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Salmonella Typhimurium LT2 Decay in Poultry Carcasses During Thermophilic Digestion

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Abstract. *The elevated levels of pathogen such as Salmonella in poultry carcasses can potentially pose risk to the public health. Controlling Salmonella in poultry carcasses prior to its disposal can help in reducing Salmonella risk to the public and environment health. In order to identify the effect composting temperature on Salmonella inactivation, here we have evaluated the impacts of thermophilic temperature on Salmonella decay during poultry carcass digestions resembling composting environment. We have studied the decay of Salmonella Typhimurium LT2 in poultry carcasses at 55, 62.5 °C. The effects of mixing and non-mixing were also evaluated on the decay of Salmonella. Considering the importance of composting for disposing poultry carcasses, we anticipate that the study presented here will provide additional insight for assessing the effects of composting environment on controlling Salmonella.*

Keywords. *Composting, Decay, Poultry carcasses, Salmonella*

I. Introduction

Taking into consideration about the importance of egg and poultry industries in the United States, which play an important role in the economy of the US, issues such as disposal of poultry carcasses are of utmost importance. The US Poultry and Egg Association (USPEA) estimates that the annual per capita egg production was 263 and 255 in 2014 and 2013, respectively generating greater than 8 billion revenue (USPEA, 2014). The US poultry industry is the largest meat producer in the world (USDA-ERS 2014). Considering the extent of the industry, concerns such as the public health and environmental pollution are challenges for the US poultry industry (Shane, 2013).

Issues such as the disposal of poultry carcasses are also a leading concern (CAST, 2009). Options of poultry carcass disposal such as incineration, rendering, burial, and composting are often applied for disposing poultry carcasses (Blake and Donald, 1992). In many places around the world, composting has been a preferred method for disposing poultry carcasses mainly due to its simplicity and the economic benefits (CAST, 2009). However, the effectiveness of composting process in *Salmonella* elimination completely is uncertain. For improving the composting process, a range of additional provisions such as providing supplementary heating has been proposed and debated (Sander et al., 2002). Identifying the cost-effective methods of poultry carcasses disposal with minimum risk to the public health and environment is important.

The primary goal of this study was to improve the understanding of *Salmonella* inactivation in poultry carcasses during composting process. In order to achieve the goal, multiple experiments were carried out at 55, and 62.5 °C (representative to composting temperature) to assess *Salmonella* inactivation. The objectives of the study were to: 1) understand the impacts of 55 °C on *Salmonella* inactivation in poultry carcasses; 2) assesse the impacts of 62.5 °C on the inactivation of *Salmonella*; and 3) assess the effects of mixing and non-mixing on *Salmonella* inactivation at 55 and 62.5 °C.

2. Materials and Methods

2.1 Poultry carcasses feedstock prepration

The frozen Specific pathogen free (SPF) layer birds were purchased from Charles River Laboratories Inc., New York, USA (www.criver.com). A day prior to beginning of the experiment, the SPF chicken carcasses were thawed overnight using a bio-safety cabinet (level II) to prevent bird contamination. The SPF chicken carcasses were defragmented into small pieces using knives. Then the pieces were blended with a residential grade blender (Ninja model BL800) to form homogenous slurry (by diluting to 4.5 x with deionized (DI) water). A detailed method poultry carcass feedstock preparation is described by Vaddella et al., 2015.

Centrifuged pure *Salmonella typhimurium LT2* culture pellets (from 2 tubes each of 25 mL) were introduced into the slurry of ground carcasses. Subsequently, the slurry was blended approximately for 2 minutes to make sure that the pellets of *Salmonella typhimurium LT2* culture were uniformly mixed in the slurry. The fine SPF slurry (each 800 g) was then added into two 1L glass beakers. Each beaker served as a reactor, one as a mixing reactor and the other beaker as a non- mixing reactor. The two beakers were later placed into an isotemp water bath (10 L capacity, Thermo-Fisher Sci.). The mixing slurry beaker received continuous mixing at 50 RPM with a compact digital mixer system (Cole-Parmer catalog #EW-50006-01) for entire duration of the experiment. While the non-stirring reactor did not receive any mixing. Each persistence study was carried out (in a bio-safety cabinet (level II)) at 55, 62.5° C temperature levels (common compost temperature ranges). Two different runs were conducted with different inactivation times.

2.2 Pathogen inoculation in SPF chicken slurry, and enumeration

To test the pathogen inactivation process, the SPF chicken slurry was inoculated with *Salmonella typhimurium LT2* pathogens as described in section 2.1. Fresh pathogen culture of *Salmonella typhimurium LT2* (ATCC #700720) was prepared in the lab prior to starting of experiment. Difco LB (Luria-Bertani) Broth Miller growth media was used to grow *Salmonella typhimurium LT2*. Two tubes

of each 25 mL fresh pathogen culture was pelletized using a centrifuge (ThermoFisher Sci.: Sorvall Legend X1R, Catalog #75004261) at 8000 RPM for 10 minutes. The pellets of pathogens from both the tubes was mixed in the slurry. After the chicken slurry was inoculated, the initial concentration of each pathogen in the slurry was enumerated using Bacteriological Analytical Manual (BAM) procedure and Difco XLD Agar (Becton, Dickinson and Company, Sparks, MD, USA) plates were used.

Before inoculating into the slurry, the *Salmonella typhimurium LT2* pathogen level in pure culture was enumerated by plating (in duplicates) 100 µL of serially diluted pure culture streaked on to Difco XLD Agar plates. To obtain the dilution, 1 mL of pure culture containing pathogens was added with 9 mL of diluent phosphate buffer solution (PBS). After plating, the plates were incubated for 24 hours at 35° C, and pathogen colonies grown in the plates were enumerated using a hand held digital colony counter (Scienceware Catalog #F37862-0002). Red colonies with black centers were enumerated as *Salmonella typhimurium LT2.*

In digestate samples, *Salmonella* colonies were enumerated at regular interval. If the colonies were absent at a higher dilution then the samples with lower dilution (until no dilution, 10^0) were streaked on the XLD agar plates. If no growth occurred at a 10^0 dilution, then the pathogen level in the sample was considered as non-detectable (ND). A pH meter (Omega Engineering, INC., Stamford, CT, USA) equipped with a pH and a temperature probe was used to measure the pH and temperature during the experiment. Also the moisture content at the beginning and at the end of the study was also determined using standard protocol (APHA, 2005).

3. Results and discussion

3.1 Mixed Condition

Salmonella inactivation at 55 °C

The log *Salmonella* inactivation plots in ground SPF chicken carcass of Run 1 mixed (R1M) and Run 2 mixed (R2M) (55 °C) are presented in Figure 1. The following observations are noted from the results of the two runs. In R1M, the initial *Salmonella* concentration had an eight orders of magnitude (CFU/mL) and at the end of the experiment (after 69 hours), the *Salmonella* concentration was reduced to about 4 orders of magnitude. From the results (R1M), about 50% of *Salmonella* was inactivated (after 70 hours) under thermophilic (55 °C) conditions. Furthermore, it took about 61 hours for the removal of 33% initial *Salmonella* concentration. The *Salmonella* inactivation process resulted in a low R^2 value of 0.34. From these results, it is evident that the *Salmonella* bacteria were able to survive for a longer duration at a thermophilic temperature of 55 °C.

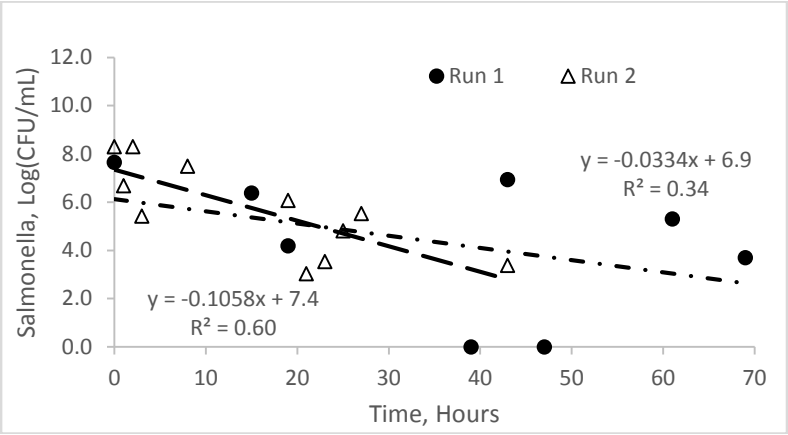

Figure 1. Inactivation of *Salmonella* pathogen at 55 °C (Mixed: R1M and R2M)

In Run 2 (Figure 1), the experiment started with an eight orders of *Salmonella* concentration (CFU/mL). At the end of the run, the remaining *Salmonella* concentration was about three orders of magnitude. In 43 hours, about 5 orders of *Salmonella* was destroyed during the R2M. The inactivation curve had a relatively better R^2 value of 0.60. By the end of the Run 2, about 60% of the *Salmonella* bacteria were removed (in 43 hours). A 33% of *Salmonella* was destroyed approximately after 19 hours. When compared both the runs, R1M showed a better *Salmonella* inactivation. However, in both runs, *Salmonella* were able to survive for a long duration (2-3 days) indicating that *Salmonella* can potentially survive greater than 2 days at 55 °C.

Salmonella inactivation at 62.5 °C

The log results from *Salmonella* inactivation experimental runs (R1M and R2M) in ground SPF chicken carcass at mixed condition (62.5 °C) are depicted in Figure 2. In run 1, the log *Salmonella* concentration (CFU/mL) at the beginning of experiment was in the orders of 7 and at end of the experiment (just in 7 hours), the *Salmonella* concentration reached to a non-detectable (ND) level. About 7 orders of *Salmonella* was removed (100% removal) in the mixed condition at 62.5 °C. About 38% of *Salmonella* bacteria were destroyed within just 3 hours after the experimental run. The *Salmonella* inactivation plot showed an excellent R^2 value of 0.99. This indicates that for current thermophilic temperature (62.5 °C), in ground SPF chicken carcass, *Salmonella* inactivation occurs in at a much quicker rate than at the 55 °C.

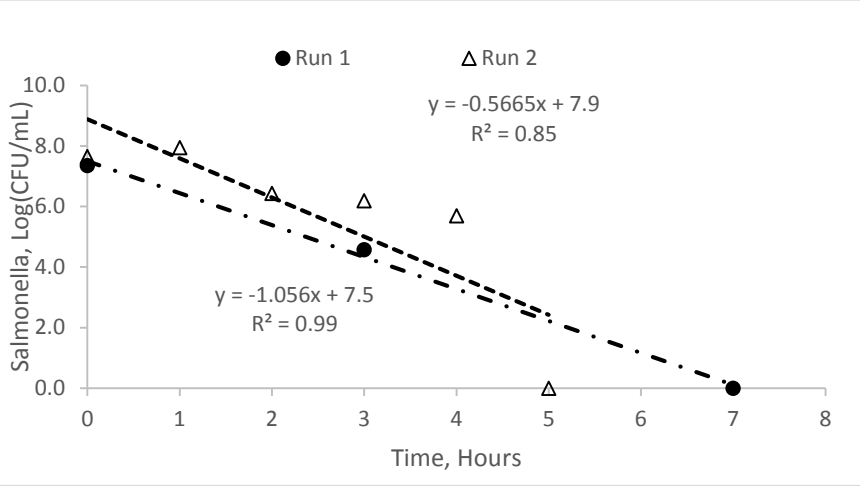

Figure 2. *Salmonella* pathogen Inactivation plots at 62.5 °C (Mixed: R1M and R2M**)**

In run 2, the initial log *Salmonella* concentration during the second run (62.5 °C) was approximately 8 orders of magnitude. During this run, the *Salmonella* concentration reached to a ND level in 5 hours duration. In this run, a 25% of *Salmonella* was removed after the 4th hour. The remaining 75% of the *Salmonella* removal (100% removal) occurred in the fifth hour of the experiment. The *Salmonella* inactivation curve (Figure 2) showed a good correlation with an R^2 value of 0.85. The results from both the runs indicate that the *Salmonella* inactivation in ground SPF chicken carcass under mixed condition, will be faster with increased thermophilic temperatures.

3.2 Non-mixed condition

Salmonella inactivation at 55 °C

The results of *Salmonella* inactivation experimental runs in log scale (R1NM, and R2NM) in ground SPF chicken carcass at non-mixed condition conducted at 55 °C are depicted in Figure 3. The duration of the R1NM was 109 hours while it was 43 hours during the R2NM. Run 1 was carried out until all the initial (about 8 orders of magnitude) *Salmonella* presence in the ground SPF chicken carcass inactivated to a ND level. For a complete *Salmonella* inactivation process (100% removal), it took 109 hours (about 4.5 days). Until first 43 hours of this experiment, there was no change in the initial *Salmonella* concentration. The reason for no inactivation until 43 hours probably attributed to non-mixing condition. Under non-mixing conditions, the temperature profile within the reactor might

not have been homogenous. This assumption could be demonstrated with the mixed condition experiments at same temperature during both the runs. Inactivation times of 69, 85, and 109 hours were observed for 33, 50, and 100% removal of *Salmonella*. When compared under mixing condition (R1NM), about 50% of *Salmonella* was removed after 70 hours. Under this non-homogenous temperature profile, *Salmonella* might have survived for a longer period without any inactivation. The *Salmonella* inactivation curve had a good correlation with an R^2 value of 0.82.

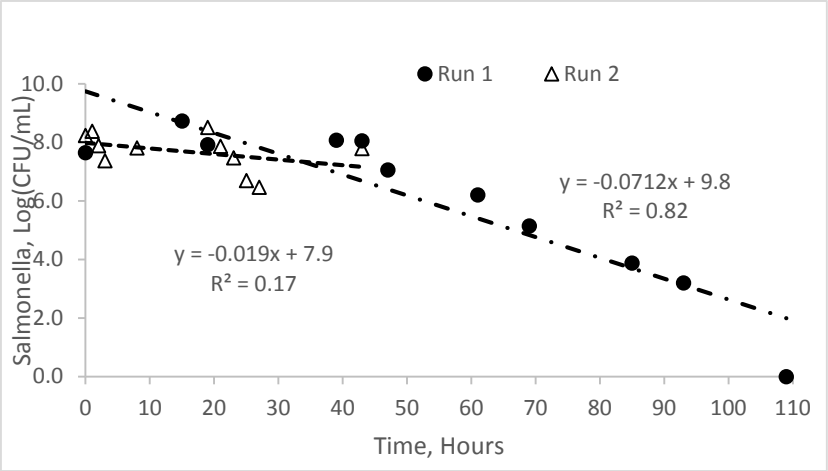

Figure 3. Inactivation of *Salmonella* pathogen at 55 °C (R1NM, and R2NM)

Run 2 (R2NM) was carried out for 43 hours of duration under non-mixed condition. Similar to the R1NM, only 5% reduction of *Salmonella* was observed in 43 hours of time. However, in mixed condition during R2NM, about 60% *Salmonella* was removed in the same duration (after 63 hours). The inactivation process had a very low value of R^2 of 0.17. When first 43 hours of inactivation from R1NM was considered for comparison (not shown in Figure 3), the R^2 value was only 0.03. These results indicate that at a thermophilic temperature of 55 °C, it is difficult to predict the *Salmonella* inactivation process under non-mixed conditions in the first 2-3 days. Results from both the runs at a temperature of 55 °C clearly indicate that the mixing condition is favorable to inactivating the *Salmonella* bacteria.

Salmonella inactivation at 62.5 °C

The results from *Salmonella* inactivation experimental runs in log scale (R1NM, and R2NM) in ground SPF chicken carcass at non-mixed condition conducted at 62.5 °C are presented in Figure 4. In Run 1, the initial *Salmonella* concentration presence (on a log scale) in this run was about 7.5 orders of magnitude. In this R1NM, the complete inactivation of *Salmonella* process completed to a ND level (100% removal) in 19 hours. The inactivation curve at a temperature of 62.5 °C temperature had a good R^2 value of 0.79. The removal of 25, 60, and 100 % of *Salmonella* bacteria in SPF ground chicken carcass occurred at 3, 15, and 19 hours, respectively. However, the complete inactivation (100%) of *Salmonella* took place just in 7 hours during the R1M under mixed conditions at same temperature.

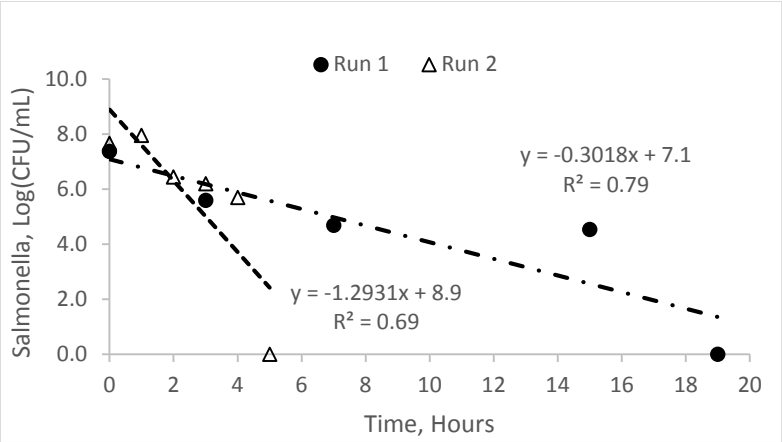

Figure 4. Inactivation of *Salmonella* pathogen at 62.5 °C (Non-mixed: R1NM, and R2NM)

The initial *Salmonella* concentration (CFU/mL) for R2NM was approximately 8 orders of magnitude and the final *Salmonella* concentration was at an ND level. This run was carried out until there was a 100% removal of *Salmonella* bacteria in ground SFP chicken carcass under non-mixed condition. The *Salmonella* inactivation curve had a fair R^2 value of 0.69. Within the first four hours of the inactivation process, there was only about 25% of the *Salmonella* was removed. However, in the fifth hour, about 75% of *Salmonella* inactivation occurred reaching to a ND level of *Salmonella* after 5 hours of inactivation. The same pattern was observed in the mixed condition also. A complete inactivation of *Salmonella* occurred in 5 hours of duration. The results clearly demonstrate that increased temperature is detrimental to the pathogenic bacteria, for this study it is *Salmonella*.

Dunkley et al. (2011) conducted a study examining the composting efficiency in breaking down the poultry carcasses and the effectiveness of composting in inactivating the *Salmonella* in both winter and summer seasons. Though Dunkley et al., 2011 reported that both winter and summer composting processes were effetcive, they could observe ciliform pathogen activity even after 10 weeks. Bharathy et al. (2012) conducted a bin compost experiment on the chicken slaughter house waste. They included poultry litter and coir pith as the compos additives along with the poultry slaughter waste. Though the authors achieved a good compost temperature (55.6 ° C) in the compost bins. They achieved a non-detectable *Salmonella* levels only after 52 days. Results from these experiments suggest that the dead bird composting process would require longer durations for a complete inactivation of *Salmonella*.

3.3 Moisture content and pH changes

Table 1. Moisture content and pH changes during the experimental runs

Temperature	Moisture, % (mean ± st. dev)		pH (mean ± st. dev)	
(°C)	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

The details of slurry pH and moisture content with time for both the experimental runs are presented in Table 1. The slurry pH consistently remained slightly acidic (around 6) throughout the experimental runs and there is no significant change in the pH with time. Based on these experimental results, it can be concluded that the slurry pH does not have significant influence on the *Salmonella* inactivation process during the experiment. The average of overall ground SPF carcass moisture content for both the experimental runs was 90.7% and maintained within the same range until the end of the experiments. This also indicates that the moisture content does not have any influence on the *Salmonella* inactivation process in the experiment. With the evidence of no influence of either moisture content or slurry pH, it could be concluded that slurry temperature alone influenced the *Salmonella* inactivation in the study.

4. Conclusions:

Two laboratory scale experiments were conducted to evaluate the *Salmonella* Typhimurium LT2 inactivation in ground SPF poultry carcasses. The following conclusions were drawn from the results of the experiments.

1. The inactivation time of *Salmonella* was inversely proportional to the temperature levels tested during the experimental trials, and
2. The slurry pH and moisture content did not show any influence on the *Salmonella* inactivation time under the experimental conditions

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