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Original Article

Three Months of Composting Are Enough to Eliminate the Fowl Typhoid Bacteria

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ABSTRACT

The composting technique has been increasingly highlighted in poultry production units, as an efficient and low-cost solution for the destination of carcasses. The process is based on the accelerated decomposition of organic material under high temperatures, associated with eliminating pathogenic microorganisms. This study aims to evaluate the effectiveness and the time necessary for the elimination of *Salmonella* Gallinarum in carcasses of poultry submitted to the composting process. The composting was carried out following the models used in the field, and microbiological analysis was performed in five different periods: 45, 90, 120, 150 and 180-days after closing the composter. After 90 days of experiment and in the subsequent analysis, the elimination of the bacteria in 100% of the samples was verified, validating the composting process as an effective method for eliminating *S*. Gallinarum in poultry carcasses, when respecting the period necessary for the elimination of the bacteria and the good quality of the structure adopted for the process.

INTRODUCTION

Fowl typhoid is an avian disease characterized by high mortality in mature flocks from broilers and semi-heavy laying hens (Oliveira *et al.*, 2005; Neto *et al.*, 2007). In general, the infection occurs orally: the bacteria crosses through the gastrointestinal pathway and evade the immune system due to its capacity to settle within avian macrophages (Huang *et al.*, 2020), triggering a systemic infection, with a focus on the liver and spleen (Barrow & Neto, 2011; Ojima *et al.*, 2021). Because of the systemic behaviour of infection, the carcasses of poultry stricken by fowl typhoid become a source of infection to those healthy ones (Berchieri *et al.*, 2000).

Throughout the poultry production chain, the solid waste obtained is composed of feed, litter with excreta, incubation scraps, and chicken carcass (Costa *et al.*, 2017), which are a source of pathogenic bacteria, endangering the triad human-animals-environment (Chiarelotto *et al.*, 2021). Thus, as a strategy for eliminating and inactivating pathogens, several procedures can be performed, such as alkaline hydrolysis, anaerobic digestion, incineration, and composting (Staroń *et al.*, 2017; Arias *et al.*, 2018; Avidov *et al.*, 2021), preventing the spread of diseases and environmental contamination (Berge *et al.*, 2009).

Composting is an easy method to perform and allows obtaining compound environmentally friendly (Singh *et al.*, 2018), which is generated due to the activity of aerobic microorganisms (Prabakaran & Valavan, 2021), and reducing the risk of nitrogen and phosphorus compounds spreading to water systems (Singh *et al.*, 2018). Therefore, in view of the correct destination of poultry residues, to minimize the environmental impact and the epidemiology of avian diseases, the present study aimed to evaluate the efficiency of the composting process in the elimination of *Salmonella* Gallinarum from bird carcasses that died as a result of fowl typhoid.

MATERIAL AND METHODS

The study was conducted at the Laboratory of Avian Pathology of the Department of Animal Pathology from the School of Agricultural and Veterinary Sciences (FCAV/UNESP), Jaboticabal, Brazil. It was carried out following the Ethical Principles on Animal Experimentation approved by the internal Ethical Committee on the Use of Animals (Process n° 017354/18).

The composting process has been performed in five 60-liter plastic barrels (Plastienvases®, Mexico) with 695 mm height x 315 mm length x 310 mm width and with removable cover, and their structural arrangement following the distribution of layers described by (Irfan *et al.*, 2020): sawdust substrate, poultry litter, poultry carcasses, poultry litter, sawdust substrate, and water (Figure 1). The proportion of each layer is as follows: 1:10 part by weight of sawdust substrate, 3 parts by weight of poultry litter, 1 part by weight of poultry carcass, and 1/2 part by weight of water. The distribution pattern in the order described was successively repeated until the complete filling of the barrel, which was sealed, with no exposure of the material to the environment, vectors, or leakage of content, until its opening for sample collection. The carcasses used were from semi-heavy lineage from commercial laying chickens 22 weeks old, which died after an experimental infection by *S*. Gallinarum carried out within controlled environmental rooms (Rubio *et al.*, 2021).

Microbiological analyses were performed at five different times: 45-, 90-, 120-, 150- and 180-days after closing the composter barrels. In each of these periods, 7 samples (5 g each) were randomly collected from each of the layers present in the barrels, totalizing 35 samples. All samples were placed into conical bottom tubes (Thermo Scientific™, Brazil) and led to the laboratory for immediate analysis.

Microbiological analyses were performed immediately after sampling at the end of each experimental period. Samples were homogenized directly into selenite broth supplemented with 0.4% of novobiocin (1:9). Then, each sample was plated on Brilliant Green agar containing nalidixic acid sodium as done previously (Rodrigues Alves *et al.*, 2018). After 24h of incubation, visual identification of characteristic colonies for the biovar *S*. Gallinarum on Brilliant Green agar plates was performed, followed by biochemical and serological confirmation (Grimont & Weill, 2007; Brenner & Farmer, 2015).

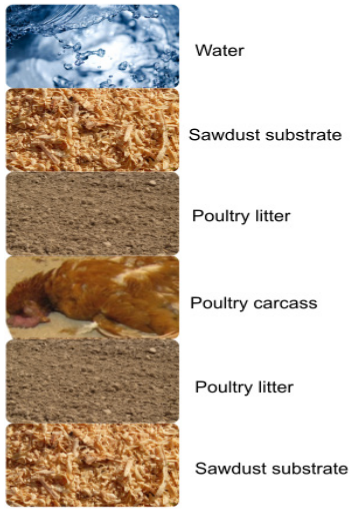

Figure 1 – Distribution of layers: sawdust, poultry litter, carcass, and water, in the compost barrels used in this study.

RESULTS AND DISCUSSION

From all 35 samples collected from five experimental days (Figure 2), the presence of *S*. Gallinarum was identified in only 1/7 (14.29%) from the first experimental day (45 days of composting), which came from the bottom of the compost barrel, where the drained liquid accumulates of the process (slurry). In the following experimental periods (90-, 120-, 150, and 180-days), no bacteria were identified on the collected samples (0/28).

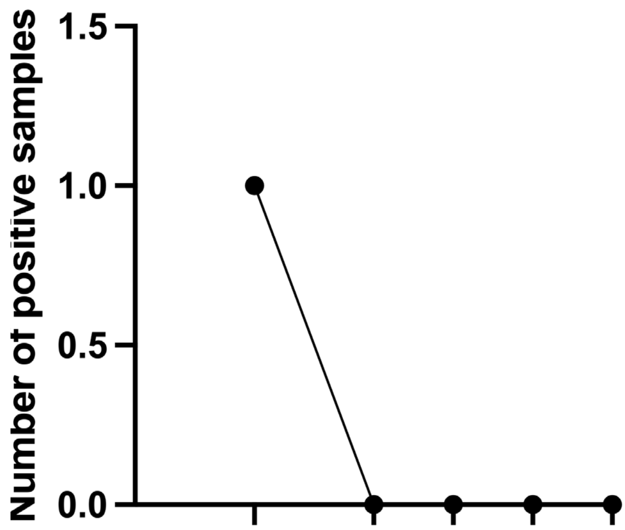

Figure 2 – Number of positive samples from composting barrels at different collection days.

Composting can be done on-farm and is applicable in cases of mass animal deaths avoiding the spread of diseases (Wilkinson, 2006). Besides, it is considered a mitigation method of pathogens for the broiler production chain (Chiarelotto *et al.*, 2021), in addition to promoting the biological conversion of carcasses into useful fertilizers products (Melo *et al.*, 2022).

The correct management in poultry farms is the most effective measure used to action control fowl typhoid disease (Celis-Estupiñan *et al.*, 2017). Standards regarding temperature, pressure, and humidity should be well-defined in the biosecurity measures when aiming for the complete elimination of pathogenic bacteria (Vaddella *et al.*, 2016).

However, concerning the composting process, the variety of protocols used are divergent in the disposal of waste, litter material used, mixing or not of the material, and the opening or not of the biodigester during the process, which may justify the variety of results previously reported. (Orrico *et al.*, 2010; Pandey *et al.*, 2016; Vaddella *et al.*, 2016). Another important factor focusing on eliminating pathogens is the period in which the composing was closed, although the efficiency period is directly linked with the temperatures reached and their maintenance during the process of pathogens elimination (Vaddella *et al.*, 2016). Despite no information on the temperature from the barrels having been recorded after the opening, our results showed that 90-days after the composting was closed was enough to completely eliminate *Salmonella* Gallinarum.

Divergent results regarding the minimum period necessary for the complete elimination of bacteria from *Salmonella* genus were reported using different methodologies. Orritco *et al.* (2010) and Esperón *et al.* (2020), needed 100-120 days after closing the compost, while in the Paiva *et al.* (2011) and Pandey *et al.* (2016) experiments, bacteria were eliminated after 20- and 8-days, respectively. These divergences reinforce the need for a standard protocol for composting poultry carcasses since the main source of both contamination and permanence of *S.* Gallinarum in the poultry industry is the management in which the carcasses are improperly discarded or that they are not removed from the sheds (Oliveira *et al.*, 2005).

Despite only one positive sample having been recovered in the present study, it has come from the last layer of 45-days closed barrel, in the slurry accumulates, which highlights another composting concern: the structure of the composter. This liquid has a high concentration of organic compounds and contains highly soluble substances, it can contaminate groundwater and, consequently, the environment around the property and other crops that may be intended for human consumption (Vadella *et al.*, 2016).

Vaz *et al.* (2019) described how this kind of environment can be propitious to the exchange of antimicrobial resistance elements between the bacteria present in that liquid when extravasated from the composter, accelerating both microbial adaptation and potentially leading to future problems involving the control of these pathogens. This reinforces the need to maintain correct composting sealing, to avoid the leakage of contaminated liquids into the environment, and to ensure the permanence time of the residues in the composter is respected.

To our knowledge, the composting process proved to be an effective method for eliminating *S.* Gallinarum in poultry carcasses. However, it is necessary to respect the period of completely closing the composter recipient to avoid environmental contamination by pathogenic microorganisms. In addition, the rigid implementation of the guidelines for the composting process can be safely adopted in poultry farms to present a financial return to the farmer without causing environmental impacts.

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