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Odor Evaluation and Gas Emission from Manure of Dairy Heifers Fed High/Low - Forage Quality and High/Low-Concentrate Diets

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Abstract. *A heifer feeding trial evaluated the impact of high/low forage quality and high/low concentrate level nutrient-balanced diets on simultaneous odor and gas emissions from the manure. Gas concentration was determined using an infrared photoacoustic analyzer over a 24-hour period using a steady-state flux chamber setup containing urine:feces as-excreted from eight individual heifers. Odorous air samples were collected from chamber headspace and evaluated by six human assessors for pleasantness, intensity and detection threshold using a forced-choice dynamic olfactometer. Ammonia emission ranged from 0.64 to 3.94 mg* NH_3 cm^{-2} d^{-1} *across diets. Average ammonia emission from the low concentrate (80% forage) diets (2.11 mg* NH_3 cm^{-2} d^{-1}*) was larger than the high concentrate (20% forage) diets (1.69 mg* NH_3 cm^{-2} d^{-1}*), but not significantly different. Carbon dioxide emission was significantly higher (p= 0.0143) in the low concentrate diets. There was a linear increase of methane emission as reduced quality forage (corn stover) was increased in the low-concentrate diet (p = 0.030). Nitrous oxide emissions were similar and low in all diets. Highest average odor emission (8.58 OU* m^{-2} sec^{-1}*) was from the low concentrate, high forage quality (80% corn silage) diet while lowest emission (5.01 OU* m^{-2} sec^{-1}*) was measured when forage quality was reduced (32% silage; 48% stover). Odor emission tended to be reduced with lower quality forage diets, but with no significant difference. The volume of feces produced from the high concentrate diet was about half that from the low concentrate diet heifers. But total manure produced by the high concentrate diet heifers was 23% higher due to increased urine production.*

Keywords. ammonia, greenhouse gas emissions, dairy heifers, odor, manure, olfactometer

Introduction

The United States and global environmental policies and regulations have increasing production and operations impacts on dairy production facilities. Cattle emit approximately 50% of the ammonia (NH_3) released to the environment from agricultural sources in the U.S. (Battye et al., 1994). Currently, the U.S. Department of Agriculture (USDA) estimates the U.S. dairy replacement population (heifers at body weight 227 kg) at approximately 4 million, or 4% of the total cattle inventory (USDA, 2007; based on 6 reports from 2005 to 2007). Cattle dietary manipulation has proven to affect the composition of manure excreted and NH_3 emissions (James et al., 1999; Misselbrook et al., 2005). However, actual emission contributions by dairy heifers fed modern diets are not available in the literature.

In dairy manure, ammonia and greenhouse gases are emitted rapidly once the feces are mixed with urine. Ammonia is released from the manure as a result of microbiological hydrolysis of urea and uric acid by urease to form ammonium (NH_4^+) and its subsequent volatilization to NH_3 (Bouwman et al., 1997). Urease is produced by microorganisms present in feces while urea and uric acid are ubiquitous in urine. Carbon dioxide is emitted to the environment by heterotrophic respiration during degradation of undigested feed protein, microbial protein and endogenous protein excreted in feces (Tamminga, 1992). Methane is produced in the manure via methanogenesis when fecal materials further decompose under oxygen-deprived conditions (Oenema et al., 2005; Smith and Conen, 2004). Nitrous oxide is generated by nitrifying and denitrifying microorganisms in manure and often enhanced by high labile nitrogen [N] (Mosier et al., 1998; Smith et al., 2007). Odorous gases are produced from fermentative degradation of fecal substances by anaerobic bacteria. There are over 200 different odorous compounds that have been identified in animal manure. These include volatile fatty acids, indoles and phenols, ammonia and volatile amines, and volatile sulfur-containing compounds (O'Neil and Phillips, 1992).

The amount and kind of feed ingested by a dairy cow affect the N excretion in manure. Our hypothesis was that feeding a high quality, restricted concentrate diet to dairy heifers should result in high efficiency of nutrient utilization in the digestion track of the animal. Consequently, an increase in nutrient efficiency means less N released to the environment. This study was conducted to evaluate the impact of high/low forage and high/low concentrate nutrient-balanced diets on odor and gas emissions of ammonia (NH_3), carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) from the manure.

MATERIALS AND METHODS

Overview

A trial was conducted at the Pennsylvania State University (PSU) dairy nutrition laboratory to assess the effect of manipulating the diet ratio of forage to concentrate (F:C) on the volatilization of NH_3 and greenhouse gases (GHG), CH_4, N_2O and CO_2 emissions, from the manure produced by dairy heifers. This study was approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University. Eight (8) Holstein heifers, age approximately 12 months (321 ± 21 kg initial BW), were selected from the PSU dairy replacement herd. These cattle were individually housed in tie stalls in an environmentally controlled barn with continuous access to fresh water. The PSU Agricultural and Biological Engineering department personnel measured and evaluated the gaseous emissions and odor. Gaseous emissions were measured using a multi-chamber steady-state gas emission detection system. Odor samples were collected from the head space of flux chamber containers that contained manure (urine and feces) collected from each heifer and evaluated by qualified human assessors for odor intensity, pleasantness, and odor detection threshold levels (in odor units, OU).

Dietary treatments

Treatments were administered according to a split plot design with diet type as the whole plot and forage quality as sub-plots in a 4-period (21 d) 4 X 4 Latin square. Periods consisted of 17 d adaptation and 4 d total fecal and urine collection. The rations were balanced for crude protein and nutrients. The experiment had two diet types depending on level of forage: a high concentrate (HC) and a low concentrate (LC) diet. The HC diet had 20% forage while the LC diet had 80% forage. The forage quality consisted of different levels of corn silage and corn stover. Diets had a constant level of forage, 20% for the HC1, HC2, HC3 and HC4 with increasing levels of corn stover, 0, 4, 8 and 12 % respectively; and 80% forage for the LC1, LC2, LC3, LC4 with increasing levels of corn stover, 0,16, 32 and 48 % respectively. Corn stover was used as a decreasing quality factor of the forage component. A full description of feed ingredients and composition is discussed in Lascano et al. (2008).

Feces and urine samples

At the end of the 17 d adaptation period, during the 4 days of total manure collection, total daily urine and feces collection occurred at 10:00 a.m. just before feeding. Separate urine and feces from the eight animals were stored in refrigerated, airtight containers. Urine was collected using non-invasive urinary devices attached to the heifers. Urine collection devices were fabricated by the Penn State Engineering Services from a urinary cup developed at the University of Missouri (Fellner et al., 1988). Urine sub-samples (250 mL) were collected without acidification for use in the multi-chamber steady-state gas emission detection system. Total feces and urine excretion from the first 2 d of each period were used to determine feces to urine (F:U) ratio (wet basis) for each heifer. Each heifer's individual F:U ratio was used to partition the 200 grams of manure sample for use in the steady-state flux chamber setup.

Ammonia and greenhouse gas measurements and calculations

Gaseous emissions were quantified using the multi-chamber steady-state gas emission detection system under temperature-controlled conditions (Wheeler et al., 2007). In brief, the flux chamber setup used a photoacoustic multi-gas field-monitor (Model 1412, Innova, Denmark) to measure gas concentrations from each of six flux chambers every 20 min over a 24-h period. Flux chambers were immersed in a 25^{o}C water bath. Each flux chamber consisted of a 3.8-L glass jar with a continuous supply of 2 liter min^{-1} filtered, sweep air. Five jars each contained 200 g of manure slurry (feces to urine) combined at the initiation of the gas emission test representing the ratio of feces to urine as excreted by each heifer. The sixth jar contained distilled water as a control, a check for cross-contamination of sampling lines, and for determining background gas concentration levels. Gas emission rates were computed using the following equation for the steady-state flux chamber setup:

$$E = \frac{Q(C_1 - C_{BLK})}{A} \quad \text{[Eq. 1]}$$

where E is gas emission rate of NH_3, CO_2, CH_4, or N_2O (mg cm^{-2} min^{-1}), Q is flow rate of filtered air supplied through each chamber (0.002 m^3 min^{-1}), C_1 is the measured gas concentration (mg m^{-3}), C_{BLK} is measured ambient gas concentration (distilled water chamber in mg m^{-3}) and A is the surface area of manure in each chamber (cm^2). The daily gas emission (mg cm^{-2} d^{-1}) was computed as the sum of emission rates for 24 hours.

Odor measurements and calculations

Within the first hour of manure placement in the multi-chamber, steady-state gas emission detection system, approximately 7.0 L of odorous exhaust sweep air was collected from each of the chambers into a 10 L preconditioned Tedlar™ bag for olfactory evaluations. All odor samples were presented to trained panelists and analyzed for detection threshold (DT) and recognition threshold (RT) levels using an Ac'Scent International Olfactometer (St. Croix Sensory, Inc., 2007), following the Triangular Forced-Choice method (EN13725:2003).

After use in the Olfactometer, the bag containing the odorous gas sample was moved to a different lab where each panelist would smell the undiluted bag contents and evaluate for hedonic tone and intensity. Hedonic tone (pleasantness) was subjectively quantified by using a 22-unit scale (-11 for extremely unpleasant to +11 for extremely pleasant). The panelists assessed the odor intensity using the Labeled Magnitude Scale method (non-linear scale ranging from 0 to 100, Green et.al. 1996). All odor panel evaluations were performed within seven hours of air sample collection, well within the 36 hour requirement specified in EN13725:2003.

Odor emission was computed using Eq. 1 where variable E is odor emission rate of manure (OU m^{-2} sec^{-1}), C_1 is odor concentration of manure (OU m^{-3}), C_{BLK} is odor concentration of preconditioned, N_2 filled Tedlar™ bag (OU m^{-3}), and Q is gas flow rate of the steady-state chamber (3.3 x 10^{-5} m^3 sec^{-1}). A is the area of manure surface in each chamber (m^2).

Statistical analysis

An analysis of the split plot design was performed with diet type as the whole plot and forage quality as sub-plots in a 4 x 4 Latin square, using statistical software (SAS, 2001). Main effects due to diet type (forage portion), forage quality, as well as interactions and random effects due to period, animals, forage portion and forage quality were analyzed using the PROC MIXED covariance test.

All data were subjected to normality testing using the Shapiro-Wilk method, and were converted to logarithmic values when normality tests failed. Least significant differences were calculated at $P < 0.05$, when the effects of dietary treatments on NH_3 and GHG emissions were found to be significant. Odor intensity and pleasantness results were computed using the arithmetic mean. Pearson and Spearman correlation coefficients and p-values of gas and odor emissions were computed (SAS, 2001). Data for gas emission versus odor emission were fitted to a simple linear regression model when the correlation was found to be significant. Relationships among average odor intensity and pleasantness scales to gas and odor emission rates were determined using Pearson and Spearman correlation analysis.

Results

Differences in gas emissions were observed across the various fed diets (Table 1). Correlations among the measured gas and odor emissions and odor characteristics are presented in Table 2. Ammonia emission rates ranged from 0.64 to 3.94 mg NH_3 cm^{-2} d^{-1} across all diet treatments. Average NH_3 emission in the low concentrate diet (2.12 mg NH_3 cm^{-2} d^{-1}) was larger than the high concentrate diet (1.68 mg NH3 cm^{-1} d^{-1}) but there was no significant difference in daily NH_3 emission rate between high and low concentrate diets (Table 1). The lowest average NH_3 emission was measured in 8% corn silage HC diet (HC3). Within each forage quality diet, increasing corn stover dry matter percentage did not affect the production of NH_3 in the manure (p=0.530).

Carbon dioxide emission was significantly higher (p= 0.0143) in the LC diet than in HC diet. Highest average CO_2 emission was measured in the LC 32% corn stover (LC3) diet, while the lowest average CO_2 emission rate in all HC diets was measured in the HC 8% corn stover treatment (Table 1). There was a positive correlation between CO_2 and NH_3 emissions (r= 0.613, p= 0.000). It is likely that both CO_2 and NH_3 emissions from fresh manure were produced during microbial degradation of urea and affected by similar environmental variables. Methane emissions were statistically the same in all diets tested regardless of forage type. Interestingly, there was a corresponding linear decrease of CH_4 emission as percent corn stover in the LC diet increased (p=0.030) (Fig.1). Nitrous oxide gas emission hovered around 0.00 mg N_2O cm^{-2} d^{-1} (Table 1) with some concentrations above minimum detectable levels for the photoacoustic gas analyzer and some below (instrument noise), resulting in some nitrous oxide emissions reported below 0.00 mg N_2O cm^{-2} d^{-1} . This is due to nitrous oxide concentrations being very low and when background levels were subtracted from the manure emission level, it resulted in a slightly negative emission rate.

Odor emission rates ranged from 2.11 to 12.7 OU m^{-2} sec^{-1} across the dietary treatments. These odor values are higher than the odor emission rates from beef cattle feed yards reported by Parker et al. (2005) who also used gas collection sample bags and an olfactometer. The high magnitude of odor emission reported in our study could be due to: different feeds and animal types (dairy vs. beef), the fact that our odor sample was collected within a few centimeters of the manure surface (vs. 1 m off ground downwind of beef feedlot) and the odor was concentrated in the headspace of an enclosed chamber equipped with a continuous supply of 2 liter min^{-1} sweep air (½ chamber volume min^{-1}). This ventilation rate was used to mimic dairy freestall conditions during mild weather ventilation and probably under estimates airflow conditions found in the beef cattle study. The highest average odor emission was measured in LC diet (L1) with 80% corn silage (0% corn stover) while the lowest odor emission was observed in the LC (LC4) with 48% corn stover (Table 1). Although odor emission rates in HC diets also tended to be reduced with high percent corn stover (HC4 and LC4), there was no significant difference (p=0.623 and 0.328) between forage quality and forage portion during the trial period. Only CH_4 emission was highly and positively correlated with odor emission (r=0.648, p=<0.000) (Table 2 and Figure 2). This suggests that emissions of odor and methane in the manure were likely produced by similar bacterial community under similar environmental conditions. There was no relationship found between NH_3 and odor emissions suggesting that other odorant gases, such as, volatile organic acids, hydrogen sulfide, phenol or indole, may be impacting the odor concentration in the chamber headspace.

Odor intensity ranged from 17 to 43 for the dietary treatments. The strength of odor intensity emitted by manure was similar in all forage quality diets tested. Mean odor intensity of the manure measured by our odor assessors was about 30 suggesting a distinguishable odor characteristic (Table 1). From a scale of -11 to +11, average hedonic tone was consistently -4 (Table 1) with this negative value describing the manure from all diets as unpleasant. Odor intensity was highly and negatively correlated with hedonic tone (r= -0.703, p=<0.0001) (Table 2), indicating that odor became more unpleasantness as intensity increased. In contrast to other reported studies, (Parker et al. 2005; Zahn et al. 2001) odor emission rates were not correlated with odor intensity and hedonic tone. All of our data appear to be evenly distributed throughout the plot suggesting no indication of trends. In our study, the methods to quantify odor intensity and hedonic tone were based on subjective judgment of each panel assessor. Addition of control intensity and control pleasantness odorants could improve the statistical evaluation of results.

Feces to Urine Ratio Production

Our preliminary findings indicate, on a wet mass basis, the HC diets produced a significantly lower mass of feces, 55.6% less, than the LC diet animals, but the total manure produced by the HC diets was 22.7% higher than the LC diet animals. Table 3 presents all means and standard errors as the Diet x Quality interaction (DxQ). Urine:feces was lower for the HC diet (0.48 vs. 2.44 ± 0.44; $P < 0.01$). More g of feces per g of urine were linearly produced as quality of forage decreased ($P = 0.02$). Total manure was significantly higher as forage quality increased ($P < 0.01$), and decreased linearly with the addition of corn stover in the diets ($P < 0.01$). Less feces were produced by the HC diets ($P < 0.01$), but there was no effect of the corn stover increments in the diet, probably because there was no DxQ interaction for feces. The higher total manure excreted by the HC group is explained by its higher urine excretion ($P < 0.01$). There was a linear decrease of urine produced with decreasing the forage quality (by increasing the corn stover % in the diet; $P < 0.01$). As the quality of the diet increased, the urine production increased ($P = 0.03$). The difference in urine:feces is mainly due to the urine output with respect to the different corn stover increments in the diet

Even though the amount of feces was cut in half in the HC fed group, the urine mass excreted by this group was almost three times (2.84 times) higher than the LC group, which increased the total manure produced. This is similar to results from other PSU trials (Lascano, 2008) where urine excretion was increased using a HC diet. It is likely that the differences in ratio of feces to urine (F:U) in the manure under HC and LC diets impacted the production of agricultural gases from fresh manure. Ammonia gas is produced from manure during microbial degradation of urea in the urine using urease found in the feces. A 50:50 F:U in dairy manure resulted in the greatest NH_3 production throughout ratios ranging from 20:80 to 80:20 (Wheeler et al. 2007) suggesting a balance between available urease and urea. A high ratio of feces in fresh manure may have produced higher CO_2 gas due to the presence of greater carbon substrate and urease content as compared to low feces volume. Further work is needed and planned to compare the dry mass basis between urine and feces produced.

Conclusions

The use of a multi-chamber steady-state gas emission detection system coupled with a forced - choice dynamic olfactometer proved valuable in simultaneously assessing emissions of ammonia, greenhouse gases and odor from dairy heifers fed diets of high and low concentrate levels with various ratios of corn silage and corn stover forages. Odor intensity and pleasantness are quite subjective but show promise to determine the over-all effectiveness of feed manipulation on manure odors.

Emissions of NH_3, CH_4, N_2O and odor were not significantly different among the eight dairy heifers fed the high concentrate and low concentrate diets and corn silage/stover based forages during this trial. It appears that changing the quantity of concentrate in the diet fed to dairy heifers for maximum nutrient efficiency was not as important as we hypothesized in the overall nitrogen and odor released from the manure to the environment. Between high and low concentrate diets, CO_2 emission rates were significantly high in HC diet. There was a positive correlation between CO_2 and NH_3 emissions ($r= 0.613$, $p= 0.000$) and a corresponding linear increase of CH_4 emission as percent corn stover in the low concentrate diet increased ($p=0.030$).

The highest average odor emission (8.58 OU m^{-2} sec^{-1}) was measured in the low concentrate diet with 80% corn silage forage, while the lowest average odor emission (5.01 m^{-2} sec^{-1}) was measured in the low concentrate diet with 32% corn silage and 48% corn stover. Odor emission rates tended to be low in high-percent corn stover for both high and low concentrate diets, but there was no significant difference (p=0.623 and 0.328, respectively) among the diets during the trial period.

An unexpected, observation noted during the trial was that the mass of manure (feces and urine) produced from the heifers on the high concentrate diet was 22.7 % higher than the mass of manure from the low concentrate diet heifers. This is mainly due to the fact that the mass of feces was more than 50 % less in the high concentrate fed group, but the urine mass excreted by this group was almost three times (2.84 times) higher than the low concentrate group, increasing the total manure produced. This suggests that the ratio of “as excreted” feces to urine might have an effect on odor and emissions and should be taken into consideration when analyzing data.

Table 1. Average gas emission rates, odor characteristics and standard errors of manure excreted by heifers fed on high concentrate (HC; 20% forage) and low concentrate (LC; 80% forage) diets.

Concentrate	Corn silage	Forage stover	Gas emission rates[1] (mg cm^{-2} d^{-1}) NH_3	CH_4	CO_2	$_2$O	Odor: Emission rates[2] (OU m^{-2} sec^{-1})	Intensity (mean)	Pleasantness (mean)
HC 1	20%	0%	1.99 ± 0.531	0.089 ± 0.020	4.80 ± 1.03*cd*	-0.001 ± 0.0	6.55 ± 0.90	34	-4.4
HC 2	16%	4%	2.09 ± 0.639	0.128 ± 0.020	5.27 ± 1.19*cd*	-0.001 ± 0.0	7.24 ± 1.28	26	-4.0
HC 3	12%	8%	1.02± 0.186	0.069 ± 0.020	4.12 ± 0.584*d*	0.000 ± 0.0	6.29 ± 0.498	32	-4.2
HC 4	8%	12%	1.64 ± 0.206	0.096 ± 0.011	4.94 ± 0.503*d* N	-0.001 ± 0.0	5.66 ± 0.967	29	-4.1
LC 1	80%	0%	1.80 ± 0.229	0.159 ± 0.040	6.47 ± 0.421*bcd*	-0.001 ± 0.0	8.58 ± 1.10	31	-4.1
LC 2	64%	16%	2.19 ± 0.123	0.133 ± 0.036	7.45 ± 0.183*abc*	-0.001 ± 0.0	6.84 ± 1.46	27	-4.1
LC 3	48%	32%	2.17 ± 0.110	0.073 ± 0.020	8.77 ± 1.159*a*	-0.001 ± 0.0	7.55 ± 2.19	32	-4.5
LC 4	32%	48%	2.30 ± 0.085	0.060 ± 0.014	8.13 ± 0.792*ab*	-0.001 ± 0.0	5.01 ± 0.425	27	-3.7

[1] Average gas emissions followed by the same letter were not significantly different at α=0.05.
[2] OU = odor unit

Table 2. Correlation matrix of gas emissions and odor characteristics of manure.

Variable	NH_3	CH_4,	CO_2	$_2$O	Odor	Intensity
CH_4, *mg cm^{-2} d^{-1}*						
	p=0.553		N			
CO_2, *mg cm^{-2} d^{-1}*	0.111	-0.072				
	p=0.000	*p=0.700*				
N_2O, *mg cm^{-2} d^{-1}*	0.613	-0.071	-0.470			
	p=<0.000	*p=0.703*	*p=0.008*			
Odor, *OU m^{-2} sec^{-1}*	-0.861	0.648	0.177	0.0298		
	0.223 *p=0.227*	***p=<0.000***	*p=0.342*	*p=0.874*		
Intensity	0.027	-0.167	0.051	0.156	0.273	
	p=0.885	*p=0.369*	*p=0.784*	*p=0.403*	*p=0.137*	
Pleasantness	-0.211	0.114	-0.080	0.146	-0.099	-0.703
	p=0.255	*p=0.541*	*p=0.669*	*p=0.434*	*p=0.596*	***p=<0.000***

Table 3. Manure production and partitioning for heifer diets.

Forage	DIET[1] HC				DIET[1] LC				SE	P-Value Contrasts[2]		
Corn Stover	0%	4%	8%	16%	0%	16%	32%	48%		Diet[3]	Quality[L]	DxQ [L]
Corn Silage	20%	16%	12%	4%	80%	64%	48%	32%				
Quality	1	2	3	4	1	2	3	4				
Parameter												
Feces :Urine	0.21	0.44	0.69	0.57	1.72	1.91	2.95	3.18	0.35	<0.01	0.00	0.02
Feces[4]	5.81	5.48	7.12	5.87	12.44	12.52	12.02	13.89	0.54	<0.01	0.24	0.67
Urine[4]	25.71	18.33	12.65	10.47	7.51	6.65	4.52	4.97	2.80	<0.01	<0.01	0.03
Total Manure[4]	31.52	23.80	19.77	16.34	19.95	19.17	16.54	18.86	2.88	0.05	0.01	0.03

[1] High concentrate (HC) or low concentrate (LC) diet with corn stover as the quality factor. As corn stover increases, quality of diet decreases from 1 to 4)
[2] DxQ =Diet x Quality interaction L=Linear
[3] Diet main effect
[4] Feces, urine and total manure expressed in Kg (wet mass basis)

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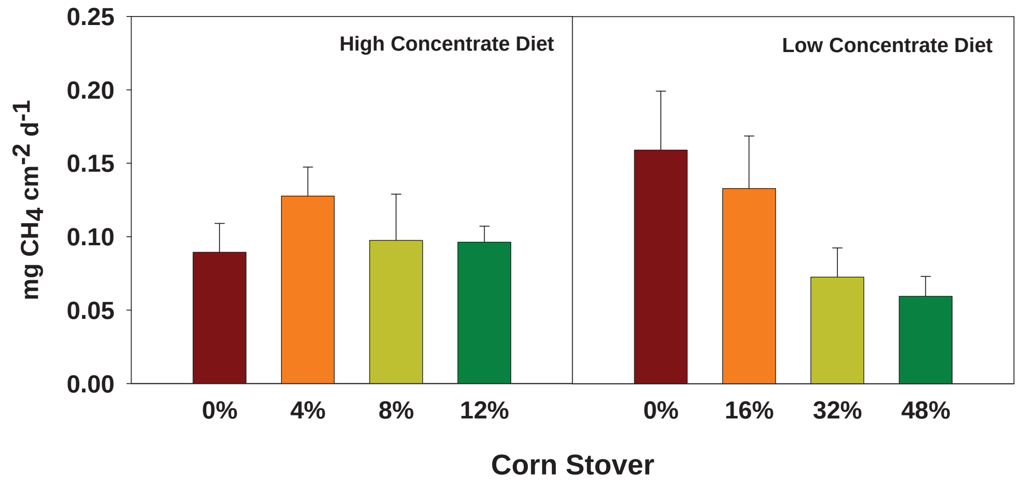

Figure 1. Average CH_4 emissions and standard errors in high (20% forage) and low (80% forage) concentrate diets with different levels of corn stover used in the forage portion.

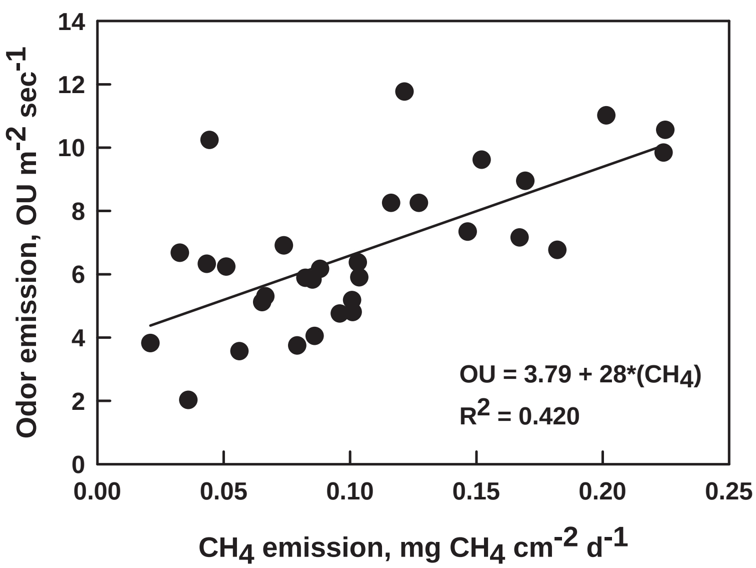

Figure 2. Relationship of odor versus methane emissions in high and low concentrate diets.

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