Dissipation of Antimicrobials in Feedlot Manure Compost after Oral Administration versus Fortification after Excretion

Inoka D. Amarakoon, Francis Zvomuya,* Srinivas Sura, Francis J. Larney, Allan J. Cessna, Shanwei Xu, and Tim A. McAllister

Abstract
Fortification of manure with antimicrobials is one approach to studying their dissipation. However, fortified antimicrobials may not accurately model dissipation that occurs after antimicrobials have been administered to livestock in feed and excreted in manure. This study examined the dissipation of antimicrobials excreted in manure versus those added directly to manure (fortified). Steers were fed a diet containing (kg feed) (i) 44 mg chlortetracycline, (ii) 44 mg each of chlortetracycline and sulfamethazine, (iii) 11 mg tylosin, and (iv) no antimicrobials (control). Fortified antimicrobial treatments were prepared by adding antimicrobials to control manure. Manure was composted for 30 d, sampled every 2 to 3 d, and analyzed for antimicrobials and compost properties. Antimicrobial dissipation followed first-order kinetics. The dissipation rate constant was significantly greater (based on 95% confidence limit) for excreted (0.29–0.54 d⁻¹) than for fortified chlortetracycline (0.11–0.13 d⁻¹). In contrast, dissipation rate constants were significantly greater for fortified sulfamethazine (0.47 d⁻¹) and tylosin (0.31 d⁻¹) than when the same antimicrobials were excreted (0.08 and 0.07 d⁻¹, respectively). On average, 85 to 99% of the initial antimicrobial concentrations in manure were dissipated after 30 d of composting. The degree of dissipation was greater (P < 0.0001) for fortified (99%) than for excreted tylosin (85%). Composting can be used to reduce environmental loading of antimicrobials before field application of beef cattle manure. Dissipation rates of fortified antimicrobials during manure composting may not accurately reflect those of antimicrobials that are consumed and excreted by cattle.

Core Ideas
• Composting enhances biodegradation of antimicrobials in manure.
• Antimicrobial dissipation follows first-order kinetics.
• Dissipation rate depends on whether the antimicrobial is excreted or fortified.

Livestock operations in North America routinely use antimicrobials to treat infections, control disease, and promote growth. Antimicrobial use in livestock in the United States is estimated to be 11 to 16 million kg yr⁻¹ (Kim et al., 2011; Sarmah et al., 2006). The amount of antimicrobials excreted after ingestion varies with feeding conditions and the type of antimicrobial and has been reported to be 65 to 75% of chlortetracycline, 90% of sulfamethazine, and 50 to 100% of tylosin (Kim et al., 2011). Manure containing excreted antimicrobials is often applied to agricultural land, resulting in the transfer of antimicrobial residues to soil, surface water, and ground water (Boxall et al., 2004; Kemper et al., 2008; Sarmah et al., 2006). Antimicrobial dispersal in the environment increases the risk of selection for antimicrobial-resistant bacteria (Cheeseman et al., 2009; Kemper et al., 2008; Zhang et al., 2009).

Composting entails the aerobic degradation of organic matter by a wide array of microorganisms. It reduces the mass and volume of manure and concentrates plant nutrients (Larney et al., 2006). Composting also reduces pathogen populations and the viability of weed seeds (Larney et al., 2003; Larney and Hao, 2007), making compost a preferred agricultural amendment over fresh manure. Composting has also shown the potential to enhance the dissipation of antimicrobials administered to livestock (Arikan et al., 2009; Bao et al., 2009; Cessna et al., 2011).

Numerous studies on the dissipation of antimicrobials have traditionally used the addition of antimicrobials to antimicrobial-free manure (Burkhardt et al., 2005; Dolliver and Gupta, 2008; Ho et al., 2013), a technique known as fortification. Manure fortification with antimicrobials is more economical and efficient than administering antimicrobials to livestock and collecting manure containing excreted antimicrobials. However, the dissipation of antimicrobials in fortified manure may not accurately reflect the dissipation of antimicrobials excreted in manure after oral administration via cattle feed. To date, a comparison of the fate of fortified versus fed antimicrobials in beef cattle manure has not been undertaken. Thus, this study was conducted to characterize the dissipation of chlortetracycline,
sulfamethazine, and tylosin in feedlot manure compost after oral administration in feed or fortification after excretion. The antimicrobials we tested are those that have relevance to human health and are most commonly fed at subtherapeutic levels to feedlot cattle (Table 1). Further, the antimicrobials were administered at concentrations used in commercial feedlots. The study also allowed assessment of the impact of these antimicrobials (both excreted and fortified) on the composting process (temperature, C, N, and dry matter losses).

Materials and Methods

Antimicrobial Administration to Cattle

Four pens housing nine steers each at the Agriculture and Agri-Food Canada Research Centre, Lethbridge, AB, Canada research feedlot were randomly assigned to four treatments (kg⁻¹ feed): (i) 44 mg of chlortetracycline (CTC) (Aureomycin-100 G, Alpharma Inc.), (ii) a mixture of 44 mg each of chlortetracycline and sulfamethazine (CTCSMZ) (Aureo S-700 G, Alpharma Inc.), (iii) 11 mg of tylosin (TYL) (Tylan, Elanco Animal Health), and (iv) no antimicrobials (control). In June 2010, the steers were fed a barley (Hordeum vulgare L.) grain–barley silage diet, in which the antimicrobials were administered as previously described (Amarakoon et al., 2014). A manure/bedding ratio of ~4:1 was maintained in each pen.

Composting Vessel Construction

Composting vessels were constructed as described by Xu et al. (2010). Briefly, cylindrical polyethylene barrels (110 L; 0.7 m in height and 0.45 m in width) and their lids were covered with a layer of polyurethane foam (~5 cm) for insulation. At the bottom of each barrel, a 0.1-m-height air plenum was created to enable passive aeration by placing a perforated (~1-cm-diam. holes) polyethylene disk 0.1 m from the bottom supported by three legs made from polyethylene pipes (~5 cm in diameter). Holes (2.5 cm diam.) were drilled on the side near the bottom of the barrel and on the lid to facilitate aeration through the air plenum created.

Experimental Design and Treatments

Manure was composted in the composting vessels described above. Factorial combinations of antimicrobial treatment (CTC, CTCSMZ, and TYL) and mode of addition to manure (excreted vs. fortified) were tested along with manure containing no antimicrobials (control), resulting in seven treatments. The experiment was conducted over two 30-d composting cycles, with each cycle containing all seven treatments and consisting of two replicates per treatment. The experimental design was a randomized complete block, with composting cycle (two 30-d periods) as the blocking factor, each of which had two replicate composting vessels per treatment.

In August 2010, manure containing excreted chlortetracycline, sulfamethazine, and tylosin was collected from the feedlot pens where steers were orally administered the corresponding antimicrobials, and manure for the control and fortification treatments was collected from pens in which steers were not administered antimicrobials. Each composting vessel was filled with 45 kg of wet manure (moisture content, 0.48–0.68 kg kg⁻¹) at the beginning of composting. Manure destined for each composting vessel was mixed separately for 5 min in a 0.34-m³ capacity mortar mixer (Model 12SGH9, Crown Construction Equipment) and immediately transferred to a composting vessel to begin composting.

For the fortified treatments, antimicrobials were sprinkled onto manure during mixing of manure from the control pens.

Table 1. Physical and chemical properties and applications of the selected antimicrobials.†

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Molecular weight g mol⁻¹</th>
<th>pKₐ ‡</th>
<th>Log Kₐ₆ §</th>
<th>Solubility mg L⁻¹</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlortetracycline</td>
<td>479</td>
<td>3.3</td>
<td>0.41</td>
<td>600</td>
<td>Active against gram-positive and gram-negative bacteria, Mycoplasma, Chlamydia, etc. Used in human and animal therapy and at subtherapeutic levels for growth promotion.</td>
</tr>
<tr>
<td>Sulfamethazine</td>
<td>278</td>
<td>2.1, 7.5</td>
<td>0.8</td>
<td>1500</td>
<td>Active against gram-positive and gram-negative bacteria. Used in human and animal therapy and at subtherapeutic levels for growth promotion.</td>
</tr>
<tr>
<td>Tylosin</td>
<td>917</td>
<td>7.5</td>
<td>3.4</td>
<td>5000</td>
<td>Active against mainly gram-positive bacteria, Vibrio, Spirochete, Coccidian, etc. Used in animal therapy and at subtherapeutic levels for growth promotion.</td>
</tr>
</tbody>
</table>

† Modified after Essington et al. (2010), Sarmah et al. (2006), and Thiele-Bruhn (2003).
‡ pKₐ, acidity constant.
§ Log Kₐ₆, octanol-water partition coefficient.
¶ Structure is for tylosin A, which accounts for 80–90% of the parent compound mixture (Sarmah et al., 2006).
The amount of antimicrobial added to each composter for fortified treatments was calculated based on the amount of antimicrobial administered to the animal in the feed, assuming the feed was 63% digestible and no antimicrobial degradation occurred in the digestive tract. For the fortified CTC treatment, 1.36 g of the chlortetracycline premix Aureomycin 220G (Zoetis Canada; 220 g chlortetracycline kg⁻¹ of premix) was added to 45 kg of wet manure. For the fortified CTCSMZ treatment, 3.89 g of Aureo700G premix (Zoetis Canada; 77 g of chlortetracycline and sulfamethazine kg⁻¹ of premix) was added to 45 kg of wet manure. The fortified TYL treatment was prepared by adding 4.69 g of Tylosin 40 premix (Bio Agri Mix; 88 g of tylosin kg⁻¹ of premix) to 45 kg of wet manure. Moisture content of the control manure used for the fortified treatments averaged 0.63 kg kg⁻¹. To facilitate uniform mixing with manure, each antimicrobial premix aliquot was mixed with approximately 100 g of purified fine sand and then sprinkled onto manure in a mortar mixer at a rate of 20 g min⁻¹ of mixing.

Mixed manure (300 g) from each treatment was placed in each of six retrievable approximately 15−× 15-cm nylon mesh bags with 3-mm-diameter holes. Polyethylene twine was attached to each mesh bag to facilitate retrieval at sampling. After filling composting vessels to 50% capacity, the six mesh bags were placed in the center of each vessel, with retrieval twines protruding from the top of the vessels. Two thermocouples (T-thermocouples, Thermo Electric) were placed in proximity to the nylon mesh bags in each composting vessel. Each vessel was then filled with a total of 45 kg of manure (including manure in the mesh bags), leaving 5 cm of headspace. The vessels were closed with insulated lids and placed in an unheated room. The temperature in the room was monitored using two thermocouples and ranged from 9 to 25°C during the 30-d experiment.

Composting Process and Sampling

In each of the two composting cycles described above, manure was composted for 30 d. On Day 0 (i.e., at the start of composting), two subsamples were collected immediately after manure mixing for each composting vessel. Manure was subsequently sampled after 3, 7, 10, 14, and 16 d of composting. On each sampling day, one nylon mesh bag was retrieved from each vessel and split into two subsamples: one was processed immediately for compost properties, and the other was freeze-dried and stored at −40°C until antimicrobial extraction. Moisture content in each vessel was adjusted to 0.5 kg kg⁻¹ dry manure by adding deionized water. To simulate windrow turning, the contents of each vessel were then transferred to a mortar mixer and mixed for 5 min. Another six nylon mesh bags were filled with compost manure from the same composter after mixing and placed in the middle of each composting vessel during refilling as described above. Further compost samples were retrieved in mesh bags on Days 18, 22, 24, 28, and 30. Temperature in each vessel was continuously measured at 1-min intervals, with 15-min averages recorded for the 30-d of composting. The mixer was thoroughly washed between treatments.

Compost Properties

Fresh manure (150 g) was placed into 500-mL Whirl-Pak bags, weighed, and stored at −25°C. Before analysis, the sample was freeze-dried for 7 d and weighed to determine moisture content. The sample was then ground to pass through a 2-mm screen using a Model 4 Wiley Mill (GMI Inc.). A subsample was further ground to <150 μm using a ball-and-capsule grinder (model MM2000, Retsch). The fine-ground subsample was used to determine total C and N by dry combustion gas chromatography (Model NC 2100, Carlo Erba).

Antimicrobial Extraction

Pressurized Liquid Extraction

Antimicrobials were extracted from freeze-dried manure samples by pressurized liquid extraction (PLE) followed by solid-phase extraction (SPE) and antimicrobial elution and analysis as previously described (Sura et al., 2014). Briefly, 2 g of freeze-dried manure were mixed with 20 g of Ottawa sand (Fischer Scientific) and placed into a stainless steel PLE cell (33 mL). The packed cell was subjected to PLE with an ASE 200 ( Dionex) using a citric acid buffer solution (pH 5.0) followed by an 80/20 mixture of methanol/citric acid buffer. Temperature was increased from room temperature (22 ± 2°C) to 75°C in 5 min, where it was held for 2 min during extraction. Each cell was flushed immediately after extraction with the series of solvents described above and purged with N₂ for 90 s. Pressure during extraction was held at 105.5 kg cm⁻². Two extraction cycles per sample generated ~44 mL of extract, which was diluted to 250 mL with Milli-Q water before SPE.

Solid-Phase Extraction

Solid-phase extraction was performed to concentrate antimicrobials extracted from manure by passing the diluted PLE extract through an assembly of an Oasis weak cation exchange cartridge (225 mg of sorbent, 60 μm particle size; Waters) stacked on top of an Oasis hydrophilic–lipophilic balance cartridge (225 mg of sorbent, 60 μm particle size; Waters) at a flow rate of 100 mL h⁻¹. The cartridge assembly was conditioned with 10 mL of methanol followed by 10 mL of Milli-Q water for SPE. After passing the 250 mL of extract through the assembly, the cartridges were rinsed with 10 mL of Milli-Q water to remove salt. The cartridges were then dried for 30 s under vacuum and maintained at −10°C until elution.

The Oasis hydrophilic–lipophilic balance cartridge was eluted with 10 mL of methanol, and the Oasis weak cation exchange cartridge was eluted with 10 mL of methanol followed by 8 mL of methanol containing 2% formic acid. Each eluate was concentrated to a volume of ~200 μL, and Milli-Q water was used to bring the volume to 1 mL, after which the contents were transferred to a 2-mL amber liquid chromatography vial through a 0.45-μm nylon membrane syringe filter (Chromographic Specialties Inc.). The extract was stored at −15°C until analysis. Each eluent was fortified with a 100 ng ¹⁵N₃−sulfamethazine as an internal standard (Cambridge Isotope Laboratories) before analysis.

Antimicrobial Analysis

Antimicrobial concentrations were determined with a liquid chromatography–tandem mass spectrometry (Waters 2965 Alliance Separation Module interfaced with a Micromass Quattro Ultima triple quadrupole mass spectrometer, Waters Canada). The conditions for liquid chromatography–tandem mass spectrometry were adapted from Cessna et al. (2011) as...
previously described (Aramakoon et al., 2014). Briefly, a C-18 stainless steel column (MS Xterra, 100-mm × 2.1-mm i.d., 3.5 μm diam. packing; Waters Canada) was used for analyte separation. Gradient elution was performed using two mobile phases, both containing 0.1% formic acid. Mobile phase A contained 10:90 acetonitrile/water (v/v), and mobile phase B contained 90:10 acetonitrile/water (v/v). Mobile phase flow rate was 200 μL min⁻¹, and the sample extract injection volume was 20 μL. Retention times were 6.83 min for tylosin, 6.92 min for *iso-*chlortetracycline, 7.11 min for sulfamethazine, and 7.11 min for *C6*-sulfamethazine (internal standard). Electrospray ionization mass spectrometry was operated in positive ion mode. Suitable multiple reaction monitoring transitions were used for conformation and quantification of antimicrobials. Data were processed using MassLynx software (v 4.1, Waters Canada). Antimicrobial concentrations in the samples were corrected for background concentrations in the control samples.

### Statistical Analysis

Mean daily temperature data were analyzed with PROC MIXED for repeated measures in SAS 9.4 (SAS Institute, 2013), with sampling time as a repeated fixed factor, antimicrobial treatment as a fixed factor, and composting cycle as a random factor. Based on the Akaike Information Criterion (Littell et al., 2006), the first-order autoregressive covariance structure was the most suitable of six covariance structures tested in the model.

Data for antimicrobial loss and for percentage losses of dry matter, C, and N, determined as described by Larney and Buckley (2007), were subjected to ANOVA using PROC MIXED, with antimicrobial treatment as a fixed factor and composting cycle as a random factor. The Tukey multiple comparison procedure was used for pairwise comparisons of treatment means. Treatment differences were considered significant at \( P < 0.05 \).

Dissipation kinetics of antimicrobials during composting were evaluated using PROC NLIN in SAS. Parameter estimates were considered significant if their 95% confidence intervals did not overlap. Dissipation half-lives were computed for each replicate, and the results were subjected to ANOVA using PROC MIXED as described above.

### Results and Discussion

#### Composting Parameters

**Temperature**

Temperature increased and peaked twice during the 30-d composting, initially during the first 4 d of composting and again during the 4-d period immediately after watering and mixing of compost on Day 16 (Fig. 1). The highest temperature ranged from 67 to 69°C and was recorded during the first 4 d of composting (data not presented). The observed increase in temperature reflects an increase in biological activity and therefore successful composting (Larney et al., 2006). The high temperature may also play a role in the degradation of organic compounds (Arikan et al., 2009).

Composting is mediated by microorganisms, and therefore the presence of antimicrobials could conceivably reduce the rate of composting due to their inhibitory properties. There was an effect of day (\( P < 0.001 \)) on composting temperature (Fig. 1), but the treatment and treatment × day effects were not significant. Further, the temperature of composting processes did not differ from any of the antimicrobial treatments, indicating that the composting process was not adversely affected by the presence of the antimicrobials themselves.

Our results corroborate previous studies, which showed that the presence of chlortetracycline at 113 mg kg⁻¹ (dry wt.) in beef cattle manure (Arikan et al., 2009) and both chlortetracycline and sulfamethazine at 100 mg kg⁻¹ in swine manure (Xun et al., 2013) did not suppress the composting process. However, in contrast, Cessna et al. (2011), using comparable antimicrobial concentrations in manure during windrow composting, observed lower mean composting temperatures in manure containing tylosin (20.5°C) compared with the control (35°C) and treatments containing either chlortetracycline (35°C) or a mixture of chlortetracycline and sulfamethazine (36°C).

#### Carbon, Nitrogen, and Dry Matter Losses

The effects of antimicrobial treatment, including method of introduction into manure (excreted vs. fortified), did not affect C (\( P = 0.45 \); mean, 400 g kg⁻¹) or N concentrations (\( P = 0.73 \); mean, 31.3 g kg⁻¹) in the final compost. Likewise, treatment effects were nonsignificant for mass losses of C (\( P = 0.22 \); mean, 17%), N (\( P = 0.28 \); mean, 7.8%), and dry matter (\( P = 0.20 \); mean, 13%) during composting. Thus, the main physical and chemical properties of compost in this study did not appear to be altered by the presence of antimicrobials or the route by which they were introduced into manure.

#### Antimicrobial Recovery

Antimicrobial recoveries, determined from freeze-dried control manure (1 g) subsamples fortified with 100 ng of each antimicrobial; CTC, chlortetracycline treatment; CTC-F, fortified CTC treatment; CTCSMZ, treatment consisting of a 1:1 mixture of chlortetracycline and sulfamethazine; CTCSMZ-F, fortified CTCSMZ treatment; TYL, tylosin treatment; TYL-F, fortified TYL treatment. Antimicrobials were either included in the diet or fortified in manure just before composting.

Fig. 1. Changes in temperature during composting of beef cattle feedlot manure. Con, control treatment (manure containing no antimicrobial); CTC, chlortetracycline treatment; CTC-F, fortified CTC treatment; CTCSMZ, treatment consisting of a 1:1 mixture of chlortetracycline and sulfamethazine; CTCSMZ-F, fortified CTCSMZ treatment; TYL, tylosin treatment; TYL-F, fortified TYL treatment. Antimicrobials were either included in the diet or fortified in manure just before composting.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>7</td>
</tr>
<tr>
<td>Temperature</td>
<td>11</td>
</tr>
<tr>
<td>Temperature</td>
<td>15</td>
</tr>
<tr>
<td>Temperature</td>
<td>20</td>
</tr>
<tr>
<td>Temperature</td>
<td>25</td>
</tr>
<tr>
<td>Temperature</td>
<td>30</td>
</tr>
</tbody>
</table>

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Initial Concentrations

Except for sulfamethazine, the amount of antimicrobial used in fortified treatments did not yield the same antimicrobial concentrations in fortified manure as was achieved when the antimicrobials were fed to and excreted by animals (Table 2). In the case of CTC, we measured iso-chlortetracycline, the main metabolite detected in beef cattle feedlot manure formed by irreversible isomerization (Cessna et al., 2011), and the lower concentration of iso-chlortetracycline in the fortified treatments compared with the excreted treatments may also be due to insufficient time for conversion to take place in the fortified treatments. Excreted CTC would have had a longer opportunity, during manure accumulation on the feedlot pen floor, to form iso-chlortetracycline, whereas fortified manure was sampled immediately after fortification and therefore had much less iso-chlortetracycline. However, these differences in antimicrobial concentrations had no significant effect on the composting process, as indicated by the compost properties described above. It is not clear if the differences in the initial concentrations altered the properties of the compost microbiome. Coefficients of variation for initial antimicrobial concentrations in manure in this study were 3 to 58% for subsamples and 7 to 45% for replicates in excreted antimicrobial treatments and were 0.04 to 95% for subsamples and 24 to 64% for replicates in fortified antimicrobial treatments (Table 2). The higher variability of fortified antimicrobials in subsamples reflects the challenge of achieving uniform mixing during the fortification of cattle manure, a problem identified by others (Carlson and Mabury, 2006; Hamscher et al., 2002).

Dissipation Kinetics

Dissipation data for chlortetracycline, sulfamethazine, and tylosin during 30 d of composting were best described by a first-order kinetic model (Table 3; Fig. 2):

\[ C_t = C_0 e^{-kt} \]

where \( C_t \) is the antimicrobial concentration (\( \mu g \text{ kg}^{-1} \)) at time \( t \) (d), \( C_0 \) is the initial antimicrobial concentration (\( \mu g \text{ kg}^{-1} \)), and \( k \) is the first-order rate constant (d\(^{-1}\)). The first-order kinetics observed in the present study were consistent with findings from a previous study using the same antimicrobials in beef cattle manure (Cessna et al., 2011). Dissipation rate constants were significantly greater for excreted (0.29 d\(^{-1}\) for CTC and 0.54 d\(^{-1}\) for CTCSMZ) than for fortified (0.11 d\(^{-1}\) for CTC and 0.13 d\(^{-1}\) for CTCSMZ) chlortetracycline. Similar to our results for chlortetracycline, excreted chlortetracycline was found to dissipate faster than fortified chlortetracycline during anaerobic digestion of swine wastewater (Huang et al., 2014). In contrast, dissipation rate constants of fortified sulfamethazine (0.47 d\(^{-1}\)) and tylosin (0.31 d\(^{-1}\)) in the present study were significantly greater than those of the excreted compounds (0.08 d\(^{-1}\) for sulfamethazine and 0.07 d\(^{-1}\) for tylosin).

Chlortetracycline shows strong sorption to clay minerals, with soil partition coefficients (\( K_s \)) of 1208 to 2386 L kg\(^{-1}\), which are greater than those reported for tylosin (66–92 L kg\(^{-1}\)) and sulfamethazine (0.6–3.2 L kg\(^{-1}\)) (Sarmah et al., 2006). However, chlortetracycline sorption has been shown to decrease with increasing organic matter content. Dissolved organic matter reduced the sorption of chlortetracycline to kaolinite and montmorillonite (Essington et al., 2010). Chlortetracycline rapidly forms complexes with clay minerals, but this affinity decreases in the presence of humic substances (Pils and Laird, 2007).

Chlortetracycline in excreted manure is exposed to microbial activity in the animal’s digestive tract and during the accumulation of manure on the feedlot pen floor. Introduction of antimicrobials selects for resistance in the microbial community (Schmitt et al., 2006; Thiele-Bruhn and Beck, 2005; Westergaard et al., 2001). Mechanisms of antimicrobial resistance in bacteria include expression of genes that are capable of enzymatically modifying and/or degrading antimicrobials (Speer et al., 1992). Thus, the comparatively greater bioavailability of chlortetracycline in manure, along with the presence of bacteria capable of breaking it down, may have resulted in the faster dissipation of the excreted than the fortified compound.

In contrast to our observations, others have reported lower dissipation percentages for excreted chlortetracycline in broiler

### Table 2. Initial antimicrobial concentrations in beef cattle manure used for composting.

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Antimicrobial formulation†</th>
<th>Method of antimicrobial introduction to manure‡</th>
<th>Antimicrobial concentration in manure before composting (( \mu g \text{ kg}^{-1} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlortetracycline</td>
<td>CTC</td>
<td>excreted</td>
<td>3138 (1003)§</td>
</tr>
<tr>
<td></td>
<td>CTC</td>
<td>fortified</td>
<td>239 (57)</td>
</tr>
<tr>
<td>Sulfamethazine</td>
<td>CTCSMZ†</td>
<td>excreted</td>
<td>3211 (212)</td>
</tr>
<tr>
<td></td>
<td>CTC</td>
<td>fortified</td>
<td>183 (94)</td>
</tr>
<tr>
<td>Tylosin</td>
<td>TYL‡</td>
<td>excreted</td>
<td>107 (49)</td>
</tr>
<tr>
<td></td>
<td>TYL</td>
<td>fortified</td>
<td>4453 (1448)</td>
</tr>
</tbody>
</table>

† CTC, chlortetracycline; CTCSMZ, a 1:1 mixture of chlortetracycline and sulfamethazine; TYL, tylosin.
‡ Excreted antimicrobials are antimicrobials excreted in manure after being fed to beef cattle; fortified antimicrobials are antimicrobials added directly to manure just before composting.
§ Concentrations are means of four replicates. Each replicate consisted of two subsamples. Values in parentheses are SD.
manure (92%) and hog manure (27%) than for fortified chlortetracycline in layer-manure (94–100%) during composting (Bao et al., 2009). In the same study, most of the fortified chlortetracycline dissipated during the first 3 d of composting, whereas most of the excreted chlortetracycline dissipated over 4 to 10 d of composting. Chlortetracycline can form strong complexes with divalent cations, such as \( \text{Ca}^{2+} \) and \( \text{Mg}^{2+} \), and subsequently can sorb strongly to organic matter via cation bridging (Parolo et al., 2012; Loke et al., 2002; Pilis and Laird, 2007), a mechanism that may result in strong sorption and contribute to the recalcitrance of excreted chlortetracycline in manure.

Sulfamethazine has been shown to form strong hydrogen bonds with organic matter (Teixido et al., 2011) and covalent (Bialk et al., 2005; Gulkowska et al., 2010) bonds with organic matter. Sulfamethazine can also form recalcitrant residual fractions with extended contact time (Forster et al., 2009), and its recovery has been shown to decrease with increasing contact time with soil and organic matter (Carstens et al., 2013; Stoob et al., 2007). Similarly, tylosin sorbs strongly to manure, with partition coefficients of 175 to 840 L kg\(^{-1}\) (Clay et al., 2005; Loke et al., 2002; Sarmah et al., 2006). Tylosin is a comparatively large organic molecule (molecular weight, 917 g mol\(^{-1}\)) with a log octanol-water partition coefficient \((K_{ow})\) of 3.41 (Sarmah et al., 2006), indicating its strong affinity for organic matter. The sorption of tylosin to manure can exceed its sorption to soil, even with tylosin being predominantly cationic at environmentally relevant pH values and readily participating in cation exchange processes (Essington et al., 2010; Sassman et al., 2007). Thus, the decrease in the rate of microbial and chemical degradability in excreted sulfamethazine and tylosin compared with the fortified compounds could have resulted from the strong sorption to manure during the extended contact time inside the animal and also on the feedlot floor where manure accumulated for 2 mo (June–August).

A wide range of values has previously been reported for the time to 50% dissipation (\( DT_{50} \)) during the composting of manure containing antimicrobials. In a laboratory composting study, Bao et al. (2009) reported a \( DT_{50} \) value of 12 d for excreted chlortetracycline in broiler manure, whereas \( DT_{50} \) values for fortified chlortetracycline were 4 to 12 d in layer manure and 87 d in swine manure. In another laboratory study, \( DT_{50} \) values of 4 to 5 d were reported for excreted chlortetracycline in beef cattle manure (Arikan et al., 2009). Indoor laboratory composting of broiler manure containing excreted antimicrobials, further fortified with the same antimicrobial, yielded \( DT_{50} \) values of 1 d for sulfadiazine and 2 d for tylosin (Ho et al., 2013). In piled and in-vessel composting studies of turkey litter fortified with antimicrobials, \( DT_{50} \) values were 1 d for chlortetracycline and 19 d for tylosin, but there was no measurable degradation for sulfamethazine (Dolliver et al., 2008). In a windrow composting study using beef cattle manure, \( DT_{50} \) values of excreted antimicrobials ranged from 14 to 27 d for chlortetracycline, from 20 to 44 d for tylosin, and from 27 to 237 d for sulfamethazine (Cessa et al., 2011).

A parallel study conducted to characterize antimicrobial resistance determinants in manure treatments tested in this study, the resistance genes \( tet(B) \), \( tet(L) \), \( tet(W) \), \( erm(A) \), \( erm(B) \), \( erm(F) \), \( erm(X) \), \( sul(1) \), and \( sul(2) \) were detected (Xu et al., unpublished data, 2015). Many of these genes had higher occurrence in excreted than in fortified compost treatments.

Field incorporation of manure containing antimicrobials can lead to the development of antimicrobial resistance in bacteria in environmental media (Esiobu et al., 2002; Ghosh and LaPara, 2007; Sengeløv et al., 2003). The development of resistant phenotypes is a complex process, and the duration for resistance to

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**Table 3. First-order kinetic model parameters for antimicrobial dissipation over 30 d in vessel composters.**

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Formulation†</th>
<th>Method of antimicrobial introduction</th>
<th>Model‡</th>
<th>( k_\gamma )</th>
<th>RMSE§</th>
<th>( DT_{50} )††</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlortetracycline</td>
<td>CTC</td>
<td>excreted</td>
<td>( C_t = 3116e^{-0.22t} )</td>
<td>0.29 (0.20–0.37)a††</td>
<td>995</td>
<td>1.9b</td>
</tr>
<tr>
<td></td>
<td>CTC</td>
<td>fortified</td>
<td>( C_t = 225e^{-0.11t} )</td>
<td>0.11 (0.08–0.14)b</td>
<td>77</td>
<td>6.3ab</td>
</tr>
<tr>
<td></td>
<td>CTC</td>
<td>excreted</td>
<td>( C_t = 3197e^{-0.54t} )</td>
<td>0.54 (0.42–0.65)a</td>
<td>920</td>
<td>2.0b</td>
</tr>
<tr>
<td></td>
<td>CTC</td>
<td>fortified</td>
<td>( C_t = 172e^{-0.33t} )</td>
<td>0.13 (0.09–1.17)b</td>
<td>63</td>
<td>5.6ab</td>
</tr>
<tr>
<td>Sulfamethazine</td>
<td>CTCSMZ</td>
<td>excreted</td>
<td>( C_t = 322e^{-0.80t} )</td>
<td>0.08 (0.06–1.01)b</td>
<td>105</td>
<td>9.0ab</td>
</tr>
<tr>
<td></td>
<td>CTCSMZ</td>
<td>fortified</td>
<td>( C_t = 781e^{-0.40t} )</td>
<td>0.47 (0.21–0.72)a</td>
<td>269</td>
<td>7.2ab</td>
</tr>
<tr>
<td>Tylosin</td>
<td>TYL</td>
<td>excreted</td>
<td>( C_t = 119e^{-0.07t} )</td>
<td>0.07 (0.04–0.10)b</td>
<td>51</td>
<td>12a</td>
</tr>
<tr>
<td></td>
<td>TYL</td>
<td>fortified</td>
<td>( C_t = 4460e^{-0.31t} )</td>
<td>0.31 (0.21–0.41)a</td>
<td>1439</td>
<td>1.5b</td>
</tr>
</tbody>
</table>

† CTC, chlortetracycline; CTCSMZ, a 1:1 mixture of chlortetracycline and sulfamethazine; TYL, tylosin.
‡ \( C_t \), antimicrobial concentration; \( t \), day of composting.
§ \( k_\gamma \), rate constant; \( \alpha \), days since the start of composting.
†† DT, time for 50% dissipation; DT, means followed by the same letter are not significantly different according to the Tukey-Kramer pairwise comparison procedure (\( P < 0.05 \)).
††† Values in parentheses are 95% confidence limits for rate constant (\( k_\gamma \)).
emerge is a function of many factors, such as the concentration of antimicrobial in the environment, the bacterial species, the type of antimicrobial, and the prevailing conditions in the soil. A study by Halling-Sørensen et al. (2005) showed an ~1 to 5% increase in the tylosin- and tetracycline-resistant population in the first set of samples collected 3 d after the field application of manure containing 30 to 50 µg kg⁻¹ of these antimicrobials in a sandy and in a sandy loam soil.

The DT₅₀ of excreted antimicrobials in this study decreased in the order tylosin > sulfamethazine > chlortetracycline from CTC > chlortetracycline from CTCSMZ, whereas that of fortified antimicrobials decreased in the order sulfamethazine > chlortetracycline from CTC > chlortetracycline from CTCSMZ > tylosin. Our results for excreted antimicrobials are consistent with those from previous studies, which showed DT₅₀ values decreasing in the order sulfamethazine > tylosin > chlortetracycline in windrow compost (Cessna et al., 2011), stockpiled beef cattle manure (Sura et al., 2014), and stockpiled and composted turkey litter (Dolliver et al., 2008). The greater persistence of excreted sulfamethazine and tylosin compared with excreted chlortetracycline could have resulted from the strong bonding of sulfamethazine (Bialk et al., 2005; Gulowska et al., 2013; Teixido et al., 2011) and tylosin (Clay et al., 2005; Loke et al., 2002; Sarmah et al., 2006) with organic matter as discussed above. The opposite was observed for fortified antimicrobials, with chlortetracycline being more persistent than tylosin. The lack of persistence of fortified tylosin as compared with that of fortified chlortetracycline may be due to insufficient time for the formation of strong bonding with manure.

Percentage Antimicrobial Dissipation

Concentrations of all antimicrobials in manure decreased by more than 85% during the 30-d indoor composting period (Table 4). We found no difference in percent dissipation (i.e., percent reduction in antimicrobial concentration relative to initial concentration) between fortified and excreted chlortetracycline (mean 98%) and sulfamethazine (mean 96%) at the end of composting. However, for tylosin, the percent dissipation was significantly greater when this antimicrobial was fortified (99%) than when it was excreted (85%). Strong sorption of tylosin to manure during the extended contact time in feedlot pens may have resulted in the lower percent dissipation of excreted tylosin.

Conclusions

Composting reduced concentrations of chlortetracycline, sulfamethazine, and tylosin in manure by 85 to 99% over a 30-d period, indicating its potential to reduce environmental loading of these antimicrobials and hence the risk of antimicrobial resistance development in bacteria within the environment. Temporal changes in concentrations of the three antimicrobials during composting were adequately described by first-order kinetics. Our results showed that first-order dissipation rate constants for excreted chlortetracycline were higher than those for fortified chlortetracycline, whereas fortification produced greater dissipation rate constants for fortified than for excreted sulfamethazine and tylosin. These results indicate that fortified chlortetracycline, sulfamethazine, and tylosin may not accurately reflect the dissipation of these antimicrobials in manure when they are administered in the feed and excreted in feces. Therefore, caution should be exercised when decision-making is based on the dissipation rates of fortified antimicrobials. To our knowledge, this is the first time that a study of this scale has examined dissipation kinetics of the three antimicrobials in excreted versus fortified beef cattle manure.

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References


Table 4. Antimicrobial dissipation (percent of initial concentration) during 30 d of composting.

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Formulation†</th>
<th>Method of antimicrobial introduction to manure</th>
<th>Dissipation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlortetracycline</td>
<td>CTC</td>
<td>excreted</td>
<td>99a‡</td>
</tr>
<tr>
<td></td>
<td>CTCSMZ</td>
<td>fortified</td>
<td>97a</td>
</tr>
<tr>
<td>Sulfamethazine</td>
<td>CTCSMZ</td>
<td>excreted</td>
<td>94a</td>
</tr>
<tr>
<td>Tylosin</td>
<td>TYL</td>
<td>excreted</td>
<td>85b</td>
</tr>
</tbody>
</table>

† CTC, chlortetracycline; CTCSMZ, a 1:1 mixture of chlortetracycline and sulfamethazine; TYL, tylosin.
‡ Means followed by the same letter are not significantly different according to the Tukey–Kramer multiple comparison procedure (P < 0.05).


