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Feedlot Manure Handling and Application Strategies on Surface Runoff of Artificial Hormones Applied to Rowcrop Fields

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Abstract. *Hormones are essential to the function and propagation of almost all organisms, yet the environmental fate of hormones is not well understood. Because these substances are so common in nature, the question is not whether they will be found, but rather at what concentrations and in what form (biologically active or inactive) will they be found. The objective of this research was to determine the effect of manure handling and application strategies on artificial hormone losses in runoff through the use of simulated rainfall. In 2008, rainfall simulations were conducted at the Haskell Agricultural Laboratory near Concord, NE (Latitude: 42° 23' 33.6" N; Longitude: 96° 57' 18.0" W). The soil at the site is of the Nora silty clay loam family. Field slope was approximately 8% and no-till practices and a corn-soybean rotation had been employed for the previous 7 years. The field study consisted of 3 replications of a check (no manure, no tillage); 2 animal treatments (w/hormones, w/o hormones); 2 manure handling practices (stockpile and compost); and two incorporation methods (moldboard plow+disk and disk). Simulated rainfall was applied within 24-hours of manure application and runoff samples were collected at five minute intervals beginning at runoff initiation. Analyses of runoff samples were conducted using liquid chromatography tandem mass spectrometry. Results of the study showed sporadic infrequent detection of very low concentrations artificial hormones among treatments with no distinct pattern. More samples were detected with treated composted manure plots compared to stockpile manure; on the other hand, moldboard plow with disk incorporation detected more samples compared to the other two methods. The mass transport and flow weighted concentrations varied within the range of 1635 µg/ha and 9.75 ng/L for melengestrol acetate to 10.73 µg/ha and 0.14 ng/L for 17α-trenbolone respectively among the treatments. It can be concluded that low levels of artificial hormones and some metabolites were detected in runoff samples after land application and, may be transported to nearby surface water sources.*

Keywords. Artificial hormones, manure management, surface runoff, tillage practices

Introduction

The methods and trends of animal production have changed fundamentally over the last 35 years in U.S. and Concentrated Animal Feeding Operations (CAFOs) are common practices for the large production facilities of meat, dairy and poultry. The increased density of animals generates higher amounts of manure often with insufficient land area for dispersal (USEPA 2004). Each year almost 15 million metric tons of cattle manures are applied to agricultural fields as a source of nutrients with minimal treatment (USEPA, 2001).

Beef cattle industries in the U.S. have been using growth promoting hormone treatments to improve growth rate, feed efficiency and lean muscle mass for years (USDA, 2000). Generally the treatments are administered via feed additives (i.e., melengestrol acetate) or small implants (i.e., zeranol, trenbolone acetate, 17 β -estradiol or estradiol benzoate) placed subcutaneously behind the ear. Based on a study conducted in the twelve states in the U.S., 97.5% of all cattle placed in large feedlots (>8,000 head) received implants compared to 88.9% of all cattle placed in small feedlots (<8,000 head) (USDA, 2000). Thus, the extensive use of exogenous hormones, in addition to the implications from endogenous forms, increases the risk of transport to surface water systems.

Natural and synthetic hormones, their metabolites and conjugates have been detected from feedlot treated cattle manure (Schiffer et al., 2001; Lorenzen et al., 2004; Hutchins et al., 2007). Entering into the environment, the hormones are subjected to a variety of transport and degradation mechanisms including sorption, desorption, biodegradation, and photodegradation (Young & Borch, 2008). According to a study conducted by Schiffer et al. (2001) in Germany, the synthetic androgen trenbolone acetate was found within the range of 5 to 75 ng/g and synthetic progesterone, melengestrol acetate was found within the range of 0.3 to 8 ng/g in treated solid manure. Casey et al. (2003, 2004) found that both natural and synthetic hormones are slightly soluble, lipophilic and highly sorptive. Shore and Shemesh (2003) and Lange et al. (2002) concluded that conjugated forms of the steroid hormones were less bioactive than the free forms but they were generally more mobile in environment. In a similar type of study Reddy et al. (2002) showed that the conjugated form can be converted back to free form in the environment. These studies indicate that these hormones may exhibit strong adsorption and desorption characteristics making them available for transport for some time after entering an surface water supply.

Two possible routes to reach water sources are either leaching through soil matrix or overland runoff from agricultural fields. In United States, treatment of animal manure is not required as long as it is not discharged directly into surface water. The U.S. Geological Survey (USGS) conducted a national reconnaissance survey from 1999 to 2000 where water samples were collected and analyzed from 139 stream sites across the United States to determine the concentrations of 95 selected organic wastewater contaminants including 14 steroid compounds (Koplin et al., 2002). Soto et al. (2004) and Kolok et al. (2007) detected both natural and synthetic androgens (17 α - and 17 β -trenbolone), estrogens (estrone, 17 β -estradiol and estriol) and progestogens (progesterone and melengestrol acetate) in different sites with livestock impact along the Elkhorn River in Nebraska. In a more recent study Shore (2009) concluded that hormones can be detected in runoff from cattle pasture and fields fertilized with manure up to 60 km away from CAFOs. The contamination is not only limited to surface water, Peterson et al. (2000) have reported endogenous hormones in spring water discharged from mantled karst aquifer in northwest Arkansas.

Previous studies of steroid hormones in runoff from agricultural fields have focused on transport from fields fertilized with chicken litter. Shore et al. (1995), Nichols et al. (1997), Finlay-Moore et

al. (2000) determined the concentration of estrogens and androgens in agricultural runoff at various application rate and rainfall intensity in different parts of U.S. Some of the recent studies on soils fertilized with chicken litter show the effects of different litter types, rainfall condition (natural or artificial) and tillage practices on detection of different natural estrogens and androgens (Haggard et al., 2005; Jenkins et al., 2008 & 2009; Dutta et al., 2010). Few studies have investigated transport of hormones used in beef cattle production. Because of the density of beef cattle operations in central North America, a study of hormone transport under representative Midwestern soil and climatic conditions is highly relevant.

The specific objectives of this study were to report the amount of free synthetic hormones and metabolites in representative runoff samples and to evaluate the effects of different manure management and application strategies on hormone detection in surface runoff.

Methodology

The study was conducted in the summer of 2008 at the Haskell Agricultural Laboratory of the University of Nebraska-Lincoln (Latitude: 42° 23' 33.6" N, Longitude: 96° 57' 18.0" W). Soils at the site were mapped as Nora silty clay loam (Fine-silty, mixed, mesic Udic Haplustoll) with 28% sand, 48% silt and 24 % clay and a field slope of approximately 8%. The area has an average annual precipitation of 672 mm/yr and average annual temperature of 8° C (46.5° F) (HPRCC, 2011).

Ninety-six heifers were purchased for the study that had not received artificial hormones. The animals were weighed and distributed in 6 feedlot pens with three pens labeled as treated animals and three pens labeled untreated animals. The treated heifers group were implanted initially with 36 mg of α -zearalanol (also known as zeranol) and then after 35 days with 140 mg of trenbolone acetate and 14 mg of 17 β -estradiol (natural estrogen). They also received 0.45 mg of melengestrol acetate in their daily feed ration throughout observation period. Trenbolone acetate normally hydrolyzed in the body to 17 β -trenbolone and trendione and excreted as 17 α -trebolone from animals (Schiffer et al., 2001). For this study, the focus was on the synthetic androgen 17 α and 17 β -trenbolone, synthetic progesterone melengestrol acetate and semi-synthetic non-steroidal growth promoter α -zearalanol (mycotoxin) and its metabolite β -zearalanol.

The untreated animals received the same regimen of feed and forage but without the additional hormones. Following the feedlot study, manure that had accumulated on the feedlot surface was removed using a box scraper and placed in two separate piles from each pen. One pile was composted and the other was stockpiled until applied to the field research plots the following summer (Table 1). The treated and untreated manure was placed on the concrete pad under a roof to prevent rainfall from entering the manure piles. The compost piles were turned periodically to facilitate proper aeration and mixing.

Table 1. Chemical properties of stockpiled and composted manure

	pH	%OM	%OC	C:N ratio	% Moisture content	% Dry matter	% Organic N
Stockpile	7.7	13.06	7.57	11.47	14.18	85.82	0.58
Compost	7.8	13.95	8.09	10.60	21.49	78.51	0.70

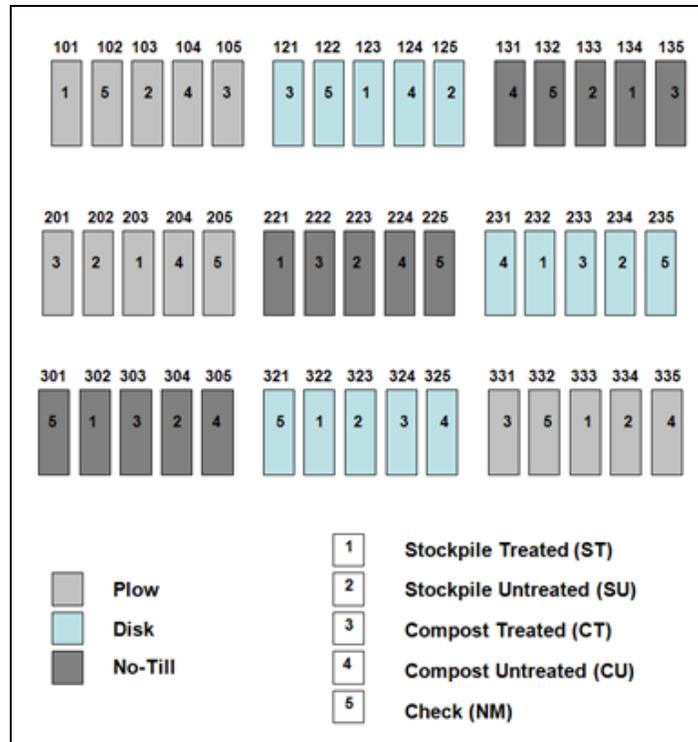


Figure 1: Experimental layout of the rainfall simulation study

In total forty-five 0.75 m wide and 1.75 m long experimental plots were prepared to evaluate the effects of five different manure sources and three different tillage practices in three replications (Figure 1). The experiment was designed as a split-plot design (Fisher, 1925) which is a blocked experiment with the blocks acting as experimental units for other subset factors. In the two levels of experimental units, the blocks are identified as whole plots and the experimental units within the blocks are referred as subplots (Jones and Nachtsheim, 2009). For this study, three different manure application strategies: surface application (NT), single disk incorporation (DK) and a moldboard plow (MP) plus single disk were randomly applied in three replications in the whole plots. Within the main plots five different manure sources: treated compost manure (CT), treated stockpile manure (ST), untreated compost manure (CU), untreated stockpile manure (SU) and a control with no manure (NM) were applied randomly in the subplots.

During the rainfall simulation study, manure was applied one day earlier at an application rate of 170 kg-N/ha to satisfy the nitrogen requirements for dryland corn. Each runoff plot was isolated on three sides by galvanized steel borders. A 150-mm I.D. PVC pipe with a 2.6 m long by 100 mm wide slot, cut lengthwise, was used to collect runoff. To prevent bypass flow around the collection pipe, a piece of galvanized steel bent in an "L" shape was pressed into the soil at the downstream end of the plot and directed runoff water into the PVC pipe. Rainfall was applied using a portable rainfall simulator designed by Humphry et al. (2002) for plot-scale runoff studies similar to that used by the National Phosphorus Research Project. The water application rate was 75 mm/h, similar rainfall intensity index (EI) of a single storm event expected to occur once every two years in the study area (Wischmeier and Smith, 1978). The amount of applied rainfall for each subplot was recorded using water meter attached to the rainfall simulator. Starting time of rainfall and runoff were also recorded for each plots. Six runoff samples were collected in 400 ml amber jars after initiation of the runoff from each plot with a time interval of 5, 10, 15, 20, 25, 30 minutes. In total, 270 runoff samples were collected and immediately frozen at -20°C within four hours of collection in Haskell Agricultural Laboratory to prevent degradation

before transferring to the Water Sciences Laboratory at UNL. The runoff initiation period varied among the plots based on soil water infiltration rate and so did the total rainfall on plots.

In the Water Sciences Laboratory samples were analyzed using on-line solid phase extraction (SPE) liquid chromatography-tandem mass spectrometry (LC/MS/MS) with a Spark Holland Symbiosys Environ automated extraction system and Waters Quattro Micro liquid chromatograph tandem mass spectrometer system with atmospheric pressure photoionization (APPI) source. The online method using APPI as source and toluene as a dopant is beneficial as it saves time and reduces inference and matrix effects due to photoionization compared to traditionally utilized electrosparry ionization (ESI).

Briefly, a 25 milliliter portion of the sample was syringe filtered (Whatman GD/X glass microfiber) into a precombusted amber vial, and fortified with internal standards (d3-testosterone and 13C-3-estradiol) and surrogate (methyltestosterone) along with 10 uL of formic acid. Twenty milliliter aqueous samples were then autoextracted using Prospekt 2/Symbiosys 2.0 x 10 mm Oasis HLB solid phase extraction cartridges. Each cartridge was eluted directly to a Waters 2695 HPLC and then to a Micromass Quattro Micro triple-quadrupole mass spectrometer. A mixture of toluene and methanol was used as the dopant to improve ionization efficiency. Detection and quantification of steroids utilized multiple reaction monitoring (MRM) with argon collision gas. A Thermo HyPurity C18 column (250 x 2 mm, 5 um, 50°C) was used for gradient separation at a flow rate of 0.35 ml/min. The gradient consisted of solvent A (0.1% formic acid water) and solvent B (0.1% formic acid methanol), with 0 to 3 min at 50% B, 3 to 14 min at 65% B, and 14 to 20 min at 95% B, with a return to initial solvent conditions for the last 10 minutes of the gradient (30 minutes total). Instrument control, data acquisition and evaluation used MassLynx 4.0 software (Waters Corporation, Milford, MA). Compound retention times, MRM transitions, and estimated method detection limits are listed in Table 2. Identification of target compounds was accomplished by comparing the retention times for the respective MRM transition in a sample to that of a standard analyzed under the same conditions. Retention times were considered to match if they were within $\pm 5\%$ of the standards.

Table 2. List of synthetic hormones column retention time, MRM transitions, method detection limit (MDL) and reporting limit

Steroid Hormone	Retention Time (min)	Precursor Ion (m/z)	Product Ion (m/z)	MDL (ng/mL)
17 α -trenbolone	9.1	271.1	253.3	0.005
17 β -trenbolone	8.7	271.1	199.2	0.007
Melengestrol Acetate	15.9	397.1	337.1	0.004
α -zearalanol	10.4	303.1	285.1	0.003
β -zearalanol	11.1	303.1	285.1	0.004

The laboratory results were then analyzed to determine the flow weighted concentrations (FWC), mass transport from plots and evaluate the effects of manure types and tillage practices. Method detection limits for these compounds were ignored for the calculations of FWC and mass transport. Flow weighted concentration (ng/L) was determined for 30 mins from the runoff samples collected using the discharge rate at 5 min intervals and the corresponding hormone concentrations using Eq.1. Mass transport ($\mu\text{g}/\text{ha}$) was then determined by the cumulative mass generated from 30 mins runoff samples in per unit plot area using Eq. 2. Statistical analysis was then run to evaluate the impact of different manure management and application strategies on individual hormones using SAS ($\alpha=0.1$) statistical software (SAS, 1990).

$$FWC = \frac{\sum(\text{Discharge rate} \times \text{Concentration} \times \text{Time interval})}{\sum(\text{Discharge rate} \times \text{Time interval})} \dots (1)$$

$$\text{Mass transport} = \frac{\sum(\text{Discharge rate} \times \text{Concentration} \times \text{Time interval})}{\text{Plot area}} \dots (2)$$

Results

The results from the rainfall simulation study showed significant ($p \leq 0.1$) difference among the treatments in runoff initiation time, calculated as the difference between sprinkler start time and the beginning of runoff (Figure 2). In general, the DK plots (average time 61.4 min) required more time to generate runoff followed by MP (average time 61.13 min) and NT (average time 56.13 min) plots.

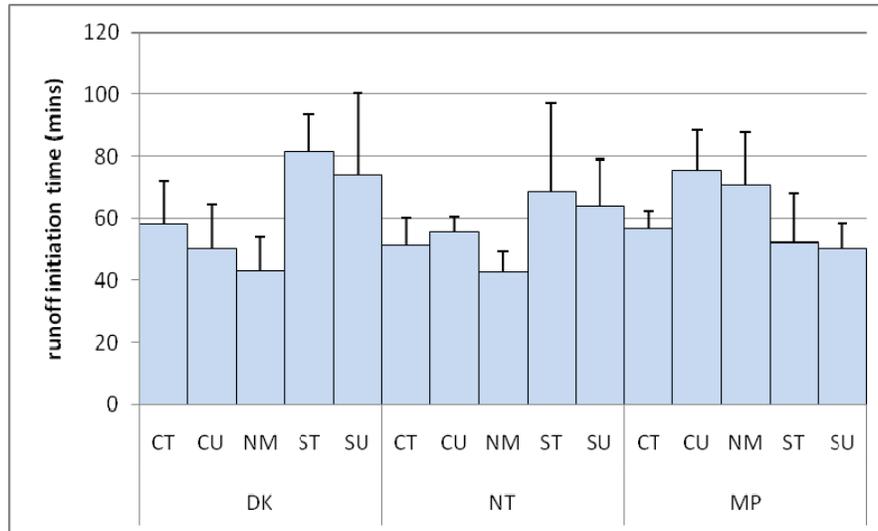


Figure 2: Runoff initiation time (average \pm SD) among treatments; Tillage: DK= single disk incorporation, NT= no till surface application, MP= moldboard plow plus single disk; Manure: CT= compost treated, CU= compost untreated, NM= no manure, ST= stockpile treated, SU= stockpile untreated.

Out of 270 runoff samples collected, only 19 samples contained detectable levels synthetic hormones or its metabolites, though most detection were near or below the detection limit (Table 3). Comparison of concentrations and compounds detected is based on samples from treatments with detected compounds. Among the tillage practices MP tillage treatment showed highest number of detection, run-off from soil fertilized with composted manure had more detection as compared to stockpiled manure. Melengestrol acetate was detected within the range from 2 to 52 ng/L, α -zearalanol was detected within 2 to 8 ng/L range, β -zearalanol was within the range of 1 to 5 ng/L, 17 α -trenbolone was within the range of 1 to 6 ng/L and 17 β -trenbolone was within the range of 1 to 2 ng/L.

Table 3. Summary of the number of hormone concentration sample analysis that provided positive detections for each manure handling and incorporation strategy, Tillage: DK= single disk incorporation, NT= no till surface application, MP= moldboard plow plus single disk; Manure: CT= compost treated, CU= compost untreated, NM= no manure, ST= stockpile treated, SU= stockpile untreated.

Tillage Practices	MP	DK	NT	Total
Manure Handling Strategy	Total Number of Synthetic Hormone Detections			

CT	8	0	0	8
ST	3	0	2	5
CU	0	1	0	1
SU	3	0	0	3
NM	0	0	2	2
Total	14	1	4	19

The flow weighted concentrations of the compounds were not significantly different among treatments ($p \leq 0.1$) and showed considerable variability among the replications (Figure 3). Among the detected values, FWC was greater in composted manure (CT) compared to stockpiled manure (ST and SU) for synthetic progesterone melengestrol acetate. The mean FWC of melengestrol acetate was 4.22 ng/L from CT manure compared to 1.89 ng/L for ST and 0.79 ng/L from SU manure from MP tillage practice. No synthetic hormone compounds were detected in run-off from the DK plots except 0.05 ng/L of β -zearalanol in CU plot which is a metabolite of α -zearalanol. This compound was also detected in FWC of 0.34 ng/L from “NT-NM” plot. In the NT treatment plots, the synthetic growth promoters, α -zearalanol had the FWC of 0.61 ng/L from ST manure. 17 α -trenbolone, one of the metabolites of trenbolone acetate, was detected in FWC of 0.22 ng/L from “NT-NM” plot and 0.05 ng/L in “MP-SU” plot. The other metabolite 17 β -trenbolone was detected only in “MP-CT” plot with FWC of 0.19 ng/L.

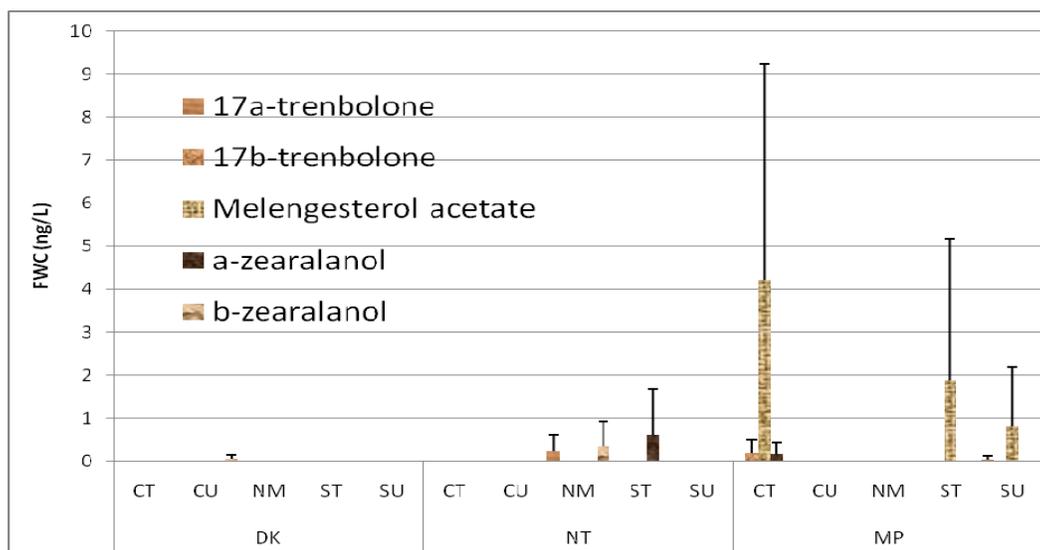


Figure 3. Flow weighted concentration (FWC) (Average \pm SD) among treatments, Tillage: DK= single disk incorporation, NT= no till surface application, MP= moldboard plow plus single disk; Manure: CT= compost treated, CU= compost untreated, NM= no manure, ST= stockpile treated, SU= stockpile untreated.

The analysis showed similar results for the mass transport with no significant difference among the treatments (Figure 4), but the plots with MP tillage showed greater mass transport compared to the other two. Unlike the FWC, melengestrol acetate had greater mean mass transport of 792.9 μ g/ha from CT manure compared to 210.9 μ g/ha from ST manure and 61.7 μ g/ha from SU manure in MP tillage plots. For α -zearalanol, the mean mass transport varied

between 41.3 $\mu\text{g}/\text{ha}$ in “MP-CT” plots to 78.7 $\mu\text{g}/\text{ha}$ in “NT-ST” plots and β -zearalanol had 9.6 $\mu\text{g}/\text{ha}$ in “DK-CU” plots to 46.3 $\mu\text{g}/\text{ha}$ from “NT-NM” plots. Besides, mass transport for 17 α -trenbolone was 3.6 $\mu\text{g}/\text{ha}$ in “MP-SU” plot to 28.3 $\mu\text{g}/\text{ha}$ in “NT-NM” plot. 17 β -trenbolone had a mass transport of 31.3 $\mu\text{g}/\text{ha}$ from “MP-CT” plot.

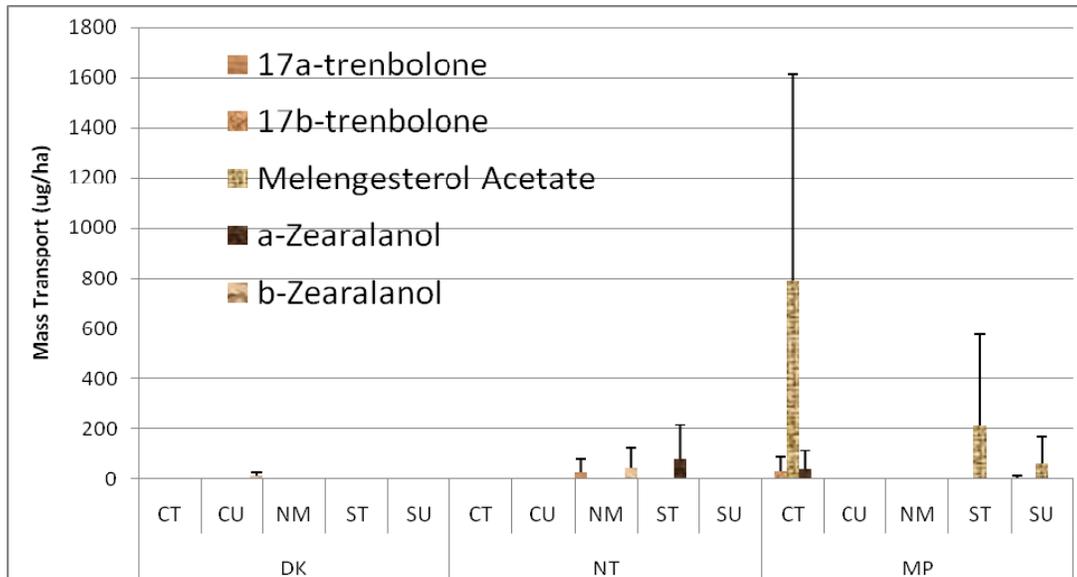


Figure 4. Mass transport (Average \pm SD) from the plots among the treatments, Tillage: DK= single disk incorporation, NT= no till surface application, MP= moldboard plow plus single disk; Manure: CT= compost treated, CU= compost untreated, NM= no manure, ST= stockpile treated, SU= stockpile untreated.

Discussion

Considering the sporadic detection of synthetic hormones in runoff samples, it is difficult to conclude the extent to which artificial hormones can be transported in surface runoff. While manure handling and tillage practices may affect different types of synthetic hormone and metabolites from soils treated with manure, their presence in surface runoff from agricultural land may impact surface water. The FWC was 9.75 ng/L for melengesterol acetate in a treated compost manure plot whereas mass transport was 1636.5 $\mu\text{g}/\text{ha}$. On the contrary, the lowest FWC and mass export were 0.56 ng/L and 93.82 $\mu\text{g}/\text{ha}$ for 17 β -trenbolone, also from a treated compost manure plot. Based on a limited number of detections, the potential for transport to surface water seems highest for melengesterol acetate followed by α - and β -zearalanol and finally 17 α - and 17 β -trenbolone.

For this study, 96 heifers were fed approximately 145 days before manure was collected, therefore it is likely that some of synthetic hormones might be degraded in the feedlot before it was composted or stockpiled and finally applied to the field during the rainfall simulation study. A related degradation study with composted manure showed that melengesterol acetate degraded from 0.72 ng/g to 0.55 ng/g within 14 days, α -zearalanol degraded from 32.3 ng/g in 21 days to 4.27 ng/g in 42 days and 17 β -trenbolone degraded from 26.7 ng/g to no-detection level from treated cattle manure (DeVivo, 2009). Trace residues melengesterol acetate and α -zearalanol were also found in their untreated compost study. Some level of synthetic hormones detection in different plots like melengesterol acetate (SU plot), β -zearalanol (CU and NM plot) and 17 α -trenbolone (NM and SU plot) was a possibility but require further investigation. Though

quit low, the traces of hormone detected in check plots (NM) may be the result of using treated manure upslope from the study plots. Cross contamination from improper feed additive or field equipment during the study period was unlikely as considerable effort was taken to minimize this during the study. The statistical relevance of analytical error and instrumental noise is highly significant at these low levels. Presence of α -zearalanol from untreated plot runoff samples can be explained by the fungal action from the *Fusarium* genus which is widely distributed in soil and associated with plants. The fungi normally grow on cereal plants like corn or barley and can produce mycotoxin like α -zearalanol. Since the field area was in a corn soybean rotation with corn planted the previous year, it is possible that excretions by fungal colonies could be the source of some hormones.

The lower detection of 17β -trenbolone can be explained by its short half life as it described by Khan et al. (2008). They reported that 17β -trenbolone had a half life of 12 hours when initial concentration was <1 mg/kg and concluded that field applied beef manure could activate the microbial communities under normal temperature and moisture conditions and its persistence was less likely. In a subsequent study, Khan et al. (2009) also showed that sorption capacity of 17β -trenbolone was twice that of 17α -trenbolone. When Schiffer et al. (2001) conducted the study in Germany, they treated cattle with trenbolone acetate and melengestrol acetate for 8 weeks and solid dung and liquid manure were spread on maize fields after 4.5 and 5.5 months of storage, respectively. The concentrations in the dung hill went from 75 ng/g to 10 ng/g for trenbolone and from 8 ng/g to 6 ng/g for melengestrol acetate. The above study also voiced the possible degradation mechanism of these synthetic hormones after land application. But the critical question might be what will happen if it rains immediately after fresh manure application.

Conclusion

Low levels of artificial hormones and some metabolites were detected in runoff samples after land application and, may be transported to nearby surface water sources. Because of the low frequency of detection, it was difficult to relate hormone occurrence to manure source and tillage method. Aerobic degradation from composting likely reduced the possible presence of artificial hormone in surface runoff and can be used as best management practices (BMP). Run-off water from single disk incorporation plots showed fewer detection compared to moldboard plow+disk and no-tillage and can be used as recommended tillage practices to control the movement of artificial hormones in runoff.

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