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Ractopamine and Other Growth-Promoting Compounds in Beef Cattle Operations: Fate and Transport in Feedlot Pens and Adjacent Environments

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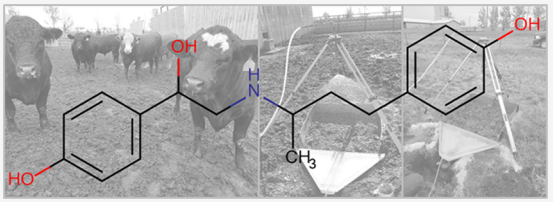
ABSTRACT: The current study represents a comprehensive investigation of the occurrence and fates of trenbolone acetate (TBA) and metabolites 17α-trenbolone (17α-TBOH), 17β-TBOH, and trendione (TBO); melengesterol acetate (MGA); and the less commonly studied β-andrenergic agonist ractopamine (RAC) in two 8 month cattle feeding trials and simulated rainfall runoff experiments. Cattle were administered TBA, MGA, or RAC, and their residues were measured in fresh feces, pen floor material, and simulated rainfall runoff from pen floor surfaces and manure-amended pasture. Concentrations of RAC ranged from 3600 ng g^{-1}, dry weight (dw), in pen floor to 58 000 ng g^{-1} in fresh feces and were, on average, observed at 3−4 orders of magnitude greater than those of TBA and MGA. RAC persisted in pen floors (manure $t_{1/2}$ = 18−49 days), and contamination of adjacent sites was observed, likely via transport of windblown particulates. Concentrations in runoff water from pen floors extrapolated to larger-scale commercial feedlots revealed that a single rainfall event could result in mobilization of gram quantities of RAC. This is the first report of RAC occurrence and fate in cattle feedlot environments, and will help understand the risks posed by this chemical and inform appropriate manure-management practices.



INTRODUCTION

In North America, growth-promoting compounds including synthetic hormones, antibiotics, and β-adrenergic agonists are administered to beef cattle to improve health and increase feed efficiency.[1] Manure generated from these feedlots not only contains beneficial nutrients and organic matter but also microorganisms, pharmaceuticals, and steroidal hormones that have the potential to adversely affect the health of wildlife or functions of the environment in general.[2−5] Veterinary pharmaceuticals, including steroidal hormones trenbolone acetate, melengestrol acetate,[1,6] β-agonist ractopamine,[7,8] and associated metabolites, are commonly used to promote the growth of cattle in North American feedlots. A key challenge for feedlot managers and policymakers is to manage manure to optimize its economic value while minimizing environmental risks from associated veterinary pharmaceuticals.[9]

Trenbolone acetate (TBA) is a synthetic, androgenic steroid used as an anabolic growth promoter administered via implants at the base of the ear and is excreted primarily as 17α-trenbolone (17α-TBOH) in feces.[1,6] Melengestrol acetate (MGA) is a synthetic progestogen orally administered to heifers as a feed supplement to improve feed conversion, promote growth, and suppress estrus.[1] MGA is excreted primarily in feces and to a lesser extent in the urine.[6] Ractopamine (RAC) is a β-adrenergic agonist used to promote feed efficiency and is administered as a feed additive for up to 42 days prior to slaughter.[7,8,10,11] RAC is eliminated rapidly in cattle and swine, with 95% of the ingested amount (by mass) excreted within 3 days.[11] While RAC is known to be eliminated in both urine (45%) and feces (55%),[11,12] quantitative measurements of metabolites in cattle have focused on urine, where the monoglucuronide conjugate comprises 95% of the compound mass excreted.[13,14] These growth-promoting compounds have been detected in feedlot manure, runoff, catch-basin water, and particulate matter, as well as surrounding surface waters and soils.[2,5−7,9,15−22] However, concerns about their toxicity to aquatic nontarget organisms such as fish have mostly focused on TBA and metabolites 17α- and 17β-TBOH.[23−27] Studies have observed altered sex ratios in zebra fish (*Danio rerio*) and fathead minnow (*Pimephales promelas*) skewed toward male fish as a result of near environmentally relevant exposures (10−25 ng L^{-1}) to 17β-TBOH.[23,26] However, to the best of our knowledge, these effects have never been observed under practical production conditions in the field.

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Ractopamine is banned or restricted in China, Russia, the European Union, and 157 other countries, while 27 countries, including the United States, Canada, Japan, Brazil, and South Korea, classify it as safe for use in livestock.[10,11] While many countries have cited concerns over data gaps relating to acceptable residues in meat,[11] the greater knowledge gap relates to RAC as a potential environmental contaminant and toxicant in nontarget organisms given its widespread use in beef and swine production in approved countries.[28] RAC is a full β_2-receptor agonist and partial β_1 agonist.[29] Adverse effects, including agonism of the trace amine-associated receptor,[28] binding to the estrogen receptor (ER)-α,[30] production of vitellogenin, and increased ER-α and -β transcription in medaka (*Oryzias latipes*),[31] have been observed in a select number of *in vitro* studies, all at concentrations in the μg L^{-1} range. One *in vivo* study of zebra fish revealed apical effects related to exploratory behavior and locomotion at exposure concentrations between 0.85 and 8.5 μg L^{-1}.[32] The same study reported induction of lipid peroxidation and elevated thiol content in the brain of zebra fish at 0.2 μg L^{-1}. FDA registration and safety trials found that RAC is not carcinogenic in chronically exposed rats and mice and that the acceptable daily intake of 1.25 μg kg^{-1} day^{-1} is not exceeded based on residues in target tissues of exposed livestock.[33] However, this did not consider residues of the major glucuronide conjugate that can be hydrolyzed *in vivo* releasing free RAC and thus contribute to total body burdens.[11]

The occurrence of RAC in the environment has been investigated by relatively few studies. groundwater associated with swine and beef cattle facilities in Nebraska found RAC concentrations ranging from 134 to 524 ng L^{-1} at swine-associated lagoons to less than the limit of quantification at cattle-associated lagoons and a single detection of 54 ng L^{-1} at one of the swine facility groundwater sites.[19] In comparison, RAC was observed in wastewater from Malaysian cattle feedlots at concentrations ranging from 140 to 500 ng L^{-1} and from swine farms between 3000 and 30 000 ng L^{-1}.[16] Only two studies have investigated the occurrence of RAC in surface waters, both reporting concentrations <1 ng L^{-1}, downstream of municipal wastewater effluents[34] and across two sampling seasons in an agriculture-dominant watershed.[22] Although mechanisms of transport and pathways of RAC and other veterinary pharmaceuticals and hormones are yet to be fully elucidated, there is evidence that deposition of particulate matter containing veterinary-use chemicals represents an important pathway leading to contamination of environments adjacent to feedlots.[7,15,18,35]

Despite the results outlined above, there was yet to be a comprehensive investigation of these additives during full-scale feeding trials, from commercial beef cattle feedlots, and their potential for transport in surface runoff. In the current study, cattle were fed the same diet in a multiyear effort to understand the relative occurrence of 17α-TBOH, 17β-TBOH, MGA, and RAC in fresh feces and pen floor material at a confined research feedlot. To assess the potential for transport via surface runoff, studies of simulated rainfall were conducted, both within cattle pens and on manure-amended pasture. Groundwater wells located within and around the research feedlot and a catch basin (runoff storage pond) were also sampled to monitor for potential contamination. Companion pen floor and catch-basin samples were also collected from commercial feedlots administering these additives in southern Alberta. This work expands upon the knowledge regarding the presence and fate of these compounds, particularly RAC, in feedlot manure, which is currently insufficient to appropriately evaluate risks posed by these compounds in environments affected by feedlots.

MATERIALS AND METHODS

Feeding Trials. Cattle were fed a standard 60% (by mass) corn (*Zea mays* L.) silage−40% barley (*Hordeum vulgare* L.) grain diet during the growing period and a 93% (by mass) barley grain−7% corn silage diet during the finishing period (beginning 112 days from the start of the trial, Tables S1 and S2) at the Agriculture and Agri-Food Canada (AAFC) Research Centre research feedlot (Lethbridge, AB, Canada) in 2017−2018 and 2018−2019. Cattle were fed for 259 days in 2017−2018 and 273 days in 2018−2019. Cattle were penned in groups of 10 with four pens per treatment (n = 4) and a total of six treatments (240 cattle total), organized in a randomized block design. Treatments were (1) control heifers (no growth promoters), (2) trenbolone acetate + estradiol-implanted heifers (TBA), (3) heifers continuously fed with melengestrol acetate (MGA), (4) control steers (no growth promoters), (5) TBA-implanted steers, and (6) steers implanted with TBA and fed ractopamine hydrochloride for the last 42 days prior to slaughter (TBA+RAC). Cattle received three TBA implants (100 mg + 100 mg + 200 mg) spaced approximately 80 days apart over the course of the feeding trials. MGA was fed over the complete duration of each feeding trial at a rate of 0.4 mg $heifer^{-1}$ day^{-1} (104−109 mg total per heifer), with respect for a mandated 24 h withdrawal period prior to slaughter. RAC-treated cattle were fed a total of 14.5 g over 42 days. Complete details of the implant and feed regimens are given in the Supporting Information.

Twenty samples of fresh feces (warm fecal pat) were sampled from each pen on 3 consecutive days, 2 weeks after each of the three TBA implants were administered. This ensured that the peak excretion of TBA in feces[36] was captured (Tables S1 and S2). Twenty samples of pen floor material (mix of manure/urine + cereal straw bedding) were collected within each pen once per month on nearly the same date each month. Each set of 20 samples from a given pen was composited into a single sample for processing and analysis. In addition to this routine schedule, in 2017−2018, further sampling was conducted in TBA+RAC pens during RAC treatment (10 sampling points in a 23 day period) and after cattle had vacated the pens (5, 7, 9, 14, 21, and 36 days post-trial). Samples were stored at −20 °C until they were freeze-dried and ground.

Commercial Feedlots. For comparison to large-scale industry practice, manure samples were also collected from four commercial feedlots at 5−6 sampling points over a 2 year period (2016−2018). Two feedlots used conventional production practices (i.e., TBA implants, MGA to heifers, RAC to steers and heifers) with implants and feed additives dosed at the same concentrations employed in the research feeding trials, and two used natural production practices with no additives administered to cattle. At each feedlot, manure samples (20) were randomly collected within 10 pens, with samples composited by pen. All samples were subject to residue analysis as described below. Samples from the two natural commercial feedlots are not discussed further, since residues of target analytes were not detected.

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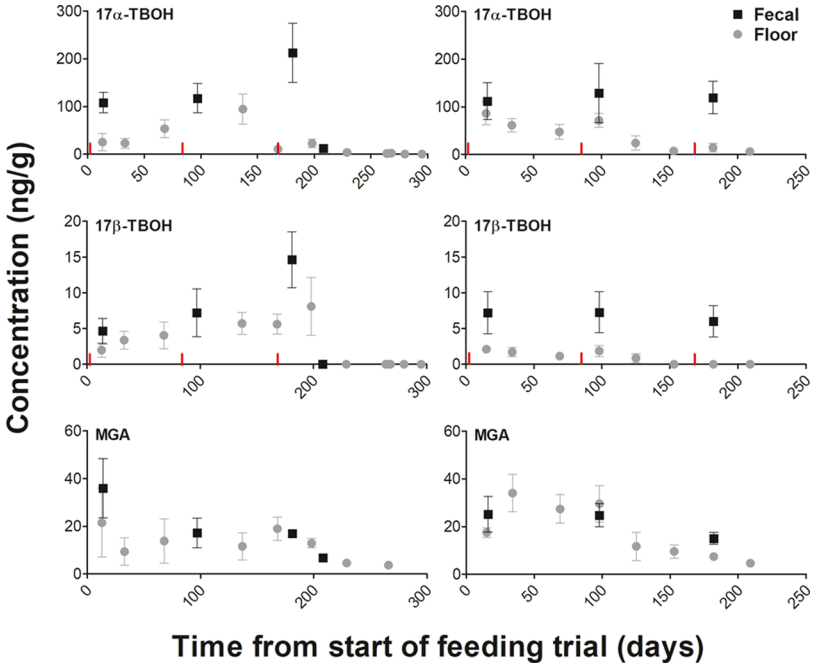

Figure 1. Concentrations of trenbolone acetate metabolites 17α-trenbolone (17α-TBOH) and 17β-TBOH and melengesterol acetate (MGA) during the 2017−2018 (left panel) and 2018−2019 (right panel) feeding trials in fresh fecal samples (Fecal) and pen floor samples (Floor). Data points represent mean ± SD from replicate treatment pens: 12× TBA floor (heifer/steer combined), 36× TBA fresh (heifer/steer, consecutive sampling days combined), and 4× MGA. Floor concentrations represent a single sampling event, while fresh concentrations are a mean of 3 consecutive days of sampling. TBA and TBO were less than the LODs in all samples. The 2017−2018 feeding trial began on October 31, 2017, and TBA implants (denoted by red lines) were administered on November 1 (day 1, 100 mg of TBA), January 23 (day 83, 100 mg of TBA), and April 17 (day 167, 200 mg of TBA). The 2018−2019 feeding trial began on December 11, 2018, and TBA implants were administered on December 12 (day 1, 100 mg of TBA), March 5 (day 84, 100 mg of TBA), and May 28 (day 168, 200 mg of TBA). MGA was fed continuously throughout each of the trials.

Simulated Runoff Experiments. To characterize concentrations of TBA and metabolites, MGA, and RAC in surface runoff from pen floors and manure-amended pasture, simulated rainfall runoff experiments were conducted in the summer of 2019 with a portable Guelph Rainfall Simulator II (Figure S1).[37] These experiments simulated the mobility of these chemicals from pen floors to catch basins and from manure-amended soils to surface water during rainfall events. Simulated rainfall water was applied at 126.5 mm h^{-1}, mimicking a 1 in 100 year rainfall event in Lethbridge, following protocols established in similar runoff experiments conducted by our group.[3,38] The commercial feedlot catch basins in Alberta are designed to hold 1 day of a 1 in 30 year rain event (https://www.agriculture.alberta.ca/app19/loadcatchbasin). Therefore, a 1 in 100 year event would not only guarantee runoff accumulation for our experiments over reasonable time frames (<1 h) but also simulate extreme rain events that may push feedlot contaminants to surface waters beyond the "closed feedlot environment". In total, 15 consecutive 1 L samples were collected for each runoff simulation. Simulations were conducted in triplicate for each treatment (TBA × 3 pens, MGA × 3 pens, RAC × 3 pens) directly in the feedlot pens and on manure-amended pasture for a total of 18 runoff experiments. Transport of these chemicals can be influenced by the moisture content of the manure prior to a rainfall event; therefore, runoff experiments were conducted at least 2 days after the last recorded rainfall event in the region. The total duration of rainfall application was 21 ± 3.5 min across all 18 experiments, depending on surface conditions of each plot, with an average of 2.1 ± 1.6 min to the start of runoff collection. Rainfall simulations on pasture were conducted within 1 h of amending the surface soil with manure taken directly from the feedlot pens and applied at a rate of 6 kg m^{-2} (wet weight, ww), consistent with practices for irrigated land (60 Mg ha^{-1}, recommended maximum rate).[39] Complete experimental and sampling details can be found in the Supporting Information.

Sampling of Groundwater and Catch Basins. Sixteen groundwater wells, located in interior alleyways and around the perimeter of the AAFC research feedlot (Figure S2), were sampled nine times at monthly intervals in 2018−2019. Details of groundwater wells and sampling can be found in the Supporting Information. Water samples were also taken to assess the movement of growth-promoting chemicals from the research feedlot into the catch basin. The feedlot site drained from south to north toward the catch basin with a 0.35% slope (Figure S2).[40] Samples could only be collected opportunistically when heavy precipitation led to runoff water in the catch basin, an event that occurred only once in October 2019. To complement this sampling event, water samples were also collected from catch basins at three commercial conventional feedlots in October 2019. These samples were highly turbid and required centrifugation prior to solid-phase extraction (SPE) (details in the Supporting Information).

Extraction of Residues. Fresh fecal and pen floor samples were freeze-dried and ground to <2 mm. All samples were shipped on ice to the University of Saskatchewan Toxicology Centre (Saskatoon, SK, Canada) for extraction and analysis. While efficiencies of extraction from all matrices were corrected for by isotope dilution (methanolic standards spiked

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in all samples at 50 ng), in addition, absolute recovery experiments in water and fecal matrices conducted without isotope dilution were ≥91 ± 8% (n = 3) for all analytes in water samples and ≥86 ± 12% (n = 3) for all analytes in fecal samples except for TBO (48 ± 4%, n = 3). All solid samples (dry weight, dw) were extracted in true replicates of four, corresponding to the four pens per treatment used in the feeding trials. Extraction of solid and aqueous samples by accelerated solvent extraction and SPE are detailed in the Supporting Information.

Instrumental Analysis. Analysis was conducted using a Vanquish UHPLC and Q-Exactive HF Quadrupole-Orbitrap mass spectrometer (Thermo-Fisher, Mississauga, ON). LC separation was achieved with a Kinetex 1.7 μm Biphenyl LC column (100 × 2.1 mm^2) (Phenomenex, Torrance, CA) by gradient elution with water and acetonitrile, both containing 0.1% formic acid at a flow rate of 0.35 mL min^{-1} and a column temperature of 40 °C. Samples were ionized by positive mode heated electrospray ionization (HESI) using a full MS/parallel reaction monitoring method at 120 000/15 000 resolution. Complete details of the chromatographic method, retention times, source parameters, inclusion list ions, and collision energies are provided in the Supporting Information. Method detection limits ranged from 0.05 to 0.8 ng mL^{-1} (Table S6).

Data Analyses. All data acquisition and processing were conducted using Xcalibur v. 4.2 and TraceFinder v. 4.1 (Thermo-Fisher), respectively. Prism v. 5.01 (GraphPad Software, La Jolla, CA) was used for graphing and statistical analysis. Estimates of variabilities are presented as standard deviations (σ; SDs) of the mean. Based on results of the Shapiro−Wilk normality test (p-value > 0.05), with the exception of RAC fresh fecal samples from 2017−2018 (p = 0.019), all of the other samples were sufficiently described by a normal distribution to allow for the use of parametric statistics. Complete details of statistical analyses can be found in the Supporting Information.

RESULTS AND DISCUSSION

Research Feedlot. *Occurrence of TBA and MGA in Feeding Trials.* Concentrations of TBA, 17α-TBOH, 17β-TBOH, TBO, and MGA were less than the limits of detection (0.029−0.10 ng g^{-1}, Table S6) in control treatments for all samples collected over both feeding trials. Also, in all samples, TBA and its metabolite, TBO, occurred at concentrations less than their respective limits of detection. The parent compound TBA is fully hydrolyzed to the 17β-TBOH metabolite, which is then oxidized to TBO and in turn reduced to the major metabolite 17α-TBOH (≈95%).[6] Detection of TBO in comparable studies investigating the occurrence of TBA metabolites in implanted cattle varies. A mass ratio of 17α-TBOH/17β-TBOH/TBO of approximately 94:4:2 has been reported,[6] while other studies did not detect TBO, reporting ratios of 98:2:0[2] and 87:13:0.[17] Averaged over 2 years, both fresh fecal and pen floor samples exhibited a 17α-TBOH/17β-TBOH/TBO ratio ranging from 93:7:0 to 95:5:0. The lack of detection of TBO might relate to the relatively poor recovery of TBO (48%), which is consistent with a previous study using similar methods.[2]

Samples of fresh feces generally contained greater concentrations of 17α-TBOH (average 41 ± 30 ng g^{-1}) and 17β-TBOH (average 3 ± 2 ng g^{-1}) than did samples of pen floor (Figure 1). Since pen floor samples consisted of feces, urine, and bedding and greater concentrations of metabolites have been observed in feces compared to urine,[41] elevated levels in fresh fecal samples were expected. In addition, over time, microbial activity on the pen floor or environmental conditions (e.g., desiccation, UV-light) could further reduce concentrations of these residues and their metabolites.[42−44] However, this systematic difference in concentrations between the two types of samples was somewhat variable for each compound, a result attributed to the heterogeneous nature of the matrices and differing distribution of metabolites between urine and feces. Concentrations of MGA were fairly consistent among sampling events and between years (Figure 1), and there was less distinction between fresh fecal and pen floor samples compared to TBA treatments, likely because MGA was continuously administered in the feed as opposed to slow-release implants used for TBA.

Most commercial implants are designed to constantly release TBA over an estimated *in vivo* lifetime of 100 days,[36,41] which is why implants were administered at ≈80 day intervals in the current study. This resulted in a fairly consistent concentration of 17α-TBOH and 17β-TBOH in fresh fecal and pen floor samples throughout the study (Figure 1 and Tables S7−S10,S13−S16). Overall, concentrations differed maximally by 2−3-fold for 17α-TBOH and 17β-TBOH in fresh samples across time points in 2017−2018, which is likely due to natural variation in feeding trials of this type.

Comparisons with concentrations in samples from commercial feedlots, measured in the current study, provide confidence in the realistic feeding trial conditions and sampling protocols used. Commercial concentrations of 17α-TBOH ranged from 1.2 to 44 ng g^{-1} and MGA from 0.50 to 15 ng g^{-1}, which agree well with measured feeding trial concentrations: 41 ± 30 ng g^{-1} 17α-TBOH, 3 ± 2 ng g^{-1} 17β-TBOH, and 18 ± 8 ng g^{-1} MGA (means over both feeding trials). A complete description of the commercial feedlot data can be found in the Supporting Information.

Concentrations of metabolites of TBA in a 40 m^3 stockpile of manure from 12 Holstein-Friesian heifers implanted with TBA over an ≈80 day period ranged from 5 to 75 ng g^{-1} 17α-TBOH and from 0.5 to 5 ng g^{-1} 17β-TBOH.[6] Additionally, 0.5−4.5 ng g^{-1} 17α-TBOH and 0.04−0.2 ng g^{-1} 17β-TBOH in liquid manure and 2−19 ng g^{-1} MGA in feces were reported.[6] In another study conducted on composite surface soil samples from a commercial feedlot in Nevada, only 17α-TBOH at 4−6 ng g^{-1} (dw) was detected.[45] During a 113 day study of metabolite excretion from TBA-implanted cattle, concentrations in manure ranged from 64 ng g^{-1} 17α-TBOH at peak, dissipating to 10 ng g^{-1} (dw) over the course of the exposure.[2] In the same study, 17β-TBOH measured 1.0−3.9 ng g^{-1} and TBO was only detected twice at 1.3 and 2.5 ng g^{-1}. In pen floor samples from cattle 28 days post-TBA implantation, mean concentrations were 21 and 3.1 ng g^{-1} (dw) for 17α-TBOH and 17β-TBOH, respectively, and TBO was not detected.[17] Concentrations in our pen floor materials (Figure 1 and Tables S7 and S18) were typically elevated but nevertheless comparable to those observed in previous studies.

Occurrence of RAC in Feeding Trials. RAC was not detected in any samples until RAC was administered in the final 42 days of the 2017−2018 feeding trial. Between the start (June 5) and the end (July 17) of RAC administration, concentrations in fresh feces ranged from 27 000 to 58 000 ng g^{-1} (Figure 2). The only pen floor sampling during the 42 day period in 2017−2018 occurred 13 days after the start of RAC administration and measured 3600 ± 700 ng g^{-1} (n = 4). The

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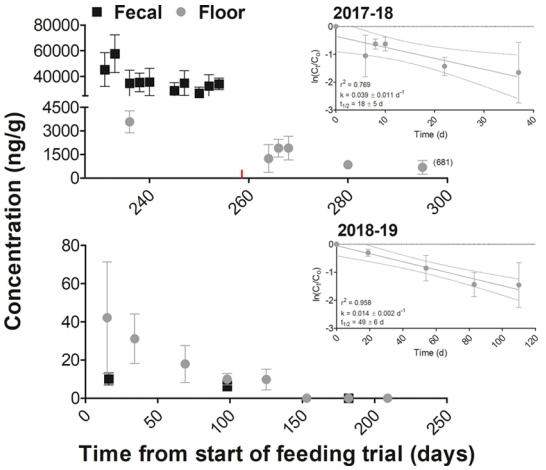

Figure 2. Concentrations of ractopamine (RAC) during 2017−2018 (top) and 2018−2019 (bottom) feeding trials in fresh fecal samples (Fecal) and pen floor samples (Floor). Data points represent mean ± SD from 4× RAC treatment pens. Fecal concentrations represent single sampling events in 2017−2018 and the mean of 3 consecutive days of sampling in 2018−2019. Floor concentrations are single sampling events in both feeding trials. RAC treatment began on June 5, 2018 (day 216, not shown on the plot) and lasted 42 days until the end of the feeding trial on July 17, 2018 (day 258, red line). Prior to the start of treatment in the 2017−2018 feeding trial, RAC was not detected in any samples (top plot begins on day 231). In 2018−2019, sampling was not conducted during RAC treatment (days 230−274), hence the narrower *y*-axis scale range. The concentrations measured in 2018−2019 reflect the residual RAC remaining from the 2017−2018 trial. Insets are the first-order kinetic time series plots of the floor concentrations. Linear regression provided the first-order rate constant and manure half-life of RAC in 2017−2018 (summer) and 2018−2019 (winter). Dashed lines represent 95% confidence intervals of the linear regression.

RAC concentrations in fresh feces were 3−4 orders of magnitude greater than concentrations of 17α-TBOH, 17β-TBOH, and MGA measured in this study. This result can be attributed to the more concentrated feed (100 g kg^{-1} or 30 mg kg^{-1} of the total diet) used for administering RAC. For comparison, the concentration of MGA in feed was ≈200 mg kg^{-1}.

Over the 42 days, RAC was detected in manure and feces at concentrations as great as 96 ng g^{-1} in >80% of TBA, MGA, and control samples, which suggested movement and contamination from the highly concentrated TBA+RAC-treated pens to the non-RAC pens. However, these concentrations were <1% of those present in RAC pens. Similar cross-contamination from TBA- and MGA-treated pens into non-TBA and -MGA pens was not observed. Contamination of environments adjacent to feedlots via windblown particulates is a well-known phenomenon and has been observed for RAC and other natural and synthetic hormones.[7,15,18,35] Concentrations observed in the non-RAC-treated pens (mean = 13 ng g^{-1}, range = 1−96 ng g^{-1}) are within the range of residues detected on wildflowers within 1 km of feedlots in Texas (40−380 ng g^{-1})[15] and in wetland sediments in close proximity to a feedlot (5.2 ng g^{-1}).[7] However, this is the first study to report concentrations of RAC in fresh fecal and pen floor samples of RAC-treated cattle, making comparisons to the source concentrations in the TBA+RAC pens difficult. Our conventional commercial feedlot data agree well with the pen floor concentrations from our feeding trials, with mean concentrations between 3000 and 4500 ng g^{-1} RAC (Tables S19 and S20). Additionally, although RAC is only administered in the final ≈40 days prior to slaughter, at large commercial feedlots, administration of RAC is almost constant as cattle rotate through the RAC finishing stage, which suggests the potential continuous release of this contaminant. This is confirmed by the fact that RAC was detected in 100% of the samples taken from the two conventional commercial feedlots during spring, summer, fall, and winter months between 2016 and 2018 (Tables S19 and S20).

Fate and Dissipation of TBA and MGA during Feeding Trials. Dissipation of the target analytes in material from pen floors is best observed in the latter portion of the 2017−2018 feeding trial (after ≈200 days) when extensive sampling was conducted in the TBA+RAC pens. Concentrations of 17α-TBOH and 17β-TBOH dissipated quickly during the summer months (June and July) following the third and final TBA implant. Where sampling was conducted postfeeding in TBA+RAC treatments (Figure 1), 17α-TBOH concentrations were ≤1 ng g^{-1} in manure samples 10 days post-trial and below detectable limits 22 days post-trial. This finding suggests a benefit to leaving manure in pens for a period after cattle vacate to facilitate dissipation of hormone residues prior to land application. Concentrations of 17β-TBOH were also less than limits of quantification in all samples taken after May in both the 2017−2018 and 2018−2019 feeding trials. While extended sampling, post-trial, was not conducted for MGA, noticeable dissipation in MGA occurred in June and July of both feeding trials, with concentrations dropping below 5 ng g^{-1}, as compared to concentrations from November to May of 15 ± 5 ng g^{-1}. The observed dissipation of TBA metabolites is consistent with observations during previous studies. During batch soil microcosm experiments conducted with clay loam and sandy soils under aerobic conditions, half-lives ranged from 3 to 12 h for 17α-TBOH and 17β-TBOH.[43] Half-lives of all three TBA metabolites as a function of temperature have been reported to range from 4 to 50 h, depending on the compound and the incubation temperature (5−35 °C).[42] As such, we expect dissipation of these chemicals to be greater during summer than winter, consistent with the results reported here (Figure 1).

Fate and Dissipation of RAC during Feeding Trials. Following sampling of pen floor materials, 13 days after the start of RAC treatment (3600 ± 700 ng g^{-1}) in 2017−2018, the five remaining sampling events occurred to track dissipation after cattle were removed from pens and RAC administration ended. Postfeeding trial dissipation of RAC in pen floor samples was observed (Figure 2), but significant amounts remained (681 ± 450 ng g^{-1}, n = 4) up to 37 days after RAC administration ended. Assuming that the concentration in pen floor materials on day 13 of RAC treatment remained relatively constant until administration ended, dissipation rates can be estimated via first-order kinetics (Figure 2, inset). Over the 37 day post-RAC sampling period (6, 8, 10, 22, and 37 days), RAC degraded with a half-life of ≈18 days over July and August of 2018 (0.04 day^{-1}, r^2 = 0.77; Table 1).

Residues of RAC from the 2017−2018 treated pens remained at the start of the 2018−2019 feeding trial, measuring 42 ng g^{-1} maximally with a mean of 15 ng g^{-1}, and dissipated slowly over the course of the feeding trial to less than the limit of detection by May 2018 (Figure 2). This

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Table 1. Ractopamine (RAC) First-Order Manure Degradation Rates Measured *In Situ* during the 2017−2018 and 2018−2019 Feeding Trials (Figure 2) and RAC *In Situ* Distribution Coefficients ($\log K_d$, L kg^{-1}) Estimated from Sediment (C_s) and Water (C_w) Concentrations in Commercial Feedlot Catch Basins[a]

feeding trial	k (day^{-1})	$t_{1/2}$ (days)	r^2
2017−2018 (July−August)	0.039 ± 0.011	18 ± 5	0.769
2018−2019 (January−April)	0.014 ± 0.002	49 ± 6	0.958
feedlot	C_s (ng g^{-1})	C_w (ng L^{-1})	$\log K_d$
commercial 1	234	4000	1.79
commercial 2	1281	27 000	1.67
commercial 3	1506	21 000	1.85

[a]The date range provided for each feeding trial indicates the months over which the first-order kinetic data apply to (i.e., summer and winter half-lives, $t_{1/2}$). Catch-basin water and suspended sediments were taken from three conventional commercial feedlots in October 2019.

second set of RAC dissipation data provides a first-order kinetic time series (Figure 2, inset) and an estimated manure degradation rate of ≈49 days from January to April of 2019 (Table 1). These differing degradation rates in manure represent a seasonal effect between 2017−2018 (summer) and 2018−2019 (winter), as rates of degradation in soils have been shown to be a strong function of temperature.[42,44] These are the first reported estimates of a half-life of RAC in feedlot pens.

A period of 126 days separated the final sampling point of the 2017−2018 trial (37 days post-trial on August 23, 2018) and the initial sampling of the 2018−2019 trial (December 26, 2018). Given that pens are cleaned (i.e., all floor material removed) prior to the start of a new feeding trial, this result suggests that the earthen-clay black base layer of the pens may harbor RAC residues for extended periods. Additionally, in contrast to observations during administration of RAC, concentrations at the start of the 2018−2019 trial were greatest in pen floor samples as opposed to feces, which further supports the contention that the source of the RAC was from previous use (i.e., 2017−2018 trial) as opposed to fresh feces from the cattle present for the 2018−2019 feeding trial. Lastly, it has been noted that anaerobic environments[42,43] of deeper soil layers in feedlot pen floors can act as potential reservoirs of veterinary chemicals.[17]

Assuming a constant half-life of 49 (winter) and 18 (summer) days over the 126 day period separating the last and first sampling point of the two feeding trials, between ≈120 and 5 ng g^{-1} RAC would remain, respectively, in the pens at the start of the new feeding trial on December 26, 2018, which agrees well with observed concentrations (range = 6.0−78.9 ng g^{-1}). These results suggest that manures containing RAC residues can serve as sources of this chemical with the potential to enter surrounding environments via runoff, windblown particulates, or when manure is land-applied. Various composting practices are often employed prior to field application and have proved effective at removing certain veterinary pharmaceuticals present in manure.[46−48] However, efficacy varies for different chemicals, and specific composting studies are yet to be published for RAC. The dissipation rates observed here (Table 1) in pen floors suggest that manure composting should be employed as the best management practice to reduce RAC residues prior to land application. Ongoing composting of manure and soil dissipation studies by our group will provide important information regarding these best management practices of RAC residues in manure.

Surface Runoff. *Simulated Rainfall Experiments.* Concentrations of residues remained relatively constant over the entire duration of rainfall simulations (15 sequential L), which suggests that there was no significant depletion of any of the target chemicals in the source pen floor material and that compounds desorbed or leached from the manure at a constant rate over the duration of the simulated rainfall, consistent with observations elsewhere for similar compounds.[38] With the exception of RAC (discussed below), this was also evidenced by the fact that concentrations measured in the pen floor material pre- and postrainfall simulations remained relatively constant. However, another explanation for the constant concentrations may be related to the leaching of chemicals from suspended solids collected in the 1 L samples. This could potentially drive concentrations in some of the initial samples prior to the establishment of mass transfer on the runoff surface. MGA was not detected in the pasture runoff experiment (Figure S3). The absence of MGA in runoff from pasture could be the result of specific physiochemical interactions, including sorption on soil surface organic matter.[49] Pasture can attenuate certain chemicals due to interactions with living or dead vegetation and soil organic matter fractions, resulting in reduced mobility and thus transport of MGA in the runoff water samples.[5,38]

Mean concentrations over the 15 L pen floor runoff experiments were 76 ± 60 ng L^{-1} 17α-TBOH, 6 ± 3 ng L^{-1} 17β-TBOH, 24 ± 17 ng L^{-1} MGA, and 6300 ± 5300 ng L^{-1} RAC. In pasture runoff, sample concentrations measured 51 ± 30 ng L^{-1} 17α-TBOH, 12 ± 6 ng L^{-1} 17β-TBOH, <LOD MGA, and 3100 ± 2100 ng L^{-1} RAC. While pen floor and pasture concentrations were not statistically different for any of the target chemicals, in general, the pasture samples appeared to have slightly reduced concentrations compared to the pen floor, likely for the same reasons discussed above for MGA. Total masses of chemicals exported in surface runoff over ≈15 L ranged from 60 ng 17β-TBOH to 63 000 ng RAC in the pen floor and from 116 ng 17β-TBOH to 31 000 ng RAC in pasture (Figure S3). Extrapolating these masses from a 1 × 1 m^2 simulation area to full pen areas (273 m^2) at the AAFC research feedlot, a rainfall event of this magnitude has the potential to mobilize up to 17.2 mg of RAC per pen or 63 μg m^{-2}, assuming a similar stocking density (10 cattle pen^{-1}) as used here. Considering that the largest commercial feedlot in Alberta has a 75 000 head capacity, it is possible that a heavy rainfall event could leach gram quantities of RAC from a single feedlot. It is important to note that these experiments captured only surface runoff. Infiltration into deeper soil layers in feedlot pens has been observed during rain events[17] and leaching experiments on manure-amended land.[5] This could result in unaccounted compound fractions in the current study, thereby underestimating the total estimated loads mobilized during simulated rainfall events.

The fraction of compound captured in these runoff events was estimated for RAC to better understand the extent of mobilization occurring from manure. This was done for the RAC pasture runoff experiments as a defined amount of manure (6 kg or 2.5 kg dw based on 58% water content[38]) was applied to the 1 m^2 plots, allowing for an accurate mass inventory pre- and postrunoff. Using the average measured

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RAC concentrations in the applied manure samples, prerunoff (435 ± 240 ng g^{-1} or 1.1 × 10^6 ng RAC per 1 m^2 plot), the total rainfall volume (36 L), and the experimental K_d values (Table 1), the fraction of RAC expected in all runoff water (retained and captured) was estimated to be 19%. This is consistent with the measured mass lost in manure samples postrunoff, which ranged from 17 to 25% (compared to initial manure concentrations prerunoff) in replicate runoff experiments. Based on calculated volumetric runoff coefficients, which provide an estimate of the fraction of incoming water (36 L) that leaves the surface as runoff versus the amount retained,[50] 43 ± 9% of total rainfall was captured in the 15 consecutive 1 L runoff water samples. Therefore, of the 19% total RAC mass, only 8% is expected in the collected runoff samples. This, compared to the measured mass of RAC captured in 15 L from the manure-amended pasture, was 30 900 ng or 2.8% of the total mass present in the 1 m^2 plot, agreeing well with the estimated 8%. A part of the uncertainty in this calculation is likely related to the assumption of an equilibrium K_d value, which is likely not applicable to the initial stages of these runoff scenarios, as the manure is saturating with rainwater and mass transfer is slow.

It should be noted that monthly groundwater samples taken from the AAFC research feedlot during the 2018−2019 feeding trial (data not shown) revealed that the target chemicals present in the feces, manure, and runoff samples described previously were not detected in groundwater over the course of sampling. This is likely a result of the hard-packed black interface layer that forms under the manure pack on the pen floors as a result of physical compaction by cattle that has been suggested to be responsible for limiting leaching and infiltration to the groundwater below feedlots.[51]

The concentrations in other simulated runoff experiments conducted in cattle feedlot pens were similar to ours, with median (range) concentrations of 34 (1−390) ng L^{-1} 17α-TBOH and 16 (5−26) ng L^{-1} 17β-TBOH.[17] However, 17α-TBOH, 17β-TBOH, or MGA were not detected in runoff samples from edge-of-field weirs where manure from beef cattle receiving TBA and MGA had been applied.[4] Conversely, in a 17 month study, maximum concentrations from different tile-drained fields receiving animal wastewater from lagoons ranged from 9.7 to 22.7 ng L^{-1} 17α-TBOH (1.0−5.7% detection rate) and 4.1 to 162 ng L^{-1} 17β-TBOH (0.4−3.5% detection rate).[21,52] Based on observations in the current study and those of past studies, it remains that concentrations of 17α-TBOH, 17β-TBOH, and MGA in runoff where these compounds are known to be present (i.e., feedlot waste effluents) occur at low ng L^{-1} concentrations to below limits of detection. While 17β-TBOH is known to be a potent androgenic toxicant at these concentrations,[23,26] detection frequencies are so low[21,52] that the risk of exposure appears to be minimal in most feedlot-associated environments, especially considering the dilution as runoff reaches a body of water.[2] This assertion is supported by the fact that these compounds were also not detected in commercial feedlot catch-basin samples, as detailed below, despite ongoing TBA and MGA treatments at these feedlots. Concentrations of RAC were up to 1000-fold greater in runoff samples compared to 17α-TBOH, 17β-TBOH, and MGA, which suggested that the RAC concentrations mobilized in feedlot runoff pose a greater potential for contamination of surrounding environments.

Catch-Basin Samples. Concentrations of all target chemicals in the AAFC research feedlot catch basin were less than the limit of detection, except for RAC, which was measured at 140 ng L^{-1}. The pens had been cleaned at the time of sampling (October 2019, between feeding trials), which meant that the major source material of the target chemicals (pen floor material) was not present. This explained the relatively low RAC concentration in water in this catch basin compared to catch basins from commercial feedlots, which had concentrations of 4000−27 000 ng L^{-1} in water and 234−1506 ng g^{-1} in sediment (Table 1). Other target compounds were not found in commercial feedlot catch-basin samples. Estimated *in situ* K_d values determined from the ratio of solid-to-water phase concentrations of each water sample were in good agreement across the three catch-basin sites, varying maximally by <0.2 log units (1.67−1.85). These values were also in good agreement with log K_d values for RAC in 10 mL batch sorption experiments, ranging from 1.58 to 1.68 in natural topsoil and from 1.53 to 1.64 in natural subsoil.[53] The study reported sorption dominated by hydrophobic interactions with soil organic matter and relevant interactions with charged complexes on clay particles.[53] Given that the pK_a of RAC (≈9.4)[53] overlaps the typical pH range of manures from our feeding trials (8.5−9.5),[47] binding to charged complexes is likely to be a relevant mechanism. Despite RAC's relatively high water solubility (31 g L^{-1}) and moderate polarity (log K_{OW} 2.4), these results suggest that RAC persists in both the solid and liquid fractions of manure, presenting multiple potential pathways into adjacent feedlot environments, consistent with our data from both the feeding trials and runoff experiments.

RAC Mass Balance. A mass balance was conducted to better inform the fate and overall fraction of RAC accounted for in our sampling and analyses. Extensive mass balance inventories in feedlot pen environments are reported elsewhere for both TBA[2,6,17] and MGA[6] and thus are not the focus of this exercise. The daily mass intake of feed (0.003% RAC wt/wt) by the TBA+RAC steers during the course of this study was 11.5 ± 0.2 kg day^{-1},[54] which equates to a RAC intake of 0.00035 kg day^{-1} per head. Reported fecal excretion rates range from 1.8 kg (dw) day^{-1} AU^{-1} (animal unit) for juvenile steers[2] and heifers weighing 379 ± 31 kg[55] to 27−40 kg (ww) day^{-1} or 3.1−4.6 kg (dw) day^{-1}, assuming a fecal moisture percentage of 88.5%.[41,56] The shrunk body weights of the TBA+RAC steers throughout the finishing period of this study were 425 ± 14 kg (initial) and 717 ± 8 kg (final),[54] the latter weight being more reflective of the cattle during the final 42 day RAC feeding period prior to slaughter. Thus, a fecal excretion rate on the higher end of the reported range (i.e., 3.1−4.6 kg day^{-1}) may be most appropriate for this RAC mass balance. Using the minimum (1.8 kg day^{-1}), median (3.1 kg day^{-1}), and maximum (4.6 kg day^{-1}) reported fecal excretion rates results in predicted RAC manure concentrations of 106 000, 60 100, and 41 400 ng g^{-1}, respectively, assuming 55% excretion in feces.[11] Fresh samples represent only recently excreted fecal pats and thus attenuation is expected to be negligible, especially given that the observed dissipation rates for RAC in this study are in the order of weeks (18−49 days, Table 1). Compared to the average fresh fecal concentrations observed over the 2017−2018 RAC sampling period (37 000 ± 8800 ng g^{-1}), these measured values account for 35% (minimum excretion), 61% (median), and 89% (maximum) of total RAC expected in cattle feces.

Floor samples represent a more uncertain matrix to account for due to dilution with bedding and mixing in the pen floor,

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potential attenuation over time, and the contribution of urine to the sample. While 45% of RAC is excreted in the urine,[11] an estimated 95% of this is the monoglucuronide conjugate.[13,14] While RAC metabolites were not targeted in this work, retrospective mining of the full-scan high-resolution (120 000) MS data did not reveal characteristic m/z ions[14] of the glucuronide metabolite in any samples, likely due to poor recoveries during extraction[57,58] (details in the Supporting Information). Additionally, the ratio of urine/feces excretion in steers on a typical high-energy diet is 1:2.75, further suggesting that feces likely represent the major contributor to floor RAC concentrations.[41] Given the reasonable mass balance reflected in the fresh fecal samples described above, RAC floor concentrations measuring approximately 10-fold lower (3600 ng g^{-1}) likely reflects the attenuation occurring in aged manure and subsequent mixing by the cattle. While we do have first-order attenuation rate constants for RAC (Table 1), given that floor manure samples were not taken frequently during RAC feeding, it is very difficult to estimate how "aged" a given floor sample was and thus the extent of attenuation that had occurred prior to the time of sampling.

Environmental Significance. This work presents the first comprehensive characterization of the occurrence and fate of RAC in cattle feedlots and adjacent environments. RAC was found at concentrations in fresh fecal and pen floor samples that were 3−4 orders of magnitude greater than those of the synthetic hormones TBA and MGA studied here. The body of knowledge regarding the occurrence and fate of TBA and MGA, here and elsewhere, suggests limited exposure risk to nontarget aquatic organisms given the small concentrations and infrequent detections in most animal manure-impacted environments. However, scenarios of low dilution in close proximity to high-density livestock operations remain a concern that warrants study on a case-by-case basis. The observed concentrations of RAC in fecal and floor samples, surface runoff from rainfall simulations, and catch basins of commercial feedlots suggest that this compound may pose specific challenges when it comes to management practices for manure intended for agricultural use and protection of edge-of-field aquatic environments. Concentrations of RAC in catch-basin water samples (4000−27 000 ng L^{-1}) exceeded levels causing behavioral effects in zebra fish (≥850 ng L^{-1})[32] by 5−32-fold. While the lack of detection of all chemicals, including RAC, in the groundwater collected in 2018 and 2019 suggests that these chemicals have limited downward mobility, further research is needed to elucidate the presence of RAC and its major glucuronide metabolite in both soil and aquatic environments adjacent to beef cattle feedlots, especially given the elevated concentrations of RAC observed in manures.

Post-trial sampling demonstrated that floor material left in the pens after cattle were removed results in dissipation of 17α-TBOH to below LODs after >10 days while significant RAC concentrations remained after 37 days post-trial (681 ng g^{-1}). From a manure-management perspective, leaving pens for approximately 2 weeks post-feeding can reduce residues of 17α-TBOH, 17β-TBOH, and MGA to below detection and potentially reduce or eliminate the requirement for manure composting prior to land application. Given the elevated concentrations of RAC observed post-trial, further research should be directed toward understanding its dissipation and fate following land-application of fresh manure, and during manure storage (stockpiling) or processing (composting), and informing optimal manure-management practices, work that is ongoing in our group.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.0c06450.

Feeding trial diets and sampling dates; simulated runoff and groundwater sampling; methodological extraction and analysis protocols including analytical detection limits; and raw tabular concentration data for feeding trials and commercial feedlot samples (PDF)

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Notes

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SUPPORTING INFORMATION

Ractopamine and Other Growth Promoting Compounds in Beef Cattle Operations: Fate and Transport in Feedlot Pens and Adjacent Environments

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Contents: 27 pages, 20 tables, 3 figures

MATERIALS AND METHODS

Feeding trials.

Steers and heifers were implanted at the beginning of the study (1st implant) with Component TE-100 with Tylan (10 mg of estradiol, 100 mg of TBA and 29 mg of tylosin tartrate; Elanco Animal Health, Division Eli Lilly Canada Inc., Guelph, Ontario, Canada) and again at the end of the growing period (84 d, 2nd implant) with Component TE-100 with Tylan. At 84 d prior to slaughter cattle were given a 3rd implant (Component TE-200; 20 mg of estradiol, 200 mg of TBA and 29 mg of tylosin tartrate; Elanco Animal Health). The MGA (100 premix; melengestrol acetate at 220 mg kg^{-1}, Zoetis Canada Inc., Kirkland, QC, Canada) was included in the diets of heifers to administer 0.40 mg of melengestrol acetate $heifer^{-1}$ day^{-1}. Ractopamine hydrochloride (Optaflexx, Elanco Animal Health) was included in the diet at 30 mg kg^{-1} (0.003%) for the final 42 days of each feeding trial, with a 24 h withdrawal prior to slaughter.

Table S1: Timeline and details of the 2017-18 feeding trial.

Date	Action
2017	
October 31	Start of feeding trial and backgrounding diet. Cattle assigned to treatment pens as per above. MGA administered continuously in feed
November 1	First TBA implant administered
November 14	Pen floor sampling (i.e. not fresh feces), mix of bedding + feces (denoted 'Floor' in figures)
November 14-16	Three consecutive days of fresh fecal sampling at ~2 wk after implant date to capture peak excretion of TBA (denoted 'fresh' in figures)
December 4	Pen floor sampling (as above)
2018	
January 8	Pen floor sampling (as above)
January 23	Second TBA implant administered. End of backgrounding. Start of transition to finishing diet
February 5-7	Three consecutive days of fresh fecal sampling at ~2 wk after implant date (as above)
February 20	Start of finishing diet
March 18	Pen floor sampling (as above)
April 17	Third TBA implant administered
April 30	Pen floor sampling (as above)
April 30-May 2	Three consecutive days of fresh fecal sampling at ~2 wk after implant date (as above)
May 28	Pen floor sampling (as above)
June 5	Start of ractopamine administration
June 20-July 13	Intensive sampling of TBA/RAC pen floors (10 times in a 23 day period: June 20, 22, 25, 27, 29; July 4, 6, 9, 11, 13) to capture RAC concentrations in the 15-38 day period after RAC was first administered
June 25	Pen floor sampling (as above)

July 17	End of feeding trial (50% of animals removed from pens)
July 18	Remainder of trial animals removed from pens
July 23-August 23	TBA/RAC pen floors sampled Days 5, 7, 9, 14, 21, and 36 to capture dissipation of TBA/RAC after cattle vacated pens

Table S2: Timeline and details of the 2018-19 feeding trial.

Date	**Action**
2018	
December 11	Start of feeding trial and backgrounding diet. Cattle assigned to treatment pens as per above. MGA administered continuously in feed
December 12	First TBA implant administered
December 26	Pen floor sampling (i.e. not fresh feces), mix of bedding + feces
December 26-28	Three consecutive days of fresh feces sampling at ~2 wk after implant date to capture peak excretion of TBA
2019	
January 14	Pen floor sampling (as above)
February 18	Pen floor sampling (as above)
March 5	Second TBA implant administered
March 6	Start of transition to finishing diet
March 18-20	Three consecutive days of fresh feces sampling at ~2 wk after implant date to capture peak excretion of TBA
March 19	Pen floor sampling (as above)
April 2	Start of finishing diet
April 15	Pen floor sampling (as above)
May 13	Pen floor sampling (as above)
May 28	Third TBA implant administered
June 4	Fresh feces sampling one week after 3rd implant. Switched fresh feces sampling to weekly instead of consecutive days to assess longer term dissipation of TBA post implantation.
June 10-12	Three consecutive days of fresh feces sampling at ~2 wk after implant date to capture peak excretion of TBA
July 8	Pen floor sampling (as above)
July 29	Ractopamine administration via feed begins
September 9-11	Feeding trial ends

Commercial feedlot sampling. Composite pen floor samples were taken from four commercial feedlots located in the surrounding Lethbridge, AB area. Two of the commercial feedlots were conventional (growth-promoters used) and two were natural (no growth-promoters used). Between August 2016 and July 2018 a total of 18 sampling events took place, split between winter (8) and summer (10) and the four commercial feedlots. During each sampling event up to 10 samples were taken from a given feedlot, depending partially on the number of pens with cattle present at the time of sampling. The purpose of these commercial feedlot samples was to provide a point of comparison for our research feeding trials. Given that our feeding trials were conducted in accordance with industry standards, we would expect concentrations of growth

promoters in pen floor samples to be in a similar range. To accomplish this goal a subset (listed in Table 2) of the hundreds of commercial samples taken were extracted and analyzed. Only composite pen floor samples were collected from commercial pens, as described above.

Table S3: List of samples analyzed from the conventional and natural commercial feedlots between 2016 and 2018.

Feedlot	Dates sampled
Conventional 1	OCT 2016; APR 2017; JUN 2017; SEP 2017; DEC 2017; MAY 2018 (6 sampling events)
Conventional 2	JAN 2017; MAY 2017; AUG 2017; JAN 2018; JUN 2018 (5 sampling events)
Natural 1	OCT 2016; APR 2017; SEP 2017; DEC 2017; MAY 2018 (5 sampling events)
Natural 2	JAN 2017; MAY 2017; AUG 2017; JAN 2018; JUN 2018 (5 sampling events)

Simulated runoff experiments. A stainless steel frame (1 × 1 m) consisting of 20-cm high walls on three sides with iron posts welded at the ends and center of each side, was driven into the pen floor to approximately 3 cm below the floor surface. This created a leak-proof barrier with the frame being positioned so that simulated rainfall runoff drained to the open side of the frame and onto a triangular shaped stainless-steel flume (Fig. S1). The flume had 5-cm sidewalls which directed drainage into a 1-L glass measuring cup. A PVC triangular cover was placed over the triangular flume to prevent direct deposition of simulated rain onto the flume. Runoff collected in the cup was transferred to 1-L amber glass bottles. The nozzle of the simulator was positioned centrally over the stainless steel frame at a height of 80 cm above the pen floor or pasture surface. De-ionized water was applied at an intensity of 126.5 mm h-1, representing a one in 100-year event for the Lethbridge area.

In all experiments, 15 consecutive 1-L runoff samples were collected. The 1st, 2nd, 3rd, 4th, 5th, 6th, 9th, and 12th litre were collected individually. The 7th, 8th, 10th, and 11th 1-L samples were combined in a large container, mixed, and a 1-L composite sub-sample was taken. A similar composite sub-sample was also taken for the 13th, 14th, and 15th 1-L samples. Each experiment resulted in a total of 10 1-L runoff samples. Immediately before and after each runoff experiment pen floors were sampled in order to determine concentrations in the source material pre- and post-rainfall simulation.

Figure S1: Photograph of the Guelph Rainfall Simulator II prior-to (left) and during (middle) a pen-runoff experiment and during a pasture-runoff experiment (right).

Groundwater sampling. Wells were installed in 1996 to an average depth of 5.87 m below ground level.[1] Fluctuations in water table elevation studied over a 4.5 – year period between 1996 – 2000 ranged from 1.23 m and 2.50 m, which followed precipitation patterns and ground water recharge events over this time.[1] Sampling of the wells was conducted with a narrow stainless steel pipe (closed on one end) that was submerged in ground water 5 – 6 times to fill a 1L sample bottle.

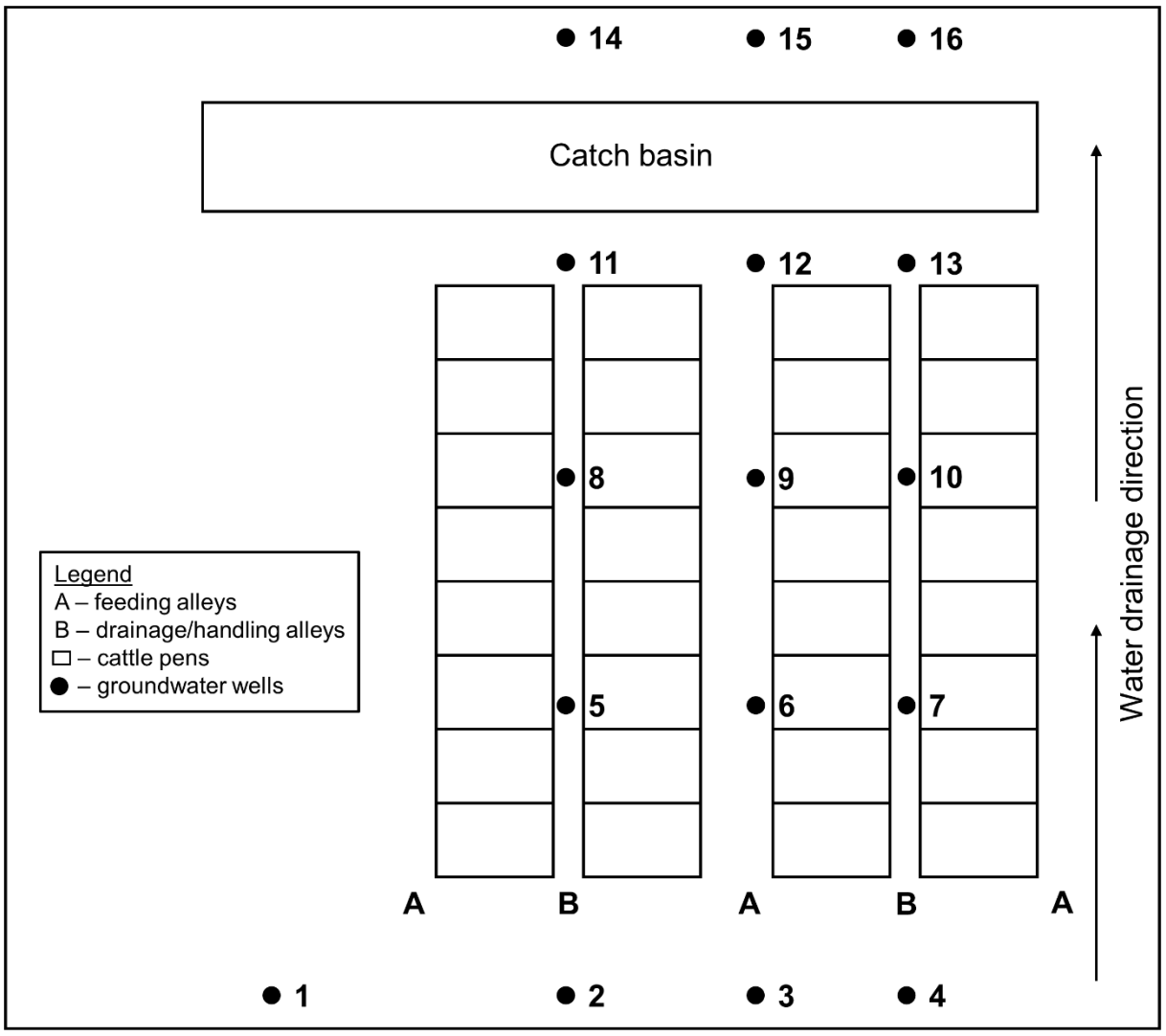

Figure S2: Layout of the Agriculture and Agri-Food Canada (AAFC) Research Centre research feedlot in Lethbridge, AB, Canada. Direction of water drainage south to north on a 0.35% slope. Figure is not to scale and the location of the groundwater wells are approximate. Figure is adapted from Olson, B.M., Miller, J.J., Rodvang, S.J., Yanke, L.J., 2005. Soil and Groundwater Quality under a Cattle Feedlot in Southern Alberta. Water Qual. Res. J. Canada 40, 131–144.

Sample extractions. *Solid samples.* Based on expected concentrations in each type of sample different sample weights were used as follows; 2 g pen floor samples, 1 g fresh fecal sample, and 0.5 g RAC samples (during the treatment period) (all dry wt.). Prior to extraction all samples (solid and aqueous) were spiked with the isotopically labelled internal standard (IS) mixture at a target concentration of 50 ng mL^{-1} in the final 1 mL extract. Each spiked extract was mixed with ≈5 g Ottawa Sand (Fisher Scientific) and loaded into 34 mL accelerated solvent extraction (ASE) cells as follows: filter paper, 8 g Ottawa Sand (Fisher Scientific), filter paper, spiked sample, filter paper, sand to top of cell, filter paper. Pressurized liquid extraction was conducted using a Dionex ASE 200 (Thermo-Fisher) with a 100% methanol solvent mixture. Two 70% volume extractions were conducted at 100^{o}C and 1500 psi for 10 min each. A blank cell (sand spiked with IS) was also extracted with each batch of samples to serve as an extraction lab blank.

The resulting 45 mL methanol extracts were diluted to 500 mL with RO water for solid-phase extraction (SPE) using OASIS™ HLB cartridges (6cc, Waters Corporation, Milford, MA). After pre-conditioning with methanol followed by water, samples were drawn through at ≈5 mL min-1, cartridges vacuum dried, and eluted with 2 × 6 mL fractions of methanol (combined). Extracts were evaporated to dryness under nitrogen in a water bath at 40^{o}C, reconstituted in 1 mL of 50:50 MeOH:H2O, and filtered through a 0.45 µm PTFE syringe filter (Pall Life Sciences, Mississauga, ON) into amber LC vials. All water samples (500 mL) employed the same method as above, beginning at the SPE stage.

Fresh fecal and pen floor RAC samples taken in the ≈40 day treatment period during the 2017-18 and 2018-19 feeding trials and commercial samples containing RAC were diluted to maintain concentrations within the linear dynamic range of the analytical method. ASE extracts of RAC were not put through SPE. Exactly 0.5 mL was taken from the ASE extract and diluted quantitatively to 1 mL with MQ H_2O. This abbreviated method resulted in a 160-fold dilution of RAC concentrations compared to the 2 g ASE-SPE method used for all other solid samples.

Water samples. Surface runoff, ground water, and catch basin samples were extracted and concentrated using the same SPE method described above for solid samples. Samples (500 mL) were spiked with IS mixture prior to extraction. The catch basin samples were highly turbid and solids were recovered by centrifugation (5,000 rpm). The resulting centrifuged solids were freeze dried, and extracted using the same method as for fresh and floor samples. From this analysis the *in-situ* distribution coefficients (K_d) between the water and sediment phase were determined for the detected analytes.

Instrumental analysis.

The Q-Exactive Orbitrap method used the following source parameters: sheath gas flow = 35; aux gas flow = 10; sweep gas flow = 1; aux gas heater = 400 ^{o}C; spray voltage = 3.8 kV; S-lens RF = 60; capillary temperature = 350 ^{o}C. A Full MS/parallel reaction monitoring (PRM) method was used with the following scan settings: 120,000/15,000 resolution, AGC target = $1x10^6/2x10^5$, max injection time = 50 ms/50 ms, full MS scan range of 80-500 m/z and PRM isolation window of 2.0 m/z and multiplexing count of 4.

Batch analyses of sets of samples were conducted by running calibration standards at the beginning and end of each sample batch along with blanks run between replicate treatment sets and single calibration standards (10, 25, or 50 µg/L) every 15-20 samples as a QA/QC protocol. An eleven point calibration curve ranging from 0.05 – 500 ng/mL and spiked with 50 µg/L IS was used for quantification by isotope dilution (linearity > 0.99 for all analytes).

Table S4. Positive mode gradient elution method. Flow rate = 0.35 mL/min, column temperature = 40^{o}C, solvent A = 100% H_2O + 0.1% formic acid and 90:10% ACN:H_2O + 0.1% formic acid.

Positive mode	
Time (min)	**%B**
0.00	10
7.00	100 (curve 8)
8.50	100
8.51	10
11.00	10

Table S5. Precursor and product ions ($[M+H]^+$), collision energy (HCD), and retention time details for the full-scan parallel reaction monitoring Orbitrap™ mass spectrometer method.

Compound Name	**Precursor Ion**	**Product Ion**	**HCD**	**Ret Time (min)**
17α-TBOH	271.1693	253.1584	60	6.05
17β-TBOH	271.1693	253.1584	60	6.30
MGA	397.2373	337.2161	35	7.52
RAC	302.1751	284.1644, 164.1069	35	2.57
TBA	313.1798	253.1582	40	7.48
TBO	269.1536	225.1272	60	6.85

Detection Limits

Method detection limits were determined using a procedural extraction blank, extracted and processed as detailed in the main text for solid and liquid samples. Each procedural blank sample was measured in seven consecutive injections. Slopes from the calibration curve run in the same batch of samples were used in the LOD and LOQ calculations.

Table S6: Method detection limits for target analytes in solid and liquid samples.

Compound Name	Solid samples		Liquid samples	
	LOD (ng/g)	LOQ (ng/g)	LOD (ng/L)	LOQ (ng/L)
17α-TBOH	0.10	0.35	0.42	1.39
17β-TBOH	0.079	0.26	0.31	1.05
MGA	0.073	0.24	0.29	0.97
RAC	0.029	0.10	0.12	0.38
TBA	0.034	0.11	0.14	0.45
TBO	0.084	0.28	0.33	1.11

*Limit of detection (LOD) = $3\sigma_{BLK}$; **limit of quantitation (LOQ) = $10\sigma_{BLK}$

Chemicals and reagents.

Methanol (LC-MS grade) from Fischer Scientific (Ottawa, ON) and 18.2 MΩ-cm Milli-Q water (EMD Milli-Pore Synergy® system, Etobicoke, ON), were used for LC solvents, analytical standards, and sample extractions. Optima LC/MS grade formic acid was purchased from Fischer Scientific for LC solvent preparation. Trenbolone acetate (TBA), 17α-trenbolone (TBOH), 17β-TBOH, trendione (TBO), 17α-TBOH-d_5, melengesterol acetate (MGA), MGA-d_3, ractopamine (RAC), and RAC-d_6 were all purchased from Toronto Research Chemicals (Toronto, ON). All analytical standards were ≥95% purity. 17α-TBOH-d_5 was used as the internal standard for TBA and related metabolites (17α-TBOH, 17β-TBOH, and TBO). All stock solutions were dissolved in 100% methanol at 100 µg/mL. Two separate stock mixtures of the 7 native standards and 4 isotopically labelled internal standards (IS) were made at 10 µg/mL as stocks for calibration standards. A 0.5 µg/mL stock IS mixture was made for spiking samples.

RESULTS

Data analyses. Effects of consecutive sampling days for fresh fecal samples (e.g., Nov. 14-16, 2017) and Heifer/Steers implanted with TBA (TBA-H, TBA-S, TBA/RAC-S) on concentrations were assessed by use of a one-way ANOVA and Tukey post-hoc test. The assumption of homogeneity of variances was met according to the Bartlett's test for equal variances. A *p*-value < 0.05 was considered significant. No significant effect was observed for consecutive sampling days (n=3 days) or treatment (TBA-Heifers, n=4; TBA-Steers, n=4; and TBA+RAC-Steers, n=4) and therefore all TBA fresh fecal samples were pooled for each sampling event (n = 3 days × 3 treatments × 4 replicates = 36) and all TBA pen floor samples were pooled (n = 1 day × 3 treatments × 4 replicates = 12). A complete summary of all un-pooled and pooled data is shown in SI (Table S7-S18).

RAC mass balance. *Glucuronide metabolites.* With known metabolism and excretion of RAC, we expect the glucuronide metabolite to contribute most significantly to floor samples due to its presence in urine. In an attempt to assess the contribution of the monoglucuronide conjugate to the overall RAC mass balance, the full-scan high resolution MS data acquired for all samples was retrospectively mined for the parent *m/z* ion 478.2065 and reported characteristic fragment ions 340.138 and 284.164.[2] No *m/z* signatures (<5ppm mass accuracy) were observed in fresh fecal or floor manure samples. Given the similarities between our chromatographic method and that of Tang et al.[2] who successfully measured RAC metabolites, we suspect the lack of observed glucuronide signatures is a result of poor recoveries during the accelerated solvent extraction, especially given the known instability and sensitivity of some glucuronide conjugates.[3,4]

Feeding Trial raw data.

Table S7: 2017-18 feeding trial concentrations (ng/g) of 17α-TBOH in individual treatment pens in fresh and floor samples.

Treatment	**TBA-H**		**TBA-S**		**TBA-H**		**TBA-S**		**TBA/RAC-S**			
Sample-date	**P25**	**P26**	**P27**	**P28**	**P29**	**P30**	**P31**	**P32**	**P37**	**P38**	**P39**	**P40**
Fresh-Nov-14	87.4	54.2	109.3	91.6	115.8	133.3	144.5	107.9	156.3	146.2	109.6	108.9
Fresh-Nov-15	81.8	100.7	118.4	127.7	97.0	94.8	86.7	92.3	102.7	108.2	86.1	89.3
Fresh-Nov-16	138.0	116.5	107.6	102.0	97.8	130.9	94.5	124.6	84.1	101.6	98.2	136.4
Floor-Nov-14	53.6	12.0	10.8	15.5	9.5	10.6	18.3	58.8	17.9	15.9	24.5	52.4
Floor-Dec-4	15.4	35.4	33.9	24.2	27.1	27.6	27.1	35.1	5.7	19.2	5.2	15.8
Floor-Jan-8	54.7	39.2	66.8	42.4	48.4	42.3	21.5	37.2	65.9	57.6	75.1	88.9
Fresh-Feb-5	122.3	75.2	88.1	111.9	28.5	53.6	112.4	101.6	122.8	86.1	102.9	100.0
Fresh-Feb-6	103.7	105.4	118.1	129.9	160.9	146.0	112.4	143.7	99.4	131.3	197.4	121.4
Fresh-Feb-7	126.6	126.0	126.8	112.5	155.7	108.1	148.7	107.1	99.1	143.2	156.5	132.0
Floor-Mar-18	146.5	56.1	130.6	95.9	124.5	79.3	80.6	57.1	67.3	134.4	80.4	78.3
Fresh-Apr-30	220.3	105.4	186.3	239.8	184.7	220.3	195.0	177.0	108.0	247.6	271.1	173.0
Fresh-May-1	NS	146.6	340.2	211.5	NS	273.8	281.8	NS	173.5	372.9	278.6	206.4
Fresh-May-2	165.8	147.3	240.2	176.4	165.6	224.6	248.4	172.6	NS	280.0	241.2	122.8
Fresh-May-28	17.2	18.7	14.8	15.1	9.0	8.5	15.6	6.9	4.5	7.8	14.4	12.8
Floor-Apr-30	6.7	7.7	11.4	5.2	24.3	11.2	17.3	7.3	8.0	8.2	9.4	8.8
Floor-May-18	19.2	39.5	22.0	22.8	15.0	35.5	15.8	15.1	NS	NS	25.9	12.1
Floor-Jun-18	3.0	2.4	0.9	4.6	1.1	1.8	NS	2.1				
Floor-Jul-23	1.2	1.3	ND	2.3	ND	1.0	1.0	1.4		NA		
Floor-Jul-25	2.6	1.0	2.2	1.0	1.1	2.1	1.0	2.4				
Floor-Jul-27	2.5	ND	0.7	1.8	1.2	4.3	1.6	1.3				

NS – not sampled. ND – not detected. NA – not analyzed; samples from TBA/RAC-S taken during RAC treatment (after June 5) were only analyzed for RAC.

Table S8: 2017-18 feeding trial average concentrations (ng/g) of 17α-TBOH in each of the three TBA treatments (n=4) and pooled together as a single TBA treatment (n=12). For fresh samples, in addition to treatments averaged together, the three consecutive fresh sampling days are also pooled (n=36).

Treatment	**TBA-H**		**TBA-S**		**TBA/RAC-S**		**TBA-H/S and fresh days pooled**	
Sample-date	**AVE**	**SD**	**AVE**	**SD**	**AVE**	**SD**	**AVE**	**SD**
Fresh-Nov-14	97.7	34.6	113.3	22.3	130.3	24.6		
Fresh-Nov-15	93.6	8.2	106.3	19.9	96.6	10.6	107.9	21.5
Fresh-Nov-16	120.8	17.7	107.2	12.8	105.1	22.2		
Floor-Nov-14	21.4	21.5	25.9	22.2	27.7	16.9	25.0	18.6
Floor-Dec-4	26.4	8.2	30.1	5.3	11.5	7.1	22.6	10.5
Floor-Jan-8	46.2	6.8	42.0	18.8	71.9	13.4	53.3	18.7
Fresh-Feb-5	69.9	39.8	103.5	11.4	102.9	15.1		
Fresh-Feb-6	129.0	28.9	126.0	13.9	137.4	42.2	117.1	30.7
Fresh-Feb-7	129.1	19.7	123.8	18.6	132.7	24.6		
Floor-Mar-18	101.6	41.3	91.1	30.8	90.1	30.1	94.2	31.6
Fresh-Apr-30	182.7	54.2	199.5	27.8	199.9	74.2		
Fresh-May-1	210.2	89.9	277.8	64.4	257.8	88.4	212.5	62.4
Fresh-May-2	175.8	33.7	209.4	40.5	214.7	81.9		
Fresh-May-28	13.4	5.3	13.1	4.1	9.9	4.6	22.3	9.1
Floor-Apr-30	12.5	8.1	10.3	5.3	8.6	0.6	10.5	5.4
Floor-May-18	27.3	12.0	18.9	4.0	19.0	9.8	22.3	9.1
Floor-Jun-18	2.1	0.8	2.6	1.8			3.7	2.7
Floor-Jul-23	1.1	0.2	1.6	0.7	NA		1.4	0.5
Floor-Jul-25	1.7	0.8	1.7	0.8			1.7	0.7
Floor-Jul-27	2.6	1.5	1.3	0.5			1.9	1.2

NA – not analyzed; samples from TBA/RAC-S taken during RAC treatment (after June 5) were only analyzed for RAC.

Table S9: 2017-18 feeding trial concentrations (ng/g) of 17β-TBOH in individual treatment pens in fresh and floor samples.

Treatment	**TBA-H**		**TBA-S**		**TBA-H**		**TBA-S**		**TBA/RAC-S**			
Sample-date	**P25**	**P26**	**P27**	**P28**	**P29**	**P30**	**P31**	**P32**	**P37**	**P38**	**P39**	**P40**
Fresh-Nov-14	1.9	ND	5.3	8.4	2.7	4.6	ND	5.4	ND	ND	3.5	4.3
Fresh-Nov-15	2.2	2.9	1.6	5.0	3.9	6.3	3.4	3.5	3.0	6.0	6.6	4.9
Fresh-Nov-16	4.2	8.1	6.5	7.1	5.5	4.0	3.7	3.3	3.7	4.6	5.1	8.0
Floor-Nov-14	8.9	2.4	2.9	2.4	2.0	3.6	4.7	2.9	6.7	4.5	3.5	3.3
Floor-Dec-4	6.6	4.4	12.8	4.4	6.2	9.1	8.8	6.5	4.1	7.1	5.1	5.8
Floor-Jan-8	6.8	7.5	5.7	5.8	3.6	5.8	12.0	3.3	10.5	15.8	11.6	9.0
Fresh-Feb-5	9.8	3.7	3.4	6.2	2.5	2.6	4.0	2.6	5.9	4.9	5.3	8.5
Fresh-Feb-6	3.2	5.2	7.2	6.7	11.1	13.1	4.0	13.9	5.6	7.9	12.0	7.0
Fresh-Feb-7	5.4	7.7	7.6	8.5	10.4	6.1	7.0	7.1	5.6	12.6	14.1	11.4
Floor-Mar-18	11.7	10.9	15.0	12.3	14.5	10.2	12.0	9.0	8.8	16.1	5.6	
Fresh-Apr-30	10.1	10.2	10.6	12.7	17.5	14.7	13.3	11.3	10.1	12.4	21.0	12.4
Fresh-May-1	NS	14.4	20.6	16.4	NS	20.2	21.4	NS	13.1	17.5	16.9	19.4
Fresh-May-2	9.5	10.8	14.1	12.3	13.4	12.3	17.1	12.4	NS	23.4	17.2	10.3
Fresh-May-28	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Floor-Apr-30	9.3	14.7	9.8	ND	16.9	14.5	11.6	9.5	10.2	9.1	10.0	8.2
Floor-May-18	22.1	34.9	20.1	13.1	16.0	11.4	14.0	14.6	NS	NS	10.9	5.3
Floor-Jun-18	ND	ND	ND	ND	ND	ND	ND	ND				
Floor-Jul-23	ND	ND	ND	ND	ND	ND	ND	ND		NA		
Floor-Jul-25	ND	ND	ND	ND	ND	ND	ND	ND				
Floor-Jul-27	ND	ND	ND	ND	ND	ND	ND	ND				

NS – not sampled. ND – not detected. NA – not analyzed; samples from TBA/RAC-S taken during RAC treatment (after June 5) were only analyzed for RAC.

Table S10: 2017-18 feeding trial average concentrations (ng/g) of 17β-TBOH in each of the three TBA treatments (n=4) and pooled together as a single TBA treatment (n=12). For fresh samples, in addition to treatments averaged together, the three consecutive fresh sampling days are also pooled (n=36).

Treatment	**TBA-H**		**TBA-S**		**TBA/RAC-S**		**TBA-H/S and fresh days pooled**	
Sample-date	**AVE**	**SD**	**AVE**	**SD**	**AVE**	**SD**	**AVE**	**SD**
Fresh-Nov-14	3.1	1.4	6.4	1.8	3.9	0.6		
Fresh-Nov-15	3.8	1.8	3.4	1.4	5.1	1.6	4.7	1.8
Fresh-Nov-16	5.5	1.9	5.1	1.9	5.3	1.9		
Floor-Nov-14	4.3	3.2	3.2	1.0	4.5	1.6	2.0	1.0
Floor-Dec-4	6.6	1.9	8.1	3.6	5.5	1.3	3.4	1.2
Floor-Jan-8	5.9	1.7	6.7	3.7	11.7	2.9	4.1	1.9
Fresh-Feb-5	4.6	3.5	4.1	1.6	6.1	1.6		
Fresh-Feb-6	8.1	4.7	8.0	4.2	8.1	2.8	7.2	3.3
Fresh-Feb-7	7.4	2.2	7.5	0.7	10.9	3.7		
Floor-Mar-18	11.8	1.9	12.1	2.5	10.1	5.4	5.7	1.5
Fresh-Apr-30	13.1	3.6	12.0	1.3	14.0	4.8		
Fresh-May-1	17.3	4.1	19.5	2.7	16.7	2.6	14.7	3.9
Fresh-May-2	11.5	1.7	14.0	2.2	17.0	6.5		
Fresh-May-28	ND	ND	ND	ND	ND	ND	ND	ND
Floor-Apr-30	13.8	3.2	10.3	1.2	9.4	0.9	5.6	1.4
Floor-May-18	21.1	10.2	15.4	3.2	8.1	3.9	8.1	4.0
Floor-Jun-18	ND	ND	ND	ND			ND	ND
Floor-Jul-23	ND	ND	ND	ND	NA		ND	ND
Floor-Jul-25	ND	ND	ND	ND			ND	ND
Floor-Jul-27	ND	ND	ND	ND			ND	ND

NA – not analyzed; samples from TBA/RAC-S taken during RAC treatment (after June 5) were only analyzed for RAC.

Table S11: 2017-18 feeding trial concentrations (ng/g) of MGA in individual pens in fresh and floor samples and average concentrations of MGA (n=4). For fresh samples the three consecutive fresh sampling days are also pooled (n=12).

Treatment	**MGA-H**					
Sample-date	**P13**	**P14**	**P15**	**P16**	**AVE**	**SD**
Fresh-Nov-14	41.8	28.9	37.1	56.8		
Fresh-Nov-15	34.0	53.5	42.5	45.2	36.0	12.5
Fresh-Nov-16	18.5	22.0	20.9	31.1		
Floor-Nov-14	30.0	29.8	4.8	NS	21.6	14.5
Floor-Dec-4	10.2	3.7	6.7	17.1	9.4	5.8
Floor-Jan-8	6.5	14.0	8.1	26.9	13.9	9.3
Fresh-Feb-5	12.1	10.5	11.9	19.1		
Fresh-Feb-6	7.7	15.0	13.6	23.6	17.3	6.2
Fresh-Feb-7	20.9	25.3	22.1	25.8		
Floor-Mar-18	17.7	10.7	6.6	NS	11.6	5.6
Fresh-Apr-30	17.9	15.6	17.5	15.4		
Fresh-May-1	18.0	18.6	14.2	16.5	17.0	1.7
Fresh-May-2	17.1	15.4	20.5	16.7		
Fresh-May-28	9.0	5.6	7.2	5.2	6.8	1.7
Floor-Apr-30	18.9	21.3	23.6	12.2	19.0	4.9
Floor-May-18	12.3	12.9	15.5	10.9	12.9	1.9
Floor-Jun-18	6.0	3.8	3.9	ND	4.6	1.2
Floor-Jul-25	3.6	2.6	3.6	5.3	3.8	1.1

NS – not sampled. ND – not detected.

Table S12: 2017-18 feeding trial concentrations (ng/g) of RAC in individual pens in fresh and floor samples and average concentrations of RAC (n=4). Prior to the initial sampling during RAC treatment (June 18) no RAC was detected in any samples.

Treatment	TBA/RAC-S					
Sample-date	P37	P38	P39	P40	AVE	SD
Floor-Jun-18	4395.5	3810.2	2750.8	3347.4	3576.0	697.5
Floor-Jul-23	806.3	835.9	775.3	2569.4	1246.7	882.2
Floor-Jul-25	2127.8	1390.0	1521.5	2592.8	1908.0	558.3
Floor-Jul-27	2906.6	1198.3	2049.0	1481.5	1908.9	753.4
Floor-Aug-8	714.1	711.8	1128.9	870.7	856.4	196.3
Floor-Aug-23	895.8	260.1	1205.2	365.5	681.7	446.3
Fresh-June-20	35905.9	37278.8	64272.9	44179.0	45409.2	13086.5
Fresh-June-22	60882.6	68734.0	65109.7	36315.9	57760.6	14652.0
Fresh-June-25	23773.5	48028.4	32740.0	34704.1	34811.5	10013.8
Fresh-June-27	32334.2	43382.3	39159.4	26881.2	35439.3	7298.7
Fresh-June-29	50153.1	25745.5	36886.7	30397.8	35795.8	10606.1
Fresh-July-4	32734.7	20756.1	27855.6	34708.6	29013.7	6213.0
Fresh-July-6	48311.6	25754.5	31386.1	34279.4	34932.9	9595.8
Fresh-July-9	23674.0	33676.7	27039.8	23615.4	27001.5	4729.3
Fresh-July-11	23575.2	27504.5	36303.4	43151.3	32633.6	8802.5
Fresh-July-13	32359.8	30786.0	40751.0	33178.3	34268.8	4434.0

Table S13: 2018-19 feeding trial concentrations (ng/g) of 17α-TBOH in individual treatment pens in fresh and floor samples.

Treatment	**TBA-H**		**TBA-S**		**TBA-H**		**TBA-S**		**TBA/RAC-S**			
Sample-date	**P25**	**P26**	**P27**	**P28**	**P29**	**P30**	**P31**	**P32**	**P37**	**P38**	**P39**	**P40**
Fresh-Dec-26	147.4	58.3	65.6	166.0	70.3	76.8	82.0	111.3	143.2	68.9	139.4	69.5
Fresh-Dec-27	109.7	96.1	238.5	86.8	76.5	134.7	96.6	84.1	143.4	110.9	165.0	NS
Fresh-Dec-28	76.3	98.1	165.2	101.4	95.6	165.9	114.1	133.2	111.8	110.4	88.3	107.7
Floor-Dec-26	91.9	75.6	89.0	68.8	109.0	111.1	NS	NS	55.3	121.1	54.3	NS
Floor-Jan-14	75.1	79.1	70.1	43.2	46.2	43.1	52.3	67.8	68.4	74.1	68.0	44.9
Floor-Feb-18	64.8	62.9	38.4	25.6	40.8	43.8	47.8	39.5	34.6	78.4	44.5	NS
Fresh-Mar-18	153.3	79.2	137.2	69.6	87.5	58.4	93.9	144.2	132.6	140.7	120.3	95.3
Fresh-Mar-19	78.8	65.6	81.0	132.0	71.5	102.2	133.4	273.5	82.4	84.6	109.2	155.4
Fresh-Mar-20	302.1	207.4	93.6	143.5	99.8	144.9	84.2	146.1	277.8	255.1	98.3	104.1
Floor-Mar-19	NS	NS	NS	NS	NS	NS	NS	NS	80.7	54.6	64.3	85.9
Floor-Apr-15	56.0	NS	18.6	18.2	24.2	39.9	30.4	6.9	25.4	28.6	9.4	5.1
Floor-May-13	14.0	3.7	6.5	5.1	2.7	3.0	4.5	6.2	8.0	12.2	7.4	13.4
Fresh-Jun-10	110.4	58.1	114.2	125.9	79.6	87.4	NS	138.6				
Fresh-Jun-11	129.8	93.6	NS	162.9	71.7	156.4	118.7	158.3				
Fresh-Jun-12	187.4	114.8	82.6	151.8	85.4	NS	135.8	140.9		NS		
Floor-Jun-11	35.1	8.9	13.1	11.0	14.8	13.7	2.7	14.1				
Floor-Jul-8	15.0	2.7	4.1	5.3	2.1	6.0	5.1	6.1				

NS – not sampled.

Table S14: 2018-19 feeding trial average concentrations (ng/g) of 17α-TBOH in each of the three TBA treatments (n=4) and pooled together as a single TBA treatment (n=12). For fresh samples, in addition to treatments averaged together, the three consecutive fresh sampling days are also pooled (n=36).

Treatment	**TBA-H**		**TBA-S**		**TBA/RAC-S**		**TBA-H/S and fresh days pooled**	
Sample-date	**AVE**	**SD**	**AVE**	**SD**	**AVE**	**SD**	**AVE**	**SD**
Fresh-Dec-26	88.2	40.2	106.2	44.1	105.2	41.6		
Fresh-Dec-27	104.2	24.5	126.5	74.9	139.8	27.2	111.7	38.6
Fresh-Dec-28	109.0	39.2	128.5	27.7	104.6	11.0		
Floor-Dec-26	96.9	16.6	78.9	14.3	76.9	38.3	86.2	24.5
Floor-Jan-14	60.9	18.9	58.4	12.8	63.9	12.9	61.0	13.9
Floor-Feb-18	53.1	12.5	37.9	9.2	52.5	23.0	47.4	15.3
Fresh-Mar-18	94.6	41.0	111.2	35.6	122.3	19.8		
Fresh-Mar-19	79.5	16.0	155.0	82.7	107.9	33.9	128.9	62.3
Fresh-Mar-20	188.5	87.6	116.9	32.5	183.8	95.9		
Floor-Mar-19	NS	NS	NS	NS	71.4	14.5	71.4	14.5
Floor-Apr-15	40.0	15.9	18.5	9.6	17.1	11.6	23.9	15.1
Floor-May-13	5.9	5.5	5.6	0.9	10.2	3.0	7.2	4.0
Fresh-Jun-10	83.9	21.6	126.2	12.2				
Fresh-Jun-11	112.9	37.7	146.6	24.3			119.3	34.4
Fresh-Jun-12	130.9	43.0	125.1	37.2	NS			
Floor-Jun-11	18.1	11.6	10.2	5.2			14.2	9.3
Floor-Jul-8	6.5	5.9	5.2	0.8			5.8	4.0

NS – not sampled.

Table S15: 2018-19 feeding trial concentrations (ng/g) of 17β-TBOH in individual treatment pens in fresh and floor samples.

Treatment	**TBA-H**		**TBA-S**		**TBA-H**		**TBA-S**		**TBA/RAC-S**			
Sample-date	**P25**	**P26**	**P27**	**P28**	**P29**	**P30**	**P31**	**P32**	**P37**	**P38**	**P39**	**P40**
Fresh-Dec-26	11.0	4.5	4.4	8.8	6.3	3.9	6.1	8.2	8.9	6.1	6.5	6.5
Fresh-Dec-27	6.2	4.9	16.0	4.1	5.5	10.8	6.3	6.5	10.6	6.9	10.0	NS
Fresh-Dec-28	4.2	4.8	9.0	6.7	6.8	16.4	7.1	6.6	5.5	6.3	4.9	5.3
Floor-Dec-26	5.3	5.4	3.7	3.7	4.4	5.3	NS	NS	2.2	3.6	4.4	NS
Floor-Jan-14	3.6	4.6	1.7	2.2	5.4	2.7	4.9	3.0	5.1	3.0	2.2	2.8
Floor-Feb-18	1.9	4.0	3.6	0.9	2.2	2.0	2.1	2.2	3.1	ND	1.0	NS
Fresh-Mar-18	9.1	9.1	8.2	3.5	5.2	3.4	8.0	12.1	5.2	13.5	7.1	7.4
Fresh-Mar-19	7.0	8.1	6.0	8.3	5.0	3.6	8.1	9.4	8.5	5.2	4.8	9.4
Fresh-Mar-20	12.7	3.9	4.8	6.3	4.9	6.9	3.9	7.1	15.5	8.3	6.8	5.6
Floor-Mar-19	NS	NS	NS	NS	NS	NS	NS	NS	4.2	1.4	4.9	4.3
Floor-Apr-15	56.0	NS	18.6	18.2	24.2	39.9	30.4	6.9	1.2	1.0	0.4	0.3
Floor-May-13	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Fresh-Jun-10	6.2	3.3	4.0	8.2	4.7	5.7	NS	6.7				
Fresh-Jun-11	7.4	4.8		7.4	4.5	5.4	5.8	5.6				
Fresh-Jun-12	13.6	6.7	3.9	6.4	3.4	NS	6.2	6.4		NS		
Floor-Jun-11	ND	ND	ND	ND	ND	ND	ND	ND				
Floor-Jul-8	ND	ND	ND	ND	ND	ND	ND	ND				

NS – not sampled. ND – not detected.

Table S16: 2018-19 feeding trial average concentrations (ng/g) of 17β-TBOH in each of the three TBA treatments (n=4) and pooled together as a single TBA treatment (n=12). For fresh samples, in addition to treatments averaged together, the three consecutive fresh sampling days are also pooled (n=36).

Treatment	**TBA-H**		**TBA-S**		**TBA/RAC-S**		**TBA-H/S and fresh days pooled**	
Sample-date	**AVE**	**SD**	**AVE**	**SD**	**AVE**	**SD**	**AVE**	**SD**
Fresh-Dec-26	6.4	3.2	6.8	2.0	7.0	1.3		
Fresh-Dec-27	6.8	2.7	8.2	5.3	9.2	2.0	7.2	3.0
Fresh-Dec-28	8.1	5.7	7.3	1.1	5.5	0.6		
Floor-Dec-26	5.1	0.5	3.7	0.0	3.4	1.1	2.1	0.5
Floor-Jan-14	4.1	1.2	3.0	1.4	3.3	1.3	1.7	0.6
Floor-Feb-18	2.5	1.0	2.2	1.1	2.0	1.4	1.2	0.5
Fresh-Mar-18	6.7	2.9	7.9	3.5	8.3	3.6		
Fresh-Mar-19	5.9	2.0	8.0	1.4	7.0	2.3	7.3	2.9
Fresh-Mar-20	7.1	3.9	5.5	1.4	9.1	4.4		
Floor-Mar-19	ND	ND	ND	ND	3.7	1.5	1.9	0.8
Floor-Apr-15	1.4	0.8	0.5	0.4	0.7	0.5	0.9	0.6
Floor-May-13	ND	ND	ND	ND	ND	ND	ND	ND
Fresh-Jun-10	5.0	1.3	6.3	2.1				
Fresh-Jun-11	5.5	1.3	6.3	0.9			6.0	2.2
Fresh-Jun-12	7.5	4.3	5.5	1.5	NS			
Floor-Jun-11	ND	ND	ND	ND			ND	ND
Floor-Jul-8	ND	ND	ND	ND			ND	ND

NS – not sampled.

Table S17: 2018-19 feeding trial concentrations (ng/g) of MGA in individual pens in fresh and floor samples and average concentrations of MGA (n=4). For fresh samples the three consecutive fresh sampling days are also pooled (n=12).

Treatment	**MGA-H**					
Sample-date	**P13**	**P14**	**P15**	**P16**	**AVE**	**SD**
Fresh-Dec-26	21.5	18.0	19.3	20.1		
Fresh-Dec-27	26.9	17.1	27.8	18.1	25.2	7.5
Fresh-Dec-28	28.2	37.1	29.4	39.0		
Floor-Dec-26	16.0	17.9	15.8	20.3	17.5	2.1
Floor-Jan-14	40.9	34.6	37.9	22.9	34.1	7.9
Floor-Feb-18	35.3	25.4	27.9	21.0	27.4	6.0
Fresh-Mar-18	27.5	17.3	16.2	27.7		
Fresh-Mar-19	29.6	28.6	25.3	20.5	24.8	4.8
Fresh-Mar-20	30.1	22.1	28.6	23.5		
Floor-Mar-19	NS	20.7	34.1	33.9	29.6	7.7
Floor-Apr-15	NS	6.5	10.4	18.1	11.7	5.9
Floor-May-13	8.3	13.2	9.6	6.9	9.5	2.7
Fresh-Jun-10	14.7	11.8	15.2	13.0		
Fresh-Jun-11	13.8	14.0	21.3	14.9	15.0	2.5
Fresh-Jun-12	16.1	17.5	15.3	12.9		
Floor-Jun-11	8.2	7.3	5.8	8.5	7.4	1.2
Floor-Jul-8	3.3	5.8	4.8	4.6	4.6	1.0

NS – not sampled. ND – not detected.

Table S18: 2018-19 feeding trial concentrations (ng/g) of RAC in individual pens in fresh and floor samples and average concentrations of RAC (n=4). Samples were not collected during RAC administration in 2018-19.

Treatment	TBA/RAC-S					
Sample-date	**P37**	**P38**	**P39**	**P40**	**AVE**	**SD**
Fresh-Dec-26	10.0	8.0	11.3	8.5	9.5	1.5
Fresh-Dec-27	9.0	7.8	11.8	NS	9.6	2.1
Fresh-Dec-28	7.6	ND	17.4	ND	12.5	6.9
Floor-Dec-26	78.9	17.0	31.3	NS	42.4	32.4
Floor-Jan-14	17.5	23.5	59.1	26.3	30.9	19.0
Floor-Feb-18	12.1	22.2	24.0	NS	19.4	6.4
Fresh-Mar-18	6.3	ND	9.3	ND	-	-
Fresh-Mar-19	ND	ND	3.9	ND	-	-
Fresh-Mar-20	ND	ND	ND	ND	-	-
Floor-Mar-19	17.5	6.0	9.7	6.1	9.8	5.4
Floor-Apr-15	9.4	9.6	ND	ND	9.5	0.2
Floor-May-13	ND	ND	ND	ND	ND	ND
Fresh-Jun-10 Fresh-Jun-11 Fresh-Jun-12	NS					
Floor-Jun-11	ND	ND	ND	ND	ND	ND
Floor-Jul-8	ND	ND	ND	ND	ND	ND

NS – not sampled. ND – not detected.

Commercial feedlot occurrence of TBA, MGA, and RAC. Concentrations of TBA, 17α-TBOH, 17β-TBOH, TBO, MGA, and RAC were less than limits of quantification in all composites of pen floor materials from both natural feedlots (data not shown). This was expected since these commercial feedlots do not use growth-promoting chemicals. TBA, 17β-TBOH, and TBO were less than limits of detection in all samples from both conventional feedlots (Conv-1 and Conv-2). MGA was detected in 45% (22/49) of Conv-1 samples and 41% (15/37) of Conv-2 samples, with average concentrations of 4.7 ng/g (range: 0.50 – 11.3 ng/g) and 8.3 ng/g (range: 1.8 – 15.0 ng/g) respectively (Fig. 3). 17α-TBOH and RAC were detected in >80% and 100% of all Conv-1 and Conv-2 samples, respectively (Fig. 3). Concentrations of 17α-TBOH were similar for the two conventional feedlots with mean concentrations of 10.8 and 8.2 ng/g and ranges of 1.2 to 43.9 and 1.1 to 43.3 ng/g respectively. Concentrations of RAC measured as high as 19,900 and 15,700 ng/g in Conv-1 and Conv-2, respectively, with mean concentrations between 3000 and 4500 ng/g.

Results of samples taken from commercial feedlots were comparable to concentrations measured in the feeding trials, which provides confidence in the realistic feeding trial conditions and sampling protocols used in the current work. However, differences in the data sets were observed. Concentrations of 17α-TBOH, MGA, and RAC measured at the two conventional commercial feedlots were more variable, compared to the feeding trial data (Fig. 3 and S4). The greater variability, especially for 17α-TBOH and RAC, could result from changing TBA-implants and RAC treatment schedules over the 1.5-year sampling period. The sampling program with the commercial feedlots was not designed to match with those used during the feeding trials and therefore differences between the data sets were expected. Regardless, summary average concentrations compared across all floor samples in each feeding trial and conventional feedlot samples are in general agreement and fall within ≈4-fold of each other, ranging from ≈10-40 ng/g for 17α-TBOH, ≈6-18 ng/g for MGA, and 2000-4000 ng/g for RAC (Fig. S4). Levels of 17β-TBOH were less than limits of detection in all commercial feedlot samples. This is not surprising given that 17α-TBOH was observed, on average, at concentrations ≈4-fold less than in samples of floor materials during the feeding trial. Assuming the same 4-fold reduction in concentrations of 17β-TBOH observed during the feeding trials, expected concentrations in commercial feedlots would be <1 ng/g and near the limit of quantification for 17β-TBOH (Table S3).

Table S19: Pen floor concentrations at Commercial Feedlot 1 at each sampling date (month-yy). A total of 15 pens were sampled over the entire monitoring campaign and a subset of those were sampled at each date, indicated by # of samples at the bottom of each column.

	17α-TBOH Concentration (ng/g)						RAC Concentration (ng/g)						MGA Concentration (ng/g)					
Sample	**Oct-16**	**Apr-17**	**Jun-17**	**Sep-17**	**Dec-17**	**May-18**	**Oct-16**	**Apr-17**	**17-Jun**	**Sep-17**	**Dec-17**	**May-18**	**Oct-16**	**Apr-17**	**17-Jun**	**Sep-17**	**Dec-17**	**May-18**
1	35.6	5.5	3.2				14711	7236	53.9				9.2	4.4				
2			2.1					3623	175.2					2.9				
3	27.2	1.2	5.4	4.3			5161	10058	344.4	256.3			4.7	1.5				
4	14.9		4.3	4.2			18803		557.0	159.5								
5		3.5	1.7					9633	286.2					6.8				
6	15.2	3.4		13.0	2.6	5.5	12397	8127		305.1	4197	1110		6.5			4.6	
7					8.7						7243	548.6					4.1	
8				31.0	14.3					21.4	10059	398.4					6.1	
9	43.9		9.0				1483		121.8									
10	33.5		13.2	4.1			15180		212.0	138.5			11.3					
11	9.2		1.7		6.9		10972		280.5		4012				2.1		4.4	
12	14.7	3.0	2.6		4.3		19858	4761	287.7		4358	430.2		3.2	4.5		4.9	
13	24.1	2.2	3.5	3.6	4.0		4087.8	1170	543.2	76.0	12579			0.8			3.5	
14		3.9			2.8			3426			10603	1484		0.5			3.6	
15				20.0	15.2	34.0		6282		558.2	3789	563.8		5.2			7.7	
AVE	24.3	3.2	4.7	11.5	7.4	19.7	1141	6036	286	216	7106	756	8.4	3.5	3.3	--	4.9	--
SD	11.7	1.3	3.7	10.6	5.0	20.1	6553	3019	164	179	3536	440	3.4	2.3	1.7	--	1.4	--
# of samples	9	9	10	7	8	6	9	9	10	7	8	6	9	9	10	7	8	6
% detect	100	78	100	100	100	33	100	100	100	100	100	100	33	100	20	0	100	0

Table S20: Pen floor concentrations at Commercial Feedlot 2 at each sampling date (month-yy). A total of 14 pens were sampled over the entire monitoring campaign and a subset of those were sampled at each date, indicated by # of samples at the bottom of each column.

	17a-TBOH Concentration (ng/g)					RAC Concentration (ng/g)					MGA Concentration (ng/g)				
Sample	**Jan-17**	**May-17**	**Aug-17**	**Jan-18**	**Jun-18**	**Jan-17**	**May-17**	**Aug-17**	**Jan-18**	**Jun-18**	**Jan-17**	**May-17**	**Aug-17**	**Jan-18**	**Jun-18**
1	6.4			10.0		3777			1343		12.2			10.4	
2	8.4	5.2	2.5	5.4	8.5	10535	516.4	1814	9859	220.6	12.7				
3	5.0	7.0		12.9	2.9	5287	2374		1395	244.3	8.5			2.9	
4	11.2	3.1	43.3	28.8	1.9	6724	909.3	307.1	495.4	310.6	15.0		6.3	3.4	
5		2.4	2.2		2.0	5819	536.9	418.4		120.8			3.2		
6		3.9	2.9				1062	232.3	640.2					8.1	
7		1.1	1.4	30.1			5552	780.8	14780					8.3	
8	9.9	1.1				1878	7380		7010					7.9	
9		2.5				2339	988.2					1.8			
10									15650						
11			15.8					752.3							
12							6.2								
13		3.7				1050	2147				11.4				
14	5.3					811.3					11.9				
AVE	7.7	3.4	11.4	17.4	3.8	4247	2147	718	6397	224	11.9	--	4.8	6.8	--
SD	2.6	1.9	16.6	11.3	3.2	3176	2425	583	6401	79	2.1	--	2.2	3.0	--
# of samples	9	10	6	8	4	9	10	6	8	4	9	10	6	8	4
% detect	67	90	100	63	100	100	100	100	100	100	67	10	33	75	0

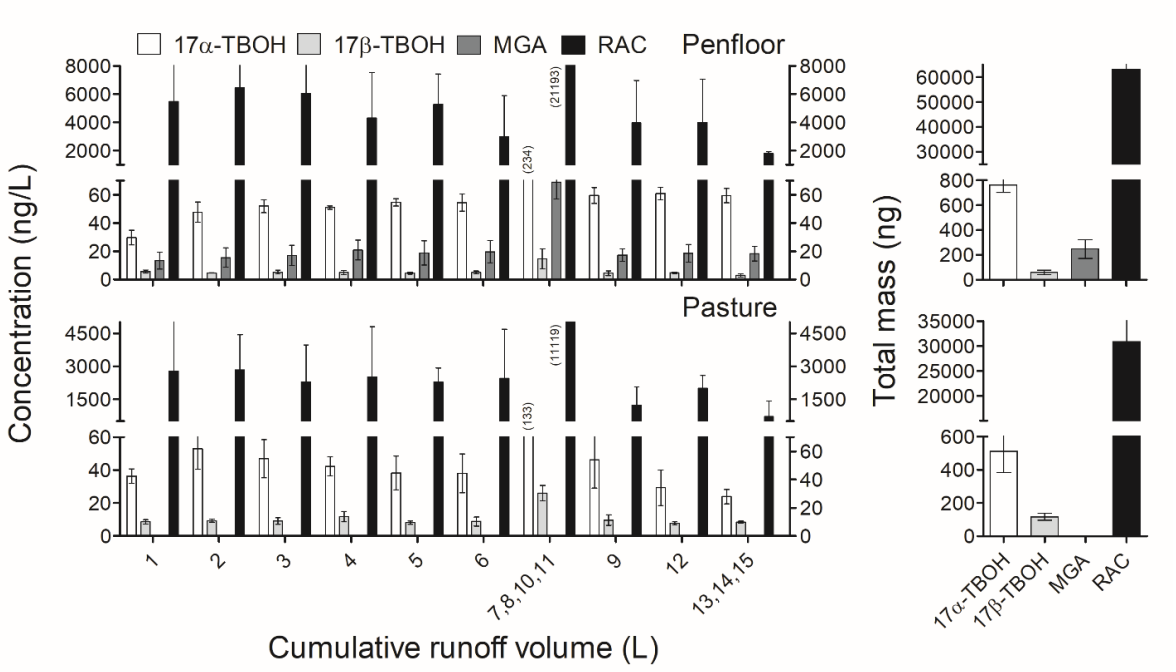

Figure S3: Concentrations of trenbolone acetate (TBA) metabolites 17α-trenbolone (17α-TBOH) and 17β-TBOH, melengesterol acetate (MGA), and ractopamine (RAC) in simulated rainfall runoff in sequential 1L runoff samples and total runoff mass in 15L from feedlot pens (top panel) and pasture (bottom panel). TBA and trendione were not detected in any samples. Litre # 7, 8, 10, 11 and 13, 14, 15 were taken as composite sub-samples. MGA was not detected in pasture runoff. Bars represent mean ± std. dev. of replicate runoff experiments (n = 3) conducted for each treatment. Numbers in brackets are concentrations where bars are cut-off by the y-axis range.

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