# MANURE AMMONIA AND GREENHOUSE GAS EMISSIONS FROM CONDENSED TANNIN FED BEEF CATTLE

by

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#### ABSTRACT

Greenhouse Gas (GHG), Ammonia (NH<sub>3</sub>), and Hydrogen Sulfide (H<sub>2</sub>S) emissions from confined animal feeding operations (CAFO) are an emerging concern due to the potential harm to human and animal health and the environment. A study was conducted to determine the effects of three levels of condensed tannins (0, 0.5, and 1.0%, DM basis)fed to 27 beef steers on NH<sub>3</sub> and GHG emissions from manure. Manure and urine were collected from two periods over 6 days. Feces and urine were placed in inert plastic containers and stored separately at -4° C until analysis. Feces and urine were placed in 16.7 x 16.7 x 17 cm plastic chambers and urine was topically added. Gas samples were collected every 24 hours for 1 week, then every 48 hours for 1 week. Headspace samples were injected into a GHG gas chromatograph (GC) for analysis. NH<sub>3</sub> concentrations were measured using a handheld electronic gas detector. H<sub>2</sub>S concentrations were measured using a Jerome 631-x hydrogen sulfide analyzer. Tannin inclusion at the 0, 0.5, and 1.0% treatment levels showed a 0, 51, and 57% reduction of  $NH_3$  concentrations in the headspace, respectively (P < 0.001). Tannin inclusion in the diet increased CO<sub>2</sub> headspace concentrations (P=0.028). There was no treatment effect on N<sub>2</sub>O emissions, ( $P\geq0.123$ ). Results indicate that condensed tannins fed to beef cattle can effectively reduce gaseous NH<sub>3</sub> emissions from confined beef animal facilities.

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#### Chapter 1

## Introduction

The United States is the world leader in the fed cattle industry. Nationwide, cattle on feed numbers as of June 1, 2016 were 10.8 million head (NCBA, 2016). This is an increase of 2% from June 1, 2015. Texas leads the nation for cattle on feed with 2.44 million head of cattle (USDA, 2016).

Greenhouse gases (GHG) are theorized to trap heat in the atmosphere, in a phenomenon known as the greenhouse effect. The greenhouse effect, along with natural causes, may contribute to global climate change, including shifts in ecosystems, intensity of severe weather events, and change in temperature and precipitation (Cubasch et al., 2013). While GHGs are essential for human life, and occur from natural sources, human activity is thought to be causing an increase in GHG concentrations present in the atmosphere. The United States 2014 GHG emissions reported by the USEPA (2016a) by gas, are carbon dioxide (CO<sub>2</sub>) 80.9%, methane (CH<sub>4</sub>) 10.6%, nitrous oxide (N<sub>2</sub>O) 5.9%, and fluorinated gases 2.6% (figure 1).

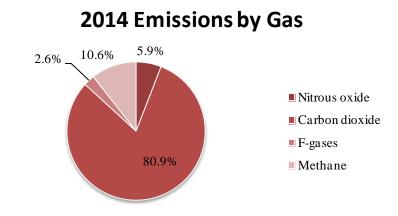


Figure 1: 2014 US Greenhouse Gas Emissions by Gas (USEPA, 2016a)

Greenhouse gas emissions as a result of human activity are: Agriculture 9.1%, Commercial and Residential 12.3%, Industry 21.3%, Transportation 26.3%, and electricity 30.3% (USEPA, 2016a)(figure 2). The breakdown of agricultural emissions is represented in figure 3. Gaseous emissions from livestock raised in Confined Animal Feeding Operations (CAFO) are considered anthropogenic emissions, because the cattle are raised by humans as a food source.

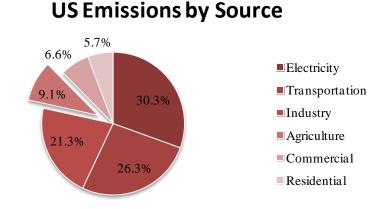


Figure 2: 2014 US Emissions by Sector (USEPA, 2016a)

# **Agricultural Emissions**

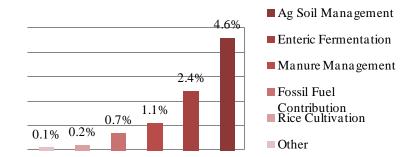


Figure 3: Break down of 9.1 % Agricultural Emissions for the US 2014

In addition to GHG, ammonia (NH<sub>3</sub>) and hydrogen sulfide (H<sub>2</sub>S) are produced in beef cattle feeding operations. Ammonia emissions, primarily from animal wastes, contribute 70-90% of all ammonia emissions in the United States (McQuilling and Adams, 2015). Emissions of NH<sub>3</sub> and H<sub>2</sub>S greater than 45.4 kg/day are subject to reporting requirements under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA; 40 C.F.R. Part 302), and the Emergency Planning and Community Right-to-Know Act, due to potential environmental and human health impacts (USEPA, 2004; Leytem et al., 2011; Dai et al., 2015). The importance of reducing gaseous emissions from beef cattle feed operations has grown as public awareness and scrutiny has increased.

#### Methane

Methane is a colorless, odorless gas, which is extremely flammable (NCBI, 2016). Methane is a natural byproduct of anaerobic bacteria decomposition in areas such as wetlands and marshes.

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According to the US Environmental Protection Agency (USEPA, 2016a) CH<sub>4</sub> from human activity, accounts for 60% of all atmospheric CH<sub>4</sub>, and is the second most prevalent greenhouse gas emitted in the United States. Methane emissions are becoming an emergent concern because CH<sub>4</sub> is more efficient than CO<sub>2</sub> at trapping radiation, and is thought to have 25 times the climate change potential of CO<sub>2</sub> (USEPA, 2016a).

Methane emissions account for 3.5% of the agricultural GHG emissions (9.1%) totals (USEPA, 2016a). Methane is produced as a part of the natural digestive process in ruminant animals, known as enteric fermentation. Beef cattle are the greatest ruminant contributors of CH<sub>4</sub> (71%), with followed by 25.5% from dairy cattle, and 3.5% from other ruminant animals (USEPA, 2016a). Ruminant animals raised in feeding facilities are thought to contribute between 15 and 33% of global CH<sub>4</sub> emissions (Bodas et al., 2012). Hydrogen produced during ruminal fermentation leads to the generation of methane (Buddle et al., 2011). Methanogens alleviate H<sub>2</sub> concentration in the rume by reducing CO<sub>2</sub>, which results in CH<sub>4</sub> formation and eradication by the animal (Bodas et al., 2012). Ruminant livestock eradicate 6% of their ingested energy as CH<sub>4</sub>, and can produce 250- 500L of CH<sub>4</sub> per day (Johnson and Johnson, 1995).

Manure storage that promotes anaerobic conditions also produces  $CH_4$ . Manure management contributes 12.1% of agricultural GHG emissions, and 8.4% of all agriculture related  $CH_4$  emissions (USEPA, 2016a). The organic matter present in manure is broken down by anaerobic bacteria and methane is a byproduct of the anaerobic breakdown. Temperature, moisture, storage, neutral PH, and residence time

effect the amount of methane produced because these factors influence the growth of bacteria responsible for CH<sub>4</sub> production (S mith et al., 2014).

## Nitrous Oxide

The global warming potential of  $N_2O$  is 298 times  $CO_2$  (Cubasch et al., 2013). Nitrous oxide is a colorless, odorless, non flammable gas (NCBI, 2016). Nitrous oxide emissions contribute 4.9% of all agricultural greenhouse gas emissions (USEPA, 2016a). Manure storage and management contributes to 5.2% of agricultural  $N_2O$  emissions. Agricultural soil management is the largest source of  $N_2O$  emissions from this sector at 78.9% (USEPA, 2016a).

Cattle consume nitrogen (N) in the form of crude protein (Powell et al., 2011), and convert into body tissue or excrete the excess in feces and urine (Parker et al., 2005). The organic N excreted in feces is made up residues that are mineralized at a much slower rate than volitilization from urea. Nearly equal proportions of N is excreted in feces and urine, with most urinary N, 50-70% present in the form of urea ( $CO(NH_2)_2$ ) (Misslebrook et al., 2005). Urea, when excreted onto soil, is converted by denitrifying bacteria to N<sub>2</sub>O, and volatized to the atmosphere (Kronberg and Liebig, 2011)(Figure 4).

 $N_2O$  losses are primarily linked to nitrification-denitrification, and volatization of  $NH_3$  (Havlin et al., 2007).

Nitrification of NH<sub>3</sub>:

$$NH_{3} + O_{2} \rightarrow NO_{2}^{-} + 3H$$
(1)  
$$NO_{2}^{-} + H_{2}O \rightarrow NO_{3}^{-} + 2H^{+} + 2e^{-}$$
(2)

Biochemical Denitrification of Nitrate (NO<sub>3</sub><sup>-</sup>):

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O^{\uparrow} \rightarrow N_2^{\uparrow}$$

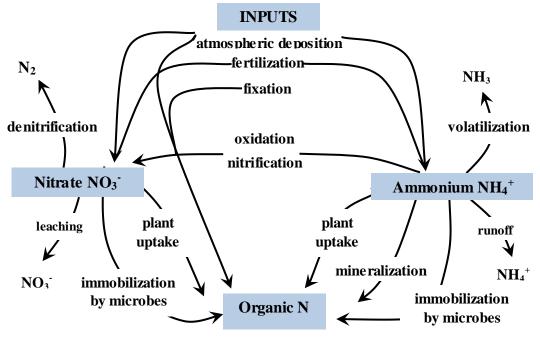


Figure 4: Nitrogen Cycle (adapted from learner.org)

## Ammonia

Ammonia is a colorless gas with a pungent, suffocating odor. Ammonia is considered a high health hazard because it is corrosive to the skin, eyes, and lungs. Exposure to 300 parts per million(ppm) NH<sub>3</sub> is immediately dangerous to life and health (NIOSH, 2016). Ammonia is produced commercially, primarily for use as a fertilizer. It is also naturally occurring, from decomposition of organic matter, including plants, animals, and animal wastes. Seventy to 90% of all NH<sub>3</sub> emissions in the United States are from livestock operations. (Pinder et al., 2004; McQuilling and Adams 2015). Ammonia produced in CAFOs comes from urine spots that have volatized from urea, and from mineralization of feces (Todd et al., 2008).

Ammonification:

 $CO(NH_2)_2 + H^+ + 2H_2O \leftrightarrow 2 NH_4^+ + HCO_3^-$ 

Hydrolysis:

 $2 \text{ NH}_4^+ + \text{HCO}_3^+ + 2\text{H}^+ \rightarrow 2\text{NH}_4^+ + \text{CO}_2\uparrow + \text{H}_2\text{O}$ 

Conversion of  $NH_3$  from Ammonium ( $NH_4^+$ ):

 $NH_4^+ + OH^- \leftrightarrow H^+ + NH_3$ 

Ammonia losses begin directly after urine deposition and continue throughout manure handling and land application (Misslebrook et al., 2005). According to Stewart (1970) 25-90% of urinary N is volatized to NH<sub>3</sub> within 48 hours of excretion. Pen surface temperature, air temp, wind speed, moisture content, pH, and N concentration at the source all effect the rate of volatization (Rhoades, 2009). Manure surface pH can be altered by the release of  $CO_2$  which can accelerate NH<sub>3</sub> emission (Ni et al., 2000; Dai et al., 2015).

Ammonia is a precursor to  $PM_{2.5}$ , which is considered hazardous to human health (Todd et al., 2008; McQuilling and Adams, 2015). Ammonia reacts with acidic compounds like sulfate and nitrate to form particulates (Todd et al., 2008) (figure 4).



Figure 5: Formation of Fine Particulate Matter From Ammonia (Hristov et al., 2011)

Concerns over potential health hazards from  $PM_{2.5}$  emissions have led to regulations by the USEPA National Ambient Air Quality. Particulate Matter has an aerodynamic diameter of < 10µM (PM<sub>10</sub>) or < 2.5µM (PM<sub>2.5</sub>) (Leytem et al., 2011; Hristov et al., 2011). According to the World Health Organization, air pollution related deaths and illnesses are most closely related to exposure to small particulate matter  $\leq$ 10µM.

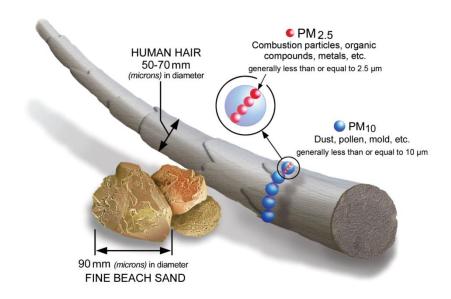


Figure 6: Graphic Showing Relative Size of Particulate Matter (USEPA 2016b)

## **Carbon Dioxide**

Carbon Dioxide is a gas that is naturally present in the Earth's atmosphere as part of the Carbon cycle. 80.9% of all CO<sub>2</sub> emissions are the result of human activity (USEPA, 2016a). Both animal waste and animal respiration contribute to the carbon cycle. Trace amounts of CO<sub>2</sub> are emitted from manure, with the majority of emissions from CAFOs being attributed to energy use from manure management (Beauchemin et al., 2010). Temperature, air velocity, and mass concentration influence CO<sub>2</sub> emission from manure (Ni et al., 2000).

## Hydrogen Sulfide

Hydrogen sulfide is a gas of concern due to the danger to human and animal health. Hydrogen sulfide has a strong odor of rotten eggs at low concentrations, is highly flammable, explosive and can be incapaciting or deadly in high concentrations (NCBI, 2016). Under anaerobic conditions, sulfate-reducing bacteria (SRB) produce H<sub>2</sub>S (Whitehead et al., 2013). Hydrogen sulfide is a concern in all CAFOs, but is an elevated concern in enclosed feed facilities, where high concentrations have led to human health issues and animal deaths (Dai et al., 2015). Regulatory agencies have proposed the measurement of sulfide levels in animal feeding operations, as a means to control odor (Whitehead et al., 2013).

#### **Oxidation Reduction Potential**

Oxidation Reduction Potential (ORP) measures a substance's capacity to either release or accept electrons (Suslow et al., 2004). Oxidation is electron acceptance, and is associated with aerobic conditions. Anaerobic conditions are associated with reduction, which is the release of electrons. Low mV readings represent reduction and high mV readings represent oxidation. Masscheleyn et al. (1993), in a study of  $CH_4$  and  $N_2O$  emissions from rice paddies, found that an ORP of -150 mV was critical for the formation of  $CH_4$  and the highest  $N_2O$  concentrations were recorded when ORP was +400mV.

#### Tannins

Tannins are a group of compounds that plants have developed to deter predation by herbivores (Krueger et al., 2010). Red hued seed coats and fruit skins can be an indication of the presence of tannins. Tannins produce an astringent taste in plants when consumed (Mezzomo et al., 2011). Tannin formation occurs as a response to stress or death in plants. Two types of tannins exist in nature, Hydrolyzable Tannins (HT) and proanthocyanidins, more commonly known as Condensed Tannins (CT) (Reed, 1995; Naumann, et al. 2013). They are typically differentiated by molecular weight with CT having a higher molecular weight than HT (Frutos et al., 2004). The higher molecular weight of CT many reduce the ability for tissue absorption. Hydrolyzable tannins can destroy ruminal microbes which can lead to potentially toxic effects in ruminants (Reed, 1995).

Condensed tannins are water soluble polyphenolic compounds synthesized by plants that bind and precipitate proteins (Hagerman et al. 1992; Naumann et al., 2013). Herbaceous plants such as alfalfa and cotton have CT present in their seed coats and hulls (Waghorn et al., 1999). Quebracho tannins are derived from the South American evergreen tree Quebracho Colorado(*Schinopsis balansae*) (Streit and Fengel 1994). Quebracho tannin is a CT produced commercially that has been used as a feed supplement to reduce gaseous emissions from ruminants. Condensed tannins are reduce the amount of ruminal NH<sub>3</sub> produced which improves the efficiency of urea recycled to the rumen (Reed, 1995). Condensed tannins reduce N excretion in urine by shifting the excreted N from the urine to feces (Kronberg and Liebig, 2011). The biological activities of condensed tannins are related to a combination of factors including molecular weight, degree of polymerization, stereochemistry, hydroxylation, and functional groups within the polyphenolic compound (Naumann et. al. 2013).

Tannin studies are increasing in utilizing tannins to decrease gaseous emissions from ruminants to improve N utilization and reduce urinary N excretion (Hagerman et et al., 1992; Powell et al., 2011). Livestock whose feed has been supplemented CT has shown a reduction in the amount of ammonia produced in the rumen, which improves the absorption of fed N by ruminants (Bodas et al., 2012).

The objective of this study was to investigate the effect of feeding beef cattle diets supplemented with condensed tannins on GHG, NH<sub>3</sub>, and H<sub>2</sub>S emissions from manure in laboratory simulated feedyard conditions.

## Chapter 2

### **Materials and Methods**

Ammonia, greenhouse gas, and hydrogen sulfide concentrations were measured from steers fed diets supplemented with condensed tannin (CT) extract, in a laboratory chamber emission study designed to simulate feedyard conditions. The experiment was located in the Kilgore Research Center Environmental Agriculture Laboratory, on the West Texas A&M University Campus in Canyon, Texas. The chamber study took place over a 45 day period beginning in February 2016. 54 samples of feces and urine, collected previously, were analyzed in three separate runs. Each run utilized 20 chambers for 15 days. Of the 20 chambers, eighteen chambers contained slurry from steers, and two chambers contained no slurry and were utilized as blanks. Headspace concentrations were sampled every 24 hours for 8 days and then every 48 hours for the remainder of the run.

### **Cattle Feeding and Sample Collection**

Cattle were fed, and feces and urine were collected from steers as described in Ebert et al. (2016). Briefly, 27 angus-cross steers (initial body weight =  $350 \pm 32$  kg) were assigned, by initial body weight, randomly to 1 of 3 CT supplementation treatments. Treatments were three levels of CT added to feed at 0% tannin, 0.5% tannin, and 1.0% tannin on a dry matter basis. Steers were fed using the Calan Broadbent Feeding System (American Calan, Northwood, N.H.) to monitor individual dry matter intake (DMI), and steers had *ad libitum* access to feed. Steers were housed in 3 pens with 9 Calan gates. The diet fed to steers contained 14.0% crude protein (CP), 1.50 NEg Mcal/kg, 8.5% roughage, 60% steam-flaked corn, 15% wet distiller's soluble and 6% fat (DM basis, Table 1). Condensed tannins were added to feed after a grain adaptation period of 21 days.

Table 1. Diet composition	
Ingredient composition	DM, %
Steam flaked com	60.3
Com wet distiller's grains	15.0
Condensed distiller's soluble	10.0
Sorghum stalks	8.5
Trace mineral premix <sup>1</sup>	3.0
Limestone	1.15
Yellow grease	0.95
Urea	0.8
Salt	0.3
Analyzed nutrient composition <sup>2</sup>	
DM, %	58.65
CP, %	14.0
Starch, %	57.5
NDF, %	16.3
ADF,%	13.4
Ether extract, %	6.1
Ca,%	0.53
P,%	0.45
S,%	0.2
NEm, Mcal/kg <sup>3</sup>	2.16
NEg,Mcal/kg <sup>3</sup>	1.47

<sup>1</sup>Provided to diets (per kg of DM): 0.1 mb Co, 6.3 mg Cu, 0.6mg 1, 37mg Fe, 27 mg Mn, 0.2 mg, Se, 40 mg Zn, 1440 IU Vitamin A, 148 IU Vitamin D, 59 mg Vitamin E, 26 mg Monesin (Elanco Animal Health, Indianaplois, IN), and 5.9 mg Tylosin (Elasnco Animal Health).

<sup>2</sup>Nutrient analysis conducted by commercial laboratory (Servi-Tech Labaoratories, Amarillo, TX.)

<sup>3</sup>Calculated from nutrient analyses according to NRC (1996).

Condensed tannins ((By-Pro: Quebracho extract), 95% tannic acid, Silvateam, Ontario, CA)) were mixed daily with a basal diet to form a premix (10% CT, and 90% basal diet), and were fed as a top dress at either 0.5 or 1% of the diet DM. Dry matter content was adjusted following ingredient sampling and complete diet samples were taken prior to mixing and feeding. Fecal and urine samples were taken over two periods. Period 1 fecal and urine samples were collected after steers were on feed for 34 days. Period 2 samples were collected after 95 days on feed. Urine and feces were collected for 6 days per period, twice daily, with a 2 hour advancement every 24 hours. Urine samples were collected by external catheter. Feces were collected by rectal palpation. Urine was frozen immediately, at -20°C to prevent nitrogen (N) loss. Steers were fed for an average of 126 days to an average final BW of  $601 \pm 50$  kg. Feces and urine samples were frozen and stored until utilized in the chamber study.

#### Chamber Apparatus Design

The design of the chamber apparatus is similar to the one described in Parker et al. (2005). The chamber design was comprised of 40 - 16.7 X 16.7 X 16.7 cm Tupperware® containers (Figure 7). Each container was attached, by equal lengths Teflon® tubing, to a NH<sub>3</sub> collection trap containing 100 mL 0.2N sulfuric acid (H<sub>2</sub>SO<sub>4</sub>). Each acid trap was connected to a common center cylinder using equal length tubing to ensure equal airflow to all chambers. The common cylinder was connected to a Marathon Electric, Wausau, WI vacuum pump. Ambient air above the manure was pulled through the acid traps by the vacuum pump (Figure 8). Air flow rate was measured with a flow meter connected to the apparatus, and maintained at 1.4 L/min in each chamber.



Figure 7: Photograph of Chamber Apparatus

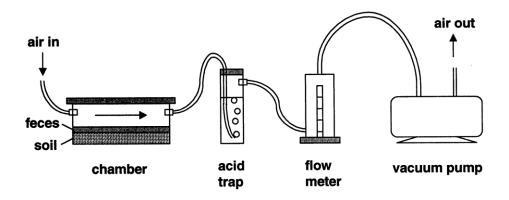


Figure 8: Schematic of Chamber System (Parker et al., 2005)

54 samples were analyzed in three separate runs. Each run utilized 20 chambers. Of the 20 chambers, eighteen chambers contained slurry from steers, and two chambers contained no slurry, and were utilized as blanks. Twenty chambers were utilized per run, with three runs total for the chamber experiment. Urine and feces were thawed, in their original containers, at room temperature for 24 hours. Urine and feces were weighed separately and combined at a urine to feces ratio of 0.5:1 by weight (Figure 9).

Initial temperature, pH, electrical conductivity (EC), and oxidation reduction potential (ORP) measurements were taken prior to adding the slurry to each chamber. Chambers containing slurry were layered with 1.11 kg of calcined clay, a sheet of Tedlar<sup>®</sup>, and urine and feces, to replicate a feed yard pen surface. Beef cattle feed yards in the Texas Panhandle are outdoors, with native soil, typically a clay variety, under a hard compacted layer. Cattle deposit urine and feces on top of the compacted layer.



Figure 9: Photograph of Urine and Feces Being Combined

Two chambers were blanks, with no feces or urine added. The clay, Tedlar<sup>®</sup>, and feces/urine mixture were approximately 6 cm thick in each chamber, leaving approximately 11 cm for headspace accumulation of gases (Figure 10).



Figure 10: Manure Added to Chambers

Chamber lids were immediately sealed and air flow was started. Urine was added to each chamber every 48 hours, on days 3, 5, 7, 9, and 11, until a urine to feces ratio of 3:1, by weight, was achieved. Urine to feces ratios were determined by the average daily urine and fecal output from the steers.

## Sampling

Air flow through the chambers was maintained throughout the sampling process. All headspace gas concentrations were sampled from each chamber within on minute of opening the container lid in order to reduce gaseous losses, and were closed immediately following the collection of samples. Chambers were sampled every 24 hours for the first 7 days, and then every 48 hours for the remainder of each 15 day run. As part of quality control/quality assurance blank air samples, and spike samples were collected and analyzed, in addition to the 2 blank chambers each sample collection day.

Ammonia concentrations were measured with a handheld Mannings Systems, Inc., EC-P2 (Honeywell Industries<sup>®</sup>, Lincolnshire, IL) gas detector. Measurements were taken from directly from chambers.

Greenhouse gas samples were collected with a Pressure-Lok<sup>®</sup> Precision Analytical Syringe (Valco Instruments Co. Inc.<sup>®</sup>, Houston, TX). 1.0 mL samples were immediately injected into a SRI 8610 C Gas Chromatograph (SRI Instruments<sup>®</sup>, Torrence, CA). Output from the GC was displayed and recorded with Peak Simple Software (SRI Intsruments<sup>®</sup>, Torrence, CA) (figure 12). Samples were analyzed for CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O concentrations.

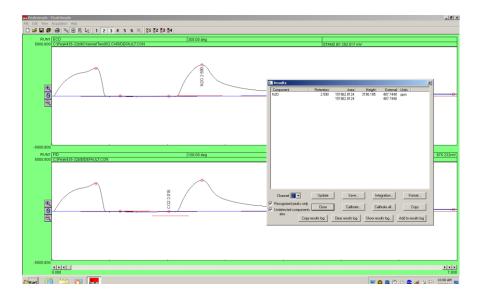


Figure 11: SRI 8610 Peak Simple Display of GHG Results

Hydrogen sulfide concentrations were measured using a Jerome<sup>®</sup> 631-X Hydrogen Sulfide Analyzer (Arizona Instrument LLC, Chandler, AZ).



Figure 12: Sampling Heads pace NH<sub>3</sub> and H<sub>2</sub>S Concentrations

Total nitrogen (TN) was calculated from  $NH_3$  concentrations and  $N_2O$  headspace concentrations present in the chambers. The concentrations of  $N_2O$  (ppb) and  $NH_3$  (ppm) were converted from ppm to mg/m<sup>3</sup>.

$$mg/m^3 = (ppmv)(MW)(atm) / (R * T(K))$$

Where:

- atm= pressure
- MW= molecular weight
  - $\circ$  N2O = 44.013
  - NH3 = 17.031
- R= ideal gas constant (0.08206 L atm/(mol K))
- T= Temperature (Kelvin)

The amount of N flux  $(mg/m^2h)$  present in the chambers was then calculated by the following equation.

$$J = \Delta CQ/A$$

Where:

- $J = flux (mg/m^2h)$
- $\Delta C = mg/m^3 (C_{out} C_{in})$
- Q = Flow Rate (L/min)
- $A = Surface Area (m^2)$

The amount of N was calculated by taking the percentage of nitrogen present in each compound and adding the N from  $NH_3$  flux calculated to N from  $N_2O$ .

 $TN = (N_2O mg/m^2h) (63.6\% N) + (NH3 mg/m2h) (82.24\% N)$ 

Initial temperature, pH, EC, and ORP was measured before the slurry was added on top of the Tedlar<sup>®</sup>, with a sensION<sup>TM</sup>+MM150 (Hach<sup>®</sup>, Loveland, CO) (figure 13). Final temperature, pH, EC, and ORP was measured at the conclusion of each run. Feces and urine mixtures were frozen for further CH<sub>4</sub> analysis.



Figure 13: Measuring Initial ORP, Temperature, EC, and pH

#### **Statistical Analysis**

Statistical analysis was performed using SPSS Version 22. Ammonia,  $H_2S$ ,  $CO_2$ , and  $N_2O$  headspace concentration differences and TN were analyzed as a general linear model in univariate analysis of variance (UNIANOVA) with two independent variables in a completely randomized design. Fixed factors were period and treatment, allowing for analysis of treatment and period effects and treatment by period interaction. Chamber served as experimental unit.

Change in pH, EC, and ORP were determined from day 1 and day 15 values. Delta pH, EC, and ORP were analyzed as a general linear model in UNIANOVA, with two independent variables. Chamber served as experimental unit. Fixed factors were period and treatment. There were no interactions between period and treatment (P<0.05). Mean separation was determined with Tukey's HSD when ANOVA indicated significant differences. Significance was determined at  $\alpha \le 0.05$ .

#### Chapter 3

### **Results and Discussion**

#### Ammonia

Mean NH<sub>3</sub> concentrations by treatment are presented in Table 2. The greatest mean concentrations were found in the control (63.95 ppm), while inclusion of CT at 0.5% and 1% resulted in significantly lower (P < 0.01) concentrations at 32.89 and 36.57 ppm, respectively. There was no difference among the 0.5% treatment and the 1% CT supplement treatment. However, the means for the 1% supplement level were greater than those in the 0.5% treatment. Headspace concentrations of NH<sub>3</sub> were decreased by 48.57% in the 0.5% treatment, and 42.81% in the 1% treatment.

Treatment means differed in between period 1 and period 2 (P < 0.01)(Table 2, Figure 14). While there was not a statistically significant decrease in urinary N (Ebert et al., 2016), there was an average 34% increase in NH<sub>3</sub> headspace concentration from period 1 to period 2 in this study. The treatment concentration means increased by 18.17% (Control), 45.03% (0.5%), and 48.17% (1%).

There was no treatment by period interaction for ammonia concentration  $(P \ge 0.79)$ .

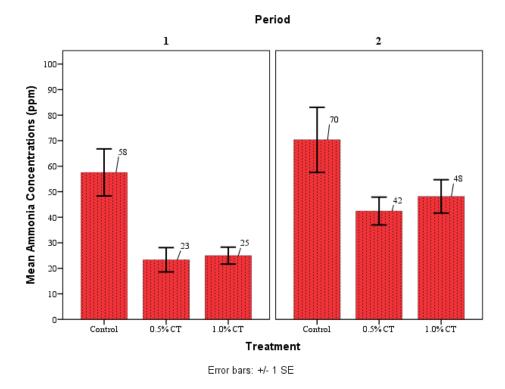


Figure 14: Mean NH<sub>3</sub> Concentrations (ppm) by Period by Treatment

These results are consistent with the findings of Kronberg and Liebig (2011) and Buddle et al. (2011) who did not observe serum urea reductions in beef cattle at or above 1.0% tannin inclusion. Powell et al. (2009) found that high levels of CT included in diet were most effective at reducing cattle manure emissions, when compared to low and medium tannin levels. Misselbrook et al. (2005) conducted a similar manure emission chamber study of cattle fed condensed tannins, and manure from cattle fed condensed tannins emitted lesser amounts of NH<sub>3</sub> than cattle not fed diets supplemented with CT.

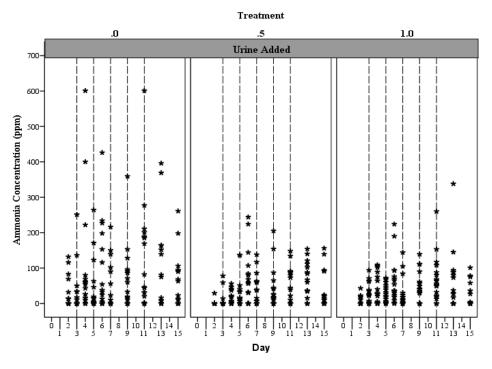


Figure 15: Ammonia Concentrations Following the Addition of Urine. Dotted lines represent days of urine addition.

Headspace concentrations of  $NH_3$  increased in all treatments the day following addition of urine. The greatest increases in headspace concentrations were seen in the control treatment, with lesser increases seen in the 0.5% and 1.0% treatments (Figure 15).

### Nitrous Oxide

Nitrous oxide concentrations did not differ among treatments ( $P \ge 0.12$ )(table 2). However, the greatest mean headspace concentration for N<sub>2</sub>O was in the 0.5% treatment (452.3 ppb). Mean concentrations per treatment were lowest in the control treatment (353.22 ppb), and was slightly increased in the 1% treatment (381.66 ppb). Hao et al. (2011) found an increase in NO<sub>3</sub>-N with the diet CT inclusion. No statistically significant differences in N<sub>2</sub>O emissions were found with tannin inclusion in diet (Hao et al., 2011). There was no interaction between treatment and period ( $P \ge 0.34$ )(table 2). There was no difference in headspace N<sub>2</sub>O concentrations among periods ( $P \ge 0.11$ ). Mean headspace concentrations were greatest in those treatments that included CTs, and were greater in period 1 contrary to the result of CO<sub>2</sub> and NH<sub>3</sub> (table 3, figure 18). In period 1 the greatest mean concentration was in the 0.5% treatment (526.49 ppm), with 0% (361.24 ppm) and 1% (396.48 ppm) having similar concentrations.

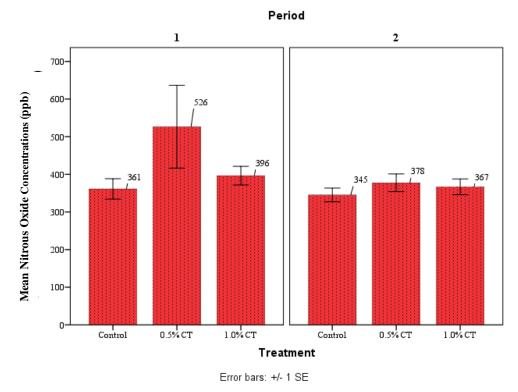


Figure 16: Mean N<sub>2</sub>O Concentrations by Treatment and by Period

Headspace concentrations of  $N_2O$  were not affected by the addition of urine (Figure 19). This could be attributed to the manure layer remaining undisturbed, with applications of urine made topically. During the composting process peak  $N_2O$  emissions are found after turning the compost pile (Maeda. et al., 2010).

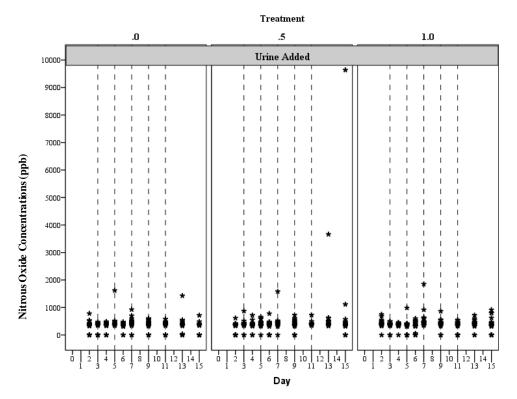
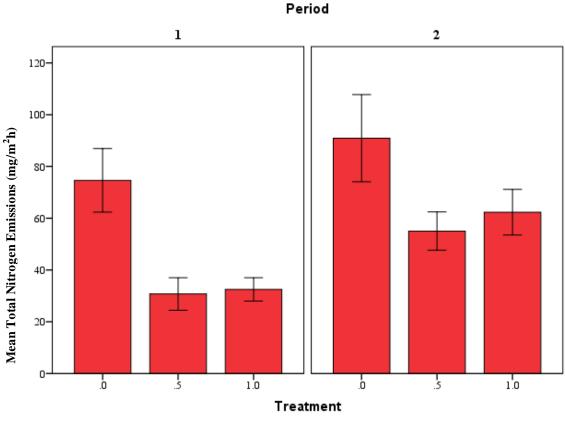


Figure 17: N<sub>2</sub>O Concentrations Following the Addition of Urine

## **Total Nitrogen**

There was no period by treatment interaction for total nitrogen ( $P \ge 0.80$ ). Total nitrogen was greater in the control treatment (82.78 mg/m<sup>2</sup>h)( $P \le 0.01$ ) than the 0.5% (42.88 mg/m<sup>2</sup>h) treatment and the 1% treatment (47.42 mg/m<sup>2</sup>h)(Table 2). There was no difference between the 0.5% and 1.0% treatments (P = 0.89). There was a difference in TN in period 1 and period 2 ( $P \le 0.01$ ). Period 2 TN (45.96 mg/m<sup>2</sup>h) treatment means were greater than period 1 (69.43 mg/m<sup>2</sup>h)(Table 3, Figure 18).



Error bars: +/- 1 SE

Figure 18: TN Emissions (mg/m<sup>2</sup>h) by Period by Treatment

## **Carbon Dioxide**

Carbon dioxide concentrations were greater at 0.5% (8,277.72 ppm)(P=0.04) than in 0% (5946.54 ppm) treatment and the 1% treatment (7394.84 ppm). The means were different among the control and the 0.5% treatment (P=0.03) (Table 2). There was no difference between the means in the 1.0% and the control (P= 0.25) or the 1.0% and 0.5% treatments (P= 0.59)(Appendix A). There was no difference between periods (P≥ 0.91) (Table 3). There was no treatment by period interaction ( $P \ge 0.57$ ).

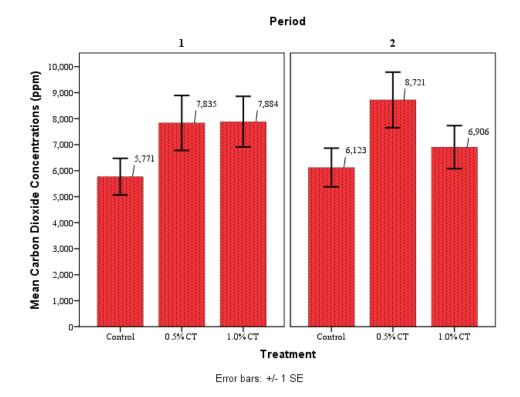


Figure 19: Mean CO<sub>2</sub>, Concentrations, By Treatment and Period

While there was no statistical difference between periods, mean concentrations of  $CO_2$  increased in period 2 (Table 3, Figure 16). In period one, the 0.5% and 1.0% means were similar, while in period 2 the 0.5% treatment mean concentrations were 21% greater than the 1.0% treatment, and 30% greater than the control mean concentrations (Table 3, Figure 16).

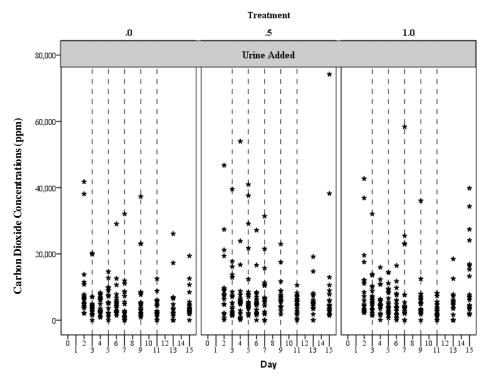


Figure 20: CO<sub>2</sub> Concentrations Following the Addition of Urine, By Treatment

The addition of urine to the chambers did not appear to have an effect on  $CO_2$  concentrations in the headspace. Urine was added to chambers following the collection of samples, and increases were noted before the addition of urine. It is possible that the  $CO_2$  concentrations found in this study were due to higher amounts of C in the feces as opposed to the urine. A study by Al-Kindi et al. (2016) found that while only slight increases were detected, fecal C concentration increased with inclusion level of quebracho tannin extract.

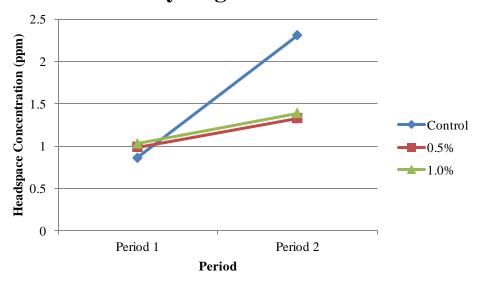
Hao et al. (2011) found that total C content of manure and compost increased with the addition of CT in cattle diets. This same study however showed no difference on  $CO_2$  emissions between diets with CT inclusion and diets without.

#### Hydrogen Sulfide

There was a treatment by period interaction for H<sub>2</sub>S ( $P \le 0.01$ )(Table 3). The interaction between treatment and period is in the control (Figure 20). It does not appear that an interaction occurs due to tannin inclusion in the diet.

There was no treatment effect ( $P \ge 0.12$ )(Table 3) on H<sub>2</sub>S headspace concentrations. The control diet had the greatest mean headspace concentration at 1.59 ppm, while the 0.5% and 1.0% treatment means were 1.16 and 1.12 ppm, respectively.

H<sub>2</sub>S concentrations were greater in Period 2 than in Period 1 (P< 0.01)(Figure 21). The mean control concentrations increased by 62.77%, 0.5% inclusion by 25.56%, and 1.0% inclusion by 25.9% from period 1 to period 2.



# Hydrogen Sulfide

Figure 21: Mean Hydrogen Sulfide Concentrations by Period

The production of  $H_2S$  from manure is primarily a result of Sulfate- reducing bacteria present in manure under anaerobic conditions (Whitehead et al., 2013). Tannins

inhibitory effect on bacteria is one of the reasons that tannins are effective at reducing  $CH_4$  emissions. There is very limited research available on the effect of CT inclusion in cattle diets on  $H_2S$  emissions. In swine manure studies, where CT is added topically to manure after excretion, the addition of 0.5% tannin decreased  $H_2S$  production by greater than 90% over the control (Whitehead et al., 2013).

The addition of urine did not affect H<sub>2</sub>S headspace concentrations (Figure 22).

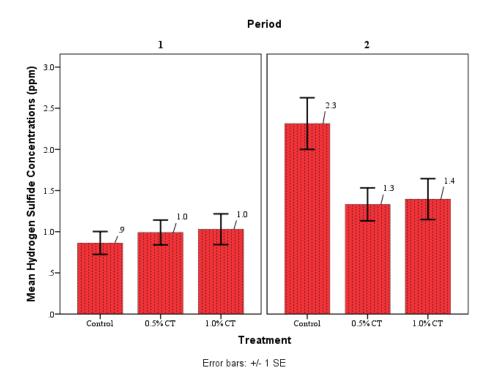


Figure 22: Mean H<sub>2</sub>S Concentrations by Period, by Treatment

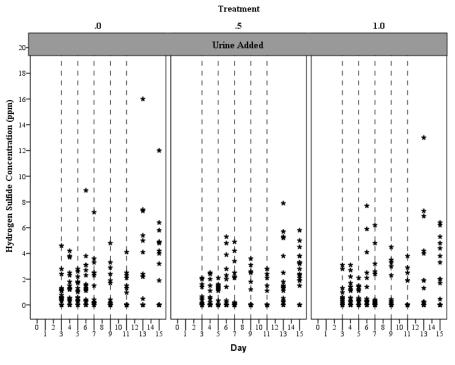


Figure 23: H<sub>2</sub>S Concentrations by Treatment With the Addition of Urine. Dashed lines represent day of urine addition.

#### **Oxidation Reduction Potential, pH, and Electrical Conductivity**

There was no difference in the change in ORP means among treatments (P= 0.22), or periods (P= 0.43). The ORP means were similar among all treatments, but the control had the lowest ORP (-281.606mV), followed by the 1% treatment (-264.728 mV), and the .5% treatment (-241.294 mV) (Table 4). The trend across all treatment and all periods was for the ORP to decrease significantly. Xing and Han (2007) found that dinitrogen-N, Nitrogen-N, and CH<sub>4</sub> emissions linearly increased as ORP was reduced. Nitrous oxide emissions were more correlated with oxidized conditions.

Electical conductivity (EC) is a measure of salinity. Salt, as a result of feed additive, is primarily excreted in the feces of ruminant animals (Azeez et al., 2012).

Change in EC was greater in the 1% tannin treatment than in the 0.5% tannin treatment  $(P \le 0.01)$  (Table 4). There was no difference between the 0% and 0.5% treatments, or Control and 1% treatments. There was no difference in  $\Delta$  EC between periods (P= 0.89).

The change in pH was not different among treatments (P= 0.79), or among periods (P= 0.05) (table 4). Initial pH was slightly acidic or neutral at the beginning of the chamber study and increased to basic levels at the final reading. The constant movement of air over manure is thought to increase pH, and some studies suggest that the increase in pH is due to CO<sub>2</sub> emission, and increased pH drives NH<sub>3</sub> emissions. (Hafner et al., 2013; Petersen et al., 2014).

#### Methane

Methane concentrations were absent through the duration of the study. There are several possibilities for this. First, the conditions inside the chambers were aerobic, due to the constant airflow through the chambers, necessary to pull ammonia emissions through the acid traps. In order for  $CH_4$  to form, conditions must be anaerobic. The ORP in the initial readings and final readings were ideal for the formation of  $CH_4$ . The second possibility is that microbes needed for decomposition were slowed when the feces was frozen and it would take longer for the microbes to regenerate in order to produce  $CH_4$  emissions. Finally it is possible that  $CH_4$  flux was too minute to detect.

	CT	CT extract, % of DM				P-value	
Item	0	0.5	1.0	SEM	Treatment	Period	Interaction
No. of observations	180	180	180				
Gas							
NH <sub>3</sub> ppm	63.95 <sup>a</sup>	32.89 <sup>b</sup>	36.57 <sup>b</sup>	7.67	< 0.001	0.003	0.792
CO <sub>2</sub> ppm	5946.54 <sup>b</sup>	8277.72 <sup>a</sup>	7394.84 <sup>ab</sup>	906.78	0.035	0.907	0.572
N <sub>2</sub> O ppb	353.22	452.03	381.66	49.58	0.123	0.110	0.338
H <sub>2</sub> S ppm	1.59	1.16	1.21	0.22	0.096	< 0.001	0.014
TN mg/m <sup>2</sup> h	82.78 <sup>a</sup>	42.88 <sup>b</sup>	47.72 <sup>b</sup>	4.24	< 0.001	< 0.001	0.801

Table 2: Treatment Mean Heads pace Concentrations of NH<sub>3</sub>, CO<sub>2</sub>, N<sub>2</sub>O, and H<sub>2</sub>S

 $^{a.b}$  means within the same row with different letters differ at  $\alpha=0.05$ 

#### Table 3: Period Mean Heads pace Concentrations of NH<sub>3</sub>, CO<sub>2</sub>, N<sub>2</sub>O, and H<sub>2</sub>S

	CText	ract, % of DM			
Item	0	0.5	1.0	SEM	P-value
No. of observations	90	90	90		
Period 1					
NH₃ ppm	57.55	23.33	24.96	3.73	0.004
CO₂ ppm	5770.57	7834.67	7884.16	534.92	0.907
N₂O ppb	361.24	526.49	396.48	38.75	0.110
H₂S ppm	0.86	0.99	1.03	0.09	<0.001
TN mg/m2h	74.64	30.73	32.50	4.95	0.005
Period 2					
NH₃ ppm	70.33	42.44	48.16	5.14	0.004
CO <sub>2</sub> ppm	6122.52	8720.76	6905.52	516.58	0.907
N₂O ppb	345.21	377.56	366.83	12.11	0.110
H₂S ppm	2.31	1.33	1.39	0.15	<0.001
TN mg/m <sup>2</sup> h	90.93	55.03	62.33	6.83	0.005

Table 4: Change in ORP, EC, and PH by Treatment and Period

	Treatment					Peri	od		
	0	0.5	1	SEM	P-value	1	2	SEM	P-value
$\Delta ORP mV$	-281.606	-241.294	-264.728	9.360	0.221	-255.115	-269.97	9.287	0.431
ΔEC ms/cm	6.749 <sup>a</sup>	-0.102 <sup>b</sup>	2.0133 <sup>ab</sup>	0.834	0.004	2.751	3.023	0.890	0.871
ΔрН	2.306	2.313	2.233	0.52	0.789	2.207	2.361	0.052	0.146

<sup>a.b</sup> means within the same row with different letters differ at  $\alpha = 0.05$ 

Treatment	Period	Initial ORP mV	Final ORP mV	Initial EC ms/cm	Final EC ms/cm	Initial pH	Final pH
Control	1	-145.53	-427.89	6.66	14.09	6.57	8.74
	2	-144.92	-425.78	6.54	12.61	6.55	8.99
0.5%	1	-139.39	-372.81	6.60	6.14	6.52	8.88
	2	-151.91	-408.33	7.06	10.42	6.68	8.92
1.0%	1	-121.96	-371.44	7.23	7.23	6.56	8.68
	2	-139.52	-396.22	8.02	11.00	6.56	8.95

Table 5: Initial and Final ORP, EC and pH by Treatment and Period

### Chapter 4

#### Conclusions

Ammonia concentrations were significantly decreased by inclusion of CTs at the 0.5% and 1.0% level as compared to the control. Period 2 ammonia headspace concentrations were significantly greater than the Period 1 concentrations. As cattle age the amount nitrogen needed by the animal decreases, meaning that more N would be excreted, simply as a factor of age. Total N emissions were decreased by CT inclusion at the 0.5% and 1.0% level as compared to the control. Total N emissions were greater in period 2 than in period 1 in The cattle were fed higher than recommended amounts of N to begin with so the amount of N excreted should be expected to increase as the steer ages.

Carbon dioxide concentrations were not decreased by any tannin treatment, and conversely increased emissions at both tannin inclusion levels.

Condensed tannin inclusion in the diet did not have an effect on Nitrous O xide concentrations in this study. Hydrogen sulfide concentrations were similar among all treatments, but increased in Period 2. There was an interaction between treatment and period in the control group.

Some studies have suggested that there is a correlation between  $CO_2$  emissions and higher NH<sub>3</sub> emissions, due to  $CO_2$  emissions increasing the surface pH of manure. That does not appear to be the case in this study. Tannin inclusion appears to have an inverse effect on NH<sub>3</sub> and GHG emission.

While indirect  $N_2O$  emissions are the result of  $NH_3$  emissions, it appears at least in this study that the main source of  $N_2O$  emissions was from the feces portion of the manure.

The combination of the results from all of the gaseous emissions agrees with the hypotheses that CT repartitions N lost from the urine to the feces. While CT are effective at reducing  $NH_3$  and TN, they are not effective at reducing  $CO_2$ ,  $N_2O$ , or  $H_2S$  emissions, and can potentially increase these emissions.

#### **Future Research**

Further research is needed to determine the effect of CT inclusion on CH<sub>4</sub> emissions from cattle manure. There are several studies available on direct emissions from enteric fermentation sources, but very little research on the effect on manure emissions. Manure CH<sub>4</sub> emissions have the potential to be a valuable resource for biogas generation with anaerobic digesters.

The emission data presented by agencies are based on model calculations and not on empirical data. Emperical data is imperative in order to find areas of weakness or discontinue exploration in areas where they are not truly needed. Laboratory research on manure emissions is essential to the research process as it is more cost effective and easier to control conditions than in the feedlot environment. Finding the cause for the

lack of  $CH_4$  emissions in this study is necessary to ensure further laboratory exploration of all GHG emissions. Utilizing fresh manure in a chamber system separate from the  $NH_3$ chamber system, in which air flow is required, would answer many of the  $CH_4$  emission questions not answered during the course of this study. Laboratory research on manure emissions could also be valuable for developing more accurate emissions models.

More research is needed to study the potential to increase GHG and other emissions with CT inclusion. There are several studies available on the effectiveness of CT to reduce NH<sub>3</sub>, but very few studies have looked into the consequences of shifting the N fraction to feces. Most studies available in this area are focusing on direct emissions from ruminants, and not from direct manure emission. However, manure N can be a very valuable source of fertilizer N for crops. If efficient methods are discovered to reduce losses from stored manure before land application, the result could be beneficial to farmers and CAFOs.

Hydrogen Sulfide emissions from cattle with CT diet inclusion needs further study. With H<sub>2</sub>S being a foremost concern in swine facilities, several researchers have focused on adding tannins topically to manure to reduce emissions. There has been a lot of success in this area and could be beneficial to study in relation to cattle manure. Where this study focused solely on emissions from manure, analyzing Sulfur and C content of the feces could have provided valuable information to accompany emission data collected. A H<sub>2</sub>S focused study with the addition of tannins to cattle manure could potentially produce interesting results.

Research will continue with respect to the amount of total N capture in acid traps

through this chamber study. Due to time constraints and equipment issues, total N from the acid was not analyzed in time to be included in this study. A correlation between total N, and ORP, EC and PH could provide interesting insight to simple monitoring of emissions through this method. Trends related to urine addition and total N captured based on NH<sub>3</sub> emissions following urine addition are likely

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# Appendix A

# Headspace Concentration Statistical Tables

# Ammonia Tables

<b>Between-Subjects Factors</b>							
		Value Label	Ν				
Treatment	.0	Control	180				
	.5	0.5% CT	180				
	1.0	1.0% CT	180				
Period	1		270				
	2		270				

# Descriptive Statistics

Descriptive Stausucs								
Dependent Variable: NH3								
Treatment	Period	Mean	Std. Deviation	Ν				
Control	1	57.556	87.3003	90				
	2	70.344	120.8696	90				
	Total	63.950	105.3301	180				
0.5% CT	1	23.333	45.1743	90				
	2	42.444	51.5362	90				
	Total	32.889	49.2651	180				
1.0% CT	1	24.967	31.4709	90				
	2	48.167	61.9571	90				
	Total	36.567	50.3624	180				
Total	1	35.285	61.4311	270				
	2	53.652	84.4258	270				
	Total	44.469	74.3312	540				

#### **Tests of Between-Subjects Effects**

Dependent Variable: NH3								
Source	Type IV Sum of Squares	df	Mean Square	F	Sig.			
Corrected Model	149230.087 <sup>a</sup>	3	49743.362	9.425	.000			
Intercept	1067822.535	1	1067822.535	202.330	.000			
Treatment	103689.937	2	51844.969	9.824	.000			
Period	45540.150	1	45540.150	8.629	.003			
Error	2828814.378	536	5277.639					
Total	4045867.000	540						
Corrected Total	2978044.465	539						

a. R Squared = .050 (Adjusted R Squared = .045)

#### **Estimated Marginal Means**

	Grand Mean
Dependent Variable:	NH3

Dependent variable. 14115							
		95% Confidence Interval					
Mean	Std. Error	Lower Bound	Upper Bound				
44.469	3.126	38.327	50.610				

#### **Multiple Comparisons**

Dependent Variable: NH3

LSD

		Mean			95% Confide	ence Interval
(I) Treatment	(J) Treatment	Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Control	0.5% CT	31.061*	7.6577	.000	16.018	46.104
	1.0% CT	27.383 <sup>*</sup>	7.6577	.000	12.341	42.426
0.5% CT	Control	-31.061*	7.6577	.000	-46.104	-16.018
	1.0% CT	-3.678	7.6577	.631	-18.721	11.365
1.0% CT	Control	-27.383*	7.6577	.000	-42.426	-12.341
	0.5% CT	3.678	7.6577	.631	-11.365	18.721

Based on observed means.

The error term is Mean Square(Error) = 5277.639.

\*. The mean difference is significant at the .05 level.

			Su	bset
	Treatment	Ν	1	2
Tukey HSD <sup>a,b</sup>	0.5	180	32.889	
	1	180	36.567	
	0	180		63.95
	Sig.		0.881	1

Means for groups in homogenous subsets are displayed

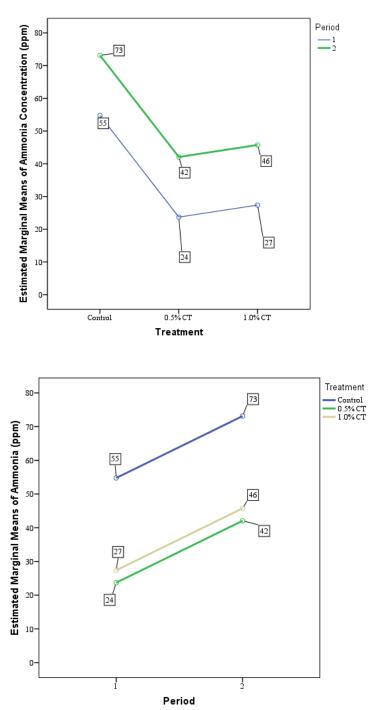
Base on observed means.

The error term is Mean Square(Error) = 5277.639

a. uses Harmonic Mean Sample Size = 180.000

b. Alpha = .05

**Profile Plots** 



# **Carbon Dioxide Tables**

Treatment	Period	Mean	Std. Deviation	Ν
Control	1	5770.571514	6666.3778732	90
	2	6122.515764	7060.1835926	90
	Total	5946.543639	6849.1723170	180
0.5% CT	1	7834.674169	10035.5128902	90
	2	8720.758044	10142.7633902	90
	Total	8277.716107	10070.8632981	180
1.0% CT	1	7884.167084	9242.1531347	90
	2	6905.521745	7839.2329574	90
	Total	7394.844414	8559.5563070	180
Total	1	7163.137589	8789.6935744	270
	2	7249.598518	8488.2125246	270
	Total	7206.368053	8632.3577178	540

**Descriptive Statistics** Dependent Variable: CO2

#### Tests of Between-Subjects Effects

Dependent Variable: CO2

	Type IV Sum of				
Source	Squares	df	Mean Square	F	Sig.
Corrected Model	499693349.563 <sup>a</sup>	3	166564449.854	2.251	.082
Intercept	28043139880.308	1	28043139880.308	378.949	.000
Treatment	498684158.119	2	249342079.059	3.369	.035
Period	1009191.444	1	1009191.444	.014	.907
Error	39665292925.722	536	74002412.175		
Total	68208126155.593	540			
Corrected Total	40164986275.285	539			

a. R Squared = .012 (Adjusted R Squared = .007)

#### **Estimated Marginal Means**

Grand Mean Dependent Variable: CO2						
		95% Confidence Interval				
Mean	Std. Error	Lower Bound	Upper Bound			
7206.368	370.191	6479.165	7933.571			

#### **Multiple Comparisons**

		Mean			95% Confide	ence Interval
(I) Treatment	(J) Treatment	Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Control	0.5% CT	-2331.172468*	906.7794793	.010	-4112.449809	-549.895126
	1.0% CT	-1448.300776	906.7794793	.111	-3229.578117	332.976566
0.5% CT	Control	2331.172468*	906.7794793	.010	549.895126	4112.449809
	1.0% CT	882.871692	906.7794793	.331	-898.405649	2664.149034
1.0% CT	Control	1448.300776	906.7794793	.111	-332.976566	3229.578117
	0.5% CT	-882.871692	906.7794793	.331	-2664.149034	898.405649

Dependent Variable: CO2 LSD

Based on observed means.

The error term is Mean Square(Error) = 74002412.175.

\*. The mean difference is significant at the .05 level.

#### **Carbon Dioxide**

			Subset		
	Treatment	Ν	1	2	
Tukey HSD					
a,b	0.5	180	5946.5436		
	1	180	7394.8444	7394.8444	
	0	180		8277.7161	
	Sig.		0.248	0.594	

Means for groups in homogenous subsets are displayed

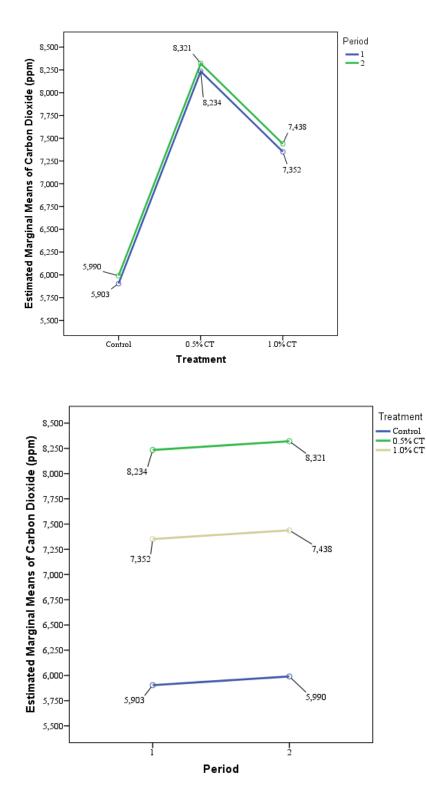
Base on observed means.

The error term is Mean Square(Error) = 74002412.175

a. uses Harmonic Mean Sample Size = 180.000

b. Alpha = .05

#### **Profile Plots**





# **Nitrous Oxide Tables**

Descriptive Statistics Dependent Variable: N2O							
Treatment	Period	Mean	Std. Deviation	Ν			
Control	1	361.241545	256.3764290	90			
	2	345.207804	173.6466339	90			
	Total	353.224674	218.4898528	180			
0.5% CT	1	526.492070	1042.9262270	90			
	2	377.560581	222.8233298	90			
	Total	452.026326	755.6929660	180			
1.0% CT	1	396.482982	234.5766224	90			
	2	366.834444	198.1641368	90			
	Total	381.658713	217.0373254	180			
Total	1	428.072199	636.3103092	270			
	2	363.200943	198.9407716	270			
	Total	395.636571	472.0972715	540			

#### **Tests of Between-Subjects Effects**

Tests of Derneen Subjects Interes								
Dependent Variable: N2O								
	Type IV Sum of							
Source	Squares	df	Mean Square	F	Sig.			
Corrected Model	1499429.484 <sup>a</sup>	3	499809.828	2.258	.081			
Intercept	84525280.037	1	84525280.037	381.904	.000			
Treatment	931311.707	2	465655.854	2.104	.123			
Period	568117.777	1	568117.777	2.567	.110			
Error	118630644.894	536	221325.830					
Total	204655354.415	540						
Corrected Total	120130074.378	539						

a. R Squared = .012 (Adjusted R Squared = .007)

#### **Estimated Marginal Means**

#### **Grand Mean**

Dependent Variable: N2O						
95% Confidence Interval						
Mean	Std. Error	Lower Bound	Upper Bound			
395.637	20.245	355.867	435.406			

### Multiple Comparisons

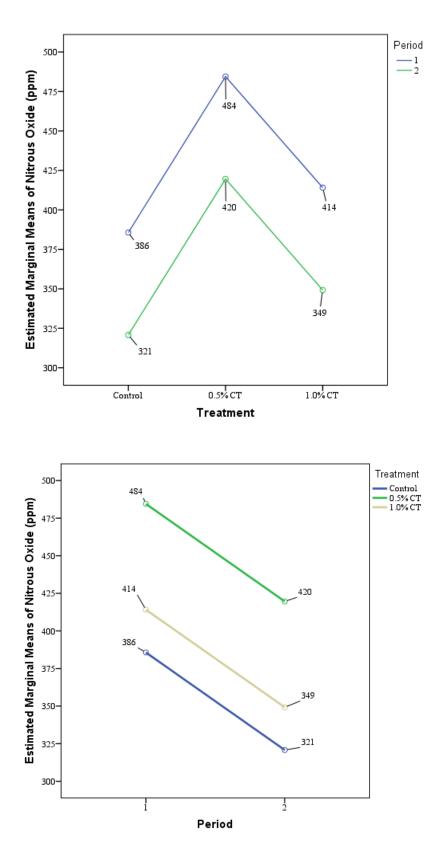
Dependent	Variable:	N2O
LSD		

		Mean			95% Confidence Interval	
		Difference (I-				
(I) Treatment	(J) Treatment	J)	Std. Error	Sig.	Lower Bound	Upper Bound
Control	0.5% CT	-98.801651 <sup>*</sup>	49.590078	.047	-196.216387	-1.386916
			5			
	1.0% CT	-28.434039	49.590078	.567	-125.848775	68.980697
			5			
0.5% CT	Control	98.801651 <sup>*</sup>	49.590078	.047	1.386916	196.216387
			5			
	1.0% CT	70.367612	49.590078	.156	-27.047123	167.782348
			5			
1.0% CT	Control	28.434039	49.590078	.567	-68.980697	125.848775
			5			
	0.5% CT	-70.367612	49.590078	.156	-167.782348	27.047123
			5			

Based on observed means.

The error term is Mean Square(Error) = 221325.830. \*. The mean difference is significant at the .05 level.

#### **Profile Plots**



# Hydrogen Sulfide Tables

Tests of Detween Subjects Effects								
Dependent Variable: H2S								
	Type IV Sum of							
Source	Squares	df	Mean Square	F	Sig.			
Corrected Model	113.068 <sup>a</sup>	5	22.614	6.047	.000			
Intercept	847.977	1	847.977	226.764	.000			
Treatment	17.616	2	8.808	2.355	.096			
Period	62.985	1	62.985	16.843	.000			
Treatment * Period	32.467	2	16.234	4.341	.014			
Error	1794.942	480	3.739					
Total	2755.986	486						
Corrected Total	1908.010	485						

#### Tests of Between-Subjects Effects

a. R Squared = .059 (Adjusted R Squared = .049)

#### **Estimated Marginal Means**

Grand Mean Dependent Variable: H2S

Dependent variable. 1125							
		95% Confidence Interval					
Mean	Std. Error	Lower Bound	Upper Bound				
1.321	.088	1.149	1.493				

#### **Multiple Comparisons**

Dependent Variable: H2S LSD

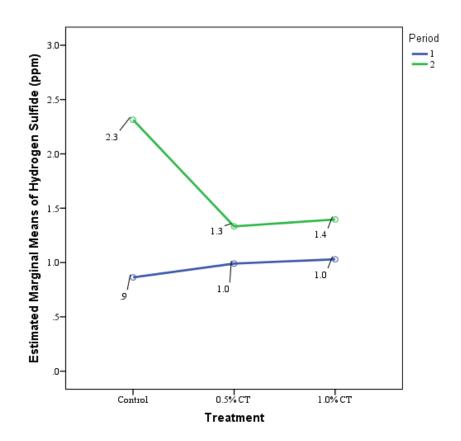
		Mean			95% Confidence Interval	
(I) Treatment	(J) Treatment	Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Control	0.5% CT	.42716*	.214863	.047	.00497	.84935
	1.0% CT	.37562	.214863	.081	04657	.79781
0.5% CT	Control	42716*	.214863	.047	84935	00497
	1.0% CT	05154	.214863	.811	47373	.37065
1.0% CT	Control	37562	.214863	.081	79781	.04657
	0.5% CT	.05154	.214863	.811	37065	.47373

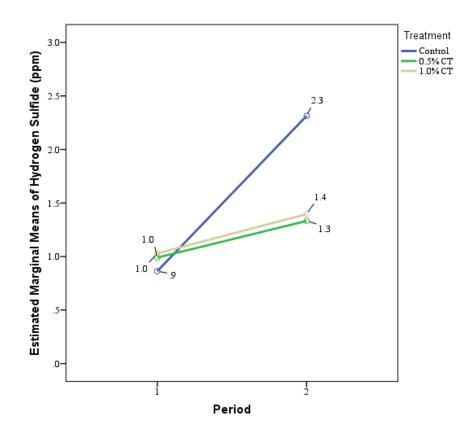
Based on observed means.

The error term is Mean Square(Error) = 3.739.

\*. The mean difference is significant at the .05 level.

#### **Profile Plots**





## **Total Nitrogen Tables**

#### Tests of Between-Subjects Effects

Dependent Variable: TN

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	300402.536 <sup>a</sup>	5	60080.507	5.322	.000
Intercept	2156878.329	1	2156878.329	191.052	.000
Treatment	206160.041	2	103080.020	9.131	.000
Period	89242.754	1	89242.754	7.905	.005
Treatment * Period	4999.741	2	2499.870	.221	.801
Error	7247861.891	642	11289.505		
Total	9705142.755	648			
Corrected Total	7548264.427	647			

a. R Squared = .040 (Adjusted R Squared = .032)

#### Multiple Comparisons

Dependent Variable: TN

			95% Confidence Interval
	(I) Treatment	(J) Treatment	Upper Bound
Tukey HSD	.0	.5	63.92
		1.0	59.38
	.5	.0	-15.88
		1.0	19.49
	1.0	.0	-11.35
		.5	28.55
LSD	.0	.5	59.98
		1.0	55.44
	.5	.0	-19.82
		1.0	15.54
	1.0	.0	-15.29
		.5	24.61

Based on observed means.

The error term is Mean Square(Error) = 11289.505.

 $\ast.$  The mean difference is significant at the 0.05 level.

#### Homogeneous Subsets

		TN			
			Subset		
	Treatment	Ν	1	2	
Tukey HSD <sup>a,b</sup>	.5	180	42.88		
	1.0	180	47.42		
	.0	180		82.78	
	Sig.		.897	1.000	

Means for groups in homogeneous subsets are displayed. Based on observed means.

The error term is Mean Square(Error) = 11289.505.

a. Uses Harmonic Mean Sample Size = 216.000. b. Alpha = 0.05.

# Appendix B

Chamber Heads pace Concentrations and Data

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	$N_2Oppb$	CH4 ppb	NH <sub>3</sub> ppm	$H_2S ppm$
Day 0	1	1	63	125.55	63.206	-126.9	7.12	6.19					
Day 1	1	1	63										
Day 2	1	1	63			•		•	11996.262	325.80876	0	0	
Day 3	1	1	63		63.206	•		•	10085.396	409.32786	0	7	
Day 4	1	1	63			•		•	10790.62	320.06818	0	0	0.55
Day 5	1	1	63		63.206				5360.7695	353.57566	0	45	1.1
Day 6	1	1	63						1526.2205	0	0	18	0.48
Day 7	1	1	63		63.206				2732.5903	482.86336	0	0	0.4
Day 9	1	1	63		63.206				2204.1143	0	0	0	0.29
Day 11	1	1	63		63.206	•		•	0	0	0	19	0.014
Day 13	1	1	63			•		•	0	418.93708	0	37	0
Day 15	1	1	63			-295	7	8.97	24097.188	799.43728	0	0	0
Day 0	1	2	23	165.199	82.713	-169.2	6.06	5.91					
Day 1	1	2	23										
Day 2	1	2	23						4742.8164	349.42622	0	0	
Day 3	1	2	23		82.713				3037.7242	492.94057	0	3	0.57
Day 4	1	2	23						4624.5561	368.14549	0	5	0.2
Day 5	1	2	23		82.713				4041.8042	327.58709	0	4	0.29
Day 6	1	2	23						645.90142	0	0	0	0.3
Day 7	1	2	23		82.713				3509.4021	446.14241	0	0	0.24
Day 9	1	2	23		82.713	•			36055.072	321.34733	0	0	0.43
Day 11	1	2	23		82.713				1697.3954	0	0	0	0.017
Day 13	1	2	23						0	474.3149	0	0	0
Day 15	1	2	23			-311	8.25	8.68	27396.23	814.2879	0	0	0

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	$N_2Oppb$	CH <sub>4</sub> ppm	NH <sub>3</sub> ppm	$H_2S ppm$
Day 0	blank	blank	1A					•					
Day 1	blank	blank	1A										
Day 2	blank	blank	1A						0	384.30645	0	0	0
Day 3	blank	blank	1A						0	426.48719	0	0	0.012
Day 4	blank	blank	1A						0	414.41326	0	0	0.042
Day 5	blank	blank	1A						0	371.26536	0	0	0.038
Day 6	blank	blank	1A						0	0	0	0	0.029
Day 7	blank	blank	1A						0	489.82069	0	0	0.021
Day 9	blank	blank	1A						0	0	0	0	0.011
Day 11	blank	blank	1A						0	0	0	0	0
Day 13	blank	blank	1A						0	474.2213	0	0	0
Day 15	blank	blank	1A			•	•		0	527.5088	0	0	0
Day 0	blank	blank	1B				•		•		•	•	•
Day 1	blank	blank	1B										
Day 2	blank	blank	1B						0	396.22438	0	0	0
Day 3	blank	blank	1B						0	371.26536	0	0	0
Day 4	blank	blank	1B						0	439.90266	0	0	0.034
Day 5	blank	blank	1B						0	414.94364	0	0	0.03
Day 6	blank	blank	1B						0	508.53995	0	0	0.029
Day 7	blank	blank	1B						0	452.38217	0	0	0.019
Day 9	blank	blank	1B					•	0	415.8796	0	0	0.009
Day 11	blank	blank	1B					•	0	0	0	0	0
Day 13	blank	blank	1B			•	•		0	0	0	0	0
Day 15	blank	blank	1B						0	393.1045	0	0	0

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	$N_2Oppb$	CH <sub>4</sub> ppm	NH <sub>3</sub> ppm	$H_2S ppm$
Day 0	1	1	55	156.969	78.609	-153.7	7.55	6.43					
Day 1	1	1	55										
Day 2	1	1	55						2354.7521	424.30327	0	11	
Day 3	1	1	55		78.609				6038.9022	368.14549	0	62	0
Day 4	1	1	55						3441.554	455.50204	0	89	0.52
Day 5	1	1	55		78.609				3818.2097	414.94364	0	60	0.49
Day 6	1	1	55						697.62753	499.18032	0	30	0.33
Day 7	1	1	55		78.609				0	424.30327	0	4	0.23
Day 9	1	1	55		78.609				2838.9293	455.50204	0	40	0.17
Day 11	1	1	55		78.609				0	327.58709	0	49	0
Day 13	1	1	55						0	404.11767	0	89	0
Day 15	1	1	55			-396	10.21	9.09	7680.3512	327.58709	0	36	0
Day 0	0	1	56	152.348	76.627	-214	8.39	7.1					
Day 1	0	1	56			•			•		•		•
Day 2	0	1	56						2180.2857	399.34426	0	83	
Day 3	0	1	56		76.627				3486.035	443.02253	0	136	1.1
Day 4	0	1	56						6108.7072	349.42622	0	400	2.3
Day 5	0	1	56		76.627				3603.2054	409.01587	0	171	1.8
Day 6	0	1	56						5449.6169	471.10143	0	227	1.2
Day 7	0	1	56		76.627				645.97426	0	0	139	0.17
Day 9	0	1	56		76.627				2031.459	486.70081	0	96	0.16
Day 11	0	1	56		76.627				1775.1254	0	0	185	0
Day 13	0	1	56						0	0	0	164	0
Day 15	0	1	56			-487	14.15	9.1	10690.277	0	0	94	0

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	N <sub>2</sub> O ppb	CH <sub>4</sub> ppm	NH <sub>3</sub> ppm	$H_2S ppm$
Day 0	0	1	42	136.15	68.163	-69.3	5.82	6.25					
Day 1	0	1	42						-			•	
Day 2	0	1	42						4870.0484	533.49897	0	0	
Day 3	0	1	42		68.163				2789.7914	377.50512	0	11	0.31
Day 4	0	1	42						835.92641	0	0	15	0.28
Day 5	0	1	42		68.163				5211.8185	396.22438	0	0	0.38
Day 6	0	1	42						2756.3287	399.34426	0	0	0.27
Day 7	0	1	42		68.163				827.25359	0	0	0	0.18
Day 9	0	1	42		68.613				37318.384	433.6629	0	0	0.17
Day 11	0	1	42		68.163				0	0	0	31	0
Day 13	0	1	42						0	0	0	0	0
Day 15	0	1	42			-318	5.43	8.72	8454.515	0	0	0	0
Day 0	0.5	1	43	131.252	65.844	-118.8	6.47	6.01					
Day 1	0.5	1	43										
Day 2	0.5	1	43						86.694168	0	0	0	
Day 3	0.5	1	43		65.844				1669.9843	383.74487	0	0	0.11
Day 4	0.5	1	43						507.06838	0	0	0	0.049
Day 5	0.5	1	43		65.844				2064.4313	433.6629	0	0	0.048
Day 6	0.5	1	43						8420.7896	418.06352	0	0	0.19
Day 7	0.5	1	43		65.844				0	467.98155	0	0	0.19
Day 9	0.5	1	43		65.844				22952.816	436.78278	0	0	0.042
Day 11	0.5	1	43		65.844				0	383.74487	0	0	0.028
Day 13	0.5	1	43						4847.0254	502.3002	0	0	0
Day 15	0.5	1	43			-389	4.49	8.68	10594.486	405.58401	0	0	0

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	$N_2Oppb$	$CH_4 ppm$	NH <sub>3</sub> ppm	$H_2S ppm$
Day 0	0	1	47	108.6466	54.412	-166	7.78	6.85					
Day 1	0	1	47										
Day 2	0	1	47						4171.7896	380.62499	0	14	
Day 3	0	1	47		54.412				4069.0901	377.50512	0	34	0.58
Day 4	0	1	47						2775.7333	358.78586	0	222	1.8
Day 5	0	1	47		54.412				1081.3147	502.3002	0	46	0.28
Day 6	0	1	47						12573.267	464.86167	0	54	1.1
Day 7	0	1	47		54.412				0	416.19159	0	56	0.18
Day 9	0	1	47		54.412				869.93298	474.2213	0	71	0.021
Day 11	0	1	47		54.412				1090.9781	467.98155	0	277	0
Day 13	0	1	47						6941.0169	545.97848	0	139	0
Day 15	0	1	47			-458	17.5	8.99	3011.6695	0	0	94	0
Day 0	0	1	65	163.155	81.148	-112.1	5.67	6.58					
Day 1	0	1	65										
Day 2	0	1	65						6128.1215	0	0	13	
Day 3	0	1	65		81.148				4929.1896	474.2213	0	17	1.2
Day 4	0	1	65						7931.9409	358.78586	0	54	1.2
Day 5	0	1	65		81.148				5231.8398	355.66598	0	0	1.7
Day 6	0	1	65						3732.3313	361.90573	0	25	1.3
Day 7	0	1	65		81.148				0	483.58094	0	0	0.41
Day 9	0	1	65		81.148				4378.5281	439.90266	0	0	0.036
Day 11	0	1	65		81.148				2629.1355	439.90266	0	82	0
Day 13	0	1	65						0	0	0	8	0
Day 15	0	1	65			-354	5.29	8.53	12563.758	371.26536	0	0	0

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	$N_2Oppb$	CH4 ppm	NH3 ppm	$H_2S ppm$
Day 0	1	2	55	219.753	109.814	-150.1	7.4	6.68	•		•		
Day 1	1	2	55										
Day 2	1	2	55						12051.072	499.18032	0	21	
Day 3	1	2	55		109.814				10238.352	386.86475	0	35	0.97
Day 4	1	2	55						1519.1316	349.42622	0	102	1.7
Day 5	1	2	55		109.814				9483.8311	383.74487	0	19	1.4
Day 6	1	2	55						7561.7729	321.34733	0	93	1.4
Day 7	1	2	55		109.814				3991.6466	371.26536	0	9	0.3
Day 9	1	2	55		109.814				7924.9483	386.86475	0	31	0.024
Day 11	1	2	55		109.814				1026.8615	545.97848	0	117	0
Day 13	1	2	55						0	315.10758	0	73	0
Day 15	1	2	55			-467	11.85	8.9	16506.708	359.75302	0	75	0
Day 0	0	1	62	160.303	80.022	-126.3	6.9	6.15	•				
Day 1	0	1	62										
Day 2	0	1	62						7728.2366	330.70696	0	0	
Day 3	0	1	62		80.022				3709.1682	380.62499	0	0	0.4
Day 4	0	1	62						6953.5346	456.12602	0	26	1.3
Day 5	0	1	62		80.022				7607.9632	474.2213	0	0	2.8
Day 6	0	1	62						10637.685	358.78586	0	0	0.41
Day 7	0	1	62		80.022				1148.6965	496.06044	0	0	0.13
Day 9	0	1	62		80.022				7698.5956	327.58709	0	0	0
Day 11	0	1	62		80.022				5637.612	439.90266	0	31	0
Day 13	0	1	62			•			0	355.66598	0	0	0
Day 15	0	1	62			-443	10.81	8	19372.775	407.23754	0	0	0

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	$N_2Oppb$	CH <sub>4</sub> ppm	$\mathbf{NH}_3$ ppm	$H_2Sppm$
Day 0	0	1	56	148.898	72.726	-112.1	6.5	6.64	•			•	•
Day 1	0	1	56						•				
Day 2	0	1	56						3726.3244	336.94672	0	69	
Day 3	0	1	56		72.726	-			0	414.94364	0	33	0.61
Day 4	0	1	56			-			1067.6984	486.70081	0	59	0.54
Day 5	0	1	56		72.726	-			0	383.74487	0	14	0.28
Day 6	0	1	56						7810.8323	393.1045	0	116	0.27
Day 7	0	1	56		72.726				8514.4036	405.58401	0	23	0.15
Day 9	0	1	56		72.726				2931.3875	399.34426	0	91	0.008
Day 11	0	1	56		72.726				4719.7843	377.50512	0	189	0
Day 13	0	1	56						1337.2426	35.25461	0	16	0
Day 15	0	1	56			-409	12.96	8.78	0	0	0	0	0
Day 0	0.5	1	48	106.815	53.856	-19.2	6.81	6.73					
Day 1	0.5	1	48										
Day 2	0.5	1	48						6396.6388	377.50512	0	0	
Day 3	0.5	1	48		53.856				3402.2107	374.38524	0	1	0.15
Day 4	0.5	1	48						91.43848	427.42315	0	0	0.21
Day 5	0.5	1	48		53.856				3429.147	467.98155	0	0	0.34
Day 6	0.5	1	48						27151.882	436.78278	0	0	0.34
Day 7	0.5	1	48		53.856				0	393.1045	0	0	0.13
Day 9	0.5	1	48		53.856				3979.3269	602.13626	0	0	0.007
Day 11	0.5	1	48		53.856				1990.2316	405.58401	0	0	0
Day 13	0.5	1	48						0	324.81039	0	0	0
Day 15	0.5	1	48			-312	4.85	8.72	74199.72	1113.7961	0	0	0

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	N <sub>2</sub> O ppb	CH <sub>4</sub> ppm	NH3 ppm	$H_2S ppm$
Day 0	1	1	68	153.622	77.02	-110.4	7.56	6.85					
Day 1	1	1	68									•	
Day 2	1	1	68						2905.7867	748.77048	0	0	
Day 3	1	1	68		77.02				4355.3658	421.1834	0	94	0.6
Day 4	1	1	68						4393.8788	430.54303	0	106	1.4
Day 5	1	1	68		77.02				2874.718	358.78586	0	51	0.38
Day 6	1	1	68						5930.7688	599.01638	0	70	0.32
Day 7	1	1	68		77.02				0	427.42315	0	0	0.15
Day 9	1	1	68		77.02				3209.2139	405.58401	0	0	0.007
Day 11	1	1	68		77.02				0	530.37909	0	153	0
Day 13	1	1	68						0	720.69159	0	28	0
Day 15	1	1	68			-404	9.09	8.84	34331.5	620.85552	0	0	0
Day 0	0	2	42	165.155	82.125	-169.2	6.06	6.6					
Day 1	0	2	42										
Day 2	0	2	42						3704.1859	781.84118	0	0	
Day 3	0	2	42		82.125				1831.8192	0	0	50	0.47
Day 4	0	2	42						2275.1962	371.26536	0	62	0.4
Day 5	0	2	42		82.125				2063.0522	374.38524	0	1	0.28
Day 6	0	2	42						4210.2443	331.45573	0	38	0.3
Day 7	0	2	42		82.155				0	385.30481	0	0	0.18
Day 9	0	2	42		82.155				23149.615	602.13626	0	0	0.007
Day 11	0	2	42		82.155				0	483.58094	0	0	0
Day 13	0	2	42						0	536.61884	0	0	0
Day 15	0	2	42			-418	7.88	8.7	5479.2461	717.57171	0	0	0

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	N <sub>2</sub> O ppb	CH <sub>4</sub> ppm	NH <sub>3</sub> ppm	$H_2S ppm$
Day 0	0	2	59	107.664	52.232	-87.9	13.34	6.26					
Day 1	0	2	59						•			•	
Day 2	0	2	59						5077.0257	349.42622	0	132	
Day 3	0	2	59		52.232				3345.6286	355.66598	0	251	2.4
Day 4	0	2	59						7668.668	455.50204	0	601	3.7
Day 5	0	2	59		52.232				2076.6053	424.30327	0	264	1.8
Day 6	0	2	59						2452.0955	324.46721	0	426	1.5
Day 7	0	2	59		52.232				0	389.98463	0	216	0.19
Day 9	0	2	59		52.232				23026.55	558.45798	0	359	0.003
Day 11	0	2	59		52.232				0	0	0	601	0.023
Day 13	0	2	59						0	368.14549	0	369	0
Day 15	0	2	59			-499	24.8	9.18	1857.3878	0	0	261	0
Day 0	1	2	58	126.908	63.613	-170.9	11.71	7.29					
Day 1	1	2	58										
Day 2	1	2	58						3315.5603	683.25306	0	43	
Day 3	1	2	58		63.613				5024.5032	368.14549	0	64	0.43
Day 4	1	2	58						4388.8671	389.98463	0	109	1.2
Day 5	1	2	58		63.613				3609.3871	396.84835	0	72	0.28
Day 6	1	2	58						3679.5369	330.70696	0	94	0.23
Day 7	1	2	58		63.613				4181.9872	352.5461	0	24	0.11
Day 9	1	2	58		63.613				2363.8717	430.54303	0	29	0.005
Day 11	1	2	58		63.613				7291.6968	0	0	55	0
Day 13	1	2	58						0	386.86475	0	17	0
Day 15	1	2	58			-461	13.8	9.04	15344.763	464.86167	0	0	0

ay	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	N <sub>2</sub> O ppb	CH <sub>4</sub> ppm	NH <sub>3</sub> ppm	H <sub>2</sub> S ppm
Day 0	1	2	63	87.167	44.54	-66.4	8.66	6.29					
Day 1	1	2	63										
Day 2	1	2	63						2617.2189	386.86475	0	22	
Day 3	1	2	63		44.54				1373.3836	492.94057	0	26	0.15
Day 4	1	2	63						60.131848	346.30635	0	21	0.29
Day 5	1	2	63		44.54				729.51688	418.06352	0	6	0.29
Day 6	1	2	63						0	59.277663	0	36	0.2
Day 7	1	2	63		44.54				4109.7688	430.54303	0	0	0.039
Day 9	1	2	63		44.54				0	355.66598	0	0	0.001
Day 11	1	2	63		44.54				8139.802	418.06352	0	64	0
Day 13	1	2	63						0	343.18647	0	0	0
Day 15	1	2	63			-357	6.23	8.97	16878.377	0	0	0	0
Day 0	0.5	2	54	225.329	122.816	-113.1	7.08	6.43					
Day 1	0.5	2	54							•	•		
Day 2	0.5	2	54						758.52662	365.02561	0	29	
Day 3	0.5	2	54		122.816				1369.897	362.8417	0	78	0.38
Day 4	0.5	2	54						822.75694	408.70389	0	21	0.48
Day 5	0.5	2	54		122.816				1356.2905	336.94672	0	34	1.1
Day 6	0.5	2	54						6972.5847	414.94364	0	0	2
Day 7	0.5	2	54		122.816				0	380.62499	0	63	0.11
Day 9	0.5	2	54		122.816				6721.5295	349.42622	0	26	0.004
Day 11	0.5	2	54		122.816				496.37061	402.46413	0	148	0
Day 13	0.5	2	54						0	402.46413	0	88	0
Day 15	0.5	2	54			-390	13.98	9.11	5996.5175	330.70696	0	91	0

Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	N <sub>2</sub> O ppm	CH4 ppb	NH3 ppm	$H_2S ppm$	$H_2S ppm$
Day 0	0	1	52	126.082	63.014	-185.7	8.57	6.78	•				•
Day 1	0	1	52			•			•				•
Day 2	0	1	52						5096.082	0	0	0	
Day 3	0	1	52		63.014				4522.1022	0	0	0	0
Day 4	0	1	52						2816.2558	344.12243	0	15	0
Day 5	0	1	52		63.014				2153.9568	417.81393	0	19	0
Day 6	0	1	52						2203.1933	427.42315	0	198	0
Day 7	0	1	52		63.014				2069.0859	403.1817	0	90	0
Day 9	0	1	52		63.014				3199.4199	427.42315	0	86	0
Day 11	0	1	52		63.014				2177.9045	402.46413	0	169	0
Day 13	0	1	52						2107.4023	371.26536	0	396	2.2
Day 15	0	1	52			-431	44.4	8.9	2543.2514	405.58401	0	106	4.9
Day 0	0.5	2	43	132.046	66.019	-113.3	9.26	6.03					
Day 1	0.5	2	43										
Day 2	0.5	2	43						46726.857	0	0	0	0
Day 3	0.5	2	43		66.019	•			1456.0234	0	0	0	0
Day 4	0.5	2	43						23890.279	717.57171	0	0	0
Day 5	0.5	2	43		66.019				37607.552	653.33344	0	12	0
Day 6	0.5	2	43						6226.416	477.34118	0	0	0
Day 7	0.5	2	43		66.019				10422.062	458.62192	0	0	0
Day 9	0.5	2	43		66.019				5536.7207	318.22745	0	15	0
Day 11	0.5	2	43		66.019				7069.3769	414.94364	0	44	0
Day 13	0.5	2	43						5594.1953	399.34426	0	84	0.22
Day 15	0.5	2	43			-443	9.16	8.67	7797.3886	324.81039	0	10	1.9

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	N <sub>2</sub> O ppb	CH <sub>4</sub> ppm	NH <sub>3</sub> ppm	$H_2S ppm$
Day 0	0.5	2	57	105.332	52.667	-250	10.02	7.93					
Day 1	0.5	2	57						•				
Day 2	0.5	2	57						4866.1836	358.53626	0	0	0
Day 3	0.5	2	57		52.667				2532.5229	511.59743	0	0	0
Day 4	0.5	2	57						53977.854	550.62709	0	18	0
Day 5	0.5	2	57		52.667				7661.557	601.85547	0	16	0
Day 6	0.5	2	57						0	0	0	59	0
Day 7	0.5	2	57		52.667				31398.187	0	0	37	0
Day 9	0.5	2	57		52.667				6091.1591	359.90901	0	25	0
Day 11	0.5	2	57		52.667				8246.4569	412.51014	0	89	0
Day 13	0.5	2	57						19149.965	507.88478	0	154	1.1
Day 15	0.5	2	57			-429	8.75	9.02	8949.3714	489.6335	0	21	2.3
Day 0	0.5	1	64	106.906	53.453	-144	7.03	6.2					
Day 1	0.5	1	64										
Day 2	0.5	1	64						1948.0061	0	0	0	0
Day 3	0.5	1	64		53.453				7431.6585	408.92228	0	0	0
Day 4	0.5	1	64						7695.2754	416.84677	0	37	0
Day 5	0.5	1	64		53.453				7776.8893	355.63478	0	40	0
Day 6	0.5	1	64						3123.5534	0	0	107	0
Day 7	0.5	1	64		53.453				5538.4449	350.45578	0	138	0
Day 9	0.5	1	64		53.453				4147.9425	384.49364	0	11	0
Day 11	0.5	1	64		53.453				3419.3561	429.41987	0	87	0
Day 13	0.5	1	64						3600.9758	399.46905	0	121	1.4
Day 15	0.5	1	64			-325	6.56	8.92	1574.038	317.0731	0	15	2.8

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	N <sub>2</sub> O ppb	CH <sub>4</sub> ppm	NH <sub>3</sub> ppm	$H_2S ppm$
Day 0	1	1	49	72.368	36.34	-98.6	7.54	6.38			•	•	•
Day 1	1	1	49										
Day 2	1	1	49						17563.857	375.60199	0	0	0
Day 3	1	1	49		36.34				1617.1439	0	0	0	0
Day 4	1	1	49						6330.2534	396.75476	0	0	0
Day 5	1	1	49		36.34				2080.5808	0	0	13	0
Day 6	1	1	49						4808.9005	457.31157	0	23	0
Day 7	1	1	49		36.34				5368.5116	918.1174	0	12	0
Day 9	1	1	49		36.34				6496.7382	534.49733	0	35	0
Day 11	1	1	49		36.34				1417.8986	0	0	22	0
Day 13	1	1	49						4586.0906	464.98647	0	94	0.25
Day 15	1	1	49			-400	8.24	8.86	3308.2385	311.36372	0	4	2
Day 0	1	2	67	62.226	31.113	-139.4	8.85	6.65					
Day 1	1	2	67										
Day 2	1	2	67						42703.634	440.40184	0	0	0
Day 3	1	2	67		31.133				6182.3521	365.43119	0	0	0
Day 4	1	2	67						15931.77	425.36403	0	75	0
Day 5	1	2	67		31.133				8347.229	307.86946	0	39	0
Day 6	1	2	67						1831.7158	0	0	190	0
Day 7	1	2	67		31.133				7589.3305	486.29523	0	144	0
Day 9	1	2	67		31.133				5310.4623	376.53796	0	139	0
Day 11	1	2	67		31.133				4731.8846	389.85983	0	260	0
Day 13	1	2	67						7771.525	376.03877	0	338	1.9
Day 15	1	2	67			-399	16.57	9.09	7107.5017	347.83509	0	58	4.8

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	N <sub>2</sub> O ppb	CH <sub>4</sub> ppm	NH <sub>3</sub> ppm	$H_2S ppm$
Day 0	1	1	51	105.501	52.514	-85.1	6.25	6.52	•			•	•
Day 1	1	1	51						•				
Day 2	1	1	51						8458.9214	439.12269	0	0	0
Day 3	1	1	51		52.514				5427.9021	389.14226	0	0	0
Day 4	1	1	51						5748.9936	412.79093	0	0	0
Day 5	1	1	51		52.514				14452.565	334.54441	0	0	0
Day 6	1	1	51						5976.4014	332.42289	0	0	0
Day 7	1	1	51		52.514				7631.0954	484.4233	0	0	0
Day 9	1	1	51		52.514				7066.3116	450.19825	0	0	0
Day 11	1	1	51		52.514				1934.2122	0	0	0	0
Day 13	1	1	51						7241.226	390.10942	0	0	0.18
Day 15	1	1	51			-426	3.2	8.52	6920.1345	425.89441	0	0	0.45
Day 0	0.5	2	44	157.504	78.752	-153.6	5.75	6.48					•
Day 1	0.5	2	44										
Day 2	0.5	2	44						7679.5657	418.74989	0	0	0
Day 3	0.5	2	44		78.752				16169.906	441.55619	0	0	0
Day 4	0.5	2	44						16739.863	339.25542	0	5	0
Day 5	0.5	2	44		78.752				21732.682	585.13293	0	0	0
Day 6	0.5	2	44						3121.8292	451.66459	0	80	0
Day 7	0.5	2	44		78.752				21537.843	1581.7152	0	39	0
Day 9	0.5	2	44		78.752				17544.124	389.42305	0	44	0
Day 11	0.5	2	44		78.752				5530.2069	348.52146	0	90	0
Day 13	0.5	2	44						3607.298	400.59221	0	102	1.5
Day 15	0.5	2	44			-433	8.22	8.82	1434.1831	0	0	11	3.2

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	N <sub>2</sub> O ppb	CH <sub>4</sub> ppm	NH3 ppm	$H_2S ppm$
Day 0	0.5	2	66	88.95	44.475	-151.8	5.91	6.54					
Day 1	0.5	2	66										
Day 2	0.5	2	66						2049.1614	0	0	0	0
Day 3	0.5	2	66		44.475				7081.0634	400.717	0	0	0
Day 4	0.5	2	66						8749.7429	343.81045	0	1	0
Day 5	0.5	2	66		44.475				40935.716	361.37535	0	0	0
Day 6	0.5	2	66						4866.1836	358.69226	0	30	0
Day 7	0.5	2	66		44.475				6805.1853	426.45599	0	0	0
Day 9	0.5	2	66		44.475				8896.8779	510.75506	0	16	0
Day 11	0.5	2	66		44.475				5502.8106	411.76137	0	10	0
Day 13	0.5	2	66						7563.0838	604.94415	0	36	0.33
Day 15	0.5	2	66			-379	4.18	9.01	2656.2848	0	0	1	1.5
Day 0	0.5	1	53	107.017	51.5085	-74.4	7.09	6.76					
Day 1	0.5	1	53						•		•		-
Day 2	0.5	1	53						19380.438	356.28995	0	0	0
Day 3	0.5	1	53		51.508				39562.839	427.76634	0	0	0
Day 4	0.5	1	53						6020.2737	369.89262	0	0	0
Day 5	0.5	1	53		51.509				29130.814	427.07996	0	51	0
Day 6	0.5	1	53						5014.4681	778.93969	0	56	0
Day 7	0.5	1	53		51.508				1849.3413	448.60711	0	7	0
Day 9	0.5	1	53		51.508				8814.8808	479.21311	0	18	0
Day 11	0.5	1	53		51.508				4392.5928	373.94846	0	39	0
Day 13	0.5	1	53						3443.4954	394.32125	0	15	0.52
Day 15	0.5	1	53			-394	7.38	8.74	3355.9424	378.62827	0	0	2.1

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	N <sub>2</sub> O ppb	CH <sub>4</sub> ppm	NH <sub>3</sub> ppm	$H_2S ppm$
Day 1	1	2	68										
Day 2	1	2	68						11049.302	383.21449	0	0	0
Day 3	1	2	68		39.328				13538.144	400.24902	0	0	0
Day 4	1	2	68						1542.2353	0	0	27	0
Day 5	1	2	68		39.328				9063.7458	0	0	31	0.0001
Day 6	1	2	68						11739.189	408.3607	0	224	0
Day 7	1	2	68		39.328				1962.7579	361.21936	0	105	0
Day 9	1	2	68		39.328				12489.999	864.73631	0	111	0
Day 11	1	2	68		39.328				5493.2315	471.44461	0	80	0
Day 13	1	2	68						5598.0269	393.35409	0	80	0.23
Day 15	1	2	68			-307	8.61	9.08	13253.645	914.40475	0	0	2
Day 0	0	2	62	124.901	62.767	-121.3	2.68	6.63					
Day 1	0	2	62										
Day 2	0	2	62						41791.896	348.86465	0	0	0
Day 3	0	2	62		62.767				1602.2005	0	0	0	0
Day 4	0	2	62						2931.5882	0	0	0	0
Day 5	0	2	62		62.767				9936.402	383.62008	0	15	0
Day 6	0	2	62						4393.1675	0	0	5	0
Day 7	0	2	62		62.767				7267.4727	702.78349	0	0	0
Day 9	0	2	62		62.767				4954.886	0	0	16	0
Day 11	0	2	62		62.767				5902.0676	368.61347	0	0	0
Day 13	0	2	62						17274.185	424.42807	0	0	0.48
Day 15	0	2	62			-444	7.56	9	2500.9118	379.59543	0	23	3.2

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	N <sub>2</sub> O ppb	CH <sub>4</sub> ppm	NH <sub>3</sub> ppm	$H_2S ppm$
Day 0	0	1	59	92.259	48.516	-107.4	6.74	6.4					
Day 1	0	1	59										
Day 2	0	1	59						13778.388	0	0	0	0
Day 3	0	1	59		48.516				6996.001	0	0	0	0
Day 4	0	1	59						2019.4662	0	0	43	0
Day 5	0	1	59		48.516				9005.6965	381.46736	0	123	0
Day 6	0	1	59						29061.27	347.83509	0	234	0
Day 7	0	1	59		48.516				32060.87	925.94829	0	102	0
Day 9	0	1	59		48.516				8496.663	428.45271	0	153	0
Day 11	0	1	59		48.516				12511.648	578.61239	0	201	0
Day 13	0	1	59						26078.529	1428.9661	0	164	2.2
Day 15	0	1	59			-445	13.63	9.09	3701.9396	364.93201	0	68	5.8
Day 0	0.5	1	61	120.881	60.44	-138.7	7.79	6.62	•				
Day 1	0.5	1	61										
Day 2	0.5	1	61						9707.653	359.47223	0	0	0
Day 3	0.5	1	61		60.44				3915.3619	352.23411	0	0	0
Day 4	0.5	1	61						2009.3123	0	0	0	0
Day 5	0.5	1	61		60.44				12395.549	470.50865	0	136	0
Day 6	0.5	1	61						2169.0917	0	0	244	0
Day 7	0.5	1	61		60.44				11334.951	397.84672	0	117	0
Day 9	0.5	1	61		60.44				11696.466	550.97028	0	154	0
Day 11	0.5	1	61		60.44				10567.856	498.40035	0	73	0
Day 13	0.5	1	61						5116.1981	358.41147	0	36	1.3
Day 15	0.5	1	61			-376	8.03	9.15	4201.5855	351.79733	0	21	3.8

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	N <sub>2</sub> O ppb	CH <sub>4</sub> ppm	NH <sub>3</sub> ppm	$H_2S ppm$
Day 0	0.5	2	64	125.628	62.814	-175.8	6.71	6.78	•				
Day 1	0.5	2	64						•				
Day 2	0.5	2	64						21175.562	0	0	0	0
Day 3	0.5	2	64		62.814				8565.2494	395.28842	0	0	0
Day 4	0.5	2	64						5724.2795	318.19626	0	41	0
Day 5	0.5	2	64		62.814				3882.793	0	0	137	0
Day 6	0.5	2	64						8077.6731	423.86649	0	224	0
Day 7	0.5	2	64		62.814				6549.0401	382.37213	0	59	0
Day 9	0.5	2	64						4174.1893	389.26705	0	205	0
Day 11	0.5	2	64		62.814				7545.8414	369.70542	0	0	0
Day 13	0.5	2	64						6116.0647	357.91229	0	67	1.8
Day 15	0.5	2	64			-449	15.21	9.02	4311.5536	350.23739	0	139	5.8
Day 0	1	1	67	195.998	97.999	-185.6	7.01	6.73				•	
Day 1	1	1	67										
Day 2	1	1	67						4249.6726	373.23089	0	0	0
Day 3	1	1	67		97.999				32042.095	396.97315	0	0	0
Day 4	1	1	67						12305.314	337.57069	0	0	0
Day 5	1	1	67		97.999				6608.6221	0	0	20	0
Day 6	1	1	67						8007.7457	359.12904	0	34	0
Day 7	1	1	67		97.999				25457.037	1852.1462	0	0	0
Day 9	1	1	67		97.999				6191.9312	434.72366	0	41	0
Day 11	1	1	67		97.999				8192.0476	457.65476	0	88	0
Day 13	1	1	67						12529.273	457.21797	0	0	1.9
Day 15	1	1	67			-356	9.23	8.19	4187.6	353.73165	0	0	3.8

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	N <sub>2</sub> O ppb	CH <sub>4</sub> ppm	NH <sub>3</sub> ppm	$H_2S ppm$
Day 1	1	1	69										
Day 2	1	1	69						19590.22	0	0	0	0
Day 3	1	1	69		58.915				13870.156	378.78427	0	0	0
Day 4	1	1	69						4226.4912	397.65952	0	0	0
Day 5	1	1	69		58.915				1661.2078	0	0	68	0
Day 6	1	1	69						9771.6414	313.82843	0	36	0
Day 7	1	1	69		58.914				58353.971	629.96556	0	18	0
Day 9	1	1	69		58.915				7925.9402	557.67801	0	57	0
Day 11	1	1	69		58.914				5512.7729	397.16034	0	52	0
Day 13	1	1	69						18482.493	0	0	0	1.3
Day 15	1	1	69			-361	8.96	8.07	39816.11	434.38047	0	0	6.4
Day 0	0.5	1	57	145.596	72.798	-198.5	7.74	6.78	•		•	•	
Day 1	0.5	1	57										
Day 2	0.5	1	57						4680.5406	329.92699	0	0	0
Day 3	0.5	1	57		72.798				5207.1996	410.8878	0	0	0
Day 4	0.5	1	57						5173.8643	357.22592	0	21	0
Day 5	0.5	1	57		72.798				4218.6363	319.75619	0	33	0
Day 6	0.5	1	57						5615.0777	362.87289	0	144	0
Day 7	0.5	1	57		72.798				2542.2935	310.92694	0	86	0
Day 9	0.5	1	57		72.798				7642.2072	726.83774	0	41	0
Day 11	0.5	1	57		72.798				7143.7107	726.77535	0	80	0
Day 13	0.5	1	57						14710.243	3662.9228	0	60	1.3
Day 15	0.5	1	57						38238.624	9638.2672	0	24	3.3

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	N <sub>2</sub> O ppb	CH <sub>4</sub> ppm	NH <sub>3</sub> ppm	H <sub>2</sub> S ppm
Day 0	blank	blank	2A				•					•	
Day 1	blank	blank	2A				•					•	
Day 2	blank	blank	2A						2666.2471	383.24569	0	0	0
Day 3	blank	blank	2A						2840.9699	410.8878	0	0	0
Day 4	blank	blank	2A				•		2562.4097	355.0108	0	0	0
Day 5	blank	blank	2A				•		2659.9249	380.25061	0	0	0
Day 6	blank	blank	2A						2344.0061	314.4836	0	0	0
Day 7	blank	blank	2A						2349.9452	387.51992	0	0	0
Day 9	blank	blank	2A						2330.5954	316.51152	0	0	0
Day 11	blank	blank	2A				•		2154.1483	323.00087	0	0	0
Day 13	blank	blank	2A						3612.2792	0	0	0	0
Day 15	blank	blank	2A				•		3920.3431	1444.8462	0	0	0.35
Day 0	blank	blank	2B										
Day 1	blank	blank	2B										
Day 2	blank	blank	2B						2699.0076	411.60537	0	0	0
Day 3	blank	blank	2B				•		0	0	0	0	0
Day 4	blank	blank	2B						2747.6695	385.74159	0	0	0
Day 5	blank	blank	2B						2667.205	427.64154	0	0	0
Day 6	blank	blank	2B						0	367.7087	0	0	0
Day 7	blank	blank	2B						1379.199	428.01593	0	0	0
Day 9	blank	blank	2B						1666.1889	387.83191	0	0	0
Day 11	blank	blank	2B						2159.5126	362.21772	0	0	0
Day 13	blank	blank	2B						1397.3993	0	0	0	0
Day 15	blank	blank	2B					•	2329.8291	436.47079	0	0	0.39

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	N <sub>2</sub> O ppb	CH <sub>4</sub> ppm	NH <sub>3</sub> ppm	$H_2S ppm$
Day 0	blank	blank	3A										
Day 1	blank	blank	3A										
Day 2	blank	blank	3A						2602.6419	390.35901	0	0	0.023
Day 3	blank	blank	3A						2132.308	416.44118	0	0	0.34
Day 4	blank	blank	3A						3735.2748	338.28826	0	0	0.19
Day 5	blank	blank	3A						2063.3385	0	0	0	0.11
Day 6	blank	blank	3A						1848.0003	0	0	0	0.28
Day 7	blank	blank	3A						1843.0191	312.73647	0	0	0.54
Day 9	blank	blank	3A						2033.2601	567.19364	0	0	0.1
Day 11	blank	blank	3A						1788.6098	0	0	0	0.12
Day 13	blank	blank	3A						1930.9553	446.3608	0	0	0.54
Day 15	blank	blank	3A						1770.0264	0	0	0	0.53
Day 0	0.5	2	61	133.654	66.827	-150.7	3.69	6.65					
Day 1	0.5	2	61										
Day 2	0.5	2	61						2094.7579	615.11495	0	0	0.32
Day 3	0.5	2	61		66.827				2162.0032	870.97606	0	59	1.6
Day 4	0.5	2	61						5705.3129	397.06675	0	56	2
Day 5	0.5	2	61		66.827				2007.0133	453.53652	0	0	1.3
Day 6	0.5	2	61						1836.6969	394.66444	0	68	3.9
Day 7	0.5	2	61		66.827				1911.7971	478.21475	0	62	3.4
Day 9	0.5	2	61		66.827				1756.9988	0	0	87	2.6
Day 11	0.5	2	61		66.827				1765.2368	357.35071	0	134	2.8
Day 13	0.5	2	61						1797.231	490.10148	0	140	5.7
Day 15	0.5	2	61			-427	-15.36	8.83	1863.1352	0	0	156	4.5

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	N <sub>2</sub> O ppb	CH <sub>4</sub> ppm	NH3 ppm	$H_2S ppm$
Day 0	1	2	69	120.561	60.208	-153.2	8.75	7.04					
Day 1	1	2	69						•				
Day 2	1	2	69						36873.793	542.39062	0	0	0.043
Day 3	1	2	69		60.208				7248.6977	338.13227	0	38	1.3
Day 4	1	2	69						6147.2926	345.61997	0	40	1.4
Day 5	1	2	69		60.208				11131.299	985.94353	0	0	0.52
Day 6	1	2	69						2830.2413	534.40373	0	65	4.1
Day 7	1	2	69		60.208				2254.7289	433.7253	0	18	3.2
Day 9	1	2	69		60.208				4842.8106	464.1441	0	90	3.3
Day 11	1	2	69		60.208				4644.7147	433.8501	0	68	2.5
Day 13	1	2	69						1648.755	0	0	88	6.9
Day 15	1	2	69			-337	12.49	8.88	2016.4009	0	0	78	4.4
Day 0	0	2	65	113.565	57.78	-132.9	7.52	6.78					
Day 1	0	2	65										
Day 2	0	2	65						38090.339	515.40368	0	0	0.21
Day 3	0	2	65		57.78				4260.9759	407.54953	0	0	1.3
Day 4	0	2	65						6564.1751	334.48201	0	69	2.5
Day 5	0	2	65		57.78				14672.693	377.31792	0	4	1.3
Day 6	0	2	65						1429.5851	0	0	33	3.1
Day 7	0	2	65		57.78				11053.134	465.61044	0	10	3.6
Day 9	0	2	65		57.78				5076.9238	545.10491	0	33	2.4
Day 11	0	2	65		57.78				6103.4203	445.51844	0	20	2.1
Day 13	0	2	65						6555.3623	363.37207	0	81	7.4
Day 15	0	2	65			-476	13.2	9.01	4738.7815	0	Õ	90	4.8

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	$N_2Oppb$	CH <sub>4</sub> ppm	NH <sub>3</sub> ppm	$H_2S ppm$
Day 0	0	2	41	102.206	51.1025	-132.5	3.21	6.33					
Day 1	0	2	41						•				
Day 2	0	2	41						11466.951	427.36075	0	0	0.49
Day 3	0	2	41		51.1025				20188.148	440.37064	0	0	0.77
Day 4	0	2	41						6455.1649	426.92397	0	9	1.2
Day 5	0	2	41		51.1025				7190.0736	378.31629	0	0	0.54
Day 6	0	2	41						5431.3506	402.80732	0	0	2.3
Day 7	0	2	41		51.1025				8669.2784	429.41987	0	0	2.5
Day 9	0	2	41		51.1025				8224.425	361.81214	0	11	1.7
Day 11	0	2	41		51.1025				8840.9359	361.81214	0	2	1.3
Day 13	0	2	41						3242.7174	371.14057	0	0	4.1
Day 15	0	2	41			-340	6.53	9.05	3275.6695	355.72838	0	13	4.9
Day 0	1	1	58	110.155	55.0775	-175	7.3	6.8					
Day 1	1	1	58										
Day 2	1	1	58						7199.2695	372.66931	0	0	0.24
Day 3	1	1	58		55.078				8854.7298	424.14728	0	71	3.1
Day 4	1	1	58						4562.9092	408.79748	0	29	2.2
Day 5	1	1	58		55.078				4795.1066	368.48867	0	0	1.2
Day 6	1	1	58						3870.3402	336.32274	0	9	2.1
Day 7	1	1	58		55.078				3647.5303	376.69395	0	0	2.4
Day 9	1	1	58		55.078				2881.3937	375.25881	0	61	3
Day 11	1	1	58		55.078				2654.1774	375.25881	0	13	1.9
Day 13	1	1	58						3638.7175	424.42807	0	0	4.2
Day 15	1	1	58			-258	9.47	8.51	3657.8757	400.40501	0	0	1.7

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	$N_2Oppb$	CH <sub>4</sub> ppm	NH <sub>3</sub> ppm	$H_2S ppm$
Day 0	0.5	2	53	141.022	70.511	-131.2	8.4	6.74					
Day 1	0.5	2	53										
Day 2	0.5	2	53						27381.862	412.19815	0	0	0.15
Day 3	0.5	2	53		70.511				1474.99	0	0	14	2
Day 4	0.5	2	53						4792.9992	406.42638	0	47	2.5
Day 5	0.5	2	53		70.511				8528.8488	392.29333	0	15	1.5
Day 6	0.5	2	53						16616.867	308.93022	0	84	4.8
Day 7	0.5	2	53		70.511				15721.605	454.97166	0	15	4.9
Day 9	0.5	2	53		70.511				5673.5102	450.26065	0	65	3.6
Day 11	0.5	2	53		70.511				5641.1329	385.95998	0	91	2.8
Day 13	0.5	2	53						8090.3175	485.79605	0	112	7.9
Day 15	0.5	2	53						12963.781	572.02945	0	94	5
Day 0	0	2	52	88.592	44.296	-145.4	8.02	6.81					
Day 1	0	2	52										
Day 2	0	2	52						7202.718	390.85819	0	116	0.12
Day 3	0	2	52		44.296				1516.7549	0	0	11	2.8
Day 4	0	2	52						4900.0936	362.24892	0	80	3.8
Day 5	0	2	52		44.296				5066.9615	360.65778	0	5	2.2
Day 6	0	2	52						5970.4624	309.05502	0	13	3.1
Day 7	0	2	52		44.296				4851.4317	338.25706	0	0	2.5
Day 9	0	2	52		44.296				1884.0177	0	0	60	2.9
Day 11	0	2	52		44.296				2167.1759	440.52663	0	44	2.3
Day 13	0	2	52						3564.3837	382.24733	0	75	7.3
Day 15	0	2	52			-384	13.72	9.12	2766.2529	350.48698	0	63	6.4

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	N <sub>2</sub> O ppb	CH <sub>4</sub> ppm	NH <sub>3</sub> ppm	$H_2S ppm$
Day 0	1	2	51	150.488	75.244	-97.8	6.49	6.19					
Day 1	1	2	51									•	
Day 2	1	2	51						5939.2345	387.42633	0	0	0.036
Day 3	1	2	51		75.244				3359.5825	0	0	0	0.48
Day 4	1	2	51						4758.3229	368.64467	0	2	1.1
Day 5	1	2	51		75.244				8388.036	402.96331	0	0	1.5
Day 6	1	2	51						16470.116	335.60517	0	2	2.5
Day 7	1	2	51		75.244				23119.161	353.88765	0	0	2.6
Day 9	1	2	51		75.244				1769.0685	0	0	0	2.3
Day 11	1	2	51		75.244				3787.1936	326.77592	0	0	1.9
Day 13	1	2	51						6003.6061	594.58616	0	0	4
Day 15	1	2	51			-449	6.41	8.6	4512.9063	0	0	0	3.3
Day 0	0.5	1	66	109.821	54.9105	-161	3.17	6.5					
Day 1	0.5	1	66						•			•	
Day 2	0.5	1	66						9332.5354	346.33755	0	0	0.21
Day 3	0.5	1	66		54.9105				13744.861	374.26044	0	0	0.65
Day 4	0.5	1	66						6551.1475	387.9879	0	0	0.6
Day 5	0.5	1	66		54.9105				7050.6019	381.09298	0	0	1.2
Day 6	0.5	1	66						4558.5028	381.06178	0	0	1.4
Day 7	0.5	1	66		54.9105				10753.308	460.02586	0	0	2.1
Day 9	0.5	1	66		54.9105	0			4028.3953	0	0	0	1.2
Day 11	0.5	1	66		54.9105				4721.5391	392.66772	0	0	1.1
Day 13	0.5	1	66						4304.6566	616.70609	0	0	2.5
Day 15	0.5	1	66			-377	3.06	8.48	3492.3488	451.4462	0	0	1.9

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	N <sub>2</sub> O ppb	CH <sub>4</sub> ppm	NH <sub>3</sub> ppm	$H_2S ppm$
Day 0	0	2	47	204.22	102.11	-200	3.32	6.56					· ·
Day 1	0	2	47									11	
Day 2	0	2	47						10797.18	384.83683	0	32	0.33
Day 3	0	2	47		102.11				7083.9371	383.62008	0	0	4.6
Day 4	0	2	47						3510.9323	376.63155	0	26	4.2
Day 5	0	2	47		102.11				12756.298	342.5001	0	63	2.6
Day 6	0	2	47						4984.7728	387.05194	0	153	8.9
Day 7	0	2	47		102.11				5497.6379	370.92218	0	150	7.2
Day 9	0	2	47		102.11				5368.1285	359.50343	0	128	4.8
Day 11	0	2	47		102.11				2748.819	389.51664	0	211	4.1
Day 13	0	2	47						4795.873	482.45778	0	152	16
Day 15	0	2	47			-478	16.55	9.07	4340.0993	487.44958	0	198	12
Day 0	0.5	1	54	102.442	51.221	-180.9	7.01	6.4					
Day 1	0.5	1	54										
Day 2	0.5	1	54						8887.1072	351.11096	0	0	0.17
Day 3	0.5	1	54		51.221				17764.061	343.03048	0	0	1.4
Day 4	0.5	1	54						5484.6104	349.17663	0	7	2.4
Day 5	0.5	1	54		51.221				5468.5175	341.84492	0	7	2.1
Day 6	0.5	1	54						5125.0109	404.99123	0	32	5.3
Day 7	0.5	1	54		51.221				6278.1431	482.52018	0	7	4.2
Day 9	0.5	1	54		51.221				7182.0271	373.13729	0	0	3.1
Day 11	0.5	1	54		51.221				6204.0009	381.84175	0	0	2.1
Day 13	0.5	1	54			-			7447.1767	470.19666	0	34	5.3
Day 15	0.5	1	54			-460	7.93	9	5880.9936	394.50845	0	0	3.8

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	$N_2Oppb$	CH <sub>4</sub> ppm	NH <sub>3</sub> ppm	$H_2S ppm$
Day 0	0.5	2	48	90.409	45.409	-127.7	6.7	6.53					
Day 1	0.5	2	48						•				
Day 2	0.5	2	48						7784.9358	358.62986	0	0	0.024
Day 3	0.5	2	48		45.409				12912.82	378.28509	0	0	0.51
Day 4	0.5	2	48						2882.1601	387.58232	0	0	1.1
Day 5	0.5	2	48		45.409				5015.6175	372.38852	0	0	1.6
Day 6	0.5	2	48						1546.6417	0	0	0	2.3
Day 7	0.5	2	48		45.409				5467.9427	521.30025	0	0	2.5
Day 9	0.5	2	48		45.409				2861.6608	0	0	0	2.5
Day 11	0.5	2	48		45.409				2616.4358	383.99446	0	22	2.4
Day 13	0.5	2	48						5059.4898	621.60429	0	0	5.2
Day 15	0.5	2	48			-386	11.14	8.81	2913.9627	357.91229	0	0	3.3
Day 0	1	1	46	101.827	50.91	-67.2	8.59	6.56					
Day 1	1	1	46										
Day 2	1	1	46						12081.929	482.55138	0	17	0.18
Day 3	1	1	46		50.91				6830.091	374.16685	0	22	2.8
Day 4	1	1	46						10852.164	361.99933	0	64	3.1
Day 5	1	1	46		50.91				14393.941	376.91234	0	26	2.1
Day 6	1	1	46						3845.6261	341.40814	0	54	5.9
Day 7	1	1	46		50.91				22923.365	591.65347	0	30	4.8
Day 9	1	1	46		50.91				3344.2559	393.38529	0	49	3.5
Day 11	1	1	46		50.91				2822.1949	375.4772	0	32	2.9
Day 13	1	1	46						3496.7552	414.53806	0	28	7.3
Day 15	1	1	46			-447	11.26	9.04	6139.6293	385.36721	0	27	5.3

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	N <sub>2</sub> O ppb	CH <sub>4</sub> ppm	NH <sub>3</sub> ppm	$H_2S ppm$
Day 0	0.5	1	44	134.25	67.125	-219	6.32	6.71					
Day 1	0.5	1	44									•	
Day 2	0.5	1	44						7450.6251	386.98954	0	0	0.24
Day 3	0.5	1	44		67.125				5152.7903	342.09451	0	0	2.1
Day 4	0.5	1	44						5161.0283	338.81864	0	0	1.5
Day 5	0.5	1	44		67.125				4552.7554	348.36547	0	0	1.4
Day 6	0.5	1	44						4628.8134	348.98944	0	0	2.8
Day 7	0.5	1	44		67.125				4381.2894	395.97479	0	0	2.2
Day 9	0.5	1	44		67.125				6052.8427	368.33268	0	0	1.8
Day 11	0.5	1	44		67.125				6091.5422	354.57402	0	0	1.5
Day 13	0.5	1	44						5513.9224	395.53801	0	0	3.8
Day 15	0.5	1	44			-339	5.9	8.83	4583.9832	382.24733	0	0	2.4
Day 0	0	2	45	79.37	39.685	-203	8.17	6.35					
Day 1	0	2	45									•	
Day 2	0	2	45						6767.252	368.92546	0	0	0.033
Day 3	0	2	45		39.685				19871.463	407.20635	0	0	0.53
Day 4	0	2	45						6157.4464	392.19974	0	0	1.5
Day 5	0	2	45		39.685				5379.4318	348.42786	0	0	1
Day 6	0	2	45						2697.0918	426.98637	0	0	3.8
Day 7	0	2	45		39.685				2385.1963	460.55624	0	0	3.3
Day 9	0	2	45		39.685				1379.3906	0	0	19	3.3
Day 11	0	2	45		39.685				1865.2426	0	0	46	2.5
Day 13	0	2	45						2256.2616	329.24062	0	0	5
Day 15	0	2	45			-384	10.25	8.98	2083.8377	375.13401	0	11	4.2

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	N <sub>2</sub> O ppb	CH <sub>4</sub> ppm	NH <sub>3</sub> ppm	$H_2S ppm$
Day 1	0	1	41										
Day 2	0	1	41						2034.4096	0	0	0	0.028
Day 3	0	1	41		39.685				2618.9264	351.82853	0	0	0.58
Day 4	0	1	41						8285.5396	419.12428	0	0	0.51
Day 5	0	1	41		39.685				9080.2219	1621.0569	0	0	1.1
Day 6	0	1	41						6717.2491	441.02581	0	0	1.6
Day 7	0	1	41		39.685				11931.346	530.34789	0	0	1.5
Day 9	0	1	41		39.685				3670.9033	322.1897	0	0	0.4
Day 11	0	1	41		39.685				1853.173	406.51997	0	0	0.96
Day 13	0	1	41						6684.8718	395.7252	0	0	2.4
Day 15	0	1	41			-440	6	8.51	3499.0542	352.20291	0	0	1.9
Day 0	0	1	45	92.405	46.205	-139.9	3.26	6.62					
Day 1	0	1	45										
Day 2	0	1	45						6479.879	325.80876	0	0	0.04
Day 3	0	1	45		46.205				2643.4488	348.11588	0	0	1.1
Day 4	0	1	45						2550.9147	392.69892	0	0	1.2
Day 5	0	1	45		46.205				9795.7808	512.97018	0	0	0.59
Day 6	0	1	45						2128.2848	404.11767	0	0	2.7
Day 7	0	1	45		46.205				2774.1078	592.21505	0	0	2.3
Day 9	0	1	45		46.205				2051.0772	383.62008	0	0	1.9
Day 11	0	1	45		46.205				4303.8903	338.47546	0	0	1.5
Day 13	0	1	45						2066.2122	364.65122	0	9	5.4
Day 15	0	1	45			-475	9.58	8.84	1944.366	354.10604	0	0	4

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	N <sub>2</sub> O ppb	CH <sub>4</sub> ppm	NH <sub>3</sub> ppm	$H_2S ppm$
Day 0	blank	blank	3B										
Day 1	blank	blank	3B								•	•	
Day 2	blank	blank	3B						8309.679	342.56249	0	0	0.012
Day 3	blank	blank	3B						1715.0423	0	0	0	0
Day 4	blank	blank	3B						4707.5536	377.78591	0	0	0.3
Day 5	blank	blank	3B						2049.9277	0	0	0	0.53
Day 6	blank	blank	3B						1849.7245	367.55271	0	0	0.48
Day 7	blank	blank	3B						1874.4386	456.78119	0	0	0.41
Day 9	blank	blank	3B						0	440.52663	0	0	0.38
Day 11	blank	blank	3B						1816.3892	360.2834	0	0	0.11
Day 13	blank	blank	3B						1779.9886	426.98637	0	0	0.61
Day 15	blank	blank	3B						1464.6446	0	0	0	0.6
Day 0	1	2	46	137.756	68.878	-142.8	6.49	6.34					
Day 1	1	2	46								•	•	
Day 2	1	2	46						2238.8276	406.05199	0	0	0.18
Day 3	1	2	46		68.878				2159.7042	339.38022	0	24	0.3
Day 4	1	2	46						1993.6026	363.06009	0	27	2.7
Day 5	1	2	46		68.878				2441.9046	408.73509	0	0	1.4
Day 6	1	2	46						3853.8641	446.4856	0	76	7.7
Day 7	1	2	46		68.878				3544.4591	613.61741	0	84	6.2
Day 9	1	2	46		68.878				5094.9325	412.72853	0	111	4.5
Day 11	1	2	46		68.878				3305.7479	352.01572	0	108	3.8
Day 13	1	2	46						5049.7191	543.91936	0	145	13
Day 15	1	2	46			-478	14.76	9.27	1666.9552	0	0	101	6.2

Day	Treatment	Period	Bunk	Feces (g)	Urine (g)	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	N <sub>2</sub> O ppb	CH <sub>4</sub> ppm	NH <sub>3</sub> ppm	$H_2S ppm$
Day 0	R1	Room	1			•	•		•	•	•		•
Day 1	R1	Room	1						•	•			
Day 2	R1	Room	1						0	0	0	0	0.002
Day 3	R1	Room	1						0	0	0	0	0.049
Day 4	R1	Room	1						0	0	0	0	0.38
Day 5	R1	Room	1						0	382.15373	0	0	0.028
Day 6	R1	Room	1						0	493.37735	0	0	0.021
Day 7	R1	Room	1						0	452.00778	0	0	0.001
Day 9	R1	Room	1						0	0	0	0	0
Day 11	R1	Room	1						0	0	0	0	0
Day 13	R1	Room	1						0	0	0	0	0
Day 15	R1	Room	1						0	0	0	0	0
Day 0	C1	Cal	1										
Day 1	C1	Cal	1										
Day 2	C1	Cal	1						353806221	0	912752027	0	0
Day 3	C1	Cal	1						272221014	0	655025523	0	0
Day 4	C1	Cal	1						21265369	462.36577	563403219	0	0
Day 5	C1	Cal	1						24936317	0	630822208	0	0
Day 6	C1	Cal	1						33751006	0	804229095	0	0
Day 7	C1	Cal	1						32321804	0	793261500	0	0
Day 9	C1	Cal	1				•		37191819	566.56966	862797406	0	0
Day 11		Cal	1						23100961	348.05348	561848845	0	0
Day 13	C1	Cal	1						12324.664	614.14779	11943.068	0	0
Day 15	C1	Cal	1			•	•	•	11452.774	0	12285.98	0	0

Day	Treatment	Period	Bunk	ORP mv	EC ms/cm	pН	CO <sub>2</sub> ppm	N <sub>2</sub> O ppb	CH <sub>4</sub> ppm	NH <sub>3</sub> ppm	$H_2S ppm$
Day 0	R2	Room	2								
Day 1	R2	Room	2						•		
Day 2	R2	Room	2				0	0	0	0	0
Day 3	R2	Room	2				4234.5376	0	0	0	0
Day 4	R2	Room	2				0	0	0	0	0
Day 5	R2	Room	2				2488.0758	0	0	0	0
Day 6	R2	Room	2				0	0	0	0	0
Day 7	R2	Room	2								
Day 9	R2	Room	2								
Day 11	R2	Room	2				0	0	0	0	0
Day 13	R2	Room	2				2041.1149	0	0	0	0
Day 15	R2	Room	2				3317.0513	0		0	0.023
Day 0	C2	Calgas	2								
Day 1	C2	Calgas	2								
Day 2	C2	Calgas	2				11511.589	0	12901.985	0	0
Day 3	C2	Calgas	2				10765.377	0	12243.091	0	0
Day 4	C2	Calgas	2				7951.6122	407.17515	10806.811	0	0
Day 5	C2	Calgas	2				9608.6051	0	13937.902	0	0
Day 6	C2	Calgas	2				10406.736		9947.6362	0	0
Day 7	C2	Calgas	2								
Day 9	C2	Calgas	2				•				
Day 11	C2	Calgas	2				9992.9187	307.49508	11980.571	0	0
Day 13	C2	Calgas	2				11666.388	0	10490.63	0	0
Day 15	C2	Calgas	2	•	•	•	4119.78	355.38519	2263.7371	0	0