

2012

# Effect of Growth Promotants on the Occurrence of Endogenous and Synthetic Steroid Hormones on Feedlot Soils and in Runoff from Beef Cattle Feeding Operations

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Bartelt-Hunt, Shannon L.; Snow, Daniel D.; Kranz, William L.; Mader, Terry L.; Shapiro, Charles A.; van Donk, Simon; Shelton, David P.; Tarkalson, David; and Zhang, Tian, "Effect of Growth Promotants on the Occurrence of Endogenous and Synthetic Steroid Hormones on Feedlot Soils and in Runoff from Beef Cattle Feeding Operations" (2012). *West Central Research and Extension Center, North Platte*. 61.

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# Effect of Growth Promotants on the Occurrence of Endogenous and Synthetic Steroid Hormones on Feedlot Soils and in Runoff from Beef Cattle Feeding Operations

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## S Supporting Information

**ABSTRACT:** Supplements and growth promotants containing steroid hormones are routinely administered to beef cattle to improve feeding efficiency, reduce behavioral problems, and enhance production. As a result, beef cattle manure will contain both synthetic steroids as well as a range of endogenous steroids including androgens, estrogens, and progestogens. A two-year controlled study was conducted in which beef cattle were administered steroid hormones via subcutaneous implants and feed additives and the occurrence of 16 endogenous and synthetic steroid hormones and metabolites was evaluated in runoff from beef cattle feedlots and in manure and soil collected from feedlot surfaces. Samples were extracted and analyzed using liquid chromatography tandem mass spectrometry for metabolites of the synthetic androgen trenbolone acetate, 17 $\alpha$ -trenbolone, 17 $\beta$ -trenbolone, for the nonsteroidal semisynthetic estrogen agonist,  $\alpha$ -zearalanol, and the synthetic progesterone melengesterol acetate, as well as a wide range of endogenous estrogens, androgens, and *fusarium* metabolites. Synthetic steroids including trenbolone metabolites and melengesterol acetate were detected in fresh manure and in feedlot surface soils from cattle administered synthetic steroids at concentrations up to  $55 \pm 22$  ng/g dry weight (dw) (17 $\alpha$ -trenbolone) and  $6.5 \pm 0.4$  ng/g dw (melengesterol acetate). Melengesterol acetate was detected in 6% of runoff samples from feedlots holding cattle administered synthetic steroids at concentrations ranging up to 115 ng/L. The presence of melengesterol acetate in runoff from beef cattle feeding operations has not been previously reported. Synthetic steroids were not detected in manure or runoff from control cattle. A wide range of endogenous hormones were detected in runoff and feedlot surface soils and manure from cattle given synthetic steroids and from control cattle, with no statistically significant differences in concentration. These results indicate that runoff from confined animal production facilities is of environmental and public health concern regardless of the use of growth promotants.



## INTRODUCTION

It is estimated that the approximately 250 000 confined beef cattle operations in the United States produce on the order of 4.5 billion metric tons of waste on an annual basis.<sup>1</sup> Operators of beef cattle production facilities routinely utilize synthetic hormones such as trenbolone acetate and melengesterol acetate to enhance livestock growth,<sup>2,3</sup> and manure produced by beef cattle feeding operations can contain these endocrine-active compounds.<sup>4</sup> There have been a limited number of studies reporting the occurrence of trenbolone acetate metabolites and melengesterol acetate in runoff and manure from beef feedlots<sup>4</sup> with concentrations of up to 75 ng/g dw and 8 ng/g dw reported for

trenbolone metabolites and melengesterol acetate, respectively. Exposure of aquatic organisms to exogenous and endogenous steroid hormones may negatively impact reproductive function and development<sup>5,6</sup> and studies have documented the reproductive impacts observed in aquatic organisms in surface waters impacted by runoff from beef cattle feeding operations or after land application of beef cattle manure.<sup>5,7-9</sup>

**Received:** August 2, 2011

**Revised:** December 22, 2011

**Accepted:** January 3, 2012

**Published:** January 3, 2012

Although numerous studies have focused on the occurrence and biologic effect of steroid hormones in land-applied confined animal feedlot wastes,<sup>10–13</sup> there have been a limited number of studies evaluating the occurrence of contaminants in runoff occurring directly from feedlot surfaces. Most previous studies evaluating runoff from feedlot surfaces have focused on conventional contaminants including sediment, nutrients and *E. coli*.<sup>14,15</sup> A limited number of studies have focused on the occurrence of biologically active organic compounds in beef cattle feedlot surface soils, manure or runoff from feedlot surfaces. Aust et al.<sup>16</sup> evaluated the distribution of three veterinary antibiotics in manure and soil in beef cattle feedlots and determined that these compounds could persist in soil and manure up to 1 year after medication. Schiffer et al.<sup>4</sup> determined the occurrence of exogenous growth promotants including trenbolone acetate and melengesterol acetate in fresh cattle manure and after manure storage within a beef cattle feeding operation. Recently, Mansell et al.<sup>17</sup> evaluated the occurrence of endogenous steroid hormones in beef cattle feedlot runoff after simulated rainfall. Endogenous steroids were detected in runoff at biologically active concentrations.<sup>17</sup> To date, no studies have concurrently investigated the occurrence of a large suite of endogenous and exogenous steroid hormones in feedlot surface soils, manure and in runoff from beef feedlot surfaces. Although much speculation exists regarding the effect of growth promotants on the occurrence of endogenous and exogenous steroid hormones in wastes from beef cattle production facilities, there is relatively little data available to document their occurrence in feedlot surface runoff.

The objective of this study was to evaluate the occurrence of endogenous and synthetic steroids and related metabolites in manure, beef feedlot surface soils and natural rainfall-produced runoff events from production facilities under conditions where the diet and exogenous hormones given to the animals were carefully controlled. No previous study has examined the occurrence of both endogenous and exogenous steroid hormones in feedlots in a direct comparison of implanted and unimplanted cattle. The data generated in this study will provide a better understanding of the potential environmental impacts from both endogenous and synthetic steroid hormones in runoff water from feedlot surfaces and in soils receiving animal waste.

## MATERIALS AND METHODS

**Site Description and Feeding Regimen.** Feeding pen studies were conducted at the University of Nebraska Haskell Agricultural Laboratory near Concord, NE. All study protocols involving animals were approved by the University of Nebraska-Lincoln Institutional Animal Care and Use Committee. Studies were conducted in 2007 and 2008 with 96 cross-bred heifers divided equally into two groups: a treatment group in which animals were administered synthetic hormones via subcutaneous implant and feed additives, and a control group with no synthetic hormone administration (i.e., no subcutaneous implant or feed additives). Animals were randomly assigned to one of six pens for a total of 16 animals per pen with an approximate animal density of 40 m<sup>2</sup> per animal. For each treatment, three replicate groups of animals were held in adjacent pens and the treated and control groups were separated throughout the study by a distance of approximately 15 m. The feedlots utilized in this study have been used previously for studies of growth performance and animal behavior in beef feedlots, and the research facility accurately simulates a commercial confined beef cattle production facility.<sup>18</sup> All cattle

were fed a feedlot finishing ration for the duration of the study. On day 1 of the study, heifers in the treated group received an implant containing 36 mg of  $\alpha$ -zearalanol (Ralgro). The expected release lifetime of Ralgro is 35–55 days.<sup>19</sup> After 35 days, the same animals received an implant containing 140 mg of trenbolone acetate (TbA) and 14 mg of 17 $\beta$ -estradiol benzoate (Revelor-H). The expected release lifetime of Revelor-H is 90–120 days.<sup>19</sup> Animals in the treated group also received 0.45 mg of melengesterol acetate per animal per day via their feed through the study from day 7 to through the end of the study period. This regimen is representative of what is typically utilized in the commercial beef production industry.<sup>20</sup> The animals were held in the pens for 112 and 141 days in 2007 and 2008, respectively. At the conclusion of the feeding study in 2007, all soil and manure was mechanically scraped from the feedlot pens down to the clay layer, and was replaced with fresh soil prior to the initiation of the 2008 study. Background concentrations of steroid hormones in the soil were evaluated in both 2007 and 2008.

**Collection of Feedlot Runoff.** Runoff was collected using tipping bucket samplers instrumented for measurement of runoff volume during each runoff event. A schematic of the tipping bucket sampler is provided in Figure S1 in the Supporting Information (SI). Runoff was directed toward the samplers via earthen berms established below each pen, and each of the three replicate pens in the treatment and control groups was sampled individually. The tipping bucket samplers consisted of a galvanized steel tank fitted with a runoff splitter, tipping bucket mechanism, event data logger, and covered sump. After each runoff event, a composite sample was manually collected within 5 h in a 250 mL amber glass sampling jar. The total volume of runoff produced during each precipitation event was calculated from the recorded number of tips and the bucket geometry. Runoff samples were stored frozen at –20 °C until analysis. A permanent weather station located at the facility provided measurements of daily rainfall and temperature during the study period.

**Collection of Feedlot Surface and Manure Samples.** Feedlot surface soil, urine-soaked feedlot surface soil, and fresh manure samples were collected in each pen at 7, 46, and 109 days (in 2007) and 7, 47, and 138 days (in 2008) after study initiation. Prior to initiation of the study, feedlot surface soil samples were collected to establish background levels of steroid hormones. To collect soil samples representative of the feedlot surface, each pen was subdivided into four equally sized zones and five 100 g samples were obtained from randomly selected locations within each zone using a stainless-steel spoon. Samples were collected from the top few centimeters of soil. The five subsamples were mixed thoroughly in a stainless steel bucket, and an approximately 250 g composite sample was frozen at –20 °C until analysis, for a total of four composite samples per pen per sampling event. Fresh manure samples and urine-soaked feedlot surface samples were collected by mixing five 100 g samples of fresh manure and five 100 g samples of urine-soaked soil. Urine-soaked soil samples were collected from the same depth as the feedlot surface soils. Samples were obtained from feedlot areas where sample collectors visually observed animals to be urinating or defecating. The five 100 g samples were mixed in a stainless-steel bucket and an approximately 250 g composite sample was frozen at –20 °C until analysis. All manure and feedlot surface sampling was conducted on days when there was no rainfall for the preceding 24 h period. To avoid cross-contamination between the control

and treatment pens, dedicated sampling equipment was used for the treatment and control samples. Personnel wore disposable boot covers during sample collection, and control pens were always sampled prior to the treatment pens. A timeline describing the timing of all surface sampling events, runoff events, and implantation activities is given in Figures S2 and S3 in the SI.

**Sample Extraction and Steroid Hormone Analysis.** All reagents used in steroid analysis were purchased from Fisher Scientific and used the highest purity available (Optima, Thermofisher Scientific, St. Louis, MO). Pure steroid standards, including  $17\beta$ -estradiol, estrone, estriol, testosterone, 4-androstenedione, androsterone,  $17\beta$ -trenbolone, and progesterone were purchased from Sigma-Aldrich (St. Louis, MO) or Acros Chemicals, whereas  $17\alpha$ -trenbolone was obtained from Hayashi Pure Chemical Industries (Osaka, Japan). Internal standards included testosterone- $d_3$ , obtained from Sigma Aldrich (St. Louis, MO), and  $^{13}C_6$ -estradiol purchased from Cambridge Isotopes (Andover, MA).

Surface runoff samples were analyzed for endogenous and synthetic steroids using online solid phase extraction liquid chromatography tandem mass spectrometry with atmospheric pressure photoionization (APPI). A 25 mL aliquot of each runoff sample was filtered using  $0.45\ \mu m$  glass microfiber syringe filters into amber glass vials and spiked using a micropipet with 500 ng/L testosterone- $d_3$ ,  $^{13}C_6$ -estradiol, and  $17\alpha$ -methyltestosterone (surrogate) and acidified with 10  $\mu L$  concentrated formic acid. Filtered samples were then immediately analyzed by online solid phase extraction (SPE) using a Spark Holland Symbiosys Environ automated extraction system. Waters Oasis HLB  $2.0 \times 10\ mm$  SPE cartridges were used for both sample preconcentration and instrument calibration. Microwave-assisted solvent extraction (MASE) was used for feedlot surface and manure samples.<sup>21,22</sup> Briefly, 2–3 g of sample was weighed into a 10 mL Teflon microwave digestion vessel, mixed with 1 mg of butylated hydroxytoluene and 5 mL of high purity methanol.<sup>22,23</sup> Twenty-five ng of testosterone- $d_3$ ,  $^{13}C_6$ -estradiol, and  $17\alpha$ -methyltestosterone was added by pipet and the contents vortexed prior to microwaving in a CEM MARS Xpress microwave at 1000 W for 10 min.

Instrumental detection and quantification of steroids from online extraction and MASE extracts utilized multiple reaction monitoring (MRM) with argon collision gas. A Thermo Hypurity C18 column ( $250 \times 2\ mm$ ,  $5\ \mu m$ ,  $50\ ^\circ C$ ) was used for gradient separation at a flow rate of 0.35 mL/min. The gradient consisted of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in methanol), with 0 to 3 min at 50% B, 3 to 14 min at 65% B, and 14–20 min at 95% B, with a return to initial solvent conditions for the last 10 min of the gradient (30 min total). Instrument control, data acquisition and evaluation used MassLynx 4.0 software (Waters Corporation, Milford, MA). 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 (SI Table S1). Retention times were considered to match if they were within  $\pm 5\%$  of the standards.

## ■ QUALITY CONTROL

Laboratory reagent blanks, fortified blanks, fortified matrix samples, and duplicates were prepared and analyzed at a rate of at least 1 in 20 samples (5%) for both LC/MS/MS methods. Testosterone- $d_3$  was used for quantification of androgens,

melengesterol acetate and progesterone, whereas  $^{13}C_6$ -estradiol served as the internal standard for steroid estrogens and resorcylic acid lactones ( $\alpha$ -zearalanol compounds) in both the online SPE and MASE LC-tandem MS methods. Recovery of the synthetic androgen ( $17\alpha$ -methyltestosterone) surrogate was calculated to evaluate individual sample extraction efficiency and possible matrix effects, but concentrations were not corrected for surrogate recovery. Though isotope dilution may correct for variability in testosterone and  $17\beta$ -estradiol source ionization, all other compounds may be affected by matrix enhancement or suppression. Online SPE method detection limits ranged between 1 and 9 ng/L (SI Table S2). Surrogate recovery of online SPE averaged  $133 \pm 38\%$  in all runoff samples, suggesting there may be some matrix enhancement in runoff samples. Average recovery in fortified blanks spiked at 500 ng/L range between 65 and 130% with most compounds averaging between 90 and 110%. Recovery in fortified matrix (runoff) samples spiked at 500 ng/L ranged between 50 and 157%. (SI Table S3). Method detection limits of the MASE method range between 0.09 ng/g and 0.7 ng/g, except for estriol (SI Table S2). In solid samples,  $17\alpha$ -methyltestosterone surrogate recovery averaged  $97 \pm 23\%$ . Fortified matrix (sand) samples spiked with 8.3 ng/g of analyte averaged between 75 and 108%, whereas fortified matrix sample recovery 59 and 112%. Laboratory duplicates were generally within  $\pm 30$ –50% and method blanks were at or below detection limits in both methods.

**Nutrient Analysis.** An aliquot of the unfiltered runoff sample was digested with a Kjeldahl procedure<sup>25</sup> and analyzed by inductively coupled plasma optical emission spectroscopy for total phosphorus and by flow injection analysis (QuickChem 8500, Lachat Instruments) for total nitrogen. A second aliquot was filtered using a  $0.45\ \mu m$  glass fiber filter and analyzed for dissolved  $NH_4$ -N and  $NO_3$ -N using flow injection analysis.<sup>26</sup> Total suspended solids concentrations were determined gravimetrically.

## ■ RESULTS

**Cattle Performance Data.** Measured indicators of cattle growth performance were consistent with previous studies evaluating cattle growth with and without the use of steroid hormone supplements. More information is provided in the SI Table S5.

**Steroid Occurrence in Runoff.** Recorded precipitation volumes for 2007 and 2008 are presented in SI Figure S4. A total of 33.7 and 41.6 cm of precipitation was recorded in 2007 and 2008, respectively. There were four rainfall events that produced measurable runoff in 2007. Approximately 1 cm of rainfall was required to produce measurable runoff, although this was highly dependent on soil moisture and the time between rainfall events. No rainfall events greater than 10 mm occurred in May through July 2007 and no measurable runoff was recorded during this period. Fourteen runoff events occurred in 2008 between May and September. Estimates of runoff volume as measured in 2008 by the tipping bucket samplers are provided in SI Figure S5. The percent occurrence and maximum concentration of steroid hormones detected in the runoff samples is presented in Table 1. Additional information on the steroid hormones detected in individual runoff events can be found in SI Tables S6. Fifteen of the 16 steroid hormones evaluated were measured in at least one runoff sample ( $n = 50$ ) over the two-year study period at concentrations of up to 24  $\mu g/L$ . The steroids that were detected in



Table 1. Occurrence and Maximum Concentration of Steroid Hormones in Runoff from Cattle Feedlots ( $n = 50$ )

	testosterone	4-andro- stenedione	andro- sterone	androstane- dionedione	17 $\beta$ - estradiol	17 $\alpha$ - estradiol	estrone	estriol	progesterone	17 $\alpha$ - hydroxy- progesterone	$\alpha$ - zearalanol	$\alpha$ - zearalanol	$\beta$ - zearalanol	melengesterol acetate	17 $\alpha$ - trenbolone	17 $\beta$ - tren- bolone
treated cattle	percent occurrence	47%	100%	92%	14%	80%	26%	29%	32%	98%	6%	90%	10%	10%	6%	0%
	maximum concentration (ng/L)	420	1050	24,300	1250	1100	720	1050	570	1070	245	5200	2930	500	115	<5
	median concentration (ng/L)	6.9	138.5	2260	<5	131	10.6	269	59.5	69.4	<5	587	<5	<5	<5	<5
control cattle	percent occurrence	25%	100%	96%	24%	77%	24%	46%	20%	100%	2%	72%	10%	16%	0%	0%
	maximum concentration (ng/L)	475	1000	17,000	540	1360	720	2600	390	1250	26	3820	1720	1440	<5	<5
	median Concentration (ng/L)	<5	102	2660	<5	103	<5	243	<5	99.1	<5	348	<5	<5	<5	<5

more than 90% of runoff samples include 4-androstenedione (detected in 100% of runoff samples from both groups); androsterone (detected in 92% and 96% of runoff samples from treated and control animals, respectively); and progesterone (detected in 98% and 100% of runoff samples from treated and control animals, respectively). Other steroids that were consistently detected in runoff samples include 17 $\beta$ -estradiol, which was detected in 80% of runoff samples from the treated group and 77% of runoff samples from the control group and  $\alpha$ -zearalanol, which was detected in 90% of samples from the treated group and 72% of samples from the control group. In addition to  $\alpha$ -zearalanol, the *fusarium* metabolites  $\alpha$ -zearalenol and  $\beta$ -zearalenol were detected in runoff samples from both the treated and control groups. Other compounds that were detected in runoff from both groups include testosterone, at maximum concentrations of 420 ng/L and 475 ng/L for the treated and control groups, respectively; androstenedione, at maximum concentrations of 1250 ng/L and 540 ng/L for the treated and control groups, respectively; 17 $\alpha$ -estradiol, detected at a maximum concentration of 720 ng/L in both groups; estrone, detected at maximum concentrations of 1050 and 2600 ng/L in the treated and control groups, respectively; estriol, detected at maximum concentrations of 570 ng/L and 390 ng/L, respectively; and 17 $\alpha$ -hydroxyprogesterone, detected at maximum concentrations of 245 ng/L and 26 ng/L in the treated and control groups, respectively. There were two steroid hormones that were detected in samples from the treated group only. The synthetic progesterone melengesterol acetate was detected in 6% of runoff samples from the treated group at concentrations up to 115 ng/L and 17 $\beta$ -trenbolone was detected in 2% of samples from the treated group at concentrations up to 270 ng/L. 17 $\alpha$ -trenbolone was not detected in any runoff samples throughout the two-year study.

Nutrients measured in runoff in 2008 are presented in SI Table S7. Concentrations of total phosphorus, total nitrogen, dissolved  $\text{NH}_4\text{-N}$ , dissolved  $\text{NO}_3\text{-N}$ , and total suspended solids are consistent with previous studies investigating nutrients and solids in feedlot runoff.<sup>27</sup> Concentrations of total phosphorus, total nitrogen, and total suspended solids were not correlated with the concentration of total steroid hormones present in the runoff samples (data not shown).

**Steroid Occurrence on Feedlot Surfaces.** Prior to placement of the cattle in the pens during each year of the study, the soil in the feedlot pens was scraped down to the clay layer and replaced with fresh soil obtained from another location at the research facility. Samples from the fresh soil were collected and analyzed for steroid hormones (Table 2 and SI Table S8, day 0). In 2007, estrone and estriol were detected in the clean soil at maximum concentrations of 0.26 ng/g dw and 6.4 ng/g dw, respectively (Table 2). In 2008, 4-androstenedione (max. concentration 1.9 ng/g dw); androsterone (max. concentration 0.82 ng/g dw);  $\alpha$ -zearalanol (max. concentration 0.34 ng/g dw); and progesterone (max. concentration 1.7 ng/g dw) were detected in the clean soil. Because the fill soil was obtained from an area of the research facility that had historically had manure applied, it is possible that this is the source of the steroids detected in the fill soil. Alternatively, the pens utilized in this study have been used for animal production for over 40 years, and although the pens were scraped down to the clay layer, some residual soil may have remained in the pens and been mixed with the background soil. There were no statistically significant differences ( $p < 0.05$ ; Mann–Whitney test) in the concentration of steroid

**Table 2. Concentrations of Steroid Hormones (ng/g dry weight) Detected in Feeding Pen Surface Samples, Urine-Soaked Surface Soil Samples, And Fresh Manure Samples in 2007<sup>a</sup>**

sample type treatment <sup>b</sup>	description	day	testo- sterone	4-androsten- edione	andro- sterone	andro- stane dienedione	17 $\beta$ - estradiol	17 $\alpha$ -estra- diol	estrone	estriol	$\alpha$ -zeara- lanol	$\alpha$ -zeara- lanol	$\beta$ -zeara- lanol	melen- gestrol acetate	proge- sterone	17 $\alpha$ -hydroxy- proge- sterone	17 $\alpha$ tren- bolone	17 $\beta$ - tren- bolone
control	feeding pen surface	day 0	ND	ND	ND	ND	ND	ND	ND	4.9 $\pm$ 0.09	ND	ND	ND	ND	ND	ND	ND	ND
		day 7	ND	2.3 $\pm$ 0.16	ND	ND	ND	ND	ND	3.2 $\pm$ 0.05	0.18 $\pm$ 0.04	0.42 $\pm$ 0.11	ND	0.06 $\pm$ 0.09	6.6 $\pm$ 0.5	ND	ND	ND
		day 46	ND	29 $\pm$ 0.99	ND	ND	1.2 $\pm$ 0.19	1.8 $\pm$ 0.15	ND	0.12 $\pm$ 0.03	14 $\pm$ 0.83	37 $\pm$ 3.9	2.8 $\pm$ 0.7	1.5 $\pm$ 1.0	68 $\pm$ 2.8	ND	ND	ND
		day 109	ND	9.5 $\pm$ 0.8	53 $\pm$ 11	ND	19 $\pm$ 4.2	0.19 $\pm$ 4.5	9.1 $\pm$ 2.6	ND	19 $\pm$ 5.0	ND	ND	ND	7.0 $\pm$ 0.7	0.16 $\pm$ 0.04	ND	ND
	urine soaked soil	day 0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	ND	ND
		day 7	ND	1.4 $\pm$ 0.4	ND	ND	ND	ND	ND	4.9 $\pm$ 0.6	3.7 $\pm$ 0.4	8.1 $\pm$ 4.3	ND	ND	3.3 $\pm$ 1.2	ND	ND	ND
		day 46	ND	19 $\pm$ 1.7	ND	ND	18 $\pm$ 2.2	0.93 $\pm$ 0.52	ND	ND	9.8 $\pm$ 2.0	83 $\pm$ 6.7	48 $\pm$ 3.4	1.2 $\pm$ 0.36	71 $\pm$ 3.9	ND	ND	ND
		day 109	ND	6.0 $\pm$ 1.3	ND	ND	ND	0.22 $\pm$ 0.1	ND	ND	3.9 $\pm$ 2.2	ND	ND	0.40 $\pm$ 0.22	5.2 $\pm$ 1.3	ND	ND	ND
	fresh manure	day 0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		day 7	ND	1.1 $\pm$ 0.14	ND	ND	ND	ND	25.6 $\pm$ 3.1	ND	72.5 $\pm$ 22	25 $\pm$ 4.6	ND	6.5 $\pm$ 0.37	2.7 $\pm$ 0.24	ND	ND	ND
		day 46	ND	0.87 $\pm$ 0.24	ND	ND	ND	8.5 $\pm$ 1.6	11 $\pm$ 3.1	ND	47 $\pm$ 5.5	46 $\pm$ 4.5	ND	3.2 $\pm$ 0.70	4.7 $\pm$ 0.36	ND	31 $\pm$ 6.0	ND
		day 109	ND	ND	ND	ND	ND	ND	ND	ND	126 $\pm$ 36	ND	ND	2.8 $\pm$ 0.94	0.84 $\pm$ 0.25	ND	ND	ND
	feeding pen surface	day 0	ND	ND	ND	ND	ND	ND	0.26 $\pm$ 0.04	6.4 $\pm$ 0.20	ND	ND	ND	ND	ND	ND	ND	ND
		day 7	ND	0.75 $\pm$ 0.05	ND	ND	ND	ND	0.45 $\pm$ 0.09	4.5 $\pm$ 0.11	0.13 $\pm$ 0.03	ND	ND	ND	4.9 $\pm$ 0.34	ND	ND	ND
		day 46 <sup>c</sup>	ND	31 $\pm$ 1.7	ND	ND	3.8 $\pm$ 0.6	1.0 $\pm$ 0.14	ND	0.43 $\pm$ 0.07	15 $\pm$ 1.6	66 $\pm$ 5.2	13 $\pm$ 2.1	ND	148 $\pm$ 9.0	1.3 $\pm$ 0.22	ND	ND
		day 109	ND	6.1 $\pm$ 0.45	8.4 $\pm$ 2.4	ND	3.5 $\pm$ 0.4	0.15 $\pm$ 0.04	0.08 $\pm$ 0.02	ND	ND	ND	ND	ND	25 $\pm$ 2.6	3.1 $\pm$ 0.9	ND	ND
	urine soaked soil	day 0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	ND	ND
		day 7	ND	2.6 $\pm$ 0.3	ND	ND	ND	ND	ND	7.0 $\pm$ 1.2	2.9 $\pm$ 1.2	ND	ND	ND	8.8 $\pm$ 1.0	ND	ND	ND
		day 46	ND	15 $\pm$ 1.6	ND	ND	16 $\pm$ 2.3	0.93 $\pm$ 0.52	ND	ND	9.8 $\pm$ 2.0	94.5 $\pm$ 8.8	43 $\pm$ 5.1	ND	106 $\pm$ 13	ND	ND	ND
		day 109	ND	9.8 $\pm$ 0.16	7.3 $\pm$ 1.0	ND	4.2 $\pm$ 0.6	ND	ND	ND	5.1 $\pm$ 0.73	ND	ND	ND	59 $\pm$ 2.8	ND	ND	ND
	fresh manure	day 0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	ND	ND
		day 7	ND	1.5 $\pm$ 0.44	ND	ND	ND	ND	ND	ND	37.7 $\pm$ 1.5	24 $\pm$ 4.8	ND	ND	6.6 $\pm$ 1.6	ND	ND	ND
		day 46	ND	1.3 $\pm$ 0.42	ND	ND	1.3 $\pm$ 0.75	ND	22 $\pm$ 2.1	ND	76 $\pm$ 11	47 $\pm$ 4.1	ND	ND	7.7 $\pm$ 0.74	ND	5.0 $\pm$ 2.8	ND
		day 109	ND	ND	ND	ND	ND	ND	ND	ND	ND	630 $\pm$ 211	ND	ND	4.5 $\pm$ 0.38	1.1 $\pm$ 0.62	ND	ND

<sup>a</sup>All concentrations presented as average  $\pm$  standard error. For feeding pen surface samples,  $n = 12$ ; for urine-soaked soil and fresh manure samples,  $n = 3$ . <sup>b</sup>Treatment = subcutaneous implants (36 mg  $\alpha$ -zearalanol, 140 mg trenbolone acetate, 14 mg 17 $\beta$ -estradiol), and 0.45 mg melengesterol acetate per day via feed additives. <sup>c</sup> $n = 9$  for 46 days feeding pen surface data.

hormones in the background soil between the treatment and control pens and concentrations of steroid hormones detected on day 0. Background subtraction was not performed on data collected from subsequent feedlot surface sampling events.

17 $\alpha$ -trenbolone and 17 $\beta$ -trenbolone were not detected in any of the dry feedlot surface samples from either year of the study, but 17 $\alpha$ -trenbolone was detected in the fresh manure samples at approximately day 45 (10 days after implantation with Revelor-H) at average concentrations of 31 ng/g dw (2007) and 55 ng/g dw (2008) (Table 2 and SI Table S8). In 2008, 17 $\beta$ -trenbolone was also detected in the fresh manure collected from the treated cattle 10 days after implantation at an average concentration of 0.5 ng/g dw. No trenbolone metabolites were detected in manure samples collected at the end of the study, 70–104 days after implantation. 17 $\beta$ -trenbolone was detected at an average concentration of 5 ng/g dw from the fresh manure sample obtained from the control pen 10 days after implantation. The anomalous occurrence of 17 $\beta$ -trenbolone in manure from control cattle may be due to accidental contamination during sample collection or animal holding. The fact that no other control samples were found to contain trenbolone metabolites or melengesterol acetate indicates that contamination was not widespread. Melengesterol acetate was detected in dry feedlot soils, urine-soaked soils and in fresh manure samples from the treated cattle in both years of the study at average concentrations ranging from 0.06 to 6.5 ng/g dw.

Of the 16 steroid hormones evaluated in this study, androstenedione was not detected in any of the samples taken from the feedlot surface during either year of the study. There were limited detections of testosterone, which was not detected in any of the dry feedlot surface soils from either the treated or control pens, but was detected in the urine-soaked soil from the treated pens at an average concentration of 3.8 ng/g dw on day 6 and in the fresh manure from the treated pens on day 139 at an average concentration of 1.5 ng/g dw in 2008 only. Other steroids detected in the feedlot soil and manure samples include 4-androstenedione, androsterone, 17 $\beta$ -estradiol, 17 $\alpha$ -estradiol, estrone, estriol,  $\alpha$ -zearalenol,  $\alpha$ -zearalenol,  $\beta$ -zearalenol, progesterone, and 17 $\alpha$ -hydroxyprogesterone (Table 2 and SI Table S8).

## DISCUSSION

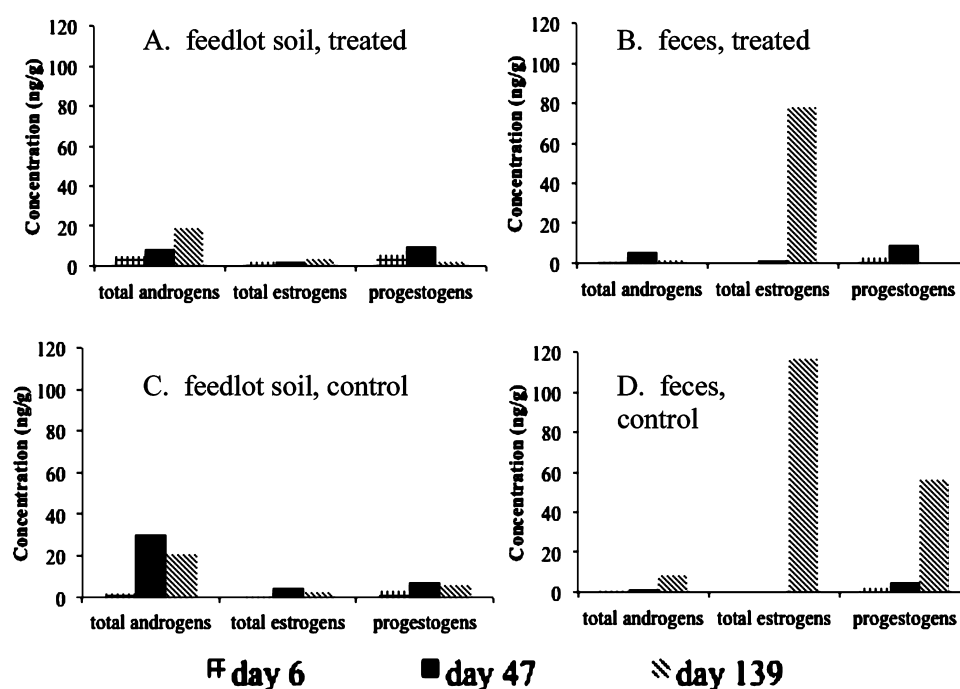
**Occurrence of Exogenous Steroids.** Exogenous steroids and steroid metabolites including 17 $\alpha$ -trenbolone, 17 $\beta$ -trenbolone, and melengesterol acetate were detected in runoff and/or feedlot surface and manure samples obtained from pens holding cattle administered growth promotants. These compounds were not detected in runoff or feedlot surface and manure samples obtained from pens holding unimplanted cattle. In both years of this study, 17 $\alpha$ -trenbolone and 17 $\beta$ -trenbolone were detected in fresh manure on day 45 of the study, approximately 10 days after cattle were administered the implant containing trenbolone acetate. No trenbolone metabolites were detected in manure samples collected at the end of the study period (day 109 or 139), which may be partially explained by the short half-lives reported for these compounds. Reported half-lives for 17 $\alpha$ -trenbolone and 17 $\beta$ -trenbolone in aerobic soils range from 4 to 50 h and from 5 to 15 h, respectively.<sup>28</sup> Although gradual, the rate at which trenbolone is excreted in cattle bile is greatest within the first 30–45 days after implantation and tapers during feeding.<sup>29</sup> The lack of detection of trenbolone metabolites during later

sampling events likely indicates a decrease in trenbolone excretion rates below detection limits in fresh manure. 17 $\alpha$ -trenbolone was the dominant metabolite identified in the manure (average concentration 30–50 ng/g dw) and concentrations of 17 $\beta$ -trenbolone were approximately 100 times lower (average concentration 0.5 ng/g dw). This is consistent with previous studies that determined 17 $\alpha$ -trenbolone was the dominant metabolite excreted in cattle waste.<sup>4,28</sup> Schiffer et al.<sup>4</sup> determined concentrations of 17 $\alpha$ -trenbolone and 17 $\beta$ -trenbolone in fresh cattle dung were between 4 and 75 ng/g dw and 0.5 to 4 ng/g dw, respectively, which is consistent with the concentrations observed in this study. Although trenbolone has been previously detected in runoff from beef feedlots,<sup>9</sup> 17 $\beta$ -trenbolone was detected in only one runoff event over a two year period in this study. One limitation of this study is that the presence of trendione was not evaluated for liquid or solid samples as commercial standards were not available. 17 $\alpha$ -trenbolone and 17 $\beta$ -trenbolone can be converted to trendione in hours or days,<sup>28</sup> and it is possible trendione was present, but unaccounted for, in liquid and solid samples obtained in this study.

Melengesterol acetate was detected in dry feedlot surface soils (average concentration 0.28–1.5 ng/g dw), in urine-soaked soil (average concentration 0.4–1.2 ng/g dw), and in fresh manure (average concentration 1.7–6.5 ng/g dw) at sampling times between day 7 (when MGA feed additives were initiated) and the end of the study period, consistent with previous studies that detected melengesterol acetate in cattle feces at concentrations of 1.6–2.5 ng/g and in soil fertilized with solid cattle manure at concentrations between 0.6 and 34 pg/g.<sup>4</sup> The higher concentrations of melengesterol acetate detected in fresh manure in this study are consistent with previous reports that melengesterol acetate is primarily excreted via bile.<sup>4</sup> We also detected melengesterol acetate in 6% of runoff events at concentrations up to 115 ng/L, indicating that melengesterol acetate can be exported from feedlot surfaces via runoff. The occurrence of melengesterol acetate in runoff from beef cattle feeding operations has not been previously reported.

**Occurrence of Estrogens, Androgens, and Progesterone.** Additional steroid hormones including testosterone, 4-androstenedione, androsterone, 17 $\beta$ -estradiol, estrone, estriol, progesterone, and 17 $\alpha$ -hydroxyprogesterone were detected in both runoff and soil and manure samples obtained from pens containing both treated and control animals. The occurrence of these endogenous steroids in runoff is consistent with a previous study investigating steroid hormone occurrence in surface waters adjacent to grazing lands and dairy farms.<sup>30</sup> The maximum concentrations of 17 $\beta$ -estradiol, estrone, testosterone, 4-androstenedione, and progesterone measured in runoff samples in the present study were 24–800 times higher than concentrations previously reported in surface waters adjacent to grazing lands in the previous study,<sup>30</sup> which implies that runoff directly from confined animal feedlot surfaces may constitute a more concentrated source of steroids to the environment compared with other types of animal production facilities. The occurrence of progesterone and progesterone degradation products including 4-androstenedione and 17 $\alpha$ -hydroxyprogesterone, in runoff, feedlot surface soils, and manure samples in this study provides additional evidence that animal waste was a likely source of the progesterone identified in surface waters adjacent to cattle grazing lands. For most compounds evaluated, there was good agreement between the frequency of detection in the surface samples and the occurrence in runoff.





**Figure 1.** Comparison of natural steroids in treated and implanted cattle. (A) feedlot soil from treated cattle pens, (B) feces from treated cattle, (C) feedlot soil from control cattle pens, (D) feces from control cattle.

However, testosterone and androsterone were detected more frequently in the runoff when compared to their detection in the feedlot surface samples. The cattle utilized in this study were heifers and would not be expected to excrete significant quantities of testosterone in urine or feces. The presence of testosterone and androsterone in the runoff samples is hypothesized to be partially due to transformation of progesterone, which was consistently detected in feedlot surface samples and in runoff samples. Androsterone is a transformation product of progesterone and is a precursor to testosterone formation. Progesterone was detected in nearly 100% of runoff samples at concentrations up to 1250 ng/L and in feedlot surface and manure samples at concentrations as high as 148 ng/g dw. Previous studies have documented androstenedione and androstenedione production from progesterone by soil bacteria<sup>31</sup> and bacterial mechanisms for production of testosterone via progesterone conversion to 17 $\alpha$ -hydroxyprogesterone to androstenedione have also been identified.<sup>32,33</sup> A recent study of steroids in cattle feedlot runoff also hypothesized that the presence of androgens may be due to progesterone degradation.<sup>17</sup> Clearly, more controlled experiments to evaluate the potential for androgen formation from progesterone in animal production facilities is warranted.

There were fewer detections of 17 $\alpha$ -estradiol in the runoff samples relative to the detections of 17 $\beta$ -estradiol. For both the treated and control cattle, 17 $\beta$ -estradiol was detected in 77–80% of the runoff samples, while 17 $\alpha$ -estradiol was only detected in 24–26% (Table 1). In both 2007 and 2008, 17 $\alpha$ -estradiol was detected in fresh manure and urine-soaked soil samples obtained approximately 10 days after implantation of the Revelor-H implant containing 17 $\beta$ -estradiol (Table 2 and SI Table 3), but was not detected at the sampling times at the end of the study. It has been previously reported that 17 $\alpha$ -estradiol is degraded to estrone over a 1 day period in dairy wastewater,<sup>34</sup> which may explain the lack of detection of 17 $\alpha$ -estradiol in runoff samples obtained in this study.

#### Comparison between Treated and Control Animals.

Statistical differences between endogenous steroid concentrations in runoff, soil, and fresh manure samples obtained from the treated and control groups collected in 2008 was evaluated using a Mann–Whitney test (GraphPad Prism version 5). There were sporadic instances when the concentrations of endogenous steroids were significantly higher ( $p < 0.05$ ) in runoff, soil and fresh manure samples from the treated cattle compared to the control animals or vice versa, but no clear trends could be identified across compounds or compound groups. Figure 1 shows the concentrations of total estrogens, total androgens, and progestogens in fresh manure and feedlot soils from the treated and control pens in 2008. This data suggests that use of growth promotants may not significantly alter the levels of endogenous steroids excreted by the cattle.

**Occurrence of  $\alpha$ -Zearalanol in Treated and Control Animals.** Although  $\alpha$ -zearalanol was administered via implant to cattle only in the treated group,  $\alpha$ -zearalanol,  $\alpha$ -zearalenol, and  $\beta$ -zearalanol were detected in runoff, feedlot soil and manure samples from both the treated and control groups.  $\alpha$ -Zearalanol, a compound derived from the mycotoxin zearalenone, has been widely used as a growth promoter in the United States to improve feeding efficiency in cattle production. Despite its frequent use in cattle production, its metabolism and excretion in cattle is not well understood. Early studies conducted with tritiated implants indicated that 10% of the implanted compound was excreted in cattle urine and 45% was excreted via feces.<sup>35</sup> It is known that in cattle,  $\alpha$ -zearalanol is metabolized into its diastereoisomer  $\beta$ -zearalanol and to a lesser extent into zearalanone.<sup>36</sup>  $\alpha$ -Zearalanol and  $\beta$ -zearalanol were identified in the urine of pasture-raised cattle and attributed to *fusarium* mold occurring in grasses.<sup>37</sup> In this study, the occurrence of  $\alpha$ -zearalanol could be attributed to excretion from implanted cattle, or also due to metabolism of zearalenone produced by *fusarium* mold which is commonly found in fermented corn<sup>38,39</sup> (SI Figure S6). In the current study,

attribution of the  $\alpha$ -zearalanol identified in manure, feedlot soils and runoff to the implant is difficult without additional information regarding  $\alpha$ -zearalanol metabolism within the animal as well as information on processes controlling environmental transformation of naturally occurring mycotoxins. Zearalenone and its metabolites  $\alpha$ -zearalenol,  $\alpha$ -zearalanol, and  $\beta$ -zearalanol have been detected in surface and wastewater samples at concentrations up to 60 ng/L.<sup>38–41</sup>

The data obtained from this study indicate that runoff from beef cattle feedlots can be a source of steroid hormones to the environment at concentrations that are above the predicted no effect concentration (PNEC) for aquatic organisms. Direct exposure to runoff from beef cattle production facilities may negatively impact the health of aquatic organisms. Endogenous steroids were detected in runoff from pens housing both treated and untreated animals at similar concentrations, indicating that runoff from confined animal production facilities is of environmental and human health concern regardless of the use of growth promotants.

Synthetic steroids including trenbolone metabolites and melengesterol acetate were detected in runoff, fresh manure and feedlot surface soils in this study. Trenbolone metabolites were only detected in fresh manure collected 10 days after administration of synthetic steroids to cattle. Additional information is needed to evaluate the occurrence of trendione in beef cattle feedlot soils. Both endogenous and synthetic steroids were associated with manure solids and surface soils indicating that land application of solid manure from beef feedlots may be an important source of synthetic and endogenous steroids to the environment.

## ■ ASSOCIATED CONTENT

### ● Supporting Information

Additional information as noted in the text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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## ■ ACKNOWLEDGMENTS

This research was funded by a U.S. EPA Science to Achieve Results (STAR) Award No. R833428. The authors would like to acknowledge Leslie Johnson, Kevin Heithold, Mike Mainz, and Mitiku Mamo for their assistance in collection of field samples and for animal care throughout the study period, and Teyona Damon for assistance in steroid hormone analysis.

## ■ REFERENCES

- (1) U.S. Environmental Protection Agency. *Risk Management Evaluation for Concentrated Animal Feeding Operations*, EPA-600-R-04-042; Office of Research and Development, National Risk Management Research Laboratory: Cincinnati, OH, 2004.
- (2) Preston, R. L. Hormone containing growth promoting implants in farmed livestock. *Adv. Drug Deliver. Rev.* **1999**, *38*, 123–138.
- (3) Duckett, S. K.; Andrae, J. G. Implant strategies in an integrated beef production system. *J. Anim. Sci.* **2001**, *79*, E110–E117.
- (4) Schiffer, B.; Daxenberger, A.; Meyer, K.; Meyer, H. H. D. The fate of trenbolone acetate and melengesterol acetate as growth promoters in cattle: Environmental studies. *Environ. Health Perspect.* **2001**, *109*, 1145–1151.
- (5) Orlando, E. O.; Kolok, A. S.; Binzcik, G. A.; Gates, J. L.; Horton, M. K.; Lambright, C. S.; Gray, J. L. E.; Soto, A. M.; Guillelte, J. L. J. Endocrine disrupting effects of cattle feedlot effluent on an aquatic sentinel species, the fathead minnow. *Environ. Health Perspect.* **2004**, *112*, 353–358.
- (6) Kidd, K. A.; Blachfield, P. J.; Mills, K. H.; Palace, V. P.; Evans, R. E.; Lazorchak, J. M.; Flick, R. W. Collapse of a fish population after exposure to a synthetic estrogen. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 8897–8901.
- (7) Jeffries, M. K. S.; Abbott, K. I.; Cowman, T.; Kolok, A. S. Occurrence and endocrine effects of agrichemicals in a small Nebraska, USA, watershed. *Environ. Toxicol. Chem.* **2011**, *30*, 2253–2260.
- (8) Soto, A. M.; Calabro, J. M.; Precht, N. V.; Yau, A. Y.; Orlando, E. F.; Daxenberger, A.; Kolok, A. S.; Guillelte, L. J.; le Bizec, B.; Lange, I. G.; Sonnenschein, C. Androgenic and estrogenic activity in water bodies receiving cattle feedlot effluent in eastern Nebraska, USA. *Environ. Health Perspect.* **2004**, *112*, 346–352.
- (9) Durhan, E. J.; Lambright, C. S.; Makynen, E. A.; Lazorchak, J.; Hartig, P. C.; Wilson, V. S.; Gray, L. E.; Ankley, G. T. Identification of metabolites of trenbolone acetate in androgenic runoff from a beef feedlot. *Environ. Health Perspect.* **2006**, *114*, 65–68.
- (10) Gall, H. E.; Sassman, S. A.; Lee, L. S.; Jafvert, C. T. Hormone discharges from a midwest tile-drained agroecosystem receiving animal wastes. *Environ. Sci. Technol.* **2011**, *45*, 8755–8764.
- (11) Nichols, D. J.; Daniel, T. C.; Moore, P. A.; Edwards, D. R.; Pote, D. H. Runoff of estrogen hormone 17 $\beta$ -estradiol from poultry litter applied to pasture. *J. Environ. Qual.* **1997**, *25*, 1002–1006.
- (12) Dutta, S.; Inamdar, S.; Tso, J.; Aga, D. S.; Sims, J. T. Free and conjugated estrogen exports in surface-runoff from poultry litter amended soil. *J. Environ. Qual.* **2010**, *39*, 1688–1698.
- (13) Finley-Moore, O.; Hartel, P. G.; Cabrera, M. L. 17 $\beta$ -estradiol and testosterone in soil and runoff from grasslands amended with broiler litter. *J. Environ. Qual.* **2000**, *29*, 1604–1610.
- (14) Miller, J. J.; Handerek, B. P.; Beasley, B. W.; Olson, E. C. S.; Yanke, L. J.; Larney, F. J.; McAllister, T. A.; Olson, B. M.; Selinger, L. B.; Chanasyk, D. S.; Hasselback, P. Quantity and quality of runoff from a beef cattle feedlot in southern Alberta. *J. Environ. Qual.* **2004**, *33*, 1088–1097.
- (15) Gilley, J. E.; Vogel, J. R.; Eigenberg, R. A.; Marx, D. B.; Woodbury, B. L. Runoff, erosion, and size distribution of sediment from beef cattle feedlots. *Trans. ASABE* **2011**, *54*, 435–440.
- (16) Aust, M. O.; Godlinski, G.; Travis, G. R.; Hao, X. Y.; McAllister, T. A.; Leinweber, P.; Thiele-Bruhn, S. Distribution of sulfamethazine, chlortetracycline, and tylosin in manure and soil of Canadian feedlots after subtherapeutic use in cattle. *Environ. Pollut.* **2008**, *33*, 1088–1097.
- (17) Mansell, D. S.; Bryson, R. J.; Harter, T.; Webster, J. P.; Kolodziej, E. P.; Sedlak, D. L. Fate of endogenous steroid hormones in steer feedlots under simulated rainfall-induced runoff. *Environ. Sci. Technol.* **2011**, *45*, 8811–8818.
- (18) Mader, T. L.; Dahlquist, J. M.; Gaughan, J. B. Wind protection effects and airflow patterns in outside feedlots. *J. Anim. Sci.* **1997**, *75*, 26–36.
- (19) Mader, T. L. Implants. *Vet. Clin. N. Am.: Food Anim. Pract.* **1998**, *14*, 279–290.
- (20) Kreikemeier, W. M.; Mader, T. L. Effects of growth-promoting agents and season on yearling feedlot heifer performance. *J. Anim. Sci.* **2004**, *82*, 2481–2488.
- (21) Camel, V. Microwave-assisted solvent extraction of environmental samples. *TrAC, Trends Anal. Chem.* **2000**, *19*, 229–248.
- (22) Labadie, P.; Cundy, A. B.; Stone, K.; Andrews, M.; Valbonesi, S.; Hill, E. M. Evidence for the migration of steroidal estrogens through river bed sediments. *Environ. Sci. Technol.* **2007**, *41*, 4299–4304.
- (23) Matejíček, D.; Houserová, P.; Kubán, V. Combined isolation and purification procedures prior to the high-performance liquid chromatographic-ion-trap tandem mass spectrometric determination of estrogens and their conjugates in river sediments. *J. Chromatogr., A* **2007**, *1171*, 80–89.
- (24) Snow, D. D.; Damon-Powell, T.; Onanong, S.; Cassada, D. A. Sensitive and simplified analysis of natural and synthetic steroids in water and sediments using on-line solid phase extraction and

microwave assisted solid phase extraction with liquid chromatography tandem mass spectrometry atmospheric pressure photoionization. (in review).

(25) *Methods for Chemical Analysis of Water and Wastes*, EPA-600/4-79-020, Revised March 1983, Method 351.2; U.S. Environmental Protection Agency: Washington, DC, 2003.

(26) Keeney, D. R.; Nelson, D. W. Nitrogen: Inorganic forms. In *Methods of Soil Analysis, Part 2—Chemical and Microbiological Properties*, 2nd ed.; Page, A.L. Ed.; American Society of Agronomy and Soil Science Society of America: Madison, WI, 1982; pp 643–698.

(27) Young, R. A.; Huntrods, T.; Anderson, W. Effectiveness of vegetated buffer strips in controlling pollution from feedlot runoff. *J. Environ. Qual.* **1980**, *9*, 483–487.

(28) Khan, B.; Lee, L. S. Soil temperature and moisture effects on the persistence of synthetic androgen 17 $\alpha$ -trenbolone, 17 $\beta$ -trenbolone and trendione. *Chemos* **2010**, *79*, 873–879.

(29) Heitzman, R. J. The absorption, distribution and excretion of anabolic agents. *J. Anim. Sci.* **1983**, *57*, 233–238.

(30) Kolodziej, E. P.; Sedlak, D. L. Rangeland grazing as a source of steroid hormones to surface waters. *Environ. Sci. Technol.* **2007**, *41*, 3514–3520.

(31) Jenkins, R. L.; Wilson, E. M.; Angus, R. A.; Howell, W. M.; Kirk, M.; Moore, R.; Nance, M.; Brown, A. Production of androgens by microbial transformation of progesterone *in vitro*: A model for androgen production in rivers receiving paper mill effluent. *Environ. Health Perspect.* **2004**, *112*, 1508–1511.

(32) Carlström, K. Mechanism of the side chain degradation of progesterone by microorganisms. *Acta Chem. Scand.* **1967**, *21*, 1297–1303.

(33) Wadhwa, L.; Smith, K. E. Progesterone side-chain cleavage by *Bacillus sphaericus*. *FEMS Microbiol. Lett.* **2000**, *192*, 179–183.

(34) Zheng, W.; Yates, S. R.; Bradford, S. A. Analysis of steroid hormones in a typical dairy waste disposal system. *Environ. Sci. Technol.* **2008**, *42*, 530–535.

(35) Sharp, G. D.; Dyer, I. A. Zearalanol metabolism in steers. *J. Anim. Sci.* **1972**, *34*, 176–179.

(36) Kleinova, M.; Zallner, P.; Kahlbacher, H.; Hochsteiner, W.; Lindner, W. Metabolic profiles of the mycotoxin zearalenone and of the growth promoter zeranol in urine, liver, and muscle of heifers. *J. Agric. Food Chem.* **2002**, *50*, 4769–4776.

(37) Erasmus, A. F.; Scahill, B. G.; West, D. M. Natural zeranol (alpha-zearalanol) in the urine of pasture-fed animals. *J. Agric. Food Chem.* **1994**, *42*, 2721–2725.

(38) Hartmann, N.; Erbs, M.; Wettstein, F. E.; Schwarzenbach, R. P.; Bucheli, T. D. Quantification of estrogenic mycotoxins at the ng/L level in aqueous environmental samples using deuterated internal standards. *J. Chromatogr., A* **2007**, *1138*, 132–140.

(39) Kolpin, D. W.; Hoerger, C. C.; Meyer, M. T.; Wettstein, F. E.; Hubbard, L. E.; Bucheli, T. D. Phytoestrogens and mycotoxins in Iowa streams: An examination of underinvestigated compounds in agricultural basins. *J. Environ. Qual.* **2010**, *39*, 2089–2099.

(40) Langaná, A.; Fago, G.; Marino, A.; Santarelli, D. Development of an analytical system for the simultaneous determination of anabolic macrocyclic lactones in aquatic environmental samples. *Rapid Comm. Mass. Spectrosc.* **2001**, *15*, 304–310.

(41) Bucheli, T. D.; Wettstein, F. E.; Hartmann, N.; Erbs, M.; Vogelgsang, S.; Forrer, H.-R.; Schwarzenbach, R. P. *Fusarium* mycotoxins: Overlooked aquatic micropollutants? *J. Agric. Food Chem.* **2008**, *56*, 1029–1034.

## **Supporting Information**

### **Effect of growth promotants on the occurrence of steroid hormones on feedlot soils and in runoff from beef cattle feeding operations**

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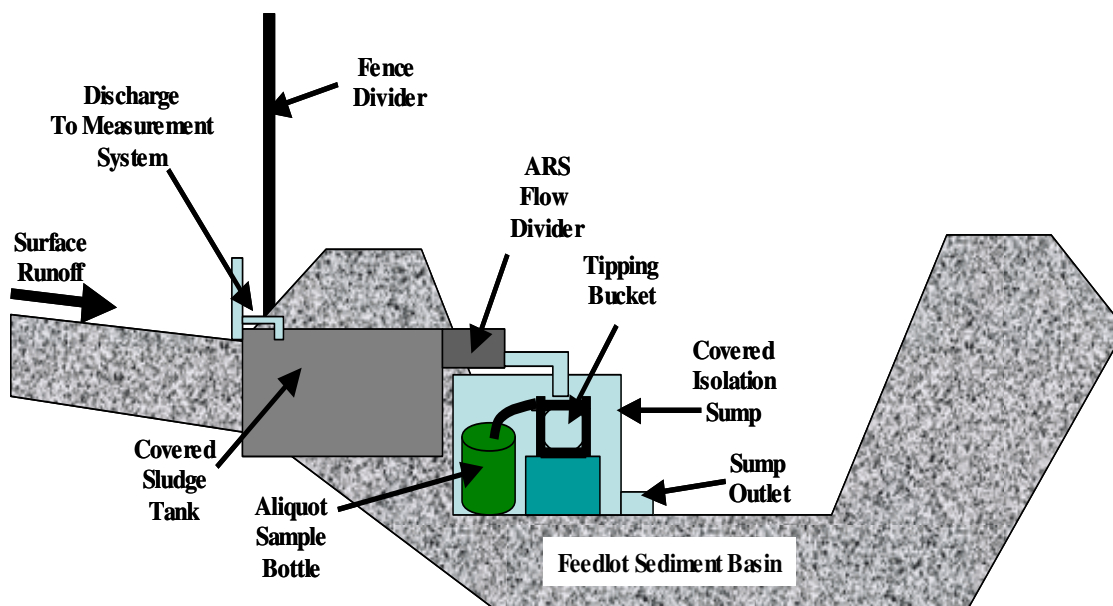
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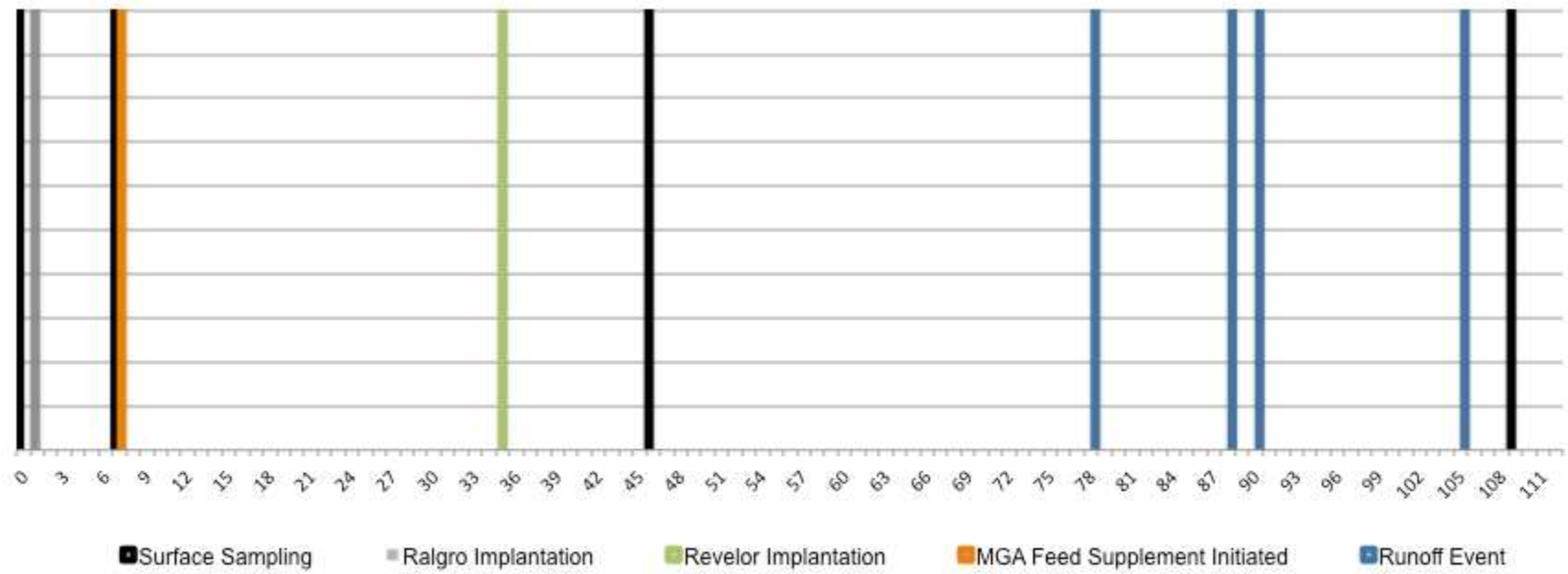
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The Supporting Information contains 23 pages, 6 figures, and 8 tables.

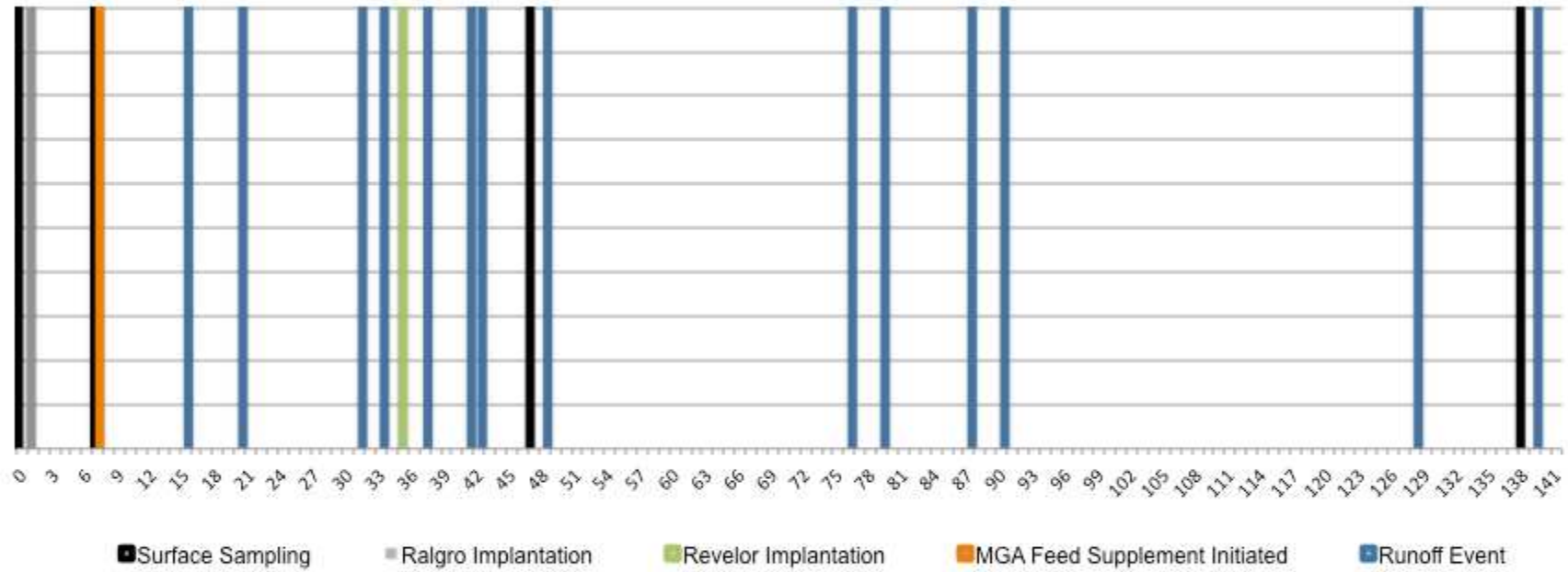


**Figure S1.** Schematic of feedlot surface runoff equipment layout for monitoring surface runoff and collecting water samples from each set of feedlot pens.

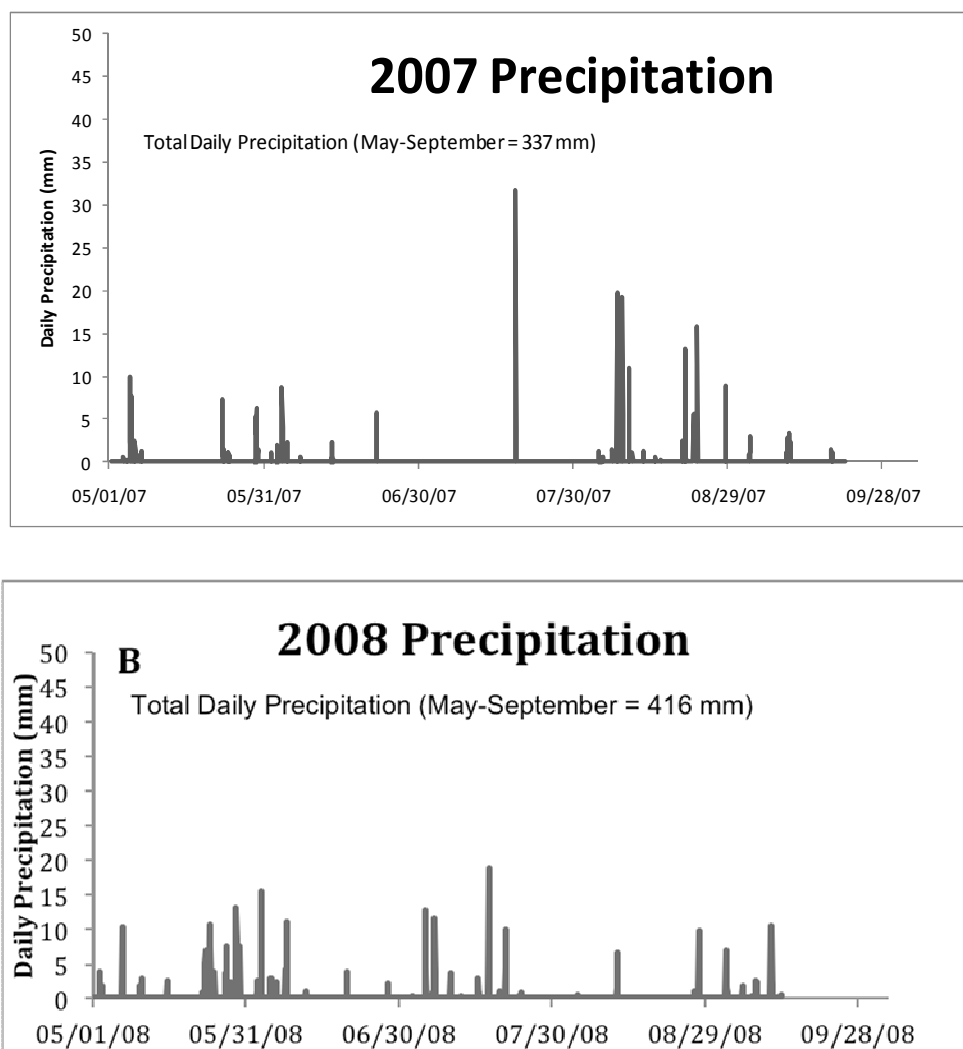




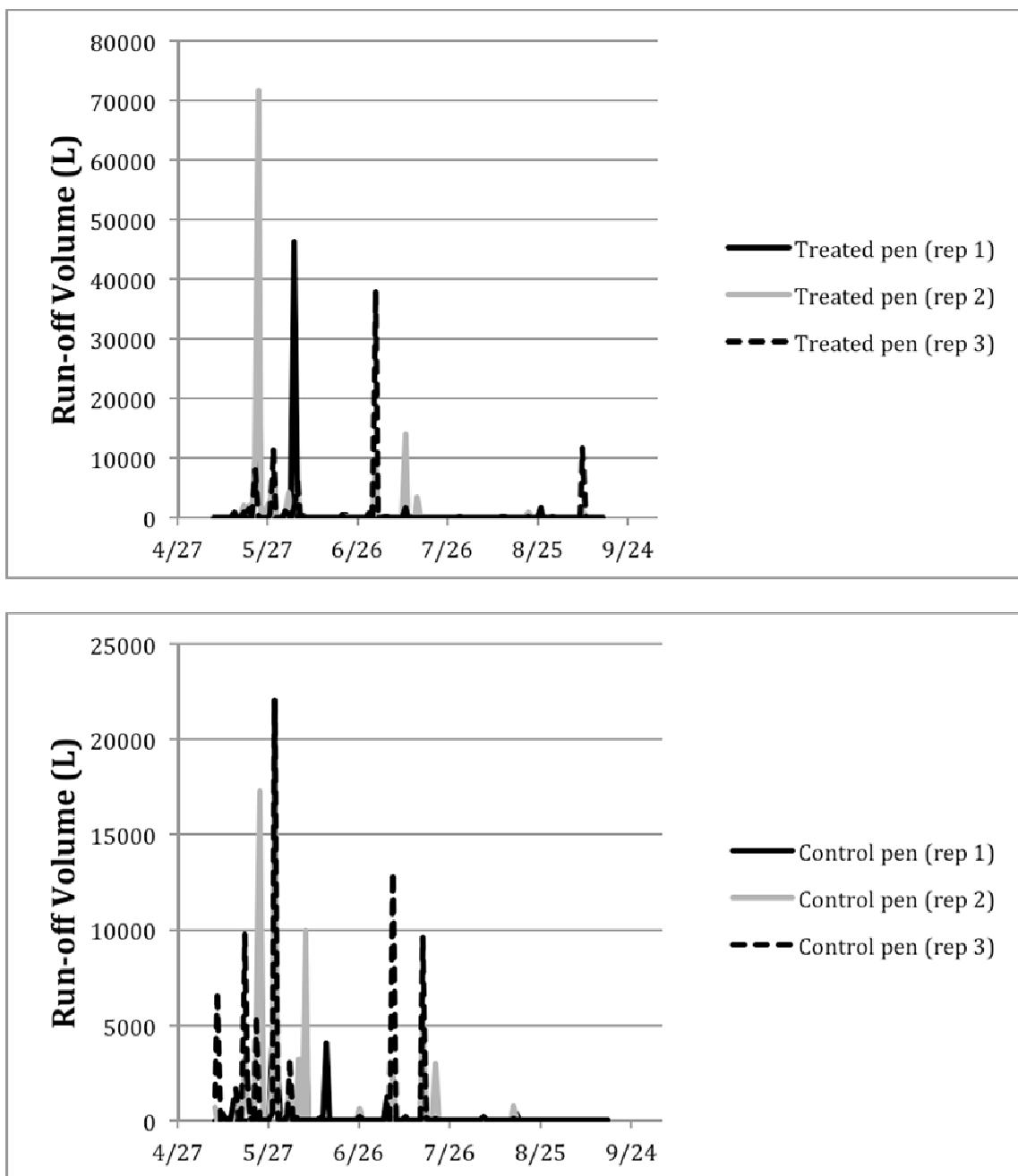
**Figure S2.** Experimental timeline in 2007



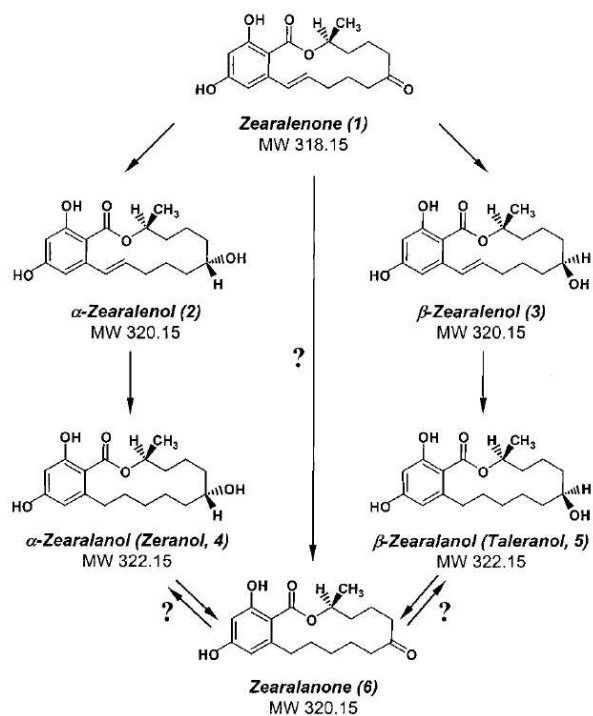
**Figure S3.** Experimental Timeline in 2008.



**Figure S4.** Precipitation occurring over the study period in 2007 (A) and 2008 (B).



**Figure S5.** Runoff volumes measured in 2008 by the tipping bucket sampler. Area contributing to runoff was 655 m<sup>2</sup> per pen.



**Figure S6.** Possible metabolic pathways of zearalenone. Implant active ingredient  $\alpha$ -zearalanol on lower left. From Kleinova et al [1].



## Analytical Methods for Steroid Hormone Analysis.

Details of the analytical methods used for steroid hormone analysis are published separately [2]. Water samples were extracted using on-line sample pre-concentration followed by atmospheric pressure photoionization (APPI) liquid chromatography-tandem mass spectrometry (LC-MS/MS). Solid samples were extracted using microwave assisted solvent extraction followed by APPI LC-MS/MS.

Briefly, water samples are filtered using 0.45µm glass microfiber syringe filters and weighed into 40 mL amber glass vials, spiked with 500 ng/L internal standards (testosterone-d<sub>5</sub>, 17β-estradiol-13C<sub>6</sub>) and a surrogate compound (17α-methyltestosterone), and acidified with 10 µL formic acid. Samples, standards, and quality control samples are loaded in a vial rack and individually extracted and eluted. Standards and samples are pumped through individual lines through an extraction cartridge loaded from 96-well format tray. The exterior of each tubing was rinsed between sample batches with 2-propanol and purified water. For surface water analysis, 30 mL of standards and samples were pumped at a rate of 2 mL/min through 10 x 2 mm cartridge packed with an Oasis HLB sorbent cartridge. Sediment samples (3 gms) were weighed out directly (no drying) into each extraction vessel and mixed with BHT in methanol, 100 µL of internal standards and the surrogate solutions (~8 ng/g of solid), and 5 mL of high-purity methanol. Each vessel was capped and agitated vigorously using a vortex mixer for 30 seconds to mix the sample and then allowed to equilibrate for at least 5 minutes. Multiple extraction vessels were loaded onto the removable microwave turntable (capacity of up to 40 samples) and placed in the microwave. A Micromass Quattro Micro triple quadrupole mass spectrometer (Waters Corporation, Milford, MA, USA) with an IonSabre<sup>TM</sup> APPI source (Syagen Technology, Inc., Tustin, CA) was used for analysis of all samples and extracts. Mass transitions, retention times, cone, and collision voltages of each compound are given in table S1. Repeller voltage was optimized at 0.9kV, extractor voltage 3V, and RF lens is 0.1 V. The source temperature was 135 °C, while the APPI probe temperature was 550 °C. Desolvation gas flow rate is 650 L/hr and the cone gas flow rate is 20 L/hr of nitrogen. Inter channel delay is 0.02 seconds and inter scan delay is 0.2 seconds. Instrument and method detection limits are listed in table S2.

**Quality Control.** Laboratory reagent blanks, fortified blanks, fortified matrix samples, and duplicates were prepared and analyzed at a rate of at least 1 in 20 samples (5%) for both LC/MS/MS methods. Testosterone-d<sub>3</sub> was used for quantification of androgens, MGA, progesterone, while 13C<sub>6</sub>-estradiol served as the internal standard for steroid estrogens and resorcylic acid lactones (α-zearalanol compounds) in both the on-line SPE and MASE LC-tandem MS methods. Recovery of the synthetic androgen (17α-methyltestosterone) surrogate was calculated to evaluate individual sample extraction efficiency and possible matrix effects, but concentrations were not corrected for surrogate recovery. Though isotope dilution may correct for variability in testosterone and 17β-estradiol source ionization, all other compounds may be affected by matrix enhancement or suppression. On-line SPE method detection limits ranged between 1 and 9 ng/L (Table S2). Surrogate recovery of on-line SPE averaged 133±38% in all runoff samples,

suggesting there may be some matrix suppression or enhancement in run-off results. Average recovery in fortified blanks spiked at 500 ng/L range between 65 and 130% with most compounds averaging between 90-110%. Recovery in fortified matrix (run-off) samples spiked at 500ng/L ranged between 50 and 157%. (Table S3). Method detection limits of the MASE method range between 0.09 ng/g and 0.7 ng/g, except for estriol (Table S2). In solid samples, 17 $\alpha$ -methyltestosterone surrogate recovery averaged 97 $\pm$ 23%. Fortified matrix (sand) samples spiked with 8.3 ng/g of analyte averaged between 75 and 108%, while fortified matrix sample recovery 59 and 112%. Laboratory duplicates were generally within  $\pm$ 30-50% and method blanks were at or below detection limits in both methods.

**Table S1.** List of method compounds by retention times, with multiple reaction monitoring (MRM) transitions, optimized cone voltage, and collision energies. From Snow et al [2].

Analyte	Mass Transition	Cone (V)	Collision (eV)	MASE extracts Retention Time (min)	On-Line SPE Retention Time (min)
Estriol	288 > 146	22	22	7.60	8.71
11-Ketotestosterone	303 > 121	30	22	8.86	10.21
$\beta$ -Zearalanol	305 > 189	28	20	9.34	10.76
Androstenedienedione	285 > 121	20	25	9.58	11.08
$\beta$ -Zearalenol	303 > 285	24	12	9.65	11.31
17 $\beta$ -Trenbolone	271 > 199	32	20	9.89	11.63
$\alpha$ -Zearalanol	305 > 189	28	20	10.29	12.42
17 $\alpha$ -Trenbolone	271 > 253	32	20	10.29	12.26
17 $\beta$ -Estradiol	255 > 159	24	20	10.52	12.66
17 $\alpha$ -Ethinylestradiol	279 > 133	20	18	10.60	12.81
4-Androstenedione	287 > 97	30	20	10.60	12.66
$\alpha$ -Zearalenol	303 > 285	24	12	10.68	12.89
Estrone	271 > 133	24	20	10.68	12.89
17 $\alpha$ -Estradiol	255 > 159	24	20	11.00	13.37
Testosterone	289 > 97	32	24	11.21	13.68
17 $\alpha$ -Hydroxyprogesterone	331 > 97	30	25	11.55	14.16
Epitestosterone	289 > 109	32	26	12.58	15.50
Melengestrol Acetate	397 > 337	24	14	14.08	17.31
Progesterone	315 > 97	30	20	14.23	17.47
Androsterone	273 > 255	25	14	14.79	18.02
<i>Internal standards and surrogates</i>					
17 $\beta$ -Estradiol- <sup>13</sup> C <sub>6</sub>	261 > 159	24	20	10.52	12.66
Testosterone-d5	294 > 100	32	24	11.20	13.60
$\alpha$ -Methyltestosterone	303 > 97	32	24	12.10	14.87

**Table S2** Instrument detection limits, method detection limits, and recoveries for on-line SPE and MASE using LC/MS/MS with APPI. Spiked analyte concentrations are 5 ng/L for on-line SPE and 0.5 ng/g for test soil. From Snow et al [2].

Compound	IDL (pg)	On-line SPE MDL (ng/L)	Average Recovery (%)	MASE MDL (ng/g)	Average Recovery (%)
11-Ketotestosterone	16.2	3.4	116.6	0.09	103.6
17 $\alpha$ -Estradiol	21.0	2.7	28.0	0.18	87.2
17 $\alpha$ -Hydroxyprogesterone	26.6	3.6	95.0	0.22	85.4
17 $\alpha$ -Trenbolone	9.4	3.2	85.4	0.16	109.9
17 $\beta$ -Estradiol	23.7	4.2	55.6	0.52	77.2
17 $\beta$ -Trenbolone	18.2	4.5	93.9	0.29	121.1
4-Androstenedione	15.2	1.3	70.2	0.12	56.1
Androstandienedione	10.2	1.9	69.8	0.14	95.9
Androsterone	48.6	2.4	94.3	0.34	51.8
Epitestosterone	21.6	2.4	64.7	0.19	94.2
Estriol	62.5	7.5	69.4	1.87	185.5
Estrone	35.1	4.9	88.8	0.60	110.8
17 $\alpha$ -Ethynylestradiol	57.5	4.7	169.9	0.49	61.8
Melengestrol Acetate	13.4	2.8	59.5	0.20	71.1
Progesterone	21.0	2.7	72.7	0.26	50.9
Testosterone	16.0	3.2	100.9	0.07	110.7
$\alpha$ -Zearalanol	33.3	9.0	82.6	0.54	135.7
$\alpha$ -Zearalenol	43.6	2.0	41.5	0.71	81.8
$\beta$ -Zearalanol	33.5	6.9	195.1	0.59	61.0
$\beta$ -Zearalenol	37.6	4.0	115.4	0.58	109.6

**Table S3.** Results of quality control water samples analyzed using on-line SPE LC/MS/MS.

Recoveries (=100xmeasured/fortified)of Laboratory Fortified Blanks (LFB) samples prepared and analyzed with Feeding Pen Run-off Samples																		
Preparation_Date	11-Keto Testosterone	17a-Hydroxyprogesterone	4-Androstenedione	17a-estradiol'	Androstosterone	17a-trenbolone'	a-Zearalanol	a-Zearalanol	17b-estradiol'	17b-trenbolone'	b-Zearalanol	b-Zearalanol	Estriol	Estrone	Ethinyl Estradiol	Melengesterol Acetate	Progesterone	Testosterone
9/10/2007	80.0	99.6	104.5	97.6	69.9	102.1	97.6	93.1	91.5	93.5	NM	85.9	68.7	94.9	79.7	87.5	119.7	86.7
7/1/2008	99.2	96.6	111.4	97.0	130.6	93.6	93.3	70.2	86.1	88.9	NM	79.5	332.9	91.2	87.1	49.0	94.9	95.2
9/11/2008	56.3	55.0	68.0	165.7	39.7	65.6	145.6	114.2	129.9	57.5	170.5	132.0	109.1	125.2	165.6	34.5	31.7	60.3
9/11/2008	106.0	111.4	91.4	101.8	59.4	105.3	132.0	125.2	112.9	92.0	154.4	143.6	155.1	94.2	131.0	94.9	79.0	90.0
9/11/2008	101.0	73.4	101.5	173.8	44.3	99.7	143.1	118.1	181.6	96.4	63.5	125.7	92.8	179.7	143.2	58.7	61.7	105.7
Average	88.5	87.2	95.3	127.2	68.8	93.3	122.3	104.2	120.4	85.7	129.5	113.4	151.7	117.0	121.3	64.9	77.4	87.6
Standard Deviation	20.5	22.7	16.9	39.0	36.6	16.0	25.1	22.4	38.4	16.0	57.7	28.8	106.1	37.6	36.9	25.6	33.3	16.9
Laboratory Reagent Blanks (LRB) samples prepared and analyzed with Feeding Pen Run-off Samples																		
9/10/2007	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	NM	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002
7/1/2008	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	NM	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002
9/11/2008	<0.002	<0.002	<0.002	0.004	0.003	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002
9/11/2008	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002
9/11/2008	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	0.003	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002
Laboratory Duplicates analyzed with Feeding Pen Samples																		
8/21/2007	<0.002	<0.002	0.212	<0.002	2.591	<0.002	0.475	<0.002	0.099	<0.002	NM	0.192	0.088	0.566	<0.002	<0.002	0.082	0.017
8/21/2007	<0.002	<0.002	0.200	<0.002	2.961	<0.002	0.390	<0.002	0.063	<0.002	NM	0.223	0.044	0.493	<0.002	<0.002	0.080	0.013
Range (ug/L)			0.013		0.370		0.085		0.036			0.031	0.044	0.073			0.002	0.004
9/10/2007	<0.002	<0.002	0.381	<0.002	2.962	<0.002	0.635	<0.002	0.139	<0.002	NM	0.388	<0.002	0.482	<0.002	<0.002	0.274	<0.002
9/10/2007	<0.002	<0.002	0.369	<0.002	3.097	<0.002	0.417	<0.002	0.189	<0.002	NM	0.439	<0.002	0.528	<0.002	<0.002	0.298	0.008
Range (ug/L)			0.012		0.135		0.218		0.051			0.051		0.046			0.024	
			0.032		0.045		0.415		0.309			0.123		0.091			0.083	
7/18/2008	<0.002	<0.002	0.163	0.155	4.738	<0.002	0.988	0.919	0.421	<0.002	0.573	<0.002	<0.002	<0.002	<0.002	<0.002	0.007	0.052
7/18/2008	<0.002	<0.002	0.257	0.192	22.589	<0.002	1.443	0.546	0.379	<0.002	0.898	<0.002	<0.002	<0.002	<0.002	<0.002	0.062	0.098
Range (ug/L)			0.094	0.037	17.852		0.455	0.373	0.042		0.324						0.055	
Recoveries (=100 x (measured-sample)/added) of laboratory fortified matrix (LFM) samples																		
9/10/2007	124.6	125.6	108.9	211.9	OR	188.2	125.8	145.3	123.5	150.9	NM	140.6	190.5	117.4	158.2	183.0	114.2	84.4
9/8/2008	32.7	100.2	92.0	97.0	OR	24.0	200.5	130.1	47.2	44.0	47.8	123.7	36.3	157.5	155.0	214.7	156.6	109.9
7/7/2008	65.2	151.9	84.3	62.4	OR	89.6	109.2	159.1	89.7	93.4	21.6	80.8	73.3	47.0	137.6	222.5	182.5	120.8
7/31/2008	108.4	89.0	104.2	184.1	47.2	105.0	186.0	172.6	192.5	96.2	89.4	155.5	131.3	191.8	176.7	65.8	63.8	110.1
Average	82.7	116.7	97.3	138.8	47.2	101.7	155.4	151.8	113.2	96.1	52.9	125.1	107.9	128.4	156.9	171.5	129.3	106.3
Standard Deviation	41.7	28.0	11.2	70.7		67.5	44.6	18.3	61.4	43.7	34.2	32.3	67.6	62.2	16.0	72.5	51.9	15.4
"OR" = Overcalibration range																		

**Table S4.** Results of quality control solid samples analyzed using MASE LC/MS/MS.

[illegible]



**Table S4.** Results of quality control solid samples analyzed using MASE LC/MS/MS, continued.

Laboratory Duplicate Samples																	
Sample_ID	Collection_Date	4-Androstenedione	Androstosterone	a-Trenbolone	b-Trenbolone	a-Zearalenol	b-Zearalenol	a-Zearalanol	Estriol	Estrone	a-Estradiol	b-Estradiol	Ethinyl Estradiol	Melengesterol Acetate	Progesterone	17a-Hydroxyprogesterone	Testosterone
0701-0012 12/13D	5/25/2007	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	6.01	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
0701-0012 12/13D LD2	5/25/2007	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	4.73	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
0701-0036 12/13D	6/7/2007	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	3.86	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
0701-0036 12/13D LD2	6/7/2007	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	2.74	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
0701-0054 4/5	6/8/2007	1.44	<0.50	1.53	<0.50	9.82	<0.50	2.02	2.95	<0.50	<0.50	<0.50	<0.50	<0.50	6.28	<0.50	<0.50
0701-0054 4/5 LD2	6/18/2007	1.79	<0.50	0.75	<0.50	10.28	<0.50	4.61	5.18	<0.50	<0.50	<0.50	<0.50	<0.50	9.66	<0.50	<0.50
0701-0071 12/13C	7/16/2007	14.14	<0.50	<0.50	<0.50	23.97	<0.50	<0.50	<0.50	<0.50	1.34	3.82	<0.50	<0.50	85.63	<0.50	<0.50
0701-0071 12/13C LD2	7/16/2007	12.27	<0.50	<0.50	<0.50	18.83	<0.50	<0.50	<0.50	<0.50	<0.50	2.69	<0.50	<0.50	74.04	<0.50	<0.50
0701-0094 8/9	7/17/2007	0.93	<0.50	33.12	<0.50	40.19	<0.50	40.93	<0.50	12.02	8.38	<0.50	<0.50	3.90	2.51	<0.50	<0.50
0701-0094 8/9 LD2	7/17/2007	1.11	<0.50	22.33	<0.50	22.81	<0.50	41.10	<0.50	14.28	8.21	<0.50	<0.50	2.92	4.09	<0.50	<0.50
0701-0106 12/13B	9/17/2007	3.71	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	13.68	<0.50	<0.50
0701-0106 12/13B LD2	9/17/2007	1.80	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	15.84	<0.50	<0.50
0701-0116 6/7D	9/18/2007	3.02	5.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	84.31	<0.50	<0.50	4.14	<0.50	0.64	<0.50	<0.50
0701-0116 6/7D LD2	9/18/2007	2.41	12.14	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	53.04	<0.50	<0.50	3.97	<0.50	1.17	<0.50	<0.50
0701-0127 16/17	9/17/2007	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	759.09	<0.50	<0.50	<0.50	99.99	<0.50	<0.50	<0.50	<0.50	<0.50
0701-0127 16/17 LD2	9/17/2007	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	525.98	<0.50	<0.50	<0.50	69.86	<0.50	<0.50	<0.50	<0.50	<0.50
0801-0007 14/15C	4/8/2008	0.29	1.26	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
0801-0007 14/15C LD2	4/8/2008	0.63	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50
0801-0014 8/9B	4/8/2008	0.87	<0.50	<0.50	<0.50	3.04	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	2.31	<0.50	<0.50
0801-0014 8/9B LD2	4/8/2008	0.93	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	2.24	<0.50	<0.50
0801-0023 4/5C	4/8/2008	2.24	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	2.39	3.57	<0.50
0801-0023 4/5C LD2	4/8/2008	3.24	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	1.97	<0.50	<0.50
0801-0076 8/9C	5/1/2008	8.91	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	1.95	<0.50	<0.50
0801-0076 8/9C LD2	5/1/2008	2.64	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	0.91	<0.50	<0.50
0801-0083 4/5B	5/1/2008	3.71	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	6.23	<0.50	<0.50
0801-0083 4/5B LD2	5/1/2008	2.67	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	4.12	<0.50	<0.50
0801-0104 14/15C	6/9/2008	2.85	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	2.18	<0.50	<0.50
0801-0104 14/15C LD2	6/9/2008	1.82	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	1.74	<0.50	<0.50
0801-0111 8/9B	6/10/2008	5.04	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	5.48	<0.50	<0.50
0801-0111 8/9B LD2	6/10/2008	7.10	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	2.65	<0.50	<0.50
0801-0120 4/5C	6/10/2008	1.91	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	1.18	<0.50	<0.50	<0.50	0.62	7.27	<0.50	<0.50
0801-0120 4/5C LD2	6/10/2008	1.80	1.56	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	0.44	6.26	<0.50	<0.50
0801-0091 4/5	5/1/2008	2.11	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	1.03	<0.50	<0.50	7.93	<0.50	<0.50
0801-0091 4/5 LD2	5/1/2008	2.90	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	1.73	<0.50	0.65	10.13	<0.50	<0.50
0801-0131 8/9	6/10/2008	<0.50	<0.50	36.05	3.89	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	3.09	1.26	<0.50	<0.50
0801-0131 8/9 LD2	6/10/2008	<0.50	<0.50	35.11	4.37	<0.50	<0.50	<0.50	<0.50	<0.50	2.73	<0.50	<0.50	2.11	1.00	<0.50	<0.50
0801-0152 6/7C	9/10/2008	1.98	11.98	<0.50	0.56	<0.50	<0.50	<0.50	<0.50	0.86	0.65	1.51	0.00	<0.50	1.13	<0.50	<0.50
0801-0152 6/7C LD2	9/10/2008	2.31	18.26	2.07	<0.50	<0.50	<0.50	<0.50	<0.50	2.42	0.68	1.05	1.67	<0.50	1.38	<0.50	<0.50

**Table S4.** Results of quality control solid samples analyzed using MASE LC/MS/MS, continued.

[illegible]

### Cattle Performance Data.

Yearling heifers with an average initial weight of 387 kg were fed an average of 125 days. Control cattle were maintained under a hormone-free regimen, while heifers in the treatment group were maintained under a hormone regimen typically utilized in the commercial feedlot industry [3]. Feeding and management regimen for both control and treated groups were similar and also typical of the commercial feedlot industry. Cattle provided hormones had 18.6% greater gains ( $P < 0.05$ ) and 7.2% better feed efficiency than hormone-free cattle. Although the hormone-free cattle had numerically greater percentage USDA choice + prime carcasses, the cattle provided hormones had 6.7% lower cost of gains. Performance and cost of gain differences between treated and control cattle are somewhat less than those reported by Coopride et al. [4] but clearly follow the same trends observed between cattle provided hormones when compared with hormone-free cattle.

**Table S5.** Effect of growth hormones on cattle performance

	Control	Treatment	Change
Average daily gain(ADG), kg/day*	1.29	1.53	18.60
Dry matter intake (DMI), kg/day*	9.32	10.26	10.10
Feed efficiency, ADG/DMI	0.139	0.149	7.20%
USDA choice & prime, %	87.50	72.92	-16.67%
USDA yield grade	2.85	2.96	3.86%
Cost/kg gain, US \$	1.64	1.53	-6.71%
*P < 0.05			

**Table S6.** Steroid Hormones detected in runoff in 2007 (ng/L)<sup>1</sup>.

Sample Type	Date	Day in Experiment	4-androstenedione	androsterone	$\beta$ -zearalenol	$\alpha$ -zearalanol	estriol	estrone	17 $\beta$ -estradiol	melengesterol acetate	progesterone
Treatment	11-Aug	78	64.0 $\pm$ 22.3	ND	ND	196 $\pm$ 78.9	ND	381 $\pm$ 535	ND	19.2 $\pm$ 22.9	24.4 $\pm$ 22.8
Treatment	21-Aug	88	197 $\pm$ 21.5	1870 $\pm$ 1020	142 $\pm$ 71.1	411 $\pm$ 90.4	45.5 $\pm$ 60.1	453 $\pm$ 160	75.1 $\pm$ 33.9	ND	62.7 $\pm$ 27.4
Treatment	23-Aug	90	305 $\pm$ 24.0	1610 $\pm$ 245.7	ND	381 $\pm$ 109	ND	523 $\pm$ 110	150 $\pm$ 73.6	ND	70.4 $\pm$ 18.0
Treatment	10-Sep	106	740 $\pm$ 94.7	5430 $\pm$ 292	449 $\pm$ 71.5	569 $\pm$ 218	35.2 $\pm$ 45.6	919 $\pm$ 193	172 $\pm$ 8.1	ND	153 $\pm$ 33.6
Control	21-Aug	88	646 $\pm$ 278	5120 $\pm$ 3560	169 $\pm$ 287	707 $\pm$ 550	241 $\pm$ 212	361 $\pm$ 375	109 $\pm$ 138	ND	558.5 $\pm$ 69.8
Control	23-Aug	90	60.4 $\pm$ 22.1	306 $\pm$ 194	12.0 $\pm$ 15.7	46.3 $\pm$ 20.9	ND	51.7 $\pm$ 42.2	33.2 $\pm$ 18.9	ND	33.2 $\pm$ 19.7
Control	10-Sep	106	381 $\pm$ 0.13	2840 $\pm$ 166	486 $\pm$ 139	568 $\pm$ 95.1	ND	628 $\pm$ 206	296 $\pm$ 222	ND	261.2 $\pm$ 18.4

<sup>1</sup> Concentrations expressed as average  $\pm$  standard deviation

**Table S6.** Steroid Hormones detected in runoff in 2008 (ng/L)<sup>1</sup>.

Sample Type	Date	Exp. Day	Testosterone	4-Androstenedione	Androsterone	androstenedione	17beta-estradiol	a-Estradiol	Estrone	Estriol	a-Zearalanol	a-Zearalenol	b-Zearalenol	Melengesterol Acetate	Progesterone	17a-Hydroxyprogesterone
Treatment	7-May	15	ND	43.2 ± 12.6	6270 ± 611	ND	378 ± 146	198 ± 337	299 ± 512	ND	880 ± 237	ND	ND	ND	28.3 ± 9.76	ND
	12-May	20	46.9 ± 77.0	108 ± 72.6	2090 ± 453	ND	149 ± 77.1	48.5 ± 78.9	ND	155 ± 264	604 ± 252	ND	ND	40.0 ± 64.1	60.6 ± 54.8	62.4 ± 103
	23-May	31	ND	60.1 ± 15.8	379 ± 189	ND	1120 ± 73.2	ND	ND	351 ± 304	740 ± 258	ND	ND	ND	59.6 ± 5.38	ND
	25-May	33	ND	123 ± 17.6	1560 ± 344	ND	88.9 ± 25.3	ND	ND	73.5 ± 122	344 ± 63.2	ND	ND	ND	58.7 ± 9.05	138 ± 123
	29-May	37	4.1 ± 1.9	107 ± 84.2	587 ± 563	ND	78.3 ± 59.4	ND	ND	185 ± 189	169 ± 124	ND	ND	ND	68.4 ± 31.4	ND
	2-Jun	41	ND	59.6 ± 0.02	964 ± 0.33	ND	173 ± 0.09	ND	337 ± 0.32	207 ± 0.18	337 ± 0.11	ND	ND	ND	30.0 ± 0.02	ND
	3-Jun	42	ND	110 ± 15.2	1370 ± 278	ND	301 ± 80.2	ND	ND	ND	217 ± 370	ND	ND	ND	101 ± 19.0	ND
	9-Jun	48	ND	57.8 ± 17.9	2260 ± 152	ND	107 ± 27.9	ND	239 ± 237	174 ± 296	109 ± 184	ND	ND	ND	30.2 ± 12.9	ND
	7-Jul	76	238	357 ± 100	19,100	3.72 ±	128 ±	341 ±	542 ±	129 ±	1800	186 ±	121 ±	ND	378 ±	ND



			±157		± 4540	1.25	134	91	252	218	± 264	318	166		41.5	
	10-Jul	79	153 ± 211	210 ± 143	6130 ± 5310	97.9 ± 164	126 ± 213	ND	325 ± 558	206 ± 186	3270 ± 2660	978 ± 1690	ND	ND	542 ± 477	ND
	18-Jul	87	84.6 ± 36.6	201 ± 74.9	13,500 ± 7820	110 ± 133	218 ± 209	248 ± 280	730 ± 972	127 ± 216	1290 ± 260	1030 ± 1090	ND	ND	72.3 ± 78.1	ND
	21-Jul	90	234.5 ± 156.3	227 ± 86.1	15,500 ± 6720	65.1 ± 108	49.9 ± 56.7	336 ± 240	193 ± 329	ND	1190 ± 420	232 ± 397	ND	ND	194 ± 70.4	ND
	28-Aug	128	90.4 ± 81.1	580 ± 451	14,200 ± 14,500	418 ± 718	134 ± 227	79.7 ± 71.1	ND	45.6 ± 73.7	1980 ± 1240	ND	ND	ND	367 ± 332	ND
	8-Sep	139	9.6 ± 11.5	154 ± 85.8	2640 ± 2420	42.1 ± 67.7	50.1 ± 50.4	21.2 ± 31.4	ND	ND	645 ± 593	ND	ND	10 ± 12.2	82.0 ± 61.0	ND
Control	7-May	15	ND	33.5 ± 27.4	1100 ± 953	ND	419 ± 107	ND	243 ± 210	ND	316 ± 302	ND	ND	ND	21.2 ± 18.7	ND
	12-May	20	ND	89.7 ± 46.2	2660 ± 424	ND	128 ± 22.3	ND	ND	ND	286 ± 86.9	ND	ND	ND	39.0 ± 18.1	ND
	23-May	31	ND	62.8 ± 20.5	112 ± 116	ND	1130 ± 306	ND	ND	ND	54.6 ± 89.4	ND	ND	ND	85.7 ± 39.9	ND
	25-May	33	ND	66.7 ± 9.1	885 ± 180	ND	103 ± 36	ND	107 ± 181	142 ± 126	99.4 ± 91.7	ND	ND	ND	34.6 ± 15.5	ND
	29-May	37	ND	45.9 ± 3.35	202 ± 188	ND	100 ± 61.4	ND	ND	33.7 ± 53.1	ND	ND	ND	ND	43.7 ± 12.8	10.8 ± 13.5
	2-Jun	41	ND	107 ± 40.4	1261 ± 188	ND	182 ± 8.17	ND	925 ± 114	131 ± 221	265 ± 227	ND	ND	ND	39.7 ± 2.50	ND
	3-Jun	42	ND	97.1 ± 30.6	625 ± 226	ND	176 ± 34.7	ND	279 ± 253	ND	150 ± 254	ND	ND	ND	99.1 ± 4.5	ND
±																

														29.1	
9-Jun	48	ND	76.7 ± 33.2	4130 ± 1060	ND	142 ± 29.6	ND	ND	ND	ND	ND	ND	ND	45.8 ± 20.0	ND
7-Jul	76	138 ± 105	177 ± 85.4	11,900 ± 3410	265 ± 128	55.6 ± 46.4	523 ± 228	1560 ± 931	10.6 ± 13.2	2150 ± 785	193 ± 329	125 ± 214	ND	942 ± 273	ND
10-Jul	79	209 ± 241	296 ± 108	12,000 ± 7620	ND	ND	88.5 ± 148	527 ± 514	131.5 ± 223	2250 ± 1620	ND	ND	ND	348 ± 27.5	ND
18-Jul	87	64.1 ± 28.2	102 ± 34.4	10,600 ± 5140	378 ± 144	72.9 ± 68.9	136 ± 176	504 ± 359	26.5 ± 40.7	907 ± 447	287 ± 493	213 ± 363	ND	573 ± 199	ND
21-Jul	90	72.1 ± 73.8	165 ± 122	8085 ± 3210	91.3 ± 97.1	92.7 ± 0.90	252 ± 99.9	ND	ND	1040 ± 403	913 ± 864	ND	ND	447 ± 271	ND
28-Aug	128	121 ± 110	789 ± 357	6580 ± 3780	279 ± 239	46.6 ± 75.5	248 ± 246	532 ± 917	ND	2540 ± 894	ND	ND	ND	1990 ± 989	ND
8-Sep	139	20.6 ± 24.6	158 ± 117	2620 ± 2270	60.4 ± 72.8	ND	ND	ND	ND	380 ± 332	325 ± 558	669 ± 722	ND	257 ± 190	ND

<sup>1</sup> Concentrations expressed as average ± standard deviation

**Table S7.** Nutrients and total suspended solids measured in runoff in 2008 (mg/L)<sup>1</sup>

Sample Type	Date	Total P	Total N	Dissolved NH <sub>4</sub> -N	Dissolved NO <sub>3</sub> -N	Total Suspended Solids
Treatment	7-May	20 ± 3	114 ± 7.8	30 ± 3.2	0.03 ± 0.01	990 ± 230
	12-May	17 ± 5.6	72 ± 37	24 ± 11	0.03 ± 0.01	1510 ± 690
	23-May	29 ± 1.4	130 ± 2.8	35 ± 6.4	0.10 ± 0.01	1950 ± 240
	25-May	40 ± 17	106 ± 24	25 ± 1.8	0.05 ± 0.03	5370 ± 3780
	29-May	47 ± 13	143 ± 21	13 ± 6.0	0.08 ± 0.04	5250 ± 1160
	2-Jun	139 ± 184	486 ± 679	35 ± 26	0.04 ± 0.01	17300 ± 26200
	3-Jun	30 ± 6.3	123 ± 23	24 ± 7.4	0.07 ± 0.05	2720 ± 530
	9-Jun	27 ± 2.9	110 ± 2.9	18 ± 5.8	0.26 ± 0.05	2050 ± 220
	7-Jul	48 ± 6.1	241 ± 33	87 ± 14	0.04 ± 0.01	2480 ± 498
	10-Jul	31 ± 2.2	173 ± 20	64 ± 20	0.09 ± 0.05	1405 ± 430
	18-Jul	30 ± 12	183 ± 92	68 ± 48	0.09 ± 0.05	1140 ± 480
	21-Jul	30 ± 2.6	189 ± 21	57 ± 8.8	0.13 ± 0.01	1350 ± 198
	28-Aug	49 ± 7.2	310 ± 78	72 ± 24	0.22 ± 0.03	2490 ± 660
	8-Sep	52 ± 9.6	342 ± 38	69 ± 13	0.37 ± 0.03	3790 ± 120
Control	7-May	26 ± 17	101 ± 23	27 ± 0.95	0.03 ± 0.01	785 ± 107
	12-May	16 ± 1.9	73 ± 6.2	19 ± 5.4	0.05 ± 0.04	1210 ± 360
	23-May	15 ± 2.0	60 ± 14	26 ± 2.5	0.1 ± 0.01	2120 ± 480
	25-May	32 ± 7.1	92 ± 16	18 ± 4.2	0.03 ± 0.02	2370 ± 690
	29-May	38 ± 24	125 ± 59	12 ± 11	0.04 ± 0.01	6100
	2-Jun	47 ± 3.6	118 ± 12	28 ± 4.1	0.04 ± 0.01	7280 ± 2660
	3-Jun	30 ± 7.5	122 ± 27	14 ± 4.0	0.04 ± 0.01	3800 ± 1060
	9-Jun	26 ± 2.2	101 ± 7.8	16 ± 2.4	0.10 ± 0.01	1970 ± 62
	7-Jul	32 ± 5.4	128 ± 27	38 ± 2.7	0.23 ± 0.08	1560 ± 97
	10-Jul	35 ± 3.3	256 ± 52	94 ± 31	0.09 ± 0.05	1605 ± 269
	18-Jul	19 ± 2.9	89 ± 13	18 ± 4.2	0.03 ± 0.01	706 ± 104
	21-Jul	23 ± 1.3	125 ± 7.9	32 ± 7.9	0.11 ± 0.01	943 ± 103
	28-Aug	28 ± 8.8	143 ± 44	43 ± 20	0.14 ± 0.03	1100 ± 290
	8-Sep	37 ± 3.6	193 ± 25	50 ± 14	4.5 ± 7.3	2060 ± 400

<sup>1</sup> concentrations expressed as average ± standard deviation

**Table S8.** Concentrations of steroid hormones (ng/g dry weight) detected in feeding pen surface samples, urine-soaked surface soil samples, and fresh manure samples in 2008<sup>1</sup>.

Sample Type	Description	Day	testosterone	4-androstenedione	androsterone	androstenedione	17 $\beta$ -estradiol	17 $\alpha$ -estradiol	estrone	estriol	$\alpha$ -zearalanol	$\alpha$ -zearalanol	$\beta$ -zearalanol	melengesterol acetate	progesterone	17 $\alpha$ -hydroxyprogesterone	17 $\alpha$ -trenbolone	17 $\beta$ -trenbolone
Treatment <sup>2</sup>	Feeding Pen surface	Day 0	ND	1.9 $\pm$ 0.14	ND	ND	ND	ND	ND	ND	ND	0.34 $\pm$ 0.09	ND	ND	1.7 $\pm$ 0.11	ND	ND	ND
		Day 6	ND	4.7 $\pm$ 0.23	ND	ND	0.78 $\pm$ 0.18	ND	0.33 $\pm$ 0.06	1.0 $\pm$ 0.28	ND	ND	1.3 $\pm$ 0.36	ND	5.6 $\pm$ 0.36	ND	ND	ND
		Day 47	ND	5.3 $\pm$ 0.7	3.3 $\pm$ 0.6	ND	1.1 $\pm$ 0.3	0.6 $\pm$ 0.14	0.66 $\pm$ 0.10	ND	0.31 $\pm$ 0.05	ND	ND	0.28 $\pm$ 0.06	10 $\pm$ 1.2	ND	ND	ND
		Day 139	ND	3.8 $\pm$ 0.3	15 $\pm$ 1.9	ND	0.63 $\pm$ 0.1	0.61 $\pm$ 0.07	2.0 $\pm$ 0.2	ND	0.47 $\pm$ 0.09	ND	ND	0.26 $\pm$ 0.05	2.2 $\pm$ 0.17	ND	ND	ND
	Urine Soaked Soil	Day 0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		Day 6	3.8 $\pm$ 0.4	ND	ND	ND	1.1 $\pm$ 0.3	ND	ND	ND	ND	ND	ND	ND	11 $\pm$ 1.1	ND	ND	ND
		Day 47	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		Day 139	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Fresh Manure	Day 0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		Day 6	ND	0.5 $\pm$ 0.3	ND	ND	ND	ND	ND	ND	ND	ND	ND	4.2 $\pm$ 0.3	2.7 $\pm$ 0.3	ND	ND	ND
		Day 47	ND	5.3 $\pm$ 2.6	ND	ND	ND	1.5 $\pm$ 0.5	ND	ND	27 $\pm$ 15	ND	ND	1.7 $\pm$ 0.6	2.4 $\pm$ 1.0	6.6 $\pm$ 3.4	55 $\pm$ 22	0.5 $\pm$ 0.3
		Day 139	1.5 $\pm$ 0.8	ND	ND	ND	2.4 $\pm$ 1.4	ND	76 $\pm$ 4.7	ND	94 $\pm$ 32	ND	ND	2.0 $\pm$ 1.1	ND	ND	ND	ND

Table S8, continued.

Sample Type	Description	Day	testosterone	4-androstenedione	androsterone	androstenedione	17 $\beta$ -estradiol	17 $\alpha$ -estradiol	estrone	estriol	$\alpha$ -zearalanol	$\alpha$ -zearalanol	$\beta$ -zearalanol	melengesterol acetate	progesterone	17 $\alpha$ -hydroxy progesterone	17 $\alpha$ -trenbolone	17 $\beta$ -trenbolone
Control	Feeding Pen surface	Day 0	ND	0.18 $\pm$ 0.03	0.82 $\pm$ 0.15	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.08 $\pm$ 0.02	ND	ND	ND
		Day 6	ND	1.8 $\pm$ 0.06	ND	ND	ND	0.2 $\pm$ 0.05	ND	ND	ND	ND	ND	ND	2.9 $\pm$ 0.24	ND	ND	ND
		Day 47	ND	4.2 $\pm$ 0.4	26 $\pm$ 3.5	ND	2.1 $\pm$ 0.46	ND	0.60 $\pm$ 0.13	2.1 $\pm$ 0.03	1.0 $\pm$ 0.29	ND	ND	ND	7.5 $\pm$ 0.6	ND	ND	ND
		Day 139	ND	3.5 $\pm$ 0.3	17.5 $\pm$ 2.1	ND	1.8 $\pm$ 0.2	ND	0.51 $\pm$ 0.1	ND	0.86 $\pm$ 0.18	ND	ND	ND	6.0 $\pm$ 0.50	ND	ND	ND
	Urine Soaked Soil	Day 0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		Day 6	ND	1.5 $\pm$ 0.05	ND	ND	ND	0.23 $\pm$ 0.11	ND	ND	ND	ND	ND	ND	3.9 $\pm$ 0.8	ND	ND	ND
		Day 47	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		Day 139	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Fresh Manure	Day 0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		Day 6	ND	0.33 $\pm$ 0.2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.3 $\pm$ 0.40	ND	ND	ND
		Day 47	ND	1.0 $\pm$ 0.3	ND	ND	ND	ND	ND	ND	5.8 $\pm$ 3.3	ND	ND	ND	4.9 $\pm$ 0.7	ND	ND	ND
		Day 139	ND	8.4 $\pm$ 2.9	ND	ND	12 $\pm$ 4.9	ND	105 $\pm$ 25	ND	61 $\pm$ 6.8	ND	ND	ND	56 $\pm$ 15	ND	ND	ND

<sup>1</sup> All concentrations presented as average  $\pm$  standard error. For feeding pen surface samples, n=12; for urine-soaked soil and fresh manure samples, n=3; <sup>2</sup> Treatment = subcutaneous implants (36 mg  $\alpha$ -zearalanol, 140 mg trenbolone acetate, 14 mg 17 $\beta$ -estradiol), and 0.45 mg melengesterol acetate per day via feed additives

## References

1. Kleinova, M.; Zallner, P.; Kahlbacher, H.; Hochsteiner, W.; Lindner, W. Metabolic profiles of the mycotoxin zearalenone and of the growth promoter zeranol in urine, liver, and muscle of heifers. *J. Agri. Food Chem.* **2002**, *50*, 4769-4776.
2. Snow, D. D.; Damon-Powell, T.; Onanong, S.; Cassada, D. A. Sensitive and simplified analysis of natural and synthetic steroids in water and solids using on-line solid phase extraction and microwave-assisted solvent extraction coupled to liquid chromatography tandem mass spectrometry atmospheric pressure photoionization. *Anal Bioanal Chem* **2012**, Submitted.
3. Kreikemeier, W. M.; Mader, T.L. Effects of growth-promoting agents and season on yearling feedlot heifer performance. *J. Anim. Sci.* **2004**, *82*, 2481–2488.
4. Coopridge, K. L.; Mitloehner, F.M.; Famula, T.R.; Kebreab, E.; Zhao, Y.; and Van Eenennaam, A.L. Feedlot efficiency implications on greenhouse gas emissions and sustainability. *J. Anim. Sci.* **2011**, *89*, 2643-2656.