Journal of Animal Prod., 37, https://rpa.reduc.edu.cu/index.php/rpa/article/view/e280

Animal Health

Original

Farm-Scale Composting: Inferred Inactivation of Selected Avian Pathogens

Magdiel Torres Villar *, Yandy Abreu Jorge,**, Beatriz Delgado-Hernández **, Damarys de las Nieves Montano Valle **, Pastor Alfonso Zamora **, Teresita de Jesús Quesada ***

*Faculty of Veterinary Medicine, Agrarian University of Havana.
**National Center for Agricultural Health (CENSA).
***National Center for Animal Health (CENASA)
Correspondence: magdietv17@gmail.com

Received: November 2025; Accepted: November 2025; Published: January 2026.

ABSTRACT

Background: Global poultry production generates large volumes of organic waste, and safe management of these residues is crucial, particularly during disease outbreaks. Composting emerges as a circular-economy technology for litter valorization, with temperature being a key factor for pathogen inactivation. **Aim.** To estimate the effectiveness of farm-scale composting for inactivating selected avian pathogens by comparing thermotolerance values reported in the literature with the temperature kinetics observed in large compost piles. **Materials and Methods:** Temperature was monitored in two compost piles (32–300 m³ each) of rice-hull duck litter during the first 240 hours. Temperature measurements were taken at the base, intermediate level, and top of each pile. Inactivation was inferred for nine pathogens (viruses, bacteria, parasites) by contrasting the measured thermal kinetics with reported thermotolerance thresholds. Data analysis included descriptive statistics and Factorial Analysis of Mixed Data (FAMD). **Results:** Mean temperature at the intermediate level was significantly higher (60–68 °C) than at the base and top (38–47 °C). Mean pile temperature exceeded 50 °C continuously for the first 144 hours. FAMD revealed a spatial thermal gradient, associating the intermediate position with the highest and most stable temperatures. **Conclusions:** The temperature kinetics observed in large compost piles permit inference of consistent inactivation of avian pathogens. The results highlight spatial heterogeneity in temperature and suggest optimal timing for turning operations, thereby improving process efficiency and biosecurity. **Keywords**: inactivation; sanitation; pathogens; valorization; litter. (Source: *AGROVOC*)

Citations (APA) Torres Villar, M., Abreu Jorge, Y., Delgado-Hernández, B., Montano Valle, D. de las N., Alfonso Zamora, P., & Quesada, T. de J. (2025). Farm-Scale Composting: Inferred Inactivation of Selected Avian Pathogens *Journal of Animal Prod*, *37*. https://rpa.reduc.edu.cu/index.php/rpa/article/view/e280

ISSN 2224-7920

INTRODUCTION

Poultry production, owing to its sustained growth, is among the most dynamic sectors of livestock farming. The proteins it supplies are of high biological value and affordable, face few cultural barriers to consumption, and consequently make a substantial contribution to global food security. However, intensification of production to meet the consumption demands of a growing human population may pose public-health and environmental challenges (Torres *et al*., 2023).

In 2021, global production of fresh or frozen chicken exceeded 31.5 billion metric tons, and egg production was approximately 12.4 billion metric tons (FAOSTAT, 2023). Such production volumes entail the generation of large amounts of organic waste and the consequent need for appropriate management. Organic solid waste represents a negative externality that should be internalized by the agroindustry, particularly in nations with intensive chicken-meat production.(Chiarelotto *et al*., 2021).

Internalizing poultry litter through composting and its subsequent agricultural application can confer multiple benefits. Converting this waste into compost adds value and confers benefits for crop productivity and soil structure (Kacprzak *et al*., 2023). Composting plays a critical role in the management of organic waste during disease outbreaks (Costa & Akdeniz, 2019; Wang & Akdeniz, 2023).

During epidemic infectious-disease outbreaks, composting is a safe and effective alternative, including for carcass disposal (Figueroa *et al*., 2021). However, pile size is a critical factor influencing temperature, the most important variable for pathogen inactivation during composting (Amuah *et al*., 2022; Sokač *et al*., 2022). Recent studies emphasize the need to validate the process's efficacy under real farm conditions (Li *et al*., 2021; Ma *et al*., 2022). The present study aimed to estimate the effectiveness of farm-scale composting for inactivating selected avian pathogens by comparing thermotolerance values reported in the literature with the temperature kinetics observed in large-volume compost piles.

MATERIALS AND METHODS

Compost pile composition and study location

The study was conducted on a commercial duck farm in November 2018. The piles consisted of rice-hull duck litter on which ducks had been reared from hatch to 90–100 days of age. Composting was carried out inside the poultry houses in two piles, each containing 32,300 m^3 of litter. The resulting piles measured approximately 1.20 m in height, 3 m in base width, and 50 m in length (Figure 1).

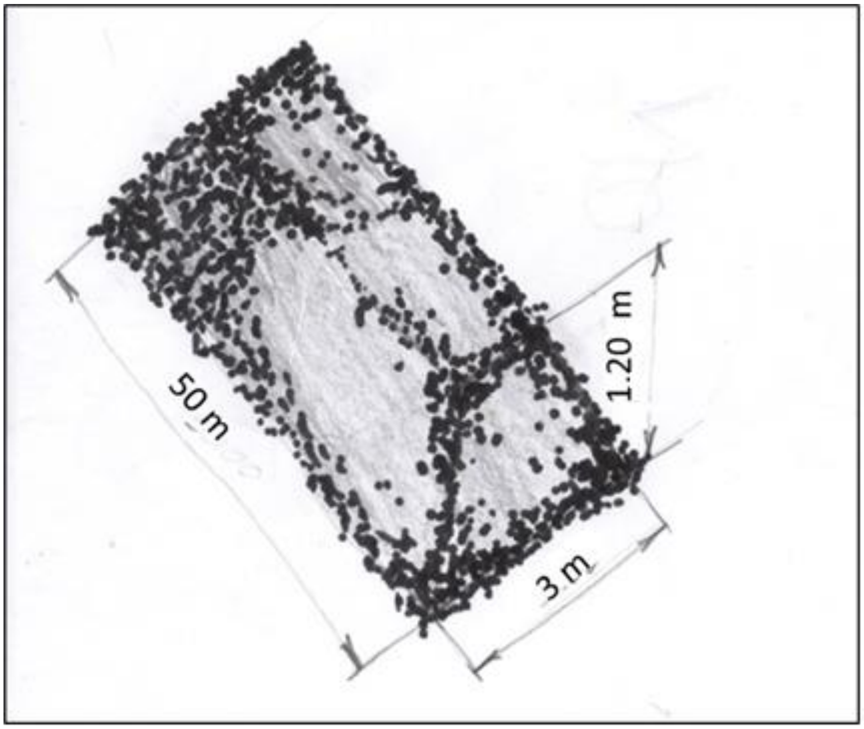

Figure 1. Compost pile schematic and dimensions

The piles were formed by progressively spraying the rice-hull litter with water using a hose while homogenizing and piling it with shovels. As a subjective criterion for adequate moisture, portions of litter were regularly hand-compressed until they remained compact without free water exuding, following commonly reported methodologies (Li *et al*., 2021). If water did exude, the wet material was mixed with dry litter until the desired consistency was achieved. Once formed, the piles were covered with polyethylene sheeting and left to rest (Figure 2).

Figure 2. Compost pile

Temperature monitoring and data collection

Beginning 24 hours after pile formation, temperature was measured at multiple points distributed across three vertical levels: base (n = 12), intermediate (n = 24), and top (n = 12). Measurements were taken at a depth of 30 cm using a stainless-steel needle thermometer with an accuracy of ±0.5 °C. Readings were recorded every 24 hours for a total monitoring period of 240 hours (10 days).

Inference of microbial inactivation

The temperature-based inactivation of nine pathogens was inferred, including viruses — avian influenza virus (AIV), Newcastle disease virus (NDV), infectious bursal disease virus (IBDV), egg-drop syndrome virus (EDS), and infectious laryngotracheitis virus (ILTV) — bacteria (*Escherichia coli*, *Salmonella* spp.) and parasites (*Eimeria* spp. and *Cryptosporidium parvum*). Thermotolerance levels as a function of time reported in the literature (primarily from the last five years) were compared with the actual temperature kinetics recorded in the large-volume litter piles. When multiple thermotolerance values were reported for the same microorganism, the highest value was used as a conservative cutoff for inferring inactivation.

Statistical analysis

Temperature data were processed using the R programming language (R Core Team, 2023). Initially, a descriptive statistical analysis (mean, standard deviation, range) was performed for each sampling position (base, intermediate, top) and for the pile-level average. Graphical visualizations were produced with the ggplot2 package (v. 3.3.3; Wickham, 2016). To compare mean temperatures among the three vertical positions, a one-way analysis of variance (ANOVA) was conducted, followed by Tukey's post-hoc test ($\alpha = 0.05$) to identify pairwise differences; analyses were implemented using R's stats package.

To identify underlying patterns in the thermal dynamics of composting, and accounting for the mixed structure of the data (continuous variables: temperature, time; categorical variables: pile position, pile identity), a Factor Analysis of Mixed Data (FAMD) was implemented using the FactoMineR and factoextra packages in RStudio (v. 4.5.0). The analysis preserved the mixed nature of the dataset by standardizing continuous variables and encoding categorical factors as indicator (dummy) variables. Emerging spatiotemporal patterns were interpreted by (1) factorial correlations ($|r| > 0.6$) between variables and latent dimensions, and (2) projection of observations onto factorial planes, taking into account each variable's relative contribution to total inertia. Observations were clustered by applying the k-means algorithm to the factorial coordinates.

RESULTS AND DISCUSSION

Temperature dynamics

Mean temperatures at all pile levels (Figure 3) consistently exceeded 40 °C, with significantly higher values ($p < 0.05$, Tukey's test) observed at the intermediate level. However, the lower end of the temperature range reached 30 °C in the coolest measurements, occurring mainly at the base and the top. The temperatures attained fell within the thermophilic range and were consistent with values reported in similar studies (Biswas *et al*., 2019; Torres *et al*., 2023). The observed heterogeneity matches the findings of Ma *et al*. (2022), who highlight the importance of management practices to homogenize pile conditions.

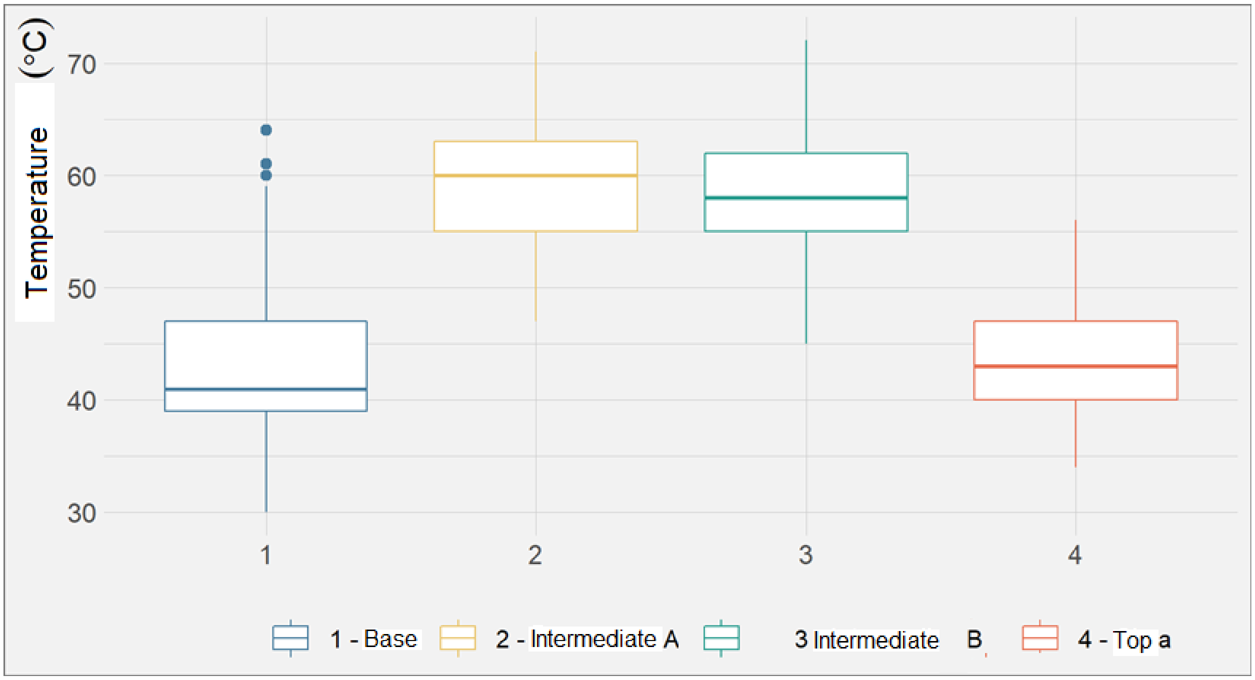

Figure 31. Mean temperatures and dispersion across compost pile levels

Pile height influences process temperature (Vaddella *et al*., 2018; Amuah *et al*., 2022). As alternatives to achieve higher temperatures than those observed here, pile turning and even water spraying — practices known to promote temperature increases — could be implemented (Li *et al*., 2021; Ma *et al*., 2022; Manga *et al*., 2023). Torres *et al*. (2023) recommend evaluating pile height (Figure 4), since temperature homogeneity is not always observed across all pile dimensions and this may compromise pathogen inactivation at some locations. Our results are consistent with those of Paterlini *et al*. (2017), who reported differences in the duration of thermophilic temperatures between pile sides and the pile surface in composting of poultry residues.

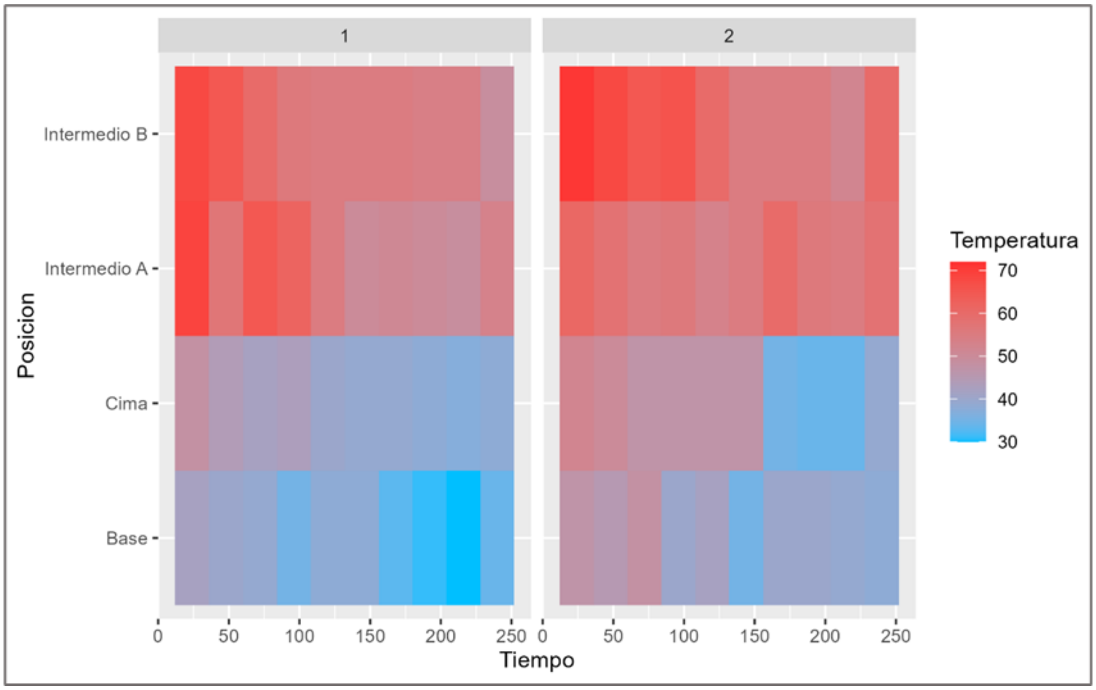

Figure 4. Sspace-time analysis of temperature behavior in each compost pile

Factor Analysis of Mixed Data (FAMD)

The FAMD identified the dimensions contributing most to the variance, with Dimension 1 accounting for 37.7% of the total variability, while Dimensions 2, 3 and 4 each contributed approximately 20% (Appendix 1). Dimensions 1 and 3 were primarily influenced by the continuous quantitative variables (time and temperature), which made the largest contributions to the formation of those factorial axes (Figure 5). Among the ordinal qualitative variables, sampling position within the pile contributed most strongly to the first factor, whereas pile identity had negligible influence, indicating good reproducibility of the thermal behavior between the two piles.

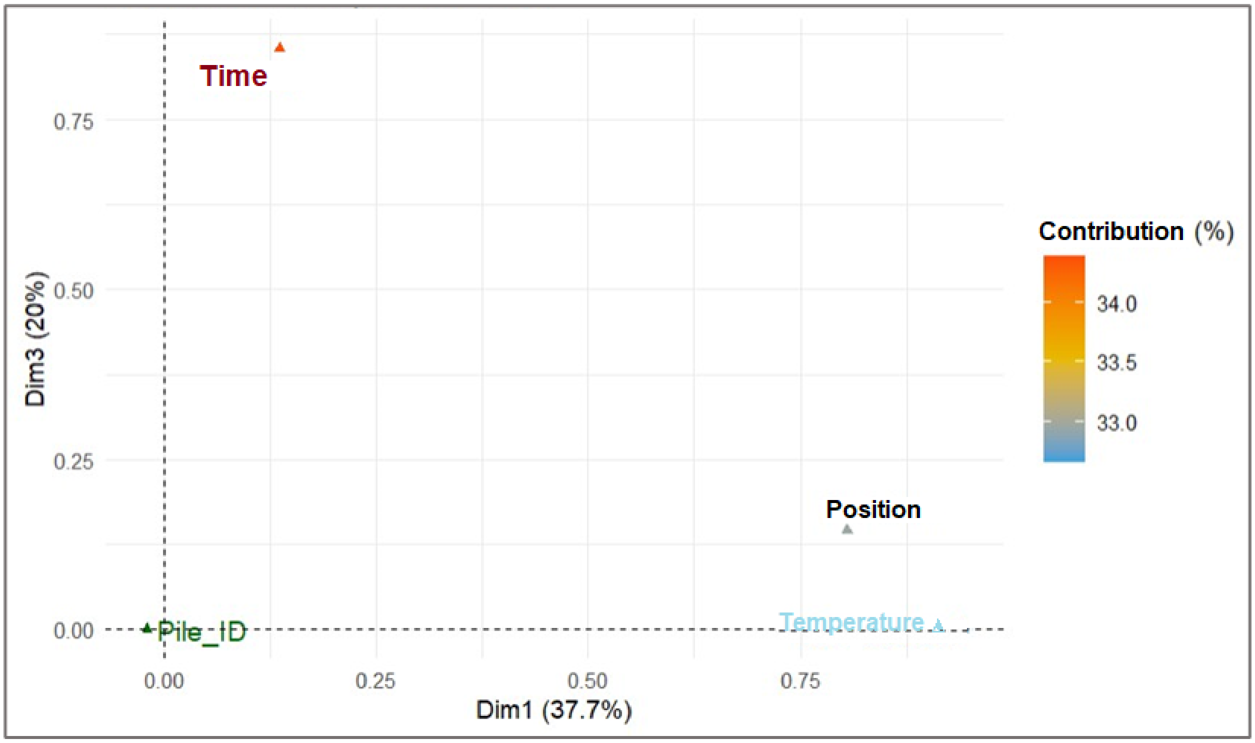

Figure 5. Analysis of the variables contributing to the variance in the factorial space

Dimension 1 is associated with a thermal gradient: high temperatures are linked to the intermediate positions (Intermedia A and B). Dimension 3 captures temporal variability, with longer elapsed times associated with the base and top positions. This suggests that the intermediate position reaches critical temperatures for viral inactivation more rapidly and maintains them more stably than the base and top positions (Figure 6), a finding consistent with the microaerophilic nature of the composting process reported by Ge *et al*. (2020).

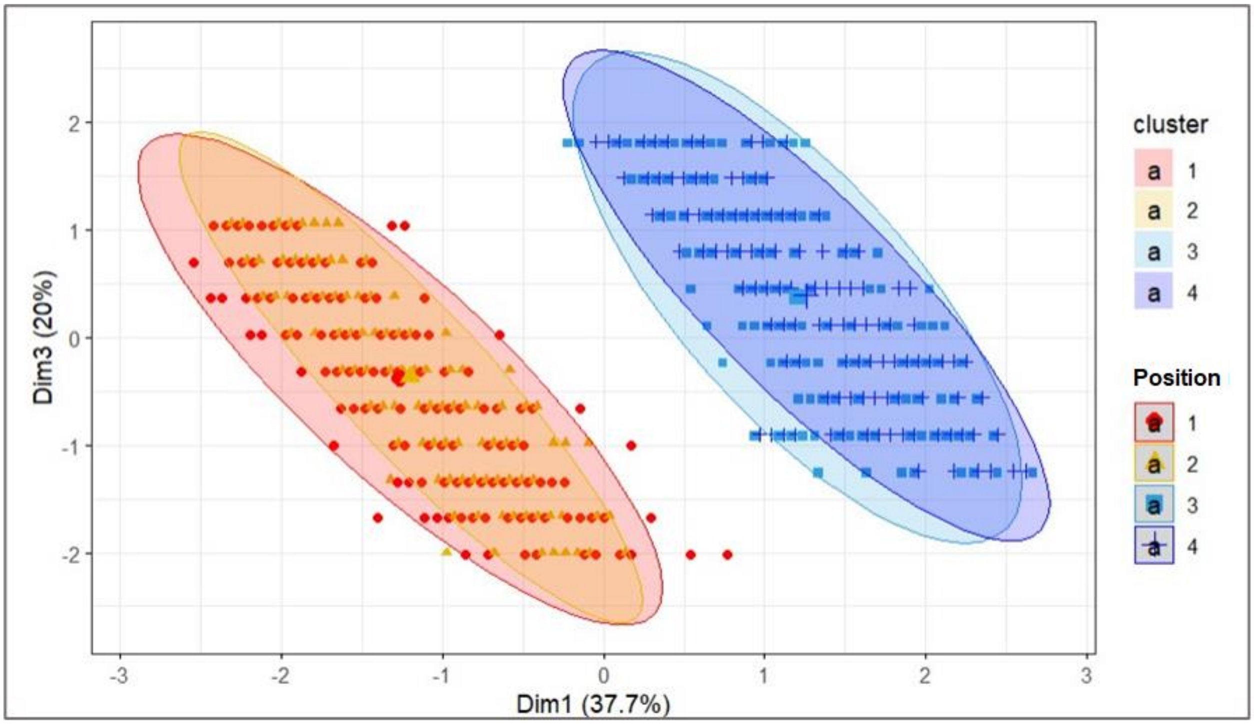

Figure 6. Cluster analysis on FAMD dimensions

A spatial heterogeneity pattern was evident with the formation of four clusters (Figure 6) corresponding to the temperature sampling positions within the compost piles. The similar behavior among the sampled positions is confirmed (Base – Top and Intermediate A – B). For positions with high temperatures (Intermediate A–B), this may be associated with greater thermal stability and lower daily fluctuation, which is consistent with effective viral inactivation. However, low-temperature zones such as those recorded at the Base and Top may reflect slower viral inactivation, requiring longer exposure times to compensate for suboptimal temperatures, which reinforces the need for turning as a management practice (Ngwabie *et al*., 2022).

Inference of pathogen inactivation

In the present study, the average temperature remained above 50 °C continuously for 144 hours (Figure 7). Despite the observed temperature variability, inactivation of various pathogens is expected in accordance with resistance levels reported in the literature (Table 1). Notably, the maximum thermal resistance times reported are usually less than two hours, a period far shorter than the duration for which pile temperatures exceeded 50 °C.

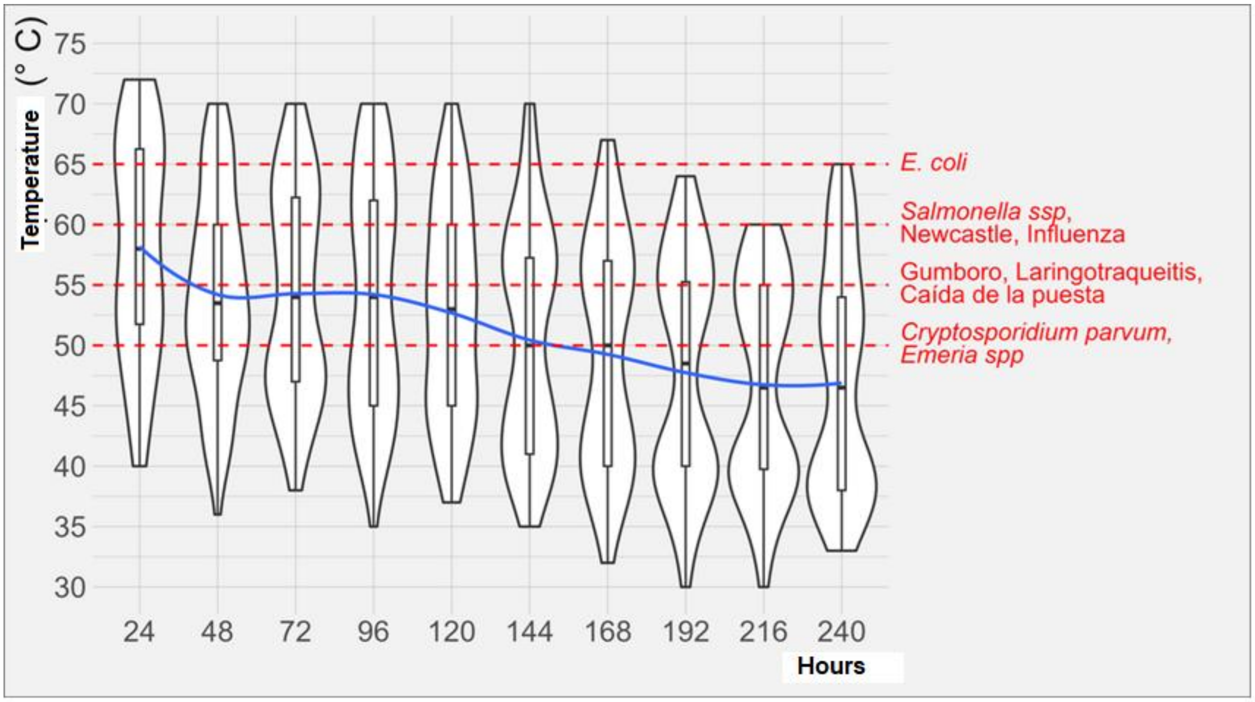

Figure 7. Expected inactivation of relevant avian pathogens based on reported thermal resistance and the compost pile's observed temperature–time kinetics

Table 1. Thermal resistance of selected avian pathogens reported in the literature

Microorganism	Thermal resistance (°C)	Time	Source
Avian influenza virus (AIV)	56-60	30 minutes	(Hessling *et al*.,2022; OMSA, 2023).
Infectious bursal disease virus (IBDV), also known as Gumboro disease virus	≥ 55	2h	(Rani & Kumar, 2015).
Newcastle disease virus (NDV)	50	30 minutes	(Ruan *et al*., 2020).
E. coli	65	1h	(Biswas *et al*., 2019).
Salmonella spp	60	1h	(Biswas *et al*., 2019).
Eimeria spp	55	1h	(Schneiders *et al*., 2020).
Infectious laryngotracheitis virus (ILTV)	55	15 minutes	(Gowthaman *et al*., 2020).
Egg drop syndrome virus	≥ 56	≥40 minutes 3h	(Suresh *et al*., 2013; Smyth, 2022).
Cryptosporidium parvum	50		Xiao *et al*., 2022.

The inferred inactivation was consistent with several reports for VEN and VIA (Costa and Akdeniz, 2019; Ruan *et al*., 2020; Figueroa *et al*., 2021), as well as for other agents (Elving *et al*., 2012), even at lower temperatures. A similar outcome was observed for the inferred inactivation of IBDV (Crespo *et al*., 2016) and for the ILTV and EDS viruses (Giambrone, Fagbohun, and Macklin, 2008). Regarding the selected parasites, oocyst inactivation is achieved at 55 °C (Schneiders *et al*., 2020), and therefore they could be eliminated.

Variations among methodologies used to demonstrate pathogen viability during composting make it difficult to determine the minimum time required for inactivation. For example, verification of inactivation for some bacterial pathogens has been reported to require 20 days at temperatures above 55 °C (Asses *et al*., 2019), whereas other studies report inactivation occurring in as little as one hour (Biswas *et al*., 2019). Nevertheless, there are reports of *Salmonella* spp. surviving at temperatures between 68 °C and 70 °C (Barrena *et al*., 2009). These discrepancies underscore the need for standardized methods to verify pathogen inactivation during composting, as well as for the availability of surrogate variables or modeling approaches to infer inactivation from time–temperature relationships, such as the approach addressed in this study.

The effectiveness of composting can be influenced by various physicochemical variables (Ge *et al*., 2020; Sharma *et al*., 2023). Turning the piles at an appropriate frequency enhances composting efficiency by improving aeration (Ngwabie *et al*., 2022). The decision to turn a pile while it may still contain viable microorganisms, beyond labor requirements, must consider biosecurity measures to prevent dissemination of the pathogen and contamination of new sites. Similarly, for pathogens with moderate thermal resistance, it may be preferable to leave the pile undisturbed. This is a risk-based decision guided by monitoring the pile's temperature.

Pile size, as another critical factor (Amuah *et al*., 2022; Sokač *et al*., 2022), can compromise both the magnitude and the homogeneity of temperatures (Asses *et al*., 2019; Miller, Miknis, and Flory, 2020) and, consequently, the effectiveness of biological agent inactivation. For these reasons, the present study adds to the body of knowledge on methods for the safe disposal of poultry production organic wastes, including during infectious disease outbreaks, despite the limitation that inactivation was inferred.

CONCLUSION

The temperature kinetics in large rice-husk bedding compost piles exhibited a sustained thermophilic phase (>50 °C for the first 144 hours), which is sufficient to infer inactivation of the main avian pathogens studied in accordance with thermal-resistance thresholds reported in the literature.

A pronounced spatial temperature heterogeneity was observed: the mid-depth region of the piles reached significantly higher and more stable temperatures (60–68 °C) than the base and surface layers (38–47 °C).

The Factor Analysis of Mixed Data (FAMD) enabled identification and characterization of spatiotemporal temperature gradients, providing an objective basis for determining optimal turning times to homogenize the pile, improve aeration, and ensure the biosecurity of the process.

REFERENCES

Amuah, E.E.Y., Fei-Baffoe, B., Sackey, L.N.A., Douti, N.B. & Kazapoe, R.W. (2022). A review of the principles of composting: understanding the processes, methods, merits, and demerits. *Organic Agriculture*, *12*(4), 547-562. https://doi.org/10.1007/s13165-022-00408-z

Asses, N., Farhat, W., Hamdi, M. & Bouallagui, H. (2019). Large scale composting of poultry slaughterhouse processing waste: Microbial removal and agricultural biofertilizer application. *Process Safety and Environmental Protection*, *124*, 128-136. https://doi.org/10.1016/j.psep.2019.02.004

Barrena, R., Artola, A., Vázquez, F. & Sánchez, A. (2009). The use of composting for the treatment of animal by-products: Experiments at lab scale. *Journal Hazardous Materials*, *161*(1), 380-386. https://doi.org/10.1016/j.jhazmat.2008.03.109

Biswas, S., Nazmi, A., Pitesky, M., Gallardo, R. & Pandey, P. (2019). Thermal Inactivation of Escherichia coli and Salmonella Typhimurium in Poultry Carcass and Litter at Thermophilic Temperatures. *Journal of Applied Poultry Research*, *28*(2), 307-317. https://doi.org/10.3382/japr/pfy072

Chiarelotto, M., Restrepo, J.C.P.S., Lorin, H.E.F. & Damaceno, F.M. (2021). Composting organic waste from the broiler production chain: A perspective for the circular economy. *Journal of Cleaner Production*, *329*(20), 129717. https://doi.org/10.1016/j.jclepro.2021.129717

Costa, T. & Akdeniz, N. (2019). A review of the animal disease outbreaks and biosecure animal mortality composting systems. *Waste Management*, *90*, 121-131. https://doi.org/10.1016/j.wasman.2019.04.047

Crespo, R., Badcoe, L.M., Williams, C. & Bary, A.I. (2016). Inactivation of Infectious Bursal Disease Virus Through Composting of Litter from Poultry Houses. *Avian Disease*, *60*(2), 506-510. https://doi.org/10.1637/11341-120615-ResNote

Elving, J., Emmoth, E., Albihn, A., Vinneras, B. & Ottosona, J. (2012). Composting for avian influenza virus elimination. *Applied and Environmental Microbiology*, *78*(9), 3280-3285. https://doi.org/10.1128/AEM.07947-11

Figueroa, A., Derksen, T., Biswas, S., Nazmi, A., Rejmanek, D., Crossley, B., Pandey, P. & Gallardo, R.A. (2021). Persistence of low and highly pathogenic avian influenza virus in reused poultry litter, effects of litter amendment use, and composting temperatures. *Journal of Applied Poultry Research*, *30*(1), 100096. https://doi.org/10.1016/j.japr.2020.09.011

Ge, M., Zhou, H., Shen, Y., Meng, H., Li, R., Zhou, J., Cheng, H., Zhang, X., Ding, J., Wang, J. & Wang, J. (2020). Effect of aeration rates on enzymatic activity and bacterial community succession during cattle manure composting. *Bioresource Technology*, *304*,122928. https://doi.org/10.1016/j.biortech.2020.122928

Giambrone, J.J., Fagbohun, O. & Macklin, K.S. (2008). Management Practices to Reduce Infectious Laryngotracheitis Virus in Poultry Litter. *Journal of Applied Poultry Research*, *17*(1), 64-68. https://doi.org/10.3382/japr.2007-00017

Gowthaman, V., Kumar, S., Koul, M., Dave, U., Murthy, T. R. G. K., Munuswamy, P., Tiwari, R., Karthik, K., Dhama, K., Michalak, I., & Joshi, S. K. (2020). Infectious Laryngotracheitis: Etiology, epidemiology, pathobiology, and advances in diagnosis and control- a comprehensive review. *Veterinary Quarterly*, *40*(1), 140-161. https://doi.org/10.1080/01652176.2020.1759845

Hessling, M., Fehler, N., Gierke, A.M., Sicks, B. & Vatter, P. (2022). Heat Inactivation of Influenza Viruses—Analysis of Published Data and Estimations for Required Decimal Reduction Times for Different Temperatures and Media. *Microbiology Research*, *13*(4), 853-871. https://doi.org/10.3390/microbiolres13040060

Kacprzak, M., Malinska, K., Grosser, A., Sobik-Szoltysek, J., Wystalska, K., Drózdz, D., Jasińska, A. & Meers, E. (2023). Cycles of carbon, nitrogen and phosphorus in poultry manure management technologies – environmental aspects. *Critical Reviews in Environmental Science and Technology*, *53*(8), 914-938. https://doi.org/10.1080/10643389.2022.2096983

Li, M.-X., He, X.-S., Tang, J., Li, X., Zhao, R., Tao, Y.-Q., Wang, C. & Qiu, Z.P. (2021). Influence of moisture content on chicken manure stabilization during microbial agent-enhanced composting. *Chemosphere*, *264*(2), 128549. https://doi.org/10.1016/j.chemosphere.2020.128549

Ma, Q., Li, Y., Xue, J., Cheng, D. & Li, Z. (2022). Effects of Turning Frequency on Ammonia Emission during the Composting of Chicken Manure and Soybean Straw. *Molecules*, *27*(2), 472. https://doi.org/10.3390/molecules27020472

Manga, M., Muoghalu, C., Camargo-Valero, M.A. & Evans, B.E. (2023). Effect of Turning Frequency on the Survival of Fecal Indicator Microorganisms during Aerobic Composting of Fecal Sludge with Sawdust. *International Journal of Environmental Research and Public Health*, *20*(3), 2668. https://doi.org/10.3390/ijerph20032668

Miller, L.P., Miknis, R.A. & Flory, G.A. (2020). Carcass management guidelines – Effective disposal of animal carcasses and contaminated materials on small to medium-sized farms.

FAO Animal Production and Health Guidelines No. 23. Rome, FAO. https://doi.org/10.4060/cb2464en

Ngwabie, N.M., Tiku, T.D., Yengong, L.F. & Manga, E.V. (2022). Effect of Wood shavings on the Temperature Profile of Livestock Waste during Composting with Daily Turning. *CIGR Journal*, *24*(2), 25-35. https://cigrjournal.org/index.php/Ejounral/article/view/7433/3849

Organización de las Naciones Unidas para la Alimentación y la Agricultura. (2023). FAOSTAT. https://www.fao.org/faostat/es/#data/

Organización Mundial de Salud Animal. Código sanitario de animales terrestres. (2023). https://www.woah.org/fileadmin/Home/esp/Health_standards/tahc/current/es_chapitre_avian_influenza_viruses.htm

Paterlini, H., González, M.V. & Piconi, L, 2017. Comparación de técnicas para compostar cama de pollo. https://www.suelos.org.ar/publicaciones/v35n2-html/vol35-n2-html/v35n2a17.htm

R Core Team., 2023. R: A Language and Environment for Statistical Computing. R FoundationforStatistical Computing, Viena, Austria. https://www.r-project.org/.

Rani, S. & Kumar, S. (2015). Evaluation of infectious bursal disease virus stability at different conditions of temperature and pH. *Biologicals, 43*(6), 515-518. https://doi.org/10.1016/j.biologicals.2015.07.005

Ruan, B., Zhang, X., Zhang, C., Du, P., Meng, C., Guo, M., Wu, Y. & Cao, Y. (2020). Residues 315 and 369 in HN Protein Contribute to the Thermostability of Newcastle Disease Virus. *Frontiers in Microbiology*, *11*, 560482. https://doi.org/10.3389/fmicb.2020.560482

Schneiders, G. H., Foutz, J. C., Fuller, A. L., Nelson, J., Rekaya, R., & Aggrey, S. E. (2020). The Effect of Increased Temperatures on Viability, Morphology, Infectivity, and Development of Eimeria Tenella, *Journal of Parasitology*, *106*(3), 428-437. https://doi.org/10.1645/19-17

Sharma, P., Sharma, S., Singh, J., Singh, A. & Katnoria, J.K. (2023). Characterization of Tectona grandis leaf litter compost: an ecological approach for converting leaf litter waste into organic product using composting. *Biomass Conversion Biorefinery*. https://doi.org/10.1007/s13399-023-04309-3

Smyth J, A. (2022). Egg Drop Syndrome ’76. Poultry - MSD Veterinary Manual. https://www.msdvetmanual.com/poultry/egg-drop-syndrome-76/egg-drop-syndrome-76

Sokac, T., Valinger, D., Benkovic, M., Jurina, T., Kljusuric, J.G., Redovnikovic, I.R. & Tušek, A.J. (2022). Application of Optimization and Modeling for the Composting Process Enhancement. *Processes, 10*(2), 229. https://doi.org/10.3390/pr10020229

Suresh, P., Shoba, K. & Johnson Rajeswar, J. (2013). Physico – Chemical and biological characterization of egg drop syndrome – 1976 (Eds –'76) virus. *Indian Journal of Veterinary Sciences and Biotechnology*, *8*(3), 64-66. https://acspublisher.com/journals/index.php/ijvsbt/article/view/3183

Torres, M., Ochoa-Álvarez, N.A., Nieto-Garibay, A., Murillo-Amador, B., P., G.L. & Alfonso, P. (2023). Inactivación de patógenos en residuos avícolas mediante el compostaje. *Revista de Investigaciones Veterinarias del Perú*, *34*(4), e24488. https://doi.org/10.15381/rivep.v34i4.24488

Vaddella, V., Pandey, P., Cao, W., Biswas, S., Chiu, C., Zheng, Y., Wu, T., Ghanem, N. & Buyuksonmez, F. (2018). Assessment of Pathogen Inactivation under Sub-composting Temperature in Lab-scale Compost Piles. *Journal of Food Research*, *7*(3), 64-75. https://doi.org/10.5539/jfr.v7n3p64

Wang, Y. & Akdeniz, N. (2023). Co-composting poultry carcasses with wood-based, distillers' grain and cow manure biochar to increase core compost temperatures and reduce leachate's COD. *Waste Management, 161,* 84-91. https://doi.org/10.1016/j.wasman.2023.02.024

Wickham, H. ggplot2. (2016). https://link.springer.com/book/10.1007/978-3-319-24277-4

Xiao, D., Lyu, Z., Chen, S., Huo, Y., Fan, W. & Huo, M. (2022). Tracking *Cryptosporidium* in urban wastewater treatment plants in a cold region: Occurrence, species and infectivity. *Frontiers of Environmental Science and Engineering, 16*(9), 112. https://doi.org/10.1007/s11783-022-1533-8

AUTHOR CONTRIBUTION STATEMENT

Research conception and design: PA, DMV, BDH, MTV; data analysis and interpretation: YAJ, DMV, BDH, MTV, TJQ; article writing: MTV, DMV, BDH.

CONFLICT OF INTEREST STATEMENT

The authors state there are no conflicts of interest whatsoever.