Online ISSN: 2251-7715 Print ISSN: 2195-3228

Dates:

Received: 12 Nov 2024

Revised: 24 Nov 2024

Accepted: 18 May 2026

DOI: 10.57647/ijrowa.fzz7.dd0188

ORIGINAL RESEARCH

Assessment of Composting Process for The Degradation of Ivermectin Residues and Elimination of Parasite Eggs in Equine Manure

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ABSTRACT

Purpose: this study aimed to evaluate the fate of veterinary pharmaceutical residues, such as ivermectin (IVM), and their impact on parasitic forms during and after composting of equine feces mixed with straw.

Method: in this study, composting trials were conducted using equine fecal matter added with IVM under controlled conditions. Physicochemical parameters of the composting process, including temperature, pH, electrical conductivity, and extractable phosphorus, were monitored over a 120-day period. High performance liquid chromatography (HPLC) was employed to quantify IVM residues in compost samples. Additionally, parasitological assessments were performed to evaluate the elimination of gastrointestinal nematodes (GIN) eggs during composting.

Results: IVM residues persisted throughout the composting period, albeit with decreasing concentrations over time. The composting process influenced physicochemical parameters, such as pH and electrical conductivity, with variations observed in response to straw additions and microbial activity. Parasitological analysis revealed the presence of GIN eggs, predominantly belonging to the *Strongylus* genus, up to day 60 of composting. However, larval development from these eggs was not observed beyond day 30, suggesting limited viability under composting conditions.

Conclusion: overall, composting reduced IVM concentrations and compromised GIN eggs viability. However, complete elimination of the compound was not achieved within the time of the study. These findings highlight the importance of further research to optimize composting strategies for pharmaceutical residue degradation and parasite control thereby enhancing the safety and efficacy of composted manure in agricultural applications.

Key words: Organic farms, Compost, Anthelmintic drug, Feces

1. Introduction

Manure is utilized worldwide as soil amendment and as a stimulant in crop production (Chaudhari et al., 2021). Its safe use implies composting before its application to the soil (Bernal et al, 2017). Compared to the soil environment, in the manure, the various species of micro-organisms present produce a wide variety of biochemical reactions, determining the rising of temperature and the transformation of the manure substances. This, in turn, leads to the death of both plant and animal pathogens (i.e., harmful bacteria and parasites, responsible for promoting food-borne diseases when vegetables are growing on fresh or incompletely composted manure) and the reduction of seed viability present in the manure (Sunar et al., 2014). However, pathogens are not the only concern in manure composting. Many medicinal drugs administered as veterinary pharmaceutical products in clinical practice are not rapidly or completely metabolized and are excreted in animal feces (Haseler et al., 2024). For these particular drugs, their fate in compost is a matter of concern. In recent years, composting process has been adopted as a strategy for the biodegradation/bioremediation of antibiotics, steroids, and antiparasitic agents (Hakk et al., 2005; Arikan et al., 2007; Dolliver et al., 2008).

In most parts of the world, there are only three drug families of anthelmintic agents available for the treatment of nematode parasites in horses: benzimidazoles, tetrahydropyrimidines, and macrocyclic lactones. Benzimidazoles act by disrupting microtubule formation through binding to β-tubulin, whereas tetrahydropyrimidines act as nicotinic acetylcholine receptor agonists, inducing spastic paralysis of nematodes The most recent class is the macrocyclic lactones, introduced in the 1980s (Nielsen et al., 2022). These compounds are highly lipophilic and, after their administration, are stored in animal adipose tissue from where they are slowly released, scarcely metabolized, and excreted mainly in feces (Taylor, 2001), determining their prolonged presence and in high concentrations in the fresh feces that will undergo the composting process. In this direction, it was found that orally administered IVM to horses remained detectable in manure for 40 days after treatment (Pérez et al., 2001). In addition, following oral administration at 200 μg/ kg in horses, ivermectin, doramectin and moxidectin reached peak dry-fecal concentrations of 19.5, 20.5 and 16.6 μg/ g respectively, at 24 h post-treatment, and were detectable in faeces for up to 8 days (Gokbulut et al., 2010). Similarly, other macrocyclic lactones such as doramectin have shown high peak faecal concentrations and prolonged persistence after oral administration in horses, with detectable residues reported for extended periods, depending on the administration route (Pérez et al., 2001). However, macrocyclic lactones are susceptible to a slow aerobic biodegradation under suitable soil conditions (Halley et al., 1993).

Despite this potential for aerobic degradation in soils, considerably less information is available regarding the behavior of IVM during composting of equine manure. Composting involves complex microbial succession, fluctuating temperatures, and high organic matter content, all of which may influence the persistence, transformation, or stabilization of macrocyclic lactones (Krogh et al., 2008). Understanding whether composting effectively reduces IVM concentrations under controlled management conditions remains essential for assessing the environmental safety of composted horse manure.

On the other hand, composting is a well-established technology for pathogen reduction (Lepesteur,2022). Composting process requires a specific time-temperature combination (within a given range) to ensure compost disinfection (Wichuk and McCartney, 2007). For helminths, reaching a temperature of 55°C for five days is sufficient for their inactivation (Koné *et al.*, 2007). However, excessively high temperatures can inhibit the growth of the microorganisms found in the decomposing substratum, even those needed for the composting process, thus slowing down organic matter decomposition (Miyatake and Iwabuchi 2005). Nevertheless, temperature is not the only factor involved in the viability of such pathogens; the combined effect with other factors, such as pathogen-to-microflora ratio, or ammonia volatilization, contributes to helminth elimination (Manga et al., 2016).

In equine production systems, manure commonly contains eggs of gastrointestinal nematodes, particularly strongylids, which may persist in the environment under favorable moisture and temperature conditions. Therefore, evaluating egg viability throughout the composting process is necessary to determine whether the resulting compost can be safely applied to agricultural soils without contributing to parasite transmission.

In this context, and considering the increasing use of composted equine manure in agricultural systems, it is necessary to evaluate both the persistence of IVM residues and the fate of parasitic forms during composting. Therefore, the aim of this study was to assess the behavior of IVM and the viability of gastrointestinal nematode eggs throughout a 120-day composting process of equine manure mixed with oat straw under controlled conditions.

2. Materials and Methods

2.1. Source of the composted material: collection, processing and laboratory analysis

Equine fecal matter (FM) was collected from the experimental farm of SINTEX S.A., located at 118 km, National Route 74, Buenos Aires province.

The experimental composting process and parasitological determinations were conducted at the Parasitology and Parasitic Diseases Laboratory, Animal Health and Preventive Medicine Department (SAMP-CISAPA), School of Veterinary Sciences, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Tandil, Buenos Aires.

The quantification of IVM in compost samples was carried out at CONICET-IPROBYQ Laboratory, Chemical Technology Area, School of Biochemical and Pharmaceutical Sciences, Universidad Nacional de Rosario (UNR), Rosario, Santa Fe.

Compost physicochemical characteristics were determined at the Soil Testing Laboratory, College of Agronomy, UNCPBA, Azul, Buenos Aires.

Detailed descriptions of the analytical and physicochemical methodologies are provided in the corresponding sections below.

2.2. Preparation of the experimental devices

Six 200-liter drums were used, each considered as an experimental unit. They were conditioned as compost drums under simulated field conditions. Throughout the 120-day trial, they were kept under a roof, protected from rain and direct sunlight. Additionally, they were covered with a mesh to prevent birds or other animals from entering.

2.3. Collection of fecal samples

FM was obtained from pregnant mares owned by Sintex S.A.; animals had not received any deworming treatment for at least two months prior to starting the trial, a sufficient time to ensure an initial compost mix free of deworming compounds. The FM was manually extracted from the rectum, avoiding contamination with elements present in the soil that could hinder any of the physical, chemical or microbiological parameters of the composted material.

2.4. Chemical reagents and addition of the FM

IVM commercial formulation (1%, Rosenbusch®, Series 113) was used to prepare the IVM stock solution. IVM was added to FM to achieve a concentration of 2,000 ng/g, a concentration reported in the literature as present in feces of IVM-treated horses (Pérez et al., 2001). The IVM-added FM was prepared as follows: feces of experimental drums were fractionated in 5-kg portions, and 100 ml of the IVM stock solution were added and mixed with an electric blender to ensure homogenization. FM of control drums was prepared in the same way, but replacing IVM stock solution with distilled water in the same volume.

2.5. Assembly of experimental units

Each drum was filled with 50 kg of equine FM (3 control drums with IVM-free FM and 3 experimental drums with IVM-added FM) along with the same volume of oat straw. For every 10 kg of FM, the same volume of straw was added and mixed until a homogeneous mass was obtained and transferred to the corresponding drum. At the

beginning of the trial, electronic sensors (Datalogger Hygrochron iButton ® Technology, Whitewater, WI, USA) were placed in each drum to register temperature and humidity.

2.6. Sampling frequency and methodology

For the physicochemical characterization of the compost, samples were taken at 30, 60, and 90 days after the start of the trial. One kg of composting material was collected from each drum to determine the carbon:nitrogen (C: N) ratio, pH, electrical conductivity (EC), ammonium:nitrate ($N\text{-}NH_4^+$: $N\text{-}NO_3^-$) ratio, and extractable phosphorus (P).

Before mixing for composting, IVM concentration was determined in FM samples. Then on days 3, 30, 60, 90, and 120, each drum was sampling at three different levels: surface, middle and bottom. At each level, five samples, equally spaced to each other, were taken. Hence, a total of 15 samples of the composting mass were collected from each drum to determine IVM concentration during the composting process.

For parasitological determinations, 20 samples were taken on day 0. Seven samples from each drum were taken on days 3, 30, 60, and 90 for egg per gram (EPG) counting and larval culture.

2.7. Determination of the physicochemical characteristics of the compost produced

The various stability indicators of the substrates were teste on air-dried samples in duplicate. Parameters related to N such as total N (Kjeldahl method, semi-micro scale, SAMLA 2004), $N\text{-}NO_3^-$ (Wetselaar et al., 1998) and $N\text{-}NH_4^+$ (Bremner and Keeney, 1966) were quantified in 2% KCl and 1N extracts, respectively. Subsequently, the $N\text{-}NH_4^+$: $N\text{-}NO_3^-$ ratio was calculated as an indicator of maturity. Total C was measured as loss on ignition using a muffle furnace (Martínez and Ardón, 2021). The C: N ratio of the substrates was calculated based on the total content of both elements. The compost reaction (pH) and the EC were determined on 1:5 water suspensions (Martínez and Ardón 2021). Extractable P was quantified using the Bray I method (Bray and Kurtz, 1945).

2.8. Extraction of IVM from compost and analysis by HPLC

The method validation for the physicochemical extraction and HPLC identification and quantification of IVM in the compost represented the technical-analytical foundation required to carry out the study. Analytical standards of IVM (97.3% purity; Vetpharma Salud Animal) and MXD (97.9% purity; Vetpharma Animal Health) were used for the preparation of stock and working solutions. Each standards was solubilized in HPLC-grade methanol (MeOH) to obtain a stock solution of 1 mg/ml. From this, successive dilutions of 1:10 in MeOH were made to obtain different concentrations used for injection into the chromatographic system. Once prepared, the dilutions were stored at -18°C and protected from light until used.

The IVM quantification in compost was performed by high-performance liquid chromatography (HPLC). The chemical extraction process from compost samples was performed using the technique described by Lifschitz et al., (2000). One gram of compost samples was homogenized, added with the internal standard (moxidectin, MXD) and let stand at room temperature. After 20 minutes, 1 ml of acetonitrile was added and shaken (Multi Tube Vortexer, VWR Scientific Products, West Chester, PA, USA) for 15 minutes. Subsequently, samples were centrifuged for 20 minutes at 1300 x g. The supernatant was transferred to another tube and the extraction process was repeated. The total supernatant was balanced with distilled water and subjected to solid-phase extraction. Firstly, it was injected into a C18 cartridge (Strata, Phenomenex, CA, USA) mounted on a Vacuum Manifold (Phenomenex, USA). The C18 cartridge was preconditioned by passing 2 ml of MeOH and 2 ml of distilled water. Then, the sample supernatant was added and impurities were removed by passing HPLC-grade distilled water (1 ml) and water/MeOH (4:1, 1 ml). The cartridge was dried for 3 minutes by increasing the pressure vacuum. For elution, 1.5 ml of HPLC MeOH was passed to each cartridge and collected. The eluted samples were evaporated to dryness under a nitrogen flow at 56°C for 30 minutes.

After evaporation to dryness, the eluate residue was derivatized to convert IVM and the internal standard molecules into their fluorescent forms. For this, 100 µl of a solution of N-methylimidazole (Sigma Chemical, St Louis, MO, USA) in acetonitrile (1:1) and 150 µl of trifluoroacetic acid solution (Sigma Chemical, St Louis, MO, USA) in acetonitrile (1:2) were used. After the reaction was completed, an aliquot of the resulting solution was

injected directly into the chromatographic equipment (Dionex Ultimate 3000, Thermo Scientific Inc). The molecules were detected using a fluorescence detector (FLD-3100 PMT), with excitation and emission wavelengths of 365 nm and 475 nm, respectively. The mobile phase consisted of an aqueous solution of acetic acid, MeOH, and acetonitrile (4/32/64). A C18 column (Kromasil, Eka Chemicals, Bohus, Sweden, 5 µm, 4.6 mm x 250 mm) was used with a flow rate of 1.5 ml/min. The data were processed using the Chromeleon program (Version 7.2 SR4).

The analytical methodologies used for the extraction and quantification of IVM in equine FM and compost were validated by determining the following parameters: limit of quantification, linearity, precision, and accuracy of the method. The respective calibration curves were determined using a least squares linear regression analysis and correlation coefficients (r) and coefficients of variation (CV) were calculated. Each calibration curve was used to determine the drug concentrations in the experimental samples using the equation of the line: $y = a\,x + b$ where a: slope of the calibration curve, b: intercept of the calibration curve on the ordinate axis, x: observed concentration, and y: detector response expressed as chromatographic area. The ratio between the areas under the chromatographic peak of IVM (analyte) and MXD (internal standard) was used to determine the concentration of IVM in the experimental samples.

The identification of IVM and MXD was based on the retention times of reference standards of analytical purity. The calibration curve constructed by the least squares linear regression method showed determination coefficients > 0.995.

Recovery percentages evaluate the efficiency of analyte extraction from biological matrices. They were calculated by comparing the areas (or area ratios) obtained after analyzing samples of the different matrices spiked with analytical standards, achieving concentrations equal to those obtained from chromatographic analysis of standards prepared in the mobile phase, MeOH, or ACN (without prior extraction). The equation used was as follows: % recovery = (area of standard in biological matrix) / (area of standard in mobile phase) x 100. In all cases, they were above 67% compared to the corresponding standards in the mobile phase.

2.9. Nematode Egg Count per Gram (EPG) of Fecal Matter

The Modified McMaster technique (Roberts and O'Sullivan, 1950) was used. From each compost sample, 3 g were weighed and 57 mL of saturated NaCl solution (density 1.2 g/mL) were added. The compost sample was disaggregated and filtered through a sieve and the resulting mixture, after agitation (avoiding bubble formation), was loaded into 2 McMaster counting chambers. The sensitivity of the technique was 20 eggs per gram. Subsequently, observation and counting of GIN eggs were carried out under an optical microscope at a magnification of 40 X.

2.10. Larval culture and recovery

Individual cultures were conducted for each of the compost samples taken. The Henriksen and Korsholm (1983) technique was employed. For this, 10 grams of sample were taken and placed in the base of a plastic cup cut in half. Before cultivation, the base of the cup was perforated many times by using a needle. Then, it was covered with a gauze and attached to the other half of the cup, ensuring it remained firm to hold the material to be cultivated. This device was placed into a second cup filled with water and cultivated in a climatic chamber for 14 days.

After the 14-day incubation period, the top part of the device was removed and placed into a conical glass filled with warm water. After 24 hours, the concentrated infective larvae settled at the bottom, and they were preserved in the refrigerator until reading. For identification, the key developed by Santos et al. (2018), was used.

2.11. Statistical analysis

Data were expressed as average ± SEM. Data normality was assayed using Brown-Forsythe and Bartlett´s test. Kruskal Wallis along Dunn Multiple Comparison or One-Way ANOVA along with Dunnett´s Multiple Comparison post hoc test was used to compare differences between experimental groups. *P* values less than 0.05

were considered statistically significant. Data were analyzed by the GraphPad Prism 8.0.2 software (GraphPad Software Inc.).

3. Results and Discussion

The temperature data were recorded over the five-month duration of the trial. Figure 1 shows that the highest temperature reached by the system was 34ºC, while the lowest was 10ºC. Most of the time, flipping or turning the material was followed by a slight increase in temperature, likely due to improved oxygen availability.

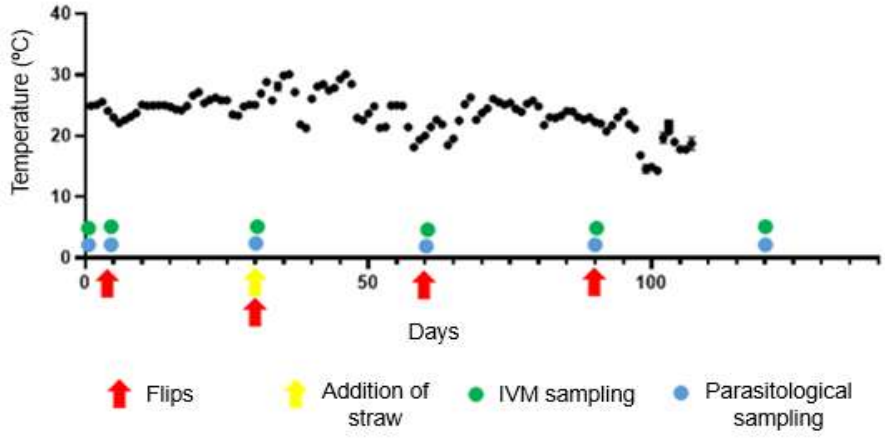

Fig. 1. Evolution of temperature during the composting process of equine fecal matter:oat straw mixture. The flips, addition of straw, and sampling times for parasitological and IVM quantification analyses are indicated by arrows and filled circles

A vertical composting system was selected due to its intrinsic advantage of allowing constant addition of fresh material. This advantage was actively exploited on day 30 of the trial by incorporating additional oat straw into the system to induce a temperature increase (Fig. 1, yellow arrow). Upon initiation of the process, an increase in system temperature is expected due to the activity of microorganisms (Mengqi et al., 2023) on the mixture of horse manure with oat straw. Fig. 1 shows that the temperature did not rise above 34°C, possibly due to high humidity in the system, and eventually difficult oxygen diffusion, causing microorganisms to decrease their metabolic activity (Escudero de Fonseca and Arias Villamizar, 2012). Another possible explanation is the low C:N ratio found at the beginning of the composting of the mixture of horse manure and oat straw. This could have caused a limitation in the provision of C for decomposing microorganisms (Ji et al., 2023). After sampling on day 30, where more straw was added to the system, an increase in the C:N ratio was observed on day 60 of the experiment. This intervention in the process led to an improvement in decomposition levels through increasing the C source (Meng et al., 2021; Ji *et al.*, 2023), but did not determine a temperature increase in the system. The issue of controlling the operating temperature of compost within the mesophilic or thermophilic range is controversial in the literature (Saludes et al., 2007). Some authors suggest that the optimal temperature is over 60°C, and this threshold is needed for pathogen and seed inhibition (Nakasaki et al., 1985; Schulze, 1962). However, another research has indicated that lower temperatures could allow greater microbial activity in the system (Suler and Finstein, 1977; McKinley and Vestal 1984; Miyatake & Iwabuchi 2005. While the temperature does not rise to achieve the thermophilic stage of the process, it does not prevent compost generation (Pezo Jácome and Barrezueta-Unda, 2023). The C: N ratios ranged from 20:1 to 26:1 at the beginning of the decomposition of the mixture of equine manure and oat straw (Fig. 2A), a value that can initially be considered moderately suitable to slightly marginal. This situation was rectified by adding oat straw after the 30-day sampling, which determined a moderate increase in the C: N ratio at day 60 of the experiment, reaching an average of 44.95:1 (±4,31) in the IVM-added experimental units. In this same sampling time, control units also showed a significant increase in the C: N ratio compared to the previous sampling time (31.6 ±2,88). This intervention (addition of a carbon source) in the composting process determined an improvement in the composting of the original material, leading to a

decrease in the C: N ratio in the subsequent sampling (90 days), being significant in the experimental units with IVM. The final value in the C: N ratio (between 33.1 and 16.1, control and IVM, respectively) suggests that the latter units would once again be limited by the carbon availability in their decomposition capacity at the end of the experiment.

Overall, the experimental units showed a pH ranging from moderate to strongly alkaline, which could be associated with ammonia production at the beginning of the experiment, as seen in the units added with IVM (Fig. 2B), showing a high $N\text{-}NH_4^+/N\text{-}NO_3^-$ ratio (Fig. 2E). This correlation between a high $N\text{-}NH_4^+/N\text{-}NO_3^-$ ratio and pH in the samples was not observed in the last sampling (Figs. 2B, 2E). The behavior of pH in the composting process showed an increase due to the formation of $N\text{-}NH_4^+$, from the degradation of organic nitrogenous material (Jian *et al.,* 2015; Montalvo *et al.,* 2018). Convergence towards neutrality indicates compost maturation, where microorganisms stabilize acidity and balance the release of decomposition products (Zainudin et al., 2022; Quadar et al., 2022).

The EC, a salinity estimator, could increase during the composting process due to mineralization of organic matter, a fact that leads to an increase in nutrient concentration (Sánchez-Monedero et al., 2001). Fig. 2C shows a sustained increase in EC in control units, while, in the IVM-added drums, EC loses salinity almost to its initial concentration, possibly due to increased moisture (Guzmán Anaya, 2018). The EC values (dS.m-1), showed a sustained increase in the control units, while in the IVM-added units, it fluctuated with a peak at day 60 of the experiment (Fig. 2C). These values suggest that decomposition ranged from moderate to strong, with a sustained increase in the catabolic activity in the control units. This observation correlates with the very low C: N ratio observed in the last sampling in the units with IVM, inferring the hindering of the decomposition due to C restraints.

The available P content expressed as mg $P.kg^{-1}$ extracted by an acidic medium showed a downward trend in almost all samplings and for both experimental groups, suggesting its conversion into recalcitrant organic forms, not extractable in the medium used (Fig. 2D).

The composting efficiencies achieved by control and experimental units were statistically non-significant after performing a Student t-test analysis (p=0.96) (Fig. 2F). The average (±SEM) for the control drums was 5.84% (±0,94) and for the IVM-added drums, it was 5.78% (±0,74).

While macrocyclic lactones undergo some metabolism, most are excreted unchanged in feces mainly via bile (Gwaltney-Brant et al., 2018; Liebig et al., 2010; Verdú et al., 2015). The horses used in this trial did not receive anthelmintic treatment for 90 days prior to its initiation, a sufficient time to ensure the absence of residues in fecal matter that could interfere with detection by HPLC. According to the literature reviewed, IVM is eliminated through the feces of animals administered orally or subcutaneously over a period that can extend up to 40 days (Cook et al., 1996; Pérez et al*.,* 2001; Pérez-Cogollo et al*.,* 2017). This study simulated the natural conditions in which feces from horses administered with a therapeutic dose of IVM, hence presenting IVM residues (Pérez et al., 2001) are added for compost formation. The IVM molecule was detected and quantified throughout the entire trial, which spanned for 120 days. Additionally, the molecule was identified at different depths of the compost drums (Figs. 3A, B, C).

The variation in IVM concentration in each treated drum is presented in Figures 3A, B and C. The initial IVM concentration was 1716 ng/g (± 416,7) of equine fecal matter: oat straw mixture. Additionally, IVM was not detected in the samples from the control group. Sample chromatograms are represented in Figure 3D. The IVM concentration varied between sampling levels in each treated drum, being higher at the surface, followed by the middle and finally by the bottom level. Figures 3B and C show that in both drums the concentration of IVM decreased in the first sampling, but then shows an increase in concentration. Conversely, in drum A, concentrations increase after the first sampling. An increase in IVM concentration was observed until the third sampling, after which it tends to decrease.

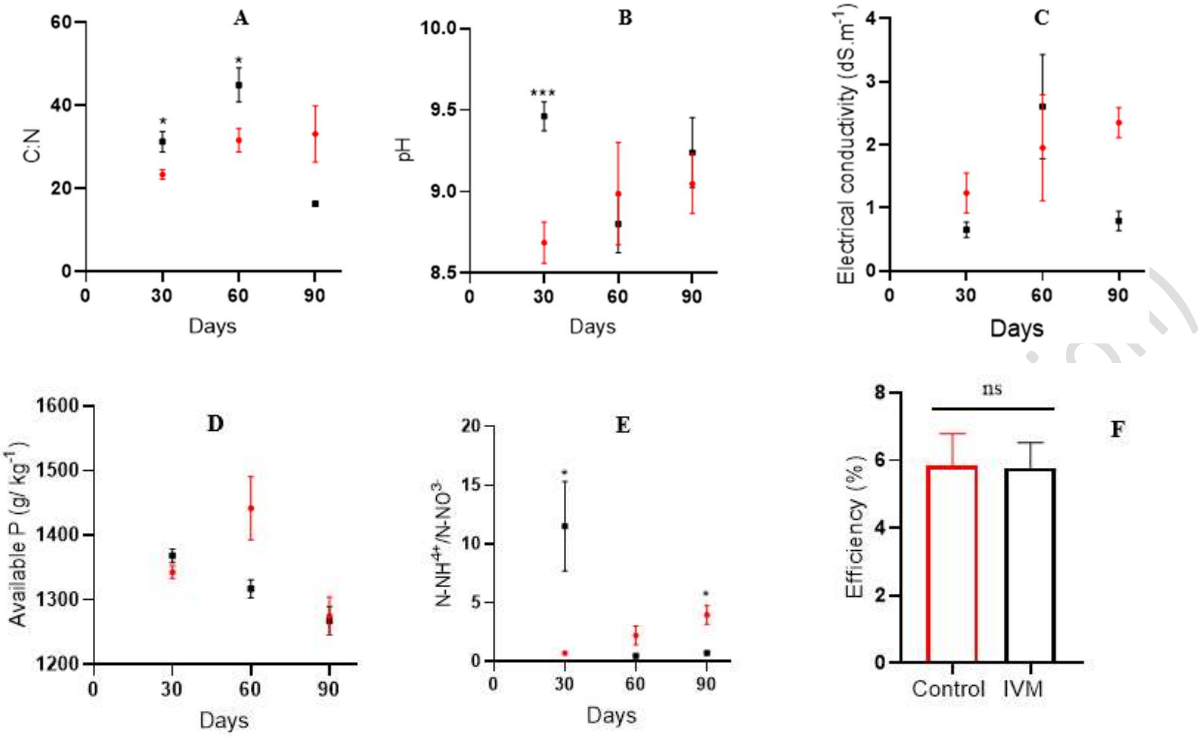

Fig. 2. Red: control / Black: treatment units. **A** Evolution of the C:N ratio during the equine manure:oat straw composting experiment. **B** pH variation during the equine manure:oat straw composting experiment. **C** EC, expressed as dS/m, during the equine manure:oat straw composting experiment. **D** P, expressed g/Kg^{-1}, during the equine manure:oat straw composting experiment. **E** N-NH_4^+/N-NH_3^- ratio during the equine manure:oat straw composting experiment. **F** Efficiency of compost sieved, expressed as percentage, in control and IVM-added drums. Each bar represents the mean ± SEM efficiency percentage. Control vs. IVM differences were statistically not significant (ns)

Given the activity of microorganisms, when optimal temperatures for the composting process are reached, the destruction of fecal matter pathogens occurs (Bonhotal et al., 2006). However, these are not the only concerns of the composting process. Various drugs used in veterinary practice are excreted through fecal matter, including IVM (Iglesias et al., 2006; Moreno-Morales et al., 2014; Pérez-Cogollo et al., 2017). For this reason, it was decided to quantify the levels of this active ingredient in composted equine manure, as soil restoration practices involving the use of livestock manure to improve fertility are increasingly common.

The present results are relevant in the use of compost as an organic amendment for crops because the transfer of this compound to plants has been confirmed and its concentrations are detected during the period of plant development (Iglesias et al., 2022). The degradation rate of IVM in stored fecal matter or soil, indoors and at room temperature, is slow, with half-lives between 93 and 240 days. The same situation occurs during winter when exposed outdoors. In contrast, when exposed to summer weather. IVM present in mixtures degrades rapidly, with half-lives of 1 to 2 weeks (Halley et al*.,* 1993; Boxal et al., 2003). The dissipation behavior of avermectins is affected by environmental conditions, primarily temperature, water content, pH, exposure to ultraviolet light and oxygen (Wohde et al*.*, 2016).

The duration of the experiment in this work was of 120 days and in all the samples collected throughout the experimental time, it was possible to detect the IVM molecule in the added drums, possibly because IVM is a highly adsorbed, binding to manure, and its loss through water is unlikely (Oppel et al., 2004). A high octanol-water partition coefficient (Kow = 1,651), a high organic carbon binding constant (Koc = 12,600-15,700) and a low water solubility are characteristic properties of IVM, determining its high affinity for organic components of

soil and fecal matter. Hence, IVM is firmly bound to soil and fecal matter (Halley et al., 1993; Carbonell-Martin et al., 2011).

A study conducted by Schwarz and Bonhotal (2015) demonstrated that the composting process is capable of reducing the concentration of IVM in fecal matter from horses. Over the six-month trial period, the presence of the molecule was quantified as 0.06 mg/kg on day 175. This means that although the composting process affects the concentration of IVM, it is not sufficient to completely downgrade it. The same result was obtained in the present work, where IVM concentration decreases over time, but it was not completely eliminated during the evaluated period. These results are consistent with those found by Halley et al., (1993), where the elimination half-life in the environment, under conditions similar to those in this work, ranged between 93-240 days.

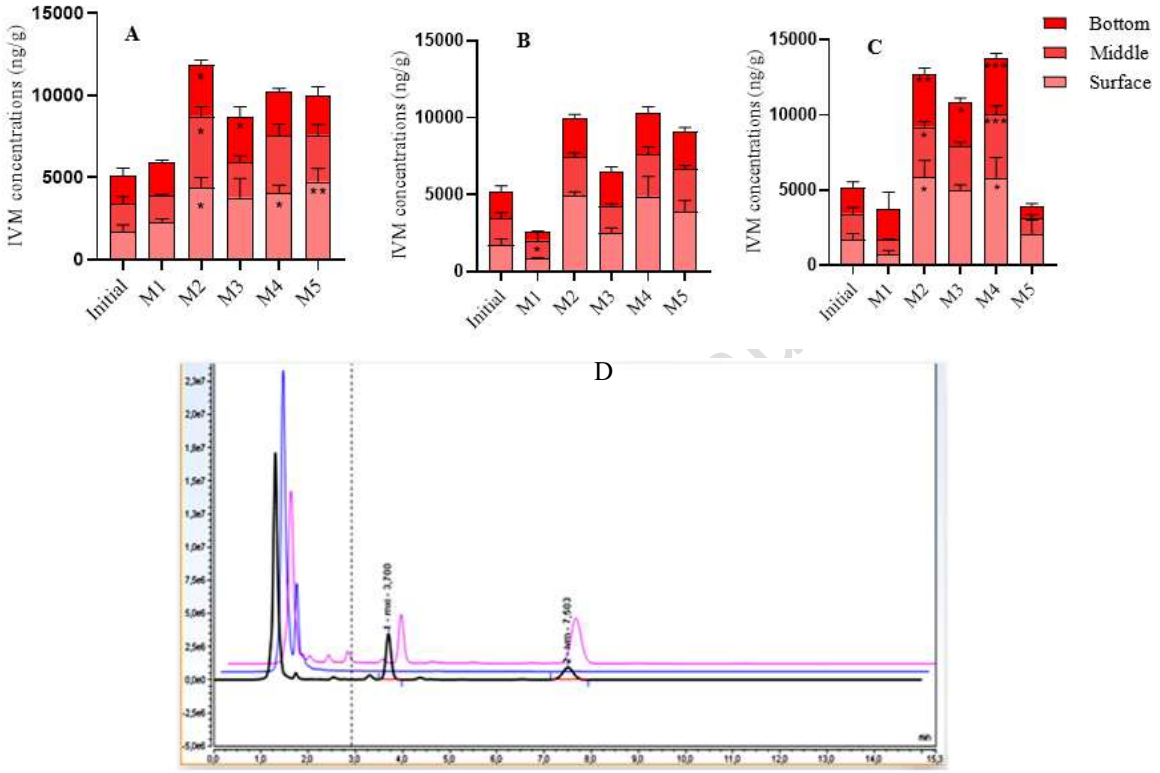

Fig. 3. Evolution of IVM concentration in drums (**A, B** or **C**) across the 5 sampling times (M1-M5). **D** Chromatograms base line (blue), analytical standards (violet) and experimental (compost) sample (black) showing IVM (retention time 7.5 min) and MXD (retention time 3.7 min) as internal standard

The presence of gastrointestinal nematode eggs was observed until day 60 of compost sampling. Subsequently, on day 90, it was decided to discontinue the samplings due to the negative results of the tests. Figure 4 shows the initial (day 0) EPG value (mean ±SEM) of the fecal matter used for both control and experimental composting drums. Mean value was 317 (±118). The next parasitological sampling was on day 3 of the experiment. In control (IVM-free) drums, an increase in the count was observed with an average of 504 (±375); however, in the following samplings, EPG values decreased to 68 (±62), 12 (±13), and 4 (±13) on days 30, 60, and 90, respectively. In experimental (IVM-added) drums, sampling on the third day showed a decrease in EPG to an average of 209 (±150), followed by an increase to 1,275 (±1579) on day 30. However, for days 60 and 90, the average was 20 (±23) and 0.9 (±40), respectively.

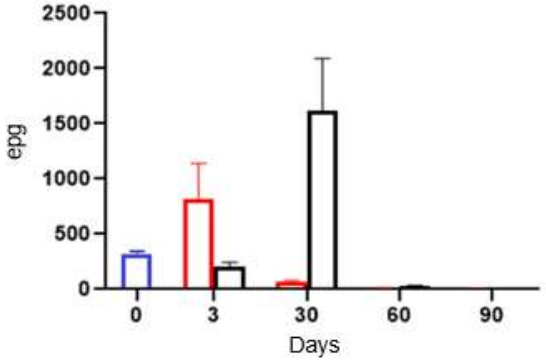

Fig. 4. Quantification of EPG present in initial (blue), control (red) and treated (black) drums. Data are represented as the mean ± SEM

Regarding larval recovery, coprocultures mainly revealed the presence of small strongyles on days 0 and 3 of sampling. For days 30, 60, and 90, there was no larval recovery.

Given that the larval culture could not be carried out, the morphology of the eggs allowed for their identification and classification into those belonging to the genera *Strongyloides*, *Strongylus*, and *Parascaris*. On day 0, 58.3% corresponded to the genus *Strongyloides,* 29.3% to the genus *Strongylus*, and 12.4% to the genus *Parascaris*.

In Table 1, the genus percentages of eggs recovered from the control and treated drums for days 3, 30, and 60 are shown.

Table 1. Percentage of occurrence of the different parasitic genera in the control and treated drums during the samplings on days 3, 30, and 60

	Control			**Treated units**		
Day	***Strongyloides***	***Strongylus***	***Parascaris***	***Strongyloides***	***Strongylus***	***Parascaris***
3	**77.8%**	**19.1%**	**3.1%**	**1.6%**	**71.8%**	**26.6%**
30	**19.4%**	**11.1%**	**69.5%**	**94.9%**	**2.1%**	**3%**
60	**0**	**41.6%**	**58.4%**	**0**	**57.1%**	**42.9%**

Horse breeding is an activity that generates large amounts of waste from stable management such as feces, urine, and bedding material. Generally, establishments dedicated to various equine activities do not have a proper waste treatment scheme; consequently, there is no standardization of management. This lack can lead to

undesirable consequences such as problems controlling parasitic diseases in animals, environmental contamination risk, and public health issues (Fujii et al., 2014).

Fig. 4 represents the presence of gastrointestinal nematode eggs in the composting fecal matter. Initially, eggs belonging to *Strongylus*, *Strongyloides*, and *Parascaris* genera were found, with an average of 317 EPG. These eggs present in equine fecal matter, if ingested, can cause health problems in horses (Romano et al., 2006).

Strongylus is the most prevalent genus in adult horses in Argentina (Fusé and Saumell 2002; Fusé et al., 2013). In this work, eggs of this genus were found up to day 60 of composting. This is considerably higher than that reported by Romano et al., (2006), who evaluated compost production during different seasons of the year, starting with 2.1 EPG in the autumn experiment and up to 6 EPG in the spring experiment. These authors reported that after 4 weeks of composting, less than 1 EPG was detected and, after 7 weeks, strongyles eggs concentrations were below the detection limit (<0.2 EPG). In our study, the period of detection of *Strongylus* eggs extended to 60 days, probably because the system did not reach the reported temperature (40°C) required for egg elimination (Ogbourne 1972; Nielsen et al., 2007). It could be assumed that system dehydration led to their elimination (Langrová et al., 2008).

From day 30 onwards, there was no larval development from the eggs. Moncol (1996) demonstrated that only 13% of larvae survive after 3 hours at 45°C, and the optimal larval development temperature is between 10 and 35°C (Mfitilodze and Hutchinson 1987). However, in the system developed in this work, the necessary temperature for larval elimination was not reached. The methodology used allows verifying the eggs' hatching capacity, but it does not evaluate larval presence in the compost, so it could be inferred that, although nematode eggs were found up to day 90, they did not hatch because the copro-cultures did not reveal larval development. Although the compost temperature was suitable for stimulating hatching (Mfitilodze and Hutchinson 1987), it can be inferred that the larvae would not be viable because as they move towards higher temperatures to be ingested since horses do not graze where they defecate (Taylor, 1954), they moved towards the compost surface, where the lower moisture affected them due to their sensitivity to desiccation (Langrová et al., 2008).

On the other hand, *Parascaris* spp eggs were identified in the samplings up to day 90. Different studies have shown that *Ascaris* eggs are inactivated by increasing temperature, reducing humidity or increasing pH (Maya et al., 2010, 2012). According to Capizzi-Banas et al*.,* (2004), *Ascaris* eggs are inactivated at 40°C for 3-10 days. In our study, eggs of this genus were exposed for weeks to temperatures of around 35°C, which possibly led to their elimination starting from day 90. *Parascaris* spp lacks a free-living larval stage as its larvae only hatch inside the host (Reinemeyer and Nielsen, 2017,), that is why larvae were not recovered from the copro-cultures.

Strongylodes eggs were found up to day 60 after the trial started. Treating mothers with IVM in the peri-partum is associated with a lower probability of *S. westeri* infection (Kirtland et al*.,* 2023). The presence of eggs of this genus is not surprising since the females received no anthelmintic treatment for at least 90 days before the trial started.

Various studies demonstrated the effectiveness of the composting system in eliminating helminth eggs based on temperature increase (Budzińska et al., 2016; Manga et al., 2016). Thus, in this work, the temperature remained in the mesophilic stage of the process, ensuring the elimination of equine gastrointestinal nematode eggs in the same way.

4. Conclusion

This study demonstrated that the composting process of equine manure under controlled conditions enables partial reduction of IVM residues and significantly decreases the viability of GIN eggs. However, while

decreases in IVM concentration were observed, complete elimination of this compound was not achieved during the 120-day period evaluated. The persistence of IVM in the compost highlights the need for additional strategies or prolonged composting times to ensure further degradation of this compound, especially when the compost is intended for organic agriculture.

Regarding parasites, a reduction in the viability of GIN eggs was observed, with complete elimination by day 60. These findings reinforce the potential of composting as a method for parasite inactivation. Though total elimination may require higher temperatures or a combination with other practices. Variations in physicochemical parameters such as C ratio, pH, and electrical conductivity reflect the dynamic nature of the composting process and underscore the importance of adjusting initial ingredients, such as straw addition, to optimize decomposition.

Overall, these results underscore the effectiveness of composting in the partial reduction of pharmaceutical residues and pathogen elimination. While suggesting the need for further research to optimize this process in agricultural settings.

Author Contribution

The authors confirm the study conception and design: Junco, M., Iglesias, L.E; data collection: Junco, M, Iglesias, L.E., Saumell, C. A; analysis and interpretation of results: Junco, M. Azcarate, F. Alonso, A., Mestelan, S. Boschetti, C., Sallovitz, J. M., Bernat, G.; draft manuscript preparation: Junco, M. The results were evaluated by all authors, and the final version of the manuscript was approved.

Funding

This work was supported by PIO- Programa de Fortalecimiento 03-PIO-61H.

Conflict of interest

The authors declare that they have no conflict of interest.

Data Availability

The data generated during and/or analyzed during the current study are available from the first author on reasonable request.

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