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

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Manure Management Update
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ure
Man tracker
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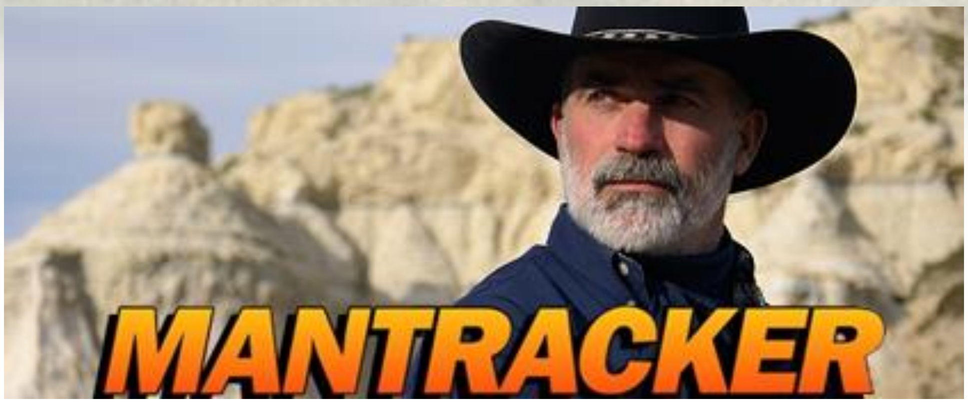

ManureTracker: Looking for hormones, antimicrobials (antibiotics) in manure

- **Needle in a haystack?**
- **Very low concs.**
- **Short half-lives of parent compounds**
- **Not much hard data for Alberta**

Hormones in beef cattle production

Natural			
Estradiol			
Testosterone			
Progesterone			

Hormones in beef cattle production

Natural	**First use**		
Estradiol	1956		
Testosterone	1950		
Progesterone	1954		

Hormones in beef cattle production

Natural	First use	Synthetic	
Estradiol	1956	Zeranol	
Testosterone	1950	Trenbolone acetate (TBA)	
Progesterone	1954	Melengestrol acetate (MGA)	

Hormones in beef cattle production

Natural	First use	Synthetic	First use
Estradiol	1956	Zeranol	1973
Testosterone	1950	Trenbolone acetate (TBA)	1975
Progesterone	1954	Melengestrol acetate (MGA)	1966

TBA (ear implant)

MGA (added in feed)

Hormones in beef cattle production

Natural	First use	Synthetic	First use
Estradiol	1956	Zeranol	1973
Testosterone	1950	Trenbolone acetate (TBA)	1975
Progesterone	1954	Melengestrol acetate (MGA)	1966

TBA

MGA

Hormones in beef cattle production

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Testosterone	1950	Trenbolone acetate (TBA)	1975
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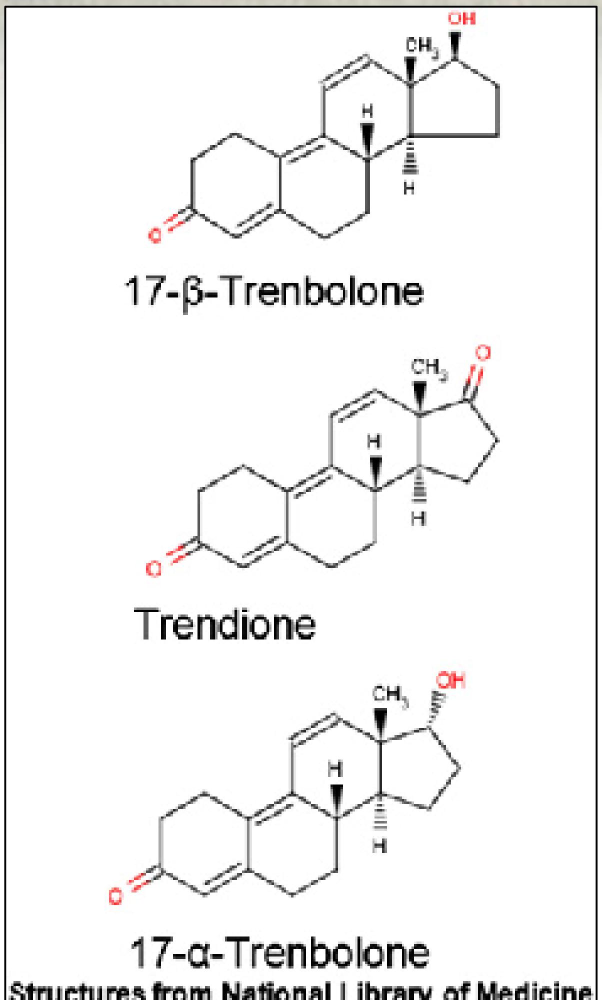
TBA

MGA

Metabolites of trenbolone acetate (TBA)



TBA first hydrolyzed to 17β-trenbolone

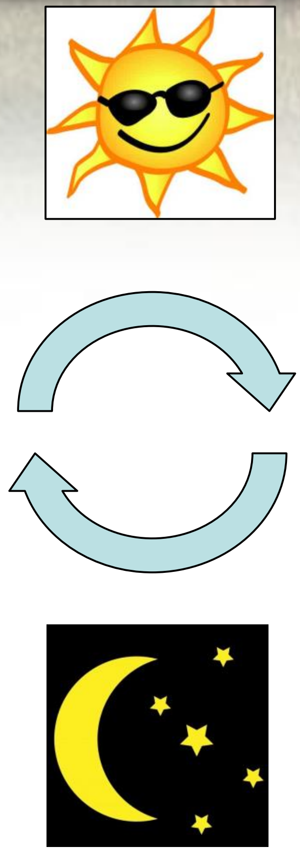
17β-trenbolone oxidized to trendione

Trendione reduced to 17α-trenbolone

Metabolites and photoproducts of TBA

Metabolite | Photoproducts

17α-trenbolone



12-hydroxy-17α-trenbolone
5-hydroxy-17α-trenbolone

- 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

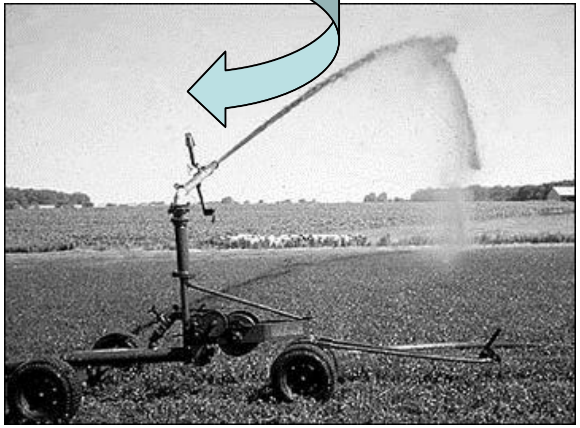
Melengestrol acetate (MGA)

- 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

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journal homepage: www.elsevier.com/locate/agee

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

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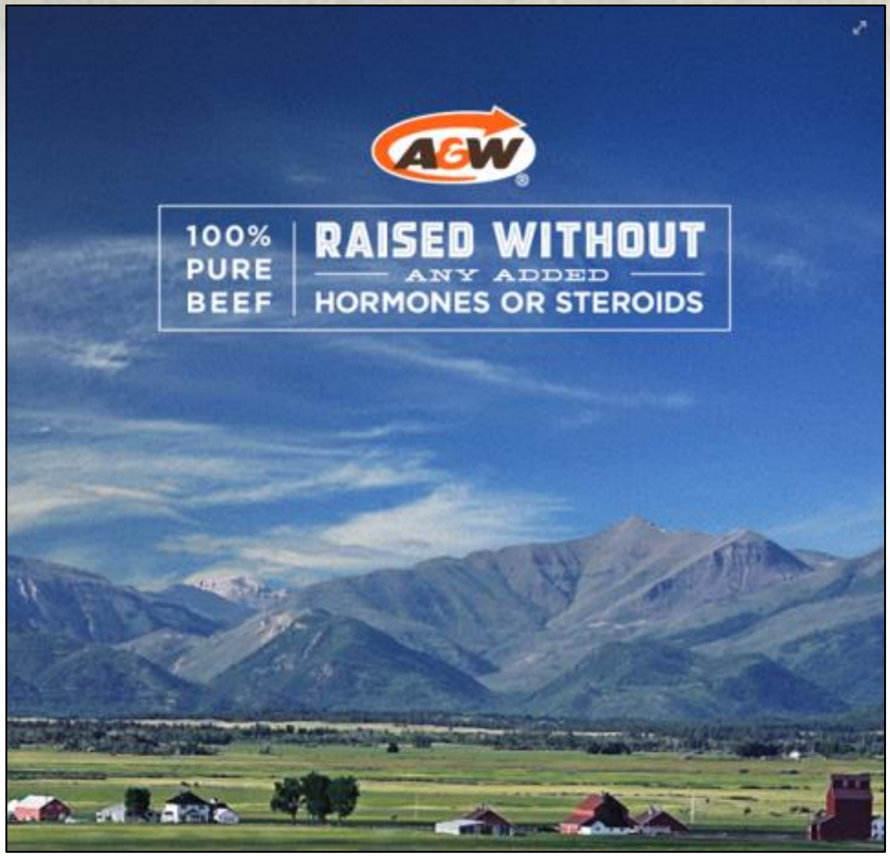
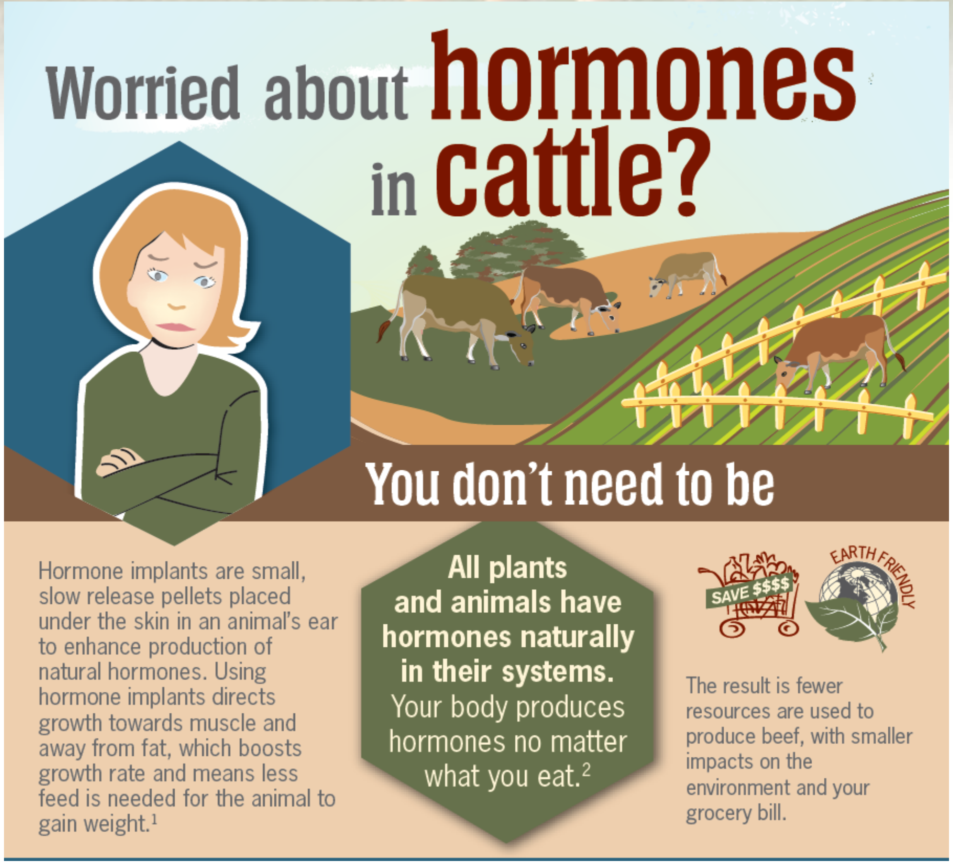
Perception *vs.* reality





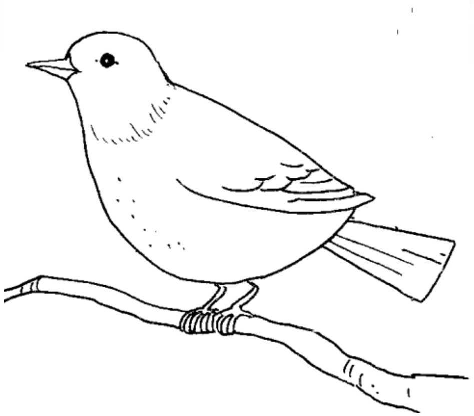
SINCE 1969
Alberta Beef Producers

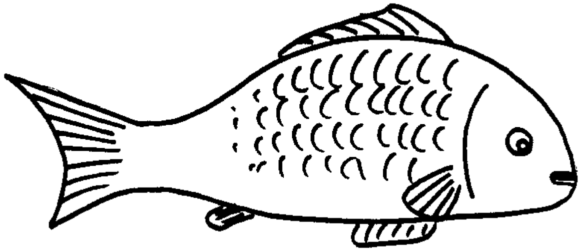
Estrogen concentrations in food

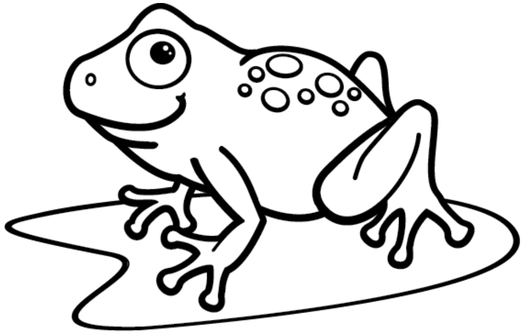
Food/supplement	Estrogen*	Servings of beef~ (75 g)
75 g beef **without** hormone implants	1.1 ng	0.65
75 g beef **with** hormone implants	1.9 ng	1
75 g chicken	2.1 ng	1.1
75 g pork	2.5 ng	1.3
355 ml beer	15 ng	7.9
355 ml milk	51 ng	26.8
75 g cabbage	2025 ng	1,065.8
1 tbsp soybean oil	28,370 ng	14,931.6

Non-target organisms

- Increasing interest in environmental transport and fate
- Effects on non-target organisms
- Fish, amphibians, birds?
- Endocrine disruption?
  - Reproductive effects
  - Behavioural effects

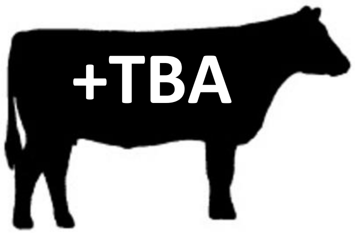


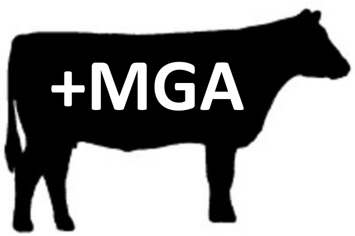


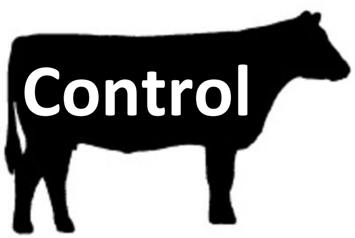


Hormone trial: LRC feedlot (Nov. 2015-Jul. 2016)

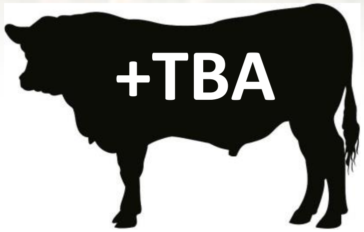
Heifers

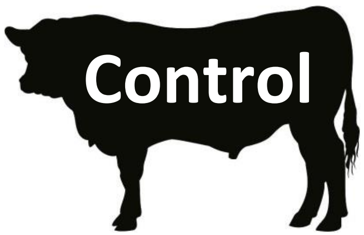






Steers





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

Fate of antimicrobials in beef cattle manure

- 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…..?

Fate of Antimicrobials

Published May 9, 2014

Journal of Environmental Quality — TECHNICAL REPORTS

WASTE MANAGEMENT

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

Veterinary antimicrobials are used in animal production, therapeutically to treat disease and sub-therapeutically 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 environ-

After 77 d in stockpiles, <1% of initial CTC, <2% SMZ, and 20% TYL remained.

Fate of Antimicrobials

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Transport of three veterinary antimicrobials from feedlot pens via simulated rainfall runoff

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HIGHLIGHTS

- Antimicrobial concentrations were higher in bedding versus non-bedding material.
- Bulk density rather than water content of pen floor material determined runoff volume.
- More runoff was generated from the non-bedding area compared to the bedding area.
- Physical properties of antimicrobials determined relative mass transported in runoff.
- Order of transport in runoff: sulfamethazine > tylosin > chlortetracycline

Antimicrobial concs. higher in bedding than non-bedding areas. Order of transport in runoff was SMZ > TYL > CTC

Fate of Antimicrobials

Journal of Environmental Quality TECHNICAL REPORTS

ORGANIC COMPOUNDS IN THE ENVIRONMENT

Runoff Losses of Excreted Chlortetracycline, Sulfamethazine, and Tylosin from Surface-Applied and Soil-Incorporated Beef Cattle Feedlot Manure

Inoka D. Amarakoon, Francis Zvomuya,* Allan J. Cessna, Dani Degenhardt, Francis J. Larney, and Tim A. McAllister

Veterinary antimicrobials in land-applied manure can move to surface waters via rain or snowmelt runoff, thus increasing their dispersion in agro-environments. This study quantified losses of excreted chlortetracycline, sulfamethazine, and tylosin in simulated rain runoff from surface-applied and soil-incorporated beef cattle (*Bos taurus* L.) feedlot manure (60 Mg ha^{-1}, wet wt.). Antimicrobial concentrations in runoff generally reflected the corresponding concentrations in the manure. Soil incorporation of manure reduced the concentrations of chlortetracycline (from 75 to 12 μg L^{-1} for a 1:1 mixture of chlortetracycline and sulfamethazine and from 43 to 17 μg L^{-1} for chlortetracycline alone) and sulfamethazine (from 3.9 to 2.6 μg L^{-1}) in runoff compared with surface application. However, there was no significant effect of manure application method on tylosin concentration (range, 0.02–0.06 μg L^{-1}) in runoff. Mass losses, as a percent of the amount applied, for chlortetracycline

Veterinary antimicrobials (VAs) are widely used by the livestock industry in North America at therapeutic levels for treatment of infections and at subtherapeutic levels to prevent disease and improve feed efficiency (Sarmah et al., 2006). Although publicly available data on VA use in livestock are scarce, recent estimates indicate that between ~11 and 16 million kg are administered annually in the United States (Kim et al., 2011; Sarmah et al., 2006).

To be approved for use in livestock, VAs must not accumulate but should rapidly dissipate from edible tissues (Thiele-Bruhn, 2003). Excretion rates in feces and urine can be up to 95% or more and vary depending on the VA, the dose administered, and the species and age of the animal (Arikan et al., 2007; Chee-Sanford et al., 2009; Sarmah et al., 2006). Excretion rates for three

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

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ORGANIC COMPOUNDS IN THE ENVIRONMENT

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 chlortetracycline, sulfamethazine, and tylosin over a 10-mo period after fall application of manure from cattle (*Bos taurus*) administered 44 mg chlortetracycline (chlortetracycline treatment [CTC]), 44 mg each of chlortetracycline and sulfamethazine (CTCSMZ), or 11 mg tylosin per kg feed daily. Antimicrobial concentrations in manured soil reflected the same relative concentrations in manure: chlortetracycline > sulfamethazine > tylosin. The first-order dissipation half-life (DT_{50}) for chlortetracycline from the CTCSMZ treatment was 77 d during

The livestock industry in North America uses antimicrobials for the treatment and prevention of disease as well as for growth promotion. The estimated annual use in the United Sates is 11 to 16 million kg (Kim et al., 2011; Sarmah et al., 2006). Approximately 75% of antimicrobials administered to animals are excreted (Chee-Sanford et al., 2009), with higher or lower values reported depending on the nature of the antibiotic, the dose, the method of administration, the breed, and the age of the animal (Kim et al., 2011).

Livestock manure has been traditionally used in agriculture as a fertilizer and a soil conditioner. When livestock manure that contains excreted antimicrobials is land applied, antimicrobials are introduced into agricultural soils (Boxall et al., 2003; Sarmah et al., 2006), which may then be transported in surface runoff

CTC had greater persistence in frozen soil - detectable up to 10 mo after application. Concs. of SMZ and TYL in manured soil were low.

Fate of Antimicrobials

JEQ Special Section (2016): Antibiotics in Agroecosystems: State of the Science

Published March 1, 2016

Journal of Environmental Quality SPECIAL SECTION

ANTIBIOTICS IN AGROECOSYSTEMS: STATE OF THE SCIENCE

Dissipation of Antimicrobials in Feedlot Manure Compost after Oral Administration versus Fortification after Excretion

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

Abstract

Fortification of manure with antimicrobials is one approach to studying their dissipation. However, fortified antimicrobials may not accurately model dissipation that occurs after antimicrobials have been administered to livestock in feed and excreted in manure. This study examined the dissipation of antimicrobials excreted in manure versus those added directly to manure (fortified). Steers were fed a diet containing (kg^{-1} feed) (i) 44 mg chlortetracycline, (ii) 44 mg each of chlortetracycline and sulfamethazine, (iii) 11 mg tylosin, and (iv) no antimicrobials (control). Fortified antimicrobial treatments were prepared by adding antimicrobials to control manure. Manure was composted for 30 d, sampled every 2 to 3 d, and analyzed for antimicrobials and compost properties. Antimicrobial dissipation followed first-order kinetics. The dissipation rate constant was

LIVESTOCK OPERATIONS in North America routinely use antimicrobials to treat infections, control disease, and promote growth. Antimicrobial use in livestock in the United States is estimated to be 11 to 16 million kg yr^{-1} (Kim et al., 2011; Sarmah et al., 2006). The amount of antimicrobials excreted after ingestion varies with feeding conditions and the type of antimicrobial and has been reported to be 65 to 75% of chlortetracycline, 90% of sulfamethazine, and 50 to 100% of tylosin (Kim et al., 2011). Manure containing excreted antimicrobials is often applied to agricultural land, resulting in the transfer of antimicrobial residues to soil, surface water, and ground water (Boxall et al., 2004; Kemper et al., 2008; Sarmah et al., 2006). Antimicrobial dispersal in the environment increases the risk of selection for antimicrobial resistant bacteria (Chee-

85-99% of initial antimicrobial had dissipated after 30 d of composting.
Dissipation rate depended on whether antimicrobial was fed or fortified.

Fate of Antimicrobials

JEQ Special Section (2016): Antibiotics in Agroecosystems: State of the Science

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ANTIBIOTICS IN AGROECOSYSTEMS: STATE OF THE SCIENCE

Transport of Three Antimicrobials in Runoff from Windrows of Composting Beef Cattle Manure

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

Abstract

Rain runoff from windrowed or stockpiled manure may contain antimicrobials with the potential to contaminate surface and ground water. To quantify the concentration of antimicrobials transported in runoff from windrowed manure, antimicrobials were administered continuously in feed to beef cattle (*Bos taurus*) as follows: 44 mg of chlortetracycline kg^{-1} feed (dry weight), a 1:1 mixture of 44 mg of chlortetracycline and 44 mg sulfamethazine kg^{-1} feed, and 11 mg of tylosin kg^{-1} feed. Cattle in a fourth treatment group received no antimicrobials (control). Manure from the cattle was used to construct two windrows per treatment. On Days 2 and 21 of composting, a portable Guelph Rainfall Simulator II was used to apply deionized water at an intensity of 127 mm h^{-1} to each windrow, and the runoff was collected. Manure samples were collected before rain simulations

ANTIMICROBIALS are routinely administered to cattle therapeutically to treat disease and subtherapeutically to prevent disease and promote growth. Generally, antimicrobials administered to cattle are partially metabolized within the animal, and the remaining portion and metabolites are excreted in feces or urine. Consequently, antimicrobials have been detected in cattle manure (Cessna et al., 2011; Sura et al., 2014, 2015; Zhao et al., 2010), commercial feedlot soils (Aust et al., 2008; Netthisinghe et al., 2013), and feedlot catch basin water (Zhang et al., 2013). The excreted parent compounds and metabolites that retain biological activity may pose a risk to nontarget organisms in the environment once these wastes are applied to agricultural land. For example, antimicrobials have also been detected in runoff from manure-amended soils

Runoff from composting windrows contained antimicrobials. Order of transport in runoff was TYL > SMZ > CTC reflecting order of decreasing solubility

Fate of Antimicrobial Resistance Genes (ARG)

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ANTIBIOTICS IN AGROECOSYSTEMS: STATE OF THE SCIENCE

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 16S rRNA genes and tetracycline [*tet*(B), (C), (L), (M), (W)] erythromycin [*erm*(A), (B), (F), (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 antimicro-

Dissipation of ARG higher in compost vs. stockpiles. ARGs declined from 0.5 to 3 log units in both composted and stockpiled manure

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?

FDA U.S. FOOD & DRUG ADMINISTRATION

Animal Drugs Expected to be VFD Drugs

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

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

FDA U.S. FOOD & DRUG ADMINISTRATION

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