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# Effect of rainfall timing and tillage on the transport of steroid hormones in runoff from manure amended row crop fields

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### HIGHLIGHTS

- Hormones and metabolites were detected in runoff from test plots fertilized with cattle manure.
- Composting can reduce the hormone concentration in manure compared to stockpiling.
- About 10% of applied hormones can be lost through the dissolved phase of runoff.

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### ABSTRACT

Runoff generated from livestock manure amended row crop fields is one of the major pathways of hormone transport to the aquatic environment. The study determined the effects of manure handling, tillage methods, and rainfall timing on the occurrence and transport of steroid hormones in runoff from the row crop field. Stockpiled and composted manure from hormone treated and untreated animals were applied to test plots and subjected to two rainfall simulation events 30 days apart. During the two rainfall simulation events, detection of any steroid hormone or metabolites was identified in 8–86% of runoff samples from any tillage and manure treatment. The most commonly detected hormones were 17 $\beta$ -estradiol, estrone, estriol, testosterone, and  $\alpha$ -zearalenol at concentrations ranging up to 100–200 ng L<sup>-1</sup>. Considering the maximum detected concentrations in runoff, no more than 10% of the applied hormone can be transported through the dissolved phase of runoff. Results from the study indicate that hormones can persist in soils receiving livestock manure over an extended period of time and the dissolved phase of hormone in runoff is not the preferred pathway of transport from the manure applied fields irrespective of tillage treatments and timing of rainfall.

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## 1. Introduction

The occurrence of natural and synthetic hormones in surface water is a concern to the scientific community due to potential

impacts from chronic exposure to aquatic organisms and public health at low nanogram per liter concentrations [1–4]. Runoff from the crop fields treated with livestock manure generated in the Concentrated Animal Feeding Operations (CAFOs) can be a potential source of hormones to the surface water. The increased density of animals in CAFOs generates greater amounts of manure often with insufficient land area for distribution at agronomic rates. Like all other animals, cattle excrete endogenous hormones through feces

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and urine as a natural physiological process. In addition to that, almost 96% of the cattle in U.S. CAFOs receive steroid hormone treatments for growth promotion through implants behind the ear or as feed additives [5]. Based on typical hormone excretion rates, each year an estimated 43 Mg of hormones can be released to the environment from approximately 15 million Mg of manure generated by feedlot cattle [6–8]. In the U.S., treatment of animal manure is not required as long as it is not discharged directly into surface water. A significant portion of this manure (if not all) is applied to agricultural fields as a source of fertilizer. Therefore, surface runoff from manure amended fields can potentially transport hormones from cropland to surface water resources [9,10].

The environmental fate of hormones and their metabolites is complex. After excretion in feces or urine, hormones and metabolites generally change forms through chemical and microbial transformation [11,12]. Both natural and synthetic hormones and their metabolites have been detected in manure, soil and wastewater of cattle feedlots [13–16]. Studies evaluating the persistence of specific hormones in soils observed that most steroid hormones and their metabolites exhibit relatively strong adsorption-desorption characteristics with low aqueous solubility [17–19]. Soil microbiology and surrounding physio-chemical characteristics are major factors controlling transformation among the steroid hormones and their metabolites. For example, Mansell et al. [20] reported the presence of parent hormones such as  $17\alpha$ -estradiol,  $17\beta$ -estradiol, and testosterone (ranging from 5 to  $175\text{ ng L}^{-1}$ ) in runoff from feedlot surface during a rainfall simulation event conducted immediately after the animals were removed from the pens but they found the presence of metabolites such as estrone, androstenedione, and progesterone (ranging from 50 to  $250\text{ ng L}^{-1}$ ) in runoff when they conducted another rainfall simulation 7 days later. They concluded that the dominant forms of hormones and metabolites were determined by changing soil microbial composition along with surrounding physio-chemical properties of the soil. Thus, the likelihood for occurrence of these common forms of hormones or metabolites in surface water is greater from soil near the vicinity of CAFOs [21–23]. Studies have detected the presence of four types of natural and synthetic hormones, namely androgens ( $17\alpha$ - and  $17\beta$ -trenbolone), estrogens (estrone,  $17\beta$ -estradiol and estriol) and progestogens (progesterone and melengestrol acetate) in river water samples and identified their adverse impacts on aquatic life (e.g., fathead minnow) at different sites impacted by livestock production facilities along the Elkhorn River in Nebraska [22,24,25]. Result from a study in Israel found that hormones can be detected in rivers up to 60 km away from a CAFO [26].

A review of the literature suggests that although the occurrence of natural and synthetic hormones at various sites in manure, soil, and water has been well documented, there are no efforts that determine the transport of all four major steroid hormone categories in runoff from crop fields under different agricultural management practices. Considering this knowledge gap, this study was designed to evaluate the performance of two common manure handling (compost and stockpiling) and three tillage practices (disk, moldboard plow with disk, and no-till) on the occurrence of steroid hormones in surface runoff from two one-month-apart rainfall events. It was hypothesized that manure treatment can reduce the concentration of hormones; the types of soil, timing of rainfall, and tillage can play a significant role to influence the transport of hormone through runoff. To verify the hypothesis, artificial rainfall was applied to row crop fields amended with beef cattle manure to determine the impact of rainfall timing, manure, and tillage practices on the concentration of hormones and their associated metabolites in surface runoff from row crop fields.

## 2. Materials and methods

### 2.1. Feedlot and manure management

This study was conducted at the Haskell Agricultural Laboratory of the University of Nebraska-Lincoln near Concord, Nebraska, U.S. ( $42^{\circ}22'52.7''\text{N}$ ,  $96^{\circ}57'31.3''\text{W}$ ). Soils at the site were mapped as a Nora silty clay loam (fine-silty, mixed, mesic Udic Haplustolls) with 28% sand, 48% silt, and 24% clay and organic carbon fraction ( $f_{oc}$ ) of 2.2% [27]. The soil has permeability in the range of  $15\text{--}50\text{ mm h}^{-1}$  and available water holding capacity of  $0.17\text{--}0.22\text{ mm mm}^{-1}$  soil [28]. The study area has an average annual rainfall of  $672\text{ mm y}^{-1}$  and average annual temperature of  $8^{\circ}\text{C}$  [29].

Ninety-six heifers (average weight 230–270 kg) that received no hormone treatments prior to the experiment were split into two groups and housed in six pens (16 animals in each). One group of three pens contained animals that was treated with Ralgr<sup>TM</sup> (36 mg of  $\alpha$ -zearalanol) and Revalor<sup>TM</sup>-H (140 mg of trenbolone acetate and 14 mg of  $17\beta$ -estradiol benzoate) and fed an additive MGA<sup>®</sup> 200 Premix (0.45 mg of melengestrol acetate per animal per day) with daily rations. The remaining group (control/untreated) of three pens contained animals that received no implants and were fed regular rations. In each pen, the accumulated manure was collected after cattle were removed from the pens after the 112 days trials. The detailed description of the cattle feeding study in the feedlot is described in Bartelt-Hunt et al. [14].

Collected manure from each pen was then split into two piles, one for composting and one for stockpiling. Twelve manure piles (six of compost piles and six of stockpiled) were stored on a concrete pad under a roof to prevent rainfall from entering the manure piles. The stockpiled manure was stored under roof and remained undisturbed before application to the test plots. For compost piles, corn silage was added to create a manure: silage ratio of 21:1 on a dry weight basis to increase the carbon content. A one-time application of 379 L of water was applied to each compost pile. The corn silage and water were thoroughly mixed using a skid loader. The compost piles were turned weekly for the first four weeks and then biweekly for the next eight weeks to facilitate proper aeration during the composting process. The temperature of the compost pile went up to  $55^{\circ}\text{C}$  during the second to third week of composting and gradually went down after that. The complete description of manure management study is supplied in Bartelt-Hunt et al. [15].

The average weight of each pile was estimated to be between 3600 and 4000 kg. Four manure types were produced: 1) composted manure from hormone treated animals (CT); 2) composted manure from untreated animals (CU); 3) stockpiled manure from hormone treated animals (ST); and 4) stockpiled manure from untreated animals (SU). At the end of composting and stockpiling period, manure samples were collected from bottom, top, center, and outer edges of a pile. Samples of a pile were then mixed in a stainless steel bucket (cleaned with methyl alcohol) to make a composite sample of each pile, and frozen before sending to the laboratory for analysis. The chemical and physical properties of the manure are provided in Table 1.

### 2.2. Rainfall simulation study

Research plots were established in a continuous corn field that had been under no-till practices for more than 5 years. Plots were oriented in the up-and-down hill direction with an average field slope of 8%. Manure was applied to a plot at either an application rate of  $193\text{ Mg ha}^{-1}$  (wet weight) of stockpiled manure or  $159\text{ Mg ha}^{-1}$  (wet weight) of composted manure to meet soil nitrogen requirements. The recommended nitrogen requirements are  $170\text{ kg-N ha}^{-1}$  for dryland continuous corn in northeast Nebraska

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