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Applied Research Note: Survival of *Escherichia coli* and temperature development during composting of chicken manure with a typically low carbon/nitrogen ratio and moisture content

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Primary Audience: Researchers, Broiler Producers, Veterinarians

SUMMARY

The presence of pathogens, e.g., *Escherichia coli* ***(E. coli)***, in chicken manure can potentially lead to serious infections and foodborne diseases when spread on land as organic fertilizer. Therefore, it is essential to inactivate these bacteria before land application. The aim of the present study was to determine the survival of *E. coli* and the temperature development in compost piles during composting of chicken manure with a typically low carbon/nitrogen (**C/N**) ratio and moisture content (**MC**). In a summer and winter trial, chicken manure piles were stacked in 1) uncovered static piles, 2) covered static piles, and 3) periodically turned piles. Samples were inoculated with a nonpathogenic *E. coli* strain at levels of 10^7 cfu/g and placed at subsurface and center locations of the piles. Within 24 h, *E. coli* were undetectable by direct count in all piles and at all sample locations. By d 28, all samples were also negative for *E. coli* by enrichment. Despite the suboptimal composting conditions with an initial C/N ratio of 10:1 and an MC below 40%, temperatures within all piles mainly exceeded 50˚C within the first 24 h. Statistical analyses showed that the sample location and the total hours at temperatures ≥50 and 55˚C in the piles had significant influences on the survival of *E. coli* in the chicken manure compost. The season and manure treatment method had no significant effects on the presence of *E. coli*.

Key words: chicken manure, composting, *Escherichia coli*, inactivation, temperature development

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DESCRIPTION OF PROBLEM

Escherichia coli ***(E. coli)*** are often found in farm animals, manure, and farm surroundings (Chen and Jiang, 2014; Blaak et al., 2015). These bacteria can be a threat to human and animal health since *E. coli* strains have the potential to cause both intestinal and extraintestinal infections. Serious gastrointestinal infections caused by Shiga toxin-producing *E. coli* (**STEC**) were seen during some large foodborne outbreaks in recent years (EFSA and ECDC, 2021). Furthermore, even antibiotic-resistant extended-spectrum β-lactamase (**ESBL**)-producing *E. coli* were detected at a

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high prevalence, in particular on European broiler farms (Blaak et al., 2015). Thus, using potentially contaminated chicken manure as organic fertilizer has been critically discussed as a source of contamination for vegetables and fruit, especially since studies have shown the prolonged survival of *E. coli* in manure (Chen and Jiang, 2014).

Appropriate manure treatment methods are necessary to reduce these bacteria before manure is applied to land. Composting is known to reduce *E. coli* effectively when certain guidelines are followed (Erickson et al., 2010; Wilkinson et al., 2011; Chen and Jiang, 2014). Hereby, temperature is considered to be the main inactivation factor for *E. coli* together with a sufficient exposure time (Chen and Jiang, 2014). However, achieving high temperatures during composting depends on further factors such as the carbon/nitrogen (**C/N**) ratio, moisture content (**MC**), substrate availability, microbial activity and oxygen level during the aerobic process (Thomas et al., 2020). Composting material should generally have a C/N ratio between 20:1 and 40:1 and an MC between 40 and 65% to achieve high microbial activity and thus high temperatures (Rynk, 1992). Several studies reported rapid reductions in pathogens during composting in static and turned piles (Erickson et al., 2010; Patel et al., 2015). However, there are also numerous studies showing that bacteria could survive for prolonged periods in manure compost, especially at subsurface and surface locations and other areas of the piles where only lower temperatures were achieved or when winter composting was performed (Erickson et al., 2010; Berry et al., 2013). Therefore, covers on compost could help increase temperatures in the outer area of compost piles and protect against major rainfall events; turning piles can accelerate the temperature increase through aeration of the piles and the bacterial inactivation by mixing particles from the outside into the hotter inside (Patel et al., 2015).

However, it is a common procedure in chicken manure waste management to only store chicken manure in piles behind barns, in halls or silos until used as fertilizer on fields. In addition, chicken manure often has a C/N ratio below 20:1 and an MC below 40% (Wilkinson et al., 2011; Thomas et al., 2020). These facts can lead to reduced microbial activity and low temperatures in chicken manure compost and therefore to potentially prolonged survival times of *E. coli*. To date, only little is known about the respective influence of various treatment methods for chicken manure—especially under suboptimal composting conditions—on the survival of *E. coli* during different seasons. In addition, there is little evidence of the differences in temperature profiles during chicken manure composting with different methods, which is essential for the inactivation of *E. coli*. However, a better understanding of the survival of *E. coli* and the temperature development during different treatments of chicken manure under field conditions is essential to adjust and improve current composting procedures in terms of sanitization. Therefore, the objective of this study was to determine the survival of a nonpathogenic *E. coli* strain and the temperature development in compost piles during composting of chicken manure in 1) uncovered static piles, 2) covered static piles, and 3) periodically turned piles at subsurface and center locations during summer and winter.

MATERIALS AND METHODS

Study Design

Approximately 15 tons of chicken manure was collected from a broiler farm in Germany on the day when the animals and manure were removed from the barns and was then transported to the test grounds in Potsdam, Germany. Both the summer and winter trials started 1 d later. The manure consisted of chicken feces and wood pellets as bedding material. On each starting day, 15 subsurface samples and 15 center samples were randomly collected from each side of the original chicken manure pile to determine the initial number of *E. coli*, the initial C/N ratio and the initial MC in the substrate. Trials were conducted from July to August 2018 and from February to March 2019. For each trial, 3 compost piles, each with a length of 5.5 m, a width of 3 m, and a height of 1.5 m, were stacked on even, impermeable ground at the test grounds and divided into 3 independent sectors.

A nonpathogenic *E. coli* strain (*E. coli* DSM 1116) was provided from the German Collection of Microorganisms and Cell Cultures GmbH (DSMZ, Braunschweig, Germany) and was used as the test strain for inoculation of the compost mixtures. Frozen cultures of the strain were incubated overnight in 5 mL Luria−Bertani (**LB**) broth (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) at 37˚C on a shaker (HSM 10, Andreas Hettich GmbH & Co.KG, Tuttlingen, Germany). Then, *E. coli* suspensions with concentrations of 10^9 cfu/mL were prepared by incubating 1 mL of the overnight suspension in 100 mL LB broth for additional 6 h at 37˚C on a shaker.

On each starting day, 10 kg of chicken manure was placed in a sanitized mixing bowl and sprayed with the *E. coli* suspension to obtain initial *E. coli* levels of 10^7 cfu/g in the mixture. After mixing by hand and 30 min of resting, 5 samples were taken for initial microbiological analyses. Then, 50 g samples of this mixture were added to bags that were permeable to water, gas, and light. These bags were then placed in a larger bag of the same consistency with 200 g of original chicken manure for later chemical analyses. Then, the bags were placed in a firm net bag and attached to colored strings to enable identification of the samples. The sample bags were randomly arranged within each sector in the center of each chicken manure pile at a height of 0.75 m above the ground and 0.5 m from the surface and at 0.5 m height and only 0.15 m below the surface for the subsurface locations. For a period of 28 d, pile 1 was treated as an uncovered static pile, and pile 2 was additionally covered with a gas-permeable compost fleece (Toptex 200 g/m^2, TenCate Industrial Fabrics, Linz, Austria). Pile 3 was not covered but turned at d 2, 7, 14, and 21, and all subsurface samples were then placed in the center of the pile and vice versa. Sampling was conducted on d 0, 1, 2, 4, 7, 14, 21, and 28. For each sampling day, 3 subsurface bags and 3 center bags of each pile were removed.

Microbiological and Chemical Analysis and Temperature Measurement

For microbiological analyses, 25 g samples were mixed immediately after collection in 225 mL LB broth using a stomacher (Seward Stomacher 400 Circulator, Worthing, UK) at 260 rpm for 2 min. Then, a serial dilution was performed in phosphate-buffered saline (**PBS**, pH = 7.3−7.5) (VWR International GmbH, Darmstadt, Germany). Each dilution was spread-plated on MacConkey No. 3 agar plates (Oxoid Deutschland GmbH, Wesel, Germany) and incubated for 24 h at 37˚C. In case no direct *E. coli* count was possible, the 1:10 dilutions prepared in LB broth were incubated for 24 h at 37˚C, and a loop-full of this enrichment was streaked on MacConkey No. 3 agar plates to determine if the sample was still positive for *E. coli*. Characteristic *E. coli* colonies were randomly chosen throughout the experiment to confirm the species using matrix-assisted laser desorption time of flight mass spectrometry (**MALDI-TOF-MS**, AXIMA Confidence, Shimadzu Deutschland GmbH, Duisburg, Germany).

Samples for the analyses of the MC and the C/N ratio were frozen at −25˚C and analyzed later as described previously (Thomas et al., 2020). Inside each of the 18 sample bags from the last sample day, a temperature data logger (Tinytag Plus 2, TGP-4017, Gemini Data Loggers Ltd, Chichester, UK) measured the temperature every hour. In addition, weather data during the trials were obtained from the weather station at the test grounds.

Statistical Analyses

E. coli concentrations were converted to logarithmic values before statistical analysis. The value “99” was used for bacterial concentrations below the detection limit. The value “0” was used when the samples were negative by enrichment. The 3 sectors within each pile were treated as replicates. Means and standard deviations of the data were determined. The effects of treatment method, season, and sample location on the log cfu were tested with a linear analysis of covariance (**ANCOVA**). The effect of the total hours at temperatures ≥50 and ≥55˚C in the piles on the presence of *E. coli* was tested with a generalized linear model with *E. coli* being a binary response variable. The MIXED and the GLIMMIX procedure of the SAS 9.4 software package (SAS Institute Inc., Cary, NC) was used for the analyses.

RESULTS AND DISCUSSION

Chemical Conditions Within the Compost Piles

The initial C/N ratio of the chicken manure was 9.11 ± 0.36 and 11.35 ± 0.36, and the initial MC was 35.70 ± 1.95% and 26.01 ± 2.16% in summer and winter, respectively. In summer, the C/N ratios at the end of the experiment ranged from 8.14 to 8.90. The final MC ranged from 28.18 to 31.93%. In winter, the final C/N ratios ranged from 9.92 to 11.66, and the final MC ranged from 21.51 to 30.78% with the lowest C/N ratios and the highest MCs in the turned pile.

Survival of E. coli *During Composting of Chicken Manure*

E. coli numbers in the original chicken manure substrate used for preparation ranged from 0 to 10^3 cfu/g in summer and were approximately 10^3 cfu/g in winter. Table 1 shows the numbers of *E. coli* during both composting trials. The mean initial levels of *E. coli* in the sample bags after spiking were 6.64 ± 0.12 cfu/g and 6.48 ± 0.26 cfu/g in summer and winter, respectively. Within 24 h, the number of *E. coli* at all sample locations was reduced to below the detection limit (<100 cfu/g) by direct count in both trials. In winter, however, *E. coli* were found again by direct count in one of the subsurface samples in the covered static pile and in the turned pile on d 2. This result could be due to a lower microbial activity and therefore cold spots within the piles. Possible reasons for the low microbial activity in these areas are a lack of oxygen or an unfavorable C/N ratio or MC. By d 28 at the latest, all samples of both trials were also negative by enrichment. These findings are in agreement with the results of previous laboratory experiments, where ESBL-producing *E. coli* were below the detection limit within 2 d when the C/N ratio of chicken manure compost was 10:1 (Thomas et al., 2020). In another field study, Erickson et al. (2010) also found no *E. coli* O157:H7 by direct count in subsurface samples of static chicken manure compost piles after 2 d, regardless of season. Wilkinson et al. (2011), on the other hand, found no significant change in *E. coli* numbers in the outer layers of both static and turned piles consisting of chicken manure over a 12-wk trial period. This difference in results may be due to a higher MC because of rainfall and temperatures below 45° C in the piles in this study.

Statistical analysis in the present study showed a significant influence of sample location on the reduction in *E. coli* during composting, whereas season and treatment method had no significant effects. Siller et al. (2020) also found a faster reduction in ESBL-producing *E. coli* and total *E. coli* for deep samples than for surface samples during a 96 h on farm storage of chicken manure in summer and winter. In addition, a seasonal influence was observed as well as an increase in *E. coli* on the surface of the piles in summer. The latter was not found in subsurface samples in the present study. The difference in results regarding the seasonal influence may be due to different environmental conditions during the experiments, as Siller et al. (2020) reported lower ambient temperatures than those in the present study. However, the results of that study suggest that pile turning can help reduce the risk of survival and regrowth of bacteria in outer regions. In addition, Berry et al. (2013) showed that in comparison to uncovered piles, covering cow manure compost piles resulted in faster *E. coli* O157:H7 reductions compared to uncovered piles. In the present study, however, no difference was seen between the covered and uncovered static piles and the turned pile, most likely due to the very fast reduction in *E. coli* in all 3 piles. The fast reductions in the subsurface areas may also have been due to desiccation, which played a role during laboratory experiments even though temperatures were not that high (Thomas et al., 2020). In addition, Thomas et al. (2020) also showed that a low C/N ratio can contribute to the reduction in *E. coli*, and this scenario might also be a reason for the fast reductions seen in the present study compared to those observed when composting other manure types.

Although the present study showed no significant benefit of turning or covering a pile, both treatment methods may become essential when the environmental conditions are different from those in the present study, e.g., more

Table 1. Detection of *E. coli* in subsurface and center samples and associated total hours (h_{total}) at a temperature ≥50°C and ≥55°C in uncovered static (pile 1), covered static (pile 2), and turned (pile 3) piles of chicken manure composted in summer and winter.

		E. coli (log_{10} cfu/g [a] or positive/negative by enrichment, n = 3)			h_{total} [b]					
					≥50˚C			≥55˚C		
Day	Location	Pile 1	Pile 2	Pile 3	Pile 1	Pile 2	Pile 3	Pile 1	Pile 2	Pile 3
Summer										
0	Subsurface		6.64 ± 0.12		0	0	0	0	0	0
	Center				0	0	0	0	0	0
0.33 (8 h)	Subsurface		*Not tested*		1	0	0	0	0	0
	Center				0	0	0	0	0	0
1	Subsurface	+ - -	+ + -	+ - -	17	10	14	0	0	0
	Center	+ + -	+ + +	+ - -	12	13	10	0	0	0
2	Subsurface	+ + +	+ - -	+ + +	20	10	30	0	0	0
	Center	- - -	+ + -	+ + -	36	37	34	0	9	0
4	Subsurface	+ + -	+ - -	+ - -	58	49	63	11	0	10
	Center	- - -	+ - -	- - -	84	85	47	43	57	0
7	Subsurface	- - -	+ - -	- - -	130	121	135	83	0	74
	Center	- - -	- - -	- - -	156	157	119	115	129	58
14	Subsurface	- - -	+ + -	- - -	298	148	136	206	0	74
	Center	- - -	- - -	- - -	324	325	273	283	297	201
21	Subsurface	- - -	+ - -	- - -	390	148	296	206	0	122
	Center	- - -	- - -	- - -	492	493	425	451	417	339
28	Subsurface	- - -	- - -	- - -	390	148	297	206	0	122
	Center	- - -	- - -	- - -	660	591	566	509	417	463
Winter										
0	Subsurface		6.48 ± 0.26		0	0	0	0	0	0
	Center				0	0	0	0	0	0
0.33 (8 h)	Subsurface	+++	3.09 ± 1.88	2.95 ± 1.28	0	0	0	0	0	0
	Center	3.30 ± 1.03	3.05 ± 0.73	2.14 ± 0.25	0	0	0	0	0	0
1	Subsurface	+ - -	+ + -	+ + -	0	12	0	0	0	0
	Center	- - -	+ - -	- - -	10	4	9	4	0	2
2	Subsurface	- - -	3.34 + -	2.43 - -	0	19	0	0	0	0
	Center	+ - -	- - -	- - -	34	28	33	28	22	26
4	Subsurface	+ - -	- - -	- - -	0	19	1	0	0	1
	Center	- - -	- - -	- - -	82	76	63	76	70	45
7	Subsurface	- - -	- - -	+ + -	0	19	1	0	0	1
	Center	+ - -	- - -	- - -	154	148	135	97	142	117
14	Subsurface	- - -	+ - -	- - -	0	19	150	0	0	142
	Center	- - -	- - -	- - -	169	228	283	97	166	258
21	Subsurface	+ - -	- - -	- - -	0	19	263	0	0	221
	Center	- - -	- - -	- - -	169	228	434	97	166	407
28	Subsurface	- - -	- - -	- - -	0	19	383	0	0	317
	Center	- - -	- - -	- - -	169	228	581	97	166	549

[a] log_{10} cfu/g is the mean ± standard deviation.
[b] Total hours.

precipitation or lower temperatures during a treatment or a difference in the substrate, in the C/N ratio or in the microbial composition of the manure.

Temperature Profile During Composting

Figure 1 shows the temperature profile within the compost piles, the ambient temperature and the precipitation during the trial periods. Temperatures within the piles increased rapidly within the first 24 h to above 50˚C in all pile locations in winter and summer, except for the subsurface location of the turned pile in winter. In both trials, in comparison to the subsurface areas, the center locations reached higher temperatures and maintained a longer high temperature phase in all piles with greater differences between both locations in the winter trial, likely due to the lower ambient

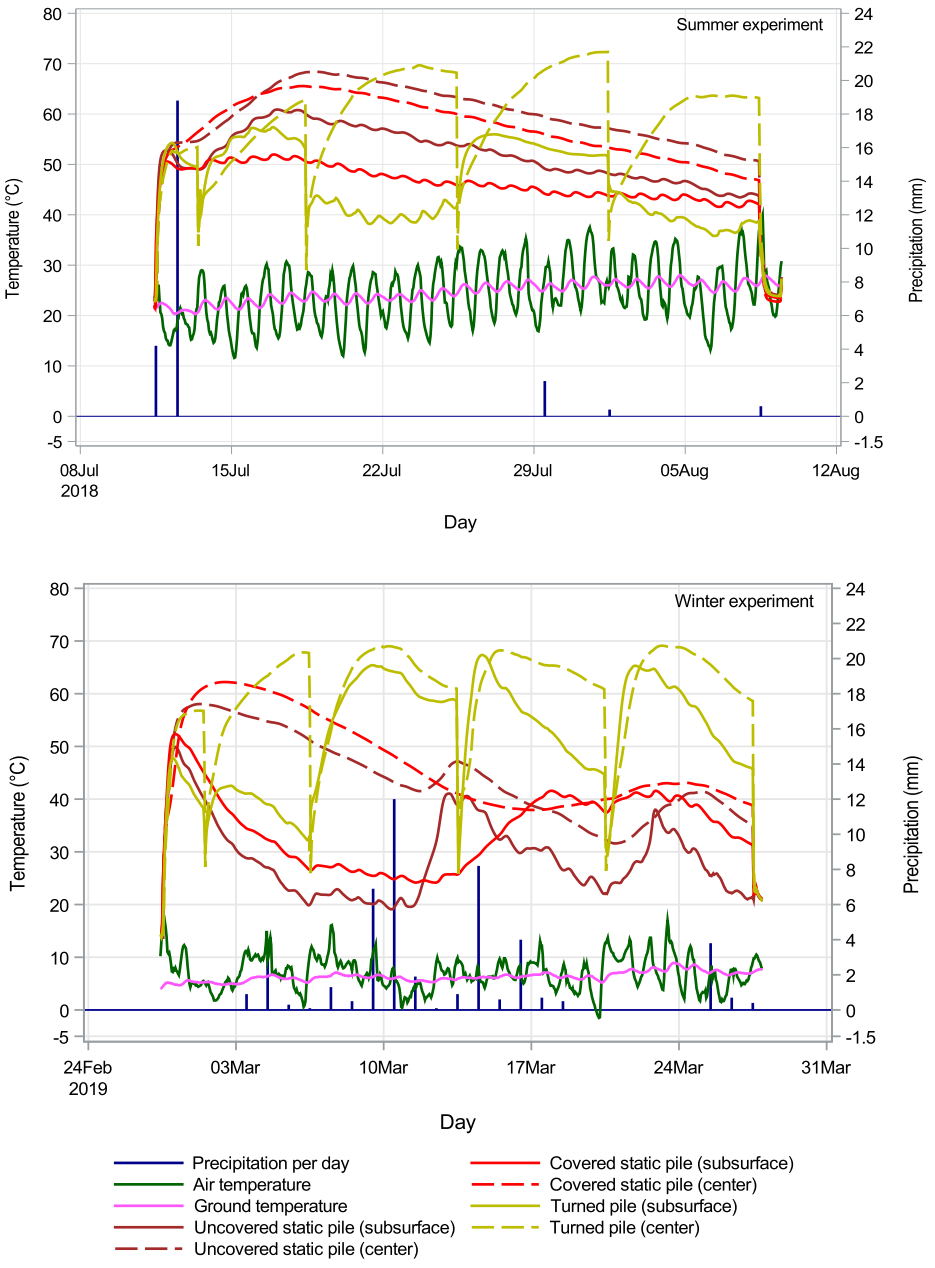

Figure 1. Weather conditions and in-pile temperatures during composting of uncovered static, covered static and turned chicken manure piles during summer and winter.

temperatures. This result is in agreement with those of Patel et al. (2015), who also found higher temperatures at internal loci than at interface loci of cow manure composts. In the summer trials, temperatures reached 70˚C and higher in all center locations, and the turned pile remained at temperatures above 60˚C until d 28, probably as a result of effective turning. In addition, the subsurface locations remained almost above 40˚C until the end of the trial. In winter, the highest temperatures were again reached in the turned pile in the center locations. In the uncovered and covered static piles, temperatures at both locations dropped faster during the winter trial than during the summer trial. However, throughout both trials, the

temperature at the subsurface and center locations remained above the ambient air temperature. The temperatures achieved in both trials were comparable to those measured in other field studies. Wilkinson et al. (2011), for example, observed temperatures above 65˚C in static and turned chicken manure windrows with consistently higher temperatures in the turned windrow. Berry et al. (2013) also found higher temperatures in cow manure compost piles that were periodically turned than in static piles.

When comparing the reduction in *E. coli* with the pile temperature, especially the first 24 h, during which temperatures of 50˚C and more were reached, were critical to the inactivation of *E. coli* in the present study. Temperature is known to be a main inactivation factor, and several laboratory experiments demonstrated that temperatures above either 50 or 55˚C were sufficient to inactivate *E. coli* in chicken manure (Wilkinson et al., 2011; Thomas et al., 2020). Therefore, the total hours ($\mathbf{h_{total}}$) at temperatures ≥50˚C and ≥55˚C at the sample locations were calculated (Table 1). In summer, the highest h_{total} values were reached in the center locations of the uncovered static pile with 660 and 509 h, respectively. In winter, the center locations of the turned pile showed the highest h_{total} with 581 and 549 h, respectively. However, since 55˚C was mainly not reached before d 2 or 4 of the experiment, 50˚C seemed to be sufficient for inactivation of *E. coli* in chicken manure.

Chicken manure is a substrate with a typically low MC and C/N ratio, both of which are too low for optimal composting results (Rynk, 1992). However, as the present study showed, these characteristics still led to rapid and high-temperature development within the piles, even though composting management was not ideal. Since chicken manure is often stored behind barns or only minimally managed, it is important that temperatures still reach levels that inactivate bacteria such as *E. coli*.

Statistical analyses showed that season and sample location both significantly influenced $h_{total} \geq 50$ and 55˚C. In summer and in the center locations, $h_{total} \geq 50$ and 55˚C was higher than during winter and in the subsurface locations. In addition, both $h_{total} \geq 50$ and 55˚C had a significant influence on the probability of finding *E. coli* in the sample. In winter, in comparison to the other 2 pile types, the turned pile also led to significantly more h at ≥50 and 55˚C. Considering the rather mild winter during the trial with the daily mean ambient temperatures ranging from 3.4 to 10.6˚C, the differences in the results could be even more dominant when ambient temperatures are lower. Thus, turning might become essential to sufficient composting processes.

In conclusion, the present study examined the survival of *E. coli* and the temperature development in chicken manure compost piles using 3 different composting treatment methods. Composting chicken manure with typically low C/N ratio and MC in static or turned piles effectively reduced the number of *E. coli* to below the detection limit within 24 h and to levels undetectable by enrichment within 28 d in summer and winter. Sample location and an $h_{total} \geq 50$ and 55˚C had a significant influence on the survival of *E. coli*, whereas treatment method and season had no significant effect. However, to increase the likelihood that all parts of the compost are exposed to high temperatures, to accelerate temperatures and to achieve sufficient composting of the material, periodic turning should be considered, especially in winter. The study is a valuable addition to laboratory studies, showing even faster reductions in *E. coli* and higher temperature profiles (Thomas et al., 2020). The results of the study also allow a better understanding of the temperature development within chicken manure compost piles, which helps to evaluate the potential of composting in terms of sanitization for other bacterial groups.

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DISCLOSURES

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