International Journal of Recycling of Organic Waste in Agriculture (2021)10: 265-273
Doi: 10.30486/IJROWA.2021.1909128.1132

ORIGINAL RESEARCH

Effect of composting on the microbiological and parasitic load in animal production wastes in Brazil

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Received: 11 September 2020 / Accepted: 22 May 2021 / Published online: 01 August 2021

Abstract

Purpose Animal production wastes are promising for use in agricultural production as a plant nutrient or soil conditioner. However, if not properly managed, they can contaminate the soil and plants, resulting in public health risks. Considering that the composting technique is recommended to ensure compost hygiene and agronomic viability, the present study aimed to evaluate the microbiological and parasitic load during the composting of residues from conventional and organic animal productions.

Method The presence of *Salmonella* sp. and/or helminth eggs and the quantification of thermotolerant coliforms were determined in horse bedding and organic and conventional poultry litter during the process of composting.

Results The initial load of thermotolerant coliforms was greater than 10^{17} MPN g^{-1} in the three raw materials. All of them showed a significant reduction in these bacteria at the end of the composting process, reaching 99.98%, 100.00%, and 99.80% in the horse bedding, organic poultry litter, and conventional poultry litter, respectively. All the fresh residues contained helminth eggs, with the horse bedding exhibiting the highest amount compared to the others. However, they all revealed an absence of helminth eggs at the end of the composting process. *Salmonella* sp. was absent in both the raw materials and the final compost.

Conclusion Composting was effective in eliminating helminth eggs and reducing thermotolerant coliform levels. However, the final composts retained a higher pathogenic microbial load than that required by the current Brazilian legislation for use in agriculture.

Keywords Horse bedding, Poultry litter, Thermotolerant coliforms, Helminth eggs, *Salmonella*

Introduction

Increases in animal production directly result in the generation of large amounts of slowly degradable wastes in some cases, and in others, by-products that can be toxic and cumulative in the environment (Schneider et al. 2012). In poultry and horse farming, one of the generated wastes is the floor covering where the animals remain. This material, known as poultry or horse bedding, is used to avoid direct contact between the animal and the floor as a substrate for water absorption and the incorporation of feces, urine, and the remains of leftover or deteriorated food (Virtuoso et al. 2015).

Such residues have the potential to be used in agricultural production as fertilizer or soil conditioners since they promote beneficial effects on the soil and plants (Komar et al. 2012; Souza and Rodrigues 2017). However, animal manure, such as poultry litter, a mixture of organic materials including feces, feed, and bedding, is a valuable nutrient-rich soil fertilizer that has also been considered an important source of pathogenic microorganisms. When handled improperly, this material can contaminate the soil and plants used for human consumption, resulting in the worsening of global threats, such as antimicrobial resistance, and

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represent a risk according to the One Health initiative (Haapapuro et al. 1997; Kyakuwaire et al. 2019; Souza et al. 2020). The term One Health refers to the idea that human and animal health are interdependent and bound to the health of the ecosystems in which they exist (OIE 2020). This concept was implemented by the World Organization for Animal Health, as well as multiple other organizations, as a collaborative global approach to understand the risks for human and animal health, and ecosystem health as a whole (OIE 2020).

Consequently, animal waste management technologies have emerged, intending to mitigate environmental risks through the previous stabilization of these residues for later use as manure in the soil (Valente et al. 2016). Some biological processes can stabilize animal production wastes, such as composting and anaerobic digestion, which use aerobic and anaerobic microorganisms, respectively (Hadin et al. 2016).

Composting is defined as the biological decomposition of organic materials, *i.e*., animal manure and plant matter, provided by the action of aerobic microorganisms (Sampaio et al. 2019). In addition to being an environmentally friendly procedure of low implementation and maintenance cost, this process stands out since it results in more stabilized organic matter, and is efficient in terms of toxic substance degradation, waste volume and matter reduction, nutrient concentration, and organic residue recovery (Costa et al. 2009; Larney et al. 2006; Larney et al. 2008). Moreover, composting is one of the best alternatives to reduce the population of potentially pathogenic microorganisms in wastes, minimizing the risk of environmental contamination and ensuring the sanitation of the final product (Sá et al. 2014). It is noteworthy that composting is a process that generates and emits gases, some of which are greenhouse gases (GHG), which favor global warming (Sánchez et al. 2015). Nevertheless, this method is also considered a mitigating technology because it reduces the emission of these gases per ton of treated waste. Solid wastes are an important source of GHG, and their treatment and/or final disposal is decisive in the amount of gas emitted (Inacio et al. 2010).

According to Pereira et al. (2013), animal manure can be used in agricultural areas without prior treatment in conventional farming. However, when this residue is obtained from non-organic production systems, it can only be utilized in organic farming after undergoing proper composting and biostabilization (Brasil 2014). Normative Instruction 46, of October 6, 2011, devised by the Ministry of Agricultural Defense, establishes in Annex VI the maximum limits of contaminants admitted in organic composts, which are: 1.000 most probable number (MPN) of thermotolerant coliforms per gram (g) of dry matter; one viable helminth egg per 4 g of total solids, and absence of *Salmonella* sp. in 10 g of dry matter (Brasil 2011). Based on these parameters, it is possible to determine a manure's suitability for agricultural use in Brazil (Duarte and Pasqualine 2017).

In Europe, animal wastes are required to come from a technical, biogas, or composting plant to be used as a fertilizer in agriculture. They must be free of *Salmonella* (no *Salmonella* in 25 g of treated product) and Enterobacteriaceae (based on the aerobic bacterial count: 1,000 CFU per gram of treated product) (European Parliament and Council 2002). In the USA, the recommendations are 0.3 MPN per gram or milliliter of analytical portion for *E. coli* O157:H7, less than 3 MPN per 4 g or mL of total solids for *Salmonella* spp., and less than 1 colony-forming unit (CFU) per 5 g or mL of analytical portion for *L. monocytogenes* (FDA 2018).

Considering that the composting of residues from animal production is a recommended technique to guarantee the sanitation and agronomic viability of organic composts, the present study aimed to evaluate the microbiological and parasitic load during the composting of residues from conventional and organic animal productions.

Materials and methods

Origins of the animal production wastes

The horse and poultry beddings from conventional and organic production systems were acquired in September 2019. The horse bedding came from a farm in Nova Friburgo (22º17'S, 42º32'W), located in the mountainous region of the state of Rio de Janeiro, Brazil. Meanwhile, the poultry litter came from two farms: one in São José do Vale do Rio Preto (22º09'S, 42º55'W) that adopts the organic production system and the other in Nova Friburgo (22º17'S and 42º32'W) that adopts the conventional production system; both also located in the mountainous region of Rio de Janeiro, Brazil. The samples were packed in plastic bags and transported to the Integrated System of Agroecological Production (SIPA), known as *Fazendinha Agroecológica* Km 47, a partnership of the Federal Rural University of Rio de Janeiro (UFRRJ), the State Agricultural Research Corporation of Rio de Janeiro (Pesagro), and the Brazilian Agricultural Research Corporation (EMBRAPA), where the composting trials were carried out.

Assembly, composting, and sample collection

The composting experiment was conducted from October 2019 to March 2020 at the Fazendinha Agroecológica Km 47, in Seropédica, located in the metropolitan region of Rio de Janeiro, at the coordinates 22º46'S and 43º41'W (Dias 2007). The climate in the area is hot and humid and is classified as Aw according to the Köppen climate classification, with high temperatures in the summer and mild temperatures in the winter. The average annual temperature in the area is 24.5°C, with a rainy period from November to March and average yearly precipitation of 1,213 mm (EMBRAPA 1999).

In a covered shed, three piles were made for each type of waste, namely, horse bedding (HB), conventional poultry litter (CPL), and organic poultry litter (OPL). With the aid of a wire mesh, cylindrical shapes measuring 1 m (height) x 1.20 m (diameter) were assembled, after which the beddings were immediately added. The material was stored in the wire mesh for better optimization of space and maintenance throughout the experiment.

The composting process was carried out for 125 days. Temperature evaluations were performed daily during the first two weeks using a culinary digital thermometer inserted at 20 cm of depth in three points in each pile, and then on alternate days until the material stabilized. The piles were irrigated to maintain humidity close to 55%, 45%, and 45% for the horse bedding and the organic and conventional poultry litter, respectively. These humidity values were stipulated based on the ease of handling and composition.

Samples were collected at 0, 14, 32, 60, 90, and 125 days and were obtained in three simple samplings conducted at the time of pile turning and at equidistant positions from the other samples. The samples were mixed, forming composite samples, then packed in plastic bags and transported to the Microbial Diversity Laboratory of the UFRRJ Veterinary Institute. Upon arrival, the samples were stored at -20ºC for further analyses.

Microbiological analysis

Microbiological analysis was performed to quantify the thermotolerant coliforms, *Salmonella*, and helminth eggs. Initially, in order to investigate the material for *Salmonella* and quantify the thermotolerant coliforms, approximately 10 g of each sample was added to 90 mL of 0.1% peptone water.

Determination of thermotolerant coliforms

The Most Probable Number (MPN) technique was used to quantify the thermotolerant coliforms (Rice et al. 2017). After homogenization of the first dilution, serial dilutions were prepared in tubes containing 9 mL of 0.1% peptone water. For the presumptive test of total coliforms, 1 mL of the respective dilutions were transferred to five tubes containing 9 mL of Lauryl Sulfate Tryptose (LST) broth and an inverted Durham tube, and incubated in an oven at 35°C for 48 h. Aliquots of positive samples (turbidity with or without gas in the Durham tube) were transferred to tubes containing *Escherichia coli* (EC) broth and were incubated in a water bath at 45ºC for 24 h. The presence of turbidity or contained gas inside the Durham tube was indicative of positivity.

Three series of five tubes were inoculated with three different serial dilutions, and the number of positive tubes from the three dilutions was compared with the MPN table. The most likely number for each sample was calculated according to the following formula:

$$\text{MPN corresponding to the table x } \frac{10}{V}$$

Where:
V = largest inoculated volume.
The results were expressed in MPN/g.

Presence/absence test for salmonella

The *Salmonella* assay was carried out according to the methodology proposed by the International Organization for Standardization (ISO 2010). After diluting the sample in peptone water, the material was homogenized and incubated at 35ºC for 24 h. Next, 1 mL was transferred to tubes containing 9 mL of tetrathionate broth, which were incubated at 35ºC for 24 h. Following incubation, an aliquot of the tetrathionate broth was inoculated on *Salmonella-Shigella* Agar medium and incubated at 35ºC for 24 h. After this period, the plates were analyzed for colonies typical of *Salmonella* (colonies with a black center). These colonies were stored and identified using the Matrix-Assisted Laser Ionization/Desorption Time-of-Flight technique

(MALDI-TOF) at the Integrated Microbiology Laboratory (LIM) of the Paulo Góes Institute of Microbiology at the Federal University of Rio de Janeiro (UFRJ).

Helminth egg quantification

The quantification of helminth eggs was performed at the Experimental Chemotherapy Laboratory in Veterinary Parasitology at the Veterinary Institute of the Federal Rural University of Rio de Janeiro (UFRRJ). For soft helminth eggs, the simple centrifuge-flotation technique was used (Figueiredo et al. 1984). The sample was initially diluted in the proportion of 2 g of sample per 10 mL of filtered water. The solution was then homogenized and filtered through a sieve and gauze and was transferred into a Falcon tube to be centrifuged at 2,500 rpm for 10 min. Soon after centrifugation, supernatant dissociation was verified, and the precipitate was resuspended in sucrose solution. Subsequently, this solution was subjected to a second centrifugation at 2,500 rpm for 10 min, after which the Falcon tube volume was completed with saturated sugar solution, forming a convex halo meniscus, where a microscopic slide was deposited. After 10 min, the slide was quickly removed, and a coverslip for microscopic viewing was placed on top of it.

Spontaneous sedimentation was conducted for dense helminth eggs (Hoffman et al. 1934). Initially, 2 g of the collected material was homogenized in water using a glass rod and filtered through gauze into a conical-bottom cup. Next, the cup was filled with water and kept at rest for two hours, favoring the residues' precipitation. Soon after, the supernatant was replaced with clean water, promoting resuspension of the precipitate. This operation was repeated twice until the supernatant became light-colored. An aliquot of the precipitate was then pipetted and deposited on the surface of a microscopic slide for visualization.

The helminth eggs were visualized by 100x and 200x power optical microscopy magnification. The identification and counting of the helminth eggs were based on the size and specific morphological characteristics of the eggs, such as shape, egg content, and thickness of the outer membrane (shell), in addition to modifications such as protuberances, spikes, polar stoppers, and operculum (Soulsby 1987; Zajac and Conboy 2012).

Results and discussion

During the composting process, the horse bedding and conventional and organic poultry litter showed very similar behavior (Fig. 1). The maximum temperature points were observed at 3, 15, 33, and 61 days after the start of the experiment. We believe this fact is related to the humidification and turnover of the windrows on the previous days (0, 14, 32, and 60 days), which provided aeration and, consequently, the maintenance of microbial activity and greater heat release. The low temperature after these peaks probably occurred due to the decrease in oxygen due to the piles' natural compaction and reduced water availability, which causes a reduction in microbial activity and, as a result, lower temperatures. Several thermophilic and mesophilic phases were observed during composting. Similar behavior was observed by Oviedo-Ocaña et al. (2015). Our results corroborate those reported by Pampuro et al. (2016) when evaluating two composting strategies (with and without turning). Note that, in general, the temperature profiles were quite similar. However, the windrows that were turned over underwent increases in average temperature after the turns. The authors stated that this is due to the rise in oxygen levels, which stimulates microbial activity.

The thermophilic phase is essential for reducing microbiological contamination, thus eliminating bacterial populations from the organic waste (Heck et al. 2013; Matos 2014). According to CONAMA resolution 481/2017, the period and temperature required for sanitizing organic solid wastes during composting in open systems is 55°C for 14 days or 65°C for three days (Brasil 2017).

The organic poultry litter exhibited temperatures above 65ºC at 33 days of composting during four consecutive days. Meanwhile, the conventional poultry litter displayed a similar temperature at 15 days of composting, which lasted three consecutive days. The horse bedding, in turn, presented a maximum temperature of 63ºC only once (16th day). Therefore, both types of poultry litter could be considered sanitized according to the temperature established in the legislation, while the horse bedding displayed similar behavior to what is deemed ideal. Compared to the horse bedding, the higher temperatures achieved in the poultry litter were probably related to the material's higher nitrogen content, favoring an increase in biological activity, and its fine granulometry, forming piles with better tempera-

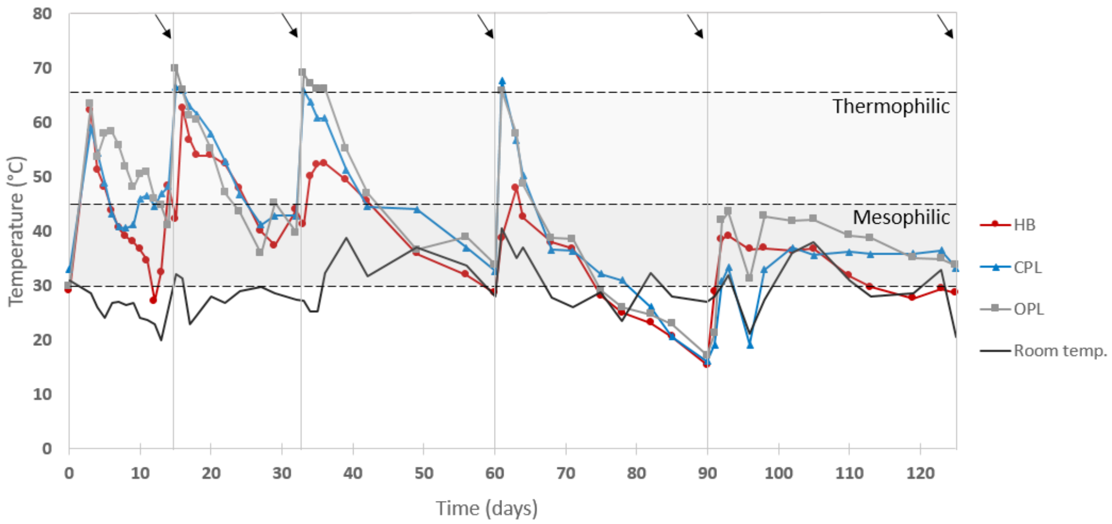

Fig. 1 Temperature profile during the composting process

HB = horse bedding; CPL = conventional poultry litter; OPL = organic poultry litter. Arrows indicate collection points

ture distribution and less heat loss. In contrast, being a coarser material, the horse bedding provides proper aeration and reaches high temperatures but is more prone to heat loss (Nascimento 2010).

After 68 days, all the materials registered average temperatures below 45ºC, remaining until the end of the composting process in the mesophilic phase. At 125 days, temperatures between 29ºC and 34ºC were recorded, close to room temperature, indicating the curing or maturation period. According to Kiehl (2004), the maturation phase indicates that the compost presents ideal physical, chemical, and biological characteristics for use as an organic fertilizer. Meanwhile, according to Bernal et al. (2009), in addition to temperature, decreases in water-soluble organic-C, NH4-N, phytotoxic effects, and microbial activity and increases in the humidification of the organic materials are indicators of progressive stabilization of the compost content, leading to an acceptable degree of maturity based on the established indices in the literature for composts of different origin.

Regarding the microbiological quality of the material, the fresh residues showed high microbial contamination (more than 10^{17} MPN g^{-1}) (Table 1). Considering that these residues contain animal waste in their composition and that animals have significant intestinal microbial diversity, such behavior was already expected and reinforces the risk of using fresh materials from animal production in agriculture, since these microorganisms can contaminate plants which, in turn, when ingested, contaminate humans and animals, rendering this practice a public health issue. This problem was reinforced by Kyakuwaire et al. (2019), who stated that the direct application of poultry litter on agricultural soil could cause damage to animal, human, and environmental health.

Composting has become an alternative to mitigate environmental risks through the previous stabilization of residues for later use in the soil as fertilizer (Valente et al. 2016). In organic farming, animal residues need to be biostabilized through composting for use (Brasil 2014). Also, according to Brazilian legislation, organic compounds are required to obey the maximum limits of microbiological contaminants in order to be used in organic agriculture: 1,000 most probable number (MPN) of thermotolerant coliforms per gram of dry matter; one viable helminth egg per 4 g of total solids, and absence of *Salmonella* sp. in 10 g of dry matter (Brasil 2011). In the present study, during the composting process, a reduction in thermotolerant coliforms was observed in all treatments. The horse bedding was the only material that showed a decrease in these microorganisms at 60 days (2.6 x 10^{11} MPN g^{-1}). However, at 90 days, this load showed a particular increase, reaching 2.7 x 10^{13} MPN g^{-1}, remaining so until the end of the experiment. Meanwhile, the levels in the poultry litter decreased only after 90 days.

All materials underwent a significant reduction in thermotolerant coliforms at the end of the composting process, reaching 99.98%, 100%, and 99.80% for the horse bedding, organic poultry litter, and conventional poultry litter, respectively. Nonetheless, despite such

Table 1 Most probable number per gram (MPN g^{-1}) of thermotolerant coliforms, *Salmonella* sp. assay, and quantification of viable helminth eggs in animal production wastes during the composting process (0, 15, 30, 60, 90, and 120 days)

	0	14	32	60	90	125	MAPA
Thermotolerant coliforms (MPN g^{-1})							
HB	> 1.6 x 10^{17}	> 1.6 x 10^{17}	1.6 x 10^{17}	2.6 x 10^{11}	2.8 x 10^{13}	2.7 x 10^{13}	
CPL	> 1.6 x 10^{17}	> 1.6 x 10^{17}	1.6 x 10^{17}	1.1 x 10^{17}	1.1 x 10^{15}	3.2 x 10^{14}	≤1000
OPL	> 1.6 x 10^{17}	> 1.6 x 10^{17}	1.6 x 10^{17}	1.6 x 10^{17}	4.3 x 10^{7}	1.4 x 10^{7}	
***Salmonella*/10g**							
HB	Absent	Absent	Absent	Absent	Absent	Absent	
CPL	Absent	Absent	Absent	Absent	Absent	Absent	Absent
OPL	Absent	Absent	Absent	Absent	Absent	Absent	
Viable helminth eggs/4g type Strongyloidea							
HB	3	1	0	0	0	0	
CPL	1	1	0	0	0	0	1
OPL	1	1	0	1	0	0	

HB = horse bedding; CPL = conventional poultry litter; OPL = organic poultry litter.

reduction, the values remained above the levels accepted by Brazilian legislation for use in organic agriculture (Brasil 2011), indicating that the composts offer a risk of transmitting pathogens to humans and cannot be used safely. It is worth mentioning that the poultry litter presented a temperature above 65ºC for three days, as recommended in the legislation for sanitizing this material (Brasil 2017). However, it was not enough to reduce the thermotolerant coliform levels to acceptable organic agriculture values, probably due to the high coliform load present in the raw material.

Vasconcelos (2019) considers that the high levels of thermotolerant coliforms in raw materials (on average 7.1 x 10^4 MPN g^{-1}) is the probable origin of this contamination at the end of the composting process of urban waste without shading screen protection (1.5 x 10^3 MPN g^{-1}) and periodic turning (1.0 x 10^4 MPN g^{-1}). Additional treatments must be performed to reduce contamination and enable the use of this material for agricultural purposes. Sá et al. (2014) reported that, although there was a significant reduction (99.9%) in the coliform population during the automated composting of swine liquid waste, the final result was greater than the limit established by the Ministry of Agriculture, Livestock and Food Supply (MAPA) for class A composts. In contrast, Souza et al. (2019) stated that composting was efficient in eliminating thermotolerant coliforms in goat and sheep waste.

Salmonella has been found in the intestinal tract of a wide array of domestic animals, namely birds (Hugas and Beloeil 2014). In poultry litter, *Salmonella*, *E. coli,* and coliforms are the most prevalent bacterial contaminants (Kyakuwaire et al. 2019). According to Brasil (2011), a large amount of *Salmonella* sp. is eliminated in the feces. Nonetheless, this microorganism was absent in the present study, since the collection of the fresh residues until the final compost of the different materials. According to Lopes et al. (2014), the possibility of intermittent excretion of this microorganism by birds under specific conditions may justify the low frequency of identification of *Salmonella* sp. in fecal samples. Moreover, the detection of low levels of this pathogen can be related to competition with other organisms in beddings (Barbour et al. 1999). Souza et al. (2019) analyzed the microbiological characteristics of goat and sheep waste composting and also observed an absence of *Salmonella* throughout the process which is according to the current Brazilian legislation.

Significant variability was observed regarding the presence and quantity of helminth eggs throughout the composting process. Considering the raw wastes, the horse bedding exhibited a larger number of helminth eggs (3 viable eggs g^{-1} type Strongyloidea) than the poultry litter (1 viable egg g^{-1} type Strongyloidea). Horses are parasitized by more than 90 species of helminths, and the eggs of these parasites are excreted in the feces (Roberts and Janovy Junior 2009). When verifying the prevalence of parasites in horses, Godéski and Pedrassani (2018) found that all the studied animals presented helminth eggs in their parasitological stool exams. However, the parasitic load was considered low (102 ± 228 eggs g^{-1} of feces). Salas-Romero et al. (2017) observed an elevated parasitic load when evaluating the excretion of helminth eggs in horses, with an average of 1,436 eggs g^{-1} of feces. Melo et al. (2019) stated that helminths are commonly reported in gastrointestinal tract infections in wild birds, releasing eggs next to the hosts' feces (Bowman 2014).

Helminth eggs were not detected in the samples on the 32nd day in any of the beddings. In the organic poultry litter, one viable egg g^{-1} type Strongyloidea was detected at 60 days, with effective helminth egg elimination only at 90 days. Likewise, the final composts showed no contamination by helminth eggs, possibly due to their lower amount in the fresh residue and the temperature above 60°C during composting. Temperatures above 55ºC for three days are adequate for eliminating helminth eggs (Wichuk and Mccartney 2007).

Thus, in the present study, the composting process was effective in eliminating helminth eggs. Similarly, Heck et al. (2013) described an absence of *Salmonella* and helminth eggs when studying the microbiological quality of a final compost comprised of solid residues. Corrêa et al. (2007) also reported a reduction rate between 93% and 100% in the number of helminth eggs in composted sewage sludge samples. In addition, our results corroborate the findings obtained by Zhu et al. (2004), who observed the elimination of helminth eggs after 63 days of composting swine manure.

Although the composts in the present study followed MAPA regulations regarding the attributes *Salmonella* sp. and helminth eggs, the most probable number (MPN) of thermotolerant coliforms showed a higher count than that recommended by the legislation, suggesting that composting alone, with reduced piles, is not enough to decrease the microbial load up to the recommended limit. However, it should be noted that the size of the pile interferes with the heat exchange with the environment, influencing its temperature. Thus, more massive piles reduce the surface exposed to the environment and, consequently, increase the sanitizing efficiency of the composting process.

Although animal waste is a sustainable alternative in agriculture, it needs to be environmentally safe. Therefore, it is necessary to use methods that guarantee its sanitation. Ensuring quality and safety in the use of agricultural wastes is essential to prevent the spread of pathogenic bacteria and, consequently, a One Health issue. Thus, more studies are needed to evaluate the use of additional techniques that guarantee the reduction of thermotolerant coliforms up to the maximum limit established for organic agriculture, such as solarization (Ozdemir et al. 2020).

Conclusion

Composting provides a significant reduction in the thermotolerant coliform populations in horse beddings and organic and conventional poultry litter. However, the final composts exhibited a higher microbial load than required by the current Brazilian legislation for organic farming.

The *Salmonella* genus was absent in the raw wastes and throughout the composting period.

The composting process was effective in eliminating viable helminth eggs from all the analyzed animal wastes.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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