

Assessing *Salmonella* typhimurium persistence in poultry carcasses under multiple thermal conditions consistent with composting and wet rendering

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ABSTRACT Mitigation of *Salmonella* associated with poultry carcasses is primarily accomplished by rendering or carcass composting. While rendering temperatures and pressures are well established for pathogen inactivation in poultry carcasses, parameters controlling composting processes are less defined in part because multiple conditions and procedures are utilized. Consequently, limited knowledge exists describing the impacts of composting with varying temperature and mixing protocols with respect to the inactivation of *Salmonella* in poultry carcasses. To improve the existing knowledge of *Salmonella* survival in poultry carcasses, inactivation of *Salmonella enterica* serovar Typhimurium (ST) LT2 was investigated. The impacts of various composting temperatures (55, 62.5°C)

and low-rendering (i.e., pasteurization) temperatures (70, 78°C) on *Salmonella* inactivation were tested in a bench-top setting using a ground carcass slurry and whole birds under mixed and non-mixed conditions. Results showed that the ground carcass slurry and the whole carcass exposed to temperatures consistent with composting had no detectable *Salmonella* after 110 h with a level of detection of one CFU/mL of ground carcass slurry and one CFU/g of whole carcasses, respectively. In addition, grinding of carcasses as opposed to whole carcasses was more predictable with respect to *Salmonella* heat inactivation. Furthermore, results showed that constant mixing decreased the overall time required to eliminate *Salmonella* under composting and low-rendering temperatures.

Key words: poultry carcass composting, ground poultry carcass slurry, *Salmonella* inactivation

2016 Poultry Science 95:705–714
<http://dx.doi.org/10.3382/ps/pev373>

INTRODUCTION

Composting of poultry carcasses is a common practice during normal mortality and whole flock disposal (Adams et al., 1994; Collins, 2009; Ritz, 2014). Several studies focusing on poultry carcass composting are currently available describing the temperature and moisture dynamics essential for composting (Lawson and Keeling, 1999; Sivakumar et al., 2008; Stentiford, 1996; USDA-NRCS, 2000; Wilkinson, 2007). However, zoonotic pathogen survivability under composting conditions is less understood in part because of multiple controlling parameters associated with composting, which include ambient temperature, water activity, pH, ammonia concentration, how the compost is mixed, and differences in the microbial ecology of the substrate material (Chen et al., 2013; Reynnells et al., 2014; Sivakumar et al., 2008). Therefore, an improved understanding of pathogen reduction during composting

is required to optimize standard operating procedures associated with poultry carcass composting.

The U.S. poultry industry has the largest number of broilers (i.e., chicken meat production) and layers (i.e., hens that produce table eggs) in the world (National Chicken Council, 2015). As broiler breeders and layer hen flocks go out of production, carcass composting is considered the most economical and practical way to depopulate those flocks and facilitate transformation of dead poultry into a value added product (i.e., compost) (CAST, 2009). *Salmonella*, which includes several zoonotic *Salmonella* (e.g., *Salmonella* Typhimurium [ST], *Salmonella* Enteritidis [SE], *Salmonella* Heidelberg [SH]) are relatively common enteric organisms present in the GI tract of poultry and other avian species (Pan and Zhongtang, 2014). Consequently, considering the importance of composted poultry carcasses as a common soil amendment in plant-based agriculture, the identification of specific composting methods associated with zoonotic pathogen control is essential.

While carcass composting is a ubiquitous practice, farm-scale differences including seasonality (i.e., ambient temperature) have a significant effect on the temperature profile and hence the ability to inactivate pathogens (Sivakumar et al., 2008). In principle,

© 2016 Poultry Science Association Inc.

Received June 10, 2015.

Accepted October 26, 2015.

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elevated compost temperatures ($>55^{\circ}\text{C}$) are associated with reduced pathogen loads (Kim et al., 2012; Himathongkham et al., 2000). In addition, elevated ambient temperatures are associated with elevated composting temperature (Sivakumar et al., 2008). However, differences in the temperature profile of the compost pile do exist, which most likely impacts pathogen survival.

While to our knowledge there have been no published studies of *Salmonella* control in composted poultry carcasses, the issue of *Salmonella* control in general composting has been studied, such as *Salmonella* inactivation in chicken litter and manure (Chen et al., 2013; Kim et al., 2012; Reynnells et al., 2014; Singh et al., 2012). However, the literature shows variation in the effectiveness of composting to control *Salmonella*. Nell et al. (1983) found complete inactivation of *Salmonella* in a pilot-scale system within 8 d versus greater than 2 wk for *Salmonella* inactivation in a field-scale windrow composting system. Other studies (Bharathy et al., 2012; Dunkley et al., 2011) have shown that a relatively longer duration (2 to 3 mo) is needed to reduce pathogen levels to a non-detectable level using composting processes.

With respect to *Salmonella* control, existing guidelines by the U.S. Environmental Protection Agency (U.S. EPA) and Canadian Council of Ministers of the Environment (CCME) assume pathogen inactivation in composting if the particles within the composting piles attain temperatures of approximately 55°C for at least 3 d (Wichuk and McCartney, 2007). However, there is some allotment for the presence of *Salmonella* in the finished composted product. For example, the U.S. EPA allows *Salmonella* to be less than 3 MPN per 4 g of total solids (dry weight basis) (U.S. EPA, 2006). Consequently, the presence or absence of *Salmonella* needs to be linked with quantification criteria.

Improperly composted poultry carcasses with a high load of *Salmonella* may be a source of *Salmonella* contamination when applied as a soil amendment to crop land. Consequently, this source material can be the root cause of *Salmonella* contamination in plant-based crops destined for human consumption. Because of elevated temperatures associated with the rendering of poultry carcasses, rendering is considered by some to be more effective in *Salmonella* control, than carcass composting. However, the logistics and cost of rendering could be prohibitive and logistically non-viable due to the lack of rendering facilities in many agricultural regions around the world. Consequently, carcass composting is the preferred method of poultry carcass disposal/recycling.

The goal of this study is to increase the understanding of *Salmonella* persistence in poultry carcasses at composting (55 and 62.5°C) and low-rendering temperatures (70 and 78°C). The primary objectives of the study are to: 1) understand the impacts of temperatures consistent with composting and low-temperature rendering on the survival of *Salmonella* in poultry car-

casses; 2) assess the effects of mixing and non-mixing conditions on *Salmonella* inactivation in ground carcass slurry; and 3) compare *Salmonella* inactivation in ground carcass slurry and whole birds under composting and low-temperature rendering temperatures. Further, observations of *Salmonella* survival at various temperature and mixing conditions were used to develop simple exponential regression models in order to estimate *Salmonella* inactivation at various temperatures and various conditions (i.e., mixing, non-mixing, grinding, and whole carcass).

MATERIALS AND METHODS

Feedstock Preparation of Ground Carcass Slurry

Pathogen persistence study experiments in poultry carcasses (Figure 1) were carried out using 6-week-old (approximately 500 g each) specific pathogen free (SPF) poultry (Charles River Laboratories Inc., Nevada - Sparks, New York [www.crriver.com]). The frozen SPF poultry carcasses for all the experiments came from one lot, were shipped overnight (at -105°C in dry ice), and stored in a laboratory freezer at -20°C immediately upon arrival. Prior to the beginning of the experiment, the SPF poultry carcasses were thawed overnight in a bio-safety cabinet. After thawing, the SPF poultry carcasses (including feathers and bones) were defragmented into small pieces using a sterile knife. Next, the pieces were blended into a homogenous slurry (by diluting to 4.5x with deionized [DI] water) with a residential grade blender (Ninja model BL800). The term “ground carcass slurry” is being used to describe the carcass that includes water. The crude fat, protein, and ash contents of the homogenous slurry were tested using the Randall modification of the standard Soxhlet extraction, combustion method and gravimetric method, respectively, at the UC Davis Analytical Lab (University of California Davis Analytical Laboratory, 2015).

Feedstock Inoculation and Sample Analysis

Prior to the experiment, the feedstock (i.e., ground SPF birds) was plated on Difco Xylose Lysine Deoxycholate (XLD) agar plates to confirm the absence of *Salmonella* (Becton, Dickinson and Company, Sparks, MD). If no growth occurred at a 10° dilution, the sample was considered as non-detectable (ND) (i.e., negative) for ST LT2. To inoculate the feedstock, ST LT2 (ATCC #700720) culture was grown in Difco LB (Luria-Bertani) Broth Miller growth media. After 24 h of incubation, 2 25 mL cultures that were determined to be 10^8 CFU/mL via standard plating on XLD were centrifuged (ThermoFisher Sci., Sorvall Legend X1R) at 8,000 rpm for 10 min to form pellets. Both pellets of ST LT2 were then mixed into the feedstock. Subsequently,

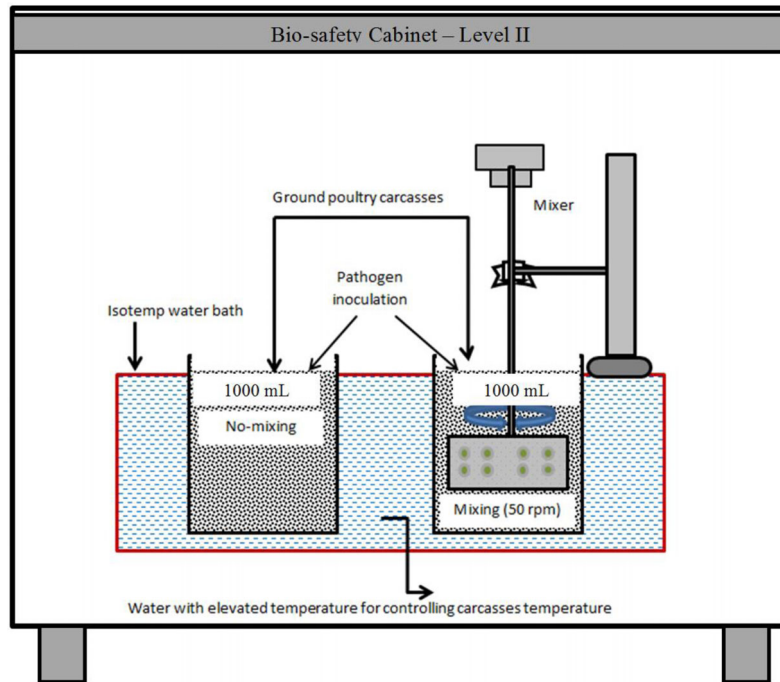


Figure 1. Schematic of experimental setup used in testing pathogen inactivation in ground poultry carcass slurry.

the feedstock was blended for approximately 2 min after inoculation to further homogenize the feedstock. During each experiment a new inoculum of *Salmonella* was grown from -80°C stock.

ST LT2 was quantified in duplicate using the standard FDA Bacteriological Analytical Manual (**BAM**) procedure, which includes a 9:1 volume/g dilution in Phosphate Buffer Solution (**PBS**) (USFDA, 2015). For Figs 2 and 3, 10^6CFU/mL was used to indicate the absence of *Salmonella* growth. In addition to ST LT2 enumeration, the pH (Omega Engineering, INC., Stamford, CT) and moisture content of the samples were measured at the same frequency based on temperature described above. The moisture content was determined using a standard protocol (APHA, 2005).

Reactor Design and Analysis

The inoculated feedstock (800 mL) was added into 2 sterile 1 L glass beakers. Each beaker served as a reactor, one as a mixing reactor and the other as a non-mixing reactor. The 2 beakers were later placed into a 10 L isotemp water bath (Thermo-Fisher Sci.) as shown in Fig. 1. In order to measure the come-up time for the slurry to reach the target temperature of the isotemp water bath, the slurry temperature was measured (shown in Fig. S1 [supplemental material]) for the experiments at 70 and 78°C . The come-up times for the slurry to reach the target temperatures of 70 and 78°C were 30 and 40 min, respectively. Since the durations of the experiments at 55 and 62.5°C were longer than 480 min (much greater than the come-up time), the come-up temperature was not measured at 55 and 62.5°C . The reactor that was being mixed received continuous

mixing at 50 rpm with a compact digital mixer system (Cole-Parmer) for the entire length of the experiment (Fig. 1). The non-stirring reactor did not receive any mixing. Each ST LT2 survival study was carried out (in a bio-safety cabinet) at 55, 62.5, 70, and 78°C . Experiments at 70 and 78°C were carried out for 60 min, while the 55 and 62.5°C experiments lasted between 44 and 8 h, respectively.

Each inactivation experiment of ground carcass slurry was conducted 2 times and named as Run 1 and Run 2. In every experiment, multiple samples were collected over time in order to generate the inactivation curve. The total number of samples of each experiment varied depending on the temperature condition. As an example, at 70 and 78°C , samples were collected at 5-min intervals, while at 62.5 and 55°C , the sample collection interval was ≥ 60 min. In summary, 12 samples were collected for each experiment at 78 and 70°C (mixed as well as non-mixed conditions), 8 samples were collected at 62.5°C , and 11 samples were collected at 55°C . However, during Run 2 of the 55°C , the inactivation study was extended because the observations of the first experiment indicated a prolonged survival of *Salmonella* at 55°C . Observations were fitted into exponential regression models in order to derive *Salmonella* persistence. Out of 2 experimental runs, the observations of one run were used for model development, and the observations of the second run were used to verify the model predictability.

Whole Carcass Experimental Design

A similar experimental design was used in order to evaluate ST LT2 inactivation in whole (i.e., not ground)

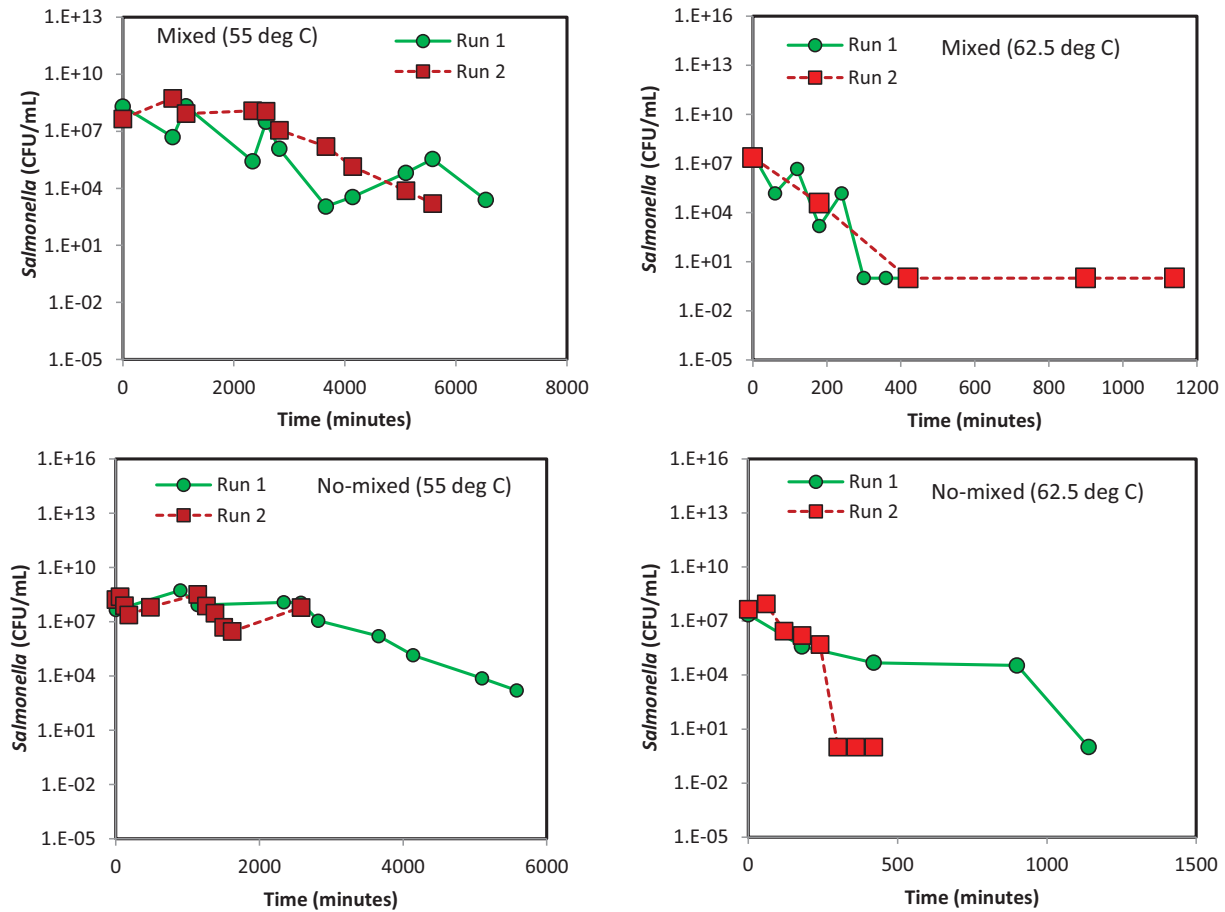


Figure 2. *Salmonella* persistence during composting at 55 and 62.5°C under mixed and non-mixed conditions (total number of samples for 2 mixed and non-mixed runs at 55°C was 42; total number of samples for 2 mixed and non-mixed runs at 62.5°C was 26).

non-mixed carcasses at composting temperatures (55 and 62.5°C). The whole bird was placed inside an isotherm lab incubator to expose the bird to the isotherm 55°C temperature. Although the incubator temperature was set to 55°C, the internal temperature of the bird was measured to be $52^\circ\text{C} \pm 0.5^\circ\text{C}$ during the length of the experiment. Consequently, the whole bird experiments were labeled based on the internal bird temperature as opposed to the isotherm temperature. The most significant differences between the 2 experiments included grinding versus not grinding, the route of ST LT2 application, and how the samples were collected for quantitative analysis. With respect to the route of application, the ST LT2 inoculum was injected approximately 25 to 30 times (total) in the breast, thigh, hock, back, cape, neck, and shank of the intact SPF poultry carcass. The pathogen injection depth was approximately one to 3 cm. In addition, the whole SPF bird carcasses were soaked in the ST LT2 inoculum (300 to 400 mL) for approximately 20 min. With respect to the quantitative analysis, tissue samples from the breast, thigh, hock, back, cape, neck, shank, and feathers were collected using a sterile knife and scissors. Compositing tissue samples were then ground and quantitative plating was done on a volumetric basis as described above.

The grinding and dilution of composite carcass samples resulted in an order of magnitude (i.e., 10x) dilution. However, if there was no growth detected at this dilution then one g of carcass was plated directly in agar plates. Over the time, carcass was degraded, and one g of degraded material was plated (i.e., smeared) directly on the agar plate. If no growth occurred in this direct plating, then pathogen level was considered non-detectable. This method resulted in setting the detection limit of 1 CFU/g of carcass.

RESULTS AND DISCUSSION

Salmonella Persistence in Temperatures Consistent With Composting

Persistence of ST at 55 and $62.5 \pm 2^\circ\text{C}$ is shown in Fig. 2 describing the impacts of temperature and the effects of mixing and non-mixing on ST persistence. At 55°C, the initial ST concentration in the feedstock was 10^8 CFU/mL in both mixed and non-mixed conditions. At the end of the experiments (after 2,580 minutes \approx 69 hours), the ST concentration was reduced to 10^3 CFU/mL and 10^4 CFU/mL for the mixed and non-mixed experiments, respectively. Using standard

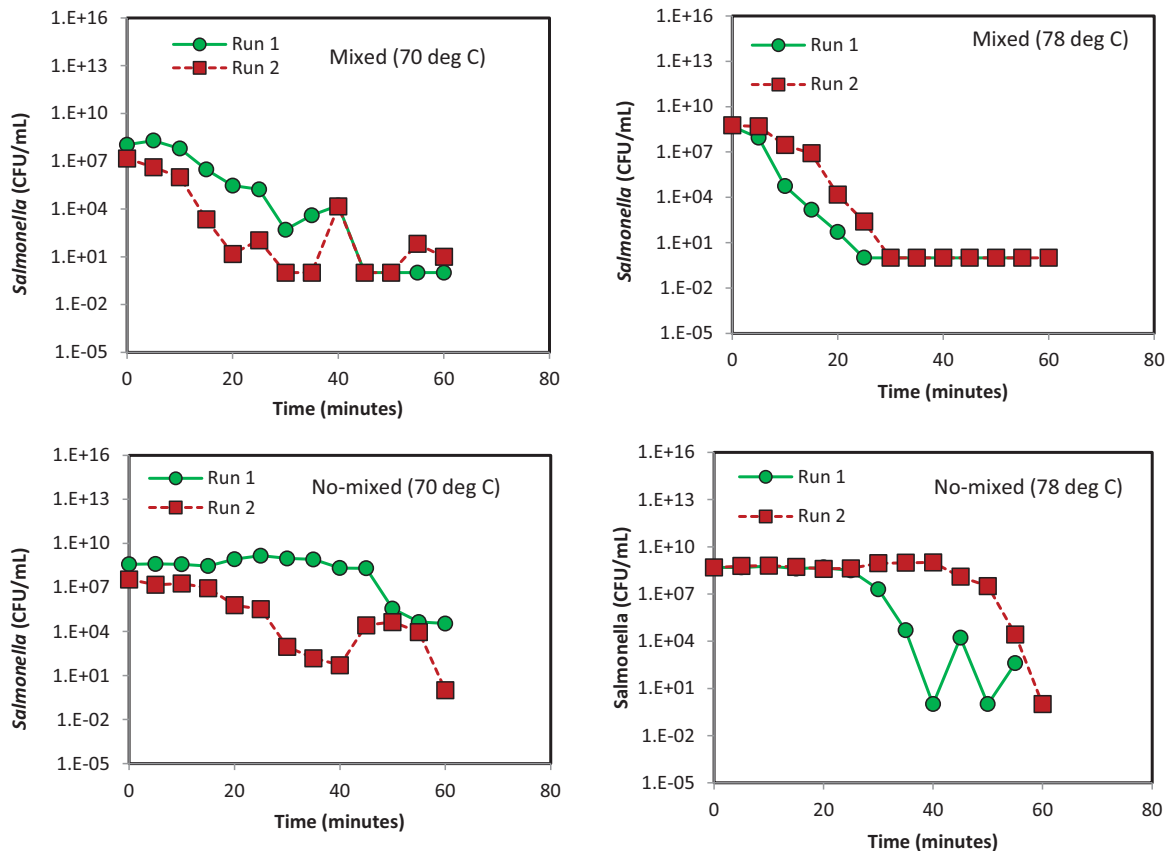


Figure 3. *Salmonella* persistence during low-temperature rendering at 70 and 78°C under mixed and non-mixed conditions (total number of samples for 2 mixed and non-mixed runs at 70°C was 24; total number of samples for 2 mixed and non-mixed runs at 78°C was 24).

regression, it was predicted that the remaining ST in the mixed and non-mixed experiments would be reduced to less than one order of magnitude after approximately 10,000 minutes (≈ 166 h) (Fig. 5). At 62.5°C, the inactivation of ST was relatively faster as shown in Fig. 2. Specifically, in both mixed and non-mixed conditions, the initial ST concentrations were 10^8 CFU/mL. At the end of both mixed and one non-mixed experiments, the ST concentration was reduced to 10^0 CFU/mL within 300 min (Fig. 2). In the second non-mixed experiment, it took 1,200 min ($20 \approx$ h) to reduce the ST concentration to 10^0 CFU/mL (Fig. 2). One potential explanation for the variability in ST reduction as a function of time in the non-mixed experiment could be the heterogeneity of each sample with respect to temperature. In order to determine if SH and SE inactivation under temperatures consistent with composting was similar to ST, pure cultures of SH, SE, and ST were heated to 58°C in Difco LB Broth. Results showed identical reduction from 10^7 CFU/mL to 10^0 CFU/mL within 2 h for SH, SE, and ST. While further experimentation using carcasses and different temperature profiles is still necessary, these initial results indicate that the survival of these 3 *Salmonella* are likely to be similar under temperatures consistent with composting. These serotypes of *Salmonella* are important because they can be found in poultry and are zoonotic in nature.

ST Persistence in Temperatures Consistent With Low Rendering

Figure 3 shows ST persistence at 70 and $78 \pm 2^\circ\text{C}$. These temperatures were selected because they are consistent with low-temperature rendering (LTR) (Australian Meat Technology, 1997) as opposed to high-temperature rendering, which is typically 120°C. Since the temperatures are lower, ST persistence may be greater in LTR and hence the experiments were focused on this type of rendering. Initial ST concentrations in the mixed and non-mixed feedstock were 10^8 CFU/mL. In the mixed conditions at 70°C, ST concentrations were non-detectable after 45 min, while in the non-mixed condition one ST experiment was non-detectable after 60 min (Fig. 3). However, the other result from that experiment showed 10^4 CFU/mL at the same time point. These inconsistent results with respect to time to ST inactivation are similar to the non-mixed composting experimental results described in Sec. 3.1.

At 78°C, ST concentrations became non-detectable within 20 min in mixed conditions, while in non-mixed conditions ST persistence lasted for 60 min. While the effects of LTR temperatures are faster on ST inactivation, mixing is also an essential component of consistent inactivation, as demonstrated by the variability of the non-mixing results (Fig. 3).

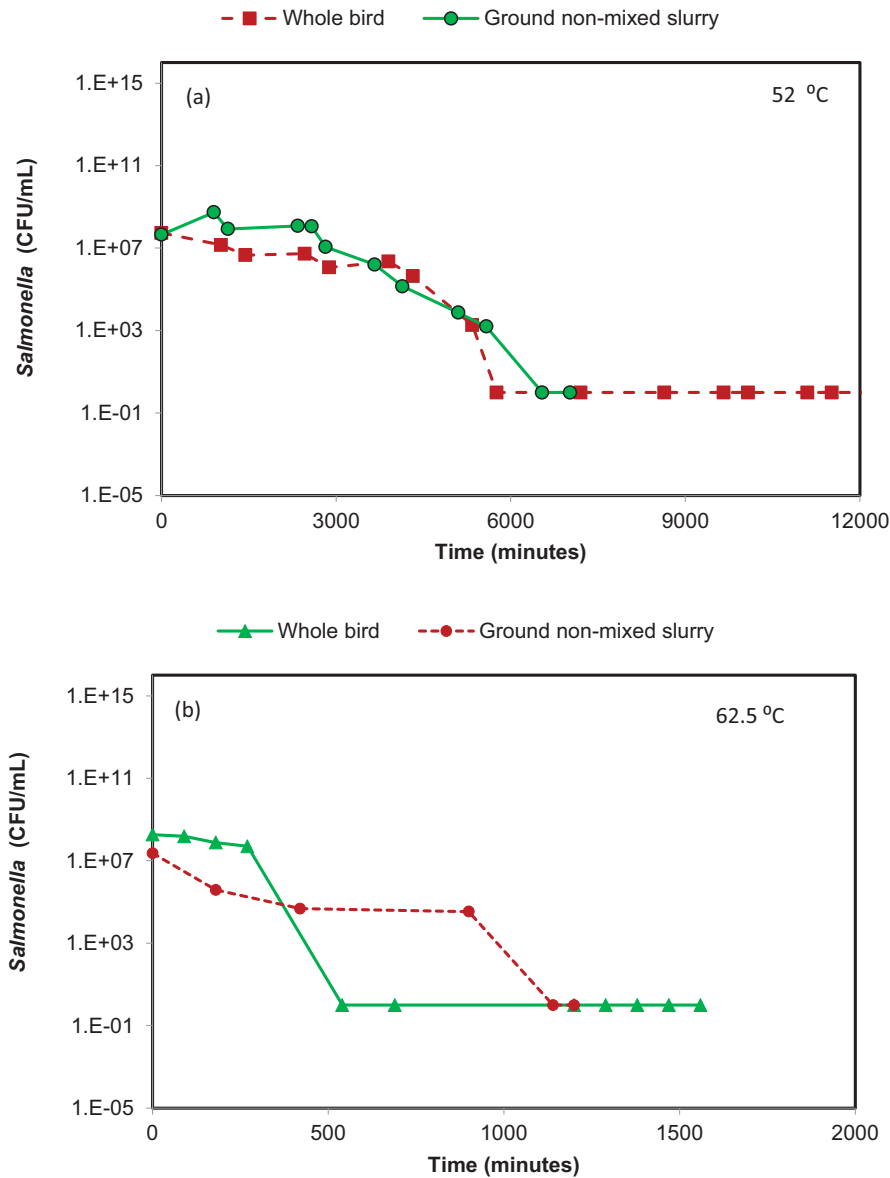


Figure 4. Comparison between *Salmonella* inactivation in whole bird and non-ground poultry carcass slurry under composting conditions (total numbers of samples at 52 and 62.5°C for whole bird were 16 and 11, and ground non-mixed carcass slurry were 12 and 6, respectively). In Fig. 4A, the whole bird actual temperature was 52°C, while non-ground poultry carcass slurry temperature was 55°C).

Comparison Between *Salmonella* Persistence in Whole Bird and Ground Carcass Slurry

Figure 4 shows the difference between ST inactivation in whole bird carcass and non-mixed ground poultry carcass slurry at 52°C and 62.5°C. At 52°C ST inactivation in both ground poultry carcass slurry and whole birds followed a similar pattern (Fig. 4). As an example, in Fig. 4A, the ST concentration for both the whole bird and the ground non-mixed bird went from 10^7 to non-detectable in 5,800 and 6,500 min, respectively (Fig. 4A). In contrast, the time to non-detection for ST at 62.5°C in the whole bird carcasses and the non-mixed ground carcasses was 570 and 1,140 min, respectively (Figure 4B).

The differences in ST reductions of non-mixed ground carcass slurry and whole birds could be due to differences in the dissemination of heat in whole birds and non-mixed ground carcass slurry. For example, in whole birds, the carcass and the bird's feathers were intact. In contrast, in the ground conditions, the carcasses and feathers were ground and mixed uniformly before the experiments were started. Consequently, in the ground poultry carcass slurry heat dissemination would be expected to be more uniform and hence *Salmonella* inactivation more predictable compared to whole carcasses due to *Salmonella* inactivation.

In addition to the differences in heat dissemination, the fat content of the carcass slurry can affect *Salmonella* inactivation (Murphy et al., 2000, 2002, 2004; Juneja et al., 2001). Specifically, Murphy

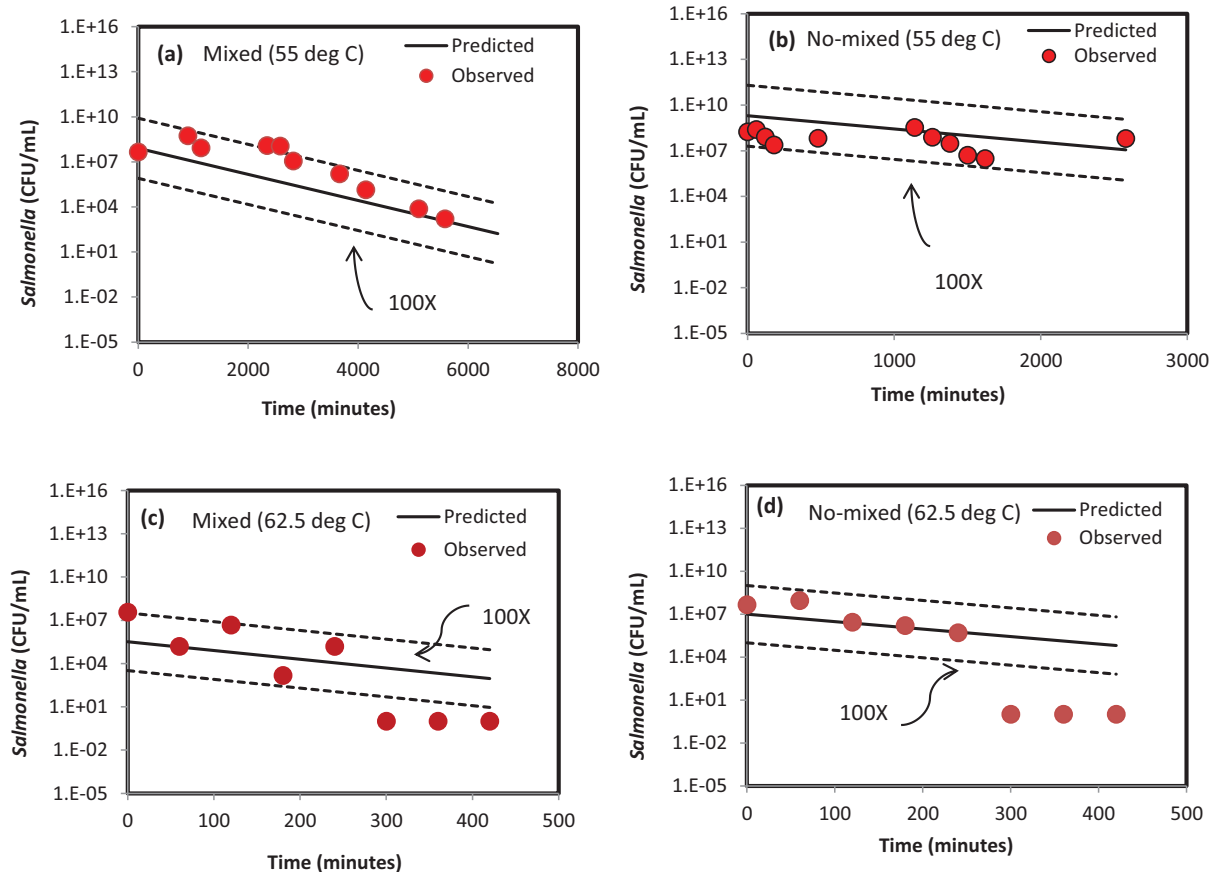


Figure 5. Comparison between observed and predicted *Salmonella* inactivation at 55°C and 62.5°C. One of the 2 runs was randomly selected to develop the predicted exponential regression equation. Subsequently, the unused data from the alternative run was plotted in order to compare the predicted regression with the data from the un-used run.

et al. and Juneja et al. tested thermal inactivation of *Salmonella* in ground chicken and thigh and leg meat and found that increased fat content resulted in increased bacterial resistance to heat (Murphy et al., 2000, 2002, 2004; Juneja et al., 2001). The fat content of the ground slurry used by Murphy et al. (2004) was 10.3% compared to 22.0% for our study. This difference is most likely due the age of the birds tested. Specifically, the birds in our study were 6-week-old SPF birds compared to sexually mature broiler breeders and laying hens, which are at least 18 wk of age. Consequently, if older birds with less body fat were used for our study, the slope of our inactivation curves would be expected to be steeper.

Exponential Regression Equations for Calculating *Salmonella* Inactivation

In order to better understand ST inactivation, exponential regression models were developed. Figure 5 shows the best-fit exponential regression of ST inactivation in mixed and non-mixed ground poultry carcass slurry at 55 and 62.5°C. The comparisons between predicted and observed ST concentrations (under mixed and non-mixed conditions) at 55°C are shown in Fig. A and B. For the mixed conditions, approximately 82% of

the predicted values were within 2 orders of magnitude of the observed values (Fig. 5A and B). The R^2 value in mixed condition was 0.75. In contrast, in the non-mixed condition, the R^2 value was relatively poor (i.e., $R^2 = 0.10$).

Figure 5C and D shows the comparison between the predicted and observed values at 62.5°C for mixed and non-mixed ground poultry carcass slurry. Under mixed conditions, the R^2 value was 0.84, while in non-mixed conditions, the R^2 was 0.49. Figure 6 shows the comparison between the predicted and observed values at 70°C for mixed and non-mixed ground poultry carcass slurry. Under mixed conditions, the R^2 value was 0.98, while in non-mixed conditions, the R^2 was below 0.1 (Fig. 6A and B). Figure 6C and D shows the comparison between the predicted and observed values at 78°C for mixed and non-mixed ground poultry carcass slurries, respectively. Under mixed conditions, the R^2 value was 0.67, while in non-mixed conditions, the R^2 was below 0.26. For all 4 temperatures, the mixed conditions have a higher R^2 value than the non-mixed conditions, indicating a higher predictability for *Salmonella* inactivation in mixed conditions versus non-mixed conditions. One possible reason for this variation could be differences in the generation and maintenance of heat during mixed and non-mixed

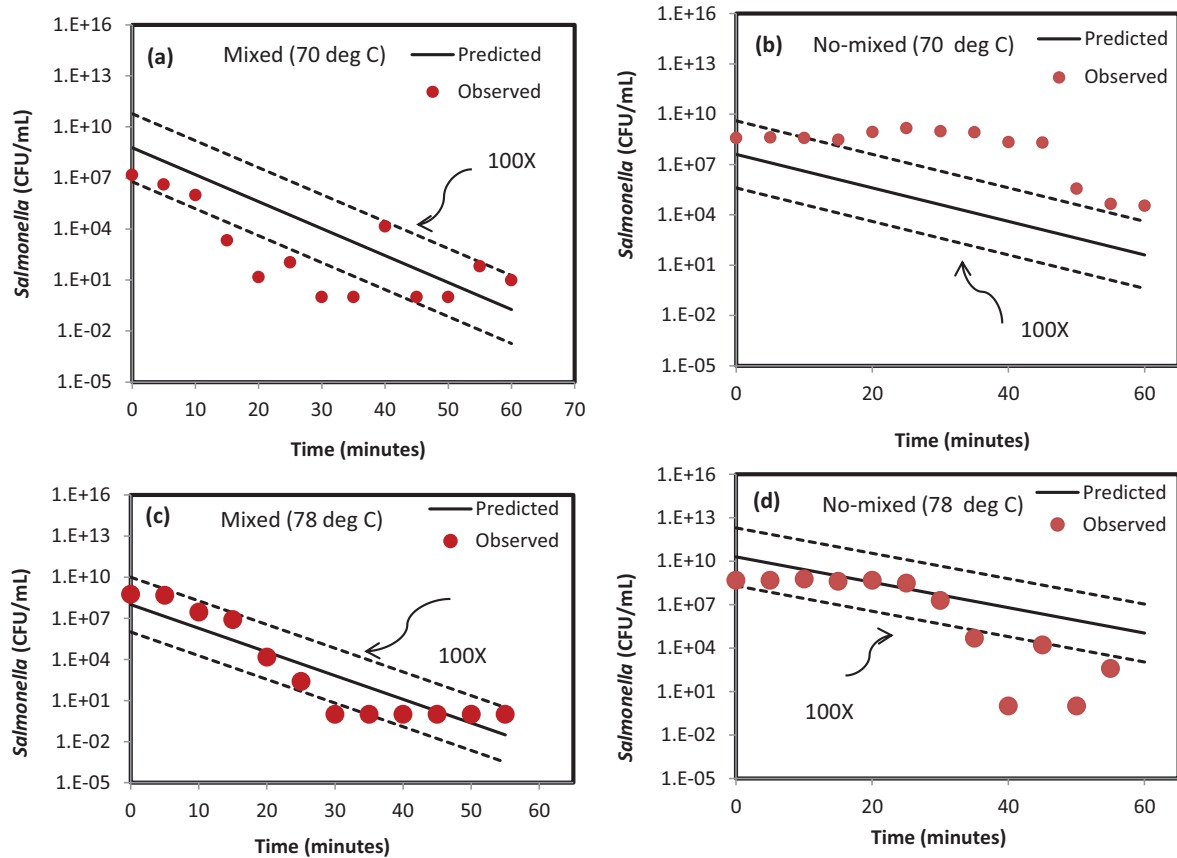


Figure 6. Comparison between observed and predicted *Salmonella* inactivation at 70°C and 78°C. One of the 2 runs was randomly selected to develop the predicted exponential regression equation. Subsequently, the unused data from the alternative run was plotted in order to compare the predicted regression with the data from the un-used run.

conditions. In mixed conditions, heat was more likely to be distributed uniformly throughout the reactor. In contrast under non-mixed conditions, heat distribution within the reactor would be expected to be less consistent in time and space. These differences in heat distribution could result in differences in *Salmonella* inactivation.

In addition to the noted differences under bench-top conditions, the literature is full of examples of differences in inactivation time under different field conditions. Lawson and Keeling (1998) reported that the temperature of mini composter changed from 36°C to 71°C on d 10. During 65 d of experiment, mini composter temperature varied from 36 to 50°C with the peak of 72°C, and *Salmonella* was fully heat-inactivated. Another study by Dunkley et al. (2011) conducted an experiment focused on examining the composting efficiency in breaking down poultry carcasses and assessing the impacts of seasonality (winter and summer) on *Salmonella* inactivation. Dunkley et al., 2011 reported that both winter and summer composting processes were effective. Bharathy et al. (2012) conducted a bin compost experiment on the chicken slaughter-house waste. They included poultry litter and coir pith as the compost additives to poultry slaughter waste. They achieved a good compost temperature (55.6°C) in the compost bins. However, it took 52 d to

have non-detectable *Salmonella* levels. While it is well known that elevated temperatures are detrimental to *Salmonella* (CDC, 2014), the types of differences noted above show a range of times for *Salmonella* inactivation, which are most likely due to additional environmental and composting factors (e.g., pH, moisture content, ambient temperature, ammonia concentration, substrate selection). Therefore, additional studies are needed to understand the heat dissemination patterns within different types of composting processes.

pH and Moisture Content

The details of carcass pH and moisture content as a function of time for both the experimental runs are presented in Table 1. The feedstock pH consistently remained acidic (~6) throughout all the experimental runs. In addition, pH did not vary as a function of time. The bench-top experiments did not get pH's below 6 most likely due to the absence of organic substrate material typically used as a carbon source in composting. The average moisture content of the ground SPF carcass slurry (i.e., feedstock) varied from 89 to 91% and did not change as a function of time during each experiment (Table 1).

Table 1. pH and moisture content during the experimental runs.

Temperature (°C)	Moisture, % (mean ± st. dev)		pH (mean ± st. dev)	
	Mixed	Non-mixed	Mixed	Non-mixed
55	89.7 ± 1.3	89.4 ± 1.7	6.2 ± 0.13	6.3 ± 0.28
62.5	91.5 ± 1.7	90.9 ± 1.6	6.3 ± 0.12	6.2 ± 0.09
70	91.7 ± 2.0	89.9 ± 1.3	6.1 ± 0.22	6.0 ± 0.14
78	93.1 ± 0.9	89.5 ± 1.8	6.2 ± 0.22	6.0 ± 0.16

CONCLUSIONS

One of the fundamental difficulties of using animal manure and carcasses as a composting substrate is the presence of pathogens including *Salmonella* in the pre-composted material. Effective composting transforms the pre-compost material into finished compost that can be used in crop-based agriculture. Regulatory surveillance of the finished compost requires that *Salmonella* levels be below 3 MPN/4 g of total solids and less than 1,000 MPN for fecal coliforms per g of total solids (EPA, 2006). In order to better understand the effectiveness of composting temperatures on the inactivation of *Salmonella* within poultry carcasses under different conditions (i.e., grinding, whole bird, mixed, non-mixed) inactivation of *Salmonella* Typhimurium LT2 was assessed. In addition, limited testing of SE and SH was done in order to determine if other *Salmonella* responded similarly. Results showed that the changes in temperature of the poultry carcasses influenced the inactivation time of *Salmonella*. Specifically, the inactivation time of *Salmonella* was inversely proportional to the temperature levels tested during the experimental trials, indicating the rapid reduction in *Salmonella* as a function of increased temperature. In addition to temperature, mixing was also determined to be a significant factor in *Salmonella* inactivation. Exponential regressions developed for calculating *Salmonella* inactivation performed well in mixed conditions compared to non-mixed conditions, indicating more predictability with respect to identifying the final inactivation. While the bench-top experiments showed that temperatures consistent with composting (i.e. 52 and 62.5°C) reduced *Salmonella* level to a non-detectable level over time, additional field scale studies are needed to better understand *Salmonella* inactivation predictability. Future studies focused on understanding the variability in temperature in composting piles will help in optimizing time and temperature with respect to pathogen inactivation under various weather conditions.

ACKNOWLEDGMENTS

The authors would like to thank the Center for Food Animal Health (CFAH), at the University of California, Davis, Pacific Egg and Poultry Association (PEPA), and the California Poultry Federation (CPF) for providing funding for this study. The authors would also like to thank the UC Davis Veterinary Medicine School

and Division of Agriculture and Natural Resources (ANR), UC Davis, for providing the support for this study.

SUPPLEMENTARY DATA

Figure S1 (supplemental information): Come-up time for the ground carcass slurry to reach the target temperature of the isotherm water bath: A) isotherm water bath temperature 78°C; B) isotherm water bath temperature 70°C.

Supplementary data is available at *PSA Journal* online.

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