Thermal Inactivation of *Escherichia coli* and *Salmonella* Typhimurium in Poultry Carcass and Litter at Thermophilic Temperatures

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Primary Audience: Poultry Farmers, Waste Management Experts, Research Scientists

SUMMARY

The disposal of by-products, such as poultry litter, and carcasses is a serious issue because of risks associated with microbial pathogens, and controlling the pathogen risks requires identifying improved pre-treatment methods capable of inactivating pathogens. As poultry litter and carcasses are known to be major reservoirs of pathogenic microorganisms such as *Salmonella* and *Escherichia coli* (*E. coli*), improvement in existing understanding of the inactivation of these pathogens in poultry litter and carcasses is needed to determine the effective treatment time and temperature. Here we conducted a study to assess the thermal inactivation of 2 common bacteria in poultry productive systems: *Escherichia coli* (*E. coli*) and *Salmonella enterica* (*Salmonella*). The inactivation study was conducted at 50°C and 60°C using 3 different feedstocks: (1) poultry carcasses, (2) poultry litter, and (3) mixture of poultry litter and carcasses. Each feedstock was inoculated with known concentrations of *E. coli* and *Salmonella* prior to thermophilic digestion experiments at 50°C and 60°C. Regardless of feedstock types, *E. coli* survival was extended beyond 3 d at 50°C. In contrast, *Salmonella* was no longer detectable within 3 d at 50°C. At 60°C, both *E. coli* and *Salmonella* were undetected within an hour. There was no significant difference (at $P < 0.05$) in pathogen survival among 3 feedstocks.

Key words: *E. coli*, *Salmonella*, poultry, carcass, litter

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DESCRIPTION OF PROBLEM

In poultry industry, millions of tons of by-product materials such as litter, manure, feathers, wasted feed, slaughter waste, and bird carcasses are generated each year [1, 2]. These poultry waste materials are used as fertilizer in croplands, because of their nutrient values. The negative aspect of litter and carcasses used as fertilizer, however, is pathogenic microorganism associated with litter and carcasses. Zoonotic pathogens present in wastes can potentially contaminate crops, and increase the risk of foodborne illness [3, 4]. As an example, *Salmonella* Typhimurium was isolated from soil amendment with untreated poultry litter after 5 to 8 months of application in a farm [3]. The same study reported the survival of *Salmonella* up to 2 and 8 months in fresh growing lettuce and parsley, respectively.

Chicken carcasses can be asymptomatic reservoirs of many zoonotic bacteria, such as *Salmonella* spp., and *Campylobacter jejuni* [5–7]. These bacteria colonize the gastrointestinal tract as commensal organisms. These pathogens can contaminate litter often resulting in condemnation. Contaminated poultry litter and condemned birds are considered to be an indirect hazard (as human foodborne illnesses), if the litter and carcasses are used as a soil amendment in agriculture land without proper treatment such as composting.

Composting is a conventional practice, and it is commonly used for treating animal waste materials. Composting is a natural process caused by different organisms to break down organic material of dead animals and plants into their substrates. During composting, the decay of organic matter raises the internal temperature that potentially eliminates pathogen [8]. However, zoonotic bacteria such as *Salmonella*, *E. coli,* and *Listeria* have been detected in poultry compost products [9, 10]. Several factors affecting composting outcomes include seasonal temperature and humidity and the composition of composted materials including moisture, carbon to nitrogen percentage, pH, and the indigenous microflora [11–23]. Therefore, it is critical to understand the factors affecting the composting process in order to develop an optimal operating condition for composting poultry waste to inactivate zoonotic pathogens.

In a typical composting practice, temperature of pile may vary between 50°C and 60°C. Therefore, in this study, we examine the impact of 2 temperatures (50°C and 60°C) on the persistence of *Salmonella enterica* serovar Typhimurium (*Salmonella*) and *Escherichia coli (E. coli)* during the composting of 3 types of chicken waste: (1) poultry carcasses; (2) poultry litter; (3) poultry litter mixed with poultry carcasses.

MATERIALS AND METHODS

Feedstock Preparation of Ground Carcass Slurry

The experiment was carried out using 6-wk-old (approximately 500 g each) specific pathogen-free (**SPF**) chickens [24]. Chickens (sex not specified) were euthanized using CO_2. During processing, the SPF whole birds were defragmented into small pieces using a sterile knife. Next, the pieces were blended with 1.5× (times the weight of chickens) deionized water to form homogenous slurry using a blender [25]. The term "chicken carcass slurry" is being used to describe the carcass that includes water. Poultry litter was autoclaved and confirmed to be *Salmonella* and *E. coli*-free and kept refrigerated until the slurry preparation. A homogenous "litter slurry" was prepared by adding deionized water (3 times the weight [gram] of chickens). The moisture content of slurry varied from 89% to 91%.

Feedstock Inoculation and Sample Analysis

Prior to the experiment, the feedstocks (i.e., ground carcass and poultry litter slurry) were serially diluted and plated on McConkey agar plates to confirm the absence of *E. coli* and Difco Xylose Lysine Deoxycholate (**XLD**) agar plates to confirm the absence of *Salmonella* [26], respectively. If no growth occurred at the undiluted sample (direct digested sample plating on agar plates), the sample was considered as non-detectable (**ND**) (i.e., negative) for both bacteria.

To inoculate the feedstock, *E. coli* and *Salmonella* strains were grown in Difco LB (Luria-Bertani) Broth Miller growth media [26]. After 24 h of incubation, 10-mL cultures of each bacteria strain with approximate concentration of 10^8 CFU/mL were then mixed into the feedstock. *Escherichia coli* and *Salmonella* were quantified by serial 10-fold dilution in PBS and plating and enumeration in duplicate (XLD agar plates), as per standard US Food and Drug Administration (**FDA**) Bacteriological Analytical Manual procedure [15]. The bacteria strains were then mixed for approximately 2 min after inoculation to further homogenize with the feedstock. Temperature was also recorded at the time of sample collection. Reactor design, feedstock preparation, and analysis are described in detail [27] in references and notes section (Fig. 1).

RESULTS AND DISCUSSION

Escherichia coli Levels in the Feedstocks

Results of the thermal inactivation of *E. coli* in studies are shown in Figure 2 at 50°C and in Figure 3 at 60°C for 3 feedstocks of poultry carcass (a), poultry litter (b), and mixed feedstock of poultry carcass and litter (c), respectively. The low thermophilic inactivation study at 50°C continued for about 66 h (≈4,000 min) and the relatively high thermophilic inactivation study at 60°C extended about 22 h (≈1,300 min).

At 50°C. Final concentrations of *E. coli* after mixing with slurry were 8.3, 9.1, and 6.7 $\log_{10}$ CFU/mL in the carcass slurry, litter, and mixed slurry, respectively (Figure 2). It took about 15 min for the feedstock samples in the 50°C water bath to reach 40°C. When the feedstocks reached 50°C, the *E. coli* levels decreased to 7.4 (0.9 log reduction), 4 (5.1 log reduction), and 7.4 (1.5 log reduction) $\log_{10}$ CFU/mL in carcass, litter, and mixed slurry, respectively.

In the carcass slurry, the *E. coli* level fluctuated during the first day of sampling but showed a decreasing trend in the second and third day of experiment. The total *E. coli* reduction at 50°C at the end of the experiment was about 1.2 $\log_{10}$ CFU/mL in poultry carcass (Figure 2a). However, in the poultry litter, the final *E. coli* level was almost unchanged (4 $\log_{10}$ CFU/mL and 4.4 $\log_{10}$ CFU/mL) (Figure 2b). In the mixed feedstock, there was decreasing trend at the end of the experiment and the total reduction was 1.6 $\log_{10}$ CFU/mL (Figure 2c). The inactivation curves of the mixed feedstock were similar to the inactivation curves of poultry carcasses. The trend follows the expected thermal inactivation profile of *E. coli* O157:H7 and *Salmonella typhimurium* in fresh chicken manure [16].

At 60°C. Final concentrations of *E. coli* after mixing were 8.1, 8.8, and 8.4 $\log_{10}$ CFU/mL in the carcass slurry, litter, and mixed slurry, respectively (Figure 3). It took about 30 min for the feedstock samples to reach 60°C. When the feedstocks reached 60°C, the *E. coli* levels decreased to 6.7 (1.4 log reduction), 7.3 (1.5 log reduction), and 5.6 (2.8 log reduction) $\log_{10}$ CFU/mL in carcass, litter, and mixed slurry, respectively (Table 1). Unlike 50°C, there was a sharp decline in *E. coli* levels at 60°C in all 3 types of feedstock. The levels of *E. coli* went to undetected levels in less than an hour (≈ 45 min) (Figure 3a–c). Compared to carcass and mixed feedstocks, the sharpest decline in *E. coli* was observed in poultry litter followed by the carcass slurry and then mixed feedstock.

Salmonella Levels in the Feedstocks

Results from the thermal inactivation of *Salmonella* in poultry feedstocks are shown in Figure 4 at 50°C and in Figure 5 at 60°C for poultry carcass (a), litter (b), and mixed feedstock (c), respectively.

At 50°C. Final concentrations of *Salmonella* after mixing were 7.7, 8.8, and 8.6 $\log_{10}$ CFU/mL in the carcass slurry, litter, and mixed slurry, respectively (Figure 2). It took about 15 min for the feedstock samples in the 50°C water bath to reach 40°C. When the feedstocks reached 50°C, the *Salmonella* levels decreased to 6.9 (0.8 log reduction), 4.1 (4.7 log reduction), and 7 (1.6 log reduction) $\log_{10}$ CFU/mL in carcass, litter, and mixed slurry, respectively (Table 1). At the end of treatment, *Salmonella* levels were undetected in all 3 feedstocks. The experiment lasted for 66–67 h. While the inactivation curve showed prevalent decline of *Salmonella* in carcass and mixed feedstocks, they were more persistence in litter (Figure 4a–c). A previous study [17] found that it took almost 100 h to reach undetected

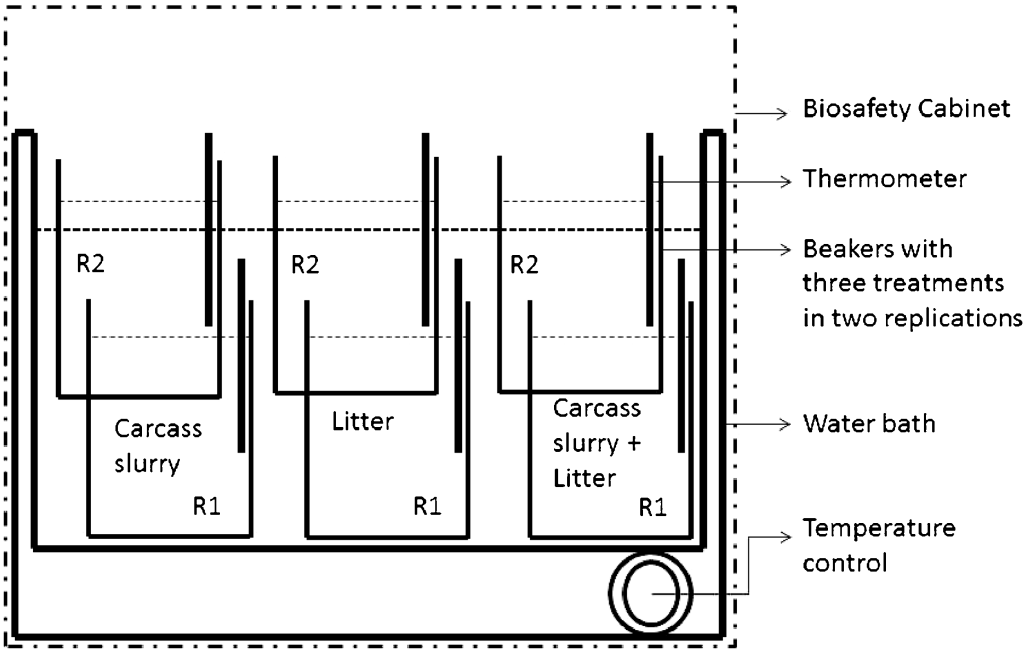

Figure 1. Schematic of the experimental setup.

Table 1. Summary Statistics of *E. coli* and *Salmonella* $\log_{10}$ CFU/mL at 50°C and 60°C in Poultry Carcass, Litter, and Mixed Feedstock.

	E. coli						*Salmonella*					
	50°C			60°C			50°C			60°C		
	Carcass	Litter	Mixed	Carcass	Litter	Mixed	Carcass	Litter	Mixed	Carcass	Litter	Mixed
Maximum	8.3	9.1	8.9	8.1	8.8	8.4	7.7	8.8	8.6	8.0	8.6	7.4
Minimum	6.2	2.9	5.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
At the start of heat treatment	7.4	4.0	7.4	6.7	7.3	5.6	6.9	4.1	7.0	6.1	5.4	4.7
Total log reduction at the end	1.2	1.1	1.6	6.7	7.3	5.6	6.9	4.1	7.0	6.1	5.4	4.7

level of *Salmonella* in non-mixed ground poultry slurry like this study at 52°C.

At 60°C. Final concentrations of *Salmonella* after mixing were 8, 8.6, and 7.4 $\log_{10}$ CFU/mL in the carcass slurry, litter, and mixed slurry, respectively (Figure 2). It took about half an hour for the feedstock samples to reach 60°C. When the feedstocks reached 60°C, the *Salmonella* levels decreased to 6.1 (1.9 log reduction), 5.4 (3.2 log reduction), and 4.7 (2.7 log reduction) $\log_{10}$ CFU/mL in carcass, litter, and mixed slurry, respectively (Table 1). At 60°C, there was a sharp decrease in *Salmonella* levels in all 3 feedstocks during the first hours (≈45 min) of heat treatment and the *Salmonella* levels were ND in less than an hour (≈45 min) (Figure 5a–c). In a similar study, it was reported that it took about 19–20 h to reach the undetected level of *Salmonella* in non-mixed ground slurry at 62.5°C [17].

The composition of carcass and litter may be the underlying reason for different inactivation patterns of *E. coli* and *Salmonella* in the feedstocks. Fat content in carcass can be a factor influencing pathogen inactivation in carcasses [18–21]. While studying fat content, a previous study examined the inactivation of *Salmonella* strain in ground chicken and found that fat content is a critical indicator of bacteria resistance. As we did not add any additional fatty material

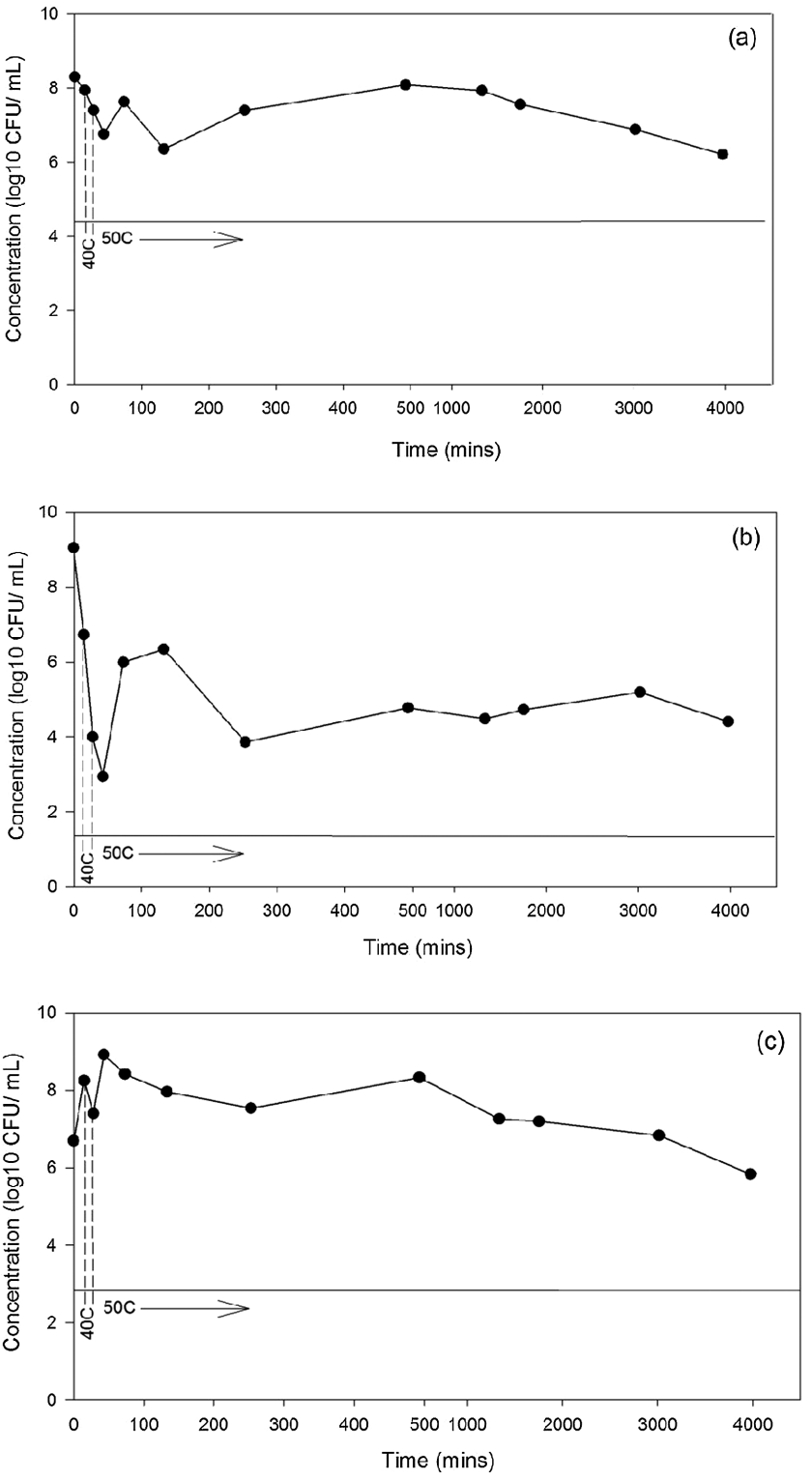

Figure 2. Thermal inactivation of *E. coli* at 50°C in (a) carcass, (b) litter, and (c) mix of carcass and litter.

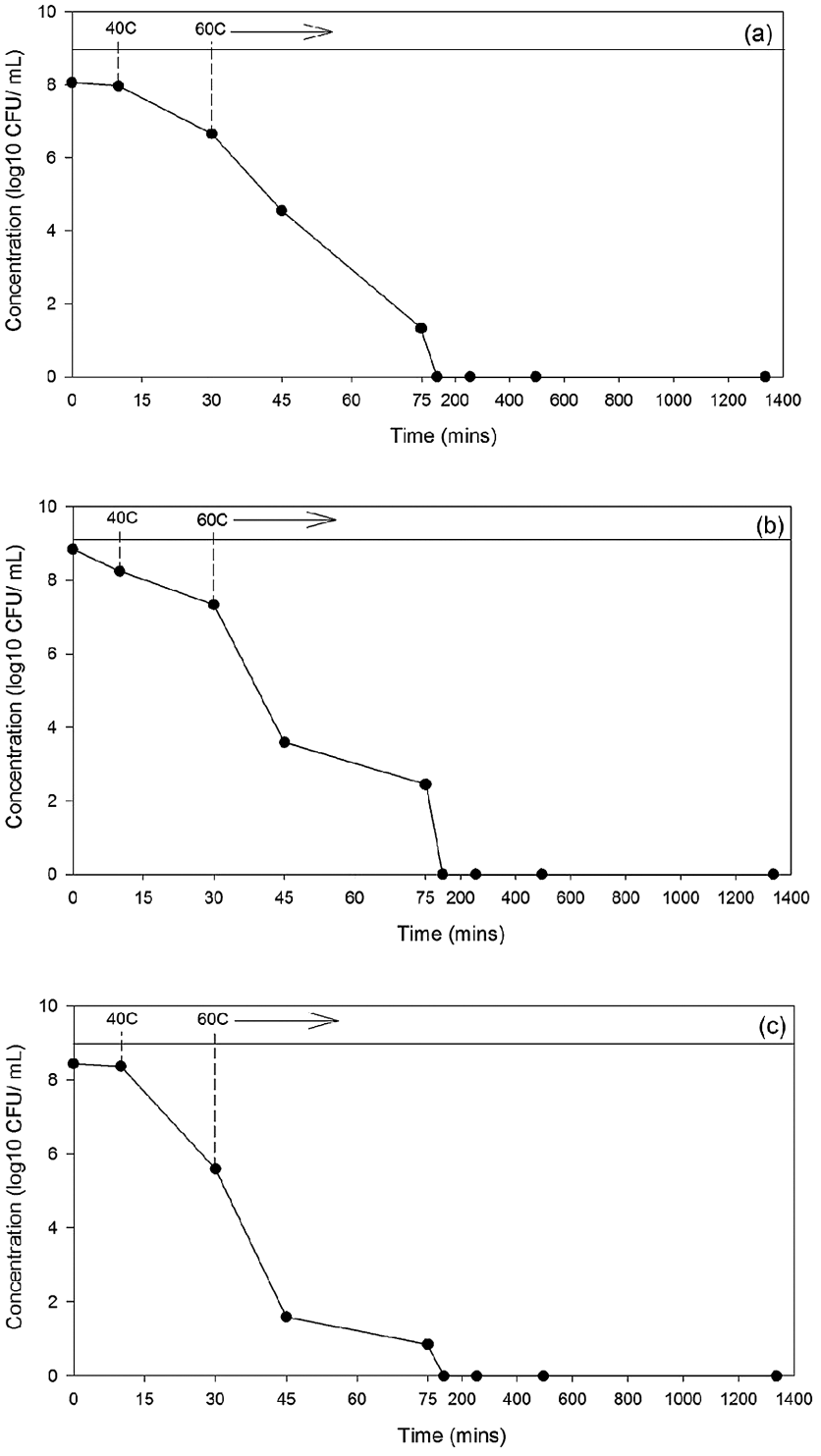

Figure 3. Thermal inactivation of *E. coli* at 60°C in (a) carcass, (b) litter, and (c) mix of carcass and litter.

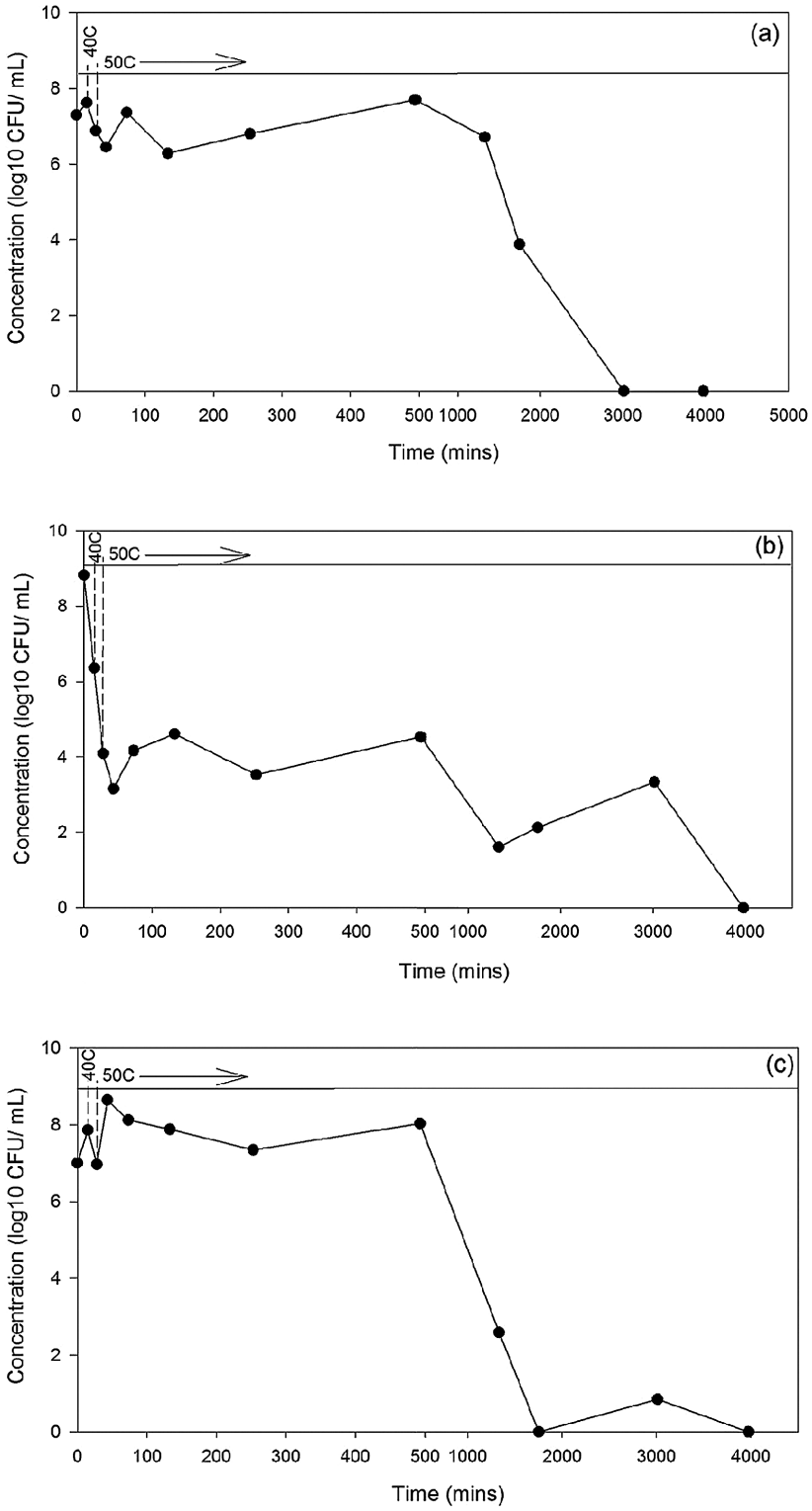

Figure 4. Thermal inactivation of *Salmonella* at 50°C in (a) carcass, (b) litter, and (c) mix of carcass and litter.

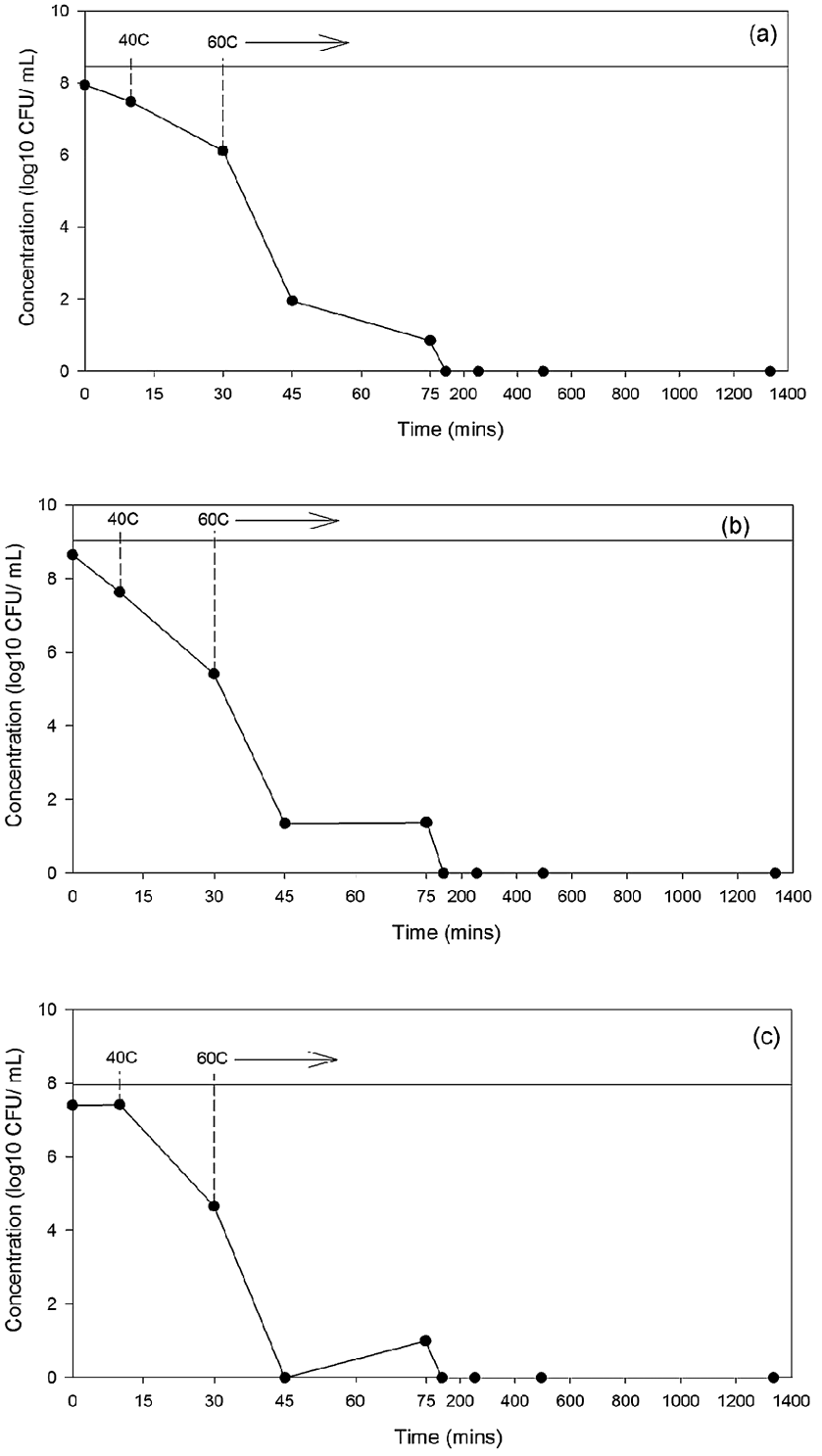

Figure 5. Thermal inactivation of *Salmonella* at 60°C in (a) carcass, (b) litter, and (c) mix of carcass and litter.

in poultry litter, there was a considerable difference in the fat content among the experiments. While analyzing the compost material of poultry farms in South Carolina [9], results showed the presence of *E. coli* and *Salmonella* spp. in compost heaps formed from chicken litter, carcass, and other organic sources even at thermophilic range (55°C). In a similar study in Australia, authors [4] found that *E. coli* and *Salmonella* counts in poultry litter were reduced by more than 99% in 1 h at 55°C or 65°C under laboratory condition but persisted for a longer period of time at 35°C. Another study [11] investigated the thermal inactivation of *Salmonella* in aged chicken litter at multiple thermophilic temperatures (70°C, 75°C, 80°C, 85°C, and 150°C) and moisture content (20%, 30%, 40%, and 50%), and results showed that *Salmonella* can survive for more than 6 h in all the litter samples with 4 levels of moisture contents at 70°C. In contrast, our results based on lab study showed that *E. coli* and *Salmonella* were not detectable at 50°C or 60°C in less than an hour. It is important to note that moisture content of slurry used in this study was relatively high (≈90%). While studying the *Salmonella* inactivation in fresh chicken litter, a previous study [22] showed a 7 log reductions of *Salmonella* level in less than 2 h at 70°C and about 5 log reductions in aged chicken litter in 3–4 h. These differences in survival of bacteria could be due to the differences in feedstock characteristics such as carbon, moisture, and fat contents of the feedstocks. A previous study [23] examined *Salmonella* survival in chicken litter under different carbon amendment conditions (carbon and nitrogen [C:N] ratios of 20:1 and 40:1) at sublethal temperature of 25°C. Results showed that there was relatively less detection of *Salmonella* positive samples after first week of incubation at low C:N ratio. The results of this study showed that the change in temperature between 50°C and 60°C has substantial impacts on the survival of both pathogens (*E. coli* and *Salmonella*).

Depending on the uses of feedstock (by-product), the desired bacterial log-reduction may vary. For example, when the efficacy of treatment methods such as heat treatment or chemicals are evaluated for food products, the requirement is that the select method should be able to reduce population of representative microorganisms by 5 log (or 99.999%) [28, 29]. In terms of standards for the biological soil amendments of animal origin and human waste, which are used for the growing, harvesting, packing, and holding of produce for human consumption, FDA guidelines require no detection of *E. coli* using a method that can detect 0.3 most probable number (**MPN**) per gram (or mL). Similarly, no detection of *Salmonella* using a method that can detect 3 MPN per 4 gm (or mL) is required [30]. With regard to composting, static composting that maintains aerobic conditions at a minimum of 55°C for 3 consecutive days followed by adequate curing is required. In turned composting, 55°C is required for 15 d, which do not have to be consecutive. Minimum 5 turning followed by adequate curing is required [31]. In general, a wide range of temperature occurs during composting process, and depending on the consistence of temperature, survival of pathogens likely to change. Therefore, frequent monitoring of temperature of a compost pile is essential to understand the progression of compost process.

CONCLUSIONS AND APPLICATIONS

1. Improved treatment methods for poultry litter and carcasses are essential for inactivating potential pathogenic organisms present in poultry litter and carcasses.
2. To determine effective temperature and time capable of reducing pathogen loads in poultry by-products, this study assessed the thermal inactivation of *E. coli* and *Salmonella* in poultry litter and carcasses at 2 thermophilic temperatures (50°C and 60°C).
3. Laboratory experiments were conducted using feedstocks prepared from poultry carcass, poultry litter, and a mixture of poultry carcass and litter.
4. *Escherichia coli* survived for more than 3 d after heat treatment at 50°C, whereas at 60°C the survival of *E. coli* survival was for less than an hour in all 3 feedstocks. *Salmonella* survived for less than 2 d of heat treatment at 50°C and, at 60°C, the survival of *Salmonella* was for less than an hour.
5. Thus, the heat treatment at thermophilic range can be helpful to reduce certain

bacteria but the duration of treatment can be a crucial factor as well.

6. Results of this study will not only help in evaluating existing treatment methods such as composting and rendering but also can be potentially useful for developing improved litter and carcass treatment methods.

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27. *Reactor Design, Feedstock preparation, and Analysis*: A series of reactors were designed for three types of feedstocks: (1) chicken carcass slurry, (2) chicken litter slurry, and (3) mixing of the carcass slurry and litter. Each bacterium-inoculated feedstock (800 mL) was added into two sterile 1 L glass beakers served as reactors. Each beaker considered as a replicate for a feedstock in a single heat treatment experiment. In total, six 1 L beakers were placed into a 20 L isotemp water bath [27] as shown in Figure 1. The experiments were conducted at 50°C and 60°C. In order to measure the come-up time for the reactors to reach the target temperature of the water bath, the feedstock temperature was measured before reaching the desired temperature of 50°C and 60°C. The come-up times for the slurry to reach the target temperatures of 50°C and 60°C were 28 and 31 min, respectively. The duration of the experiments at 50°C was about 3

days (4000 min) and at 60°C was about 1 day (1305 min). In each heat treatment 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. Samples were collected at 0, 15, 30, 60, 120, 240, 465 min interval during the first day of experiment at both temperatures and then twice (morning and evening) in the second day and once in the third and fourth day of experiment at 50°C. In total, 51 samples were collected from thee feedstocks in replicate during the experiment at 60°C and 69 samples were collected at 50°C.

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