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Contribution of Chemical and Physical Factors to Zoonotic Pathogen Inactivation during Chicken Manure Composting

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ABSTRACT

Land application is a common method for disposal of manure and litter that accumulate during poultry production; however, zoonotic pathogens residing in the manure may contaminate either directly or indirectly ready-to-eat produce crops. Aerobic composting of animal manure is a beneficial process treatment that inactivates these pathogens. Although heat is considered to be the primary contributing factor to inactivation, ammonia and volatile acids may also serve antimicrobial roles during composting. This study was designed to determine the relative contributions of chemicals and heat to the inactivation of *Salmonella* and *Listeria monocytogenes* in chicken manure-based compost mixtures formulated to give initial carbon:nitrogen (C:N) ratios of 20:1, 30:1 and 40:1. The different initial C:N ratio formulations of the compost mixtures had no effect on pH or the cumulative heat generated. In general, there was within all compost mixtures an initial decline in pH followed by an increase in pH that coincided with an increase in temperature. Levels of ammonia and volatile acids were higher in compost mixtures formulated to an initial C:N ratio of 20:1 than in other C:N formulations. The inactivation rates of *Salmonella* and *L. monocytogenes* within 20:1 C:N formulations were higher than in other formulations. Regression models derived from the data revealed that volatile acid levels, in addition to heat, played a major role in pathogen inactivation. Therefore, it may be advantageous to formulate compost mixtures containing chicken litter to an initial C:N of 20:1 to take advantage of the antimicrobial activity of volatile acids generated when sub-lethal temperatures occur.

Keywords: manure, litter, composting, chicken, heat, ammonia, volatile acids, pH, *Listeria monocytogenes*, *Salmonella*

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INTRODUCTION

Poultry production in the United States is a major enterprise having had a combined value in broiler, egg, and turkey production of $38 billion in 2012 (USDA, 2012). Although meat and eggs are the major outputs from this enterprise, a substantial amount of manure (generated from layer and turkey operations) and litter (mixture of manure, bedding material, wasted feed, feathers, and soil generated from broiler operations) is also produced. For example, the estimated tons of manure produced from poultry operations in the U.S. in 2007 was 81 million tons (US EPA, 2013). To dispose of this waste, land application has offered the best solution (Moore *et al.*, 1998; Ritz and Merka, 2013). Poultry manure can harbor zoonotic pathogens such as *Salmonella*, *Listeria monocytogenes*, and *Campylobacter* (Chinivasagam *et al.*, 2010; Hutchison *et al.*, 2004, 2005), and if applied to fields growing ready-to-eat produce, these pathogens may contaminate those crops. Once excreted from the animal, pathogen survival is dependent on storage conditions (Goss *et al.*, 2013; Leifert *et al.*, 2008; Ziemer *et al.*, 2010). If left undisturbed, Williams and Benson (1978) determined that *Salmonella* Typhimurium survived for at least 18 months in chicken litter at 11 or 25°C, and 13 days at 38°C. Decimal reduction times for *S.* Typhimurium in poultry manure are not only affected by storage temperature, but also by the type of matrix, being greater in manure slurries compared to manure piles (Himathongkham *et al.*, 2000). According to USDA, only 5% of all U.S. cropland in 2006 was fertilized with manure, with most chicken manure being applied to peanut and cotton fields (MacDonald *et al.*, 2009). Although this mode of disposal would appear to have a minimal food safety risk, natural waterways and irrigation ponds in the Southeastern U.S. have been found contaminated with *Salmonella* and *Campylobacter*, especially after precipitation events (Gu *et al.*, 2013b; Haley *et al.*, 2009; Luo *et al.*, 2013) and likely occurred from pathogen runoff of peanut and cotton fields amended with poultry manure. These water sources are frequently used to irrigate fields growing ready-to-eat produce (Gu *et al.*, 2013a), hence animal manure should be treated to inactivate pathogens prior to land application.

A treatment that is often recommended to inactivate vegetative bacterial pathogens in manures is thermophilic aerobic composting. In this process, manure is mixed with one or more carbon amendments to produce a nutrient-rich environment favorable for the metabolism of thermophilic microorganisms. The major factor responsible for inactivating pathogens in such systems is heat generated by the metabolic activity of these thermophilic microorganisms (Erickson *et al.*, 2010; Wichuk and McCartney, 2007)). As a result, process conditions that are based on time and temperature have been promulgated in regulations or guidelines worldwide (Hogg *et al.*, 2002). For example, guidelines within the U.S. include either a minimum temperature of 55°C for 3 days in aerated static piles or in-vessel systems or 55°C for 15 days in windrow systems (narrow trapezoidal elongated rows) during which time the piles must be turned a minimum of 5 times to ensure that all material is subjected to the necessary thermal conditions (US EPA, 1999).

Although heat is the primary mechanism for inactivating pathogens during aerobic composting, temperature stratification within static piles can result in extended survival of pathogens at the surface as well as extended survival of pathogens at interior sites of piles composted during the winter (Berry *et al.*, 2013; Erickson *et al.*, 2010; Shepherd *et al.*, 2007). In addition, exposure of the pathogen to nonlethal heat or selected moisture conditions could lead to metabolic alterations in the pathogen that makes them more resistant to the thermal conditions encountered during the thermophilic phase of composting (Chen *et al.*, 2013; Shepherd *et al.*, 2010; Singh *et al.*, 2011, 2012). As evidence of this potential activity, *Salmonella*, *Escherichia coli* O157:H7, and *Listeria* survived in poultry manure-based compost piles when exposed to temperatures above 55°C for more than 8 days (Hutchison *et al.*, 2005). Hence, other factors, either chemical or biological, may provide a greater contribution

to pathogen inactivation under those conditions. For example, accumulation of free ammonia in poultry manure has been reported to contribute to inactivation of *S.* Typhimurium and *E. coli* O157:H7 in poultry manure (Himathongkham *et al.*, 2000). Alternatively, volatile acids generated during the early phase of composting in cow manure systems formulated to have an initial carbon:nitrogen (C:N) ratio of 20:1 were suggested to be bactericidal agents effective against *Salmonella* but not *Listeria* (Erickson *et al.*, 2009a,b). Given that there are differences in microbial and raw material composition between cow and chicken manure (De Bertoldi *et al.*, 1987; Lynch, 1987; Wang *et al.*, 2007), it was the objective of this study to determine the relative contributions of heat, volatile acids, and ammonia to the inactivation of *Salmonella* and *L. monocytogenes* in chicken manure-based compost mixtures formulated to C:N ratios ranging from 20:1 to 40:1.

MATERIALS AND METHODS

Pathogen Strains and Their Preparation for Experimental Trials

Five strains of *Listeria monocytogenes* (101M, 12443, F6854, G3982, and H7550) from the culture collection housed at the Center for Food Safety, University of Georgia were used for these studies. In addition, three strains of *Salmonella enterica* serovar Enteritidis (ME-18, H4639, and H3353) and one strain of *S. enterica* serovar Newport (11590) were also used from the culture collection. All strains had been labeled with the green-fluorescent plasmid (GFP), but for *L. monocytogenes* strains, the plasmid also contained an erythromycin-resistant marker, whereas *Salmonella* strains contained an ampicillin-resistant marker. Previously, plasmid stability of these GFP-labeled strains was reported to range from 8 to 52% and 15 to 77% plasmid loss after 20 generations for the *L. monocytogenes* and *Salmonella* strains, respectively (Ma *et al.*, 2011).

To prepare the pathogen strains for challenge studies, frozen cultures of *L. monocytogenes* and *Salmonella* were thawed and individually streaked onto plates containing brain heart infusion agar (Becton Dickinson, Sparks, MD) with 8 µg/mL of erythromycin (BHIA-E) and tryptic soy agar (Difco Laboratories, Detroit, MI) with 100 µg/mL ampicillin (TSA-A), respectively. Following incubation of plates at 37°C for ca. 24 h, individual colonies were removed and subsequently streaked onto a second plate and held at 37°C for an additional 24 h. From this second set of plates, individual colonies of *L. monocytogenes* and *Salmonella* were removed and inoculated into 10 ml of brain heart infusion broth (Becton Dickinson) containing 8 µg/mL erythromycin (BHIB-E) and tryptic soy broth containing 100 µg/mL ampicillin (TSB-A), respectively. These suspensions were incubated for ca. 24 h at 37°C with agitation (150 rpm) before harvesting the bacteria by centrifugation (4,050 x g, 15 min, 4°C). The pelleted cells were washed three times in 0.1% peptone water (Difco) and resedimented by centrifugation before reconstituting in 0.1% peptone water to an optical density at 630 nm of ca. 0.5 that corresponded to a concentration of ca. 10^9 CFU/mL. The five strains of *L. monocytogenes* were then combined in equal proportions to make one 5-strain stock culture mixture, whereas the four strains of *Salmonella* were combined for one 4-strain stock culture mixture. Each of these stock culture mixtures was then diluted 10-fold with deionized water for mixtures of 10^8 CFU/mL that were used to spray chicken litter. Immediately after preparation of the spray mixtures, *L. monocytogenes* and *Salmonella* was enumerated by plating serial dilutions (1:10) on modified oxford medium (Acumedia Manufacturers, Lansing, MI) containing 10 mg/mL buffered colistin methanesulfonate, 20 mg/mL buffered moxalactam solution, and 8 µg/mL erythromycin (MOX-E) and TSA-A, respectively. *Salmonella* colonies emitted a bright green fluorescence when plates were held under a handheld UV light (365 nm) and the fluorescent colonies were counted as *Salmonella*. Fluorescent *L. monocytogenes* colonies were smaller than *Salmonella* colonies and required a Leica MZ16 FA stereo fluorescence microscope (Bannockburn, IL) for visualization and counting.

Compost Ingredients, Preparation, and Experimental Design

Fresh chicken litter was collected from a broiler production facility located in Orchard Hill, GA. Batches were collected at different times for each independent replicate trial. Following transport, the litter was mixed thoroughly and a portion of the litter was removed for compositional analysis. The remainder of the litter was frozen to kill insect eggs and then held at -20°C until which time it was ready to be composted. Wheat straw and cottonseed meal were purchased from a local feed store and served as the major carbon sources for the compost mixtures.

Chicken litter was added to a 28-L sanitized bowl and sprayed manually using a spray bottle with both the *Salmonella* and *L. monocytogenes* inocula for populations approximating 10^7 CFU/g. This inoculated mixture was then mixed with a Hobart mixer (model D320:0.75 h.p.). Wheat straw, cottonseed meal, and water were then added in such quantities that compost mixtures had an initial moisture content of 60% and a C:N ratio of either 20:1, 30:1, or 40:1. Immediately after mixing, the compost mixtures were sampled for chemical and microbiological analysis. The remainder of the compost mixture was then placed in one of three bioreactors.

In this experimental study, three independent trials were conducted wherein each trial consisted of three bioreactor systems containing one compost mixture each of the 20:1, 30:1 and 40:1 C:N ratio mixtures. The compost mixtures were composted for up to 6 days and were sampled on days 1, 2, 3, and 6 to measure microbiological and chemical parameters.

Composting Apparatus and Sampling

Bioreactors (46 cm high x 32 cm diameter) were constructed from PVC plastic pipe. Tightly fitting PVC covers had holes drilled into their center such that the bottom cover hole allowed condensate to drip into an attached bottle and the top cover hole allowed compressed air (155 ml/min) to be delivered to the system. Within the biochamber, a perforated shelf was supported 5 cm above the bottom. Two sampling ports (3 cm diameter) at heights of 6 to 9 cm and 10 to 13 cm above the PVC shelf and a hole (0.5 cm diameter) at a height of 6.5 cm above the shelf for insertion of a thermocouple wire were drilled into the sides of the bioreactors. Bioreactors were housed within a Precision 30 Mechanical Convection incubator (Thermo Fisher Scientific, Waltham, MA) that was maintained at a temperature of 40°C. Trapped air in the incubator was vented to a filtered exhaust system.

Compost material (ca. 5 kg) was placed into each bioreactor after which a type T thermocouple wire was inserted through the small hole to a site designated as the bottom center (16 cm from bioreactor wall). An additional thermocouple was inserted to a depth of 10 cm into the top center of the compost mixture. All thermocouples (two per bioreactor) were connected to a multi-channel HotMux data logger (DCC Corp., Pennsauken, NJ) that was programmed to record temperatures at the 6 locations at 30-min intervals. Cumulative heat > 40°C (degree-days) was calculated as the product of time (days) and temperature (°C above the ambient incubator temperature of 40°C). Oxygen levels in the bioreactor system were measured on all sampling days using a Demista OT-21 oxygen probe (Arlington Heights, IL) prior to removing duplicate samples (25 g) with a sanitized grabbing tool at both the bottom center and top center locations.

Chemical and Microbiological Analyses

All compost ingredients (chicken litter, wheat straw, and cottonseed meal) as well as the initial compost mixtures were analyzed for carbon, nitrogen, and moisture contents. Carbon content was determined on the basis of ash content obtained after combustion of samples at 550°C. The University of Georgia's Soil Testing Laboratory (Athens, Georgia) was used for analysis of nitrogen content via a macro-Kjeldahl method. Moisture levels were based on residual weights of vacuum dried samples.

Ammonia concentrations in compost samples

(5 g) was determined with a phenol-hypochlorite spectrophotometric procedure (Weatherburn, 1967), whereas the Hach spectrophotometric Method 8196 test kit (Loveland, CO) as adapted by Montgomery *et al*. (1962) was used to measure volatile acid concentrations in compost samples. Measurement of pH was made with an Acumet Basic pH meter (Fisher Scientific, Pittsburgh, PA) on compost samples (5 g) dispersed in 250 ml of deionized water.

Salmonella and *L. monocytogenes* were enumerated by direct plate counts (limit of detection was 2 log CFU/g) or detected by selective enrichment culture (limit of detection was 1 log CFU/g). In either case, compost samples (5 g) placed in a Whirl-Pak bag were first pummeled in a Stomacher 400 Circulator (Seward Ltd., West Sussex, UK) for 1 min after adding 45 mL of 0.1% peptone water. Diluted (1:10) aliquots of this homogenate were applied to either TSA-A plates to enumerate *Salmonella* or MOX-E plates to enumerate *L. monocytogenes*. Enrichment cultures of *Salmonella* and *L. monocytogenes* consisted of adding 1 mL of the homogenate to 9 ml of selective enrichment medium (TSB-A or BHIB-E, respectively) and incubating this mixture for 24 h at 37°C. Aliquots of these enriched samples were then streaked onto TSA-A or MOX-E plates to determine the presence or absence of fluorescent *Salmonella* or *L. monocytogenes* colonies, respectively.

Statistical Analyses

The StatGraphics Centurion XVI software, version 16.1.03 (StatPoint Technologies, Inc., Herndon, VA) was used for statistical analysis of the collected data; however, pathogen populations were first converted to logarithmic values prior to conducting these operations. When samples did not yield any colonies during plate count enumeration but did have fluorescent colonies on plates streaked from enrichment cultures, a value of 1.0 log CFU/g, corresponding to the limit of detection by enrichment culture, was assigned to that sample. Otherwise, samples yielding negative results for both plate counts and enrichment cultures were assigned a value of 0.0 log CFU/g. After conversion of enrichment culture data, all data were subjected to general linear models analysis of variance (GLM ANOVA) to determine the significance of experimental variables over all sampling times examined in the study. To differentiate treatments at individual sampling times, the data were subjected to one-way ANOVA and when statistical differences were observed ($P < 0.05$), sample means were differentiated using the least significant difference test. Multiple linear regression analysis was also conducted on data from each sampling day and treatment in an attempt to relate the total pathogen loss in the mixtures to the independent variables of pH, cumulative heat, and concentrations of volatile acids and ammonia.

RESULTS AND DISCUSSION

Chicken litter, collected from broiler houses, was mixed with wheat straw, cottonseed meal, and water in combinations to give mixture treatments varying in their initial C:N ratio. Following analysis of these initial compost mixtures, the C:N ratios that were measured for the 3 independent replicate trials averaged 20.6 ± 1.7, 32.4 ± 2.4, and 43.6 ±1.7, respectively.

Initial moisture contents in the 20:1, 30:1, and 40:1 C:N ratio formulations were 62.7 ± 1.9, 60.8 ± 1.7, and 60.5 ± 2.8%, respectively. Continued monitoring of moisture contents on days 2 and 6 revealed that compost mixtures were generally above 40% moisture during this time and thus aerobic microbial activity would not have been inhibited (Rynk, 1992). Oyxgen concentrations during composting were also well above the 5% level that is considered to limit aerobic microbial activity (Rynk, 1992).

All compost mixtures were initially characterized as slightly alkaline (Table 1). After one day of composting, the pH of all mixtures had decreased from 1.5 to 2.2 units and declines were greater as the C:N ratio of the compost formulation decreased. After this point in time, the pH of all mixtures increased. Overall, there were no significant differences in pH with the different C:N ratio treatments throughout the composting period ($P < 0.05$).

Table 1. pH (mean ± S.D.) in compost mixtures formulated with chicken litter, wheat straw, and cottonseed meal to different initial C:N ratios

	Initial C:N ratio		
Days	20:1	30:1	40:1
0	7.69 ± 0.48 c-e[1]	7.89 ± 0.19 d-f	7.79 ± 0.22 d-f
1	5.50 ± 0.39 a	5.96 ± 1.36 a	6.30 ± 1.37 ab
2	6.47 ± 1.68 a-c	7.52 ± 2.00 d	7.40 ± 1.80 cd
3	8.09 ± 0.95 d-f	7.37 ± 1.48 cd	7.26 ± 1.58 b-d
6	9.00 ± 0.22 f	8.55 ± 0.78 ef	8.12 ± 1.05 d-f

[1] Levels followed by a different letter are significantly different ($P < 0.05$)

Table 2. Volatile acid concentrations (mg/g, mean ± S.D.) in compost mixtures formulated with chicken litter, wheat straw, and cottonseed meal to different initial C:N ratios

	Initial C:N ratio		
Days	20:1	30:1	40:1
0	5.49 ± 2.37 a-c[1]	4.27 ± 1.32 a	3.68 ± 1.32 a
1	11.68 ± 3.52 d-g	12.42 ± 3.26 fg	9.08 ± 4.04 c-e
2	12.08 ± 3.59 e-g	11.34 ± 4.91 d-g	8.79 ± 3.88 b-d
3	13.26 ± 6.88 g	9.76 ± 4.49 d-f	5.79 ± 4.28 ab
6	8.80 ± 4.20 b-d	4.79 ± 2.67 a	4.85 ± 2.99 a

[1] Levels followed by a different letter are significantly different ($P < 0.05$)

Table 3. Ammonia concentrations (µg/g, mean ± S.D.) in compost mixtures formulated with chicken litter, wheat straw, and cottonseed meal to different initial C:N ratios

	Initial C:N ratio		
Days	20:1	30:1	40:1
0	131.6 ± 24.7 a-c[1]	114.2 ± 23.8 ab	99.7 ± 30.2 ab
1	218.6 ± 94.3 c-e	215.9 ± 74.2 c-e	132.5 ± 23.4 b
2	271.0 ± 98.9 e	217.0 ± 56.4 c-e	145.3 ± 19.9 bc
3	379.4 ± 158.6 f	185.8 ± 47.1 b-d	123.9 ± 73.8 ab
6	246.6 ± 37.9 de	168.8 ± 216.7 bc	54.3 ± 57.3 a

[1] Levels followed by a different letter are significantly different ($P < 0.05$)

These results were in contrast to those that were observed when compost mixtures were formulated to different C:N ratios with dairy manure as the nitrogen source (Erickson *et al*., 2009a, b). In those studies, compost mixtures formulated to a C:N ratio of 40:1 did not decline in pH during the first day of composting.

Volatile acids, including acetate, butyrate, and propionate, are produced during the early phases of aerobic composting and digestion (Beck-Friis *et al*., 2003; Ugwuanyi *et al*., 2005a,b) and may be potential contributors to the pH declines observed in this study during the first day of composting of chicken litter. This suggestion was corroborated by the observed increase in volatile acid levels that occurred in the chicken litter compost mixtures during the first day (Table 2). The greatest increase in volatile acid concentrations was observed in the 20:1 C:N compost mixtures, whereas the least increase occurred in the 40:1 C:N compost mixture. Furthermore, as composting continued, volatile acid levels declined in all compost mixtures, but the decrease was slower in 20:1 C:N compost mixtures than in the 40:1 C:N compost mixtures. Generally, facultative anaerobic microorganisms produce volatile acids in response to low oxygen concentrations (Brinton, 1998); however, it would appear that the nutrient conditions provided in the 20:1 C:N compost mixtures were more conducive than the other compost mixture formulations for generating such compounds.

Ammonia is another common byproduct produced during the degradation of chicken manure or chicken litter (Bush *et al*., 2007; Himathongkham *et al*., 2000). There were significant differences in the ammonia concentrations of the different formulations of the chicken compost mixtures (Table 3). Specifically, when all sampling days were taken into account, the 20:1 C:N ratio compost mixture had the highest ammonia concentrations, whereas the lowest levels were in the 40:1 C:N ratio compost mixtures ($P < 0.05$). In addition, during the composting process, the ammonia concentrations were continuously shifting, with maximal levels found in the 20:1 C:N mixtures on day 3, whereas maximal levels in

Table 4. Cumulative metabolic heat > 40°C (degree-days[1], mean ± S.D.)during composting of mixtures formulated with chicken litter, wheat straw, and cottonseed meal to different initial C:N ratios

	Initial C:N ratio		
Days	20:1	30:1	40:1
1	2.90 ± 2.00 a[2]	2.84 ± 2.36 a	3.48 ± 3.17 a
2	6.42 ± 4.53 ab	9.14 ± 10.67 a-c	9.16 ± 7.79 a-c
3	15.96 ± 10.02 cd	16.72 ± 16.09 b-d	19.05 ± 11.17 d

[1] Accumulated product of temperature (°C above the ambient incubator temperature of 40°C) and composting time (days)

[2] Levels followed by a different letter are significantly different (P < 0.05)

Table 5. Fate of *Salmonella* and *L. monocytogenes* populations (log CFU/g, mean ± S.D.) in compost mixtures formulated with chicken litter, wheat straw, and cottonseed meal to different initial C:N ratios

	Salmonella			*L. monocytogenes*		
Day	20:1 C:N	30:1 C:N	40:1 C:N	20:1 C:N	30:1 C:N	40:1 C:N
0	7.43 ± 0.25 f	7.22 ± 0.26 f	7.38 ± 0.44 f	7.53 ± 0.17 g	7.21 ± 0.12 g	7.14 ± 0.13 g
1	3.64 ± 0.83 d	3.73 ± 1.96 d	5.31 ± 1.16 e	3.49 ± 1.22 e	3.79 ± 1.09 e	4.90 ± 0.97 f
2	1.58 ± 1.82 bc	0.48 ± 0.89 a	2.57 ± 2.33 c	1.21 ± 1.48 c	0.99 ± 0.88 bc	2.40 ± 1.83 d
3	0.28 ± 0.66 a	0.28 ± 0.66 a	1.82 ± 2.42 c	0.00 ± 0.00 a	0.28 ± 0.66 ab	1.12 ± 1.05 c
6	0.00 ± 0.00 a	0.00 ± 0.00 a	0.57 ± 0.84 ab	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a

[1] Populations for each pathogen followed by a different letter are significantly different (P < 0.05).

both the 30:1 and 40:1 C:N mixtures were detected on day 2. The increased generation of both ammonia and volatile acids in compost mixtures initially formulated to low C:N ratios agrees with the models presented by Delgado-Rodríguez *et al.* (2010) that demonstrated a higher level of volatile compounds present during municipal solid waste composting at low C:N ratios.

Temperatures during composting of the mixtures were monitored throughout the period when samples were collected from the bioreactors. To assess the cumulative heat exposure above the ambient incubator temperature of 40°C, time-temperature curves were integrated using 40°C as the baseline. Results for the first 3 days of composting, expressed as cumulative heat > 40°C (degree days), are presented in Table 4 and, although heat generation in compost mixtures was slightly greater as the C:N ratio increased, it was not significantly different ($P > 0.05$). Heat generation within each bioreactor was fairly homogeneous, as location was not a significant factor affecting the cumulative levels ($P > 0.05$). In contrast, over the three independent replicate trials, the level of heat accumulated in the compost mixtures was significantly different from each other ($P < 0.05$). As the manure source for each of these independent trials was collected at separate times from the broiler houses, a plausible explanation is that the chicken litter had been collected in the houses at different periods of time before being removed for composting. Aged manure used in composting mixtures produces less heat than fresh manure (Berry *et al.*, 2013; Li *et al.*, 2008). Such variability in manure age and subsequent variability in heat generation in this study would likely have contributed to an inability to detect a significant effect of C:N ratio on heat generation. A similar situation was also likely responsible for the inconsistent response of heat generation in compost mixtures formulated to different C:N ratios when using dairy manure (Erickson *et al.*, 2009b). In that study, no statistical differences occurred in the heat generated for the different C:N formulations in the bioreactor trials inoculated with *E. coli* O157:H7, whereas in bioreactor trials inoculated with *L. monocytogenes*, 20:1 formulations were statistically different for the 30:1 C:N formulations.

During composting of chicken litter with different C:N ratio mixtures, pathogen levels were monitored (Table 5). Using ANOVA on data collected shortly after composting was initiated (days 1 and 2 only), it was revealed that the C:N ratio had a significant effect on inactivation of *L. monocytogenes* and *Salmonella* ($P < 0.05$). For both pathogens, the levels of the pathogen were higher in mixtures formulated to a C:N ratio of 40:1 than in those mixtures formulated to either 20:1 or 30:1. Slower inactivation had been observed previously for *Salmonella* in dairy manure compost mixtures of formulations having a C:N ratio of 40:1 compared to ratios of 20:1 and 30:1 (Erickson, 2009a), whereas the C:N ratio in dairy manure formulations did not affect the inactivation of *L. monocytogenes* (Erickson *et al.*, 2009b). Pathogen inactivation, however, was not log-linear, but was characterized as biphasic. Hence, to determine if the C:N treatment affected inactivation during tailing, the number of days to complete inactivation of the pathogen was recorded for each replicate trial. For *Salmonella*, the days to complete inactivation ranged from 2 to 4, 2 to 5, and 3 to 8 for 20:1, 30:1, and 40:1 C:N formulations, respectively, whereas the days to complete inactivation of *L. monocytogenes* ranged from 2 to 3, 2 to 4, and 4 to 5, respectively. Given that only three values for each treatment were available, ANOVA applied to the days to inactivation data failed to reveal any significant effect by the C:N ratio of the mixture for either pathogen ($P > 0.05$). Despite this negative response, there is a trend of increasing days to inactivation with an increasing initial C:N ratio of the compost mixture and if explored in the future with a larger number of trial replicates, could prove to be significant.

Comparison of *Salmonella* and *L. monocytogenes* responses in the composting mixtures revealed no significant differences in the rate of inactivation or days to inactivation (Table 5, $P > 0.05$). The similarity in responses contrast to those reported for composting of rural sewage sludge with straw (Pourcher *et al.*, 2005) and composting of swine manure (Grewal *et al.*, 2007), in which *L. monocytogenes* persisted for longer periods of time than *Salmonella*. Dif-

ferences in comparative pathogen response in this study and others may have arisen due the different isolates used or to the different formulations used for composting.

To understand the contribution of potential chemical and physical factors to pathogen losses during composting on any one sampling day, models were derived using backward stepwise regression. Both cumulative heat and levels of volatile acids were factors included in those models, described below, and explained 19.7% and 28.9 % of the variability in the data for *Salmonella* and *L. monocytogenes*, respectively.

Salmonella losses = 1.455 + (0.121*cumulative heat>40°C) + (0.221* volatile acid concentration)
(P = 0.0123)

L. monocytogenes losses = 0.076 + (0.215*cumulative heat>40°C) + (0.317* volatile acid concentration)
(P = 0.0018)

These models reveal that volatile acids, in addition to heat, have a bactericidal role in chicken litter compost mixtures, particularly in those formulations (i.e. 20:1 C:N compost mixtures) in which high concentrations of volatile acids are produced.

CONCLUSIONS

In summary, chicken litter was mixed with wheat straw and cottonseed meal to give formulations having initial C:N ratios of 20:1, 30:1 or 40:1. Although the pH decreased in all formulations during the first day of composting, the C:N ratio of the formulation did not have a significant effect on pH ($P > 0.05$) nor did it have a significant effect on the cumulated heat generated in the mixtures during composting ($P > 0.05$). In contrast, the different C:N formulations did have a significant effect on ammonia concentrations and volatile acids produced during composting, with the greatest amounts of these antimicrobials being generated in the 20:1 C:N compost mixtures and the least in the 40:1 C:N compost mixtures ($P < 0.05$). Moreover, in the 20:1 C:N compost mixtures, the inactivation rates of both *Salmonella* and *L. monocytogenes* were higher as well as the days required to achieve complete inactivation were in general sooner, than in compost mixtures formulated to either 30:1 or 40:1. Multiple linear regression models that were derived from fitting pathogen losses to cumulative heat and volatile acid levels were significant and explained 20 to 29% of the variability in the data. Hence, in conditions where heat may be insufficient to inactivate pathogens (winter composting or at the surface of unturned static compost piles), it may be advantageous to formulate the initial C:N ratio of chicken litter compost mixtures to values approaching 20:1, as higher volatile acid concentrations in these mixtures provide additional antimicrobial activity.

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