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FINAL PROJECT REPORT

Project Title

Validating *Salmonella* inactivation during thermal processing of the physically heat-treated chicken litter as soil amendment and organic fertilizer

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Objectives

1. *Validating the thermal inactivation of Salmonella spp. at different temperatures in broiler chicken litter*
2. *Evaluating the effect of type and freshness of chicken litter on thermal resistance of Salmonella spp.*
3. *Developing a two-step heat treatment for chicken litter to expedite Salmonella inactivation.*

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FINAL REPORT

Abstract

Chicken litter, commonly used as soil amendment and organic fertilizer, may contain harmful human pathogens such as *Salmonella* spp. Although the physically heat-treated chicken litter has been recommended and used by produce growers, there is a lack of scientific data to prove if the heating processes in terms of time-temperature combination are adequate to kill *Salmonella*. The objective of this study was to validate *Salmonella* inactivation during the heating processes as recommended for the physically heat-treated chicken litter by taking consideration of several factors such as type, dryness and freshness of chicken litter, and develop a two-step heat treatment for rapid pathogen inactivation. In this proposed study, first, we evaluated several recovery media for allowing heat-injured cells to resuscitate fully. Then, a procedure to develop desiccation adaptation of *Salmonella* spp. in the finished compost was optimized. Afterwards, the thermal resistance of desiccation-adapted *Salmonella* cells (a mixture of 4 serotypes) in partially composted chicken litter was exposed to heat treatments at 70, 75, 80, 85 and 150°C. Our results demonstrated that the thermal resistance of *Salmonella* in aged chicken litter was increased significantly when the cells were adapted to desiccation, and the reduced moisture levels in chicken litter contributed to the better survival of *Salmonella* during heat treatment (**Objective I**). The thermal resistance of *Salmonella* in both broiler chicken and egg-laying hen litter was compared, and the change of *Salmonella* heat resistance as affected by different storage ages of the broiler chicken litter collected from the same farm was also determined. The metabolic fingerprint pattern was investigated of chicken litter microflora during long term storage were analyzed with Principal Component Analysis (PCA). Our results showed that the desiccation-adapted *Salmonella* survived longer in aged broiler chicken litter than in fresh laying hen litter at 70, 75, 80, and 150^{o}C. A field study confirmed that the desiccation-adapted cells became increased resistance to lethal temperatures as the storage time was extended. Some changes in moisture level, ammonia, electrical conductivity, heavy metals, and indigenous microbial community of aged chicken litter could contribute to this difference in the thermal resistance of *Salmonella*. Moreover, there were some changes in the metabolic fingerprint pattern of microbial communities over time during 9-month storage of chicken litter (**Objective II**). Furthermore, the effectiveness of a two-step heat treatment for aged chicken litter on elimination of desiccation-adapted *Salmonella* was also evaluated. Based on our results, a two-step heating technique consisting of a moist-heat treatment for 1 h at 65°C and a sequential dry-heat treatment for 1 h at 85°C can be sufficient for achieving >5.5-log reductions of *Salmonella* in chicken litter with moisture content of ≥40%. Moisture contents in the range of 20 to 50% in chicken litter samples were all reduced to <12% after drying process. The increased moisture contents in chicken litter contributed to the better killing effects of *Salmonella* during moist-heat treatment (**Objective III).** Our results clearly demonstrated that the thermal resistance of *Salmonella* in chicken litter can vary significantly depending on moisture level, types and freshness of chicken litter, physiological stage of the pathogen, and type of heat source. The desiccation-adapted *Salmonella* in fresh chicken litter was more susceptible to heat inactivation as compared in aged chicken litter of the same type or different type. By applying moist heat to the contaminated chicken litter first followed by dry heat, this two-step heat treatment not only ensures the fast inactivation of *Salmonella* but also produce more stable and nutrient dense finished products. Results generated from this study, after actual processing plant validation, will help the chicken litter processors to modify their existing process parameters to produce microbiologically safe organic fertilizers and soil amendments, thereby reducing the possible source of produce contamination on farm.

Background

Chicken litter is a waste by-product of poultry production, which consists of bedding materials such as sawdust, wood shavings, straw, peanut or rice hulls, feathers, and manure. Approximately 14 million tons of litter and manure, most of which was broiler litter (68%) was produced on US poultry farms in 1990 and over 90% of poultry litter is applied to agricultural land (Moore et al., 1995). However, the direct application of this waste material to agricultural land can be harmful to the environment due to nutrient and pathogenic microorganisms in runoff (Giddens and Barnett, 1980; Schiffman et al., 2000). The prolonged survival of *Salmonella* in the environment has been reported. For example, Davies and Breslin (2003) studied the persistence of *Salmonella* Enteritidis PT 4 in the free-range chicken farm which had been depopulated following detection of the organism in breeding birds. *Salmonella* persisted in litter, dried feces and feed for 26 months. Islam et al. (2004) reported that *Salmonella* Typhimurium persisted for 161 and 231 days in the poultry manure compost-amended soil where lettuce and parsley were grown, respectively, and it survived for 63 and 231 days on lettuce and parsley, respectively. Therefore, *Salmonella*-contaminated litter can be a potential source of produce contamination in the field.

Some microorganisms become acclimatized to desiccation stress under dry environment, and induction of desiccation stress response in bacterial cells makes them more resistant to the dry condition they are present (Wesche et al., 2009). Most importantly, exposure to a single stress is found to be associated with the development of cross-tolerance to multiple unrelated stresses (Gruzdev et al., 2011). Using laboratory models, various researchers have demonstrated that the desiccated cells exhibit increased thermal resistance (Breeuwer et al., 2003; Gruzdev et al., 2011; Hiramatsu et al., 2005). Previous thermal inactivation studies on bacterial pathogens in chicken litter have used only non-stressed cells (Kim et al., 2012; Wilkinson et al., 2011). Therefore, to simulate real-world conditions, thermal inactivation of desiccation-adapted cells should be evaluated as they are present in the chicken litter during stockpiling. To our knowledge, there have been no available reports studying the thermal inactivation of desiccation-adapted pathogens in compost and manure.

In order to reduce the microbiological risks associated with the use of animal wastes as a soil amendment or fertilizer, physical heat treatments are recommended to reduce or eliminate potential pathogenic microorganisms. At Perdue AgriRecycle, chicken litter is exposed to the temperature up to 107^{o}C for 10~12 min by running through an Aztec dryer with a Hauck burner (MacDonald, 2011). Pelletization of the chicken litter which heating and dehydration process are involved has been widely used (Cox et al., 1986; Lopez-Mosquera, 2008). Pelletization increases the bulk density and the uniformity of particle size in chicken litter allowing a more nutrient dense litter for land application (McMullen et al., 2005). In pelletizing industry, regardless of heat source, temperature, and equipment, pellets leave the die at temperatures of 60~95°C and moisture contents of 12~18% (Kaddour and Alvai, 2008). In general, for long-term storage, the final moisture content of the pellets should be less than 12~13% (Maier et al., 1992; Robinson, 1984). Being investigated during a 2006 *Escherichia coli* O157:H7 outbreak associated with Dole pre-packaged spinach, True Organic Products Inc. produced the chicken pellets by heating the composted chicken manure mixed with feather meal at 180-200^{o}F (82~93^{o}C) for approximately 30 min with the heated air (CFERT, 2007). Several studies have investigated the value of pelletized poultry litter as a fertilizer (Hadas et al., 1983; Hamilton and Sims, 1995; McMullen et al., 2005; Wilhoit et al., 1993). However, no research has been done to evaluate if heat treatment during pelletizing is enough to kill pathogenic microorganisms. In addition, there is no official guideline for processing the pelletized chicken litter.

The non-spore-forming microorganisms can be inactivated much quicker by moist heat than dry heat. Wilkinson et al. (2011) reported that *Salmonella* Typhimurium in fresh chicken litter containing rice hulls with 30~65% of moisture was completely eliminated within 1 h at both 55 and 65^{o}C of water bath. In another study, when *S.* Typhimurium in pine shavings-chicken litter was exposed to steam for 5, 30, and 120 min, it died off completely with 30 or 120 min of steaming as compared with a 3-log reduction with 5 min of steaming (Stringfellow et al., 2010). Carrington (2001) recommended time-temperature regimes of heating at 80°C for 10 min, 75°C for 20 min, 70°C for 30 min, or 55°C for 4 h using moist heat followed immediately by anaerobic mesophilic digestion for producing pathogen-free sludge. Other studies also suggested a pasteurization system should be designed to provide a minimum temperature of 63°C for at least 30 min for sewage sludge (Bagge et al., 2005; Nissen et al., 1996; Pike et al., 1988). In contrast, there are very few quantitative data demonstrating the effectiveness of dry heat on pathogen inactivation in animal wastes (Bruch, 1964; Springthorpe and Sattar, 2004). Davey (1990) calculated that the time required to inactivate bacteria in dry heat is 19 times more than in moist heat. Apparently, in order to inactivate human pathogens by thermal processing, the use of moist heat should be considered preferably over the use of dry heat.

Research Methods and Results

Objective 1: Validating the thermal inactivation of Salmonella *spp. at different temperatures in broiler chicken litter*

Fresh chicken litter was collected from chicken barn of Bovan laying hens raised at Morgan Poultry Center, Clemson, SC, whereas the aged chicken litter was sourced from Cobb broiler chickens (Organic Farms, Livingston, CA). To prepare the aged chicken litter, the litter inside the chicken house was removed annually followed by a partial windrow composting of 7-10 d. After composting, the litter was screened out of rice hulls and ready for subsequent heat treatment. Commercially available dairy compost (Black Gold Compost Co., Oxford, FL) and poultry compost (Black Gold Compost Co., Oxford, FL) were purchased from a local supermarket. All the compost samples were dried under the fume hood until moisture contents were reduced to less than 20%, and then screened to the particle size of less than 3 mm using a sieve. Sufficient samples were collected for the entire experiment and stored in a sealed container at 4°C until use. Moisture content was determined with a moisture analyzer (model IR-35, Denver Instrument, Denver, CO), whereas water activity was measured with a dew-point water activity meter (Aqualab series 3TE, Decagon Devices, Pullman, WA). The pH value and ammonia content in compost were measured according to the methods as described by U.S. Composting Council (2002) and Weatherburn (1967), respectively.

Salmonella enterica serovars Enteritidis H2292 and Heidelberg 21380 (kindly provided by Dr. Michael Doyle, University of Georgia, Griffin, GA), Senftenberg ATCC 43845, and Typhimurium 8243 [genotype: *thyA deo polA2 zie-3024*::Tn*10*(dTc)*zag-1256*::Tn*10*(dKm), derived from *S.* Typhimurium LT2 by Dr. John Foster, University of South Alabama, Mobile, AL, and kindly provided by Dr. Roy Curtiss III, Washington University, St. Louis, MO)] were used for the thermal inactivation study. *S.* Typhimurium 8243 was used for the optimization of recovery media for heat-injured cells and selection of compost for desiccation adaptation. All the strains were induced to rifampin resistance (100 µg of ml^{-1}) using the gradient plate method (Smith et al., 1982). Each *Salmonella* strain was grown overnight at 37°C in tryptic soy broth containing 100 µg of rifampin ml^{-1} (TSB-R). The overnight cultures were washed and resuspended in 0.85% saline to desired cell concentrations by measuring the optical density at 600 nm.

<u>*Optimization of recovery media for heat-injured* Salmonella.</u> Fresh chicken litter was used for the optimization of recovery media for heat-injured *Salmonella*. Chicken litter with moisture

adjusted to 30% (a_w, 0.93) was inoculated with overnight grown *Salmonella* cells (1:100, v/w) using a sterile spray nozzle and thoroughly mixed to a final concentration of ca. 10^7 cfu g^{-1}.

About 20 g of samples were distributed evenly inside an aluminum pan (I.D. 10 cm), placed in three different locations (close to the door, center, and far away from the door) on the shelf of a controlled convectional oven (Binder Inc., Bohemia, NY), and then exposed to 75°C up to 1 h. Temperature was monitored constantly using T type thermocouples (DCC Corporation, Pennsauken, NJ), with one cord kept inside the oven chamber and others inserted into the bottom of litter samples of three different locations. The temperature was initially set at a higher set point of 80ºC to minimize the come-up time. When the interior of the litter reached the desired temperature, the temperature setting of the oven was readjusted to maintain at the designated experimental temperature. Samples were taken out at 0.5 and 1 h, and placed immediately in an ice water bath. Samples were then diluted serially with 0.85% saline and transferred in triplicate to different media to evaluate the recovery efficiency with these media. Samples taken at the beginning of heat treatment (0 h) were used to determine the initial populations. These experiments were performed in three separate trials.

Tryptic soy agar (TSA) was used as a nonselective medium, while TSA with 100 µg of rifampin ml^{-1} (TSA-R) and xylose lysine Tergitol-4 agar with 100 µg rifampin ml^{-1} (XLT-4-R) were used as selective media. The following media were used for the recovery of heat-injured *Salmonella* cells:

1) TSA supplemented with 100 µg rifampin ml^{-1};

2) XLT-4 supplemented with 100 µg rifampin ml^{-1};

3) Modified two-step overlay (OV) method (OV/TSA-R and OV/XLT-4-R) (Kang and Fung, 2000): Heat-injured cells were plated directly onto TSA. After incubation at 37°C for 3 h to allow recovery of injured cells, 7 ml of TSA-R or XLT-4-R was overlaid onto TSA. Plates were incubated at 37°C for another 21 h and then colonies were counted;

4) Modified thin agar layer (TAL) method (TAL/TSA-R and TAL/XLT-4-R) (Kang and Fung, 2000): After solidification of 25 ml TSA-R or XLT-4-R in the plate, 14 ml of melted TSA (48°C) was overlaid. Heat-injured cells were plated onto TAL media, which were then incubated at 37°C for 24 h;

5) TSA supplemented with 100 µg rifampin ml^{-1} and 1% sodium pyruvate (P/TSA-R);

6) TSB supplemented with 100 µg rifampin ml^{-1} and 1% sodium pyruvate (P/TSB-R): After heat treatment, 1 ml of heat-injured cells was transferred into 9 ml of P/TSB-R and incubated at 37°C for 3 h, followed by plating onto TSA-R and incubated for another 21 h.

Among eight media tested for recovering heat-injured *S.* Typhimurium, the highest populations of this pathogen was enumerated on P/TSB-R ($P<0.05$) (Table 1, Appendix A). However, the high level of microbial counts for P/TSB-R may not only be attributed to the repair of injured cells but may also reflect the multiplication of non-injured cells during the 3-h incubation. For 0.5- and 1-h heat treatments, no significant differences ($P>0.05$) in the enumeration of heat-injured *S.* Typhimurium occurred among TSA-R, XLT-4-R, OV/TSA-R, OV/XLT-4-R, TAL/TSA-R, TAL/XLT-4-R, and P/TSA-R. Among media containing TSA-R, TAL/TSA-R media recovered slightly more *Salmonella* cells, which were 3.96±0.35 and 2.80±0.39 log cfu g^{-1} at 0.5 and 1 h, respectively. With respect to media containing XLT-4-R, higher numbers of *S.* Typhimurium cells were observed on OV/XLT-4-R media, which were 3.80±0.22 and 2.75±0.45 log cfu g^{-1} at 0.5 and 1 h, respectively. **Therefore, TAL/TSA-R and OV/XLT-4-R were selected as recovery media for following experiments on thermal inactivation of *Salmonella* spp.**

Selection of compost matrix for desiccation adaptation of Salmonella. To select the compost matrix for desiccation adaptation of *Salmonella*, dairy compost, fresh poultry compost, old poultry compost, and aged chicken litter were compared based on changes in bacterial populations before and after desiccation adaptation. The overnight grown *S.* Typhimurium was washed, resuspended, and further concentrated 100 times (ca. 10^{11} cfu ml^{-1}) by centrifuging. Afterwards, the culture was added separately (1:100, v/w) into 300 g of above four different composts with the moisture content of 30% (a_w values of dairy compost, fresh poultry compost, old poultry compost, and aged chicken litter were 0.980, 0.916, 0.938, and 0.943, respectively) at a final concentration of ca. 10^9 cfu g^{-1} for a 24-h desiccation adaptation. Before and after desiccation adaptation, samples were homogenized and serial dilutions of homogenates were plated in duplicate onto XLT-4-R for enumeration. Two trials were conducted for each experiment.

As shown in Table 2 (Appendix A), *S.* Typhimurium counts in all four composts decreased during the 24-h desiccation adaptation at room temperature. The populations in fresh and old poultry composts, and aged chicken litter decreased more rapidly than those in dairy compost ($P<0.05$). The *Salmonella* reductions in dairy compost, fresh poultry compost, old poultry compost, and aged chicken litter were 0.45, 2.99, 2.18, and 2.87 log cfu g^{-1}, respectively. The levels of ammonia (average of 820.64 μg NH_4-N g^{-1}) and pH (average of 8.77) in fresh poultry compost, old poultry compost, and aged chicken litter were much higher ($P<0.05$) compared with dairy compost (ammonia content of 22.64 μg NH_4-N g^{-1} and pH of 7.70). **Therefore, dairy compost was selected as the matrix for desiccation adaptation of *Salmonella* in the following thermal inactivation experiments.**

Thermal inactivation of desiccation-adapted Salmonella *in aged chicken litter.* Compost selected for desiccation adaptation and aged chicken litter samples were all adjusted to desired moisture contents, 20 (a_w, 0.87), 30 (a_w, 0.94), 40 (a_w, 0.98), and 50% (a_w, 0.99), with sterile tap water. Each of four *Salmonella* serotypes was grown separately overnight at 37°C, washed, and mixed in equal volume as inoculum. Both desiccation-adaptation and inoculation were performed as described above. Thermal inactivation study was carried out as described in Fig. 1 (Appendix A). The temperatures used for this study were 70, 75, 80, 85, and 150°C. For heat treatments at 70, 75, 80, and 85°C up to 6 h, at predetermined time intervals, duplicate samples were taken out, homogenized, and plated on recovery media. The samples which were negative by direct plating recovery method (detection limit: 1.30 log cfu g^{-1}) were pre-enriched in universal pre-enrichment broth (UPB) followed by a secondary enrichment in Rappaport-Vassiliadis (RV) broth supplemented with 100 μg rifampin ml^{-1}. After 24 h incubation at 42°C, enriched samples were then plated onto XLT-4-R. Presumptive-positive colonies on XLT-4-R were further confirmed as *Salmonella* using immunolatex agglutination test (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom). For heat treatment at 150°C, duplicate samples were withdrawn every 10 min up to 60 min and enriched in UPB directly to test if *Salmonella* is alive. To serve as controls, washed *Salmonella* cultures (ca. 10^9 cfu ml^{-1}) kept at room temperature for 24 h were aseptically added to aged chicken litter (20% moisture content) in a ratio of 1:100 (v/w), and exposed to above temperatures as the desiccation-adapted cultures were.

Plate count data were converted to log cfu g^{-1} in dry weight. SigmaPlot 12.3 (Systat Software Inc., San Jose, CA, USA) was used for data analysis. Analysis of variance (ANOVA), followed by the least significant differences (LSD) test, was carried out to determine whether significant differences ($P<0.05$) existed among different treatments.

For the thermal inactivation kinetics study, the parameters for the exponential model were estimated using maximum likelihood that accounted for censored observations that were not detectable by plating. The censored observations were within the interval of 0 to 1.30 log cfu

g^{-1}. Separate regression models were used for each moisture-temperature combination. Because of the censoring, a pseudo-R^2 was calculated for each regression model as described by Magee (1990). The pseudo-R^2 was calculated as

$$\text{pseudo-}R^2=1-\exp[(x_2-x_1)/n]$$

where x_1=-2 log likelihood (model with no independent variables), x_2=-2 log likelihood (current model), and n is sample size.

Linear contrasts were used for all comparisons and the Type I error rate was controlled at P=0.05 using the Bonferroni method. The NLMIXED procedure of the Statistical Analysis System 9.3 (SAS Institute Inc., Cary, NC, USA) was used for all calculations in thermal inactivation kinetics study.

Table 1 Exposure time required for reducing 3 and 5 logs of control and desiccation-adapted *Salmonella* spp. in aged chicken litter at 70, 75, 80, and 85°C

3 logs	Sample	Moisture content (%)	Exposure time (h)	
			3 logs	5 logs
70	Control	20	0~0.5	1.5~2
	Desiccation-adapted cells	20	1~1.5	>6
		30	0.5~1	>6
		40	0~0.5	5~6
		50	0~0.5	4~5
75	Control	20	0~0.5	1~1.5
	Desiccation-adapted cells	20	0.5~1	>6
		30	0~0.5	4~5
		40	0~0.5	3~4
		50	0~0.5	2~3
80	Control	20	0~0.5	0.5~1
	Desiccation-adapted cells	20	0.5~1	4~5
		30	0~0.5	4~5
		40	0~0.5	1.5~2
		50	0~0.5	1~1.5
85	Control	20	0~0.5	<0.5
	Desiccation-adapted cells	20	0~0.5	3~4
		30	0~0.5	2~3
		40	0~0.5	0.5~1
		50	0~0.5	0.5~1

The come-up times for heating aged chicken litter with different moisture contents at 70, 75, 80, 85, and 150ºC ranged from 0.42 to 2.53 h (data not shown). Our results showed that the higher initial moisture content of chicken litter required the longer come-up time. At 70, 75, 80, and 85^{o}C, *Salmonella* levels in aged chicken litter decreased in all samples during heat treatment; however, the difference in the populations of control and desiccation-adapted *Salmonella* was significant (P<0.05) (Table 1; Fig. 2, Appendix A). For example, at 70^{o}C, in aged chicken litter with the moisture content of 20%, the control cells survived for 1.5~2 h as detected by enrichment, whereas the desiccation-adapted cells survived for more than 6 h of heat exposure (Fig. 2A, Appendix A). The desiccation-adapted cells were inactivated much faster when the moisture content of chicken litter was increased. For example, at 80^{o}C, there were still more than 2 log cfu g^{-1} counts in chicken litter with 20% moisture content after exposure to heat

treatment for 6 h, whereas *Salmonella* cells survived for less than 3 h at 50% moisture content as detected by enrichment (Fig. 2C, Appendix A). As compared to control, there were ca. >3, >4, 4~10, and >6 folds increases in the exposure times required for reducing 5 logs of desiccation-adapted *Salmonella* cells at 70, 75, 80, and 85°C, respectively.

At 150°C, desiccation-adapted *Salmonella* cells still displayed extended survival as compared to the non-adapted control (Table 3, Appendix A). Control and desiccation-adapted cells in aged chicken litter at 20% moisture content were detectable by enrichment up to 10 and 50 min, respectively. Desiccation-adapted cells in chicken litter had a shorter duration of survival with the increase of moisture content. Viable *Salmonella* cells in chicken litter could still be detected up to 50 min at 20 and 30% moisture contents, whereas they were only detectable within 40 min at 40 and 50% moisture contents.

All the parameter estimates obtained from fitting the experimental observations into an inactivation model and the pseudo-R^2 values were shown in Table 4 (Appendix A). The exponential model used in this study was appropriate for fitting all the inactivation curves and permitted the modeling of an extended tail for desiccation-adapted *Salmonella*, which was supported by the good performance in goodness-of-fit (Pseudo-R^2). The α values of desiccation-adapted cells were higher as compared to those of control ($P<0.05$), which was attributed to the tailing part in the inactivation curves and thus reflected a higher level of population remaining viable at the end of thermal treatment. Meanwhile, β values of desiccation-adapted cells were lower than those of control ($P<0.05$), which suggested a lower population reduction and that they were more heat-resistant at longer exposure times. For desiccation-adapted cells, as temperature increased from 70 to 85°C and moisture content increased from 20 to 50%, there seemed to be a trend in temperature and moisture content dependencies for these two parameters, as α values decreased while β values gradually became higher. Interestingly, the moisture content threshold to achieve a long-term log count (α value) of zero for desiccation-adapted cells decreased with an increase in temperature. At 70°C, there was no moisture content threshold, but at higher temperatures, including 75, 80, and 85°C, the moisture content thresholds were 50, 40, and 30%, respectively. It should be remarked that the λ values (decay rates) obtained from the inactivation model exhibited no dependencies on either non-adapted control or desiccation-adapted cells, temperature, or moisture content, and it is thus difficult to draw a definite conclusion from this parameter. **In conclusion, desiccation-adapted cells had significantly higher populations throughout heat treatment and also survived much longer as compared to control cultures. The reduced moisture levels in chicken litter contribute to the better survival of *Salmonella* during heat treatment.**

Characterization of Salmonella *spp. in aged chicken litter by PFGE.* Bacterial colonies were randomly selected from the TAL recovery media with the longest survival (defined as the last sampling time that *Salmonella* could be detected by direct-plating) in chicken litter with 20% moisture content after exposure to the heat treatment at 80ºC. The selected colonies (n=12) for each sample was transferred on TSA for two times, and then characterized by PFGE as described by CDC/PulseNet (2004). The band patterns of these isolates were compared with the genetic profiles of four serotypes used in this study.

Colonies that exhibited the longest survival based on growth on TAL recovery media (0.5 h, and 6 and 24 h for control and desiccation-adapted cells, respectively) at 80°C were characterized using PFGE (Table 5, Appendix A). For the non-adapted control, 3 and 6 of 12 isolates were identified as *S.* Senftenberg and *S.* Typhimurium, respectively, whereas 7 and 3 of 12 isolates from desiccation-adapted cells were identified as *S.* Senftenberg and *S.* Typhimurium, respectively. Our results also showed that *Salmonella* could still be detected by enrichment after 24 h at 80ºC in chicken litter with 20% moisture content, with all 12 isolates

identified as *S.* Senftenberg. An obvious variability in heat resistance among *Salmonella* serotypes was observed during thermal exposure of aged chicken litter, since *S.* Senftenberg and *S.* Typhimurium exhibited higher resistance profiles than the other two serotypes. To characterize the serotypes that could only be detectable by enrichment after a longer period of time, we also carried out a 24-h heat treatment at 80ºC for chicken litter with 20% moisture content. *S.* Senftenberg, the most heat-resistant *Salmonella* serotype, could even survive for up to 24 h at 80°C. **At this point, a significant practical consequence is that serotypes with robust thermal inactivation characteristics, such as *S.* Senftenberg, may be used as indicator microorganisms to assure microbial risk assessment of the 'worst-case scenario' when evaluating the thermal processing of chicken litter in further heat challenge studies.**

Objective 2: *Evaluating the effect of type and freshness of chicken litter on thermal resistance of* Salmonella *spp.*

Effects of type and freshness of chicken litter on thermal resistance of desiccation-adapted Salmonella *spp.* Fresh chicken litter was collected from chicken barn of Bovan laying hens raised at Morgan Poultry Center, Clemson, SC. Samples were prepared in the same way as described in **Obj. 1.** The chemical characteristics of chicken litter samples are presented in Table 1 (Appendix B). Four *Salmonella* serovars Enteritidis H2292, Heidelberg 21380, Senftenberg ATCC 43845, and Typhimurium 8243 were used and adapted to desiccation for the following heat treatments as described in **Obj. 1**.

Finished dairy compost inoculated with desiccation-adapted *Salmonella* mixture was added (1:100, w/w) into fresh chicken litter with the moisture contents of 30 and 50% for heat treatment. The temperatures used for this study were 70, 75, 80, and 150°C. For heat treatments at 70, 75, and 80°C up to 6 h, at predetermined time intervals, duplicate samples were taken out, homogenized, and plated onto recovery media (OV/XLT-4-R and TAL/TSA-R). The samples which were negative by direct plating recovery method (detection limit: 1.60 log cfu g^{-1}) were enriched and then plated onto XLT-4-R. The heat treatment at 150°C was performed as described in **Obj. 1**. Two trials were performed for each experiment.

At 70, 75, 80, and 150°C, *Salmonella* levels in fresh chicken litter decreased in all samples during heat treatment; however, pathogen survival was significantly different ($P<0.05$) for control and desiccation-adapted *Salmonella* cells during thermal exposure (Table 2). For example, at 70°C, in fresh chicken litter with the moisture content of 30%, more than 6 h was required to obtain a 5-log reduction for desiccation-adapted cells, while only 2~3 h was needed for the non-adapted control cells. Moreover, exposure time required to obtain 3- and 5-log reductions in the desiccation-adapted cells gradually became shorter as temperature and moisture content were increased. The ammonia levels were also monitored during thermal inactivation at 70, 75, 80, and 150°C, which decreased from ca. 200 to ca.100 μg g^{-1} after all the heat treatments.

The desiccation-adapted *Salmonella* at 70, 75, and 80°C was inactivated much slower in fresh chicken litter at the beginning of heat treatment, as compared to the results we have obtained on aged chicken litter from **Obj. 1**(Fig. 1, Appendix B; Fig. 2, Appendix A). Exposure time required to achieve a 3-log reduction of the desiccation-adapted cells in fresh chicken litter was longer at higher temperatures and moisture contents. However, longer thermal exposure time was needed for *Salmonella* cells in aged chicken litter to achieve a 5-log reduction (Table 1). Moreover, *Salmonella* inactivation was much quicker in fresh chicken litter at 150°C in comparison to aged chicken litter (Table 3, Appendix A; Table 2, Appendix B).

Table 2 Exposure time required for reducing 3 and 5 logs of control and desiccation-adapted *Salmonella* spp. in fresh chicken litter at 70, 75, and 80°C

Temperature (°C)	Sample	Moisture content (%)	Exposure time (h)	
			3 logs	5 logs
70	Control	30	0.5~1	2~3
		50	0~0.5	1~1.5
	Desiccation-adapted cells	30	1.5~2	>6
		50	0.5~1	2~3
75	Control	30	0~0.5	1~1.5
		50	0~0.5	0.5~1
	Desiccation-adapted cells	30	0.5~1	4~5
		50	0~0.5	1.5~2
80	Control	30	0~0.5	0.5~1
		50	0~0.5	0.5~1
	Desiccation-adapted cells	30	0.5~1	3~4
		50	0~0.5	1~1.5

Chicken litter is a heterogeneous waste by-product without constant compositions. For the origin of chicken litter samples, the majority of fresh chicken litter collected for this study was feces from layers, whereas aged chicken litter from **Obj. 1** was partially composted (windrow composting of 7-10 d) and consisted of feces from broilers, as well as small amounts of feeding and bedding materials. The chemical properties of aged and fresh chicken litter were also different such as organic matter, P, K, Na, & some heavy metals (Table 1, Appendix B). In addition, there was a significant difference between the initial ammonia contents of fresh (ca. 200 μg g^{-1}) and aged chicken litter (ca. 850 μg g^{-1}), which could be due to the fact that the content of crude protein in the diets of layers (16-19%) were relatively lower as compared to that of broilers (19-23%). The higher initial ammonia contents of aged chicken litter may contribute to rapid reduction of *Salmonella* at the beginning of heat exposure at 70, 75, and 80^{o}C. **Overall, desiccation-adapted *Salmonella* in fresh chicken litter was more susceptible to heat inactivation as compared in aged chicken litter of different type.**

Effect of aging of broiler chicken litter on thermal resistance of Salmonella *spp.* The chicken litter was collected from Mendel Stone Farm, SC, where the same breed of broiler chickens was fed with the same diet, and litter was piled up inside the chicken house. After the chicken litter was removed from the chicken house at the completion of chicken growing season, it was kept in a stack pile under a covered area for up to 9 months (May, 2013 ~ February, 2014). The chicken litter samples were taken at 0, 3, 6, and 9 months of storage. The raw chicken litter samples without moisture adjustment were used for desiccation-adaptation of *Salmonella*. Procedures for desiccation adaptation, inoculation, and heat treatment at 75, 80, 85, and 150^{o}C were the same as described in **Obj. 1**. For microbiological analysis, 10 g of chicken litter were mixed with 90 ml of PBS containing 1% sodium pyruvate, kept at room temperature for 30 min to allow cell recovery and samples were then serially diluted and plated in triplicate. If the plating method (detection limit: 1.52 log cfu g^{-1}) failed to recover any cells, enrichment procedure was conducted to detect the presence of *Salmonella* cells.

The electrical conductivity was determined using the Orion™ VERSA STAR™ conductivity meter (Thermo Fisher Scientific Inc., Waltham, MA) based on the method described by U.S. Composting Council (2002).

The metabolic fingerprint pattern was investigated with the EcoPlate method (Biolog Inc., Hayward, CA). Litter samples were diluted in 0.85% saline to reach a bacterial concentration of approximately 10^3 cfu ml^{-1} then wells consisting of 31 carbon sources and 3 blanks were inoculated. Plates were incubated at room temperature for 7 days and the development of

purple color was monitored by measuring optical density at 590 nm every 24 h. The analysis was performed on data collected after 5 days since there was no further significant color formation after that time point. The Principal Component Analysis (PCA) was performed using XLSTAT 2010 (Addinsoft Inc., New York, NY) to compare and analyze the changes in the community reaction pattern.

During storage on farm, chicken litter was subject to a gradual decline in electrical conductivity, moisture, populations of mesophiles, thermophiles, *Enterobacteriace*, and *Actinomycetes*, and ammonia (Table 3&4, Appendix B). However, the concentrations of most heavy metals were elevated and reached a plateau after 3~6 months, but decreased afterwards.

At 75, 80, 85, and 150°C, *Salmonella* levels decreased in all chicken litter samples during heat treatment; however, pathogen survival was significantly different ($P<0.05$) for different storage ages of chicken litter (Fig. 2 & Table 5, Appendix B). Inactivation of desiccation-adapted cells became slower in chicken litter when storage time was extended. However, there was not a significant difference in thermal resistance of *Salmonella* in chicken litter with storage time as 6 or 9 months indicating a threshold for thermal resistance being reached. For the same storage time, exposure time required to achieve a 5-log reduction of the desiccation-adapted cells in chicken litter was longer at lower temperatures. It is possible that different physical and chemical characteristics (Table 3 & 4, Appendix B), and different microbial communities of these samples contributed to the difference in survival profiles. Ammonia content in fresh chicken litter which is known as one of factors enhancing thermal destruction of bacterial cells was higher ($P<0.05$) than in aged chicken litter. Therefore, the initial high level of ammonia (279.13 μg NH_4-N g^{-1}) in the fresh chicken litter (0 month) gives a higher ammonia emission during subsequent heat treatment, accelerating destruction of *Salmonella* in these samples. **In conclusion, as compared to in fresh chicken litter, the increased thermal resistance of *Salmonella* in aged chicken litter was more pronounced ($P<0.05$) at lower lethal temperatures (70 and 75°C).**

The cluster diagram based on the EcoPlate data revealed a distinct clustering in relation to sampling times (Fig. 3, Appendix B). Only six carbon sources oxidized at the highest rates (Changes in $OD_{590}>0.7$ after 5 days based on EcoPlate data) by microbial communities in broiler chicken litter during 9-month storage were used for PCA analysis, including Tween 40, Glycogen, N-Acetyl-D-Glucosamine, D-Glucosaminic Acid, Glucose-1-Phosphate, and D, L-α-Glycerol Phosphate (Table 6, Appendix B). PCA analysis revealed the separation of four sampling times, and showed that the oxidization rates of these above six carbon sources were indeed most responsible for the observed separation of four sampling times. Moreover, Tween 40, Glycogen, N-Acetyl-D-Glucosamine, D-Glucosaminic Acid, and Glucose-1-Phosphate were oxidized at a higher rate by 9-month microflora, while L-α-Glycerol Phosphate was oxidized at a higher rate by 3-month microflora. According to the Pearson correlation analysis, there were good correlations between the oxidation rates of Tween 40 and D-Glucosaminic Acid (R^2=0.997), and also between those of Glycogen and N-Acetyl-D-Glucosamine (R^2=1.000) (Table 7, Appendix B). **Therefore, our results showed that there were some changes in the metabolic fingerprint pattern of microbial communities over time during 9-month storage of chicken litter.**

Objective 3: Developing a two-step heat treatment for chicken litter to expedite Salmonella *inactivation*

Salmonella serovars Enteritidis H2292, Heidelberg 21380, Senftenberg ATCC 43845, and Typhimurium 8243 as described in **Obj. 1** were used for the two-step heat treatment. The

aged chicken litter from Organic Farms, CA was prepared and inoculated with desiccation-adapted *Salmonella* as described in **Obj. 1.**

For **Obj. 3,** *Salmonella* cultures were desiccation-adapted in the aged chicken litter instead of dairy compost as used for **Obj. 1** study. To lower the ammonia content in order to minimize the population reduction during desiccation adaptation, the aged chicken litter used for desiccation adaptation was exposed to greenhouse conditions for 15 d. *Salmonella* cultures were added (1:100, v/w) into 300 g aged chicken litter with lower ammonia content to obtain the moisture content of 20% (water activity [a_w], 0.86) at a final concentration of ca. 10^9 cfu g^{-1} for a 24-h desiccation adaptation. Afterwards, aged chicken litter with lower ammonia content inoculated with desiccation-adapted cells was mixed (1:100, w/w) with aged chicken litter adjusted to the desired moisture contents of 20, 30, 40, and 50% with sterile tap water. Controls were washed *Salmonella* cells (ca. 10^9 cfu ml^{-1}) kept at room temperature for 24 h that were added to aged chicken litter (20% moisture content) in a ratio of 1:100 (v/w).

About 20 g of inoculated aged chicken litter were distributed evenly in an aluminum pan, placed into a metal tray immersed in a water bath (water temperature, 70^{o}C), and treated by moist heat at 65°C for 1 h. Litter samples were then dry-heated in a convectional oven set at 85°C for 1 h to the desired moisture content of <12%. Temperature was initially set at a higher set point of 100ºC to minimize the come-up time for dry-heat treatment. Temperature was determined with T type thermocouples (DCC Corporation, Pennsauken, NJ), with one cord inserted into litter samples throughout two-step treatment and another cord exposed to the air inside the water bath or the oven. During dry-heat treatment, when the interior of the litter reached the desired temperature, the temperature of the oven was readjusted to keep at the pre-designated target temperature. The relative humidity (RH) in the water bath was constantly monitored with a thermocouple data logger (Lascar Electronics Inc., Erie, PA). At predetermined time intervals, duplicate samples were taken out and placed immediately in an ice water bath. Samples were then analyzed for *Salmonella* population in the same way as described in **Obj. 1**. Each experiment was performed in two separate trials.

Table 4 Survival of desiccation-adapted *Salmonella* spp. in aged chicken litter during two-step heat treatment

Sample	Moisture content (%)	a_w	*Salmonella* population (log cfu g^{-1}) after heat treatment for (min)					
			Moist-heat treatment				Dry-heat treatment	
			0	20	40	60	30	60
Control	20	0.87	6.71±0.22a[a]	-	-	-	-	-
Desiccation-adapted *Salmonella*	20	0.87	6.68±0.18a	3.75±0.12a	3.42±0.22a	3.31±0.15a	3.04±0.13a	2.97±0.27a
	30	0.94	6.74±0.23a	3.25±0.08b	3.02±0.31a	3.00±0.13a	2.64±0.06b	2.57±0.16a
	40	0.98	6.72±0.20a	+[b]	+	-[c]	-	-
	50	0.99	6.67±0.14a	+	-	-	-	-

[a]Data are expressed as means±SD of two trials. Means with different letters in the same column are significantly different (P<0.05).
[b]+, detectable by enrichment.
[c]-, not detectable by enrichment.

RH increased from 52 to 100% within 15 min during moist-heat treatment (Fig. 1, Appendix C), and temperatures inside chicken litter samples reached 65°C after 1-h moist-heat treatment regardless of moisture content. The higher initial moisture content of chicken litter required the longer come-up time. The come-up times for heating aged chicken litter with 20,

30, 40, and 50% moisture contents to reach the target temperature of 85°C were 90, 105, 120, and >120 min. Table 4 presents the comparison of survival profiles of desiccation-adapted and non-adapted *Salmonella* cells in aged chicken litter with 20% moisture content during two-step heat treatment. *Salmonella* counts in aged chicken litter decreased in all samples during two-step heat treatment; however, desiccation-adapted *Salmonella* displayed extended survival as compared to the non-adapted control. Control cells in aged chicken litter were not detectable by enrichment after 20 min of moist-heat treatment. In contrast, desiccation-adapted *Salmonella* cells presented a much longer duration of survival and the population of viable desiccation-adapted cells was 2.97 log cfu g^{-1} after two-step heat treatment. Moisture levels in all samples decreased gradually during moist-heat treatment, and after 1-h drying process, dropped dramatically from the initial moisture contents of 20, 30, 40, and 50% to 4.1, 4.6, 6.2, and 8.3%, respectively, which were lower than the desired level of 12% (Fig. 2, Appendix C).

Salmonella counts in aged chicken litter with the moisture contents of 20, 30, 40, and 50% decreased during two-step heat treatment; however, the populations of *Salmonella* cells in aged chicken litter with different moisture contents varied. After moist-heat treatment, the populations of *Salmonella* in aged chicken litter at 20 and 30% moisture contents decreased from ca. 6.70 log cfu g^{-1} to 3.31 and 3.00 log cfu g^{-1}, respectively, and afterwards, the populations further decreased to 2.97 and 2.57 log cfu g^{-1}, respectively, by subsequent dry-heat treatment. As a comparison, *Salmonella* cells in litter samples at 40 and 50% moisture contents could only be detected by enrichment for 40 and 20 min of moist-heat treatment, respectively.

Our results revealed that the two-step heat treatment consisting of a moist-heat treatment for 1 h at 65°C and a sequential dry-heat treatment for 1 h at 85°C was effective in reducing heat-resistant desiccation-adapted *Salmonella* in aged chicken litter. Clearly, a >5-log reduction of *Salmonella* in chicken litter with moisture content of ≥40% can be achieved by this two-step heating technique.

Outcomes and Accomplishments

Objective 1: Validating the thermal inactivation of Salmonella *spp. at different temperatures in broiler chicken litter*

The thermal inactivation of desiccation-adapted *Salmonella* spp. in aged chicken litter and potential cross-tolerance of desiccation-adapted *Salmonella* spp. to heat treatment was investigated. A 5-log reduction of the desiccation-adapted *Salmonella* cells in chicken litter with 20% moisture content required >6, >6, 4~5, and 3~4 h exposure at 70, 75, 80, and 85°C, respectively, whereas the same reduction in non-adapted control with 20% moisture content was achieved within 1.5~2, 1~1.5, 0.5~1, and <0.5 h at 70, 75, 80 and 85°C, respectively. Time required to obtain a 5-log reduction in desiccation-adapted cells gradually became shorter as temperature and moisture content were increased. At 150°C, desiccation-adapted *Salmonella* survived for 50 min in chicken litter with 20% moisture content, whereas control cells were detectable by enrichment until only 10 min. In addition, *S.* Senftenberg, was found as the most heat resistant one among 4 serotypes tested in this study.

Objective 2: *Evaluating the effect of type and freshness of chicken litter on thermal resistance of* Salmonella *spp.*

The thermal resistance profiles of *Salmonella* in both broiler chicken and egg-laying hen litter were compared, and the change of *Salmonella* heat resistance as affected by different storage ages of broiler chicken litter collected from the same farm was also determined. At 70, 75, and

80°C, inactivation of desiccation-adapted cells was slower in fresh chicken litter at the beginning of heat treatment, as compared to the results we have obtained on aged chicken litter from our **Obj. 1** study. However, longer thermal exposure time was needed for *Salmonella* cells in aged chicken litter to achieve a 5-log reduction. Furthermore, *Salmonella* inactivation was much quicker in fresh chicken litter at 150°C in comparison to aged chicken litter. For our field study, inactivation of desiccation-adapted cells became slower in chicken litter when storage time was extended. However, there was not a significant difference in thermal resistance of *Salmonella* in chicken litter with storage time as 6 or 9 months indicating a threshold for thermal resistance being reached. Interestingly, physical, chemical and microbiological changes in chicken litter during long-term storage showed some correlation with the thermal resistance of *Salmonella*.

Objective 3: Developing a two-step heat treatment for chicken litter to expedite Salmonella *inactivation*

The effectiveness of a two-step heat treatment for aged chicken litter on elimination of desiccation-adapted *Salmonella* was evaluated. After moist-heat treatment, the populations of *Salmonella* in aged chicken litter at 20 and 30% moisture contents decreased from 6.7 log cfu g^{-1} to 3.3 and 3.0 log cfu g^{-1}, respectively, and after subsequent dry-heat treatment, the populations decreased further to 3.0 and 2.6 log cfu g^{-1}, respectively. *Salmonella* cells in litter samples at 40 and 50% moisture contents were only detectable by enrichment for 40 and 20 min of moist-heat treatment, respectively. Moisture contents in all samples were reduced to <12% after drying process. Our results demonstrated that the two-step heat treatment was highly effective in reducing heat-resistant desiccation-adapted *Salmonella* in aged chicken litter. The increased moisture contents in chicken litter contributed to the rapid killing of *Salmonella* during moist-heat treatment.

Summary of Findings and Recommendations

Objective 1: Validating the thermal inactivation of Salmonella *spp. at different temperatures in broiler chicken litter*

Our results demonstrated that the thermal resistance of *Salmonella* in aged chicken litter was increased significantly when the cells were adapted to desiccation or dry chicken litter were heat-treated. In addition, pronounced tailing was also observed in the survival curves of desiccation-adapted *Salmonella* at 70, 75, 80 and 85°C. Our observation implies that desiccation-adapted cells from the tailing in survival curves should be considered sufficiently by chicken litter processors when applying thermal treatment to chicken litter. Otherwise, inadequate processing would lead to the survival of a few heat-resistant *Salmonella* cells that could contaminate produce in the field. In addition, the use of *S.* Senftenberg, verified as the most heat resistant serotype in this study, as indicator microorganism can assure microbial risk assessment of the ‘worst-case scenario’ when evaluating the thermal processing of chicken litter in future heat challenge studies. Overall, our findings have important implications for the chicken litter processors to validate and modify their heating process depending on the conditions of incoming raw chicken litter in order to eliminate *Salmonella* that may be subjected to dry stress during storage.

Objective 2: *Evaluating the effect of type and freshness of chicken litter on thermal resistance of* Salmonella *spp.*

Our results revealed that desiccation-adapted *Salmonella* in different types and storage ages of chicken litter displayed different survival profiles during heat treatment, and changes in moisture level, ammonia, electrical conductivity, heavy metals and indigenous microbial community of these samples could contribute to this difference. Overall, the desiccation-adapted *Salmonella* in fresh chicken litter was more susceptible to heat inactivation as compared in aged chicken litter of the same type or different type. Therefore, our recommendation to chicken litter processing industry is to process the chicken litter as soon as possible since the presence of ammonia and moisture in fresh chicken litter can enhance the inactivation rate of *Salmonella* during thermal processing.

Objective 3: Developing a two-step heat treatment for chicken litter to expedite* Salmonella *inactivation

Due to the increased resistance of *Salmonella* in dried chicken litter during thermal processing, a few heat resistant cells may survive current physical heat processing and result in the contamination of the finished products. In order to provide temperature-time recommendations for processing physically heat-treated chicken litter, the most heat-resistant form of *Salmonella*, desiccation-adapted cells, was used to simulate the 'worst-case scenario'. We have demonstrated that a two-step heat treatment by applying moist heat to the contaminated chicken litter first followed by dry heat, can not only ensure the fast inactivation of *Salmonella* but also produce more stable and nutrient dense finished products. Based on our results, a two-step heating technique consisting of a moist-heat treatment for 1 h at 65°C and a sequential dry-heat treatment for 1 h at 85°C can be sufficient for achieving >5.5-log reductions of *Salmonella* in chicken litter with moisture content of ≥40%. Results generated from this study, after actual processing plant validation, will help the chicken litter processors to modify their existing process parameters to produce microbiologically safe organic fertilizers and soil amendment, thereby reducing the possible source of produce contamination on farm.

APPENDICES

Publications and Presentations (required)

Chen, Z. J. Diao, M. Dharmasena, C. Ionita, and X. Jiang. 2013. Thermal inactivation of desiccation-adapted *Salmonella* spp. in aged chicken litter. Appl. Environ. Microbiol. 79:7013-7020.

Chen, Z. and X. Jiang. 2014. Microbiological safety of chicken litter or chicken litter-based organic fertilizers: A Review. Agriculture 4:1-29.

Chen, Z. and X. Jiang. 2014. Developing a two-step heat treatment for inactivating desiccation-adapted *Salmonella* in aged chicken litter. Abs. 101th Annu. Mtg. Intern. Assoc. Food Prot., Indianapolis, IN, August 3-6. (submitted)

Chen, Z., J. Diao, C. Ionita, and X. Jiang. 2013. Thermal inactivation of desiccation-adapted *Salmonella* spp. in aged chicken litter. Abs. 100th Annu. Mtg. Intern. Assoc. Food Prot., Charlotte, NC, July 28-31.

Jiang, X. Invited presentation: On-farm produce safety: biological soil amendments. Department of Biology seminar series, Clemson University, November 15, 2013.

Jiang, X. Validating *Salmonella* inactivation during thermal processing of the physically heat-treated chicken litter as soil amendment and organic fertilizer. Produce Safety Symposium, UC Davis Center for Produce Safety, June 26, 2013. Rochester, NY.

Jiang, X. Invited presentation: Produce Safety. University of Food Technology, Bulgaria, December 15, 2012.

Chen, Z. and X. Jiang. Thermal inactivation of desiccation-adapted *Salmonella* spp. in aged chicken litter. American Society for Microbiology – South Carolina Branch Meeting, Columbia, SC, October 2012 (Oral presentation).

Copies of Publications:

Microbiological safety of chicken litter or chicken litter-based organic fertilizer: A review (Agriculture, 2014)

Chen, Z., Diao, J., Ionita, C., Jiang, X

Abstract: Chicken litter or chicken litter-based organic fertilizers are usually recycled into the soil to improve the structure and fertility of agricultural land. As an important source of nutrients for crop production, chicken litter may also contain a variety of human pathogens that can threaten humans who consume the contaminated food or water. Composting can inactivate pathogens while creating a soil amendment beneficial for application to arable agricultural land. Some foodborne pathogens may have the potential to survive for long periods of time in raw chicken litter or its composted products after land application, and a small population of pathogenic cells may even regrow to high levels when the conditions are favorable for growth. Thermal processing is a good choice for inactivating pathogens in chicken litter or chicken litter-based organic fertilizers prior to land application. However, some populations may become acclimatized to a hostile environment during build-up or composting and develop heat resistance through cross-protection during subsequent high temperature treatment. Therefore,

this paper reviews currently available information on the microbiological safety of chicken litter or chicken litter-based organic fertilizers, and discusses about further research on developing novel and effective disinfection techniques, including physical, chemical, and biological treatments, as an alternative to current methods.

Thermal inactivation of desiccation-adapted *Salmonella* spp. in aged chicken litter (AEM, 2013)

Chen, Z., Diao, J., Dharmasena, M., Ionita, C., Jiang, X., Rieck, J

Abstract: Thermal inactivation of desiccation-adapted *Salmonella* spp. in aged chicken litter was investigated in comparison with non-adapted control to examine potential cross-tolerance of desiccation-adapted cells to heat treatment. A mixture of four *Salmonella* serovars was inoculated into the finished compost with 20, 30, 40, and 50% moisture contents for a 24-h desiccation adaptation. Afterwards, the compost with desiccation-adapted cells was inoculated into the aged chicken litter with the same moisture content for heat treatments at 70, 75, 80, 85 and 150°C. Recovery media were used to allow heat-injured cells to resuscitate. A 5-log reduction of the desiccation-adapted cells in aged chicken litter with 20% moisture content required >6, >6, 4~5, and 3~4 h exposure at 70, 75, 80, and 85°C, respectively. As a comparison, a 5-log reduction of non-adapted control in the same chicken litter was achieved within 1.5~2, 1~1.5, 0.5~1, and <0.5 h at 70, 75, 80, and 85°C, respectively. Exposure time required to obtain a 5-log reduction in the desiccation-adapted cells gradually became shorter as temperature and moisture content were increased. At 150°C, desiccation-adapted *Salmonella* survived for 50 min in chicken litter with 20% moisture content, whereas control cells were detectable by enrichment until only 10 min. Our results demonstrated that the thermal resistance of *Salmonella* in aged chicken litter was increased significantly when the cells were adapted to desiccation. This study also validated the effectiveness of thermal processing being used for producing chicken litter free of *Salmonella* contamination.

Developing a two-step heat treatment for inactivating desiccation-adapted *Salmonella* in aged chicken litter (IAFP, 2014)

Chen, Z., J. Diao, C. Ionita, and X. Jiang. Clemson University, SC 29634

Introduction: Chicken litter may contain a variety of human pathogens, such as *Salmonella*, that can potentially contaminate fresh produce as an organic fertilizer. Some bacterial cells become acclimatized to desiccation condition in stockpiled chicken litter and develop cross-protection to subsequent dry-heat processing. Dry-heat treatment alone may not readily decrease the desiccation-adapted pathogenic cells to safe levels, resulting in the survival of some heat-resistant desiccation-adapted cells.

Purpose: The objective of this study was to evaluate the effectiveness of a two-step heat treatment for aged chicken litter on elimination of desiccation-adapted *Salmonella*.

Methods: Aged chicken litter with 20, 30, 40, and 50% moisture contents was inoculated with a mixture of four *Salmonella* serotypes for a 24-h desiccation adaptation. Afterwards, the chicken litter with desiccation-adapted cells was added into litter with the same moisture contents for a 1-h wet-heat treatment at 65°C and 100% RH inside a water bath. The inoculated litter was then dry-heated in a convectional oven at 85°C for 1 h to the desired moisture level (<12%).

Results: After wet-heat treatment, the populations of *Salmonella* in aged chicken litter at 20 and 30% moisture contents decreased from 6.7 log cfu g^{-1} to 3.3 and 3.0 log cfu g^{-1}, respectively,

and after subsequent dry-heat treatment, the populations decreased to 3.0 and 2.6 log cfu g^{-1}, respectively. *Salmonella* cells in litter samples at 40 and 50% moisture contents were only detectable by enrichment for 40 and 20 min of wet-heat treatment, respectively. Moisture contents in all samples were reduced to <8% after drying process.

Significance: Our results demonstrated that the two-step heat treatment was effective in reducing >5.5 logs of desiccation-adapted *Salmonella* in chicken litter with moisture content at or above 40%. Therefore, the findings from this study provide the industry with a cost-effective heat treatment method for processing chicken litter.

Thermal inactivation of desiccation-adapted *Salmonella* spp. in aged chicken litter (IAFP, 2013)

Chen, Z., Diao, J., Ionita, C., Jiang, X

Introduction: Heat-treated chicken litter is recycled as an organic fertilizer or soil amendment for agricultural production. However, chicken litter may contain loads of human pathogens, such as *Salmonella*. Some populations become acclimatized to desiccation environment during stockpiling and develop heat resistance during subsequent high temperature processing.

Purpose: The objective of this study was to investigate the thermal inactivation of desiccation-adapted *Salmonella* spp. in aged chicken litter and to examine potential cross-tolerance of desiccation-adapted *Salmonella* spp. to heat treatment.

Methods: A mixture of four *Salmonella* serotypes was inoculated into the finished compost with 20, 30, 40, and 50% moisture contents for a 24-h desiccation adaptation. Afterwards, the compost with desiccation-adapted cells was added into the aged chicken litter with the same moisture contents for heat treatments at 70, 75, 80, and 150°C. Recovery media were used to allow injured cells to resuscitate.

Results: A 5-log reduction of the desiccation-adapted *Salmonella* cells in chicken litter with 20% moisture content required > 6, > 6, and 4~5 h exposure at 70, 75, and 80°C, respectively, whereas the same reduction in non-adapted control with 20% moisture content was achieved within 1.5~2, 1~1.5, and 0.5~1 h at 70, 75, and 80°C, respectively. Time required to obtain a 5-log reduction in desiccation-adapted cells gradually became shorter as temperature and moisture content were increased. At 150°C, desiccation-adapted *Salmonella* survived for 50 min in chicken litter with 20% moisture content, whereas control cells were detectable by enrichment until only 10 min.

Significance: Our results demonstrated that the thermal resistance of *Salmonella* in aged chicken litter was increased significantly when the cells were adapted to desiccation. Therefore, the chicken litter processors need to validate and modify their heating process in order to eliminate *Salmonella* that may be subjected to dry stress.

Thermal inactivation of desiccation-adapted *Salmonella* spp. in aged chicken litter (ASM – South Carolina Branch Meeting)

Chen, Z., Jiang, X

Introduction: Heat-treated chicken litter is recycled as an organic fertilizer for direct application to agricultural land as a soil amendment. However, chicken litter may contain loads of human pathogens, such as *Salmonella*. Some populations become acclimatized to desiccation

environment during stockpiling and develop heat resistance during high temperature processing.

Purpose: The objective of this study was to investigate thermal inactivation of desiccation-adapted *Salmonella* spp. in aged chicken litter and to examine potential cross-tolerance of desiccation-adapted *Salmonella* spp. to heat treatment.

Methods: Dairy compost was used for desiccation adaptation. Combined inocula of *Salmonella* serovars Enteritidis H2292, Heidelberg 21380, Typhimurium 8243, and Senftenberg ATCC 43845 were inoculated (1:100, v/w; final concentration: ca. 10^9 cfu g^{-1}) into dairy compost with 20, 30, 40, and 50% moisture contents for a 24-h desiccation adaptation at room temperature. Afterwards, compost with desiccation-adapted cells was added (1:100, w/w; final concentration: ca. 10^7 cfu g^{-1}) into aged chicken litter with the same moisture content for heat treatments at 70, 75, and 80°C up to 6 h. Two recovery media, OV/XLT-4-R and TAL/TSA-R, were used to allow injured cells to resuscitate. The samples which were negative by the recovery method (detection limit: 1.60 log cfu g^{-1}) were enriched and then plated on XLT-4-R. Control was served as *Salmonella* cells kept as cell suspension (ca. 10^9 cfu ml^{-1}) at room temperature for 24 h, and inoculated into aged chicken litter with 20% moisture content in a ratio of 1:100 (v/w; final concentration: ca. 10^7 cfu g^{-1}).

Results: *Salmonella* level decreased during heat treatment in all samples. Thermal inactivation curves for desiccation-adapted cells were non-linear, with extensive tailing as detected by enrichment. A 5-log reduction of the desiccation-adapted *Salmonella* cells in chicken litter with 20% moisture content required >6, >6, and 4~5 h exposure at 70, 75, and 80°C, respectively. As a comparison, a 5-log reduction of *Salmonella* in control with 20% moisture content was achieved within 1.5~2, 1~1.5, and 0.5~1 h at 70, 75, and 80°C, respectively. Time required to obtain a 5-log reduction in desiccation-adapted cells gradually became shorter as temperature and moisture content increased.

Significance: Our results indicate that *Salmonella* spp. adapted to desiccation condition were able to resist higher temperatures for longer periods than were the non-adapted controls. This study also validates the effectiveness of thermal processing being used for producing chicken litter free of *Salmonella* contamination and provides the industry with cost-effective heat treatment method.

On-farm Produce Safety: Biological Soil Amendments

Xiuping Jiang. Clemson University, SC 29634

Abstract

Fruits and vegetables are vital to maintain good human health. However, there are increasing numbers of foodborne illness outbreaks associated with consumption of fresh produce contaminated with human pathogens. To minimize the risk for foodborne illnesses, the Food Safety Modernization Act (FSMA) has directed the U.S. Food and Drug Administration (FDA) to establish science-based minimum standards for the safe production and harvesting of fruits and vegetables that may be consumed raw. One key area of concern is land application of biological soil amendments. This seminar will review the microbiological safety issues related to biological soil amendments and the proposed standards. Current research on identifying the factors contributing to growth and survival of some major foodborne pathogens in biological soil amendments will be presented. Furthermore, development and validation of practical strategies for pathogen control during composting of animal manure and subsequent storage and handling of finished products will be discussed.

Budget Summary (required)

The fund provided by CPS was adequate for us to carry out the project. The breakdown of the grant funds spent by category is:

Grad	$33,226.54
Wages	$40,713.17
Fringe	$13,953.29
GAD	$7,671.00
Travel	$26.30
Other	$42,075.00
Indirects	$4,342.43
Total	$142,007.73

Travel unspent for future use $5,283.70

Suggestions to CPS (optional)

We enjoyed the close contact with CPS, and all those activities such as attending research symposiums and making industry contacts, which helps us to refine our research approaches in order to develop the effective solutions for produce industry.

Tables and Figures (optional)

Please see **Appendices A, B, & C** in following pages.

Appendix A

Table 1 Comparison of recovery media for heat-injured *S.* Typhimurium in fresh chicken litter

Recovery media	Population of *S.* Typhimurium (log cfu g^{-1}) after exposure to 75ºC for (h)		
	0	0.5	1
TSA-R	6.71±0.06b[a]	3.83±0.38b	2.77±0.24ab
XLT-4-R	6.14±0.29c	3.74±0.45b	2.42±0.70ab
OV/TSA-R	6.69±0.15b	3.82±0.52b	2.20±0.51b
OV/XLT-4-R	6.61±0.02b	3.80±0.22b	2.75±0.45ab
TAL/TSA-R	6.68±0.12b	3.96±0.35b	2.80±0.39ab
TAL/XLT-4-R	6.19±0.42c	3.66±0.40b	2.44±0.68ab
P/TSA-R	6.69±0.18b	3.78±0.47b	2.73±0.35ab
P/ TSB-R	7.59±0.33a	4.78±0.32a	3.33±0.71a

[a]Data are expressed as means±SD of three trials. Means with different letters in the same column are significantly different (P<0.05).

Table 2 Populations of *S.* Typhimurium before and after desiccation adaptation in different composts, and their pH and ammonia levels

Sample	Population of *S.* Typhimurium (log cfu g^{-1})		pH	NH_4-N (μg g^{-1})
	Before desiccation adaptation	After desiccation adaptation		
Dairy compost	8.81±0.01a[a]	8.36±0.13a	7.70±0.05d	22.64±3.22d
Fresh poultry compost	8.78±0.06a	5.79±0.14c	9.09±0.02a	1142.55±100.27a
Old poultry compost	8.76±0.04a	6.58±0.03b	8.26±0.01c	465.82±3.86c
Aged chicken litter	8.75±0.05a	5.88±0.25c	8.97±0.04b	853.55±72.64b

[a]Data are expressed as means±SD of two trials. Means with different letters in the same column are significantly different (P<0.05).

Table 3 Survival of control and desiccation-adapted *Salmonella* spp. in aged chicken litter at 150ºC

Sample	Moisture content (%)	Survival with exposure time (min)					
		10	20	30	40	50	60
Control	20	+[a]	-[b]	-	-	-	-
Desiccation-adapted cells	20	+	+	+	+	+	-
	30	+	+	+	+	+	-
	40	+	+	+	+	-	-
	50	+	+	+	+	-	-

[a]+, Detectable by enrichment.
[b]-, Not detectable by enrichment.

Table 4 Parameter estimates of the inactivation model for control and desiccation-adapted *Salmonella* spp.

Temperature (ºC)	Sample	Moisture content (%)	Long-term log count (α)	Long-term reduction in log count (β)	Decay rate (λ)	Pseudo-R^2
70	Control	20	-0.11±0.18B	6.98±0.32A	1.06±0.11B	0.96
	Desiccation-adapted cells	20	3.58±0.05Aa	3.35±0.12Bb	1.95±0.19Aa	0.97
		30	2.98±0.08b	3.95±0.18ab	1.67±0.18a	0.96
		40	2.50±0.21b	4.43±0.42ab	1.70±0.50a	0.83
		50	1.50±0.51b	5.00±0.51a	0.83±0.35a	0.83
75	Control	20	-0.05±0.08B	7.00±0.18A	1.49±0.09A	0.98
	Desiccation-adapted cells	20	2.39±0.09Aa	4.51±0.19Bb	1.60±0.17Aa	0.96
		30	1.62±0.35a	4.95±0.46ab	1.07±0.37a	0.84
		40	1.11±0.36a	5.43±0.46ab	1.02±0.30a	0.86
		50	0.00±0.38b	6.29±0.49a	0.75±0.17b	0.88
80	Control	20	-0.02±0.01B	7.06±0.03A	2.19±0.02A	0.99
	Desiccation-adapted cells	20	2.28±0.15Aa	4.54±0.30Bc	1.46±0.27Ba	0.92
		30	1.78±0.20a	5.14±0.39bc	1.73±0.40a	0.87
		40	0.04±0.23b	6.55±0.38ab	0.96±0.14a	0.92
		50	-0.02±0.12b	6.94±0.25a	1.26±0.11a	0.96
85	Control	20	-0.02±0.01B	7.11±0.03A	2.79±0.04A	0.99
	Desiccation-adapted cells	20	1.77±0.11Aa	5.18±0.24Bb	1.53±0.19Bab	0.95
		30	0.63±0.36b	5.98±0.50ab	1.08±0.32b	0.87
		40	-0.06±0.05b	7.12±0.11a	1.51±0.06b	0.99
		50	-0.06±0.04b	7.09±0.09a	2.06±0.07a	0.99

[a]Data are expressed as means±SE of two trials. Within each temperature, for desiccation-adapted cells, means with different lowercase letters in the same column are significantly different (P<0.05), while means of control and desiccation-adapted cells (20% moisture content) with different uppercase letters in the same column are significantly different (P<0.05).

Table 5 Characterization by PFGE of *Salmonella* spp. (n=12) in aged chicken litter with 20% moisture content after thermal inactivation at 80ºC

Sample	Serotype
Control (after 0.5-h heat treatment)	*S.* Enteritidis (n=2[b]), *S.* Heidelberg (n=1), *S.* Senftenberg (n=3), *S.* Typhimurium (n=6)
Desiccation-adapted cells (after 6-h heat treatment)	*S.* Enteritidis (n=2), *S.* Heidelberg (n=0), *S.* Senftenberg (n=7), *S.* Typhimurium (n=3)
Desiccation-adapted cells (after 24-h heat treatment)	*S.* Enteritidis (n=0), *S.* Heidelberg (n=0), *S.* Senftenberg (n=12), *S.* Typhimurium (n=0)

[a]Number of colonies.

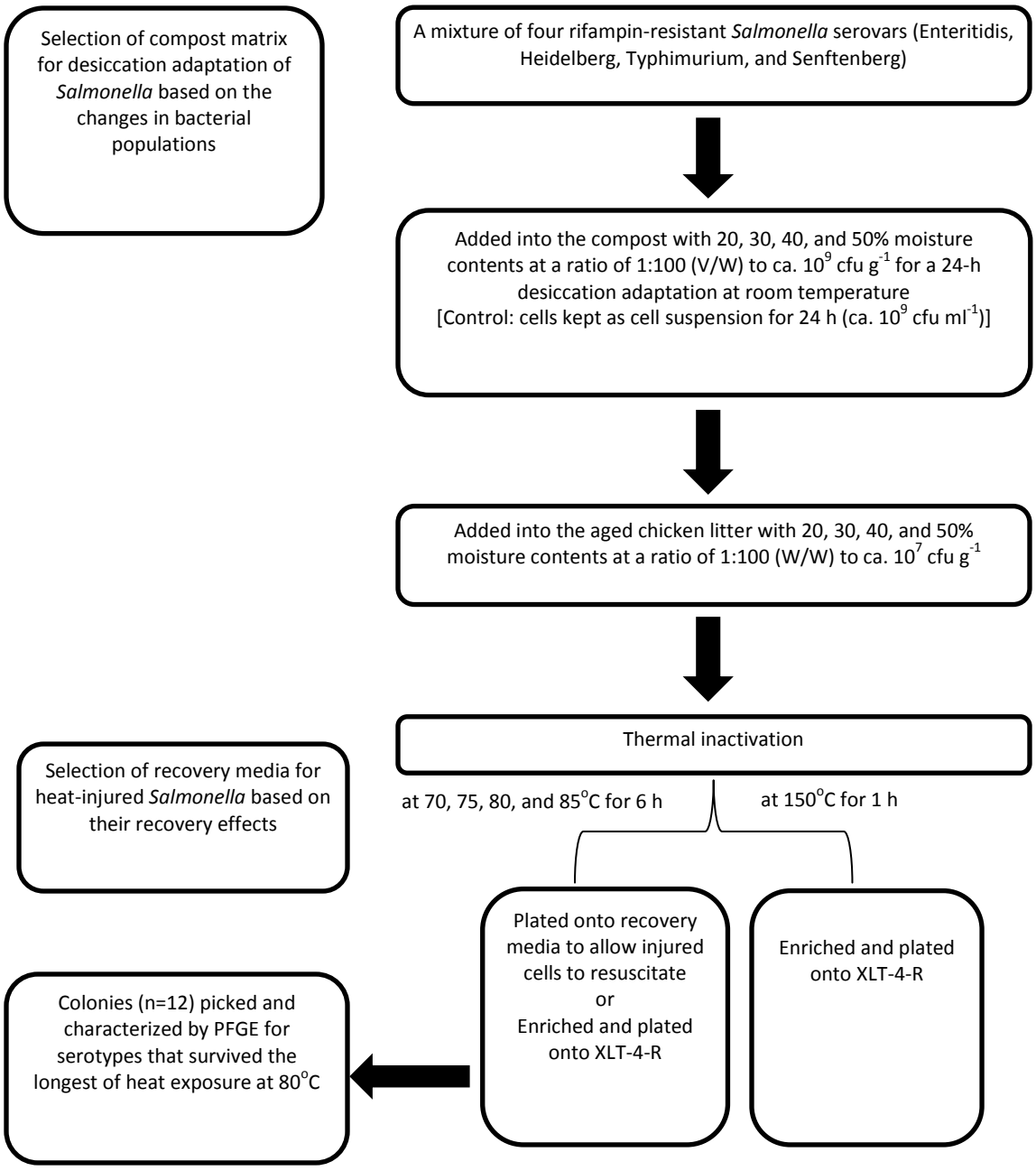

Fig. 1 Flow chart of the experimental procedure.

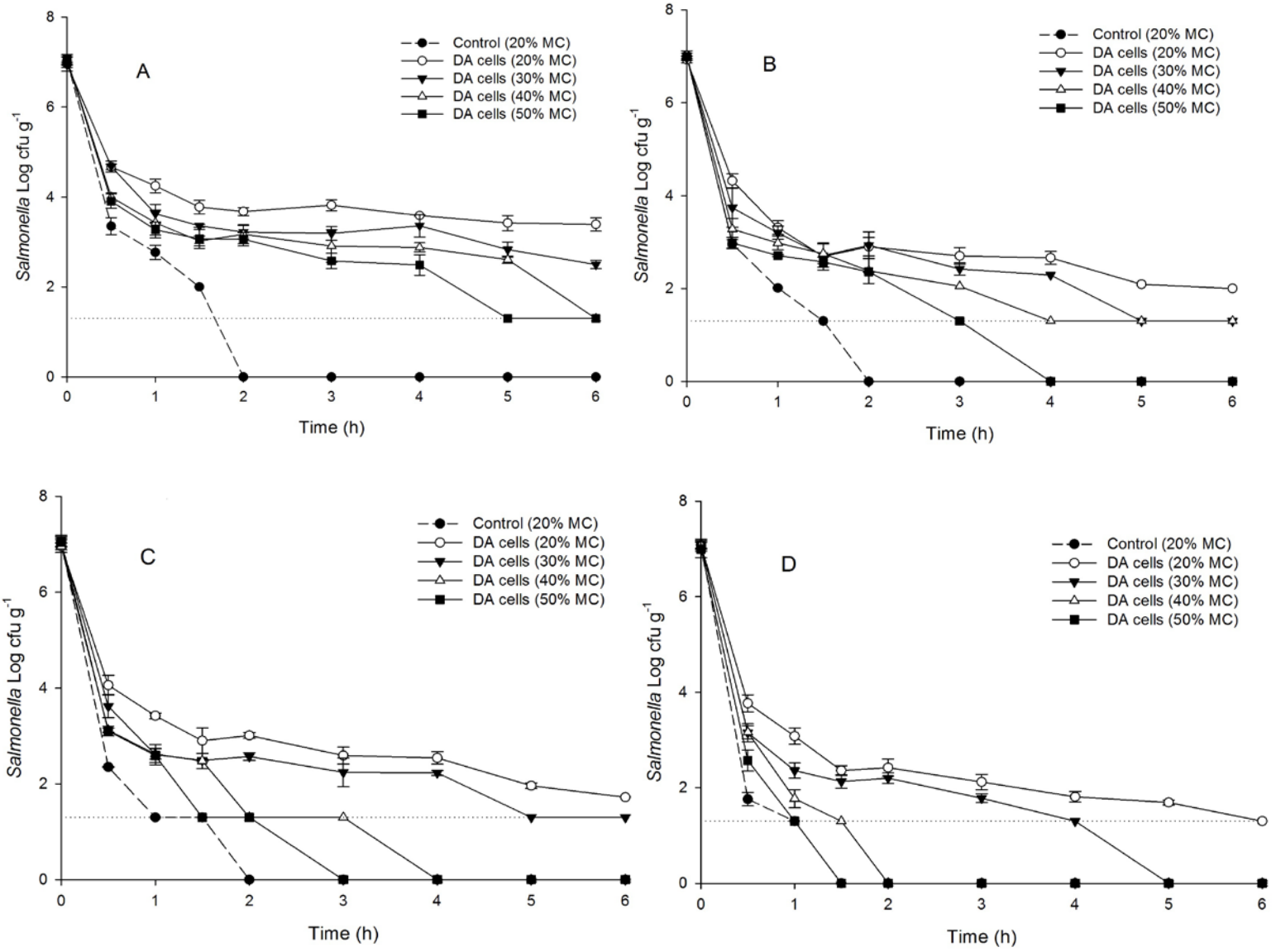

Fig. 2 Survival of control and desiccation-adapted (DA) *Salmonella* spp. in aged chicken litter with 20, 30, 40, and 50% moisture contents (MCs) at (A) 70, (B) 75, (C) 80, and (D) 85ºC. The dotted line indicates that *Salmonella* was detectable only by enrichment (detection limit by plating: 1.30 log cfu g^{-1}).

Appendix B

Table 1 Chemical characteristics of chicken litter samples

Sample	Nutrient (%)										Metal (μg g^{-1})				
	OM[b]	C	N	C/N	P	K	Ca	Mg	S	Na	Zn	Cu	Mn	Fe	Al
Aged chicken litter	55.10± 1.41b[a]	28.00± 0.02b	2.93± 0.04b	9.56± 0.10a	0.99± 0.02b	2.38± 0.08b	2.17± 0.13b	0.51± 0.02b	0.61± 0.02a	0.41± 0.01b	346.00± 36.77b	141.50± 7.78a	484.00± 24.04b	3780± 158.39a	4196.50± 283.55a
Fresh chicken litter	77.12± 0.49a	39.18± 0.16a	4.36± 0.02a	8.99± 0.09b	2.06± 0.08a	2.65± 0.10a	8.72± 0.49a	0.59± 0.02a	0.38± 0.02b	4.07± 0.25a	688.13± 26.06a	78.56± 3.30b	519.87± 1.15a	1655.59 ±88.61b	1544.89± 70.75b

[a]Data are expressed as means±SD of two samples. Means with different letters in the same column are significantly different ($P<0.05$). The values of nutrients and metals are all based on dry-weight.

[b]OM, organic matter.

Table 2 Survival of control and desiccation-adapted *Salmonella* spp. in fresh laying hen litter at 150°C

Sample	Moisture content (%)	Survival with exposure time (min)					
		10	20	30	40	50	60
Control	30	-[b]	-	-	-	-	-
	50	-	-	-	-	-	-
Desiccation-adapted cells	30	+[a]	+	+	+	-	-
	50	+	+	+	-	-	-

[a]+, detectable by enrichment.

[b]-, not detectable by enrichment.

Table 3 Chemical characteristics of broiler chicken litter during 9-month storage

Sampling time (Month)	Nutrient (%)						Metal (μg g^{-1})								pH	NH_4-N (μg g^{-1})
	OM[b]	C	N	C/N	P	K	Ca	Mg	S	Na	Zn	Cu	Mn	Fe	Al		
0	72.35± 0.07a	36.68± 0.21a	3.72± 0.01a	9.85± 0.01b	4.10± 0.11c	3.98± 0.11c	2.89± 0.05c	0.79± 0.03c	0.88± 0.02b	0.86± 0.02c	605.00± 14.14c	180.50± 4.95c	670.50± 16.26c	2768.50± 48.79c	3832.50± 6.36c	8.74 ±0.02a	279.13± 11.92a
3	67.65± 0.50b	33.98± 0.57b	3.32± 0.01c	10.24± 0.23a	4.78± 0.06b	4.74± 0.15b	3.26± 0.08b	0.94± 0.04b	1.03± 0.03a	1.00± 0.01a	744.00± 18.39a	301.00± 32.53a	829.00± 28.28a	3734.00± 288.50b	5274.00± 309.71b	8.61 ±0.01b	98.01± 0.26b
6	63.65± 2.48c	32.70± 0.29c	3.52± 0.06b	9.30± 0.08c	5.21± 0.35a	4.89± 0.08a	3.58± 0.11a	1.01± 0.03a	1.01± 0.13a	1.03± 0.01a	761.00± 25.46a	305.50± 6.36a	881.50± 44.55a	4068.00± 429.92a	6197.50± 286.38a	8.56 ±0.00c	65.05± 0.35c
9	68.30± 1.13b	33.70± 0.54b	3.83± 0.10a	8.80± 0.38d	4.56± 0.25b	4.45± 0.13b	3.13± 0.16b	0.89± 0.04b	0.84± 0.04b	0.93± 0.02b	672.00± 8.49b	225.50± 4.95b	754.50± 34.65b	3428.50± 577.71b	4884.50± 740.34b	8.31 ±0.01d	56.37± 0.28d

[a]Data are expressed as means±SD of two samples. Means with different letters in the same column are significantly different ($P<0.05$). The values of nutrients and metals are all based on dry-weight.

[b]OM, organic matter.

Table 4 Moisture content, electrical conductivity, and microbial counts of broiler chicken litter during 9-month storage

Sampling time (Month)	Electrical conductivity (ms cm^{-1})	Moisture content (%)	Mesophile (cfu g^{-1})	Thermophile (cfu g^{-1})	Enterobacteriaceae (cfu g^{-1})	Yeasts (cfu g^{-1})	Actinomycetes (cfu g^{-1})
0	20.25±0.36a	27.77±0.11a	7.80±0.07a	7.66±0.19a	3.10±0.21	3.86±0.12a	5.03±0.32a
3	19.12±0.48b	19.81±0.09b	6.78±0.33b	6.77±0.33b	-[a]	3.12±0.01c	3.59±0.12c
6	17.18±0.36c	19.53±0.08b	6.66±0.07b	4.84±0.01c	-	3.43±0.15b	3.94±0.12b
9	15.95±0.09d	18.20±0.00c	6.55±0.12b	4.10±0.23d	-	3.68±0.15a	3.40±0.20c

[a]-, not detectable (detection limit of 1.52 log cfu g^{-1}). Means with different letters in the same column are significantly different ($P<0.05$).

Table 5 Survival of *Salmonella* spp. in broiler chicken litter stored for 0, 3, 6, and 9 months at 150ºC

Sampling time (Month)	a_w	Survival with exposure time (min)			
		15	30	45	60
0	0.88±0.00	1.50±0.91b	+[b]	-[c]	-
3	0.79±0.00	2.16±0.21b	+	+	+
6	0.74±0.01	3.29±0.28a	+	+	+
9	0.74±0.00	3.63±0.28a	+	+	+

[a]Data are expressed as means±SD of two trials. For survival data after 15 min, means with different letters in the same column are significantly different (P<0.05).

[b]+, Detectable by enrichment.

[c]-, Not detectable by enrichment.

Table 6 Carbon sources used for the metabolic fingerprint for microbial communities in broiler chicken litter stored for 0, 3, 6, and 9 months

Carbon sources oxidized at a higher rate[a]	Carbon sources oxidized at a lower rate
Pyruvic Acid Methyl Ester	α-Cyclodextrin
Tween 40	D-Cellobiose
Tween 80	α-D-Lactose
Glycogen	β-Methyl-D-Glucoside
D-Xylose	i-Erythritol
D-Mannitol	D-Galactonic Acid γ-Lactone
N-Acetyl-D-Glucosamine	D-Galacturonic Acid
D-Glucosaminic Acid	2-Hydroxy Benzoic Acid
Glucose-1-Phosphate	4-Hydroxy Benzoic Acid
D, L-α-Glycerol Phosphate	γ-Hydroxybutyric Acid
L-Serine	Itaconic Acid
	α-Ketobutyric Acid
	D-Malic Acid
	L-Arginine
	L-Asparagine
	L-Phenylalanine
	L-Threonine
	Glycyl-L-Glutamic Acid
	Phenylethylamine
	Putrescine

[a]Carbon sources oxidized at higher rates are defined as carbon sources showing changes in OD_{590}>0.1 after 5 days based on EcoPlate data.

Table 7 Correlation matrix [Pearson (n)] for six carbon sources oxidized at the highest rates (Changes in OD_{590}>0.7 after 5 days based on EcoPlate data) by microbial communities in broiler chicken litter stored for 0, 3, 6, and 9 months

Variables	Correlation coefficient R^2					
	Tween 40	Glycogen	N-Acetyl-D-Glucosamine	D-Glucosaminic Acid	Glucose-1-Phosphate	D, L-α-Glycerol Phosphate
Tween 40	**1**	0.865	0.855	0.997[a]	0.892	-0.120
Glycogen	0.865	**1**	1.000	0.899	0.822	0.091
N-Acetyl-D-Glucosamine	0.855	1.000	**1**	0.890	0.809	0.087
D-Glucosaminic Acid	0.997	0.899	0.890	**1**	0.910	-0.063
Glucose-1-Phosphate	0.892	0.822	0.809	0.910	**1**	0.335
D, L-α-Glycerol Phosphate	-0.120	0.091	0.087	-0.063	0.335	**1**

[a]Highlighted data are those with higher levels of Pearson correlation.

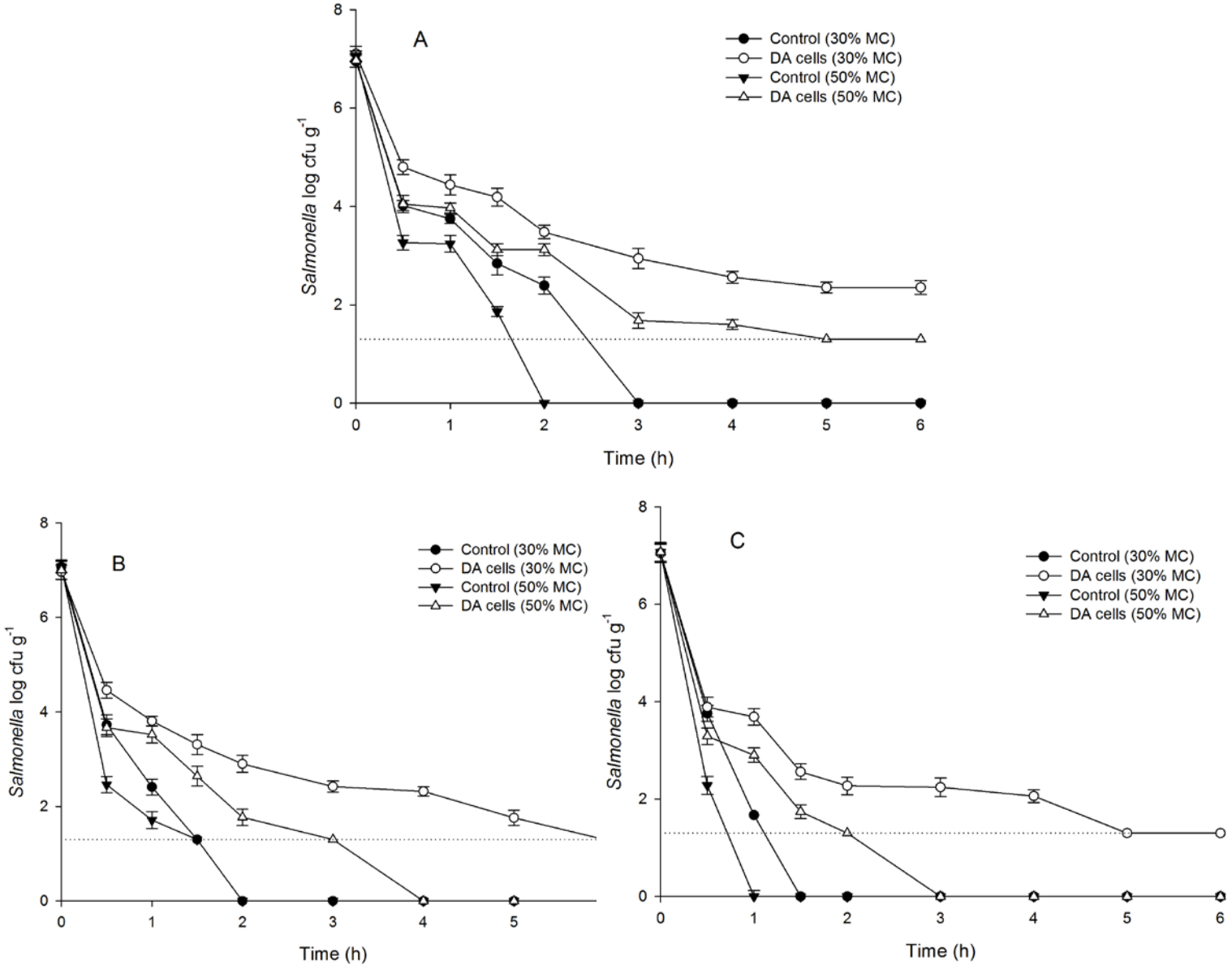

Fig. 1 Survival of control and desiccation-adapted (DA) *Salmonella* spp. in fresh laying hen litter with 30 and 50% moisture contents (MCs) at (A) 70, (B) 75, and (C) 80. The dotted line indicates that *Salmonella* was detectable only by enrichment (detection limit by plating: 1.30 log cfu g^{-1}).

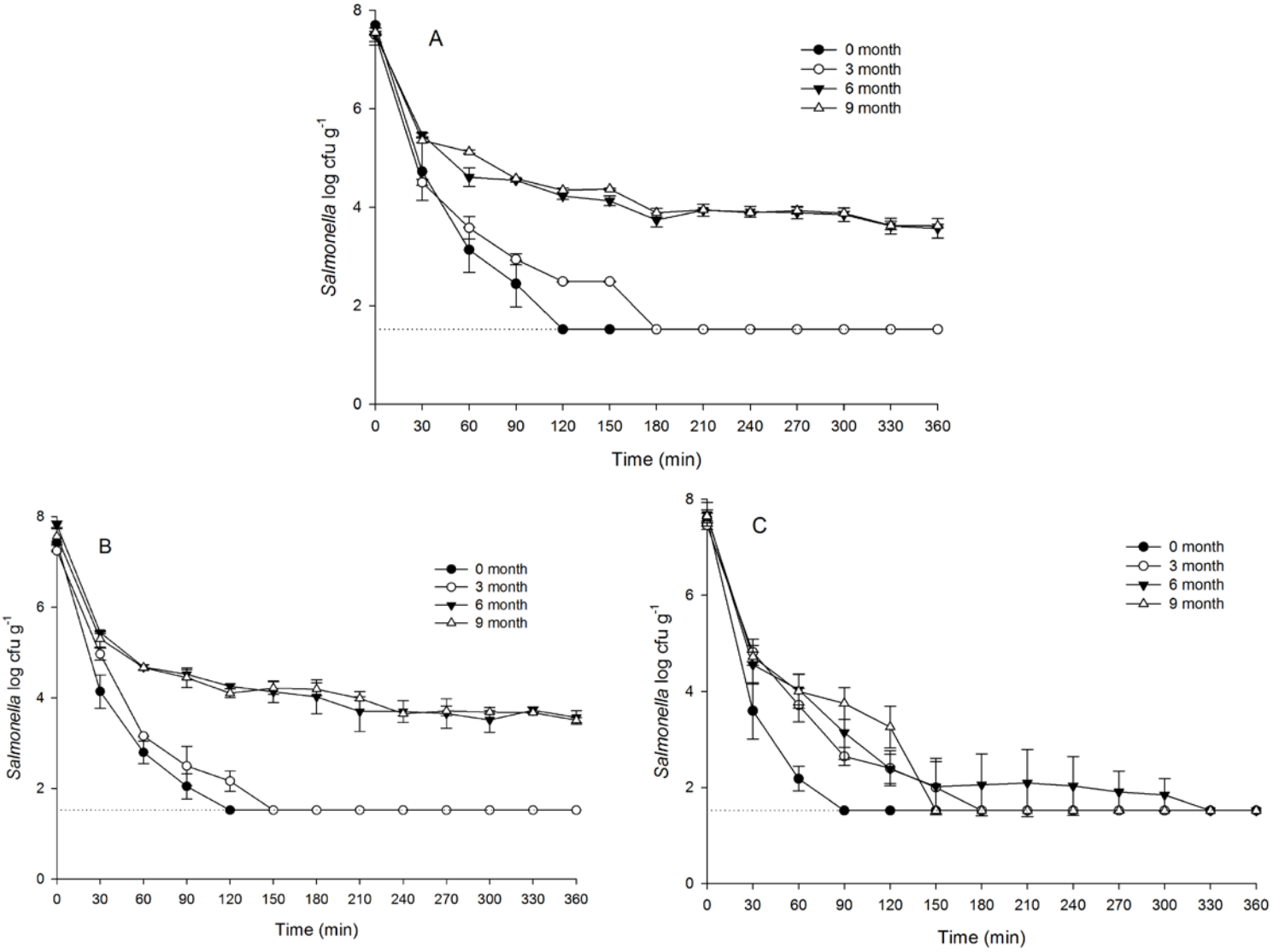

Fig. 2 Survival of *Salmonella* spp. in broiler chicken litter stored for 0, 3, 6, and 9 months at (B) 80 and (C) 85ºC. The dotted line indicates that *Salmonella* was detectable only by enrichment (detection limit by plating: 1.52 log cfu g^{-1}).

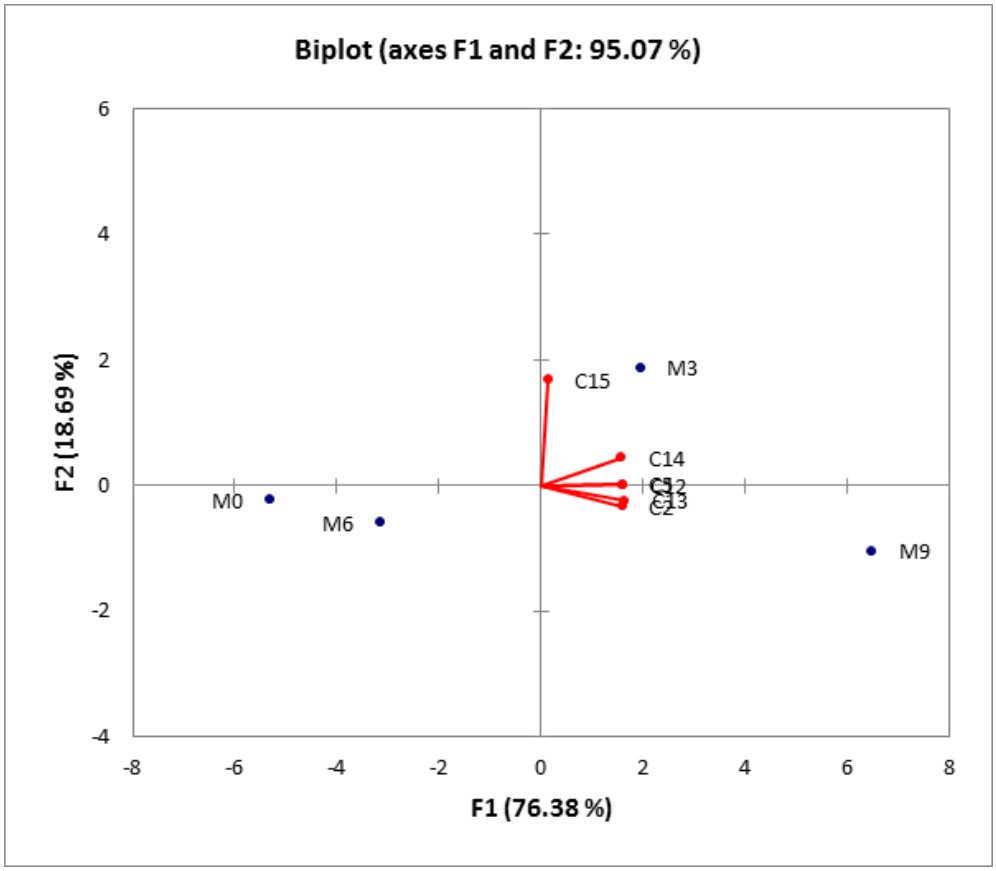

Fig. 3 Principal component analysis (PCA) ordination diagram of EcoPlate data for six carbon sources (C2, Tween 40, C5, Glycogen, C12, N-Acetyl-D-Glucosamine, C13, D-Glucosaminic Acid, C14, Glucose-1-Phosphate, and C15, D, L-α-Glycerol Phosphate) oxidized at the highest rates (Changes in OD_{590}>0.7 after 5 days based on EcoPlate data) by microbial communities in broiler chicken litter stored for 0 (M0), 3 (M3), 6 (M6), and 9 (M9) months.

Appendix C

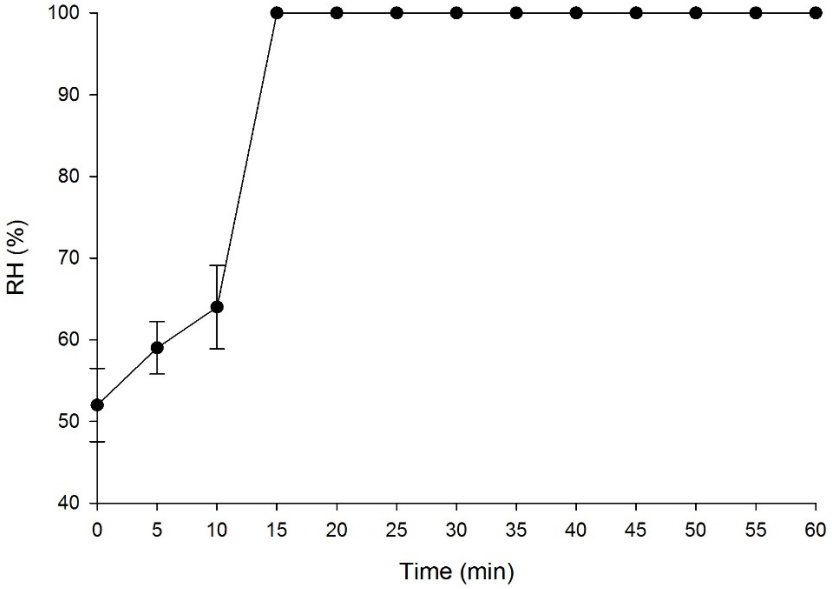

Fig. 1 Change of RH inside the water bath during moist-heat treatment.

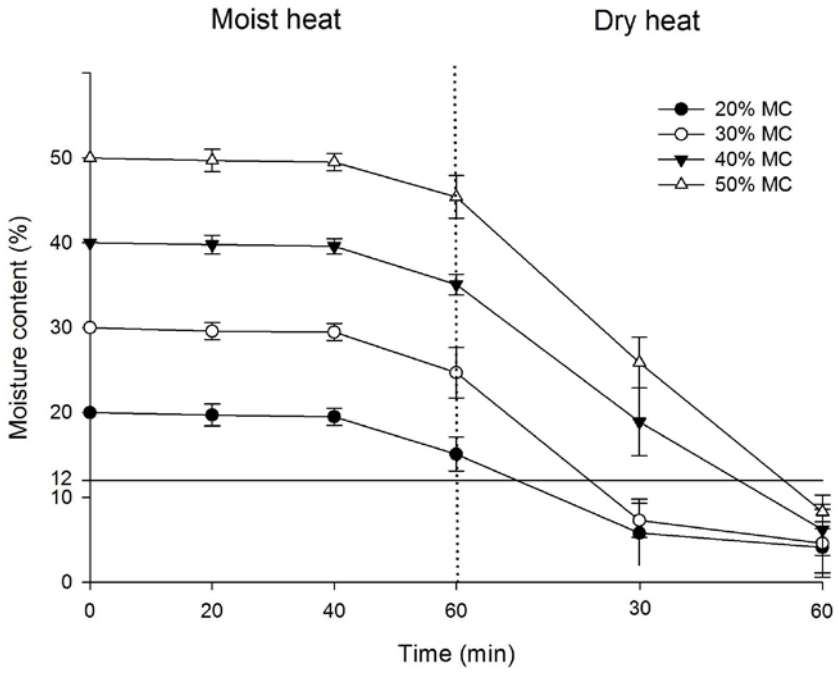

Fig. 2 Change of moisture content (MC) in aged chicken litter during two-step heat treatment. The horizontal solid line represents the target moisture content (<12%) to reach after two-step heat treatment.