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# Impact of Dietary Carbohydrate and Protein Source and Content on Swine Manure Foaming Properties

Mark B. Van Weelden
*Iowa State University*, markvw@iastate.edu

Daniel S. Andersen
*Iowa State University*, dsa@iastate.edu

Brian J. Kerr
*United States Department of Agriculture*

Steven L. Trabue
*United States Department of Agriculture*

Kurt A. Rosentrater
*Iowa State University*, karosent@iastate.edu

See next page for additional authors

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**Abstract**

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**Keywords**

Anaerobic digestion, Dietary carbohydrate, Dietary protein, Foaming, Methane production, Swine manure

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**Authors**

Mark B. Van Weelden, Daniel S. Andersen, Brian J. Kerr, Steven L. Trabue, Kurt A. Rosentrater, Laura M. Pepple, and Tania M. B. dos Santos

# Impact of Dietary Carbohydrate and Protein Source and Content on Swine Manure Foaming Properties

M. B. Van Weelden, D. S. Andersen, B. J. Kerr, S. L. Trabue,
K. A. Rosentrater, L. M. Pepple, T. M. B. dos Santos

**Abstract.** *Diet ingredients are thought to contribute to foaming problems associated with swine manure stored in deep-pit systems. Two experiments explored the impact of protein and carbohydrate sources and levels in swine diets on the physicochemical properties, methane production potential, and foaming potential of swine manure. The first experiment was specific to protein and evaluated the impact of dietary protein level and source on manure properties, while the second experiment focused on evaluating the impact of different dietary carbohydrate sources on manure foaming properties. Manure from the animals was tested for total and volatile solids, methane production rate and biochemical methane potential, surface tension, foaming capacity and stability, and microbial community structure. No single diet yielded manure with all of the anticipated qualities associated with foaming manure. However, manure collected from pigs fed diets containing soy hulls and distillers dried grains with solubles (DDGS) exhibited higher methane production rates (0.95 ±0.20 and 0.96 ±0.20 L $CH_4$ $kg^{-1}$ VS, respectively) and biochemical methane potential (322 ±25 and 269 ±22 mL $CH_4$ $g^{-1}$ VS, respectively) when compared to manure obtained from pigs fed the other diets. Additionally, the results showed that both protein level and source exhibited greater influence over the microbial community than carbohydrate source, with manipulations in the protein diet leading to positive correlations with specific microbial community and higher methane production rates, foaming capacity, and foam stability. In this study, these parameters appeared to be tied to higher levels of corn, or corn protein, in the diet. Although some of the microbial community was explained by diet, this study also demonstrated that factors other than diet have significant influence on microbial community.*

**Keywords.** *Anaerobic digestion, Dietary carbohydrate, Dietary protein, Foaming, Methane production, Swine manure.*

The accumulation of foam on the surface of deep-pit swine manure storages is a serious concern. On a practical level, foam can significantly reduce the amount of space available for manure storage, which may force farm managers to apply manure during untimely seasonal windows or seek other means of manure storage. Foam accumulation also impacts safety at swine production facilities, as it captures gases (i.e., methane) produced by the anaerobic decomposition of swine manure; when the foam layer is broken, these gases are released. In worst-case scenarios, this dispersion of methane may be rapid enough for explosive concentrations to occur in the barn. Numerous facilities have reported flash fires or explosions due to the combination of foam layer breakage and an externally provided spark or flame (Moody et al., 2009). Although commercial defoamers are available, their use has met with mixed results; they reportedly knock the foam down (Robert et al., 2011), but it often quickly redevelops worse than the previous condition.

One conceptual framework that has been used to study the occurrence of biological foam in other industries, particularly municipal wastewater treatment, is the evaluation of foam as a three-phase system (i.e., gas, surface-active agent, and stabilizer). Davenport and Curtis (2002) suggested that initiation of foam occurs as a result of the gas and liquid phases working together to capture bubbles produced within the system. In anaerobic systems, such as deep-pit manure storages, the gas phase is a result of biogas production from microbial activity, such as methanogenesis. Research has shown that deep-pit foam consists of 60% to 70% methane, but other gases, such as carbon dioxide, ammonia, and hydrogen sulfide, may contribute (Moody et al., 2009). When appropriate concentrations of surface-active agents are present in the liquid layer, they facilitate foam production by lowering the surface tension of the solution with respect to water (Glaser et al., 2007; Davenport et al., 2008). Finally, hydrophobic solids are thought to stabilize the foam by pre-

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The authors are **Mark B. Van Weelden,** Graduate Student, and **Daniel S. Andersen, ASABE Member,** Assistant Professor, Department of Agricultural and Biosystems Engineering, Iowa State University, Ames, Iowa; **Brian J. Kerr,** Swine Nutritionist, and **Steven L. Trabue,** Research Chemist, USDA-ARS National Laboratory for Agriculture and the Environment, Ames, Iowa; **Kurt A. Rosentrater,** Associate Professor, Department of Agricultural and Biosystems Engineering, Iowa State University, Ames, Iowa; **Laura M. Pepple, ASABE Member,** Extension Specialist, and **Tania M. B. dos Santos,** Visiting Scholar, Department of Agricultural and Biological Engineering, University of Illinois, Urbana-Champaign, Illinois. **Corresponding author:** Daniel S. Andersen, 3348 Elings Hall, Iowa State University, Ames, IA 50011; phone: 515-294-4210; e-mail: dsa@iastate.edu.

venting or reducing liquid drainage from the foam and holding the bubbles in a stabilized structure (Bindal et al., 2002; Horozov, 2008; Heard et al., 2009).

There are three primary inputs to deep-pit manure storages: animal feces and urine, wasted feed and water, and wash water during times of barn cleaning. This creates a well-established link between feed composition and the physical and chemical characteristics of the manure (Kerr et al., 2006; Jarret et al., 2011; Trabue and Kerr, 2014). For example, Miller and Varel (2003) found that the composition of the manure and the potential release of nutrients and volatile emissions into the environment from livestock operations are partially controlled by dietary inputs. Similarly, diet can influence manure properties that lead to greater methane production potential (Cahn et al., 1998; Masse et al., 2003; Hansen et al., 2007; Jarret et al., 2011) or potentially the stabilization of foam on the surface of the manure (Kougias et al., 2013). For example, Moen (2002) and Massart et al. (2006) suggested that these stabilization agents are present in complex feed mixtures (such as fats, oils, greases, and polymers), or they may be intermediate degradation compounds, such as volatile fatty acids, as proposed by Westlund et al. (1998).

The objective of this study was to evaluate the impact that diet has on swine manure foaming characteristics and evaluate how these characteristics compared to parameters recorded at swine production barns with foaming manures. In particular, we evaluated the impacts of the protein level and source and the carbohydrate source in swine diets on manure properties related to gas production, manure physicochemical properties, and foaming potential. The characteristics reported were selected based on their use in describing differences between foaming and non-foaming manure samples collected from swine production barns in Iowa (Van Weelden et al., 2015). Comparisons between the dietary trial manures and manures from production farms were used to better understand which dietary components were related to foam production.

# Materials and Methods

Two studies, conducted at the Iowa State University Swine Nutrition Farm in Ames, Iowa, were performed to evaluate how diets formulated to vary in dietary crude protein (CP) level or source (denoted the protein study) and carbohydrate source (denoted the carbohydrate study) impacted manure foaming properties. The diet formulations are shown in tables 1 and 2. The protein study was conducted with two sets of pigs fed in the late spring and summer of 2012, and the carbohydrate study was conducted in the late fall of 2012 and winter of 2013. The swine feeding facility consisted of 24 metabolism crates with a corresponding manure storage tank for each metabolism crate. Two groups of 24 growing gilts initially weighing 119.5 kg (SD = 8.9 kg) each were fed 1.5 kg of the designated diet twice per day (0700 and 1900 h), and water was supplied *ad libitum* through nipple drinkers. Pigs were fed a daily amount of feed that approximated 3% of their body weight, which is considered near full feed but was slightly reduced to limit spillage from the feeders. The diets were typical for pigs of this body weight and were formulated to be adequate in all nutrients (NRC, 2012). Ambient temperature in the metabolism room was maintained at approximately 21°C, and lighting was provided continuously.

In the protein study (table 1), the level or source of dietary CP varied among the diets, while the energy and minerals were held relatively constant. Six diets were formulated. The C-SBM/AA diet was formulated with corn and soybean meal with economically available crystalline amino acids, while C/AA was formulated with only corn and crystalline amino acids (subsequently lower in CP than C-SBM/AA). The C-SBM diet was formulated with only corn and soybean meal and no crystalline amino acids (subsequently higher in CP than C-SBM/AA). The C-CM, C-CGM, and C-PM diets used alternative protein sources (canola meal, corn gluten meal, and poultry meal, respectively) in place of soybean meal while maintaining CP at 17.6%, similar to C-SBM. Be-

**Table 1. Composition of experimental diets, protein source study.**

| | | Protein Diet[a] | | | | | |
|---|---|---|---|---|---|---|---|
| Ingredient | | C/AA | C-SBM/AA | C-SBM | C-CM | C-CGM | C-PM |
| Corn (%) | | 94.83 | 78.47 | 71.29 | 61.13 | 77.72 | 81.53 |
| Soybean meal (%) | | - | 18.31 | 25.90 | - | - | - |
| Canola meal (%) | | - | - | - | 36.14 | - | - |
| Corn gluten meal (%) | | - | - | - | - | 18.30 | - |
| Poultry meal (%) | | - | - | - | - | - | 17.35 |
| Dicalcium phosphate (%) | | 1.22 | 1.09 | 1.03 | 0.77 | 1.19 | - |
| Limestone (%) | | 1.28 | 1.14 | 1.07 | 0.95 | 1.28 | - |
| Sodium chloride (%) | | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 |
| Trace mineral/vitamin mix (%) | | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 |
| L-lysine·HCl (%) | | 0.83 | 0.24 | - | 0.30 | 0.71 | 0.29 |
| L-threonine (%) | | 0.31 | 0.06 | - | - | 0.04 | 0.06 |
| L-tryptophan (%) | | 0.08 | - | - | - | 0.07 | 0.03 |
| DL-methionine (%) | | 0.17 | - | - | - | - | - |
| L-isoleucine (%) | | 0.28 | - | - | - | - | 0.03 |
| L-valine (%) | | 0.28 | - | - | - | - | - |
| Total | | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Calculated composition | ME (kcal $kg^{-1}$) | 3,306 | 3,313 | 3,314 | 3,323 | 3,386 | 3,299 |
| | Crude protein (%) | 8.7 | 14.8 | 17.6 | 17.6 | 17.6 | 17.6 |
| | NDF (%) | 8.3 | 9.3 | 9.7 | 19.6 | 13.7 | 7.1 |

[a] All diets formulated to 0.255% standardized lysine per 1,000 kcal of metabolizable energy, 0.78% calcium, and 0.25% available phosphorus. C/AA = corn plus amino acids, C-SBM/AA = corn-soybean meal plus amino acids, C-SBM = corn-soybean meal, C-CM = corn-canola meal, C-CGM = corn-corn gluten meal, and C-PM = corn-poultry meal.

cause the protein sources were derived from different origins, dietary fiber was allowed to vary. In the carbohydrate study (table 2), the C-SBM formulation was approximately the average between the C-SSB/AA and C-SBM formulations used in the protein study. The five remaining diets were formulated to be higher in dietary fiber, as measured by neutral detergent fiber (NDF), but differed in their source of fiber. The levels of dietary CP and minerals were held constant in all diets.

After each feeding session, feces and urine were collected and deposited in the manure storage tanks. Urine was collected from a bucket to which all liquid produced within the feeding crate continuously drained, while feces were collected by scrapping the solids captured on a sloped pan under the floor into the bucket of urine. The entire contents of the bucket were dumped into the manure tank. Manure tanks were designed to have a similar air-liquid surface area as used for pigs maintained in grow-finish barns with deep-pit manure storage systems. Feeding trials were conducted for 42 days, based on the length of time for maturation of manure from previous feeding trials (Trabue et al., 2016). Manure samples were thoroughly mixed prior to sampling, and samples were collected in 1 L plastic bottles and stored at 4°C before analysis. After the first trial, the tanks were emptied and cleaned by scrubbing, removing all organic residue, and then rinsing. The trial was repeated with diets randomized over an additional 24 pigs, thus giving 48 experimental units for each dietary study (each diet fed to four pigs per trial, i.e., eight per study).

### Total and Volatile Solids

The total solids and volatile solids contents of manure samples were tested according to the Standard Methods 2540B and 2540E (APHA, 2000). Total solids and volatile solids are reported as percentages of the total sample mass.

### Biochemical Methane Potential

The biochemical methane potential (BMP) defines the anaerobic biodegradability of a given material (Owen et al., 1979). Specifically, the BMP is the total volume of methane that a given substrate (in this case, manure) is able to produce. For this study, the BMP allowed comparisons of methane production potential between the manures produced by pigs fed different diet formulations, shedding light on how animal diet affects the gas phase of foaming systems. Samples with higher BMP indicate a greater ability for microbes to convert the specific substrate into biogas, which is the driving force of foaming. Assessment of the BMP of the swine manure samples was performed according to Moody et al. (2011).

### Methane Production Rate

The procedure for determining methane production rate was described in detail by Andersen et al. (2015). Briefly, the test indicates the rate at which bacteria already present in the manure produce methane from the available substrates. The MPR test involved adding approximately 100 mL of well-mixed sample to a 250 mL serum bottle (No. 223950, Wheaton Science Products, Millville, N.J.), sealing with sleeve stopper septa (Z564729, Sigma-Aldrich, St. Louis, Mo.), incubating at room temperature (approx. 23°C) for seven days, and then recording the amount of biogas produced and the concentration of methane in the biogas.

### Surface Tension

Surface tension is an important parameter that quantifies the impact of surface-active agents present in solution. In this study, surface tension was tested using a CSC Precision Ring Tensiometer (CSC Scientific Co., Fairfax, Va.). Samples were brought to room temperature, agitated gently by shaking to homogenize the solids and surfactants, and then poured into the sample tray. Following standard protocol for the tensiometer, the duNouy ring was placed below the surface of the liquid, and the ring was slowly pulled upward through the surface of the liquid until it overcame the surface tension of the sample. The force needed the break the liquid interface was recorded directly from the circular scale of the instrument in dyne $cm^{-1}$ (equivalent to mN $m^{-1}$) and is reported in N $m^{-1}$.

**Table 2. Composition of experimental diets, carbohydrate source study.**

| Ingredient | | Carbohydrate Diet[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | C-SBM | B | BP | DDGS | SH | WB |
| Corn (%) | | 75.17 | - | 54.09 | 61.75 | 62.14 | 57.21 |
| Barley, pearled (%) | | - | 83.27 | - | - | - | - |
| Soybean meal (%) | | 22.31 | 14.35 | 21.85 | 7.40 | 20.85 | 17.55 |
| Beet pulp (%) | | - | - | 22.00 | - | - | - |
| DDGS (%) | | - | - | - | 28.05 | - | - |
| Soybean hulls (%) | | - | - | - | - | 14.65 | - |
| Wheat bran (%) | | - | - | - | - | - | 22.91 |
| Dicalcium phosphate (%) | | 1.06 | 0.79 | 1.07 | 0.24 | 1.06 | 0.69 |
| Limestone (%) | | 0.66 | 0.79 | 0.27 | 1.23 | 0.51 | 0.82 |
| Sodium chloride (%) | | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 |
| Trace mineral/vitamin mix (%) | | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 |
| L-lysine·HCl (%) | | 0.11 | 0.10 | 0.02 | 0.50 | 0.09 | 0.12 |
| L-threonine (%) | | - | - | - | 0.09 | - | 0.01 |
| L-tryptophan (%) | | - | - | - | 0.05 | - | - |
| Total | | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Calculated composition | ME (kcal $kg^{-1}$) | 3,329 | 2,912 | 3,138 | 3,290 | 3,215 | 3,076 |
| | Crude protein (%) | 16.3 | 16.3 | 16.3 | 16.3 | 16.3 | 16.3 |
| | NDF (%) | 9.6 | 17.0 | 17.0 | 17.0 | 17.0 | 17.0 |

[a] All diets formulated to 0.255% standardized lysine per 1,000 kcal of metabolizable energy, 0.78% calcium, and 0.25% available phosphorus. C-SBM = corn-soybean meal, B = pearled barley-soybean meal, BP = corn-soybean meal-beet pulp, DDGS = corn-soybean meal-distillers dried grains with solubles, SH = corn-soybean meal-soybean hulls, and WB = corn-soybean meal-wheat bran.

### FOAMING CAPACITY AND STABILITY

The foaming capacity and stability were tested according to the method described by Van Weelden et al. (2015). In brief, air was passed through a 300 mL manure sample at 0.2 L $min^{-1}$ until a constant foam height was recorded. This height was divided by the initial height of the manure to create a foaming capacity. Foam stability was then measured by recording the descending height of the foam at pre-selected time intervals (0, 1, 2, 5, 10, 15, 30, 60, and 120 min). A first-order decay equation was fit to the height data to determine the foam half-life.

### BACTERIAL COMMUNITY COMPOSITION

To determine the microbial community composition in the manure, genomic DNA was extracted from 200 mg manure samples using the FastDNA SPIN Kit for Soil (MP Biomedicals, Santa Ana, Cal.). Bacterial community composition was assessed using automated ribosomal integenic spacer analysis (ARISA), as described previously (Yannarell and Triplett, 2005; Kent et al., 2007). The ARISA method uses polymerase chain reaction (PCR) to amplify the internal transcribed spacer region of the bacterial rRNA operon. Different lengths of this intergenic spacer region represent different bacterial populations and can be used to develop a DNA fingerprint of the microbial community that is analogous to a census of microbial populations. Determination of DNA fragment sizes was carried out using GeneMarker 1.95 (SoftGenetics, State College, Pa.). Patterns of similarity among bacterial communities were assessed using Bray-Curtis similarity and non-metric multidimensional scaling (NMDS) analysis implemented in PRIMER 6 for Windows (PRIMER-E, Ltd., Plymouth, U.K.). Analysis of similarity (ANOSIM) was used to evaluate patterns of microbial community similarity among groups of samples (Clarke and Green, 1988). ANOSIM generates test statistics (R values) whose magnitude indicates the degree of difference between groups of samples, with a value of 1 indicating completely different assemblages among samples and 0 indicating no distinction in composition among samples. Canoco ordination plots were used to determine the correlation between microbial community composition and physical foaming characteristics.

### STATISTICAL ANALYSES

For each study, 48 pigs were used. These pigs were fed in two groups of 24 pigs with diet randomly assigned to each pig (four pigs fed each diet during each group). After 40 days, manure samples were collected, and the crates and manure tanks were cleaned. A new set of 24 pigs was assigned to each crate, and diets were again randomized over the tanks. Statistical analysis was performed in JMP Pro 10 (SAS Institute, Inc., Cary, N.C.) using the standard least squares procedure, with differences tested at $\alpha = 0.05$. Data were analyzed as a randomized complete block design with the individual manure storage tank as the experimental unit. In this analysis, diet was considered a fixed effect, with group as a blocking variable that accounted for differences based on when the trial was conducted.

## RESULTS AND DISCUSSION

### TOTAL AND VOLATILE SOLIDS CONTENT

In the protein study, the total and volatile solids contents of the manure samples were strongly correlated (r = 0.9625) to one another (tables 3 and 4). The volatile solids content increased by approximately 0.65 g for every 1.0 g increase in total solids content. Consequently, total solids content was considered an excellent surrogate of volatile solids. Dietary protein had a significant ($p < 0.0001$) impact on total solids content, with higher protein levels leading to significantly higher solids contents ($p < 0.05$) (table 4). For the C/AA, C-SBM/AA, and C-SBM diets, total solids content of the manure was most strongly correlated with the protein content of the diet (slope = 0.8 g TS $L^{-1}$ manure for every 1% CP in the diet, $R^2 = 0.99$). However, when other protein sources were considered, the total solids content of the manure was most strongly correlated with metabolizable energy (slope = 0.12 g TS $L^{-1}$ manure for every 1 kcal $kg^{-1}$ feed, $R^2 = 0.53$). The protein source significantly impacted total solids content of the manure, as pigs fed the C-SBM diet produced manure having significantly lower solids content ($\alpha = 0.05$) than pigs fed the diet containing corn gluten meal.

Statistical analysis of the total and volatile solids concentrations in the carbohydrate study also indicated that the effect of diet was highly significant ($p < 0.01$) (tables 5 and 6). Pigs fed the WB and DDGS diets produced manure with the highest total solids content, while pigs fed the C-SBM/AA and B diets yielded manure with the lowest total solids content. In this study, the solids content could not be attributed to either the CP content or NDF content of the diet, as these amounts were consistent among diets. Instead, the differ-

**Table 3. Manure properties for different protein sources, including total and volatile solids concentrations, pH, methane production rate (MPR), biochemical methane potential (BMP), surface tension, foaming capacity, and foam half-life. Standard error of the mean (SEM) is provided at the bottom of each column. Values in the same column followed by different letters are significantly different ($\alpha = 0.05$).**

| Protein Source[a] | Total Solids (%) | Volatile Solids (%) | pH | MPR ($L\ CH_4\ L^{-1}\ d^{-1}$) | MPR ($L\ CH_4\ kg^{-1}\ VS\ d^{-1}$) | BMP ($mL\ CH_4\ g^{-1}\ VS$) | Surface Tension ($N\ m^{-1}$) | Foaming Capacity (%) | Foam Half-Life (min) |
|---|---|---|---|---|---|---|---|---|---|
| C/AA | 1.37 d | 0.86 d | 7.22 c | 0.009 | 0.99 ab | 272 | 0.051 a | 29 c | 0.12 |
| C-SBM/AA | 1.82 bc | 1.08 cd | 8.04 ab | 0.013 | 1.34 a | 335 | 0.052 a | 51 abc | 0.18 |
| C-SBM | 2.12 c | 1.27 bc | 8.34 a | 0.008 | 0.63 b | 344 | 0.049 a | 37 bc | 0.12 |
| C-CM | 1.50 cd | 1.02 cd | 7.69 bc | 0.006 | 0.59 b | 339 | 0.042 b | 19 c | 0.04 |
| C-CGM | 2.76 a | 1.83 a | 8.32 a | 0.011 | 0.62 b | 346 | 0.051 a | 66 ab | 0.54 |
| C-PM | 2.06 b | 1.48 b | 7.95 ab | 0.013 | 0.90 ab | 265 | 0.043 b | 90 a | 0.54 |
| SEM | 0.12 | 0.09 | 0.35 | 0.002 | 0.18 | 46 | 0.001 | 13 | 0.17 |

[a] C/AA = corn with amino acids, C-SBM/AA = corn-soybean meal with amino acids, C-SBM = corn-soybean meal, C-CM = corn-canola meal, C-CGM = corn-corn gluten meal, and C-PM = corn-poultry meal.

**Table 4. Pearson correlations among variables measured in the protein study. Relationships significant at α = 0.05 are shown in bold.**

| Variable[a] | TS | VS | pH | MPR | BMP | ST | FC | FS |
|---|---|---|---|---|---|---|---|---|
| TS | 1 | - | - | - | - | - | - | - |
| VS | **0.9625** | 1 | - | - | - | - | - | - |
| pH | **0.8447** | 0.7394 | 1 | - | - | - | - | - |
| MPR | 0.4083 | 0.4164 | 0.2912 | 1 | - | - | - | - |
| BMP | **0.9026** | **0.9469** | 0.6921 | 0.2667 | 1 | - | - | - |
| ST | 0.2205 | 0.0186 | 0.0991 | 0.296 | -0.0273 | 1 | - | - |
| FC | 0.6382 | 0.7309 | 0.439 | **0.8193** | 0.5431 | -0.0665 | 1 | - |
| FS | 0.7568 | **0.8645** | 0.4206 | 0.6861 | 0.7376 | -0.0047 | **0.933** | 1 |
| ME | 0.7301 | 0.7017 | 0.4686 | 0.0508 | **0.8441** | 0.3316 | 0.1405 | 0.4344 |
| CP | 0.6029 | 0.6388 | **0.7879** | 0.0353 | 0.682 | **-0.5065** | 0.3556 | 0.3468 |
| NDF | -0.0467 | 0.0048 | 0.0116 | -0.5865 | 0.3095 | -0.3703 | -0.4999 | -0.3001 |

[a] TS = total solids, VS = volatile solids, MPR = methane production rate (L $CH_4$ $L^{-1}$ $d^{-1}$), BMP = biochemical methane potential (L $CH_4$ $L^{-1}$ manure), ST = surface tension, FC = foaming capacity, FS = foam stability, ME = metabolizable energy of the diet, CP = crude protein of the diet, and NDF = neutral detergent fiber in the diet.

**Table 5. Manure properties for different carbohydrate sources, including total and volatile solids concentrations, pH, methane production rate (MPR), biochemical methane potential (BMP), surface tension, foaming capacity, and foam half-life for different. Standard error of the mean (SEM) is provided at the bottom of each column. Values in the same column followed by different letters are significantly different (α = 0.05).**

| Carbohydrate Source[a] | Total Solids (%) | Volatile Solids (%) | pH | MPR (L $CH_4$ $L^{-1}$ $d^{-1}$) | MPR (L $CH_4$ $kg^{-1}$ VS $d^{-1}$) | BMP (mL $CH_4$ $g^{-1}$ VS) | Surface Tension (N $m^{-1}$) | Foaming Capacity (%) | Foam Half-Life (min) |
|---|---|---|---|---|---|---|---|---|---|
| C-SBM/AA | 3.78 cd | 2.33 cd | 8.22 b | 0.023 | 1.31 | 395 a | 0.046 bc | 67 abc | 3.4 |
| B | 3.26 d | 2.09 d | 8.34 a | 0.017 | 0.87 | 360 ab | 0.048 ab | 106 ab | 1.3 |
| BP | 4.40 bc | 2.94 bc | 8.12 c | 0.028 | 1.08 | 155 d | 0.048 ab | 124 a | 4.1 |
| DDGS | 5.21 ab | 3.70 b | 7.98 d | 0.034 | 0.96 | 269 c | 0.048 ab | 63 bc | 0.7 |
| SH | 4.42 bc | 3.08 bc | 8.20 bc | 0.03 | 0.95 | 320 bc | 0.051 a | 104 ab | 1.5 |
| WB | 6.23 a | 4.52 a | 8.16 bc | 0.033 | 0.72 | 181 d | 0.044 c | 30 c | 0.2 |
| SEM | 0.37 | 0.27 | 0.06 | 0.004 | 0.2 | 23 | 0.001 | 21 | 1.5 |

[a] C-SBM = corn-soybean meal, B = barley, BP = beet pulp, DDGS = distillers dried grains with solubles, SH = soy hulls, and WB = wheat bran.

**Table 6. Pearson correlations among variables measured in the carbohydrate study. Relationships significant at α = 0.05 are shown in bold.**

| Variable[a] | TS | VS | pH | MPR | BMP | ST | FC | FS |
|---|---|---|---|---|---|---|---|---|
| TS | 1 | - | - | - | - | - | - | - |
| VS | **0.9965** | 1 | - | - | - | - | - | - |
| pH | -0.0699 | -0.0935 | 1 | - | - | - | - | - |
| MPR | **0.8837** | **0.8876** | 0.0255 | 1 | - | - | - | - |
| BMP | -0.007 | -0.0052 | -0.4767 | 0.2897 | 1 | - | - | - |
| ST | -0.4349 | -0.3836 | 0.1659 | -0.0562 | 0.1962 | 1 | - | - |
| FC | -0.6986 | -0.6746 | 0.5802 | -0.462 | -0.2534 | 0.7731 | 1 | - |
| FS | -0.5306 | -0.5814 | 0.7015 | -0.3755 | -0.1631 | 0.1588 | 0.5788 | 1 |
| ME | 0.1601 | 0.1208 | -0.0705 | 0.4551 | 0.7295 | 0.0573 | -0.2373 | 0.283 |

[a] TS = total solids, VS = volatile solids, MPR = methane production rate (L $CH_4$ $L^{-1}$ $d^{-1}$), BMP = biochemical methane potential (L $CH_4$ $L^{-1}$ manure), ST = surface tension, FC = foaming capacity, FS = foam stability, and ME = metabolizable energy of the diet.

ences were attributed mainly to the digestibility of the various fiber sources, with less-digestible sources resulting in greater manure solids (NRC, 2012).

The total and volatile solids contents in the carbohydrate study were substantially higher than in the protein study. This was due in large part to the trial timing, as the protein trials took place in late spring and summer, while the carbohydrate trials occurred in the fall and winter. This resulted in either greater water consumption or use of the watering system as supplemental cooling by the pigs during the warmer months. The solids concentrations measured in both trials were lower than those found during sampling of deep-pits throughout Iowa, which averaged around 8.3% total solids and 6.4% volatile solids (Van Weelden et al., 2015), compared to the 1.9% TS (1.3% VS) and 4.5% TS (3.1% VS) observed for the protein and carbohydrate feeding trial manures, respectively, in this study. In summary, our results for total and volatile solids appear to be related to the fiber content and digestibility of the carbohydrate source (NRC, 2012), with less-digestible ingredients leading to higher solids content. In the protein study, our results indicate that higher solids content was positively correlated with foaming capacity and stability; however, in the carbohydrate study, we noted the opposite effect.

### METHANE PRODUCTION

The average methane production rates are shown in tables 3 and 5 for the protein and carbohydrate diets, respectively. On average, the samples from the protein study had lower methane production rates (0.010 L $CH_4$ $L^{-1}$ manure $d^{-1}$) than those from the carbohydrate study (0.027 L $CH_4$ $L^{-1}$ manure $d^{-1}$), which is likely related to the lower solids concentration in the protein study (tables 3 and 5). This seems to be confirmed by normalization for volatile solids content, which made the averages similar (0.84 L $CH_4$ $kg^{-1}$ VS $d^{-1}$ compared to 0.98 L $CH_4$ $kg^{-1}$ VS $d^{-1}$) between the two studies. In the protein study, the impact of diet on methane production rate was not significant (table 3). Of the dietary factors, i.e., crude protein content, NDF content, and metabolizable energy, the methane production rate was most strongly correlated to NDF in the diet, with methane production rate decreasing by 0.0004 L $CH_4$ $L^{-1}$ manure $d^{-1}$ for every 1% increase in NDF in the diet ($R^2 = 0.344$). When methane production was normalized to the mass of volatile solids to account for differences in solids concentrations, the impact of diet was significant ($p < 0.05$), with pigs fed the C-SBM/AA diet generating manures with greater rates of methane production compared to pigs fed the C-SBM, C-CGM, or C-CM diet. In the carbohydrate study, no differences were found for methane production rate on a per unit volume basis ($p = 0.09$) or when normalized per gram of volatile solids in the manure ($p = 0.4057$). However, the methane production rate per unit of volatile solids in the manure was positively correlated with the metabolizable energy of the feed (0.0008 L $CH_4$ $kg^{-1}$ VS in the manure for every unit increase in kcal $kg^{-1}$ feed), suggesting that increased energy content of the feed increased digestibility by microbes in the manure.

As MPR was found to be very different between foaming manures (0.148 ±0.004 L $CH_4$ $L^{-1}$ manure $d^{-1}$) and non-foaming manures (0.049 ±0.003 L $CH_4$ $L^{-1}$ manure $d^{-1}$) at swine production facilities throughout Iowa (Van Weelden et al., 2015), it seems to be a useful parameter. However, the values of methane production rate in samples from production barns were about 2 to 5 times higher than the values found in our dietary trials, making direct comparison between the dietary trials and production manures difficult. When methane production rate was normalized based on volatile solids content in the manure, the field samples had methane production rates of 3.12 ±0.11 and 1.05 ±0.08 L $CH_4$ $kg^{-1}$ VS $d^{-1}$ for foaming and non-foaming pits, respectively. In the dietary trials, the methane production ranged from 0.59 ±0.18 to 1.34 ±0.18 L $CH_4$ $kg^{-1}$ VS $d^{-1}$, which puts the methane production rate in a similar range as reported for non-foaming manure pits; however, none reached the level of foaming manures. The failure to achieve higher methane production rates over the broad range of diets tested here may indicate that more time is necessary to generate the active microbial community necessary to generate the high methane production rates associated with foaming. As methane production is a relatively complex process, requiring an assortment of microbes to perform the individual steps, cultivating the right balance may require longer time than was allowed in this study, especially when starting with no inoculation population of microbes.

Average values of BMP with standard errors of the mean are shown in tables 3 and 5. The protein study did not yield any significant differences among dietary treatments; on average, the samples had a BMP of 313 ±22 mL $CH_4$ $g^{-1}$ VS. However, despite the lack of significant differences between treatments, we found correlations between ME, CP, and NDF in the feed and the BMP (mL $CH_4$ $g^{-1}$ VS), with the relationship to ME being statistically significant. Our results indicated that the methane potential per unit volume of manure increased by approximately 0.032 L $CH_4$ $L^{-1}$ manure for every unit increase in ME in the diet. The relationships with ME, CP, and NDF were enhanced when only the C/AA, S-SBM/AA, and C-SBM diets were considered (removing impacts of digestibility by the animal), with methane production potential increasing by 0.144, 0.139, and 0.880 L $CH_4$ $L^{-1}$ manure for every unit increase in ME or 1% increase in dietary CP or NDF in the feed. This indicates that dietary fiber is critical for providing enhanced methane potential in the manure (based on the high positive response in methane potential with increased NDF), but an active microbial community must develop to convert this potential into actual production (as NDF in the diet was negatively correlated to methane production rate).

The BMP results in the carbohydrate study were significantly different ($p < 0.0001$) between treatments. Pigs fed the C-SBM diet produced manure with the highest BMP values (395 ±23 mL $CH_4$ $g^{-1}$ VS), while the pigs fed the BP diet (155 ±23 mL $CH_4$ $g^{-1}$ VS) and WB diet (181 ±31 mL $CH_4$ $g^{-1}$ VS) produced manure with the lowest BMP. Pigs fed the barley, DDGS, and soy hull diets produced intermediate amounts of methane. This result appears to be correlated with the metabolizable energy of the feed, as a unit increase in the feed's metabolizable energy (kcal $kg^{-1}$) increased the biochemical methane potential of the manure by 0.018 mL $CH_4$ $g^{-1}$ manure ($R^2 = 0.53$).

The results of the BMP test give a different measure of the gas phase of anaerobic systems in comparison to the MPR, as the BMP test provides information on the potential to produce methane rather than the current production rate based on microbial activity level. In general, the BMP magnitudes reported in our study were substantially higher than the magnitudes reported for samples collected from deep-pit production swine barns. Van Weelden et al. (2015) reported an average value of 121 ±86 mL $CH_4$ $g^{-1}$ VS for both foaming and non-foaming samples, with foaming samples showing a slightly lower average value; in our study, BMP values of 155 to 395 mL $CH_4$ $g^{-1}$ VS were found. The current BMP results were comparable to other studies, which showed that fresh swine manure ranged between 244 to 480 mL $CH_4$ $g^{-1}$ VS (Burton and Turner, 2003; Møller et al., 2004; King et al., 2011) depending on ration. This indicates that, in our study, due to the short duration of the feeding trials and the fact that the manure storages started completely clean, (i.e., no inoculation), little decomposition of the manure organics occurred during storage based on the low reduction in methane production potential. Field sampling results have indicated that increased rates of methane production are related to foam accumulation, so a diet that has both increased MPR and BMP may be indicative of potential for foaming, as it would indicate both high microbial activity and the potential to sustain it. In this regard, no single diet stood out; however, pigs fed the C-SBM, DDGS, and SH diets tended to produce manure that had both high MPR and BMP compared to other diets. More importantly, our results indicated that diets with higher NDF contents tended to increase the methane production potential of solids in the manure (0.880 L $CH_4$ $L^{-1}$ manure for 1% increase in NDF, $r = 0.3095$), but this relationship is complicated by the digestion of these materials within the pig and requires development of a microbial community capable of converting this potential to result in increased rates of methane production.

### SURFACE TENSION

Differences in surface tension were noted between diets in both the protein study ($p < 0.05$) and carbohydrate study ($p = 0.001$). In the protein study, pigs fed the C-CM and C-PM diets produced manures with significantly ($p < 0.05$) lower surface tension values compared to pigs fed the other diets. This appears to be partly related to the protein content of the diet, as we saw a decrease of 0.0006 N $m^{-1}$ in surface tension for every 1% increase in dietary protein content ($R^2 = 0.26$). In particular, the surface tension of the manure was negatively correlated to both the crude protein content and the NDF content of the diet. Proteins are often thought of as potential surfactants, providing a direct link to surface tension. Similarly, NDF degradation within the animal and the manure can lead to volatile fatty acids, which may serve as surfactants and lower the surface tension.

In the carbohydrate study, manure from pigs that were fed soy hulls had significantly higher surface tension than the C-SBM/AA or WB diet manure. This did not appear to be correlated with specific dietary properties but instead appeared to be correlated to pH, with higher pH related to higher surface tension. In manure, the pH value tends to be driven by ammonia concentration and volatile fatty acid content. As all diets were balanced for protein content in the carbohydrate study, pH was presumably driven by VFA content, i.e., higher VFA content leads to lower surface tension. A similar relationship between pH, VFA content, and surface tension was found in swine manures from production barns in Iowa (Van Weelden et al., 2015). As a point of comparison, Van Weelden et al. (2015) found that non-foaming manures had surface tensions of 0.0495 N $m^{-1}$, while foaming manures had surface tensions of 0.0515 N $m^{-1}$. In this study, four manures that approximated the surface tension of foaming manures were generated, including the SH diet from the carbohydrate study and the C/AA, C-SBM/AA, and C-CGM diets from the protein study.

### FOAMING PROPERTIES

Testing the manures for foaming capacity and foaming stability determined the effect of diet on the potential of manure to foam. Table 3 shows the results for foaming capacity and foaming stability for the protein study manures, while table 5 shows results for the carbohydrate study manures. There were significant differences ($p < 0.05$) in the foaming capacity of manures in the protein study, where manures from the C-PM diet had significantly greater foaming capacity than manures from the C/AA, C-CM, or C-SBM diets (table 3). We originally hypothesized that surface tension would be correlated to foaming capacity, but this did not hold true in the protein study ($r = -0.0665$). Instead, foaming capacity in this study appeared to be most strongly related to the solids content of the manure, as well as the microbial activity level as measured by the methane production rate. The manure with the lowest foaming capacity (C-CM) had the lowest surface tension, but the manure with next lowest surface tension (C-PM) showed the highest foaming capacity. Overall, surface tension was a poor predictor of differences in manure foaming capacity for the protein study. However, we found that foaming capacity was significantly correlated to the methane production rate of the manure, which may indicate that some sort of microbial exudate is related to the capacity of manure to foam.

Foaming capacity results from the carbohydrate study again indicated that diet had a significant ($p < 0.05$) impact on the foaming capacity of the manure. Pigs fed the BP diet produced manure with the highest average foaming capacity, which was significantly higher ($p < 0.05$) than the manure produced from pigs fed the WB and DDGS diets. Pigs fed the BP diet produced manure with a foaming capacity similar to that of foaming manures from commercial facilities (Van Weelden et al., 2015). In this study, surface tension appeared to be a reasonable means to predict foaming capacity, as the two properties were correlated ($r = 0.7731$). However, unlike our original hypothesis that lower surface tension would lead to greater foaming capacity, we found that higher surface tension led to higher capacity to foam. This relationship between foaming capacity and surface tension, i.e., higher surface tension leading to greater foaming capacity, has been reported for production barns with foaming pits. That is, foaming barns typically had higher surface tension than their non-foaming counterparts. This would seem to indicate that, in this manure system, lowering of the surface tension by surface-active agents, as others have hypothesized for wastewater foaming (Glaser et al., 2007; Davenport et al., 2008; Ganidi et al. 2009), may not be the responsible mechanism. In our field study results, foaming manures had foaming capacities of 150, while non-foaming manures had capacities of around 100. In these studies, all recorded foaming capacities were more similar to those from non-foaming manures.

In terms of foam stability, the protein study showed no significant differences in foam half-life ($p = 0.208$) as related to diet type, with all half-lives being very short, indicating that no mechanism was present to stabilize the bubbles, despite some capacity to foam. However, in our correlation analysis, we noted that foam stability was correlated to volatile solids content and the biochemical methane potential of the manure, indicating that some solid particles that are biologically degradable may play a role in bubble stabilization. Similarly, samples from the carbohydrate study showed no significant differences in foam half-life due to diet, although some of the samples exhibited substantially longer foam half-lives than samples from the protein study. There was great variability among values, and in all cases foam stability was significantly lower than the values of around 100 min found in foaming samples collected from commercial deep-pits and tended to be more similar to those for non-foaming manures at 5 min (Van Weelden et al., 2015).

### MICROBIAL COMMUNITY

The microbial community is a key component in foam formation in anaerobic digesters (Kougias et al., 2013). Consequently, knowing how the microbial communities in the manure samples are affected by diet is important. In this study, the microbial communities in each manure sample from each diet were compared for differences (R values shown in table 7). The manure with the largest distinctions among the protein diets was for pigs fed the C/AA diet, with R values ranging between 0.219 and 0.557. This diet had the lowest CP and NDF inclusion rates (table 1) of any of the

Table 7. ANOSIM microbial community comparisons for the protein and carbohydrate studies.

| Protein Study[a] | | Carbohydrate Study[b] | |
|---|---|---|---|
| Diet | R Value | Diet | R Value |
| C/AA vs. C-SBM/AA | 0.291 | C-SBM vs. B | 0.173 |
| C/AA vs. C-SBM | 0.308 | C-SBM vs. BP | 0.171 |
| C/AA vs. CM | 0.557 | C-SBM vs. DDGS | 0.000 |
| C/AA vs. C-CGM | 0.218 | C-SBM vs. SH | 0.169 |
| C/AA vs. C-PM | 0.434 | C-SBM vs. WB | 0.182 |
| C-SBM/AA vs. C-SBM | 0.000 | B vs. BP | 0.327 |
| C-SBM/AA vs. C-CM | 0.142 | B vs. DDGS | 0.157 |
| C-SBM/AA vs. C-CGM | 0.087 | B vs. SH | 0.273 |
| C-SBM/AA vs. C-PM | 0.099 | B vs. WB | 0.455 |
| C-SBM vs. C-CM | 0.035 | BP vs. DDGS | 0.013 |
| C-SBM vs. C-CGM | 0.140 | BP vs. SH | 0.142 |
| C-SBM vs. C-PM | 0.000 | BP vs. WB | 0.325 |
| C-CM vs. C-CGM | 0.312 | DDGS vs. SH | 0.055 |
| C-CM vs. C-PM | 0.000 | DDGS vs. WB | 0.204 |
| C-CGM vs. C-PM | 0.120 | SH vs. WB | 0.201 |

[a] Protein diets: C/AA = corn with amino acids, C-SBM/AA = corn-soybean meal with amino acids, C-SBM = corn-soybean meal, C-CM = corn-canola meal, C-CGM = corn-corn gluten meal, and C-PM = corn-poultry meal.

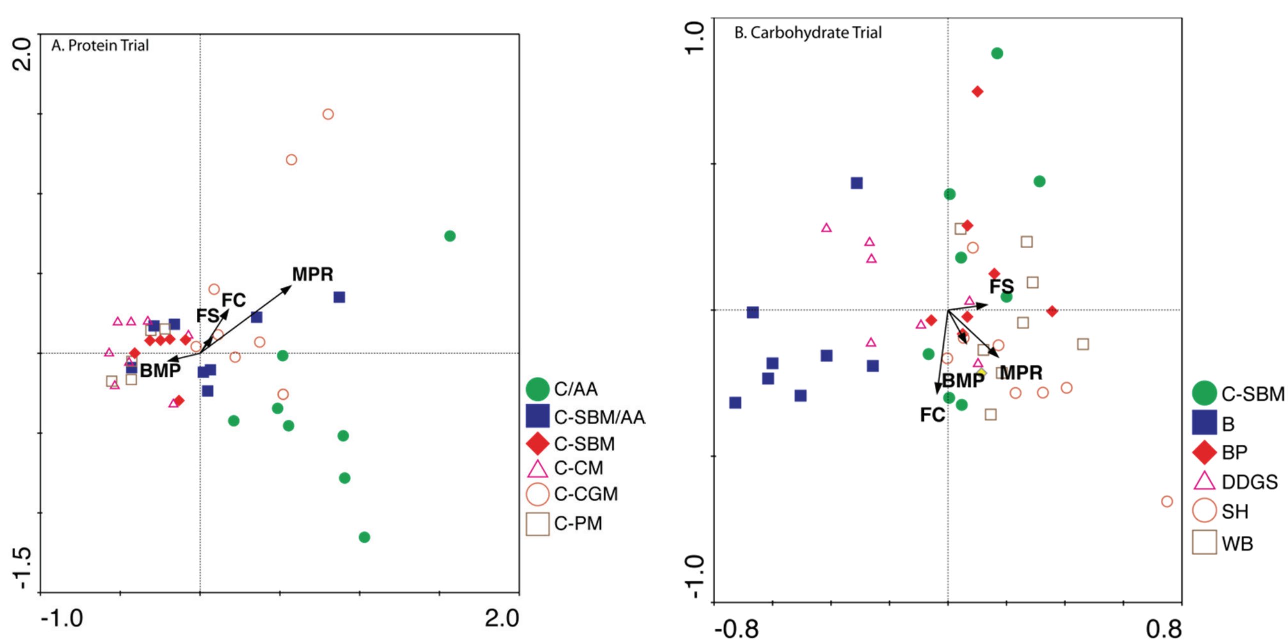
[b] Carbohydrate diets: C-SBM = corn-soybean meal, B = barley, BP = beet pulp, DDGS = distillers dried grains with solubles, SH = soy hulls, and WB = wheat bran.

diets, which may have driven these differences in microbial community. The source of the CP did not seem to greatly affect the difference among diets because the R value of manure from pigs fed the C-SBM diet compared with other protein diets ranged between 0.000 and 0.142. Consequently, crude protein level was the strongest driver of change in the microbial community.

In terms of microbial community structure in the manure, there appear to be some trends, as shown in figure 1a. In particular, it appears that a crude protein gradient exists within the figure and runs from lower right to upper left. Focusing on the C/AA (lower right), C-SBM/AA (middle), and C-SBM (upper left) diets, we see a spread of microbial community based on protein content. In the lower right is the manure from pigs fed the C/AA diet. This diet had a very low CP content and a high lysine supplementation. In comparison, the C-CGM diet had a high amount of protein, but due to the amino acid profile of the CGM, which is a good source of cysteine but often deficient in lysine, was also to the right on this plot, but separated from the C/AA diet presumably due to the higher protein content as well as NDF content in the diet. Most of the other diets are grouped close together, especially the S-SBM/AA, C-SBM, and C-PM diets, which also had similar NDF contents, protein contents, and similar amino acid profiles.

In the carbohydrate diets, manure from pigs fed the B and WB diets showed the greatest differences among all diets, ranging between 0.157 and 0.455 and between 0.182 and 0.455, respectively. These diets were relatively similar, as they were balanced for crude protein level. In the carbohydrate study, it was difficult to identify a characteristic or trend to explain the microbial community structure. The B diet was the most different, which may be partly explained by the absence of corn in this diet, but other diet microbial communities tended to group together.

A correspondence analysis was used to relate foaming capacity, foaming stability, biochemical methane production, and methane production rate of the manure to the microbial communities. Foaming manure storages typically have higher methane production rates, foaming capacity, and foam stability, so microbial community compositions that are positively correlated with these parameters may have the potential to




(a) (b)

Figure 1. Correspondence analysis used to relate differences in microbial community composition in manure samples from each diet with physical parameters that could be used to indicate a diet's potential to foam when used in a commercial production facility: (a) protein study (C/AA = corn with amino acids, C-SBM/AA = corn-soybean meal with amino acids, C-SBM = corn-soybean meal, C-CM = corn-canola meal, C-CGM = corn-corn gluten meal, and C-PM = corn-poultry meal) and (b) carbohydrate study (C-SBM = corn-soybean meal, B = barley, BP = beet pulp, DDGS = distillers dried grains with solubles, SH = soy hulls, and WB = wheat bran). Foaming capacity (FC), foaming stability (FS), biochemical methane production (BMP), and methane production rate (MPR) are shown as arrows.

generate foam if the community structure has more time to develop. Based on the ordination plots in figure 1, it appears that microbial community composition was strongly correlated with the methane production rate of the manure sample and moderately correlated with the foaming capacity and foam stability. Microbial community compositions that tended to have higher methane production rates also tended to be present in manures that had higher foaming capacity and longer foam stability. In general, MPR, FC, and FS appeared to be driven at least in part by the amount of corn protein in the diet, as C-CGM had the highest probability of higher MPR and foaming characteristics, followed by the other diets higher in corn, including C/AA and C-SBM/AA. The other item to note is that although the presence of corn protein appeared to be a driver of potential foaming, it alone was not always sufficient to cause a foaming population, that is, the microbial community composition appeared to be driven by more than just dietary composition (fig. 1a). The Pearson correlations (table 4) indicated that foaming capacity, foam stability, and methane production were all positively correlated in this study, which appears to indicate that dietary protein level and source play an important role in driving the microbial community toward a structure that will simultaneously increase methane production and foaming characteristics. Moreover, it appears that the probability of increasing these parameters increases with greater corn inclusion in the diet (either corn fiber or corn protein).

The results of the correspondence analysis for the carbohydrate study are less clear (fig. 1b), potentially because the diets had balanced levels of crude protein and NDF. Based on the Pearson correlations, there was a negative relationship between methane production rate and foaming characteristics in this study, but the results also indicated that methane production rates were mostly related to solids content and thus digestibility both within the pig and then subsequently by microbes in storage. What is clear from figure 1b is that by not including corn in the ration, the B diet produced a relatively distinct microbial community, while most of the other diets tended to be relatively similar in composition. These results contrast slightly with the results of Van Weelden et al. (2016), who found that the microbial community was driven by both a carbon gradient within the manure and by the fiber source. However, fewer carbohydrate sources were used in that study, which allowed viewing of the carbon gradient without having that information confounded by the digestibility of the material within the pig as well as its microbial biodegradability, as was the case in this study.

Overall, these results suggest that the dietary ingredients used at the levels tested here are not directly responsible for foam formation during storage of feces and urine because none of the manures generated spontaneous foam formation during storage or exhibited all the required foaming properties. However, certain diets exhibited enhancement of some of the properties related to foam formation, and diet impacted the development of different microbial community structures. We hypothesize that, given sufficient time, these differences in microbial community structure would continue and cause stronger development of foaming characteristics in the manures. Moreover, these results indicated that, while dietary composition is an important factor in microbial community composition, other unknown factors also play a role, as different diets often resulted in similar microbial community structures, and these community structures were then associated with the development of manure foaming characteristics. Our results further suggest that crude protein level and source in the diet provides a more direct link to microbial community composition than carbohydrate source, as larger differences between diets were noted in the ANOSIM and more organized patterns were found in the correspondence analysis.

# Conclusions

This study sought to elucidate the effect of swine diet composition on the physicochemical and biological characteristics of swine manures that impact foaming. No diet stood out as one that exhibited enhanced methane production, physical characteristics similar to foaming manures, and enhanced foaming capacity and stability. However, in the protein study, we found that diets higher in methane production rate also tended to be higher in foaming capacity and foam stability, and this appears to be related to higher levels of corn or corn protein in the diet. In the carbohydrate study, we found negative correlations between methane production rate and foam capacity and stability. This appears to indicate that dietary protein level and source play an important role in driving the microbial community toward a structure that will simultaneously increase methane production and foaming characteristics. However, this was not the case when maintaining consistent protein levels and varying carbohydrate sources. In the protein study, foaming capacity was not driven by reduction in surface tension, as previously proposed, and in fact surface tension measures were poorly correlated to measured foaming capacity. In the carbohydrate study, foaming capacity and surface tension were correlated, but as in the protein study, foaming capacity did not appear to be driven by the presence of surfactants but rather their absence, as higher surface tension led to higher foaming capacity. In terms of methane production potential in the manure, our results indicated that diets with increased metabolizable energy, crude protein, and most importantly NDF increased the methane production potential of the manure. However, no variable was found that drove the measured methane production rate. This study also evaluated microbial community composition. The results indicated that microbial community composition appeared to be related to dietary composition, especially protein level but also inclusion of corn, but other factors may also be relevant, as diets often exhibited significant variability in microbial community.

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