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Occurrence and Persistence of Antibiotics Administered to Cattle in a Newly Established Feedlot

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Occurrence and Persistence of Antibiotics Administered to Cattle in a Newly Established Feedlot

By

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Occurrence and Persistence of Antibiotics Administered to Cattle in a Newly Established Feedlot, 2018 – 2020

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The common practice of using therapeutic antibiotics in livestock farming is a worldwide phenomenon. Over the last decade, there has been a growing concern of antibiotics entering the environment via animal manure. Similar studies have focused on the occurrence and biological effects of antibiotics in land-applied animal feedlots; however, limited research has been conducted on the occurrence and persistence of antibiotics in animal feedlots. A study was conducted to investigate the occurrence and persistence of four injected antibiotics (ceftiofur enrofloxacin, florfenicol, and tulathromycin) and two continuously fed antibiotics (monensin and tylosin) in feedlot sediment, runoff, and sediment runoff. For antibiotics that were injected, concentrations were >20ng/g in feedlot sediment and >0.65 µg/L in runoff; there was no statistical significance found (p-value > 0.05). Monensin and tylosin were detected at the highest concentrations in both feedlot sediment and runoff, at 298 ng/g and 8.8 µg/L and at 129 ng/g and 2.68 µg/L, respectively. Statistical significance was detected with antibiotics continuously fed (p-value <0.01). Mean concentrations in feedlot sediment for monensin and tylosin were 5 and 33 times higher in pens; while, the mean concentrations in runoff were 3.5 and 1.2 times higher, respectively. This study suggests that the antibiotics that are continuously administered through animal feed will be persistent and remain in feedlot

sediment and runoff, while those that are injected will be ingested by the animal and degrade more quickly.

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Dedication

I dedicate this thesis to my loving mother and father, Maria Arellano, and Daniel Trejo. Thank you for your endless love, support, and words of encouragement throughout my academic journey. Thank you for all your sacrifices that both of you made over the years that allowed me to have a clear path to a higher education. Gracias por todo mama y papa; sin ti, nada fue posible. Los amo mucho.

Acknowledgements

I would like to give a special thank you to both my advisors, Dr. Tiffany Messer and Dr. Shannon Bartelt-Hunt, for their help and guidance throughout my time at the University of Nebraska-Lincoln. They both served as impeccable mentors throughout my academic career. I would also like to thank the USDA-NIFA for funding this project (Grant Number: 2018-68003-27545).

Chapter 1: Introduction and Objectives

Section 1.1: Introduction

Antibiotics have been at the forefront for improving human and animal health since the development of penicillin in 1928 (Zhang et al. 2015). The usage of antibiotics in animals have historically been used in feedlots in order to increase the growth rate of animals, improve feed efficiency, and treat infectious diseases (Addison 1984, Pareek et al. 2015). Antibiotics have become essential to modern animal and human health, agricultural production, and the livestock industry (Sarmah et al. 2006).

Antibiotics are a naturally occurring substance found in the earth's soil; for example, β-lactams, aminoglycosides, and streptomycins are naturally produced by soil bacteria (Kümmerer 2009). These natural concentrations, in soil and water, range from a few ng/kg soil to hundreds of ng/kg soil (Grenni et al. 2018); however, antibiotics have been found to range between 500 to 900,000 ng/kg in soil (Kemper 2008). Once antibiotics are released into the environment from anthropogenic sources, these compounds are often transformed in chemical composition (Kemper 2008), which leads, to concern regarding potential environmental implications.

In the US, there were approximately 2,277,046 kg of medically important antibiotics and 3,139,331 kg of other antibiotics that were approved for the use for in food producing animals in 2017 ("2017 Summary Report on Antimicrobials Sold or Distrubted for Use in Food-Producing Animals" 2017). In 2018, the number of approved antibiotics for food-producing animals significantly increased by 50%; there were 6,036,140 kg medically important antibiotics and 5,530,784 kg of other antibiotics that were approved for the use for in food producing animals in 2018 ("2018 Summary Report on Antimicrobials Sold and Distributed for Use in Food-Producing Animals" 2018).

The amount of antibiotics approved for livestock is approximately four times greater than that for humans (Jechalke et al. 2014). Many of these antibiotics are administered to cattle on feedlots due to a variety of reasons including the prevention of liver abscesses, increased weight gain, and treatment for illnesses (Martinez et al. 2017). Antibiotics are administered through various methods including feed, injection, intermammary, oral, topical, and/or water ("FDA Drug Report 2017" 2017).

Table 1: Adaption of FDA 2018 Cattle Antibiotics Estimated Sales

Ingredient Class	2017 Estimated Annual Totals (kg)
Aminoglycosides	293,298
Amphenicols	56,056
Cephalosporins	31,448
Fluoroquinolones	23,350
Lincosamides	125,514
Macrolides	473,038
Penicillins	731,863
Sulfas	278,562
Tetracyclines	3,974,179
NIR[1]	48,832

Antibiotics ingested by bovine and other animals are excreted through urine and fecal matter (Aust et al. 2008). An estimated 4.5 billion metric tons of bovine manure is produced on an annual basis (US EPA 2004), which leads to concern for the potential environmental impacts potentially occurring due to the animal's excretion. Depending on the antibiotic, the excreted antibiotic may be completely metabolized or may be released as the original antibiotic with active or non-active metabolites (Aust et al. 2008).

[1] NIR = Not Independently Reported. Antimicrobial classes for which there were fewer than three distinct sponsors are not independently reported. These classes include the following: Aminocoumarins, Glycolipids, Orthosomycins, Pleuromutilins, Polypeptides, and Quinoxalines ("2017 Summary Report on Antimicrobials Sold or Distrubted for Use in Food-Producing Animals" 2017)

Over the last decade, a growing concern has been raised about the possibility of antibiotics entering the environment via animal manure (Sarmah et al. 2006). With the focus being on bovine manure, where antibiotics typically enter the environment following manure application via leaching into the soil and runoff (Sarmah et al. 2006). A plethora of studies have focused primarily on the occurrence and biological effects of antibiotics in land-applied animal feedlots (Bartelt-Hunt et al. 2012), with majority of findings demonstrating the presence of antibiotics in the soil due to animal excretion (Arikan et al. 2006; Mina et al. 2017; Zhao et al. 2010). A majority of feedlot studies have concentrated on conventional contaminants that include, but are not limited to, sediment, nutrients, and *E. coli* (Gilley et al. 2011; Miller et al. 2004). Unfortunately, there has been limited research on the transport of antibiotic from agricultural fields via runoff and sediments and even fewer studies focused on feedlot runoff (Bartelt-Hunt et al. 2012; Davis et al. 2006).

Section 1.2: Objectives

The importance of developing a better understanding of the fate and transport of antibiotics continues to be a central concern for the future of both human and animal health. Therefore, this study was designed to provide new insight into the fate and transport of antibiotics from a field scale feedlot operation. Specifically, my ***objective*** was to identify and quantify commonly used antibiotics administered to bovine in feedlot soils, sediment and runoff from a newly established feedlot that had not previously received cattle.

Chapter 2: Literature Review

Section 2.1: Definition of Antibiotics

Antibiotics are a type of antimicrobial which targets bacteria or fungi in the human/animal host (Grenni et al. 2018). According to the Federal Drug Administration (FDA), ten ingredient classes of antibiotics are medically important drugs and approved for use in food-producing animals. The ten classes include: aminoglycosides, amphenicols, cephalosporins, fluoroquinolones, lincosamides, macrolides, penicillins, sulfonamides, tetracyclines, and NIR ("FDA Drug Report 2017" 2017). The two main uses for veterinary antibiotics are therapeutic and non-therapeutic treatment. Therapeutic treatment of antibiotics refers to handling of ill animals, while non-therapeutic treatment refers to growth promotion and prevention of prophylaxis and metaphylaxis ("Antimicrobials: An Introduction" 2011).

Section 2.2: Antibiotic Classes and Antibiotic Type

Antibiotics are defined by a multitude of heterogeneous compounds that are distinguished by their different field usage, molecular structures and diverse chemical and physical properties (Thiele-bruhn 2003). For the purpose of this study, we will be focusing on four major metaphylaxis antibiotics that were administered by injection and two that were given in dietary feed daily: ceftiofur (injection), enrofloxacin (injection), florfenicol (injection), tulathromycin (injection), monensin (feed), and tylosin (feed).

Ceftiofur

Ceftiofur (Figure 1) is a cephalosporin's class that is a beta-lactam, antimicrobial agent that interrupts the cell wall synthesis (Cheng et al. 2018). Cephalosporins are the largest class of antibiotics, which aid in the treatment of a wide range of diseases (Cheng et al. 2018). Cephalosporin is related to the β-lactam antimicrobial agent (which shares

similar molecular makeup as penicillin) that has a broad spectrum of activity, low rates of toxicity, and has an ease of administration (Chandrasekhar et al. 2019). Cephalosporins are derivatives of 7-amino heterocycle, which is structural differerent from penicillin (Thiele-bruhn 2003).

Ceftiofur is a third-generation cephalosporin (Shaw 2014) and is administered to cattle to control and treat bacterial infections in the respiratory tract (European Agency for the Evaluation of Medicinal Product (EMEA) 1999). It is administered intramuscularly to cattle, which include lactating cows, in doses of up to 2 mg/kg bw/day for up to five days (European Agency for the Evaluation of Medicinal Product (EMEA) 1999). Ceftiofur is poorly absorbed when orally administered and is rapidly absorbed after intramuscular administration, with a high plasma concentration of 6 µg equivalents/mL after 30 minutes (European Agency for the Evaluation of Medicinal Product (EMEA) 1999). When ceftiofur is intramuscularly administered, more than 95% of the dosage is excreted within 24 hours; furthermore, 60 to 80% of the excretion is in the form of urine and the remainder is in manure (European Agency for the Evaluation of Medicinal Product (EMEA) 1999).

Table 2: Ceftiofur Physical Parameters

Analyte	CAS Number	Mol. Formula	Mol. Weight (g/mol)	Solubility in water mol/L	pKa	LogKow
Ceftiofur	80370-57 6	$C_{19}H_{17}N_5O_7S_3$	523.56	0.016 at pH = 7 2.8E-3 at pH = 4	2.62, 1.70 at 25°C	-2.02 at 25°C, when pH = 7

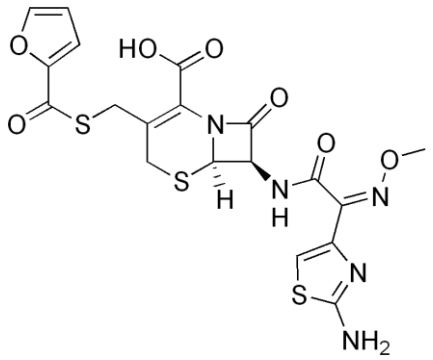


Figure 1: Molecular Structure of Ceftiofur

Enrofloxacin

Enrofloxacin (Baytril or Bayer) is a synthetic fluoroquinolone, which is relatively chemically stabile being insensitive to hydrolysis and increased temperatures; however, it easily degrades by photolysis (Thiele-bruhn 2003). Quinolone (also referred to as fluoroquinolone) is a family of broad-spectrum synthetic antibiotics developed in the 1970s (Cuprys et al. 2018).

Enrofloxacin has a recommended dosage of 2.5 to 5 mg enrofloxacin/kg bw/day for three to five days for cattle and is administered either by subcutaneous injection or intramuscular injection (European Agency for the Evaluation of Medicinal Product (EMEA) 1998). The antibiotic is also used to treat respiratory infections and alimentary tract infections in cattle (European Agency for the Evaluation of Medicinal Product (EMEA) 1998). Elimination of enrofloxacin is rapid in both urine and feces with excretion of the antibiotic within 24 hours and has a poor adsorption rate with approximately 76 – 77% of the antibiotic being retained and strongly bonded to the cattle manure (European Agency for the Evaluation of Medicinal Product (EMEA) 1998).

Table 3: Enrofloxacin Physical Parameter

Analyte	CAS Number	Mol. Formula	Mol. Weight (g/mol)	Solubility in water mol/L	pKa	LogKow
Enrofloxacin	93106-60-6	$C_{19}H_{22}FN_3O_3$	359.4	3.8E-4 at pH = 7 0.032 at pH = 4	6.43, 7.76 at 25°C	1.18 at 25°C, when pH = 7

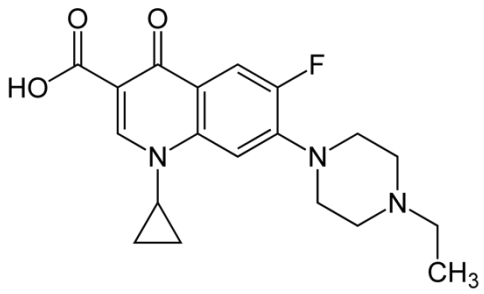


Figure 2: Molecular Structure of Enrofloxacin

Florfenicol

Florfenicol (Nuflor®) is a synthetic, broad-spectrum antibiotic that is active against gram-positive and gram-negative bacteria and is under the phenicol antibiotic class (Corp. 2017). Florfenicol is considered a bacteriostatic drug, which treats and controls bovine respirator disease (BRD), resists against pathogens such as *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilius somni,* and resists other strains such as *M. haemolytica* and *H. somni* (Corp. 2017).

Florfenicol is administered via single subcutaneous injection at a dosage rate of 40 mg/kg body weight (6 mL/100 lbs); furthermore, it may be intramuscular injected. However, intermuscular injection could result in inedible tissue (Corp. 2017). Elimination of florfenicol is within 2-3 hours after administration; however, it can be prolonged up to 18 hours when injected intramuscularly (Papich 2016). When it is subcutaneously administered, the antibiotic can take up to 27 hours before it is excreted (Papich 2016).

Table 4: Florfenicol Physical Parameters

Analyte	CAS Number	Mol. Formula	Mol. Weight (g/mol)	Solubility in water mol/L	pKa	LogKow
Florfenicol	73231-34-2	$C_{12}H_{14}Cl_2FNO_4S$	358.21	2.20E-03	10.73, 1.79 at 25°C	1.17 at 25°C, when pH = 7

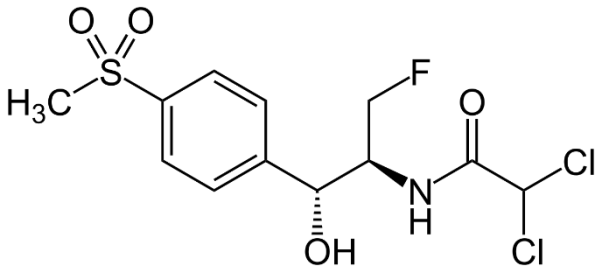


Figure 3: Molecular Structure of Florfenicol

Monensin

Monensin is a polyether carboxylic ionophore antibiotic that is used to treat ruminants and alters the volatile fatty acid production in cattle ("Diseases of the Alimentary Tract–Ruminant" 2017). Monensin displays both antimicrobial and anticoccidial activity and is used to treat gram-positive bacteria (Schering-Plough Animal Health Corp 2007). Monensin is administered orally through controlled release capsules that release the antibiotic at 400 mg/day (Schering-Plough Animal Health Corp 2007). It is rapidly absorbed and metabolized, mainly in the liver and is excreted in the bile (Schering-Plough Animal Health Corp 2007). The excretion time for monensin ranges between 3.1 to 5.6 hours (Friedlander and Sanders 2002). In the bile of bovine, approximately 35% of monensin is recovered compared to the recovery rate of 40% in rats (Schering-Plough Animal Health Corp 2007).

Table 5: Monensin Physical Parameters

Analyte	CAS Number	Mol. Formula	Mol. Weight (g/mol)	Solubility in water (mol/L)	pKa	$LogK_{ow}$
Monensin	17090-79-8	$C_{36}H_{62}O_{11}$	670.87	0.088 at pH = 7	4.26, at 25°C	1.30 at 25°C, when pH = 7

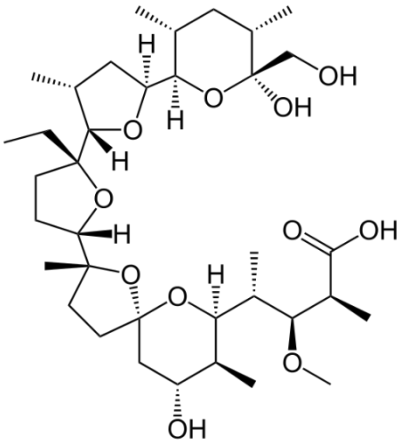


Figure 4: Molecular Structure of Monensin

Tulathromycin

Tulathromycin is in the macrolide antibiotic class and has a lactone structure with cycles of 10-C atoms or more (Thiele-bruhn 2003). It has weak bases and is unstable in acids (Thiele-bruhn 2003) and is used to help eliminate gram-positive infections, but can also be used to treat gram-negative bacteria (Feßler et al. 2018).

Tulathromycin (also known as Draxxin) is used to treat BRD in cattle caused by *Mannheimia haemolytica*, *Pasteurella multocida, Histophilus somni* and *Mycoplasma bovis* ("Draxxin Injectable Solution for Animal Use - Drugs" n.d.). The dosage of tulathromycin given to the bovine depends on bovine weight, with generally a single injection of 2.5 mg/kg bodyweight and is subcutaneous injected (Agency and Medicines 2004). The excretion rate of tulathromycin is relatively slow, where about 70% is recovered within 47 days (Agency and Medicines 2004); additionally, the excretion dosage is nearly equal with the recovery rate at 40% in urine and 32% in feces (Agency and Medicines 2004)

Table 6: Tulathromycin Physical Parameters

Analyte	CAS Number	Mol. Formula	Mol. Weight (g/mol)	Solubility in water (mol/L)	pKa	$LogK_{ow}$
Tulathromycin	27500-96-4	$C_{41}H_{79}N_3O_{12}$	806.08	1.24 at pH = 8 5E-4 at pH = 10	13.19, 10.2, at 25°C	-0.26 at 25°C, when pH = 7 2.41 at 25°C, when pH = 9

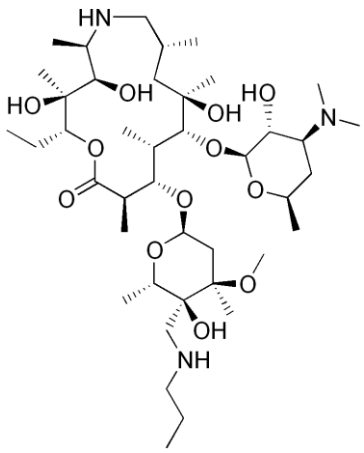


Figure 5: Molecular Structure of Tulathromycin

Tylosin

Tylosin is another antibiotic from the macrolide class and is most active against gram-positive bacteria and mycoplasmas (Committee for Veterinary Medicinal Products (CVMP) 1997). It is comprised of four tylosin derivatives produced by the Streptomyces fradiae strain, where the main component is tylosin A (factor A), but also includes tylosin factor B (desmycosin), tylosin factor C (macrosin), and tylosin factor D (relomycin) (Committee for Veterinary Medicinal Products (CVMP) 1997). It is administered both orally and by intramuscular injection with a dosage of 10 to 40 mg/kg bw and 5 to 20 mg/kg bw per day, respectively (Lewicki et al. 2004). Tylosin has a fast absorption rate, where cattle reach peak concentration at 2 to 4 hours (Committee for Veterinary Medicinal Products (CVMP) 1997). When tylosin is orally administered the elimination time ranges from 1.6 to 2.8 hours, while when intramuscularly administered it has an

elimination time of 2.2 to 3.2 hours (Committee for Veterinary Medicinal Products (CVMP) 1997).

Table 7: Tylosin Physical Parameters

Analyte	CAS Number	Mol. Formula	Mol. Weight (g/mol)	Solubility in water (mol/L)	pKa	$LogK_{ow}$
Tylosin	1401-69-0	$C_{46}H_{77}NO_{17}$	916.1	5.5E-6 at pH = 7	7.73 at 25°C	1.63 at 25°C, when pH = 7

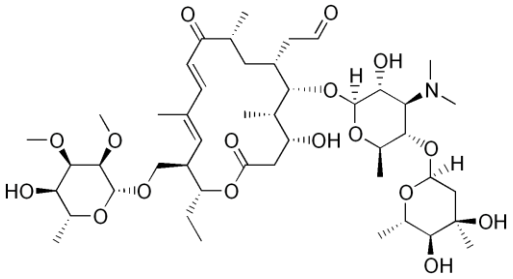


Figure 6: Molecular Structure of Tylosin

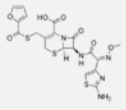
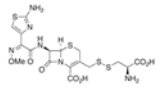
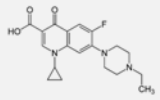
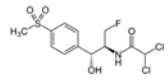
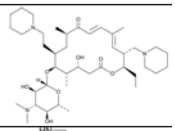
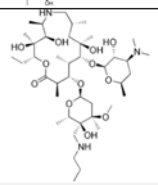
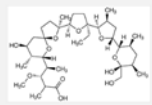
Section 2.3: Antibiotic Production

In the US, the information regarding the total production and usage of antibiotics, in both human and agriculture, is generally not available to the public (Sarmah et al. 2006). However, a study completed by the Union of Concerned Scientists (UCS) revealed that in the US approximately 16 million kg of antibiotics are used annually for animal treatment; in addition, 70% of those antibiotics are used for non-therapeutic purposes (Mellon et al. 2001). Globally, the amount of antibiotics consumed annually is between 100,000 – 200,000 kgs (Wise 2002). It is estimated that the consumption of antibiotics will rise by 67% by 2030 in the United States (US) and nearly double in the countries of Brazil, Russia, India, China, and South Africa (Van Boeckel et al. 2015).

Sweden was the first country to ban the use of all growth-promoting antibiotics in 1986 (Casewell et al. 2003; Sarmah et al. 2006). Denmark followed in banning avoparcin (1995) and avoparcin (1998) (Casewell et al. 2003; Sarmah et al. 2006). In 1999, the EU

banned the use of the antibiotics known as the “Precautionary Principal”, which included bacitracin (polypeptide), spiramycin, tylosin (macrolides), and Virginiamycin (Casewell et al. 2003; Sarmah et al. 2006). After the ban of antibiotic growth promoters, there was an increase of in sales; for example, the total sales went from 383 tons in 1999 to 437 tons in 2000 (Casewell et al. 2003). The increase in total sales went up due to the of increase of sales of tetracycline (approximately 36 tons), trimethoprim/sulphonamides (approximately 12 tons), and macrolides (approximately 12 tons) (Casewell et al. 2003).

Table 8: Complete Overview of all Analyte Physical Parameters

Analyte	CAS Number	Mol. Formula	Mol. Weight (g/mol)	Solubility in water	pKa	Log Kow	Structure
Ceftiofur	80370-57-6	$C_{19}H_{17}N_5O_7S_3$	523.56	0.016 mol/L @ pH=7 2.8E-3 mol/L @ pH=4	2.62, 1.70 @ 25C	-2.02 @25C pH=7	
Desfuroyl Ceftiofur Cysteine Disulfide (DCCD)	158039-15-7	$C_{17}H_{20}N_6O_7S_4$	548.64	0.030 mol/L @ pH=7 2.8E-3 mol/L @ pH=4	2.02, 8.38 @ 25C	-2.73 @25C pH=7	
Enrofloxacin	93106-60-6	$C_{19}H_{22}FN_3O_3$	359.4	3.8E-4 mol/L @ pH=7 0.032 mol/L @ pH=4	6.43, 7.76 @ 25C	1.18 @25C pH=7	
Florfenicol	73231-34-2	$C_{12}H_{14}Cl_2FNO_4S$	358.21	2.2E-3 mol/L	10.73, -1.79 @ 25C	1.17@25C pH=7	
Tildipirosin	328898-40-4	$C_{41}H_{71}N_3O_8$	734.0	1.36 mol/L @ pH=7 6.4E-3 mol/L @ pH=9	13.18, 9.51 @ 25C	0.32 @25C pH=7 3.81 @25C pH=9	
Tulathromycin	217500-96-4	$C_{41}H_{79}N_3O_{12}$	806.08	1.24 mol/L @ pH=8 5E-4 mol/L @ pH=10	13.19, 10.28 @ 25C	-0.26 @25C pH=7 2.41 @25C pH=9	
Monensin	17090-79-8	$C_{36}H_{62}O_{11}$	670.871	0.088 @pH=7	4.26 @25°C	1.30 @25°C pH=7	

Section 2.4: The Transport of Antibiotics

Unfortunately, due to poor digestion in the animal's gut, approximately 30-90% of the parent compound of the antibiotics are excreted following administration (Sarmah et al. 2006). Sources of antibiotic dissemination into our environment is not only due to excretion of unabsorbed medication, but rather a plethora of sources (Pareek et al. 2015). These sources include, but are not limited to, the natural environment, manure runoff, direct application of antibiotic-laden manure to fields, disposal of unused or expired compounds, and grazing animals (Pareek et al. 2015; Sarmah et al. 2006; Tasho and Cho 2016).

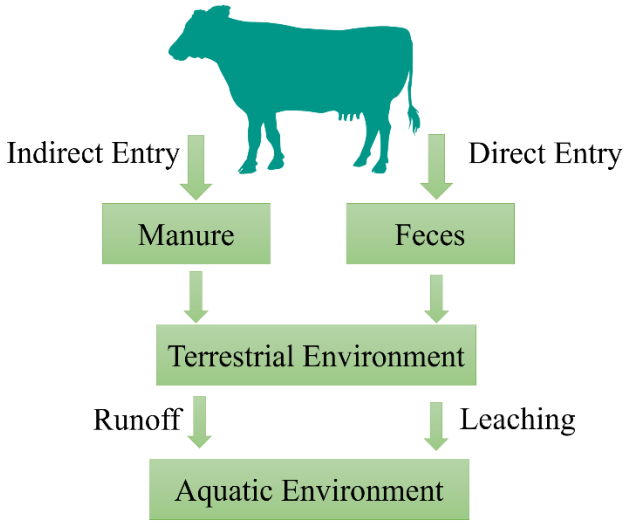


Figure 7: Adaption of Tasho and Cho 2016 potential antibiotics pathways

Antibiotics in the Natural Environment

Antibiotics are produced from naturally occurring organisms found in the earth's soil. For example, antibiotics such as β-lactams, aminoglycosides, and streptomycins are naturally produced by soil bacteria (Kümmerer 2009). The natural concentration of antibiotics in soil or water range from a few nanograms to hundreds of nanograms per liter or kg of soil (Grenni et al. 2018). These natural antibiotics have elements that will eliminate high concentration of human applied antibiotics due to certain microbial

functions (i.e. cell homeostasis, signal trafficking, and metabolic enzymes) (Martínez-Carballo et al. 2007; Martinez et al. 2009). Unfortunately, the strong increase of antibiotics in the natural environment, due to human activities, has shifted the original functions of the natural antibiotics (Martinez 2009). With human influence, the change not only effects the selection of antibiotic resistant microorganisms, but the structure and physiology of the natural microbial population (Martinez 2009).

Livestock Farming and Waste Management

Antibiotics have been used since the 1950s to control bacterial diseases in animal production (Pareek et al. 2015). The beef production industry is the third largest meat industry, globally (Cameron and McAllister 2016). In 2015 the major beef producing countries were the US, Brazil, the 28 member countries of the European Union (EU), China, and India; these countries have a global cattle population exceeding one billion (Cameron and McAllister 2016). Studies show antibiotics are not eliminated in the animal's gut, since most antibiotics are water-soluble (Kemper 2008; Zhao et al. 2010). Subsequently the dosage of antibiotics (varies from 3 to 220 g Mg^{-1} in feed) is dependent on the type and size of the animal and the amount of antibiotics excreted will vary with antibiotic type (Tasho and Cho 2016).

The application of bovine manure to agriculture fields is a common practice, since the manure provides essential nutrients for crops and adds organic matter to soils (Mina et al. 2017). In the mid-Atlantic US, manure is mainly applied in the spring and fall; however, manure may be applied year round (Mina et al. 2017). However, there are inconsistent regulations across the states with winter manure applications; in fact, across the US there are restrictions and/or complete bans of winter manure application

depending on state/region (Mina et al. 2017). Several studies have shown the influence of animal manure application and the availability of nutrients (Mina et al. 2017); however, many have also shown the negative impacts of manure application not related to antibiotic resistance. There are many factors that influence the fate and transport of manure, which include the type of manure, timing of application, method and history of application, hydrologic processes and biogeochemical cycling (Mina et al. 2017).

Unlike human biosolids, manure generated on farms does not undergo tertiary wastewater treatment (Kim et al. 2011). Therefore, our environmental concern of antibiotic resistance has primarily focused solely on the anthropogenic origin, the excretion of bovine and the applications of the manure (Kim et al. 2011). Due to absent and/or limited regulation or "manure management" practices, antibiotics are able to enter the environment at higher rates (Kim et al. 2011). For example, after the administration of antibiotics, the antibiotic is excreted via manure and/or urine within a few days (Winckler and Grafe 2000). Over a longer period of time, some individual animals are still releasing antibiotic compounds such as tetracycline (Winckler and Grafe 2000). Improper manure management and other sources are potential pathways for antibiotics to enter soil, ground water, surface water, and other sensitive ecosystems via runoff.

Runoff and Leaching From Soils

Antibiotic transport from soil to groundwater and surface water can occur by both runoff and leaching (Kim et al. 2011); however, little research has been published in regards to the transport of antibiotics from soil (Davis et al. 2006). Mobility of antibiotics are dependent on several factors, which include but are not limited to water solubility, dissociation constants, sorption-desorption processes, partitioning coefficients at different

pHs, temperature, moisture content of soil, timing of manure application, and weather (Kreuzig and Holtge 2005; Sarmah et al. 2006).

For instance, soils and sediments have binding characteristics, which delay biodegradation of antibiotics and allows a greater concentration of antibiotics to be transferred via runoff absorbed to soil. Kreuzig and Holtge (2005) discovered the manner in which agricultural lands were cultivated, determined the amount of runoff. In their study the recovery rate of antibiotics in manured grassland plots ranged from 13 to 23% of the initial amount applied; furthermore, the recovery rate, in arable (cultivated) land ranged from 0.1 to 2.5% (Kreuzig and Holtge 2005). Due to the large array of transport sources for antibiotics, a public health problem of antibiotic resistance grows in urgency (Mellon et al. 2001).

Section 2.5: The Ecological Effect of Antibiotics

Since 1987 there have been no new discoveries of antibiotic classes; as a result the development of antibiotic resistance may eliminate effectively treating diseases (Tasho and Cho 2016). Even if antibiotic concentrations are below the minimal inhibitory concentrations (MICs), antibiotics are able to have toxic effects on the environment (Grenni et al. 2018). When microbes begin adapting to antibiotics with each application, the potential for resistant microbes are developed (Grenni et al. 2018; Tasho and Cho 2016); thus creating the potential of antibiotic resistance to be transported from "farm to home" due to unregulated consumption of agricultural produce (Tasho and Cho 2016).

Antibiotics in Soil

Land application of manure is a very common practice used in agriculture in the US (Kumar et al. 2005). Generally, manure application is applied to corn; however, other

agricultural crops may receive manure treated fertilizer (Mina et al. 2017). Once antibiotics are applied to soil, via manure application, the antibiotics in the manure begin to interact with the soil in the solid phase (Jechalke et al. 2014). Thus, causing concern for potential uptake by edible crops. Antibiotics present in soil cause a reduction in microbial biodiversity and potentially influence the growth and enzyme activity of existing bacterial communities via biomass production and nutrient transfer (Grenni et al. 2018). Furthermore, different compounds, such as sulfonamides, may re-transform metabolites into the original parent compound (Jechalke et al. 2014). Although many antibiotics (such as tylosin) have half-lives of just days, other antibiotics are transferred to soil and may transport off the site through runoff to surface waters (Heuer et al. 2011; Martínez-Carballo et al. 2007) .

Antibiotic Uptake by Crops

Similarly, antibiotics with longer half-lives have the potential to accumulate into edible crops (Heuer et al. 2011). Batchelder (1982), showed tetracycline bio-accumulated into pinto bean plants, the production yield was reduced. A variety of vegetables have also been found to uptake antibiotics from applied manure. Specifically, cabbage, corn, and green onions have a chlortetracycline recovery rate of 0.34, 0.64, and 1.04%, respectively, with the amount of chlortetracycline absorbed by each plant increasing with increasing antibiotics concentration in manure-soil mixes (Kumar et al. 2005).

Section 2.6: Antibiotic Resistance

Although direct knowledge of animal-to-human antibiotic resistance is still relatively limited, recent studies demonstrate the transfer of these antibiotics is possible. Marshall and Levi (2011), was the first to report the spread of antibiotic resistant bacteria

from animals to humans by discovering the same tetracycline-resistant *E. coli* strains in the gut of chicken caretakers and chickens. The rise of antibiotic resistant bacteria among farm animals and consumer meat/fish products has also been well documented (Marshall and Levy 2011). This is the extent of our knowledge of antibiotic resistance and its transport. ***Therefore, the objective for my research project was to identify and quantify commonly used antibiotics administered to bovine in feedlot soils, sediment and runoff from a newly established feedlot that had not previously received cattle.***

Chapter 3: Materials and Methods

Section 3.1: Site Location

The project was located at the U.S. Meat Animal Research Center (USMARC) in Clay Center, Nebraska (Figure 8). The mission of USMARC is to develop new scientific information and technology to solve high priority problems that the US is facing in regards to beef, sheep, and the swine industries ("U.S. Meet Animal Research Center: Clay Center, NE" n.d.). USMARC is in a cooperative program with the University of Nebraska and other land-grand universities that focuses on six research units ("U.S. Meet Animal Research Center: Clay Center, NE" n.d.). The six research units are animal health, environmental management, genetics and breeding, meat safety and quality, nutrition, and reproduction ("U.S. Meet Animal Research Center: Clay Center, NE" n.d.).

Figure 8: Overview of USMARC Location

Section 3.2: Site Design

USMARC developed a newly renovated feedlot, B43 Facility (Figure 9) that was dedicated to antibiotic research. The feedlot had never been exposed to antibiotics over the last five years to serve as control for baseline levels of natural antibiotics in the environment**.** The facility was renovated from the skeleton framework and installed partially covered concrete pads with dirt floors on the south end of the feedlot pens. The feedlot had a total of 18 pens. These concrete pads were open to the natural environment (direct sunlight and rainfall) for seven months in order to remove any contaminants that remained following installation. Lastly, all soil areas were excavated and repacked with fresh clay soil, while a new water system and fence were installed. The clay soil was excavated on site, directly 3219 m west and 1609 m south from the study site (Figure 9). Production animals did not have access to the area prior to excavation.

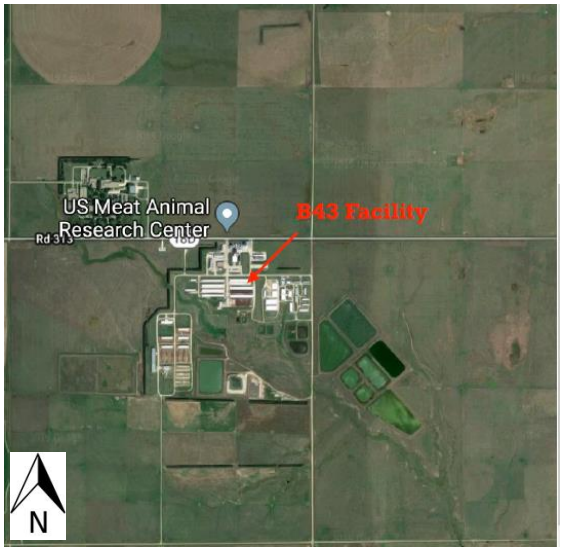


Figure 9: Project Location (B43 Facility)

Figure 10: Aerial Image of soil excavation site

Section 3.3: Bovine Selection and Treatment

The study was initiated during April of 2018. Approximately 168 Angus steers were released into the feedlot during weaning at the USMARC in 2018. Two groups of cattle were identified for the project. 84 steers, which never received antibiotics, were selected based on dame and calf herd health records. There were approximately 4 steers per pen. The second group of cattle were treated with antibiotics 2 ½ months prior to arrival to the study site, which was in October 2018. All bovine diets consisted of 40% corn silage, 36% alfalfa hay, 20% earlage, 4% vitamin and mineral supplement, and dry matter basis. However, the treated cattle (cattle given antibiotics) were also fed a mineral supplement of monensin and a month later tylosin was introduced into the daily feed and given dietary antibiotic prior to the injection date of April 9th, 2018. If any antibiotic-free

cattle required treatment with antibiotics, the bovine was immediately removed and placed into the treated steer pen group.

Section 3.4: Sample Collection

There was a total of 18 feedlot pens with four cattle per pen. Nine of the feedlot pens were used for conventional cattle and the remaining nine pens were used for antibiotic-free cattle. The pens were then separated into two different groups with Group 1 positioned on the west side of the pens and were identified with the following pen numbers: 18, 19, 20, 22, 23, 24, 26, 27, or 28; while Group 2 was positioned on the east side of the pens and were identified with the following pen numbers: 2, 3, 4, 6, 7, 8, 10, 11, 12.

The start date of the experiment was April 2nd, 2018, and a composite of each individual pen was taken. Samples were collected in 100 mL plastic test tubes and immediately placed inside a freezer until samples were transferred to the Nebraska Water Sciences Laboratory (WSL, Lincoln, NE). During the sample transfer phase, amber jars were used in order to prevent degradation of the antibiotics within the manure. A composite of the three samples collected per pen were placed into the amber jars. Samples were then transferred from USMARC to the University of Nebraska – Lincoln WSL for analysis. Additional runoff samples were collected from each pen by using a collection tub at the south end of the pen. Generally, runoff samples were collected a couple of days following a precipitation event.

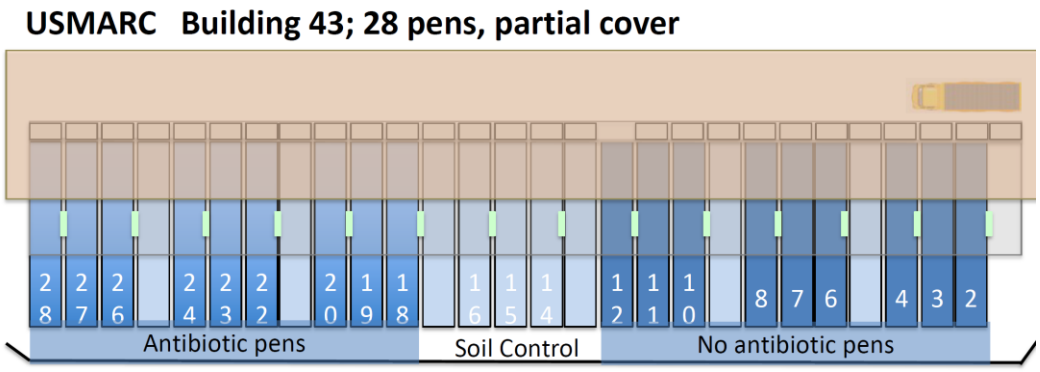


Figure 11: Pen Overview

Section 3.5: Sample Analysis

Both runoff and soil samples were analyzed using the Veterinary Pharmaceutical (VP) MacPen Analysis Method provided by WSL with modification from the Environmental Protection Agency (EPA) (EPA 2007). Both water and soil analysis had a laboratory reagent blank and a laboratory fortified blank; specifically, to provide a source of quality control measures to ensure no contamination was present.

Internal standards for this process consisted of roxithromycin, penicillin V potassium, salinomycin sodium, DCCD-d3, and tildipirosin-d10 and were purchased from Sigma-Aldrich (St. Louis, MO), Honeywell Fluka (Charlotte, NC), MP Biomedicals (Santa Ana, CA), TRC Canada and Tildipirosin-d10 (Winnipeg, Manitoba, Canada), respectively. For the surrogate, oleandomycin from ICN Biomedicals (Santa Ana, CA) was used. Finally, the analytes for this experiment were penicillin g potassium from Honeywell Fluka, penillic acid (Sigma-Aldrich), ampicillin (Honeywell Fluka), ceftiofur sodium (Honeywell Fluka), erythromycin (Sigma-Aldrich), novobiocin sodium (Honeywell Fluka), tiamulin (Honeywell Fluka), tylosin tartrate (ICN Biomedicals), virginiamycin MA (Sigma-Aldrich), monensin Na hydrate (Honeywell Fluka),

desfuroylceftiofur cysteine disulfide (DCCD) from TRC Canada, enrofloxacin (Sigma-Aldrich), tildipirosin (TRC Canada), tulathromycin A from Chem Cruz (Santa Cruz, CA), and florfenicol (Honeywell Fluka).

Water Analysis

The analysis process began by weighing approximately 100 g of sample and placing the sample into an amber vial. The liquid samples were then acidified, by adding approximately 20 to 35 drops of formic acid to obtain a pH of 2 ± 0.5. Once acidified, all samples were spiked with 100 µL of 1 ng/µL VP MacPen Surrogate spike and 0.1 g of ethylenediamine tetraacetic acid (EDTA). Samples were then capped, shook, and left to equilibrate for one hour.

After equilibration, the samples were eluted through a solid phase extraction (SPE) line and collected into an Oasis® Hydrophilic-Lipophilic-Balanced (HLB) cartridge (FIGURE 12). The HLB cartridges were preconditioned with 20 mL of methanol and 6 mL of pH of 2 ± 0.5 water. After extraction, the cartridges were placed on Visiprep DL (disposable liner) Teflon holders, were each sample eluted 12 mL of methanol into 15 x 85 mm disposable culture tubes. Then samples were blown down to an approximate volume of 500 µL and were spiked with 100 µL of 1 ng/µL VP MacPen Internal Standard and 300 µL of 4 g/L ammonium acetate buffer solution (Figure 12). Lastly, samples were vortexed and transferred to GC fitted vials with salinized conical

spring inserts using disposable glass pipets.

Figure 12: Left figure SPE Line and Right figure features elution and blowdown of sample

Soil Analysis

The solids or soils samples of this experiment were analyzed using the VP MacPen Soils Method from the Jansen et. al., (2019). First, 2 g of manure sample and 2 g of sand were added into a 50 mL centrifuge tube. This was done in order to facilitate mixing. Then each sample was spiked 100 µL of 1 ng/µL VP MacPen Surrogate spike and 4 mL of 0.125% trifluoric acetic acid (TFA) in acetonitrile (ACN) solution and placed on wrist action shaker for 10 minutes (Figure 13). An additional 4 mL of the McIlvain-EDTA buffer was added to the samples and samples were placed on the wrist action shaker again for an additional 15 minutes.

Figure 13: Wrist Shaker

Upon completion of the wrist action shaker, 2 mL of 200 g/L of lead acetate solution was added to the centrifuge tubes and placed inside a centrifuge for 10 minutes at 2000 rotation per minute (rpm)**.** Once the centrifuge came to a complete stop, the tubes were removed and decanted into RapidVap tubes. At this step all antibiotics were transformed into a liquid for analysis. The RapidVap tubes were then placed inside the RapidVap machine to evaporate samples to a volume of approximately 4 mL. Then an additional 13 mL of 0.2 M EDTA solution was added to each sample and was prepared for extraction.

In order to extract the samples, samples were placed inside reversed-phase polymeric SPE cartridges (200 mg, 6 mL Strata-X). Each cartridge was preconditioned with 5 mL of methanol and 5 mL of McIlvain-EDTA buffer. After the extraction of samples, the cartridges were rinsed with 5 mL of distilled deionized water (DDI) and vacuumed for 5 minutes. The cartridges were then placed in the elutriation device where each cartridge was eluted with 5 methanol into 15 x 85 mm disposable culture tubes. Samples were then blown down to near dryness.

Afterwards, 400 µL ACN was added to each culture tube and spiked with 100 µL of 1 ng/µL VP MacPen Internal Standard. The culture tubes were then placed back into the blowdown machine until samples reached approximately a volume of 100 µL. Once each sample reached the approximate volume, an additional 300 µL of 4 g/L ammonium acetate buffer solution was added and each sample was vortexed. Finally, each sample was transferred from the culture tubes into GC fitted vials with salinized conical spring inserts using disposable glass pipets.

Section 3.4: Determining Antibiotic Concentration

Once all samples were processed and placed into the GC fitted vials, they were then analyzed to determine the concentration of antibiotics present. Samples were analyzed using a light chromatography-mass spectrometer (LC-MS). The LC-MS allows identification of chemical compositions of particular or several complex mixtures (De O. Silva et al. 2019). One of the main components of the LC-MS is samples are converted into ions (positive or negative) and are immediately accelerated towards the mass analyzer (De O. Silva et al. 2019). The main mode of ionization used to determine antibiotic concentrations is based on the Atmospheric Pressure Ionization (API), which includes Chemical Ionization the Atmospheric Pressure (APCI) and the Electrospray Ionization (ESI) (De O. Silva et al. 2019)

Chapter 4: Results and Discussion

Section 4.1: Overview of Data

The presence of antibiotics in manure from cattle whom received antibiotic treatment compared to cattle whom did not receive antibiotic treatment was assessed. Fourteen antibiotics were tested and detected in both runoff and sediment samples; however, concentrations (converted to a dry basis to correct for organic matter content) of antibiotics administered by injection were low compared to antibiotics that were constantly administered in animal feed (tylosin and monensin). Further, statistically significant differences were not found between the treatment and control pens of the injected antibiotics, while statistical differences were observed between the treatment and control pens with constantly fed antibiotics ($\alpha = 0.05$). Due to these observations, antibiotics not considered for further statistical assessment included ampicillin, ceftiofur, erythromycin, erythromycin anhydro-, florfenicol, novobiocin, penicillin g, penillic acid, tiamulin, tulathromycin, tildipirosin, and virginiamycin M1. These twelve antibiotics were considered at "background" concentrations. Universally, there is not an accepted background concentrations for antibiotics and limited literature exists to determine background concentrations of antibiotics (Rothrock et al. 2016). Therefore, the term *background* will be defined as the concentration in the environment that is not influenced by local human activity (Franklin et al. 2016; Rothrock et al. 2016).

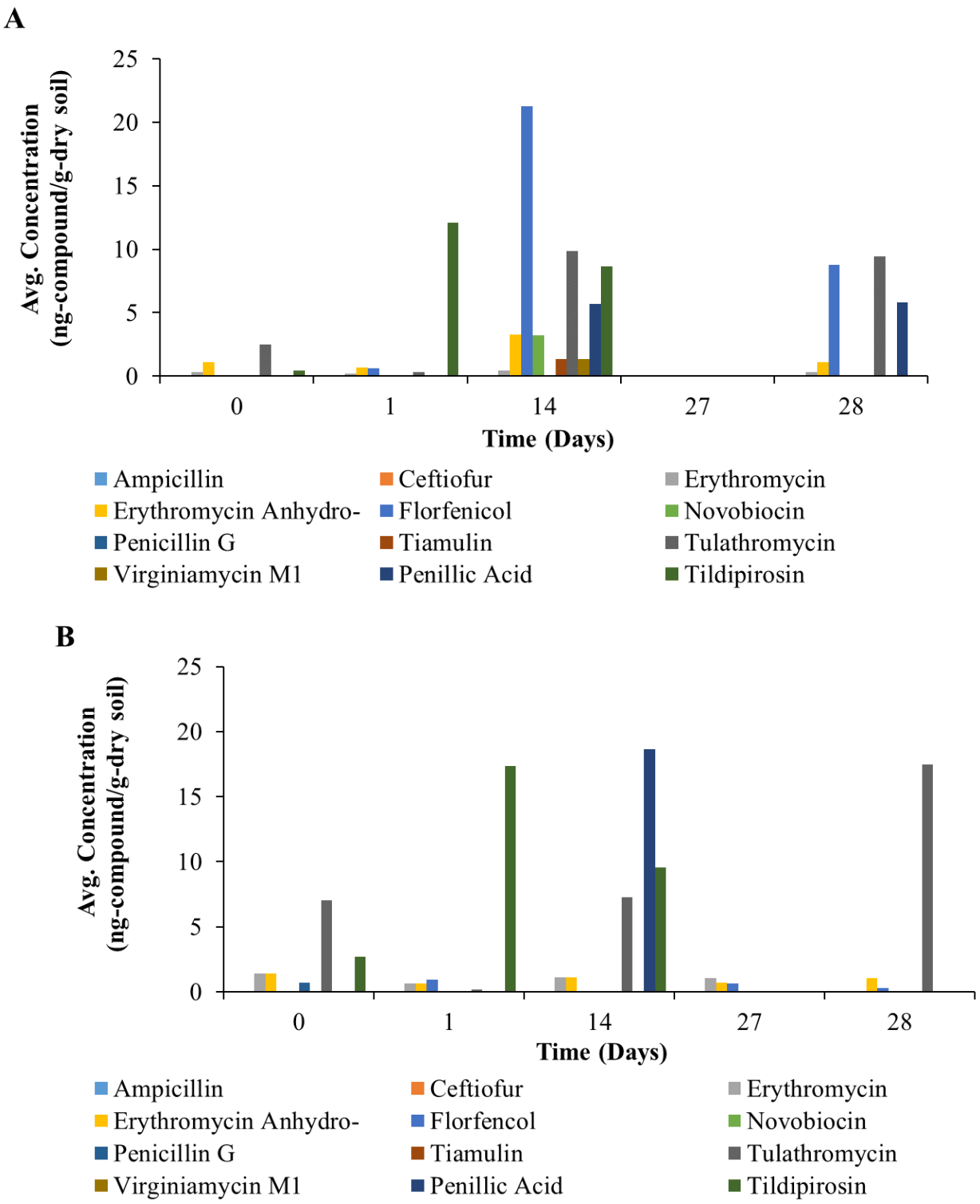


Figure 14: Occurrence of injection administered antibiotics in feedlot soil of A) treatment pens and B) control pens.

Section 4.2: Occurrence of Antibiotics in Feedlot Sediment

Two antibiotics were detected at concentrations above the established background concentrations (monensin and tylosin) and were both feed additives. Monensin was detected in feedlot soils in pens with cattle receiving antibiotics at concentrations ranging from 140 to 300 ng-monensin/g-dry soil; in addition, tylosin was detected in feedlot soils in pens with cattle receiving antibiotics at concentrations ranging from 30 to 130 ng-tylosin/g-dry soil (Figure 15)**.** It is important to note that both monensin and tylosin were administered daily throughout the experiment in the cattle's feed. Monensin was mixed into the feed at a rate of 300 mg/animal/day and tylosin was added to the feed at a rate of 80 mg/animal/day. Additionally, the excretion rate for monensin ranges between 3.1 to 5.6 hours, while the excretion rate for tylosin ranges from 1.6 to 2.8 hours (Committee for Veterinary Medicinal Products (CVMP) 1997; Friedlander and Sanders 2002). Furthermore, the half-lives on monensin and tylosin in fresh manure and soil ranges between 3 to 8 days (Dolliver and Gupta 2008b; Ingerslev and Halling-Sørensen 2001; De Liguoro et al. 2003).

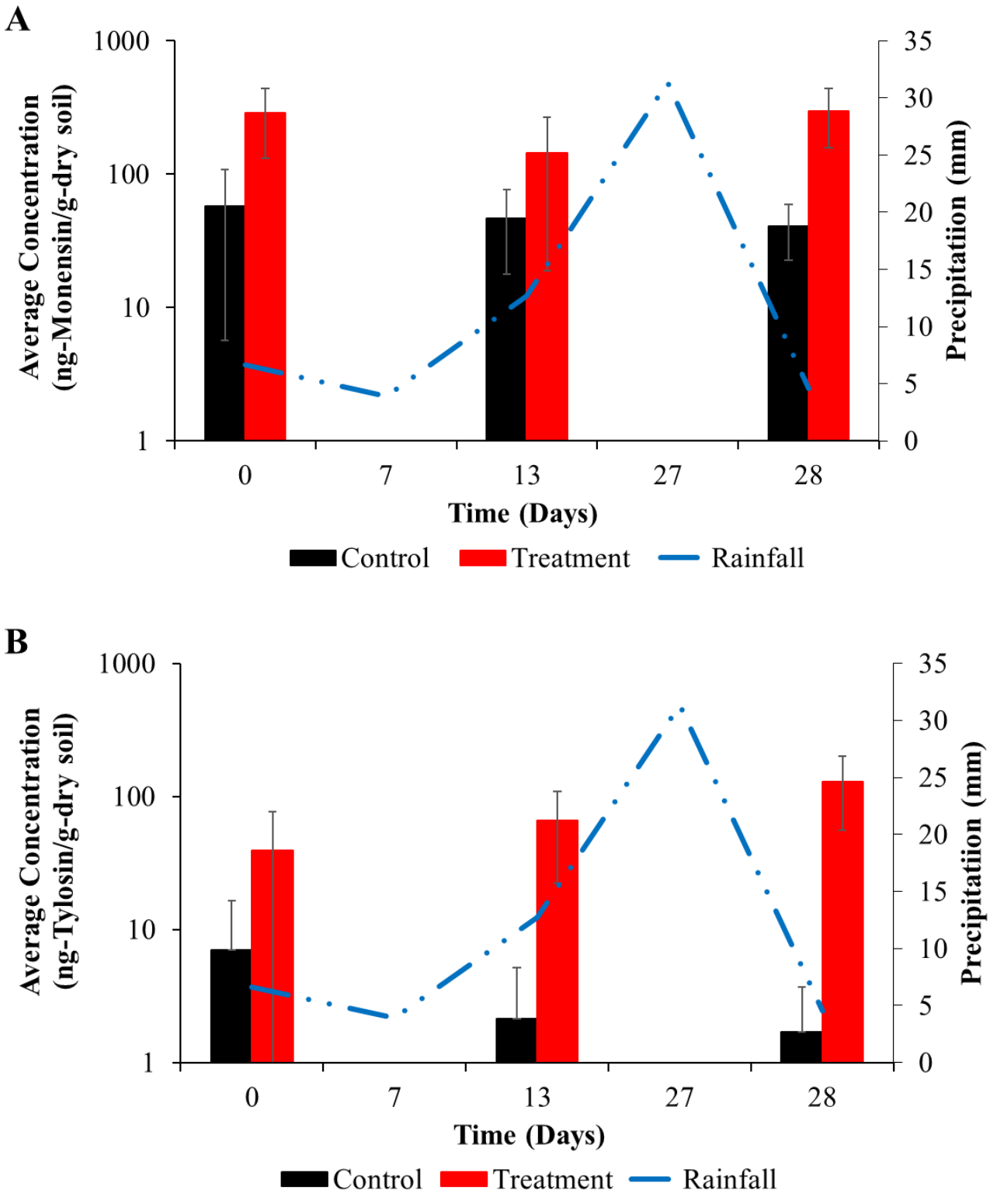


Figure 15: Feedlot soil mean concentrations of A) monensin and B) tylosin with Standard Error

In order to evaluate and discuss the concentration of antibiotics present in both feedlot sediment and runoff, an analysis of precipitation was conducted. The closest weather station was Dane, NE (KNECLAYC3), which was located approximately 1609 m east of the study site. Precipitation data was collected from the Prism Climate Group based in Oregon (**prism.oregonstate.edu)**. The total precipitation throughout the study period of April 1st, 2018, to May 12th, 2018, was approximately 59 mm. The first rainfall event during the experiment occurred on initiation day of the project when steers were introduced into the pens and the first set of samples were collected from the feedlot surface.

Table 9: Rainfall Data for Feedlot Sediment

Sampling Dates	Days Between Sampling Events	Number of Rainfall Events	Total Precipitation (mm)
4/2/2018	0	1	6.6
***4/9/2018**	7	2	3.9
4/23/2018	14	5	12.7
***5/6/2018**	13	5	31.3
5/7/2018	1	2	4.6
Total	**35**	**15**	**59.2**
***Denotes Soil Runoff Sample Collection Only**			

At the beginning of the experiment, the concentration of monensin in treated cattle was approximately 290 ng-monensin/g-dry soil, which is almost six times greater than the concentration found in the control pens. Due to a short excretion time and the absence of degradation, a higher concentration of monensin was expected at the beginning of the experiment (Yoshida et al. 2013). The change in concentration from day 0 to day 13, in monensin, can be explained by the rapid degradation of monensin in manure and the variability between sample times and excretion (Donoho 1984; Yoshida

et al. 2013). Manure is classified by its high bacterial load and high humidity, both properties that increase biodegradation of monensin (Yoshida et al. 2013; Žižek et al. 2011). Subsequently, the low-fiber diets, low animal density and a feedlot surface that was scraped every four to eight weeks throughout the experiment likely resulted in the observed increase in monensin concentrations. Similarly, Yoshida et al., (2013), observed feedlots with low animal density and low-fiber diets yielded higher concentration results in monensin compared to those who had high-fiber diets with a higher animal density (Yoshida et al. 2013).

The concentration of tylosin in treated cattle was approximately 40 ng-tylosin/g-dry soil, which is almost four times greater than the concentration found in the control pens. Ray et. al., (2017), found similar initial concentrations when determining tylosin concentration in static and turned beef compost, with the concentrations being 49.3 and 36.1 ng-tylosin/g-dry soil, respectively. Sura et. al., (2014), found initial tylosin concentrations at 80 μg-tylosin/kg-soil and Dolliver and Gupta (2008) found initial tylosin concentrations at less than 10 mg/kg-dry weight. Nonetheless, tylosin for Sura et. al., (2014), Ray et. al., (2017 and Dolliver and Gupta (2008) began to decrease or became undetectable over time during their experiments.

In contrast, during this study tylosin concentrations in the treatment pens continuously increased over time rather than decreased. Ray et. al., (2017), results indicated both static and turned beef compost had the tylosin mean concentration increase during the first week and then declined in both compost types. Similarly, Sura et. al., (2014) tylosin concentrations decreased over time and had residues stabilized at approximately 11 μg/kg. Although both Ray et. al., (2017) and Sura et al., (2014), fed

cattle tylosin over an extended period (210 days and 145 days, respectively), the lack of resemblance between Ray et. al., (2017) and Sura et. al., (2014) degradation trend between their studies and this study can possibly be explained by the feeding rate differences between the experiments. Ray et. al. (2017) and Sura et. al., (2014), both had a feeding rate of 11 mg/kg-feed; while, in this study our feed rate of tylosin was 80 mg/animal/day.

A similar inconsistent degradation trend was observed by Dolliver and Gupta (2008), in which there was an increase in Tylosin during the second portion of the experiment. The inconsistent upward trend in tylosin can be explained by the possible change in manure characteristics (Dolliver and Gupta 2008a). Sura et. al., (2014), Ray et. al., (2017), and Dolliver and Gupta (2008) all considered manure properties, such as pH, moisture and temperature. In this study, however, these factors were not taken into consideration during the experiment. Even though there was no significant difference between sampling dates for tylosin (p value = 0.1963), the "increase" in concentration may have been affected by manure characteristics delaying degradation. Factors that may affect degradation for tylosin include organic matter content, pH, moisture, temperature, oxygen status, and soil texture (Cycoń et al. 2019) and should be considered in future field experiments.

Section 4.3: Occurrence of Antibiotics in Runoff and Sediment Runoff

Monensin and tylosin were also detected at concentrations above the established background concentrations in feedlot runoff. Monensin was detected in runoff with treated cattle at concentrations ranging from 0.5 to 9 μg-monensin/L; while, tylosin was

detected in feedlot runoff in pens with treated cattle at concentrations ranging from 0.01 to 3 µg-tylosin/L (Figure 16).

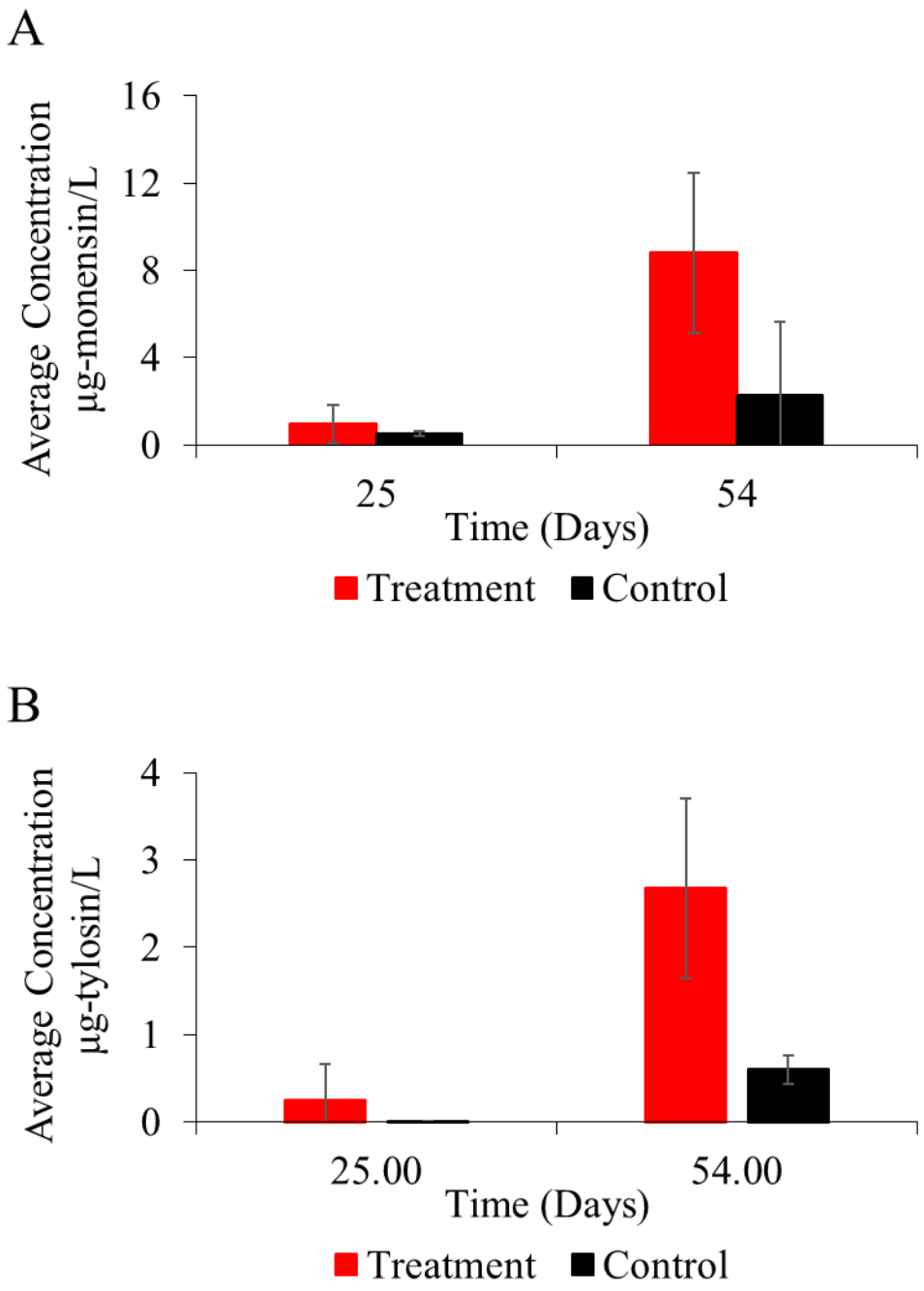


Figure 16: Feedlot runoff mean concentrations of A) monensin and B) tylosin with Standard Error

An additional evaluation of precipitation was conducted to evaluate the impact of precipitation on observed concentrations of antibiotics present in feedlot runoff. The precipitation data was collected from the same rain station in Dane, NE (KNECLAYC3) from the Prism Climate Group based in Oregon (prism.oregonstate.edu). The total precipitation throughout the study period of May 1st, 2018, to June 4th, 2018 was approximately 84 mm.

Table 10: Rainfall Data for Feedlot Runoff

Sampling Dates	Days Between Sampling Events	Number of Rainfall Events	Total Precipitation (mm)
5/3/2018	0	1	2.3
6/2/2018	30	12	81.3
Total	**30**	**13**	**83.6**

Statistically significant differences were not observed between the treatment and control pens for both monensin (p-value=0.53) and tylosin (p-value=0.43) during the first sampling event on May 3rd, 2018. This was potentially due to feedlot management procedures, such as feedlot scraping. During the experiment, the feedlot surface was scraped every four to eight weeks. If the feedlot was scraped before the initial runoff sampling date, the initial concentration of manure would be significantly lower, thus reducing the amount of antibiotic runoff. Similar observations were made by Dolliver and Gupta (2008), where the concentration of monensin in runoff was higher during experiment one compared to experiment two due a higher initial concentration in manure.

In contrast, statistically significant differences were observed for both monensin (p-value<0.01) and tylosin (p-value<0.001) on June 2nd, 2018. Results from this study were similar to those observed by Davis et al. (2006) and Dolliver and Gupta (2008).

Dolliver and Gupta (2008), performed a similar field scale study to determine the concentration of antibiotics present in runoff from both beef and hog manure over three years. Monensin and tylosin were detected in approximately 20% of all samples collected and the highest concentrations found throughout the study were 57.5 and 1.9 μg/L, respectively. The highest concentrations for monensin and tylosin during the experiment was 3175 μg/L and 2544 μg/L, respectively (Dolliver and Gupta 2008a).

Monensin and tylosin were also detected in sediment runoff during the experiment. It is important to note that, due to design error, there was no separation of the trough in the control and treatment pens. All sediment runoff was collected from the same trough at the bottom of the pens; therefore, Figure 17, demonstrates the concentration of sediment runoff for monensin and tylosin for both treatment and control pens combined. Monensin was detected in sediment runoff with treated cattle at concentrations ranging from 6.5 to 16 ng-monensin/g-dry soil; while, tylosin was detected in feedlot runoff in pens with treated cattle at concentrations ranging from 0.4 to 1.2 ng-tylosin/g-dry soil (Figure 17).

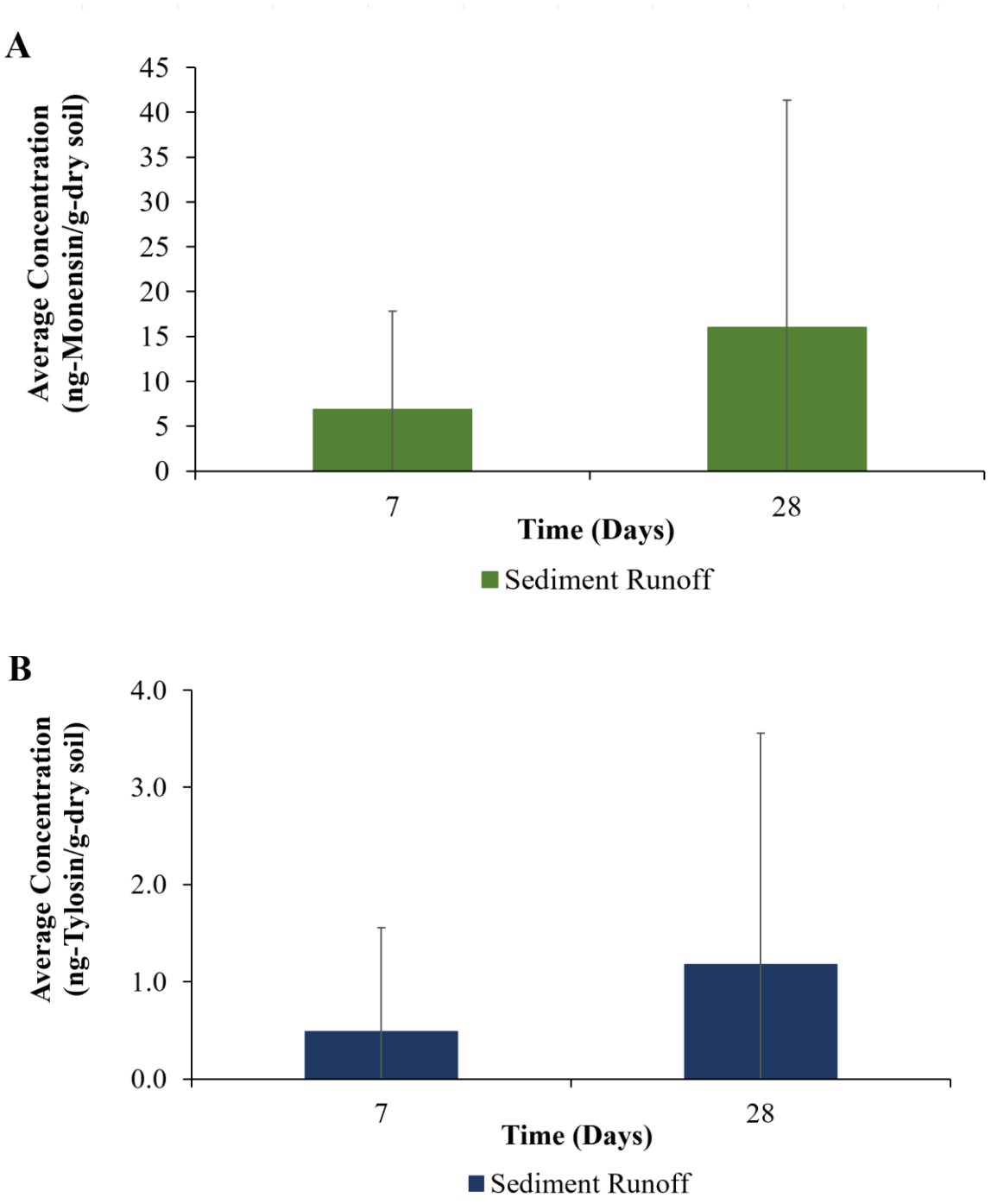


Figure 17: Sediment Runoff mean concentrations of A) monensin and B) tylosin with Standard Error

Davis et al. (2006), determined a correlation was present between the transport of antibiotics via runoff. Dolliver and Gupta (2008), assessed antibiotic loss from unprotected manure stockpiles and observed a strong, positive correlation ($r^2 = 0.7$)

between antibiotic concentration and runoff. Davis et al. (2006), observed a significant difference in the concentration of antibiotic concentrations in runoff due to the contribution of precipitation at 10 to 20 min and 60 min sampling times in soils. For the 10 to 20 min time interval, erythromycin had the highest concentration followed by monensin and tylosin; while at the 60 min mark tylosin was the highest (Davis et al. 2006). Due to the observation and detection of both monensin and tylosin in sediment runoff, results from this study, along with other studies (Sura et al., 2015), indicate runoff from feedlots are a potential source of transport for antibiotics. Proper manure management and a well-maintained catchment basin will potentially aid in the stopping of antibiotic runoff transfer (Dolliver et al. 2008; Sura et al. 2015).

Monensin and tylosin were detected in the control pens of during this experiment; however, at significantly smaller concentrations compared to the treatment pens. Wind is known to carry antibiotics causing cross contamination. For example, Sandoz et al., (2018) detected at least one veterinary pharmaceutical in all playa wetland soil samples near a beef cattle feed yard, with monensin and tylosin having the highest detected concentrations (up to 853 nm/g and 84 μg/L, respectively). Additionally, monensin was the most frequently detected and had the highest concentration (Sandoz et al. 2018). The detection of monensin and tylosin on control plots were likely from cross contamination due to the design of the test pens. However, the pens in this study had structural barriers installed between pens in an effort to minimize antibiotic transfer from wind, which has also been used in previous studies (Heuer et al. 2011).

Section 4.4: Mass Balance

A mass balance (Equation 1) was conducted for monensin and tylosin within the feedlot to determine the amount of antibiotic mass that remained in the feedlot, dissipated, or left the feedlot in the runoff. An analysis was performed using no reaction and with dissipation following first-order reaction kinetics. The analysis performed assuming no reaction only considered that antibiotics would remain in the soil or be transported off the feedlot via runoff. Since the mass balance was time sensitive and due to spatially variability in sample collection, the mass balance analysis was performed only for May 3rd, 2018. Furthermore, the main objective of the mass balance was to determine the percent of antibiotic remaining in soil, percent of antibiotic transported in runoff, and the percent of antibiotic that dissipated.

Equation 1: Mass Balance

$$X = M_{in}C_{in} - M_{out}C_{out} \pm RXN$$

Where X is the amount of monensin or tylosin that remained on the pen soil (mg-antibiotic), M is the mass of the antibiotic, C is the concentration of the antibiotic, and RXN is the kinetic reaction. Modifications were made from the Equation 1 (base equation) in order to determine X.

Equation 2: Modified Mass Balance

$$X = SC * depth_{soil} * A * \rho_{dry}$$

Where X is previously defined in Equation 1; SC is the soil concentration (ng-antibiotic/dry g)(concentration found from Section 4.1), $depth_{soil}$ is the depth of the soil on the feedlot (cm), A is the area of the pen (m), and ρ_{dry} is the dry bulk density of soil (kg/m^3) (Larney et al. 2000). It is important to note that unit conversion is required to

obtain the correct units of mg. antibiotic. In order to determine the percent of antibiotic remaining in the soil, calculations of the total antibiotic intake was required (Equation 3).

Equation 3: Total Cattle Intake

$$I = F_{intake} * No.cows\ per\ pen * t$$

Where I is the total intake of antibiotic (mg), F_{intake} is the amount of antibiotic given in feed (mg/animal/day) for either monensin or tylosin, and t is the duration of study period (days). Once the overall intake was determined the percent of antibiotic remaining in soil was calculated by Equation 4.

Equation 4: Percent of Soil Remaining in Soil

$$PS = \frac{X}{I}$$

Once the percent of soil remaining was calculated, the amount of antibiotic unaccounted for was calculated (Equation 5).

Equation 5: Mass of Antibiotic Unaccounted for

$$UA = I - X$$

Where UA is the mass of the antibiotic unaccounted for (mg). In order to determine what happened to the unaccounted antibiotic mass, runoff was calculated to determine if they were transported. The runoff was calculated using the SCS Runoff Curve Number Method (USDA 1986) .

Equation 6: SCS Runoff Curve Number Method (USDA 1986)

$Q = \frac{(P-0.2S)^2}{P+0.8S}$Where Q is the amount of runoff (in), P is the precipitation of the study period (in), and S is the potential maximum retention after runoff (in) (USDA 1986). Precipitation was calculated by gathering data from Prism Climate Group

(**prism.oregonstate.edu);** the data was collected from April 1, 2018 until May 3rd, 2018. In order to determine the value of S, an additional equation (Equation 7) was used.

Equation 7: Curve Number Method

$$S = \frac{1000}{CN} + 10$$

S is related to the soil and cover conditions of the pen through the curve number (CN) (USDA 1986). To determine the curve number and average was taken from CN of the upper and mid portion of the pen and from the bottom of the pen. The upper and mid portion of the pen was made of concrete (CN 98) and the bottom portion of the pen was considered dirt (CN 86) (Schmidt and Wilson 2011); therefore, the CN used to determine S was 94. Table 11 shows the runoff (inches) that was determined based on the calculations from above.

Table 11: Runoff Analysis Inputs

Inputs	May 3rd, 2018
P (in)	2
CN (unitless)	94
S (in)	0.64
Q (in)	2.45

The amount of runoff was calculated in order to determine the amount of antibiotic transported via runoff. The runoff was calculated using a similar method as Equation 2; however, modifications were made to incorporate runoff (Equation 8).

Equation 8: Antibiotic Mass in Runoff

$$Y = WC * A * Q$$

Where Y is the amount of antibiotic in runoff (µg), WC is the water concentration of antibiotic (µg/L)(concentration found in Section 4.2), A is the pen area (m^2), Q is the runoff calculated using the SCS Curve Number Method (in). It's important to note that

additional unit conversions were needed to obtain the amount of antibiotic in runoff to obtain the units of µg. To determine the percent amount of antibiotic in runoff Equation 3 was used; however, the units were changed from mg to µg. Then Equation 4 was used to determine the percent of antibiotic transported in runoff.

Upon analysis of the total amount of runoff for the experiment, it was concluded that the percent of both monensin and tylosin transported via runoff was <0.01%. This was because the amount of antibiotic found in runoff was 26X's smaller than what was given in the feed. Therefore, the transport of antibiotics via runoff was not significant and further supports that the unaccounted for antibiotics likely dissipated.

Equation 9: Dissipation Calculation

$$D = 1 - C_s - T_s - C_r - T_r$$

Where D is the amount of antibiotic that dissipated (%), C_s and T_s are the amount of antibiotic in soil (%) and C_r and T_r are the amount of antibiotic transported via runoff (%). Dissipation includes the control pens, due to the possibility of cross contamination.

Dolliver et. al. (2008), performed a similar study to determine the decay rates for monensin and tylosin in turkey manure. During their study they observed monensin and tylosin followed a first-order decay rate function (Dolliver et al. 2008). It is important to note that due to limited research on the fate and transport of monensin and tylosin directly on a beef feedlot, the decay rate is unknown and was considered outside of the scope of this study.

Equation 10: First-Order Decay Function

$$C = C_o e^{-kt}$$

Where C is the measured concentration (ng-antibiotic/dry-g) at time (t) (28 days, full study period), C_o is the initial antibiotic concentration (ng-antibiotic/dry-g), and k is the degradation rate for the antibiotic (1/day). In-order to calculate k, the half-life equation was used to determine k (Dolliver et al. 2008). Half-lives for both monenin and tylosin range between 3 to 8 days (Dolliver et al. 2008). The k-values were based off of Dolliver et al. (2008) experiment.

Table 12: Degradation Rate for Monensin and Tylosin (Dolliver et al. 2008)

Type	k-value
Monensin	0.032
Tylosin	0.029

When accounting for degradation the dissipation amount for both monensin and tylosin increased. Monensin dissipation rate increased by 3.4% and was approximately at 99.3%, while the amount remaining soil decreased by 3.5% and was now 0.7%. Similarly, dissipation in tylosin increased by 3.8% and was approximately 97%, while the amount the remained on the feedlot decreased by 3.8% and was approximately 3%.

The results that were found are similar to Dolliver et. al. (2008) and Yoshida et. al. (2010). Dolliver et. al. (2008) found that both monensin and tylosin had a gradual decline with reduction ranging from 54 to 76%. Further analysis of the degradation was not conducted due to the lack of data.

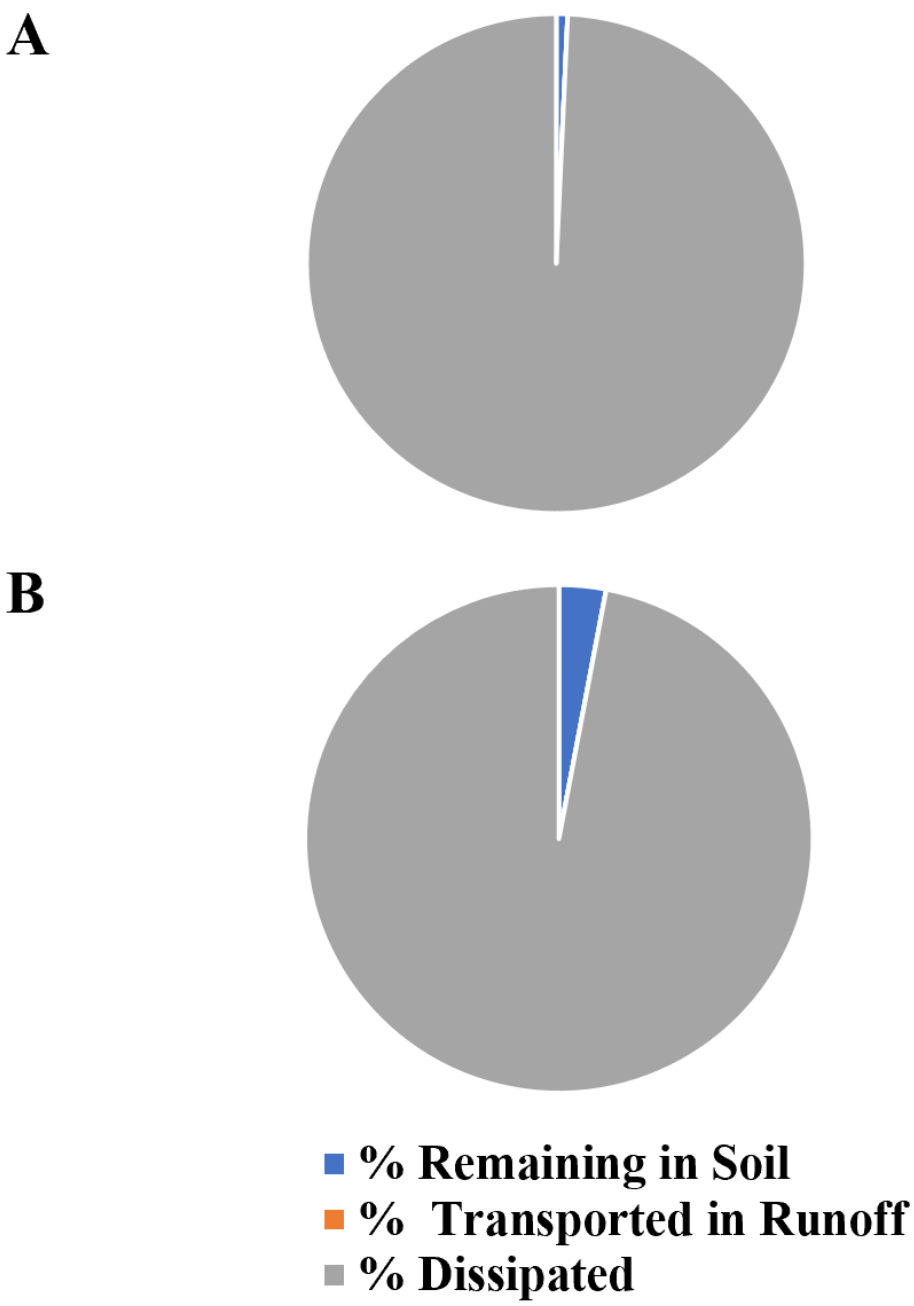


Figure 18:Monensin remaining in runoff, in soil, and dissipated and B) Tylosin remaining in runoff, in soil, and dissipated with first-order decay

Chapter 5: Conclusion

Currently, there is limited literature that observes the fate and transport of antibiotics from agricultural feedlot operations. Additionally, there is even less literature that quantifies commonly used antibiotics administered to bovine in both feedlot sediment and runoff. Therefore, the purpose of this thesis was to detect the concentration of antibiotics being administered sub-therapeutically in feed or by injection to promote feed efficiency and prevent disease (Lee et al. 2007) in the Midwest, US. For the purpose of this thesis, there were four major metaphylaxis antibiotics that were administered by injection (ceftiofur, enrofloxacin, florfenicol, tulathromycin) and two that were given in dietary feed daily (monensin, tylosin).

This research study sought to provide insight into the agricultural industry and bring light to antibiotic usage. The results presented in this thesis implicate that antibiotics being fed daily over time will not be metabolized by bovine or degraded on the feedlot as efficiently as hypothesized, thus these antibiotics are expected to be detected in both feedlot sediment and runoff as they are continued to enriched in animal feed. Contrarily, antibiotics that are injected will be metabolized in the bovine, degraded efficiently on the feedlot surface, and are unlikely to be undetectable. The results of this thesis were compared with published literature and found to be within boundaries and similar trends of previously reported experiments.

While a definitive conclusion was drawn from this research, the following actions are proposed for future research.

1. Develop a more rigorous sampling method that includes a more consistent sampling time and collection. Samples feedlot samples should be collected at

consistent sampling intervals. This study did not distribute sampling dates spatially, which potentially caused higher variability in antibiotic concentration.

2. Create a more rigorous sampling method that includes runoff sample collection within 24 hours following a precipitation event.
3. Complete measurements of manure and runoff factors that are known to result in high variability in degradation of antibiotics during sediment and runoff collection including pH, organic matter content, and temperature.

Works Cited

"2017 Summary Report on Antimicrobials Sold or Distrubted for Use in Food-Producing Animals." (2017). Elsevier Ltd, 65(5).

"2018 Summary Report on Antimicrobials Sold and Distributed for Use in Food-Producing Animals." (2018). 2(December 2019).

Agency, E. M., and Medicines, V. (2004). "Tylathromycin Summer Report (2)." (January).

"Antimicrobials: An Introduction." (2011). 23.

Arikan, O. A., Sikora, L. J., Mulbry, W., Khan, S. U., Rice, C., and Foster, G. D. (2006). "The fate and effect of oxytetracycline during the anaerobic digestion of manure from therapeutically treated calves." *Process Biochemistry*, 41(7), 1637–1643.

Aust, M., Godlinski, F., Travis, G. R., Hao, X., Mcallister, T. A., and Leinweber, P. (2008). "Distribution of sulfamethazine , chlortetracycline and tylosin in manure and soil of Canadian feedlots after subtherapeutic use in cattle." 156, 1243–1251.

Bartelt-hunt, S. L., Snow, D. D., Kranz, W. L., Mader, T. L., Shapiro, C. A., Donk, S. J. Van, Shelton, D. P., Tarkalson, D. D., and Zhang, T. C. (2012). "Effect of Growth Promotants on the Occurrence of Endogenous and Synthetic Steroid Hormones on Feedlot Soils and in Runoff from Beef Cattle Feeding Operations."

Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B. T., Levin, S. A., Robinson, T. P., Teillant, A., and Laxminarayan, R. (2015). "Global trends in antimicrobial use in food animals." *Proceedings of the National Academy of Sciences of the United States of America*, 112(18), 5649–54.

Cameron, A., and McAllister, T. A. (2016). "Antimicrobial usage and resistance in beef production." *Journal of Animal Science and Biotechnology*, Journal of Animal Science and Biotechnology, 7(1).

Casewell, M., Friis, C., Marco, E., Mcmullin, P., and Phillips, I. (2003). "The European ban on growth-promoting antibiotics and emerging consequences for human and animal health." (July), 159–161.

Chandrasekhar, D., Chinnu, P. B., and Mundadan, C. T. (2019). "Antibiotics : Need for new fences " , a prospective utilization evaluation of cephalosporin ' s and its combination in tertiary care referral hospital." (December 2018).

Cheng, L. Y., Hu, Y. J., Li, M., and Bai, A. M. (2018). "Probing the interaction of cephalosporin with bovine serum albumin : A structural and comparative perspective." (April 2017), 209–218.

Committee for Veterinary Medicinal Products (CVMP). (1997). "TYLOSIN." (April), 1–8.

Corp., M. S. & D. (2017). “Nuflor® (FLORFENICOL) Injectable Solution 300 mg/mL.” *NADA #141-063, Approved by FDA*.

Cuprys, A., Pulicharla, R., Kaur, S., Drogui, P., Verma, M., and Surampalli, R. Y. (2018). “Fluoroquinolones metal complexation and its environmental impacts.” *Coordination Chemistry Reviews*, Elsevier B.V., 376, 46–61.

Cycoń, M., Mrozik, A., and Piotrowska-Seget, Z. (2019). “Antibiotics in the soil environment—degradation and their impact on microbial activity and diversity.” *Frontiers in Microbiology*, 10(MAR).

Davis, J. G., Truman, C. C., Kim, S. C., Ascough, J. C., and Carlson, K. (2006). “Antibiotic Transport via Runoff and Soil Loss.” *Journal of Environment Quality*, 35(6), 2250.

“Diseases of the Alimentary Tract–Ruminant.” (2017). *Veterinary Medicine*, 436–621.

Dolliver, H. A. S., and Gupta, S. C. (2008a). “Antibiotic losses from unprotected manure stockpiles.” *Journal of Environmental Quality*, 37(3), 1238–1244.

Dolliver, H., and Gupta, S. (2008b). “Antibiotic losses in leaching and surface runoff from manure-amended agricultural land.” *Journal of Environmental Quality*, 37(3), 1227–1237.

Dolliver, H., Gupta, S., and Noll, S. (2008). “Antibiotic degradation during manure composting.” *Journal of Environmental Quality*, 37(3), 1245–1253.

Donoho, A. L. (1984). “Biochemical Studies on the Fate of Monensin in Animals and in the Environment.” *Journal of Animal Science*, 58(6), 1528–1539.

“Draxxin Injectable Solution for Animal Use - Drugs.” (n.d.). .

Epa. (2007). “Method 1694 : Pharmaceuticals and Personal Care Products in Water , Soil , Sediment , and Biosolids by HPLC / MS / MS.” *EPA Method*, (December), 77.

European Agency for the Evaluation of Medicinal Product (EMEA). (1998). “Maximum Residue Limit. Enrofloxacin. Summary Report.” (July), 1–6.

European Agency for the Evaluation of Medicinal Product (EMEA). (1999). *Ceftiofur summary report*.

“FDA Drug Report 2017.” (2017). 1–52.

Feßler, A. T., Wang, Y., Wu, C., and Schwarz, S. (2018). “Plasmid Mobile macrolide resistance genes in staphylococci.” *Plasmid*, Elsevier, 99(February), 2–10.

Franklin, A. M., Aga, D. S., Cytryn, E., Durso, L. M., McLain, J. E., Pruden, A., Roberts, M. C., Rothrock, M. J., Snow, D. D., Watson, J. E., and Dungan, R. S. (2016). “Antibiotics in agroecosystems: Introduction to the special section.” *Journal of Environmental Quality*, 45(2), 377–393.

Friedlander, L. G., and Sanders, P. (2002). “Monensin.” *Triclabendazole monograph*, 43–44.

Gilley, J. E., Vogel, J. R., Eigenberg, R. A., Marx, D. B., and Woodbury, B. L. (2011). "Runoff, Erosion, and Size Distribution of Sediment from Beef Cattle Feedlots." 54(1), 435–440.

Grenni, P., Ancona, V., and Barra Caracciolo, A. (2018). "Ecological effects of antibiotics on natural ecosystems: A review." *Microchemical Journal*, Elsevier B.V., 136, 25–39.

Heuer, H., Schmitt, H., and Smalla, K. (2011). "Antibiotic resistance gene spread due to manure application on agricultural fields." *Current opinion in microbiology*, 14(3), 236–43.

Ingerslev, F., and Halling-Sørensen, B. (2001). "Biodegradability of metronidazole, olaquindox, and tylosin and formation of tylosin degradation products in aerobic soil-manure slurries." *Ecotoxicology and Environmental Safety*, 48(3), 311–320.

Jansen, L. J., Schans, M. G. V. D., Boer, D. D., Bongers, I. E., Schmitt, H., Hoeksma, P., and Berendsen, B. J. (2019). "A new extraction procedure to abate the burden of non-extractable antibiotic residues in manure." *Chemosphere*, 224, 544–553.

Jechalke, S., Heuer, H., Siemens, J., Amelung, W., and Smalla, K. (2014). "Fate and effects of veterinary antibiotics in soil." *Trends in Microbiology*, Elsevier Ltd, 22(9), 536–545.

Kemper, N. (2008). "Veterinary antibiotics in the aquatic and terrestrial environment." *Ecological Indicators*, 8(1), 1–13.

Kim, K. R., Owens, G., Kwon, S. I., So, K. H., Lee, D. B., and Ok, Y. S. (2011). "Occurrence and environmental fate of veterinary antibiotics in the terrestrial environment." *Water, Air, and Soil Pollution*, 214(1–4), 163–174.

Kreuzig, R., and Holtge, S. (2005). "Investigations on the fate of sulfadizine in manured soil: laboratory experiments and test plot studies." *Environmental Toxicology and Chemistry*, 24(4), 771–776.

Kumar, K., Gupta, S. C., Baidoo, S. K., Chander, Y., and Rosen, C. J. (2005). "Antibiotic Uptake by Plants from Soil Fertilized with Animal Manure." *Journal of Environment Quality*, 34(6), 2082.

Kümmerer, K. (2009). "Antibiotics in the aquatic environment - A review - Part I." *Chemosphere*, Elsevier Ltd, 75(4), 417–434.

Larney, F. J., Olson, A. F., Carcamo, A. A., and Chang, C. (2000). "Physical changes during active and passive composting of beef feedlot manure in winter and summer." *Bioresource Technology*, 75(2), 139–148.

Lee, L. S., Carmosini, N., Sassman, S. A., Dion, H. M., and Sepúlveda, M. S. (2007). "Agricultural Contributions of Antimicrobials and Hormones on Soil and Water Quality." *Advances in Agronomy*, 93(SUPPL.), 1–68.

Lewicki, J., Reeves, P. T., and Swan, G. E. (2004). *Tylosin*.

De Liguoro, M., Cibin, V., Capolongo, F., Halling-Sørensen, B., and Montesissa, C.

(2003). “Use of oxytetracycline and tylosin in intensive calf farming: Evaluation of transfer to manure and soil.” *Chemosphere*, 52(1), 203–212.

Marshall, B. M., and Levy, S. B. (2011). “Food animals and antimicrobials: Impacts on human health.” *Clinical Microbiology Reviews*, 24(4), 718–733.

Martínez-Carballo, E., González-Barreiro, C., Scharf, S., and Gans, O. (2007). “Environmental monitoring study of selected veterinary antibiotics in animal manure and soils in Austria.” *Environmental Pollution*, 148(2), 570–579.

Martinez, J. L. (2009). “Environmental pollution by antibiotics and by antibiotic resistance determinants.” *Environmental Pollution*, Elsevier Ltd, 157(11), 2893–2902.

Martinez, J. L., Fajardo, A., Garmendia, L., Hernandez, A., Linares, J. F., Martínez-Solano, L., and Sánchez, M. B. (2009). “A global view of antibiotic resistance.” *FEMS Microbiology Reviews*, 33(1), 44–65.

Martinez, J. L., Fajardo, A., Garmendia, L., Hernandez, A., Linares, J. F., Martínez-Solano, L., Sánchez, M. B., Regassa, T. H., Educator, A. E., Koelsch, R. K., Dean, A., Briyne, N. De, Atkinson, J., Pokludová, L., Borriello, S. P., Marshall, B. M., Levy, S. B., Mina, O., Gall, H. E., Saporito, L. S., Elliott, H. A., Kleinman, P. J. A., Thompson, C. S., Traynor, I. M., Fodey, T. L., Faulkner, D. V, Crooks, R. H., Heuer, H., Schmitt, H., Smalla, K., Knapp, C. W., Dolfing, J., Ehlert, P. a I., Graham, D. W., Wise, R., McEwen, S. A., Fedorka-Cray, P. J., Kümmerer, K., Kumar, K., Gupta, S. C., Baidoo, S. K., Chander, Y., and Rosen, C. J. (2017). “Antimicrobial resistance: Priorities for action.” *Clinical Microbiology Reviews*, Elsevier Ltd, 14(4), 120–125.

Mellon, M., Benbrook, C., and Benbrook, K. L. (2001). “Hogging it.” *Estimates of antimicrobial abuse in livestock*, (January), 7–9.

Miller, J. J., Handerek, B. P., Beasley, B. W., Olson, E. C. S., Yanke, L. J., Larney, F. J., Mcallister, T. A., Olson, B. M., Selinger, L. B., Chanasyk, D. S., and Hasselback, P. (2004). “Quantity and Quality of Runoff from a Beef Cattle Feedlot in Southern Alberta.” 1097, 1088–1097.

Mina, O., Gall, H. E., Saporito, L. S., Elliott, H. A., and Kleinman, P. J. A. (2017). “Agriculture , Ecosystems and Environment Relative role of transport and source-limited controls for estrogen , TDP , and DOC export for two manure application methods.” *Agriculture, Ecosystems and Environment*, Elsevier, 247(April), 308–318.

De O. Silva, R., De Menezes, M. G. G., De Castro, R. C., De A. Nobre, C., Milhome, M. A. L., and Do Nascimento, R. F. (2019). “Efficiency of ESI and APCI ionization sources in LC-MS/MS systems for analysis of 22 pesticide residues in food matrix.” *Food Chemistry*, Elsevier, 297(June), 124934.

Papich, M. G. (2016). “Florfenicol.” *Saunders Handbook of Veterinary Drugs*, 327–329.

Pareek, S., Mathur, N., Singh, A., and Nepalia, A. (2015). “Review Article Antibiotics in

the Environment : A Review." 4(11), 278–285.

Rothrock, M. J., Keen, P. L., Cook, K. L., Durso, L. M., Franklin, A. M., and Dungan, R. S. (2016). "How Should we be determining background and baseline antibiotic resistance levels in agroecosystem research?" *Journal of Environmental Quality*, 45(2), 420–431.

Sandoz, M. A., Wooten, K. J., Clendening, S. L., Hensley, L. L., Smith, L. R., and Smith, P. N. (2018). "Transport mechanisms for veterinary pharmaceuticals from beef cattle feedyards to wetlands: Is aerial deposition a contributing source?" *Agriculture, Ecosystems and Environment*, Elsevier, 252(September 2017), 14–21.

Sarmah, A. K., Meyer, M. T., and Boxall, A. B. A. (2006). "A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment." *Chemosphere*, 65(5), 725–759.

Schering-Plough Animal Health Corp. (2007). *ENVIRONMENTAL ASSESSMENT FOR AQUAFLOR FOR FRESHWATER-REARED SALMONIDS*.

Schmidt, D., and Wilson, B. (2011). "Montana Feedlot Annualized Runoff Model (MontFARM)." (November), 1–41.

Shaw, S. P. (2014). "β-lactam antimicrobials." *Small Animal Critical Care Medicine, Second Edition*.

Sura, S., Degenhardt, D., Cessna, A. J., Larney, F. J., Olson, A. F., and McAllister, T. A. (2015). "Transport of three veterinary antimicrobials from feedlot pens via simulated rainfall runoff." *Science of the Total Environment*, Elsevier B.V., 521–522, 191–199.

Tasho, R. P., and Cho, J. Y. (2016). "Veterinary antibiotics in animal waste, its distribution in soil and uptake by plants: A review." *Science of the Total Environment*, Elsevier B.V., 563–564(3), 366–376.

Thiele-bruhn, S. (2003). "Pharmaceutical antibiotic compounds in soils - a review."

"U.S. Meet Animal Research Center: Clay Center, NE." (n.d.). U.S. Department of Agriculture.

US EPA. (2004). "Risk assessment Evaluation for concentrated animal feeding operations." *National Service Center for Environmental Publications (NSCEP)*.

USDA. (1986). "Urban Hydrology for Small Watersheds." *Soil Conservation*, (Technical Release 55 (TR-55)), 164.

Winckler, C., and Grafe, A. (2000). "Use of Veterinary Drugs in Intensive Animal Production Evidence for Persistnece of Tetracylines in Pig Slurry." *Methods*, 1(2), 66–70.

Wise, R. (2002). "Antimicrobial resistance: Priorities for action." *Journal of Antimicrobial Chemotherapy*, 49(4), 585–586.

Yoshida, N., Cirelli, A. F., and Castro, M. J. L. (2013). "Effect of feedlot design and

management on the fate and distribution of monensin." *Chemistry and Ecology*, 29(4), 379–390.

Zhao, L., Dong, Y. H., and Wang, H. (2010). "Residues of veterinary antibiotics in manures from feedlot livestock in eight provinces of China." *Science of the Total Environment*, Elsevier B.V., 408(5), 1069–1075.

Žižek, S., Hrženjak, R., Kalcher, G. T., Šrimpf, K., Šemrov, N., and Zidar, P. (2011). "Does monensin in chicken manure from poultry farms pose a threat to soil invertebrates?" *Chemosphere*, 83(4), 517–523.