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Fate and effect of antibiotics in beef and dairy manure during static and turned composting

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ABSTRACT

Manure composting has general benefits for production of soil amendment, but the effects of composting on antibiotic persistence and effects of antibiotics on the composting process are not well-characterized, especially for antibiotics commonly used in dairy cattle. This study provides a comprehensive, head-to-head, replicated comparison of the effect of static and turned composting on typical antibiotics used in beef and dairy cattle in their actual excreted form and corresponding influence on composting efficacy. Manure from steers (with or without chlortetracycline, sulfamethazine, and tylosin feeding) and dairy cows (with or without pirlimycin and cephapirin administration) were composted at small-scale (wet mass: 20-22 kg) in triplicate under static and turned conditions adapted to represent US Food and Drug Administration guidelines. Thermophilic temperature (>55°C) was attained and maintained for 3 d in all composts, with no measureable effect of compost method on the pattern, rate, or extent of disappearance of the antibiotics examined, except tylosin. Disappearance of all antibiotics, except pirlimycin, followed bi-phasic first-order kinetics. However, individual antibiotics displayed different fate patterns in response to the treatments. Reduction in concentration of chlortetracycline (71 to 84%) and tetracycline (66 to 72%) was substantial, while near-complete removal of sulfamethazine (97 to 98%) and pirlimycin (100%) was achieved. Tylosin removal during composting was relatively poor. Both static and turned composting were generally effective for reducing most beef and dairy antibiotic residuals excreted in manure, with no apparent negative impact of antibiotics on the composting process, but with some antibiotics apparently more recalcitrant than others.

Keywords: antibiotics, beef and dairy manure, static and turned composting

CORE IDEAS

- Antibiotics excreted in their natural forms did not influence manure composting.
- Antibiotic transformation did not always follow single-phase first-order kinetics.
- Composting enhanced antibiotic removal from manure, but tylosin was recalcitrant.

INTRODUCTION

Antibiotics are the most commonly used drugs in livestock production and are administered to treat bacterial infection, prevent disease, or promote growth. According to the US Food and Drug Administration (FDA), 13.5 million kg of antibiotics were sold in the US for livestock use in 2011, accounting for about 70% of total antibiotic sales (FDA, 2012; 2014a). Antibiotics are known to be excreted in feces and urine with up to 90% of administered antibiotics remaining as parent compound, metabolites, or both (Kemper, 2008). Excreted antibiotic residues can enter the environment via land application of manure, which is a growing concern to animal, human, and environmental health. Antibiotics have been shown to persist in stored manure, soil, and water and, even at subinhibitory concentrations, play a role in stimulating, selecting, and disseminating antibiotic resistance among bacteria (Beaber et al., 2004; Gullberg et al., 2011; Kuchta and Cessna, 2009; Kumar et al., 2005; Lamshoft et al., 2010). Antibiotics in soil may also be taken up by plants and deposited in roots, stems, leaves, and fruits, making consumption of raw produce a potential contributor to unintended exposure of humans to antibiotics (Bassil et al., 2013; Dolliver et al., 2007; Kang et al., 2013).

Among antibiotics commonly used in the dairy and beef industry, macrolides are considered “critically important” in human medicine by the World Health Organization (WHO), while cephalosporins, sulfonamides, and tetracyclines are considered “highly important” (Collignon et al., 2009). Cephapirin accounts for an estimated 31% of dry cow therapy administrations in the US (USDA, 2008) and pirlimycin is used to treat ~20% of mastitis infections (Pol and Ruegg, 2007; USDA/APHIS/VS/CEAH., 2008). Antibiotics are administered to feedlot cattle via feed or water to prevent diseases, treat respiratory and hepatic disorders, and improve average daily gain and feed conversion efficiency. Common classes of antibiotics used in beef cattle are tetracycline, macrolides, sulfonamides, and aminoglycosides (Mathew et al., 2007; Sarmah et al., 2006; USDA, 2000). Tylosin, a macrolide, is fed to cattle on about 20% of all feedlots, and the combination of chlortetracycline and sulfamethazine is used on 17% of all feedlots (USDA, 2000).

Recycling nutrients to soil by land application of treated manure is considered to be environmentally-friendly, with guidelines under development to ensure the health and safety of manure treatments. For example, in the most recent FDA Food Safety Modernization Act (FDA FSMA) proposed guidelines, a manure treatment processes is acceptable if it can reduce the number of specified pathogens before land-application (FDA, 2015). However, the efficiency of FDA-approved manure treatment processes in removal of antibiotics has not been assessed. Storage of manure in lagoons or pits is one low-cost management approach that has indicated some success for reducing antibiotics before land application, but long-term storage may not always be feasible and could require long-distance transport (Boxall et al., 2004; Chee-Sanford et al., 2009; Kemper, 2008). Anaerobic digestion can also be effective for reducing antibiotic loads in manure (Arikan et al., 2006), but there are reports that residual antibiotics can disrupt this sensitive microbial process (Beneragama et al., 2013; Poels et al., 1984; Stone et al., 2009). Composting is also a process driven by microbiological activity and is a preferred manure and biosolid management strategy for stabilizing nutrients, reducing mass and volume, killing pathogens, and reducing odor (Larney and Hao, 2007; Larney et al., 2003; Michel et al., 2004). Composting has also been used effectively to stimulate biodegradation of chemicals of emerging concerns such as pharmaceuticals, personal care products, pesticides, and hormones (Bartelt-Hunt et al., 2013; Büyüksönmez et al., 2000; Ho et al., 2013; Xia et al., 2005). Therefore composting of manure has been suggested to reduce environmental loading of antibiotics from livestock farms, and has demonstrated success in removing some antibiotics (Cessna et al., 2011; Dolliver et al., 2008b; Mitchell et al., 2015), but the efficiency is often inconsistent. Dissipation (rate and extent) of antibiotics during composting has been observed to vary with respect to type of antibiotics, type of feedstocks (i.e. type of manure and amendments), size of compost pile, and composting approach (turning vs. no turning) (Cessna et al., 2011; Dolliver et al., 2008b; Storteboom et al., 2007). Therefore it is difficult to form generalizable conclusions about the efficiency of composting in reducing or removing antibiotics from livestock manure.

While there is no report on the effect of composting on dissipation of cephalosporin and lincosamide antibiotics in dairy manure, there are some reports on the dissipation of macrolide, sulfonamode, and tetracycline in beef manure during composting. However, the majority of studies used beef manure spiked with antibiotics to evaluate the efficiency of composting in the removal of antibiotics. To better understand real-world conditions, considertion of actual excreted antibiotics is ideal. There is also a paucity of information about the effect of composting approach on disappearance of antibiotics in manure, with benchmarking against recent FDA guidelines of particular interest. Therefore, the objective of this study was to determine the effect of static and turned composting of beef and dairy manures, collected during peak excretion following antibiotic administration, on the disappearance of cephalosporin, lincosamide, macrolide, sulfonamide, and tetracycline antibiotics. Of further interest was the effect of antibiotics and type of manure on efficacy of the composting process.

MATERIALS AND METHODS

Animal Experiment and Manure Collection

To generate manure for composting experiments, nine healthy yearling Hereford steers (body weight: 341 ± 35 kg) were selected for homogeneity of body weight, housed in individual pens and adapted to a grain-based diet gradually over 28 days. None had a history of antibiotic treatment. After the diet adaptation period, the steers were fed a basal diet containing corn silage (45%) and non-medicated or medicated grain mix (55%) for seven days and offered free choice water. Three steers were fed chlortetracycline plus sulfamethazine at 350 mg of each antibiotic/steer d^{-1} and three steers were fed tylosin at 11 mg kg^{-1} feed. The three remaining steers were fed the basal diet containing non-medicated grain mix. The steers were fed a restricted amount of feed (~9 kg dry weight) to ensure complete consumption of antibiotic doses. Total collection (feces and urine) was conducted from d 3 to 7 post-treatment and manure from d 3 (when peak excretion of antibiotic resistance genes was expected) was used for the composting experiment. Manure from control steers served as control beef manure.

To generate dairy manure, six healthy, peak lactation dairy cows and three cows at the end of their current lactation cycle were used. Three peak lactation cows were treated therapeutically with pirlimycin (intramammary dose typical for clinical mastitis; two doses of 50 mg each, 24 h apart) and three end of lactation cows received cephapirin (intramammary dry cow therapy; single dose of 300 mg into each of four quarters). The three remaining healthy lactating cows were used as negative controls with no antibiotic treatment. Experimental cows were selected for homogeneity of body weight and stage of lactation, and none had received antibiotic treatment in the current lactation.

All cows were offered free choice water and *ad libitum* total mixed ration and were housed in tie stalls (1.25 × 2.25 m) throughout the study. After 24 h of acclimation period, the cows were treated with the assigned antibiotic. Total (24 h) collection of feces and urine was conducted on d 3 post treatment. Feces and urine from 3 cows of each treatment were composited and mixed to achieve homogeneous dairy manure. Manure from cephapirin and pirlimycin treated cows were mixed on wet weight basis (1:1, w/w) to get composited dairy manure containing both antibiotics. Manure from control cows contained no antibiotics and served as control dairy manure.

Composter Set up

Compost tumblers [71 cm (L) × 64 cm (dia.)] were used in this experiment. The composters were equipped with 20 holes to facilitate natural aeration and placed in a temperature controlled room (average room temperature: 27°C). Four different types of manure 1) dairy control, 2) dairy antibiotics (cephapirin and pirlimycin), 3) beef control, and 4) beef antibiotics (chlortetracycline, sulfamethazine, and tylosin) were composted using either static or turned composting methods. Raw materials used to prepare compost mixtures were dairy or beef manure, alfalfa hay, mulch (pine bark), and sawdust with proportions set to achieve a C:N ratio of 25-30 and moisture content of 55 to 65%. Dairy manure was mixed with alfalfa hay, mulch, and sawdust at a ratio of 5:1:3.3:1.5 (w/w, wet weight basis). Beef control and antibiotic manures were mixed with alfalfa hay, mulch, and sawdust at a ratio of 5:1:3.8:2 and 5.5:1:3.8:1.5 (w/w, wet weight basis). It may not be a standard practice to use alfalfa hay and pine bark as raw materials in commercial compost facilities, but the presence of these materials is not uncommon in dairy or beef farm waste, given that alfalfa hay and sawdust are commonly used feed ingredients and bedding materials, respectively. It is assumed that the addition of these high lignocellulosic materials may prolong the persistence of organic matter (OM) during short-term small-scale composting and thus may reduce antibiotic degradation by providing more sorption sites on OM for antibiotics (Lynch and Wood, 1985; Zhang et al., 2012).

Each manure type × composting approach combination was replicated 3 times. Static composters were not turned after initial mixing and loading into the composters. Static composters were aerated using an air pump (Model: DOA-P704-AA, GAST, MI) at a flow rate of 0.1 CFM. The pump was on for 5 min every hour during the thermophilic phase and then for 1 min every hour during the mesophilic phase. Turned composters were turned four times daily during the thermophilic phase, and once daily during the mesophilic stage. The composters were insulated (R 21 Double reflective Insulation, Reflectix, Markleville, IN), except for the holes. Temperature was monitored by placing two temperature sensors at the depth of 7.5 and 22.5 cm and recorded every 15 min using HOBO temperature data loggers (HOBO UX120-006M, Onset Computer Corp., Bourne, MA).

Sampling and Analysis

Raw Materials and Compost Properties

Sub-samples of raw materials were collected and analyzed for moisture, total C, total N, and pH. Compost samples were collected on day 0, 4, 7, 14, 21, 28, and 42. Because of the heterogeneous nature of compost, samples were collected from several locations at different depths, and then composited and mixed. Two sets of sub-samples were collected, with one set immediately frozen at -80°C and freeze-dried (for antibiotic analysis), and another set frozen (-20°C) to evaluate compost characteristics (moisture, total C and total N). Additional samples were collected on d 0 and 42 and stored frozen for ash analysis or used to measure pH and EC immediately. Detailed sample analysis plan can be found in supplemental materials (Sample Analysis).

UPLC-MS/MS Quantification of Antibiotics

Freeze-dried samples of dairy and beef compost were respectively analyzed for cephapirin and pirlimycin using the methods described previously (Ray et al., 2014a; b) and for sulfamethazine, tylosin, chlortetracycline, and tetracycline using a method modified from published methods (Jacobsen et al., 2004). Freeze-drying of a sample prior to extraction to remove water interference is a common protocol used to target total recovery of organic compounds in solid environmental samples for analysis (Jacobsen and Halling-Sørensen, 2006; Khairnar et al., 2012). Freeze-dried compost samples were extracted using methanol: phosphate buffer (70:30, v/v) or methanol: McIlvaine buffer (50:50, v/v) and extracts were clarified using solid phase extraction (SPE). Clarified extracts were analyzed using UPLC-MS/MS (Agilent 1290 UPLC coupled with Agilent 6490 Triple Quad tandem mass spectrometry) for antibiotics. Detailed antibiotic quantification is available in supplemental materials.

Statistical Analysis

All data were analyzed using the GLIMMIX procedure in SAS (SAS Institute Inc., Cary, NC) with composter (n = 3) as the experimental unit. The effects of manure type, composting approach, day of composting, and their interactions on compost properties were evaluated using a mixed statistical model which included manure type and composting method as fixed effects with day as a repeated factor and composter as random variable. Data from day 0 were used as a covariate in the model. Antibiotic concentration and reduction data were analyzed using a mixed model with composting approach as a fixed effect and day as a repeated factor. The effect of composting approach on antibiotic half-life and dissipation rate constants was evaluated using a mixed model with composting method as a fixed effect. Means of main effects were separated using a multiple comparison test following the Tukey-Kramer method. Data were reported as least square means and standard errors, and statistical significance of difference was declared at $P < 0.05$.

RESULTS AND DISCUSSION

Temperature

Thermophilic temperature (≥ 55°C) was achieved and maintained for 3 d in all composts, as recommended by US FDA Food Safety and Modernization Act (FSMA) guidelines (FDA, 2014b). Thermophilic phase duration was not influenced by manure type (dairy or beef), antibiotic content (with or without antibiotics) or composting approach (static or turned; Fig. 1). With or without antibiotics, the temperature during static composting of dairy manure reached ≥55°C by d 2 of composting, continued to increase for the next 24 h to attain peak temperature, and then gradually declined below 55°C by d 5. Lack of any negative influence of residual antibiotics in manure on the temperature profiles during composting confirms that microbial activity and exothermic processes during composting were not compromised. This is similar to the observation when composting swine and poultry manure, where the presence of antibiotic residues did not influence temperature profiles (Hu et al., 2011). The temperature profile during static composting of beef manure (with or without antibiotics) was similar to that during static composting of dairy manure, but thermophilic temperature was achieved on the 4th d of beef manure composting. Temperature in beef compost gradually increased for the next 36 h to reach peak temperature and declined below 55°C by day 7.

The temperature profile during turned compost of dairy or beef manure with or without antibiotics followed the same pattern observed during static composting of the respective manure type. A similar lack of effect of manure type (poultry vs. swine) on temperature profiles during composting was reported by others (Bao et al., 2009; Hu et al., 2011).

Turning the compost did not extend the duration of the thermophilic phase, which was not expected based on some previous reports (Cáceres et al., 2006; Derby et al., 2011). The ~3 d thermophilic phase achieved for turned composting in this study would not meet the criteria for turned composting (15 d at 55°C) in the recent FSMA recommendation (2011). Therefore, the present study provides insight into the effect of turning itself as a more high-intensity manure management approach, benchmarked against static-composting achieving FSMA standards. Lack of an extended thermophilic phase during turned composting in this study was likely due to the relatively small size of the composters, which was necessary to compare a variety of manures in a replicated and head-to-head fashion. During composting of dairy manure with sawdust, high temperature (>40°C) in larger windrows was maintained longer than in smaller windrows (Tirado and Michel, 2010). The effect of compost size on temperature evolution was also observed during a small scale (5 kg dry wt. of manure) composting of broiler manure with hay (Ho et al., 2013).

Physico-chemical Parameters

Moisture content was influenced by the interaction of manure type and composting approach ($P < 0.05$; Supplemental Table S1). Average moisture content across all sampling days (59 to 61%) did not differ between manure types during turned composting and was consistent with a range reported to be optimal for biodegradation during composting (Richard et al., 2002). Static composted beef manure with no antibiotics had lower moisture content compared to static composted dairy manure with no antibiotics (48 vs. 56%; $P < 0.05$). The interactions of manure type by day and composting approach by day also influenced the moisture content ($P < 0.05$; Supplemental Table S1). Moisture content in dairy compost did not vary with time, but beef control compost was wetter on day 0 than other sampling days. Moisture content was not influenced by composting approach from d 0 through 4, but static compost was drier than turned compost thereafter. Moisture content did not vary substantially in turned compost throughout the entire study period, but initial moisture content (d 0 and 4) in static compost was higher compared to all subsequent sampling days. This more uniform moisture profile in turned compost is in agreement with the results of a swine manure composting experiment and could be attributed to the turning process (Derby et al., 2011).

Manure type and composting approach did not influence total carbon (TC) concentration (Supplemental Table S). The average concentration of TC (averaged across all manure types and composting approaches) decreased sharply (3.22% of initial concentration) within the first 4 days of composting, overlapping with the thermophilic phase, and then decreased gradually for the next 38 days. The concentration of total nitrogen (TN) was influenced by composting approach (Supplemental Table S1). Static compost had higher concentrations of TN compared to turned compost (2.11 vs. 2.04%), suggesting greater TN loss as ammonia during the turning process (Cook et al., 2015; Tirado and Michel, 2010). Loss of TC was more rapid than volatilization of ammonia, as indicated by increasing TN concentration from d 0 through d 14. This was followed by a phase of decline in TN until d 42. A similar temporal pattern of TN concentration change was observed during composting of poultry (Ho et al., 2013) and cattle manure (Michel et al., 2004; Parkinson et al., 2004). In the current study, temporal variation in some physico-chemical parameters such as total P and K (Supplemental Table S1), pH, EC, and ash content was observed, but there was no major influence of antibiotic residues or composting method (Supplemental Table S2). Overall, temperature and physico-chemical data indicate that the presence of antibiotic residues in the manure did not have any major negative influence on the composting process.

Transformation Patterns: Beef Antibiotics

Initial (d 0) concentrations of chlortetracycline in static and turned compost derived from antibiotic-treated steers were 1,198 and 675 ng g^{-1} dry compost, respectively. The transformation of chlortetracycline was rapid during first 2 weeks of composting (Fig. 2). In static compost, chlortetracycline concentration was reduced by 33 and 60% of its initial concentration after 4 and 14 d of composting, respectively (Supplemental Table S3). Turned composting was effective in removing chlortetracycline by 54 and 73% of d 0 concentration after 4 and 14 d, respectively. After 2 weeks, removal was relatively slower in both static and turned composting with chlortetracycline concentration reduced by 71% and 84% of the initial concentration after 42-d composting. The initial concentrations of tetracycline in antibiotic-static and antibiotic-turned compost were 96.9 and 81.6 ng g^{-1} dry compost, respectively.

Although tetracycline was not intentionally fed in this study, it was detected in the feces of antibiotic-fed steers over a range of 91.1 to 102 ng g^{-1} dry manure, likely indicating that tetracycline was present as an impurity in antibiotic mix used to prepare the medicated grain. The transformation of tetracycline followed a temporal pattern similar to that of chlortetracycline (Supplemental Fig. S1). By day 4 of composting, the reduction in tetracycline concentration was 28 and 19% of the initial concentration for static and turned compost, respectively (Supplemental Table S3). In static and turned compost, the extent of reduction in tetracycline concentration after 14 d of composting was 57 and 45% of the initial concentration, respectively. Relatively slower transformation after 14 d resulted in 63 and 66% removal of tetracycline in static and tuned compost, respectively.

Initial (d 0) concentrations of sulfamethazine in static and turned antibiotic beef compost were 1200 and 992 ng g^{-1} dry compost, respectively. The transformation pattern for sulfamethazine was similar to those observed for chlortetracycline and tetracycline (Supplemental Fig. S1). Removal of sulfamethazine was >90% of initial concentration in static and turned compost after 14 and 7 d of composting, respectively (Supplemental Table S3). However, relatively slower transformation after 2 weeks resulted in only 1 and 5% additional decline in sulfamethazine concentration during static and turned composting, respectively. By the end of composting (d 42), over 95% of sulfamethazine was removed in all compost. By contrast, Dolliver et al. (2008a) did not observe any transformation of sulfamethazine during 35-d composting of turkey litter, which could have been affected by lack of microbial adaptation or a strong adsorption effect preventing biological transformation. However, our results were consistent with a recent study examining the transformation of sulfamethazine in turned vessel composting of beef manure (Amarakoon et al., 2016), where 93 to 99% of the initial sulfamethazine concentration in fortified and excreted manure was transformed after 30 d.

The initial concentrations of tylosin in static and turned antibiotic beef compost were 49.3 and 36.1 ng g^{-1}, respectively. The mean concentration of tylosin increased in the first week and then declined in both compost types (Fig. 2). Similarly, the concentration of tylosin in spiked turkey manure increased during compost (Dolliver et al., 2008b). Deconjugation of conjugated tylosin or transformation of metabolites to their parent compound (tylosin) during composting might have contributed to the increase in tylosin concentration. In the current study, the concentration of tylosin in static and turned compost increased by 138 and 356% of the initial concentration and reached a peak (116 and 161 ng g^{-1}, respectively) by 7 days. The removal of tylosin after 14 d of static composting was 43% of tylosin concentration observed on d 7, while the removal in turned compost was 79% of d 7 concentrations (Supplemental Table S4). In static and turned compost, the tylosin concentration was reduced by 63 and 81%, respectively, relative to their d 7 concentration, after 4 weeks of composting. The concentration of tylosin in finished static and turned compost (45.7 and 23.1 ng g^{-1}) was comparable to their initial concentration. Overall reduction in chlortetracycline, tetracycline, sulfamethazine, and tylosin after 42-d composting ranged from 71 to 84, 66 to 72, 97 to 98, and 62 to 86%, respectively.

Parallel to the composting study, beef antibiotic manure was stored at 4°C for 42 d, which resulted in relatively less reduction in chlortetracycline, tetracycline, sulfamethazine, and tylosin (59, 22, 50, and 47%, respectively). Thus, the transformation of antibiotics could be contributed by abiotic and biotic process associated with composting, both of which rely largely on temperature. The observations here were consistent with the corresponding temporal pattern during composting. During composting, thermophilic conditions (>55°C) were attained and maintained for 3 d, and then gradually reduced to a range of 30-40°C by d 14. Arikan (2008) reported transformation of chlortetracycline due to abiotic process(es). With a higher temperature, the frequency of molecular collision increases and more molecules hold energy to overcome the barrier for reaction activation. Also, microbial activity is strongly temperature-dependent, with the slowdown of transformation observed here consistent with trends in reduction of overall heterotrophic bacterial plate counts (data no shown). A correlation between temperature and transformation of chlortetracycline and tetracycline was reported by Loftin et al. (2008). Increasing temperature greatly accelerated the transformation of chlortetracycline in both manure and soils (Zhang and Zhang, 2010). In the present study, significant differences ($P < 0.05$) in transformation were observed in the second phase between different composting approaches. In turned compost, transformation of antibiotics continued even after the temperature reached steady-state, while the antibiotics were relatively stable in static compost. Given that there was no difference in temperature at steady-state between static and turned composting, this suggests that biotic processes might have played a role in the removal of sulfamethazine during the second phase. In particular, oxygen availability is an important factor for biodegradation (Ali et al., 2013). It is possible that more oxygen was supplied using the turned approach and resulted in increased microbial activities during the second phase of turned composting.

Transformation Kinetics: Beef Antibiotics

Transformation of the four beef antibiotics followed a bi-phasic pattern, except for tylosin in static compost (Fig. 2, Supplemental Fig. S1). Each phase followed first order kinetics, with distinct transformation rate constants (Table 1; Supplemental Table S5) and half-lives (Table 2; Supplemental Table S6) noted for each phase. Transformation rate constants did not differ between static and turned compost, except in the case of tylosin (Table 1). The transformation rate constant for tylosin was higher during the first phase of turned composting compared to static composting (0.223 vs. 0.047 d^{-1}). Overall, transformation rate constants in the first phase were higher than those in the second phase, consistent with a general slow-down of transformation after 2 weeks (Table 1).

Other than sulfamethazine, the half-lives of beef antibiotics were not influenced by composting approach (Table 2). The half-life of sulfamethazine during the second phase of static composting was higher compared to turned composting (73.7 vs. 21.5 d; Supplemental Table S6). Overall, the half-lives of antibiotics in the first phase were significantly shorter compared to those in the second phase, consistent with their higher transformation rate constants (Table 2). During static composting, the half-life of sulfamethazine for the first phase was shorter compared to the second phase (2.03 vs. 73.7 d^{-1}; Supplemental Table S6).

In this study, the half-lives of chlortetracycline in the first phase (8.68 and 6.12 d for static and turned compost, respectively) were comparable to a half-life of 8.2 d reported for composting of swine manure (Arikan, 2008). However, Dolliver et al. (2008b) observed a relatively shorter half-life of 1 d during composting of turkey manure. The difference in degradation rates might be due to either the activities of microbes or abiotic factors, both of which rely on the environmental factors such as temperature. In the current study, half-lives of sulfamethazine observed in first phase (2.03 and 2.78 d for static and turned, respectively) were comparable to those reported in swine manure-amended soils under aerobic conditions (1.2-6.6 d^{-1}) (Lertpaitoonpan et al., 2015). A short half-life of 1.4 d for sulfadiazine, a structurally similar sulfonamide, was also noted during composting of broiler manure (Ho et al., 2013) . Relatively faster dissipation of sulfadiazine was also observed during composting of swine manure (complete removal within 3 days) (Selvam et al., 2012). Dolliver et al. (2008b) did not observe any degradation of sulfamethazine during 35-day composting of turkey litter, with the persistence of sulfamethazine likely due to the lack of microbial adaptation or a strong adsorption effect preventing biodegradation. Reported half-lives of tylosin ranged from less than 2 to 30 d (Dolliver et al., 2008b; Ho et al., 2013; Ingerslev and Halling-Sorensen, 2001; Lee et al., 2001; Loke et al., 2000). In the current study, the half-life of tylosin was 18 d, which was comparable to a half-life of 19 d during composting of turkey manure spiked with tylosin (Dolliver et al., 2008b).

Transformation Patterns and Kinetics: Dairy Antibiotics

Cephapirin was present in d 0 compost samples, but was not detected thereafter. Initial concentrations of cephapirin in static and turned dairy compost were 11.0 and 14.2 ng g^{-1} dry compost, respectively. Rapid disappearance of cephapirin was not surprising considering the instability of cephapirin at high temperature. In aqueous solution, degradation of cephapirin at 37°C was 40% after 24 h (Berendsen et al., 2009). Pirlimycin was detected in d 0 compost samples at comparatively higher concentrations (154 and 109 ng g^{-1} dry compost for static and turned compost, respectively) and, as observed generally for beef antibiotics, its reduction was not influenced by composting approach (Supplemental Table S4). In static and turned compost, the reduction in pirlimycin concentration was 32 and 48% of initial concentrations, respectively, by d 4. The decline in pirlimycin concentration was almost 70% of initial concentration by d 7 of composting. In both static and turned compost, the removal of pirlimycin was more than 90% of initial concentration after 14 days and was almost complete by 42 days (99.8 and 99.9% of initial concentration for static and tuned compost, respectively). While near complete removal was achieved during composting, only 55% reduction in pirlimycin concentration was observed after 42-d storage of dairy antibiotic manure at 4°C.

It is likely that that disappearance of pirlimycin during composting involved both biotic and abiotic process. Pirlimycin was transformed to its nucleotide adducts by microflora in dairy cow feces (Hornish et al., 1992). In addition to abiotic degradation, adsorption of pirlimycin to organic matter (such as humic acid) might have also contributed to reducing the concentration of pirlimycin as adsorption sites were generated during composting (Hartlieb et al., 2003). Most likely such adsorption would be strong and irreversible, given that a strong solvent extraction method was employed in this study. Since composting is an aerobic process, oxidation of pirlimycin to pirlimycin sulfoxide and pirlimycin sulfone should also be considered as a plausible explanation for reduction. The concentrations of pirlimycin in the final product of static and turned composting were 0.26 and 0.06 ng g^{-1} dry compost, respectively. The transformation of pirlimycin in both static and turned compost followed first-order kinetics (Fig. 3), with no significant difference in transformation rate constants between static and turned compost (Table 1). Similarly, the half-life of pirlimycin was not influenced by composting approach, with values of 4.67 and 4.41 d for static and turned compost, respectively (Table 2).

Limitations and Suggested Further Studies

It is important to note that the present study focused on the fate of parent antibiotic compounds fed to the cattle. It is likely that many antibiotics are transformed into metabolites that retain antimicrobial activity. Isochlortetracycline has been reported to be the primary metabolite during degradation of chlortetracycline in swine manure (Shelver et al., 2012), while tylosin B and D were believed to be major and the minor degradation products of tylosin A (the type of tylosin analyzed in this study), respectively (Loke et al., 2000). Almost complete dissipation of sulfamethazine observed in the current study might be partly or completely due to its transformation into the metabolite N^4-acetylsulfamethazine (Grant et al., 2003). Therefore, it cannot be assumed based on the present study that loss of antimicrobial activity is equivalent to the dissipation of the parent compound.

Phase partitioning and bioavailability could also affect the residual antimicrobial activity in the compost with time. In this study we employed a bulk extraction approach to recover the total residual parent compound. However, this approach may not represent the bioavailable fraction of antibiotics because compost is rich in organic carbon (OC), which can bind to antibiotics and influence their activity. Hydrophobic antibiotics are more likely to partition to organic matter, with water-OC partition coefficients (K_{OC}) for sulfamethazine and tylosin reported to range from 82-208 and 553-7990 L kg^{-1}, respectively (Sarmah et al., 2006). Given that the concentration of OC in soils is very low (1 to 6%) compared to compost (≈50%), extrapolation of soil K_{OC} values to compost predicts availability of sulfamethazine and tylosin in the range of 0.6 to 1.6% and 0.01 to 0.2%, respectively. However, when normalizing to OC, the effect of hydrophilic interactions (e.g., as a result of ionic functional groups) is not taken into account and such predictions of availability may not be accurate.

It is also important to acknowledge that small-scale composting is not a perfect representation of full-scale because parameters such as heat accumulation and loss, moisture, aerobic or anaerobic conditions, and substrate compaction vary with the scale of composting (Petiot and Guardia, 2004). The overall effect of smaller scale tends to be accelerated reaction rates. Therefore, while the general patterns reported in this study at small-scale are expected to translate to full-scale, the precise rates of antibiotic transformation may differ. For example, Dolliver et al. (2008) reported slightly slower rate of chlortetracycline dissipation in full-scale composting compared to smaller scale vessel composting. In contrast, first-order degradation rate constants of organic micro-pollutants were not different between bench-scale and full-scale composting (Sadef et al., 2015). In future large scale composting experiment should be conducted where metabolites of antibiotics in addition to parent compounds should be quantified. In addition to total extracted antibiotics any effect of composting on bioavailable fraction of antibiotics should be monitored.

Conclusions and Implications for Composting Manure with Antibiotics

Overall temperature profile, physico-chemical properties, and temporal patterns of nutrient concentrations were not influenced by manure type and indicated that presence of antibiotics did not negatively influence the process of composting. While the static compost condition achieved federal time × temperature guidelines for pathogen reduction, the turned condition did not achieve the recommended extended thermophilic stage, which is likely related to the small-scale employed in this study. Under the conditions of this study, the transformation of antibiotics was not strongly affected by static versus turned composting; both static and turned compost resulted in complete removal of cephalosporin, lincosamide, and sulfonamide antibiotics while removal of tetracycline antibiotics ranged from 66 to 84%. Removal of tylosin was poor over the 42 d of composting. The transformation of all antibiotics, except lincosamide followed, bi-phasic first-order kinetics. Antibiotic transformation rates generally decreased from first to second phase, corresponding to the shift in thermophilic to mesophilic conditions. Overall it is concluded that composting is promising for the reduction of downstream impacts of antibiotics from livestock to crops and the environment, but future studies should verify that the benefits carry over to metabolites and verify rates at full-scale.

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Fig. 1. (A) Temporal pattern (28 days) of temperature during static composting of dairy control, dairy antibiotic, beef control, and beef antibiotic manure. (B) Temporal pattern (28 days) of temperature during turned composting of dairy control, dairy antibiotic, beef control, and beef antibiotic manure. Dairy and beef control: No antibiotic in manure; Dairy antibiotic: Manure from cows after intramammary infusion of cephapirin and pirlimycin at 1200 mg and 100 mg per cow, respectively. Temperature data from only 28 days is presented because temperature was similar to ambient temperature after 28 days.

Fig. 2. Dissipation kinetics of (A) chlortetracycline and (B) tylosin during static and turned small-scale composting of beef manure. Manure was collected from steers fed chlortetracycline sulfamethazine each at 350 mg d^{-1} and tylosin at a daily dose of 11 mg kg^{-1} feed.

Fig. 3. Dissipation kinetics of pirlimycin in dairy manure during static and turned small-scale composting. Manure was collected from dairy cows after intramammary infusion of cephapirin and pirlimycin at 1200 and 100 mg per cow, respectively.

Table 1. Transformation rate constants of different antibiotics during static and turned small-scale composting of beef and dairy manure

	Chlortetracycline	Tetracycline	Sulfamethazine	Tylosin†	Pirlimycin‡
	----------------	----------------	d^{-1}	----------------	----------------
Composting					
Static§	0.049 ± 0.014	0.042 ± 0.007	0.188 ± 0.031	0.047 ± 0.027a	0.154 ± 0.005
Turned§	0.072 ± 0.014	0.030 ± 0.007	0.149 ± 0.031	0.223 ± 0.027b	0.162 ± 0.005
Phase					
First	0.106 ± 0.012a¶	0.052 ± 0.006a	0.316 ± 0.031a	0.223 ± 0.026a	-
Second	0.015 ± 0.012b	0.019 ± 0.006b	0.021 ± 0.031b	0.010 ± 0.026b	-
			***P* value**		
Composting	0.32	0.32	0.43	<0.05	0.34
Phase	<0.05	<0.05	<0.05	<0.05	-
Composting × Phase	0.52	0.12	0.24	-	-

† Effect of composting reflects first phase data and effect of phase reflects turned composting data for tylosin transformation.

‡ Pirlimycin transformation followed single phase first order kinetics.

§ Static and turned are static and turned composting approaches.

¶ Within antibiotic, means followed by different letters are significantly different ($P < 0.05$).

Table 2. Half-lives of different antibiotics during static and turned small-scale composting of beef and dairy manure

	Chlortetracycline	**Tetracycline**	**Sulfamethazine**	**Tylosin†**	**Pirlimycin‡**
	---------------------	------------	d	------------	---------------------
Composting					
Static§	86.9 ± 25.3	26.8 ± 4.88	37.9 ± 4.31	18.0 ± 4.21	4.51 ± 0.13
Turned§	20.4 ± 25.3	27.6 ± 4.88	12.1 ± 4.31	3.32 ± 4.21	4.30 ± 0.13
Phase					
First	7.40 ± 24.8a¶	14.9 ± 3.87a	2.41 ± 4.31#	3.31 ± 23.1a	-
Second	100 ± 24.8b	39.5 ± 3.87b	47.6 ± 4.31	88.9 ± 23.1b	-
			***P* value**		
Composting	0.14	0.91	<0.05	0.07	0.34
Phase	<0.05	<0.05	<0.05	<0.05	-
Composting × Phase	0.14	0.29	<0.05	-	-

† Effect of composting reflects first phase data and effect of phase reflects turned composting data for tylosin transformation.

‡ Pirlimycin transformation followed single phase first order kinetics.

§ Static and turned are static and turned composting approaches.

¶ Within antibiotic, means followed by different letters are significantly different ($P < 0.05$).

Mean separation is not provided if Composting × Phase is significant.

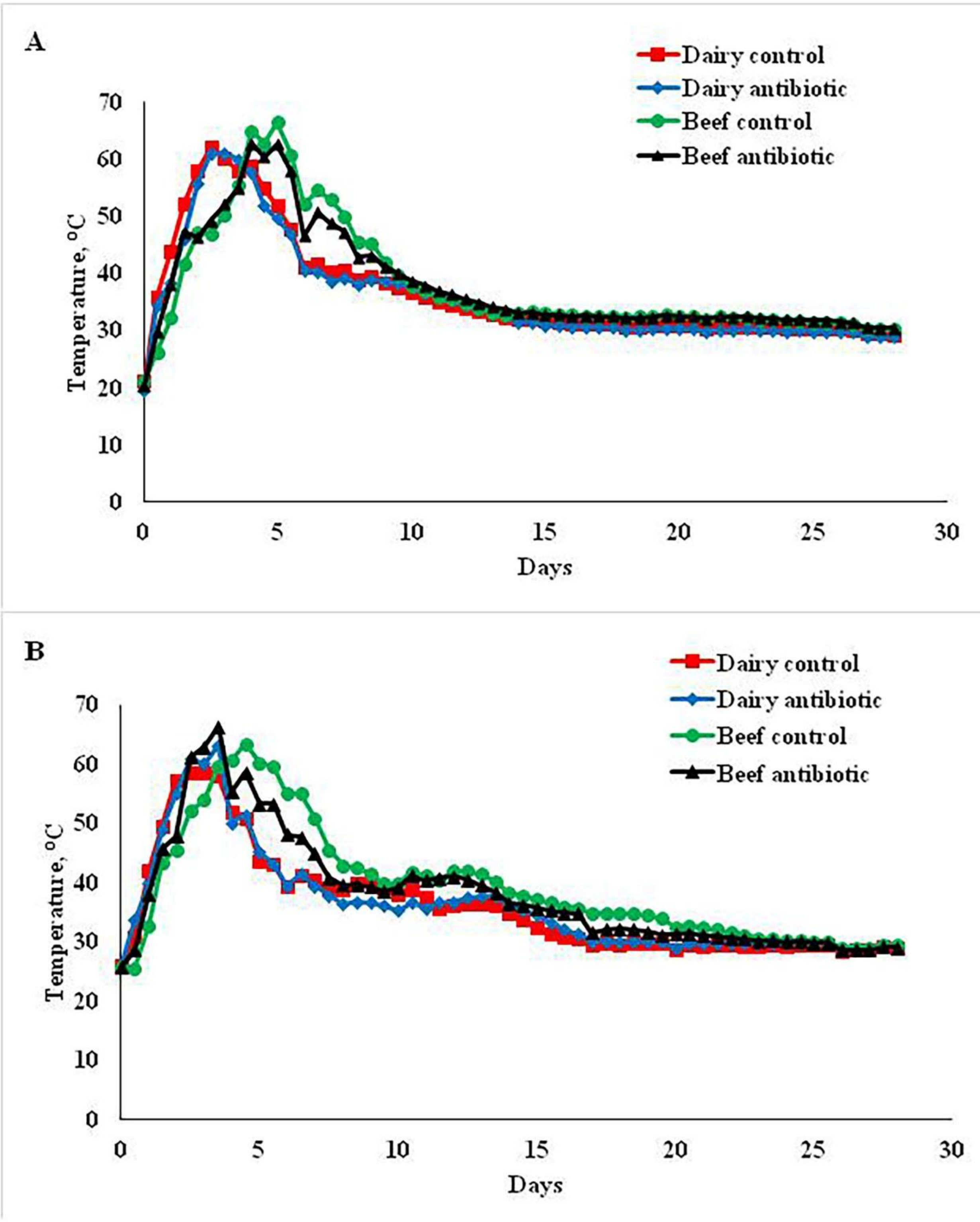

A
Dairy control
Dairy antibiotic
Beef control
Beef antibiotic
Temperature, °C
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
Days
B
Dairy control
Dairy antibiotic
Beef control
Beef antibiotic
Temperature, °C
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
Days

Fig 1.

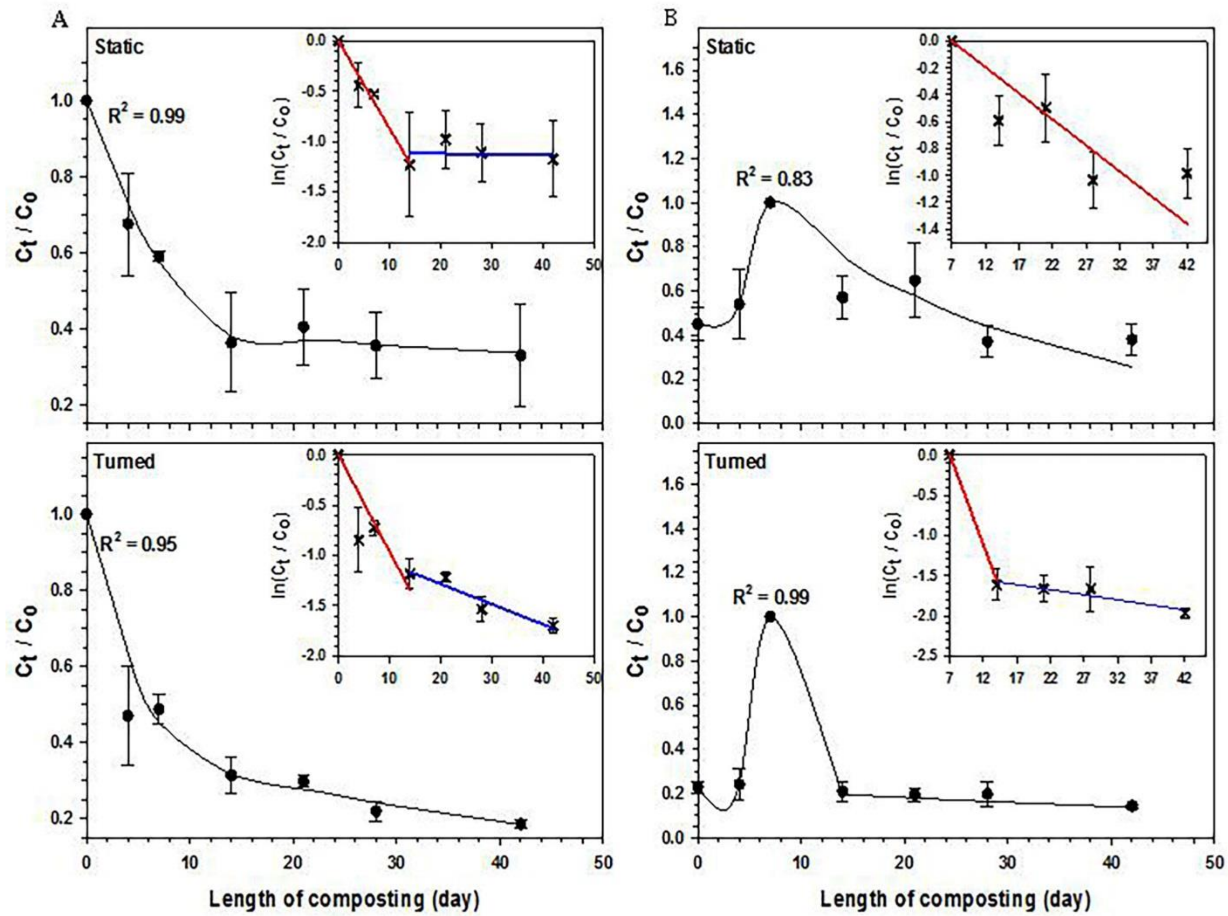
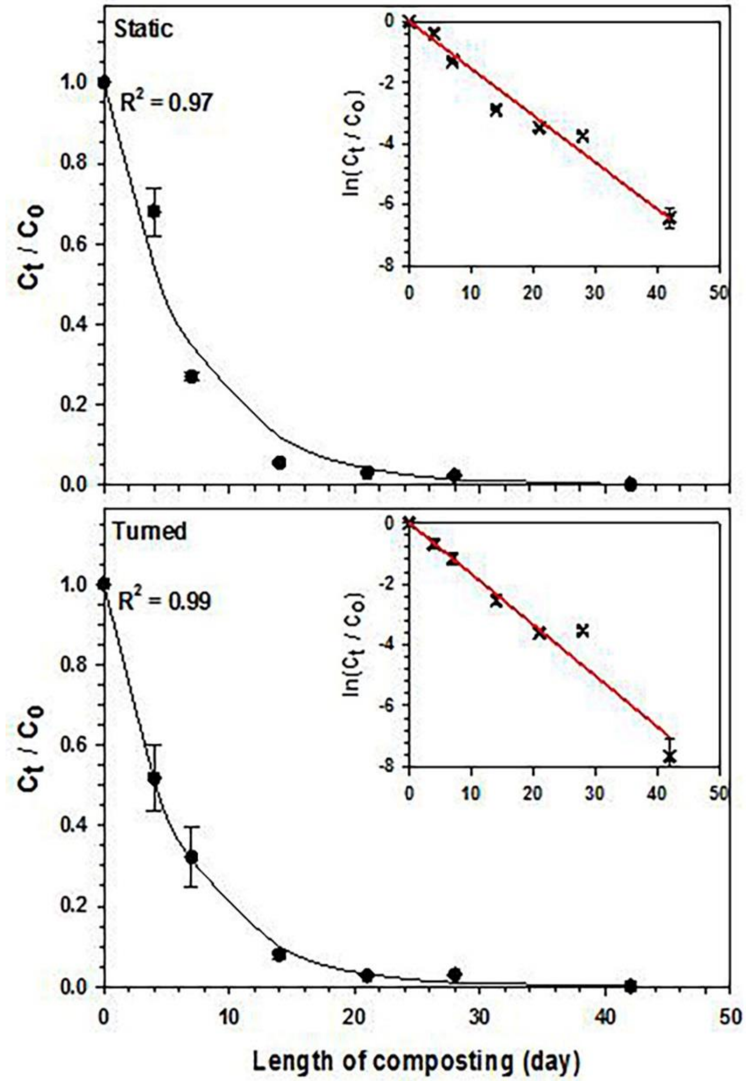

A
Static
R^2 = 0.99
Turned
R^2 = 0.95
B
Static
R^2 = 0.83
Turned
R^2 = 0.99
C_t / C_0
ln(C_t / C_0)
Length of composting (day)

Fig 2.



Static
R^2 = 0.97
Turned
R^2 = 0.99
C_t / C_0
$\ln(C_t / C_o)$
Length of composting (day)

Fig 3.