ManureTracker: On the Trail of Hormones, Antimicrobials and Antimicrobial Resistance Genes

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ManureTracker: Looking for hormones, antimicrobials (antibiotics) in manure
- Needle in a haystack?
- Very low concs.
- Short half-lives of parent compounds
- Not much hard data for Alberta
## Hormones in beef cattle production

<table>
<thead>
<tr>
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- **TBA (ear implant)**
- **MGA (added in feed)**
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TBA

MGA
Metabolites of trenbolone acetate (TBA)

- TBA first hydrolyzed to 17β-trenbolone
- 17β-trenbolone oxidized to trendione
- Trendione reduced to 17α-trenbolone
Metabolites and photoproducts of TBA

- Other TBA metabolites can also regenerate under darkness
- Can persist longer in water because of regeneration
- Metabolites retain biological activity
- Photoproducts retain some biological activity
Melengestrol acetate (MGA)

- Administered in feed
- Estrus suppression in heifers
- Mostly excreted in parent form with very low metabolite concs.
- Considered mobile in agricultural soils but has affinity for soil organic matter
- Can persist in soil for up to 6 months
Fate of hormones in feedlots: main transport pathways

Manure

Water
Another pathway: transport in dust

Airborne particulate matter collected near beef cattle feedyards induces androgenic and estrogenic activity in vitro

Kimberly J. Wooten, Brett R. Blackwell, Andrew D. McEachran, Gregory D. Mayer, Philip N. Smith

The Institute of Environmental and Human Health, Department of Environmental Toxicology, Texas Tech University, Lubbock, TX, USA
Perception vs. reality

Worried about hormones in cattle?

You don’t need to be

Hormone implants are small, slow release pellets placed under the skin in an animal’s ear to enhance production of natural hormones. Using hormone implants directs growth towards muscle and away from fat, which boosts growth rate and means less feed is needed for the animal to gain weight.¹

All plants and animals have hormones naturally in their systems. Your body produces hormones no matter what you eat.²

The result is fewer resources are used to produce beef, with smaller impacts on the environment and your grocery bill.

Since 1969

Alberta Beef Producers
<table>
<thead>
<tr>
<th>Food/supplement</th>
<th>Estrogen*</th>
<th>Servings of beef (75 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>75 g beef without</td>
<td>1.1 ng</td>
<td>0.65</td>
</tr>
<tr>
<td>75 g beef with</td>
<td>1.9 ng</td>
<td>1</td>
</tr>
<tr>
<td>75 g chicken</td>
<td>2.1 ng</td>
<td>1.1</td>
</tr>
<tr>
<td>75 g pork</td>
<td>2.5 ng</td>
<td>1.3</td>
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<tr>
<td>355 ml beer</td>
<td>15 ng</td>
<td>7.9</td>
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<tr>
<td>355 ml milk</td>
<td>51 ng</td>
<td>26.8</td>
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<tr>
<td>75 g cabbage</td>
<td>2025 ng</td>
<td>1,065.8</td>
</tr>
<tr>
<td>1 tbsp soybean oil</td>
<td>28,370 ng</td>
<td>14,931.6</td>
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Non-target organisms

- Increasing interest in environmental transport and fate
- Effects on non-target organisms
- Fish, amphibians, birds?
- Endocrine disruption?
  - Reproductive effects
  - Behavioural effects
Hormone trial: LRC feedlot (Nov. 2015-Jul. 2016)

- **Heifers**
  - +TBA
  - +MGA
  - Control

- **Steers**
  - +TBA
  - Control

5 treatments x 4 reps = 20 pens
Sampling protocols: LRC hormone trial

- Pen floor samples: manure/bedding mix
  - Literature indicates 17α-trenbolone peaks 2 wk post-implant
  - Trendione and 17β-trenbolone peak 2-4 wk post-implant
  - MGA continuously fed
- Pen runoff study – generate rainfall on pen floors and collect runoff.
- Composting study – clean pens at end of trial and sample compost windrows over 90 d
- Sample groundwater wells installed at LRC feedlot
- Sample catch basin water (if available)
- Screen select water samples for endocrine-disrupting activity: recombinant yeast assays
Research conducted from 2010-15

All of this work has been published in peer-reviewed journals including three papers in a Special Section of the Journal of Environmental Quality (JEQ): “Antibiotics in Agroecosystems: State of the Science”

Focus on chlortetracycline (CTC), sulfamethazine (SMZ) and tylosin (TYL)

What results did we find.....?
Dissipation of Three Veterinary Antimicrobials in Beef Cattle
Feedlot Manure Stockpiled over Winter

Srinivas Sura,* Dani Degenhardt, Allan J. Cessna, Francis J. Larney, Andrew F. Olson, and Tim A. McAllister

Dissipation of veterinary antimicrobials is known to occur during aerated windrow composting of beef cattle manure. However, it is unclear if a similar dissipation occurs during stockpiling. Chlortetracycline, tylosin, and sulfamethazine are three of the most commonly used veterinary antimicrobials in beef cattle production in western Canada. Their dissipation in stockpiled manure was investigated over 140 d during winter in Alberta, Canada. Beef cattle housed in pens were administered 44 mg of chlortetracycline kg⁻¹ feed (dry weight), 44 mg of chlortetracycline + 44 mg sulfamethazine kg⁻¹ feed, 11 mg of tylosin kg⁻¹ feed, or feed without antimicrobials (control). Manure samples were extracted using pressurized liquid extraction, and the extracts were analyzed for chlortetracycline, sulfamethazine, and tylosin by LC-MS-MS. Dissipation of all three antimicrobials in the manure was explained by exponential decay kinetics. Times for 50% dissipation (DT₅₀) were 1.8 ± 0.1 d for chlortetracycline,

Veterinary antimicrobials are used in animal production, therapeutically to treat disease and subtherapeutically to prevent disease and promote growth. During the last decade, the use of veterinary antimicrobials has received increased attention because of growing bacterial resistance to antimicrobials used in human medicine (Chee-Sanford et al., 2001; Kim et al., 2010b; Schwartz et al., 2003) and the impact that this may have on the treatment of infectious diseases (Goss et al., 2013). Most antimicrobials are partially metabolized by the animal or its microbial population, with the residues being excreted in feces or urine either as the parent compound or its metabolites. There is very limited information about the effects of antimicrobials or their metabolites on the environment and human health (Becuval et al., 2003). Contamination of

After 77 d in stockpiles, <1% of initial CTC, <2% SMZ, and 20% TYL remained.
Antimicrobial concs. higher in bedding than non-bedding areas. Order of transport in runoff was SMZ > TYL > CTC
Runoff mass losses (% of applied) of CTC were significantly reduced by soil incorporation: from 6.5% to 1.7%. Mass losses of SMZ and TYL were not affected by application method.
Fate of Antimicrobials

Dissipation of Antimicrobials in a Seasonally Frozen Soil after Beef Cattle Manure Application

Inoka D. Amarakoon, Srinivas Sura, Francis Zvomuya, Allan J. Cessna, Francis J. Larney, and Tim A. McAllister

Abstract

Land application of manure containing antimicrobials results in the dispersion of the antimicrobials in agro-ecosystems. Dissipation of excreted antimicrobials in seasonally frozen agricultural soils has not been fully characterized under field conditions. This study investigated the field dissipation kinetics of chlorotetracycline, sulfamethazine, and tyllosin over a 10-mo period after fall application of manure from cattle (*Bos taurus*) administered 44 mg chlorotetracycline (chlorotetracycline treatment [CTC]), 44 mg each of chlorotetracycline and sulfamethazine (CTCSMZ), or 11 mg tyllosin per kg feed daily. Antimicrobial concentrations in manured soil reflected the same relative concentrations in manure: chlorotetracycline > sulfamethazine > tyllosin. The first-order dissipation half-life (DT₀) for chlorotetracycline from the CTCSMZ treatment was 37 d during

CTC had greater persistence in frozen soil - detectable up to 10 mo after application. Concs. of SMZ and TYL in manured soil were low.
85-99% of initial antimicrobial had dissipated after 30 d of composting. Dissipation rate depended on whether antimicrobial was fed or fortified.
Runoff from composting windrows contained antimicrobials. Order of transport in runoff was TYL > SMZ > CTC reflecting order of decreasing solubility.
Dissipation of Antimicrobial Resistance Determinants in Composted and Stockpiled Beef Cattle Manure

Shanwei Xu, Srinivas Sura, Rahat Zaheer, George Wang, Alanna Smith, Shaun Cook, Andrew F. Olson, Allan J. Cessna, Francis J. Larney, and Tim A. McAllister*

Abstract

Windrow composting or stockpiling reduces the viability of pathogens and antimicrobial residues in manure. However, the impact of these manure management practices on the persistence of genes coding for antimicrobial resistance is less well known. In this study, manure from cattle administered 44 mg of chlortetracycline kg⁻¹ feed (dry wt. basis) (CTC), 44 mg of CTC and 44 mg of sulfamethazine kg⁻¹ feed (CTCSMZ), 11 mg of tylosin kg⁻¹ feed (TYL), and no antimicrobials (control) were composted or stockpiled over 102 d. Temperature remained ≥55°C for 35 d in compost and 2 d in stockpiles. Quantitative PCR was used to measure levels of 165 rRNA genes and tetracycline [tet(B), (C-L), (M-H)], erythromycin [erm(A), (B, E, X)], and

In North America, antimicrobials are routinely administered to livestock at therapeutic levels for disease treatment and at subtherapeutic levels to improve feed efficiency and promote growth. It has been estimated that 1.6 million kg of antimicrobials are used every year in the Canadian livestock industry (Public Health Agency of Canada, 2008). In the United States, annual usage for livestock production is 10 times higher than in Canada, with 70% being used at subtherapeutic levels (Ji et al., 2012). However, 30 to 90% of administered antimicrobials can be excreted either as the original compounds and/or as active metabolites in feces and urine (Sarmah et al., 2006). Once in manure, these antimicrobials may select for antimi-
New Veterinary Feed Directive (VFD): US

- **Background:**
  - Judicious use of therapeutic antimicrobials is an integral part of good veterinary practice. In 2013 US FDA moved to maximize therapeutic efficacy and minimize selection of resistant microorganisms.
  - Transition antimicrobial drugs with importance in human medicine, that are used in the feed or drinking water of food-producing animals, to veterinary oversight and eliminate the use of these products in animals for production (e.g., growth promotion) purposes.

- **January 1, 2017**
  - Many medicated livestock feeds, minerals and feed additives no longer available over-the-counter
  - Drug administration under the control of a veterinarian via a Veterinary Feed Directive (VFD)
  - New labelling: Must cease all growth promotion claims
  - Only for therapeutic use, including prevention

- **Will Canada follow suit?**
  - Decision coming summer 2017?
Animal Drugs Expected to be VFD Drugs

Apramycin (not marketed)  Oxytetracycline
Avilamycin (new VFD)      Penicillin (will disappear)
Chlortetracycline         Streptomycin
Erythromycin (not marketed) Sulfadimethoxine:Ormetoprim
Florfenicol (already VFD)  Tilmicosin (already VFD)
Hygromycin B              Tylosin
Lincomycin                Sulfamerazine
Neomycin                  Sulfamethazine
Oleandomycin (not marketed) Sulfaquinoxaline

List of affected products:
http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/JudiciousUseofAntimicrobials/ucm390429.htm
Acknowledgements

Allan Cessna, Inoka Amarakoon, Francis Zvomuya, Andrew Olson, Tony Curtis, Kyle Shade, Wendi Smart, Gabriel Ribeiro, Krysty Munns