.14	6.47[a]	0.14	6.85[a]	0.14	0.1268
	12	6.75[a]	0.14	6.41[a]	0.14	6.80[a]	0.14	0.0849
	24	6.44[a]	0.14	6.27[a]	0.14	6.56[a]	0.14	0.3172
	48	6.19[a]	0.14	6.08[a*]	0.14	6.09[a*]	0.14	0.8131

SEM: standard error of the mean.
[a-c]: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$).
Detection limit = 1 log CFU/ml
*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column and within each culture medium.

Table 13. *Salmonella* spp. populations (log CFU/ml) recovered from poultry fat with a 0.5% impurity level and high moisture content (3.9%), incubated at 25˚C for up to 48 h.

Medium	Time (h)	*Salmonella* Enteritidis		*Salmonella* Senftenberg		*Salmonella* Typhimurium		*P-value*
		LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	0	6.92[a]	0.14	6.76[a]	0.14	7.08[a]	0.14	0.2364
	2	6.88[a]	0.14	6.80[a]	0.14	7.08[a]	0.14	0.3080
	5	6.89[a]	0.14	6.82[a]	0.14	7.03[a]	0.14	0.5465
	8	6.92[a]	0.14	6.80[a]	0.14	7.00[a]	0.14	0.5889
	12	6.96[a]	0.14	6.77[a]	0.14	7.00[a]	0.17	0.4697
	24	6.84[a]	0.14	6.86[a]	0.14	6.95[a]	0.14	0.8081
	48	6.74[a]	0.14	6.77[a]	0.14	6.91[a]	0.14	0.6345
XLD	0	6.94[a]	0.14	6.59[a]	0.14	6.98[a]	0.14	0.0824
	2	6.92[a]	0.14	6.67[a]	0.14	7.06[a]	0.14	0.1202
	5	6.82[a]	0.14	6.66[a]	0.14	6.90[a]	0.14	0.4327
	8	6.84[a]	0.14	6.72[a]	0.14	6.95[a]	0.14	0.4732
	12	6.81[a]	0.14	6.60[a]	0.14	6.94[a]	0.17	0.2589
	24	6.71[a]	0.14	6.64[a]	0.14	6.76[a]	0.14	0.8166
	48	6.50[a]	0.14	6.40[a]	0.14	6.52[a*]	0.14	0.7877

SEM: standard error of the mean.
[a-c]: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$).
Detection limit = 1 log CFU/ml
*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column and within each culture medium.

Table 14. Effect of time and moisture content (low: 0.5%; medium: 2.1%; high: 3.9%) on *Salmonella* Enteritidis populations (log CFU/ml) recovered from poultry fat with a 0.5% impurity content, incubated at 45˚C for up to 48 h.

Medium	Time (h)	Low moisture		Medium moisture		High moisture		*P-value*
		LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	0	5.82^{b}	0.21	6.87^{a}	0.21	6.92^{a}	0.21	<0.0001
	2	1.39^{b*}	0.21	6.14^{a*}	0.21	6.31^{a}	0.21	<0.0001
	5	1.41^{b*}	0.21	4.93^{a*}	0.21	5.43^{a*}	0.21	<0.0001
	8	1.77^{b*}	0.21	2.38^{b*}	0.21	3.42^{a*}	0.21	<0.0001
	12	1.20^{a*}	0.21	1.40^{a*}	0.21	1.45^{a*}	0.21	0.6389
	24	<1.00^{b*}	0.21	<1.00^{b*}	0.21	1.30^{a*}	0.21	0.4692
	48	<1.00^{b*}	0.21	1.10^{a*}	0.21	<1.00^{b*}	0.21	0.9191
XLD	0	5.68^{b}	0.21	6.75^{a}	0.21	6.82^{a}	0.21	<0.0001
	2	<1.00^{b*}	0.21	5.78^{a*}	0.21	6.00^{a*}	0.21	<0.0001
	5	<1.00^{b*}	0.21	4.19^{a*}	0.21	4.78^{a*}	0.21	<0.0001
	8	<1.00^{c*}	0.21	1.88^{b*}	0.21	3.53^{a*}	0.21	<0.0001
	12	<1.00^{b*}	0.21	<1.00^{b*}	0.21	1.30^{a*}	0.21	0.4692
	24	<1.00^{a*}	0.21	<1.00^{a*}	0.21	<1.00^{a*}	0.21	1.0000
	48	<1.00^{a*}	0.21	<1.00^{a*}	0.21	<1.00^{a*}	0.21	1.0000

SEM: standard error of the mean.

$^{a\text{-}c}$: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$).

Detection limit = 1 log CFU/ml

*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column and within each culture medium.

Table 15. Effect of time and moisture content (low: 0.5%; medium: 2.1%; high: 3.9%) on *Salmonella* Senftenberg populations (log CFU/ml) recovered from poultry fat with a 0.5% impurity, incubated at 45˚C for up to 48 h.

Medium	Time (h)	Low moisture		Medium moisture		High moisture		*P-value*
		LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	0	5.40[b]	0.21	6.71[a]	0.21	6.79[a]	0.21	<0.0001
	2	1.48[b*]	0.21	6.35[a]	0.21	6.64[a]	0.21	<0.0001
	5	<1.00[b*]	0.21	5.74[a*]	0.21	5.89[a*]	0.21	<0.0001
	8	<1.00[b*]	0.21	4.55[a*]	0.21	5.09[a*]	0.21	<0.0001
	12	1.68[c*]	0.21	2.60[b*]	0.21	3.66[a*]	0.21	<0.0001
	24	<1.00[a*]	0.21	<1.00[a*]	0.21	<1.00[a*]	0.21	1.0000
	48	<1.00[a*]	0.21	<1.00[a*]	0.21	<1.00[a*]	0.21	1.0000
XLD	0	5.08[b]	0.21	6.52[a]	0.21	6.78[a]	0.21	<0.0001
	2	1.38[b*]	0.21	6.03[a]	0.21	6.12[a]	0.21	<0.0001
	5	<1.00[b*]	0.21	5.12[a*]	0.21	5.43[a*]	0.21	<0.0001
	8	1.16[b*]	0.21	3.98[a*]	0.21	4.62[a*]	0.21	<0.0001
	12	<1.00[c*]	0.21	2.24[b*]	0.21	3.09[a*]	0.21	<0.0001
	24	<1.00[a*]	0.21	<1.00[a*]	0.21	<1.00[a*]	0.21	1.0000
	48	<1.00[a*]	0.21	<1.00[a*]	0.21	<1.00[a*]	0.21	1.0000

SEM: standard error of the mean.
[a-c]: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$).
Detection limit = 1 log CFU/ml
*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column and within each culture medium.

Table 16. Effect of time and moisture content (low: 0.5%; medium: 2.1%; high: 3.9%) on *Salmonella* Typhimurium populations (log CFU/ml) recovered from poultry fat with a 0.5% impurity content, incubated at 45˚C for up to 48 h.

Medium	Time (h)	Low moisture		Medium moisture		High moisture		*P-value*
		LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	0	6.45[a]	0.21	7.09[a]	0.21	7.10[a]	0.21	0.0313
	2	1.28[b*]	0.21	6.18[a*]	0.21	6.56[a]	0.21	<0.0001
	5	<1.00[b*]	0.21	5.13[a*]	0.21	5.55[a*]	0.21	<0.0001
	8	<1.00[b*]	0.21	3.45[a*]	0.21	3.89[a*]	0.21	<0.0001
	12	<1.00[b*]	0.21	<1.00[b*]	0.21	1.16[a*]	0.21	0.8092
	24	<1.00[a*]	0.21	<1.00[a*]	0.21	<1.00[a*]	0.21	1.0000
	48	<1.00[a*]	0.21	<1.00[a*]	0.21	<1.00[a*]	0.21	1.0000
XLD	0	6.24[b]	0.21	7.03[a]	0.21	7.00[a]	0.21	0.0075
	2	1.28[b*]	0.21	5.78[a*]	0.21	6.15[a*]	0.21	<0.0001
	5	<1.00[b*]	0.21	4.45[a*]	0.21	4.73[a*]	0.21	<0.0001
	8	<1.00[b*]	0.21	3.19[a*]	0.21	3.47[a*]	0.21	<0.0001
	12	<1.00[b*]	0.21	<1.00[b*]	0.21	1.30[a*]	0.21	0.4692
	24	<1.00[a*]	0.21	<1.00[a*]	0.21	<1.00[a*]	0.21	1.0000
	48	<1.00[a*]	0.21	<1.00[a*]	0.21	<1.00[a*]	0.21	1.0000

SEM: standard error of the mean.
[a-c]: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$).
Detection limit = 1 log CFU/ml
*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column and within each culture medium.

Table 17. *Salmonella* spp. populations (log CFU/ml) recovered from poultry fat with a 0.5% impurity level and low moisture content (0.5%), incubated at 45˚C for up to 48 h.

Medium	Time (h)	*Salmonella* Enteritidis		*Salmonella* Senftenberg		*Salmonella* Typhimurium		*P-value*
		LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	0	5.82^{ab}	0.21	5.40^{b}	0.21	6.45^{a}	0.21	0.0011
	2	1.39^{a*}	0.21	1.48^{a*}	0.21	1.28^{a*}	0.21	0.7760
	5	1.41^{a*}	0.21	$<1.00^{b*}$	0.21	$<1.00^{b*}$	0.21	0.2463
	8	1.77^{a*}	0.21	$<1.00^{b*}$	0.21	$<1.00^{b*}$	0.21	0.0074
	12	1.20^{a*}	0.21	1.68^{a*}	0.21	$<1.00^{b*}$	0.21	0.0469
	24	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	1.0000
	48	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	1.0000
XLD	0	5.68^{ab}	0.21	5.08^{b}	0.21	6.24^{a}	0.21	0.0003
	2	$<1.00^{b*}$	0.21	1.38^{a*}	0.21	1.28^{a*}	0.21	0.3746
	5	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	1.0000
	8	$<1.00^{b*}$	0.21	1.16^{a*}	0.21	$<1.00^{b*}$	0.21	0.8092
	12	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	1.0000
	24	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	1.0000
	48	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	1.0000

SEM: standard error of the mean.

$^{a-c}$: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$). Detection limit = 1 log CFU/ml

*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column and within each culture medium.

Table 18. *Salmonella* spp. populations (log CFU/ml) recovered from poultry fat with a 0.5% impurity level and medium moisture content (2.1%), incubated at 45˚C for up to 48 h.

Medium	Time (h)	*Salmonella* Enteritidis		*Salmonella* Senftenberg		*Salmonella* Typhimurium		*P-value*
		LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	0	6.87^{a}	0.21	6.71^{a}	0.21	7.09^{a}	0.21	0.4034
	2	6.14^{a*}	0.21	6.35^{a}	0.21	6.18^{a*}	0.21	0.7216
	5	4.93^{b*}	0.21	5.74^{a*}	0.21	5.13^{ab*}	0.21	0.0117
	8	2.38^{c*}	0.21	4.55^{a*}	0.21	3.45^{b*}	0.21	<0.0001
	12	1.40^{b*}	0.21	2.60^{a*}	0.21	<1.00^{c*}	0.21	<0.0001
	24	<1.00^{a*}	0.21	<1.00^{a*}	0.21	<1.00^{a*}	0.21	1.0000
	48	1.10^{a*}	0.21	<1.00^{b*}	0.21	<1.00^{b*}	0.21	0.9191
XLD	0	6.75^{a}	0.21	6.52^{a}	0.21	7.03^{a}	0.21	0.1996
	2	5.78^{a*}	0.21	6.03^{a}	0.21	5.78^{a*}	0.21	0.5942
	5	4.19^{b*}	0.21	5.12^{a*}	0.21	4.45^{ab*}	0.21	0.0035
	8	1.88^{c*}	0.21	3.98^{a*}	0.21	3.19^{b*}	0.21	<0.0001
	12	<1.00^{b*}	0.21	2.24^{a*}	0.21	<1.00^{b*}	0.21	<0.0001
	24	<1.00^{a*}	0.21	<1.00^{a*}	0.21	<1.00^{a*}	0.21	1.0000
	48	<1.00^{a*}	0.21	<1.00^{a*}	0.21	<1.00^{a*}	0.21	1.0000

SEM: standard error of the mean.

$^{a\text{-}c}$: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$).

Detection limit = 1 log CFU/ml

*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column and within each culture medium.

Table 19. *Salmonella* spp. populations (log CFU/ml) recovered from poultry fat with a 0.5% impurity level and high moisture content (3.9%), incubated at 45˚C for up to 48 h.

Medium	Time (h)	*Salmonella* Enteritidis		*Salmonella* Senftenberg		*Salmonella* Typhimurium		*P-value*
		LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	0	6.92[a]	0.21	6.79[a]	0.21	7.10[a]	0.21	0.5479
	2	6.31[a]	0.21	6.64[a]	0.21	6.56[a]	0.21	0.4670
	5	5.43[a*]	0.21	5.89[a*]	0.21	5.55[a*]	0.21	0.2438
	8	3.42[b*]	0.21	5.09[a*]	0.21	3.89[b*]	0.21	<0.0001
	12	1.45[b*]	0.21	3.66[a*]	0.21	1.16[b*]	0.21	<0.0001
	24	1.30[a*]	0.21	<1.00[b*]	0.21	<1.00[b*]	0.21	0.4692
	48	<1.00[a*]	0.21	<1.00[a*]	0.21	<1.00[a*]	0.21	1.0000
XLD	0	6.82[a]	0.21	6.78[a]	0.21	7.00[a]	0.21	0.6953
	2	6.00[a*]	0.21	6.12[a]	0.21	6.15[a*]	0.21	0.8453
	5	4.78[ab*]	0.21	5.43[a*]	0.21	4.73[b*]	0.21	0.0216
	8	3.53[b*]	0.21	4.62[a*]	0.21	3.47[b*]	0.21	<0.0001
	12	1.30[b*]	0.21	3.09[a*]	0.21	1.30[b*]	0.21	<0.0001
	24	<1.00[a*]	0.21	<1.00[a*]	0.21	<1.00[a*]	0.21	1.0000
	48	<1.00[a*]	0.21	<1.00[a*]	0.21	<1.00[a*]	0.21	1.0000

SEM: standard error of the mean.

[a-c]: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$).

Detection limit = 1 log CFU/ml

*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column and within each culture medium.

Table 20. Effect of time and moisture content (low: 0.7%; medium: 3.0%; high: 4.8%) on *Salmonella* Enteritidis populations (log CFU/ml) recovered from poultry fat with a 1.0% impurity content, incubated at 25˚C for up to 48 h.

Medium	Time (h)	Low moisture		Medium moisture		High moisture		*P-value*
		LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	0	5.84[b]	0.15	6.86[a]	0.15	7.00[a]	0.15	<0.0001
	2	3.39[b*]	0.15	6.82[a]	0.15	6.98[a]	0.15	<0.0001
	5	1.00[b*]	0.15	6.77[a]	0.15	7.03[a]	0.15	<0.0001
	8	1.05[b*]	0.18	6.80[a]	0.15	7.05[a]	0.15	<0.0001
	12	1.05[b*]	0.18	6.83[a]	0.15	7.12[a]	0.15	<0.0001
	24	<1.00[c*]	0.15	6.71[b]	0.15	7.48[a*]	0.15	<0.0001
	48	<1.00[c*]	0.15	6.73[b]	0.15	7.73[a*]	0.15	<0.0001
XLD	0	5.72[b]	0.15	6.89[a]	0.15	6.98[a]	0.15	<0.0001
	2	3.47[b*]	0.15	6.79[a]	0.15	6.90[a]	0.15	<0.0001
	5	1.60[b*]	0.15	6.74[a]	0.15	6.98[a]	0.15	<0.0001
	8	1.43[b*]	0.15	6.74[a]	0.15	6.94[a]	0.15	<0.0001
	12	1.15[b*]	0.17	6.62[a]	0.15	6.94[a]	0.15	<0.0001
	24	<1.00[c*]	0.15	6.66[b]	0.15	7.45[a]	0.15	<0.0001
	48	<1.00[c*]	0.15	6.61[b]	0.15	7.95[a*]	0.15	<0.0001

SEM: standard error of the mean.
[a-c]: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$).
Detection limit = 1 log CFU/ml
*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column and within each culture medium.

Table 21. Effect of time and moisture content (low: 0.7%; medium: 3.0%; high: 4.8%) on *Salmonella* Senftenberg populations (log CFU/ml) recovered from poultry fat with a 1.0% impurity content, incubated at 25˚C for up to 48 h.

Medium	Time (h)	Low moisture		Medium moisture		High moisture		*P-value*
		LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	0	5.73[b]	0.15	6.76[a]	0.15	6.75[a]	0.15	<0.0001
	2	4.12[b*]	0.15	6.65[a]	0.15	6.64[a]	0.15	<0.0001
	5	3.37[b*]	0.15	6.58[a]	0.15	6.72[a]	0.15	<0.0001
	8	3.13[b*]	0.15	6.64[a]	0.15	6.80[a]	0.15	<0.0001
	12	2.50[b*]	0.15	6.54[a]	0.15	6.82[a]	0.15	<0.0001
	24	2.20[c*]	0.15	6.66[b]	0.15	7.18[a]	0.15	<0.0001
	48	1.81[c*]	0.15	6.51[b]	0.15	7.73[a*]	0.15	<0.0001
XLD	0	5.50[b]	0.15	6.66[a]	0.15	6.67[a]	0.15	<0.0001
	2	3.79[b*]	0.15	6.57[a]	0.15	6.56[a]	0.15	<0.0001
	5	3.08[b*]	0.15	6.51[a]	0.15	6.54[a]	0.15	<0.0001
	8	2.79[b*]	0.15	6.60[a]	0.15	6.60[a]	0.15	<0.0001
	12	2.13[b*]	0.15	6.38[a]	0.15	6.61[a]	0.15	<0.0001
	24	1.97[c*]	0.15	6.41[b]	0.15	7.08[a]	0.15	<0.0001
	48	1.20[c*]	0.15	6.30[b]	0.15	7.74[a*]	0.15	<0.0001

SEM: standard error of the mean.
[a-c]: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$).
Detection limit = 1 log CFU/ml
*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column and within each culture medium.

Table 22. Effect of time and moisture content (low: 0.7%; medium: 3.0%; high: 4.8%) on *Salmonella* Typhimurium populations (log CFU/ml) recovered from poultry fat with a 1.0% impurity content, incubated at 25˚C for up to 48 h.

Medium	Time (h)	Low moisture		Medium moisture		High moisture		*P-value*
		LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	0	6.14^{b}	0.15	7.10^{a}	0.15	7.00^{a}	0.15	<0.0001
	2	4.45^{b*}	0.15	7.05^{a}	0.15	6.98^{a}	0.15	<0.0001
	5	3.53^{b*}	0.15	6.95^{a}	0.15	7.03^{a}	0.15	<0.0001
	8	3.26^{b*}	0.15	6.94^{a}	0.15	7.05^{a}	0.15	<0.0001
	12	1.16^{b*}	0.15	7.02^{a}	0.15	7.12^{a}	0.15	<0.0001
	24	$<1.00^{b*}$	0.15	7.10^{a}	0.15	7.48^{a*}	0.15	<0.0001
	48	$<1.00^{c*}$	0.15	6.80^{b}	0.15	7.73^{a*}	0.15	<0.0001
XLD	0	6.23^{b}	0.15	7.03^{a}	0.15	6.98^{a}	0.15	<0.0001
	2	4.36^{b*}	0.15	6.95^{a}	0.15	6.90^{a}	0.15	<0.0001
	5	2.33^{b*}	0.15	7.09^{a}	0.15	6.98^{a}	0.15	<0.0001
	8	$<1.00^{b*}$	0.15	7.02^{a}	0.15	6.94^{a}	0.15	<0.0001
	12	1.26^{b*}	0.15	6.76^{a}	0.15	6.94^{a}	0.15	<0.0001
	24	$<1.00^{c*}$	0.15	6.81^{b}	0.15	7.45^{a}	0.15	<0.0001
	48	$<1.00^{c*}$	0.15	6.69^{b}	0.15	7.95^{a*}	0.15	<0.0001

SEM: standard error of the mean.

$^{a-c}$: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$). Detection limit = 1 log CFU/ml

*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column and within each culture medium.

Table 23. *Salmonella* spp. populations (log CFU/ml) recovered from poultry fat with a 1.0% impurity level and low moisture content (0.7%), incubated at 25˚C for up to 48 h.

Medium	Time (h)	*Salmonella* Enteritidis		*Salmonella* Senftenberg		*Salmonella* Typhimurium		*P-value*
		LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	0	5.84[a]	0.15	5.73[a]	0.15	6.14[a]	0.15	0.1181
	2	3.39[b*]	0.15	4.12[a*]	0.15	4.45[a*]	0.15	<0.0001
	5	1.00[b*]	0.15	3.37[a*]	0.15	3.53[a*]	0.15	<0.0001
	8	1.05[b*]	0.18	3.13[a*]	0.15	3.26[a*]	0.15	<0.0001
	12	1.05[b*]	0.18	2.50[a*]	0.15	1.16[b*]	0.15	<0.0001
	24	<1.00[b*]	0.15	2.20[a*]	0.15	<1.00[b*]	0.15	<0.0001
	48	<1.00[b*]	0.15	1.81[a*]	0.15	<1.00[b*]	0.15	<0.0001
XLD	0	5.72[b]	0.15	5.50[b]	0.15	6.23[a]	0.15	0.0017
	2	3.47[b*]	0.15	3.79[b*]	0.15	4.36[a*]	0.15	0.0001
	5	1.60[c*]	0.15	3.08[a*]	0.15	2.33[b*]	0.15	<0.0001
	8	1.43[b*]	0.15	2.79[a*]	0.15	<1.00[c*]	0.15	<0.0001
	12	1.15[b*]	0.17	2.13[a*]	0.15	1.26[b*]	0.15	<0.0001
	24	<1.00[b*]	0.15	1.97[a*]	0.15	<1.00[b*]	0.15	<0.0001
	48	<1.00[b*]	0.15	1.20[a*]	0.15	<1.00[b*]	0.15	0.5291

SEM: standard error of the mean.

[a-c]: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$).

Detection limit = 1 log CFU/ml

*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column and within each culture medium.

Table 24. *Salmonella* spp. populations (log CFU/ml) recovered from poultry fat with a 1.0% impurity level and medium moisture content (3.0%), incubated at 25˚C for up to 48 h.

Medium	Time (h)	*Salmonella* Enteritidis		*Salmonella* Senftenberg		*Salmonella* Typhimurium		*P-value*
		LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	0	6.86[a]	0.15	6.76[a]	0.15	7.10[a]	0.15	0.2176
	2	6.82[a]	0.15	6.65[a]	0.15	7.05[a]	0.15	0.1429
	5	6.77[a]	0.15	6.58[a]	0.15	6.95[a]	0.15	0.1967
	8	6.80[a]	0.15	6.64[a]	0.15	6.94[a]	0.15	0.3560
	12	6.83[a]	0.15	6.54[a]	0.15	7.02[a]	0.15	0.0627
	24	6.71[a]	0.15	6.66[a]	0.15	7.10[a]	0.15	0.0693
	48	6.73[a]	0.15	6.51[a]	0.15	6.80[a]	0.15	0.3284
XLD	0	6.89[a]	0.15	6.66[a]	0.15	7.03[a]	0.15	0.1978
	2	6.79[a]	0.15	6.57[a]	0.15	6.95[a]	0.15	0.1676
	5	6.74[ab]	0.15	6.51[b]	0.15	7.09[a]	0.15	0.0191
	8	6.74[a]	0.15	6.60[a]	0.15	7.02[a]	0.15	0.1190
	12	6.62[a]	0.15	6.38[a]	0.15	6.76[a]	0.15	0.1751
	24	6.66[a]	0.15	6.41[a]	0.15	6.81[a]	0.15	0.1473
	48	6.61[a]	0.15	6.30[a]	0.15	6.69[a]	0.15	0.1365

SEM: standard error of the mean.
[a-c]: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$).
Detection limit = 1 log CFU/ml
*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column and within each culture medium.

Table 25. *Salmonella* spp. populations (log CFU/ml) recovered from poultry fat with a 1.0% impurity level and high moisture content (4.8%), incubated at 25˚C for up to 48 h.

Medium	Time (h)	*Salmonella* Enteritidis		*Salmonella* Senftenberg		*Salmonella* Typhimurium		*P-value*
		LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	0	6.88[a]	0.15	6.75[a]	0.15	7.00[a]	0.15	0.4658
	2	6.83[a]	0.15	6.64[a]	0.15	6.98[a]	0.15	0.2654
	5	6.78[a]	0.15	6.72[a]	0.15	7.03[a]	0.15	0.2796
	8	6.82[a]	0.15	6.80[a]	0.15	7.05[a]	0.15	0.4116
	12	6.88[a]	0.15	6.82[a]	0.15	7.12[a]	0.15	0.2963
	24	7.53[a*]	0.15	7.18[a]	0.15	7.48[a*]	0.15	0.1773
	48	7.92[a*]	0.15	7.73[a*]	0.15	7.73[a*]	0.15	0.5607
XLD	0	6.88[a]	0.15	6.67[a]	0.15	6.98[a]	0.15	0.3088
	2	6.79[a]	0.15	6.56[a]	0.15	6.90[a]	0.15	0.2460
	5	6.74[a]	0.15	6.54[a]	0.15	6.98[a]	0.15	0.1052
	8	6.80[a]	0.15	6.60[a]	0.15	6.94[a]	0.15	0.2488
	12	6.83[a]	0.15	6.61[a]	0.15	6.94[a]	0.15	0.2547
	24	7.55[a*]	0.15	7.08[a]	0.15	7.45[a]	0.15	0.0568
	48	7.94[a*]	0.15	7.74[a*]	0.15	7.95[a*]	0.15	0.5026

SEM: standard error of the mean.

[a-c]: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$).

Detection limit = 1 log CFU/ml

*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column and within each culture medium.

Table 26. Effect of time and moisture content (low: 0.7%; medium: 3.0%; high: 4.8%) on *Salmonella* Enteritidis populations (log CFU/ml) recovered from poultry fat with a 1.0% impurity content, incubated at 45˚C for up to 48 h.

Medium	Time (h)	Low moisture		Medium moisture		High moisture		*P-value*
		LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	0	4.79^{b}	0.21	6.81^{a}	0.21	6.90^{a}	0.21	<0.0001
	2	1.35^{b*}	0.21	6.35^{a}	0.21	6.59^{a}	0.21	<0.0001
	5	<1.00^{b*}	0.21	5.93^{a*}	0.21	6.40^{a}	0.21	<0.0001
	8	<1.00^{b*}	0.21	5.62^{a*}	0.21	5.96^{a*}	0.21	<0.0001
	12	<1.00^{c*}	0.21	4.94^{b*}	0.21	6.14^{a*}	0.21	<0.0001
	24	<1.00^{c*}	0.21	1.59^{b*}	0.21	5.36^{a*}	0.21	<0.0001
	48	<1.00^{b*}	0.21	<1.00^{b*}	0.21	4.90^{a*}	0.25	<0.0001
XLD	0	4.59^{b}	0.21	6.75^{a}	0.21	6.86^{a}	0.21	<0.0001
	2	<1.00^{b*}	0.21	6.31^{a}	0.21	6.55^{a}	0.21	<0.0001
	5	<1.00^{c*}	0.21	5.67^{b*}	0.21	6.35^{a}	0.21	<0.0001
	8	<1.00^{c*}	0.21	5.23^{b*}	0.21	6.28^{a}	0.21	<0.0001
	12	<1.00^{c*}	0.21	4.47^{b*}	0.21	5.97^{a*}	0.21	<0.0001
	24	<1.00^{c*}	0.21	1.51^{b*}	0.21	5.68^{a*}	0.21	<0.0001
	48	<1.00^{b*}	0.21	<1.00^{b*}	0.21	4.05^{a*}	0.25	<0.0001

SEM: standard error of the mean.

$^{a-c}$: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$). Detection limit = 1 log CFU/ml

*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column and within each culture medium.

Table 27. Effect of time and moisture content (low: 0.7%; medium: 3.0%; high: 4.8%) on *Salmonella* Senftenberg populations (log CFU/ml) recovered from poultry fat with a 1.0% impurity content, incubated at 45˚C for up to 48 h.

Medium	Time (h)	Low moisture		Medium moisture		High moisture		*P-value*
		LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	0	4.84[b]	0.21	6.72[a]	0.21	7.04[a]	0.21	<0.0001
	2	1.38[b*]	0.21	6.41[a]	0.21	6.96[a]	0.21	<0.0001
	5	<1.00[b*]	0.21	6.20[a]	0.21	6.86[a]	0.21	<0.0001
	8	<1.00[b*]	0.21	6.01[a]	0.21	6.67[a]	0.21	<0.0001
	12	<1.00[b*]	0.21	5.59[a*]	0.21	6.26[a*]	0.21	<0.0001
	24	<1.00[c*]	0.21	4.25[b*]	0.21	5.00[a*]	0.21	<0.0001
	48	<1.00[c*]	0.21	1.10[b*]	0.21	4.51[a*]	0.25	<0.0001
XLD	0	4.28[b]	0.21	6.58[a]	0.21	7.17[a]	0.21	<0.0001
	2	<1.00[b*]	0.21	6.31[a]	0.21	6.79[a]	0.21	<0.0001
	5	<1.00[b*]	0.21	6.00[a]	0.21	6.79[a]	0.21	<0.0001
	8	<1.00[b*]	0.21	5.70[a*]	0.21	6.53[a]	0.21	<0.0001
	12	<1.00[c*]	0.21	5.22[b*]	0.21	6.05[a*]	0.21	<0.0001
	24	<1.00[c*]	0.21	3.91[b*]	0.21	5.00[a*]	0.21	<0.0001
	48	<1.00[b*]	0.21	<1.00[b*]	0.21	3.48[a*]	0.25	<0.0001

SEM: standard error of the mean.
[a-c]: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$).
Detection limit = 1 log CFU/ml
*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column and within each culture medium.

Table 28. Effect of time and moisture content (low: 0.7%; medium: 3.0%; high: 4.8%) on *Salmonella* Typhimurium populations (log CFU/ml) recovered from poultry fat with a 1.0% impurity content, incubated at 45˚C for up to 48 h.

Medium	Time (h)	Low moisture		Medium moisture		High moisture		*P-value*
		LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	0	5.54^{b}	0.21	6.72^{a}	0.21	7.04^{a}	0.21	<0.0001
	2	1.10^{b*}	0.21	6.41^{a}	0.21	6.96^{a}	0.21	<0.0001
	5	<1.00^{b*}	0.21	6.20^{a}	0.21	6.86^{a}	0.21	<0.0001
	8	<1.00^{c*}	0.21	6.01^{b}	0.21	6.67^{a}	0.21	<0.0001
	12	<1.00^{c*}	0.21	5.59^{b*}	0.21	6.26^{a*}	0.21	<0.0001
	24	<1.00^{c*}	0.21	4.25^{b*}	0.21	5.00^{a*}	0.21	<0.0001
	48	<1.00^{c*}	0.21	1.10^{b*}	0.21	4.51^{a*}	0.25	<0.0001
XLD	0	5.48^{b}	0.21	6.58^{a}	0.21	7.17^{a}	0.21	<0.0001
	2	<1.00^{b*}	0.21	6.31^{a}	0.21	6.79^{a}	0.21	<0.0001
	5	<1.00^{c*}	0.21	6.00^{b}	0.21	6.79^{a}	0.21	<0.0001
	8	<1.00^{c*}	0.21	5.70^{b*}	0.21	6.53^{a}	0.21	<0.0001
	12	<1.00^{c*}	0.21	5.22^{b*}	0.21	6.05^{a*}	0.21	<0.0001
	24	<1.00^{c*}	0.21	3.91^{b*}	0.21	5.00^{a*}	0.21	<0.0001
	48	<1.00^{b*}	0.21	<1.00^{b*}	0.21	3.48^{a*}	0.25	<0.0001

SEM: standard error of the mean.
$^{a-c}$: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$).
Detection limit = 1 log CFU/ml
*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column and within each culture medium.

Table 29. *Salmonella* spp. populations (log CFU/ml) recovered from poultry fat with a 1.0% impurity level and low moisture content (0.7%), incubated at 45˚C for up to 48 h.

Medium	Time (h)	*Salmonella* Enteritidis		*Salmonella* Senftenberg		*Salmonella* Typhimurium		
		LSMean	SEM	LSMean	SEM	LSMean	SEM	*P-value*
TSAp	0	4.79^{b}	0.21	4.84^{b}	0.21	5.54^{a}	0.21	0.0144
	2	1.35^{a*}	0.21	1.38^{a*}	0.21	1.10^{a*}	0.21	0.5579
	5	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	1.0000
	8	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	1.0000
	12	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	1.0000
	24	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	1.0000
	48	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	1.0000
XLD	0	4.59^{b}	0.21	4.28^{b}	0.21	5.48^{a}	0.21	<0.0001
	2	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	1.0000
	5	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	1.0000
	8	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	1.0000
	12	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	1.0000
	24	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	1.0000
	48	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	$<1.00^{a*}$	0.21	1.0000

SEM: standard error of the mean.
$^{a-c}$: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$).
Detection limit = 1 log CFU/ml
*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column and within each culture medium.

Table 30. *Salmonella* spp. populations (log CFU/ml) recovered from poultry fat with a 1.0% impurity level and medium moisture content (3.0%), incubated at 45˚C for up to 48 h.

Medium	Time (h)	*Salmonella* Enteritidis		*Salmonella* Senftenberg		*Salmonella* Typhimurium		
		LSMean	SEM	LSMean	SEM	LSMean	SEM	*P-value*
TSAp	0	6.81[a]	0.21	6.72[a]	0.21	7.01[a]	0.21	0.5611
	2	6.35[a]	0.21	6.41[a]	0.21	6.55[a]	0.21	0.7698
	5	5.93[a*]	0.21	6.20[a]	0.21	6.27[a*]	0.21	0.4596
	8	5.62[a*]	0.21	6.01[a]	0.21	5.86[a*]	0.21	0.3868
	12	4.94[a*]	0.21	5.59[a*]	0.21	5.31[a*]	0.21	0.0722
	24	1.59[c*]	0.21	4.25[a*]	0.21	3.01[b*]	0.21	<0.0001
	48	<1.00[b*]	0.21	1.10[a*]	0.21	<1.00[b*]	0.21	0.9201
XLD	0	6.75[a]	0.21	6.58[a]	0.21	7.02[a]	0.21	0.2974
	2	6.31[a]	0.21	6.31[a]	0.21	6.28[a*]	0.21	0.9899
	5	5.67[a*]	0.21	6.00[a]	0.21	5.77[a*]	0.21	0.4937
	8	5.23[a*]	0.21	5.70[a*]	0.21	5.72[a*]	0.21	0.1452
	12	4.47[b*]	0.21	5.22[a*]	0.21	4.92[ab*]	0.21	0.0312
	24	1.51[c*]	0.21	3.91[a*]	0.21	2.33[b*]	0.21	<0.0001
	48	<1.00[a*]	0.21	<1.00[a*]	0.21	<1.00[a*]	0.21	1.0000

SEM: standard error of the mean.
[a-c]: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$).
Detection limit = 1 log CFU/ml
*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column and within each culture medium.

Table 31. *Salmonella* spp. populations (log CFU/ml) recovered from poultry fat with a 1.0% impurity level and high moisture content (4.8%), incubated at 45˚C for up to 48 h.

Medium	Time (h)	*Salmonella* Enteritidis		*Salmonella* Senftenberg		*Salmonella* Typhimurium		*P-value*
		LSMean	SEM	LSMean	SEM	LSMean	SEM	
TSAp	0	6.90[a]	0.21	6.82[a]	0.21	7.04[a]	0.21	0.7274
	2	6.59[a]	0.21	6.56[a]	0.21	6.96[a]	0.21	0.3028
	5	6.40[a]	0.21	6.35[a]	0.21	6.86[a]	0.21	0.1456
	8	5.96[b*]	0.21	6.28[ab]	0.21	6.67[a]	0.21	0.0433
	12	6.14[a*]	0.21	6.19[a]	0.21	6.26[a*]	0.21	0.9097
	24	5.36[a*]	0.21	5.56[a*]	0.21	5.00[a*]	0.21	0.1440
	48	4.90[a*]	0.25	4.44[a*]	0.21	4.51[a*]	0.25	0.3234
XLD	0	6.86[a]	0.21	6.64[a]	0.21	7.17[a]	0.21	0.1775
	2	6.55[a]	0.21	6.42[a]	0.21	6.79[a]	0.21	0.4213
	5	6.35[a]	0.21	6.26[a]	0.21	6.79[a]	0.21	0.1390
	8	6.28[a]	0.21	6.06[a]	0.21	6.53[a]	0.21	0.2566
	12	5.97[a*]	0.21	5.95[a]	0.21	6.05[a*]	0.21	0.9327
	24	5.68[a*]	0.21	5.49[ab*]	0.21	5.00[b*]	0.21	0.0491
	48	4.05[a*]	0.25	4.17[a*]	0.21	3.48[a*]	0.25	0.0850

SEM: standard error of the mean.
[a-c]: Least squares means (LSMean) within a row without a common superscript letter differ ($P < 0.05$).
Detection limit = 1 log CFU/ml
*. Least squares means (LSMean) with significance below $\alpha = 0.05$ comparing with 0 h within a column and within each culture medium.

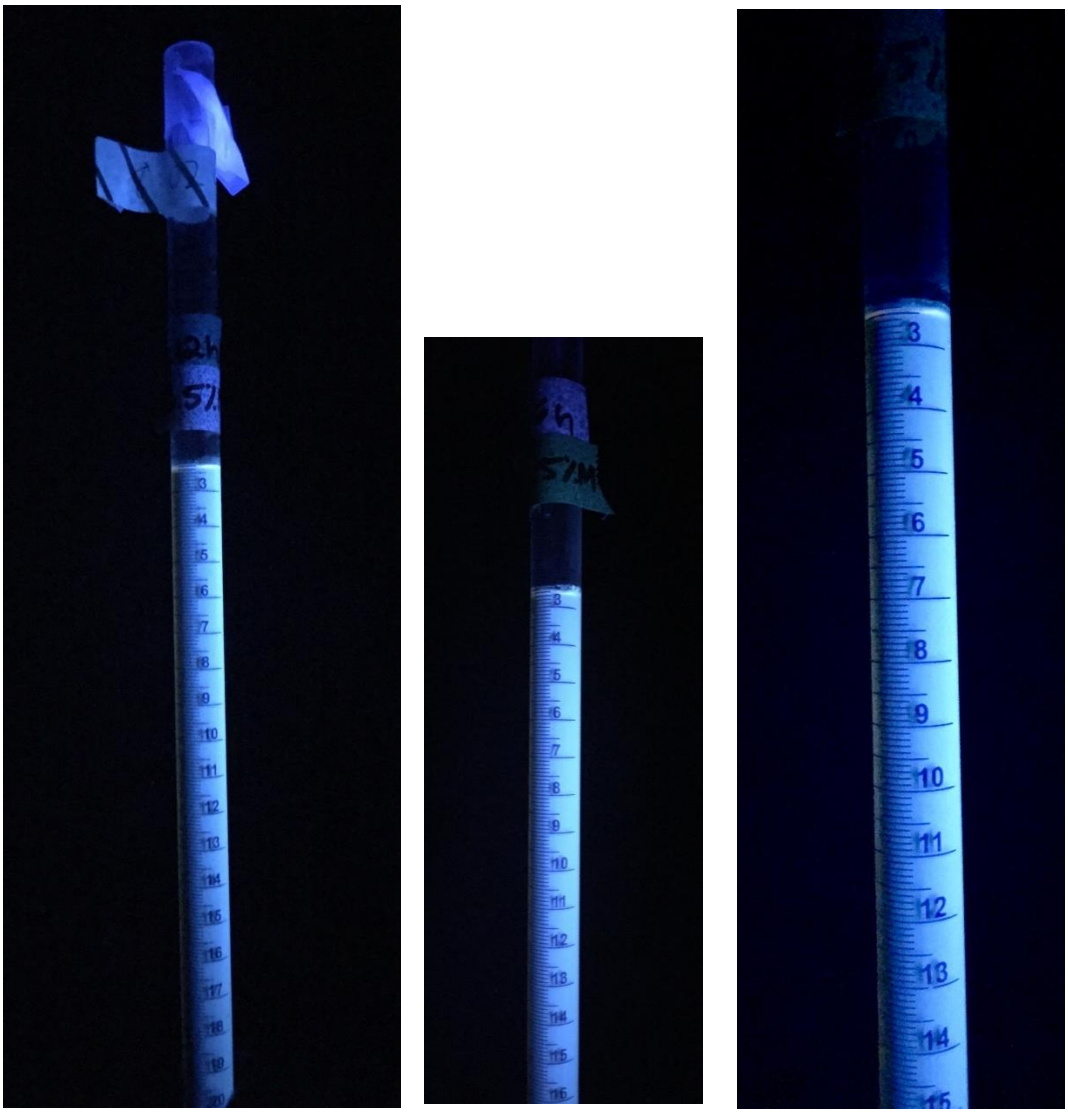

Figure 1. Photographs of burettes after inoculation. Fluorescence was noted at the point of inoculation (i.e., approximately at the 3.5 cm marking of the burette).

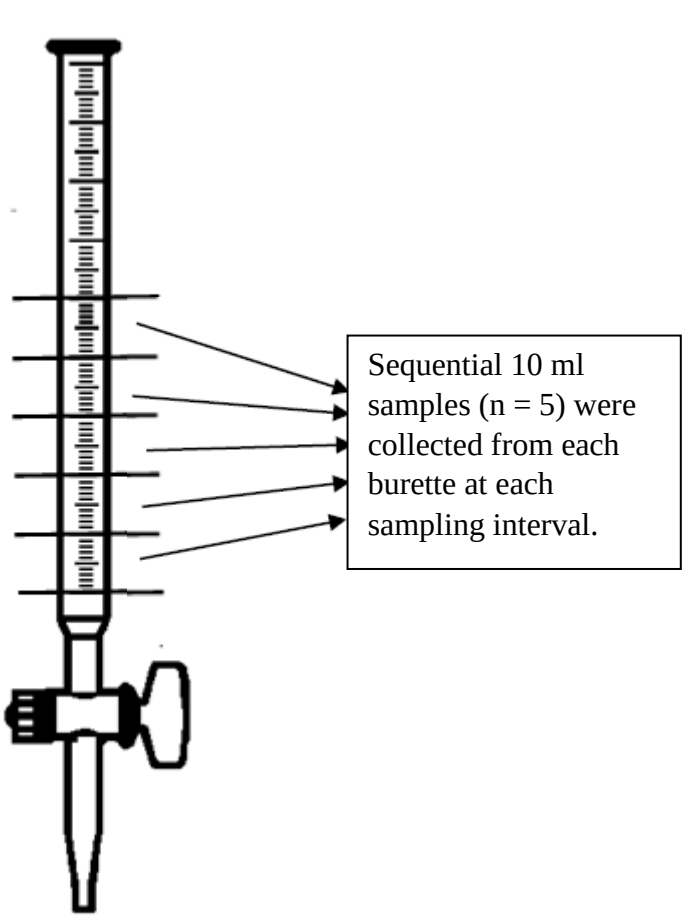

Figure 2. Microbiological sampling schematic for poultry fat at five sampling intervals.

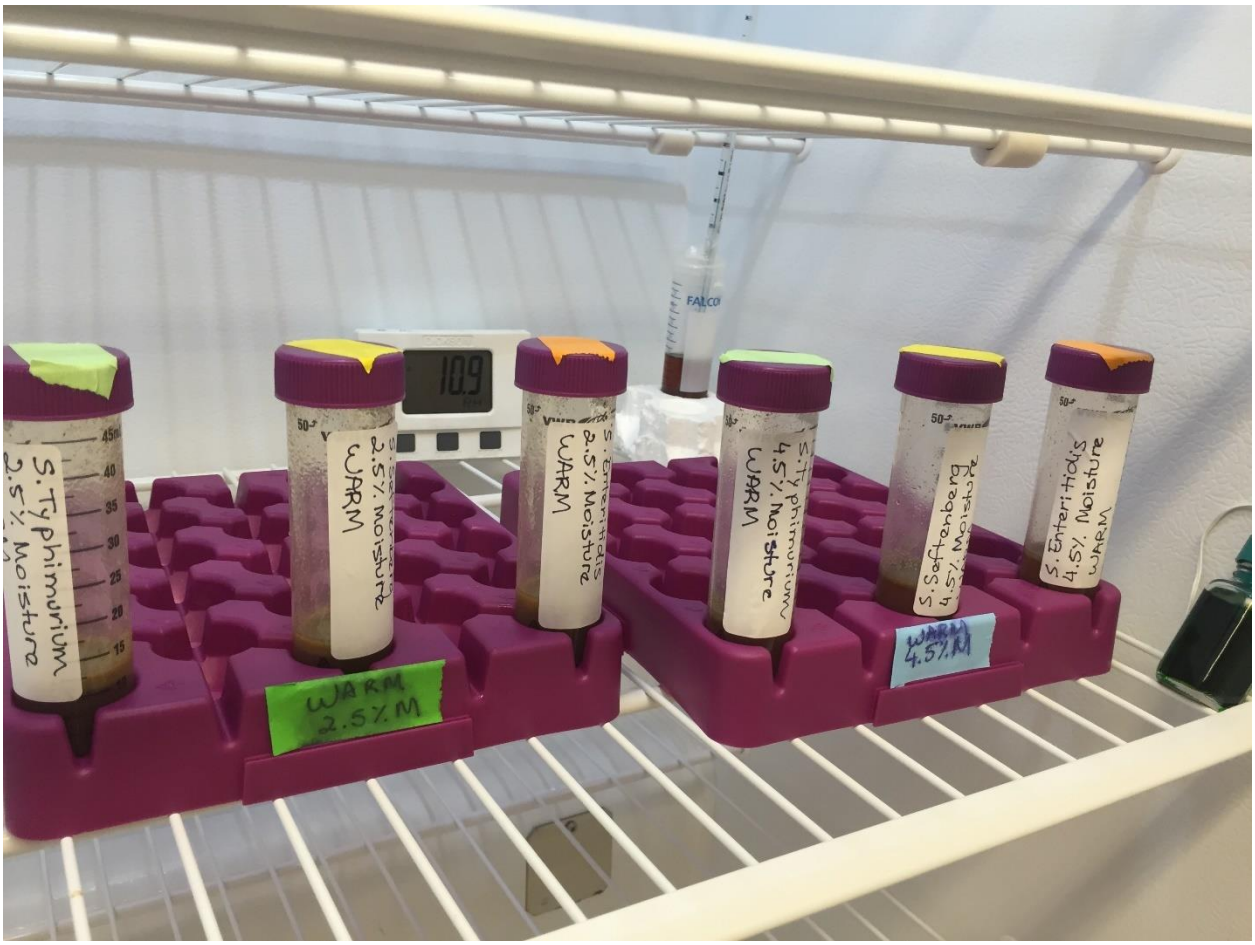

Figure 3. Inoculated poultry fat samples in 50 ml conical centrifuge tubes stored in a 45˚C incubator.

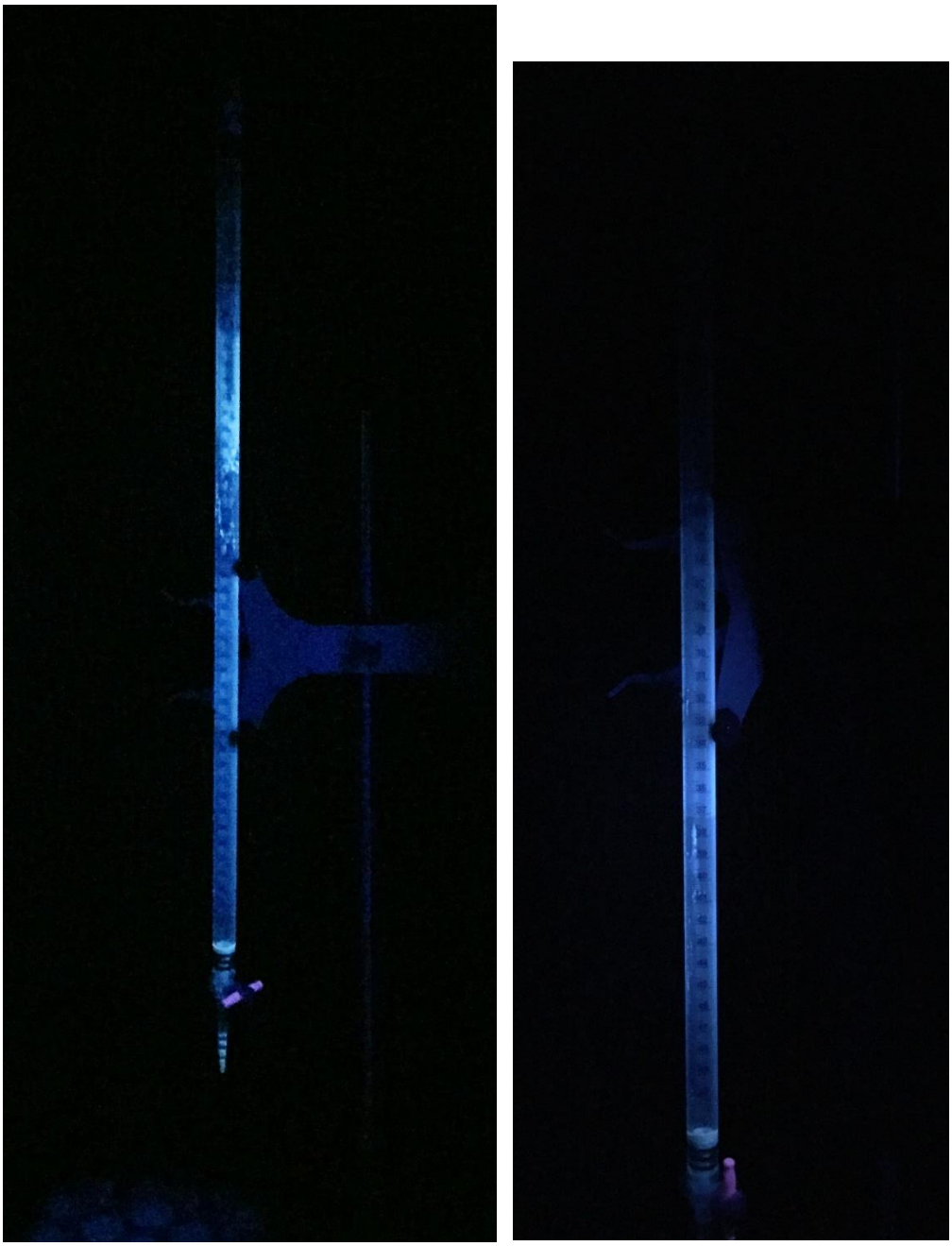

Figure 4. Photographs of burettes after sampling was completed. Fluorescence was noted along the sides of the empty burettes.

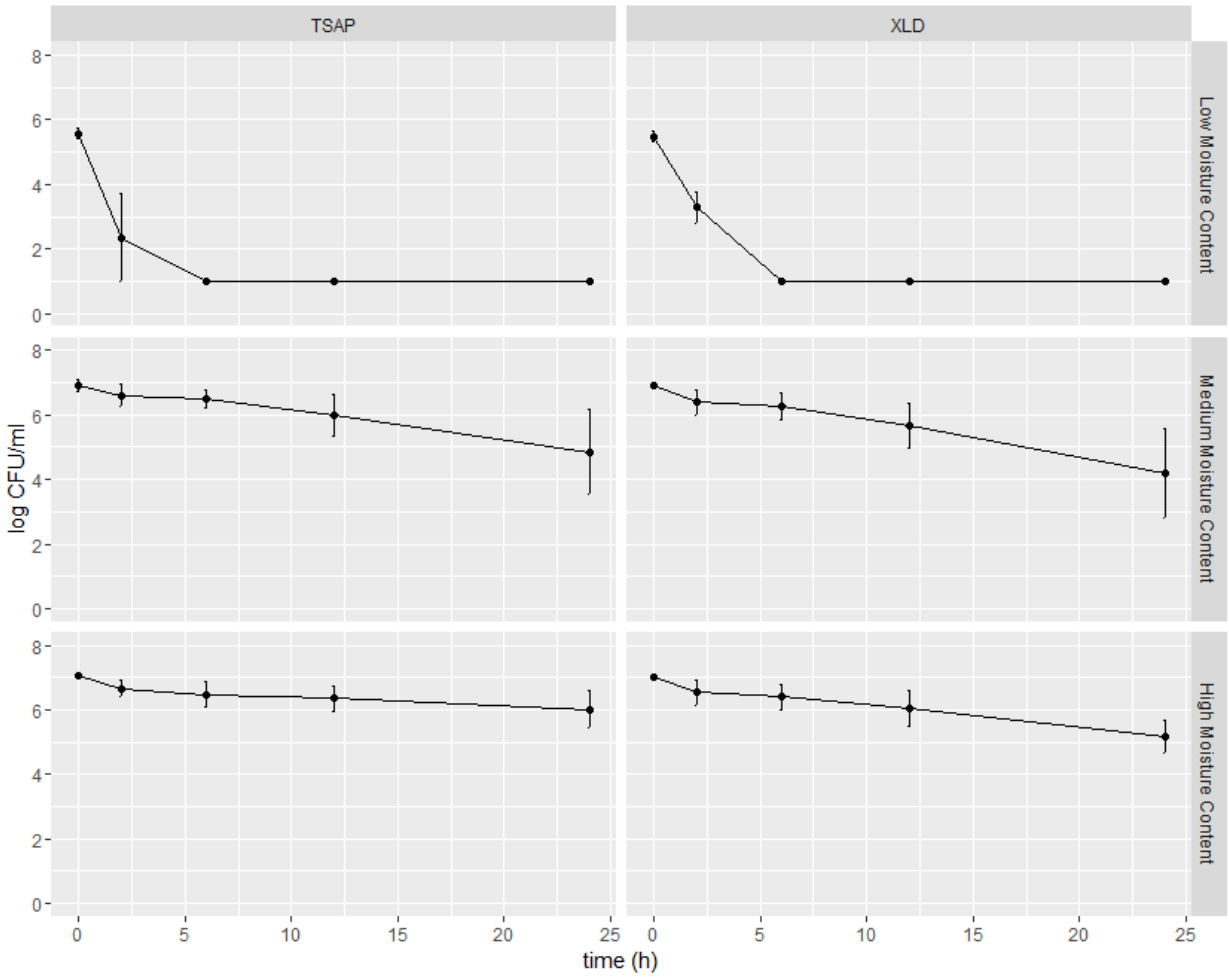

Figure 5. The survival (log CFU/ml) of *Salmonella* Typhimurium in 0.2% impurity level poultry fat incubated at 25˚C for 24 h. Poultry fat samples were formulated to represent one of three targeted moisture contents (low: 0.5 ± 0.2%; medium: 2.2 ± 0.9%; high: 4.5 ± 0.1%).

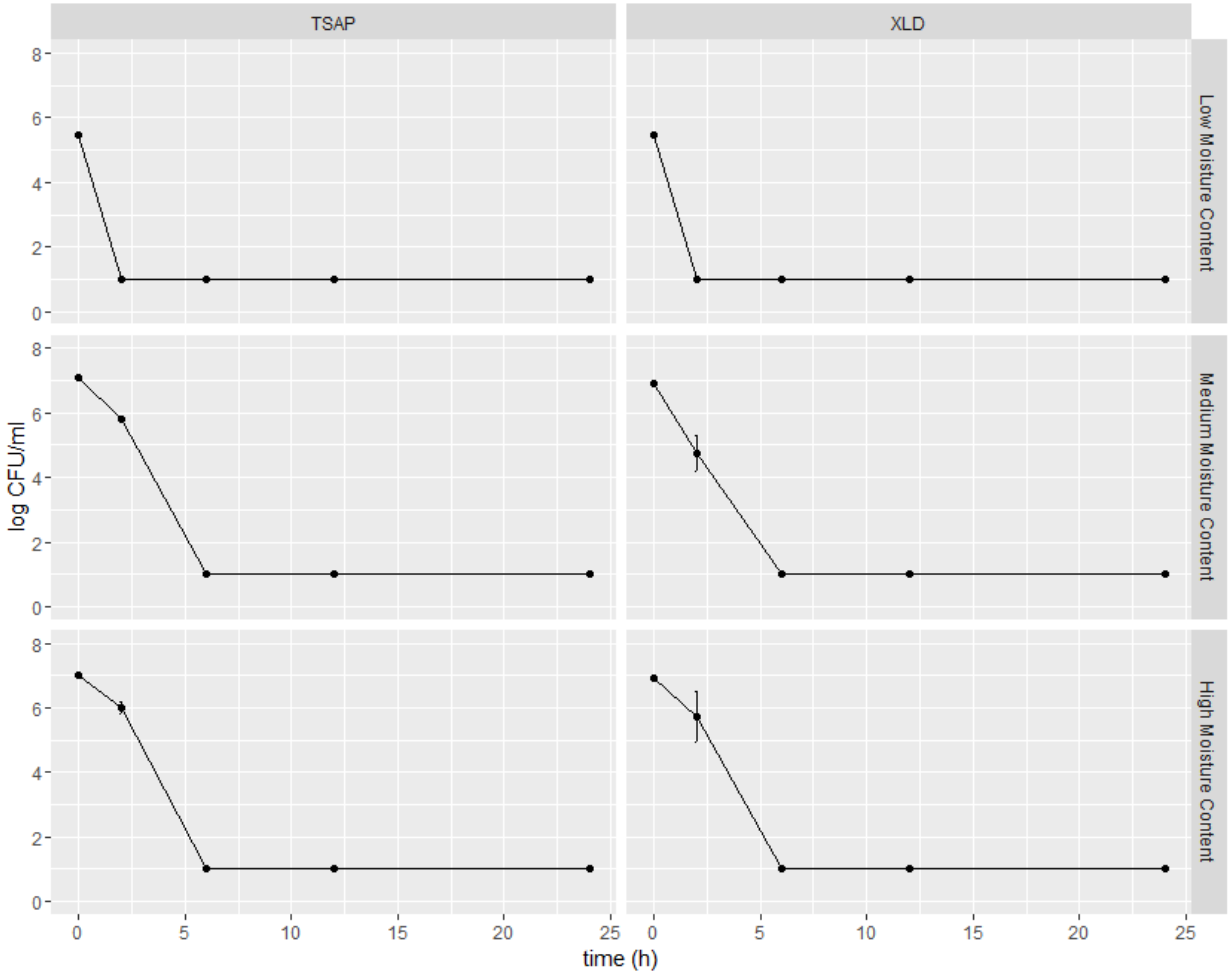

Figure 6. The survival (log CFU/ml) of *Salmonella* Typhimurium in 0.2% impurity level poultry fat incubated at 45˚C for 24 h. Poultry fat samples were formulated to represent one of three targeted moisture contents (low: 0.5 ± 0.2%; medium: 2.2 ± 0.9%; high: 4.5 ± 0.1%).

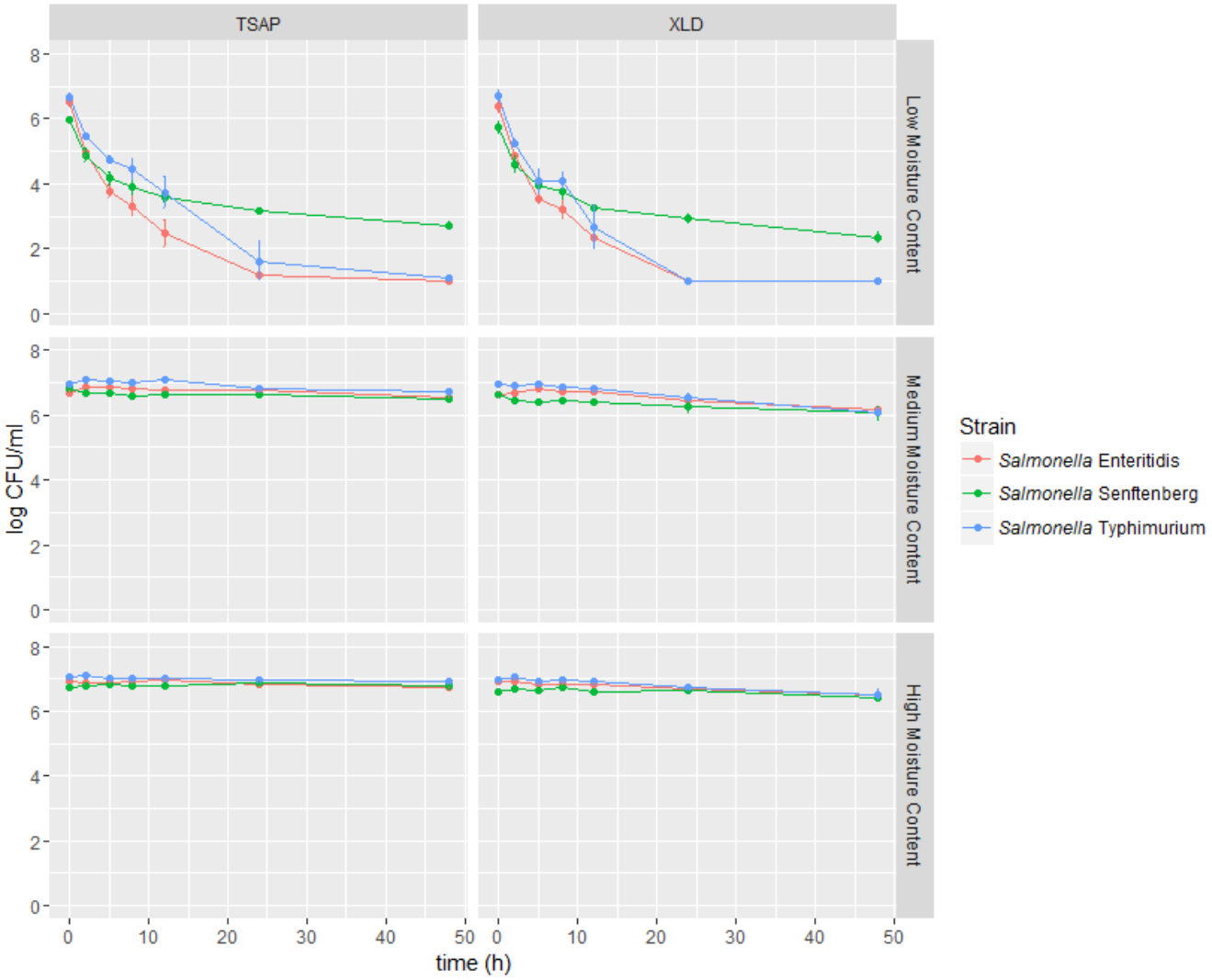

Figure 7. The survival (log CFU/ml) of *Salmonella* Enteritidis, *Salmonella* Senftenberg, and *Salmonella* Typhimurium in 0.5% impurity level poultry fat incubated at 25˚C for 48 h. Poultry fat samples were formulated to represent one of three targeted moisture contents (low: 0.5 ± 0.1%; medium: 2.1 ± 0.5%; high: 3.9 ± 0.5%).

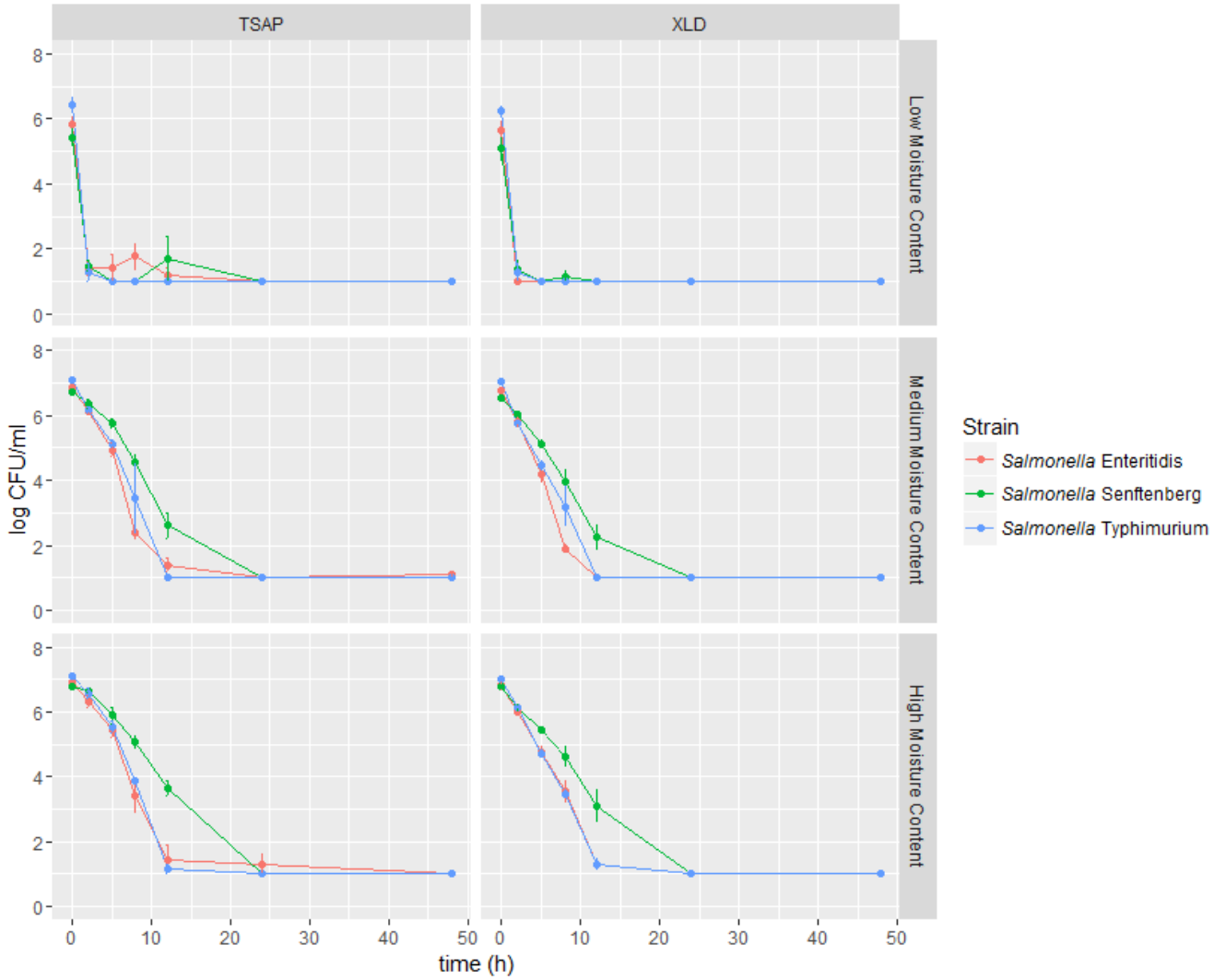

Figure 8. The survival (log CFU/ml) of *Salmonella* Enteritidis, *Salmonella* Senftenberg, and *Salmonella* Typhimurium in 0.5% impurity level poultry fat incubated at 45˚C for 48 h. Poultry fat samples were formulated to represent one of three targeted moisture contents (low: 0.5 ± 0.1%; medium: 2.1 ± 0.5%; high: 3.9 ± 0.5%).

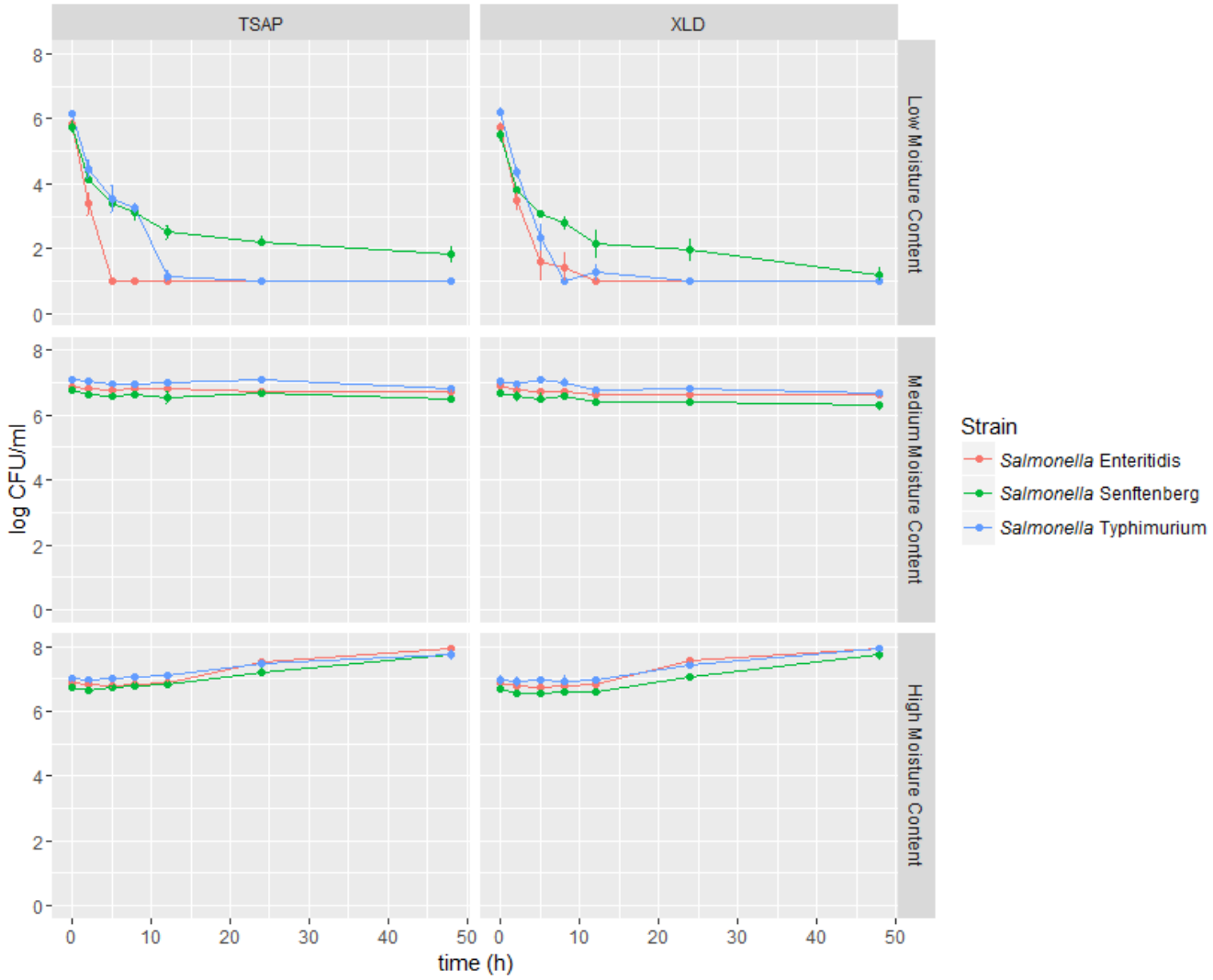

Figure 9. The survival (log CFU/ml) of *Salmonella* Enteritidis, *Salmonella* Senftenberg, and *Salmonella* Typhimurium in 1.0% impurity level poultry fat incubated at 25˚C for 48 h. Poultry fat samples were formulated to represent one of three targeted moisture contents (low: 0.7 ± 0.2%; medium: 3.0 ± 0.2%; high: 4.8% ± 0.3%).

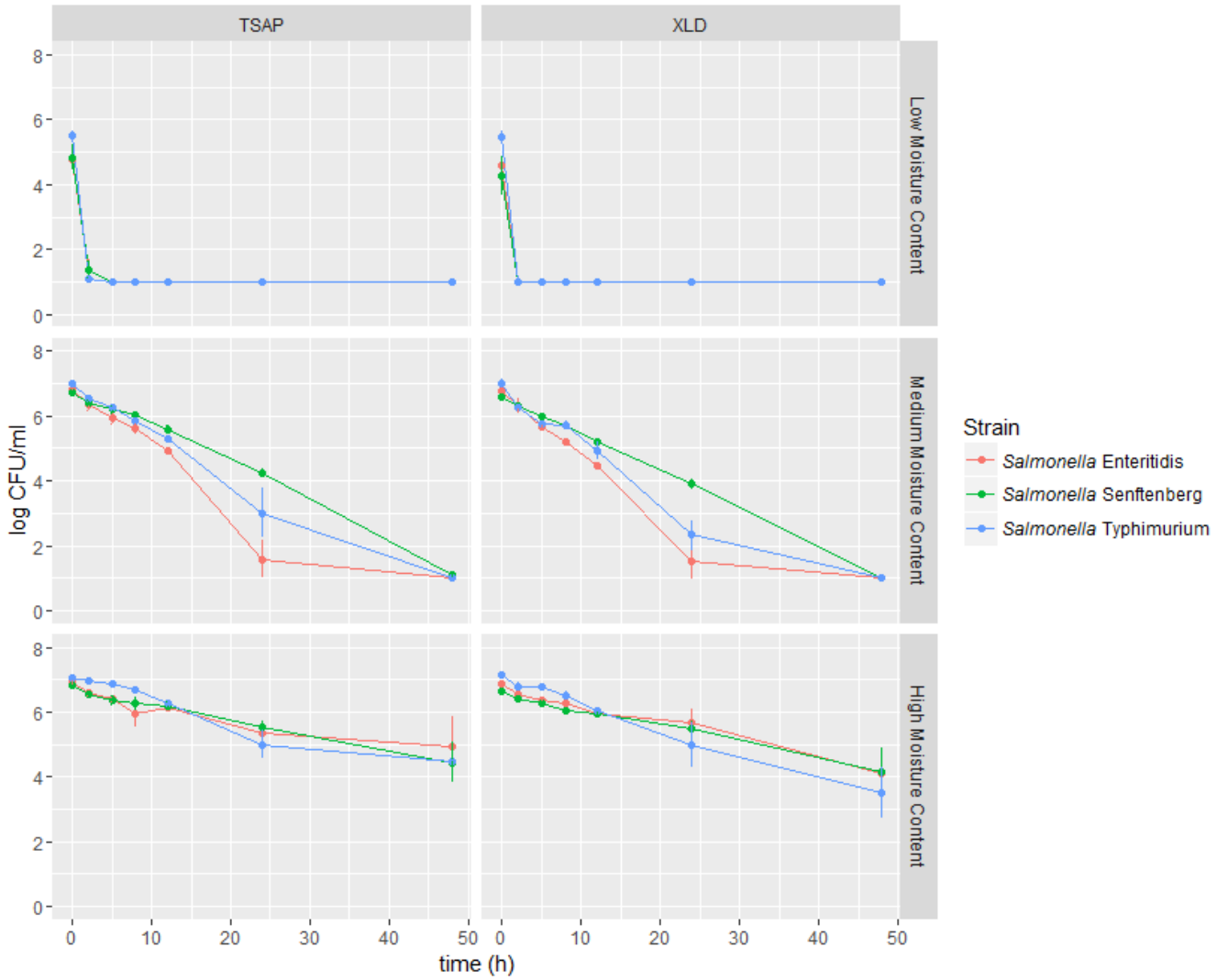

Figure 10. The survival (log CFU/ml) of *Salmonella* Enteritidis, *Salmonella* Senftenberg, and *Salmonella* Typhimurium in 1.0% impurity level poultry fat incubated at 45˚C for 48 h. Poultry fat samples were formulated to represent one of three targeted moisture contents (low: 0.7 ± 0.2%; medium: 3.0 ± 0.2%; high: 4.8% ± 0.3%).

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