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Impact of fiber source and feed particle size on swine manure properties related to spontaneous foam formation during anaerobic decomposition

M. B. Van Weelden
Iowa State University, mrkvnwld@gmail.com

D. S. Andersen
Iowa State University, dsa@iastate.edu

B. J. Kerr
United States Department of Agriculture, brian.kerr@ars.usda.gov

S. L. Trabue
United States Department of Agriculture

L. M. Pepple
University of Illinois at Urbana-Champaign, pepple@iastate.edu

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Abstract

Foam accumulation in deep-pit manure storage facilities is of concern for swine producers because of the logistical and safety-related problems it creates. A feeding trial was performed to evaluate the impact of feed grind size, fiber source, and manure inoculation on foaming characteristics. Animals were fed: (1) C–SBM (corn–soybean meal): (2) C–DDGS (corn–dried distiller grains with solubles); and (3) C–Soybean Hull (corn–soybean meal with soybean hulls) with each diet ground to either fine (374 μm) or coarse (631 μm) particle size. Two sets of 24 pigs were fed and their manure collected. Factors that decreased feed digestibility (larger grind size and increased fiber content) resulted in increased solids loading to the manure, greater foaming characteristics, more particles in the critical particle size range (2–25 μm), and a greater biological activity/potential.

Keywords

Manure, Foaming, Anaerobic digestion, Feedstock composition, Microbial community

Disciplines

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Comments

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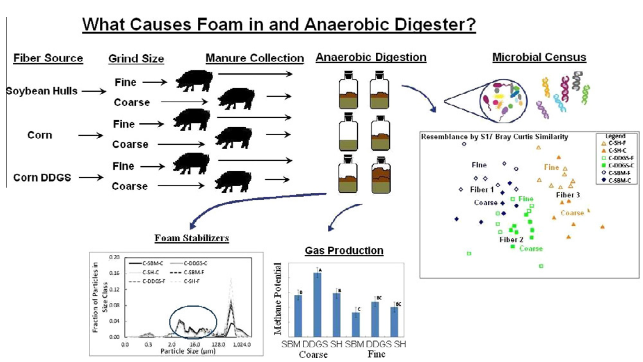
M.B. Van Weelden [a], D.S. Andersen [a,*], B.J. Kerr [b], S.L. Trabue [b], L.M. Pepple [c]

[a] Agricultural & Biosystems Engineering, Iowa State University, Ames, IA 50011, USA
[b] USDA-ARS-National Lab for Agriculture and the Environment, Ames, IA 50011, USA
[c] Ag. & Biological Engineering, University of Illinois, Urbana-Champaign, IL 61801, USA

HIGHLIGHTS

- Finer grinding of feed results in reduced methane production potential.
- Grind size and fiber source influenced the microbial community.
- Feeding DDGS increased biogas production potential of swine manure.
- Fine particles are important in foam stabilization.
- Less digestible fiber resulted in more fine particles in manure.

GRAPHICAL ABSTRACT



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ABSTRACT

Foam accumulation in deep-pit manure storage facilities is of concern for swine producers because of the logistical and safety-related problems it creates. A feeding trial was performed to evaluate the impact of feed grind size, fiber source, and manure inoculation on foaming characteristics. Animals were fed: (1) C–SBM (corn–soybean meal): (2) C–DDGS (corn–dried distiller grains with solubles); and (3) C–Soybean Hull (corn–soybean meal with soybean hulls) with each diet ground to either fine (374 µm) or coarse (631 µm) particle size. Two sets of 24 pigs were fed and their manure collected. Factors that decreased feed digestibility (larger grind size and increased fiber content) resulted in increased solids loading to the manure, greater foaming characteristics, more particles in the critical particle size range (2–25 µm), and a greater biological activity/potential.

1. Introduction

The Midwestern United States is responsible for more than 50% of pork produced in the U.S. Finishing swine operations in this region typically utilize deep-pits to store manure produced until land application can occur. Deep-pit manure storages are located within the swine production building, beneath a slatted floor on which the pigs are raised. This allows the manure to fall through slatted floors into the storage below, where it is held for up to a year before being utilized as crop nutrients. These manure storage systems were adopted by producers in the late 1970s and today represent more than 50% of swine finishing operations in the U.S. (Key et al., 2011). Even though these systems improve nutrient content and manageability of the stored manure, there are concerns that have than arisen since their implementation.

* Corresponding author at: 3348 Elings Hall, Ames, IA 50011, USA. Tel.: +1 515 294 4210.
E-mail address: dsa@iastate.edu (D.S. Andersen).

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In 2009, swine producers began observing a brown, viscous foam forming on the manure surface in their deep-pit storages. Foam production in deep-pit manure storages has significant implications on facility management and safety and is a serious concern for Midwestern U.S. pork producers. The accumulation of foam can significantly reduce the volume of the manure storage, causing producers to seek alternative acres for application during untimely seasonal windows to prevent the overflow of storages. As deep-pit storages are anaerobic environments the breakdown of organic matter in swine manure will occur. This decomposition produces biogas (i.e., methane, carbon dioxide, and hydrogen sulfide). When foam is present, it traps these gases, storing hydrogen sulfide and methane; a major safety concern for animals and farm employees (Moody et al., 2009). This has resulted in increased occurrences of poisoned swine and flash fires at facilities where foam is present, disturbed, and then a spark occurs. Thus, determining the root cause of manure foam in these systems is necessary to develop mitigation options.

Similarly, foaming has been reported to be a serious problem in many biogas plants (Kougias et al., 2013; Ross and Ellis, 1992). As reported by Oether et al. (2001) this is often a deep brown, extremely viscous layer with higher solids content, making it very similar in description to the foam forming on deep-pit manure storage. These foams can result in poor gas recovery, creation of dead zones in the digester, and blockages of gas meters (Ganidi et al., 2009). In some cases, foaming has been reported due to the feedstock composition, with Kougias et al. (2014) showing that feedstock composition could alter the microbial ecology. This has made determining the cause and developing potential mitigation approaches a topic of major interest to the anaerobic digestion industry (Pagilla et al., 1997).

The inputs to deep pit manure storages consists of animal feces and urine, wasted feed and water, and wash waters generated from cleaning between groups of animals. 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). Van Weelden (Unpublished Results) found that this was also true for properties of manures thought to be related to foam formation, where they reported that manures from pigs fed varying sources and levels of carbohydrate or proteins resulted in manure with different microbial community structures, different methane production characteristics, and different capacities to form and stabilize foam.

The results from those diet trials, along with results from their analysis of manures from foaming and non-foaming commercial production facilities (Van Weelden et al., 2015) helped provide direction for the experiments discussed in this manuscript. In brief, they showed: (a) foaming manures make methane at faster rates than their non-foaming counterparts, (b) foam stability was drastically different between foaming and non-foaming manures with fine particles appearing to be important in the stabilization of the foam bubble structure, and (c) foaming barns have lower concentrations of volatile fatty acids and higher surface tension. Taken together, these results indicated that the microbial community in foaming manures appears to be more active than non-foaming manures as a greater amount of the manure substrates have been converted to methane, which then appears to be adjusting physical properties of surface tension and the amount of fine particles.

Based on these results, along with changes in the swine feeding industry where finer particle size grinds and diet formulations with a greater amount of high fiber ingredient inclusion have become common, further study focused on the impact of diet physical properties (grind size) as well as fiber source/content were justified. Consequently, the purpose of this study was to develop a greater understanding of the role of dietary inputs and feed formulations on manure properties and the microbial community. This study was designed to understand the role of fiber content, manure inoculation, and diet grind size has on manure properties associated with foaming.

2. Methods

2.1. Animal management and manure storage

A feeding trial was conducted at the Iowa State University Swine Nutrition Farm (Ames, IA) utilizing two groups of 24 growing gilts; average individual weight initially 1195.5 kg 119.5 (SD = 8.9 kg). Pig were fed one of three diets: (1) corn–soybean meal (C–SBM); (2) corn–dried distillers grains with solubles (C–DDGS); or (3) corn–soybean meal with soybean hulls (C–SH) and each diet was ground to a particle size of either 374 (fine) or 631 μm (coarse) (see Tables 1 and 2 for ingredient and nutrient content of the animal diets). Within each group of 24 pigs, 8 received diets of each fiber source, 4 received fine grind and 4 received coarse ground. The entire trial was then replicated with an additional 24 pigs, so that the impact of inoculating the manure could be evaluated. That is, trial 1 represented un-inoculated manure and trial 2 inoculated manure (manure in trial 2 was inoculated with manure from the same diet/grind and was intended to provide a starter culture of bacteria).

All diets were balanced for metabolizable energy, digestible lysine per unit of energy, calcium, and phosphorus, but differed in their lipid and fiber contents. In particular, the C–SH diet had roughly 3 times the neutral detergent fiber content of the C–SBM diet, while the C–DDGS diet had twice the neutral detergent fiber level of C–SBM diet. Similarly, both the C–DDGS and C–SH diets had approximately 50% higher lipid content than the C–SBM diet to balance the diets to a similar metabolizable energy level. Soybean hulls were utilized as they represents a fiber from legumes and contain proportionally more cellulose than hemicellulose, while DDGS represents a fiber from a cereal grain contains proportionally more hemicellulose than cellulose; both fibers being prevalent in diets fed to pigs throughout the U.S.

Pigs were randomly allotted to individual metabolism crates (1.2 × 2.4 m) that allowed for total collection of feces and urine. Crates were equipped with a stainless steel feeder and a nipple waterer to which the pigs had ad libitum access. Ambient temperature in the metabolism room was maintained at approximately 18.4 °C, and lighting was provided continuously. Diets were typical for pigs of this body weight and were formulated to be adequate in all nutrients (NRC, 2012). Pigs were fed twice daily (0700 and

Table 1
Ingredient concentration of diets.

Ingredient	Diets		
	C–SBM[a]	C–DDGS[b]	C–SH[c]
Ingredient, as-fed basis, %			
Corn	79.72	62.50	57.34
Soybean hulls	0.00	0.00	20.75
Soybean meal	18.00	0.00	16.80
Soybean oil	0.30	0.00	3.32
Distiller's dried grains with solubles	0.00	35.10	0.00
Limestone	0.87	1.15	0.60
Monocalcium phosphate (21% P, 17% Ca)	0.41	0.10	0.49
Sodium chloride	0.35	0.35	0.35
Vitamin mix	0.20	0.20	0.20
Trace mineral mix	0.15	0.15	0.15
L-Lys-HCl	0.00	0.39	0.00
L-Trp	0.00	0.03	0.00
L-Thr	0.00	0.03	0.00

[a] C–SBM = corn–soybean meal.
[b] C–DDGS = corn–distiller's dried grains with solubles.
[c] C–SH = corn–soybean meal–soybean hulls.

Table 2
Particle size and analyzed nutrient composition of diets.

Particle size	300 μm			600 μm		
Diet composition	C–SBM[a]	C–DDGS[b]	C–SH[c]	C–SBM[a]	C–DDGS[b]	C–SH[c]
Average particle size, as-fed basis, μm						
Trial 1	373	344	394	624	605	668
Trial 2	354	360	421	605	601	681
Analyzed nutrient composition, dry matter basis						
Nitrogen, %	2.65	2.79	2.66	2.57	2.73	2.58
Carbon, %	44.62	45.89	45.47	44.71	45.70	45.31
Sulfur, %	0.21	0.24	0.22	0.20	0.23	0.20
Ether extract, %[d]	4.56	6.19	6.70	4.70	6.22	6.87
Neutral detergent fiber, %[e]	7.22	13.89	20.40	7.10	13.59	19.54

[a] C–SBM = corn–soybean meal.
[b] C–DDGS = corn–distiller's dried grains with solubles.
[c] C–SH = corn–soybean meal–soybean hulls.
[d] EE (Ether extract) refers to lipid content.
[e] NDF (neutral detergent fiber) refers to fiber content.

1900 h) an amount of feed that approximated 3% of their BW, which is considered near full feed, but slightly reduced to limit feed spillage from the feeders. Each group of animals was fed for a 49-day period. After each feeding, feces and urine from each of the 24 metabolism crates were collected and added to its assigned enclosed manure storage container (one crate assigned to its corresponding storage container).

At the completion of the feeding trial for the first set of animals, manure within the tanks was thoroughly agitated and a 2-L sample collected from each tank. These samples were subsequently stored at 4 °C until were analyzed. Manure in the tanks were then allowed to settle (3 d) and top layer removed so only 12-cm depth of manure remained. Manure was pumped from the top of the tank to mimic a pump-out event. Following removal of top layer, a second set of pigs were brought in and placed in the metabolism crates; each crate received the same diet as before. Consequently, each manure storage vessel received manure from animals fed the same diet (fiber × grind size) as in the first group of pigs and the residual manure within the tank was used to inoculate the new manure produced during the second phase of the feeding trial, similar to what would occur in deep-pit swine production system. At the conclusion of the second phase of the feeding trial, the manure was again agitated and a 2-L subsample collected. Manure samples were assayed for total solids, volatile solids, biochemical methane production potential, methane production rate, surface tension, particle size, and foaming capacity and stability.

2.2. Total solids and volatile solids

The total solids and volatile solids contents of manure samples were tested according to the Standard Methods for the Examination of Water and Wastewater 2540B and 2540E (APHA, 1998). In brief, 30 mL of sample was added to a pre-weighed porcelain dish, mass recorded, and heated to 104 °C for 24 h. Samples were cooled in a desiccator and dried mass recorded. The crucible was then placed in a muffle furnace at 550 °C for 12 h, cooled in a desiccator, and final mass recorded. Total and volatile solids were reported in percentage of total sample mass.

2.3. Particle size analysis

A Cilas 1190 laser particle size analyzer was used to assay the particle size distribution of solids present in the manure samples. Measurements were made in liquid dispersion mode. Prior to sample analysis, it was determined that 1 mL sample would give an acceptable obscuration measurement of between 8% and 22%. In brief, 1 mL of sample was introduced into the sample cup using a modified pipette tip (tip partially removed to allow larger particles in sample to be collected) where it was sonicated for two minutes before sample entered the laser chamber. A background calibration was run at the start of the sample analysis, and repeated every five samples thereafter. The Cilas 1190 instrument was capable of measuring particle sizes between 0.04 and 2500 μm. Seven of the 48 manure samples were run in duplicate and showed good repeatability, with an average particle size difference of 3% or less.

2.4. Biochemical methane potential assay

A biochemical methane potential test was used to measure the total volume of methane a given material is able to produce. In this study, the procedure was similar to that developed by Owen et al. (1979). In brief, 10–15 g of sample was added to a 250 mL serum bottle (Wheaton Science Products No. 223950) along with 50 mL of an active anaerobic digester inoculum, maintained in the Manure Management Lab at Iowa State University. This volume of inoculum was added to achieve a 2:1 mass ratio of volatile solids from the manure to inoculum. The vessel was then sealed with a sleeve stopper septa (Sigma–Aldrich Z564729), incubated at 35 °C under constant agitation on an orbital shaker. Biogas production was checked regularly by inserting the needle of a gas-tight syringe (Micro-Mate interchangeable hypodermic Syringe 50 mL Lock Tip, Popper & Sons, Inc. New Hyde Park, New York) through the septa; this allowed the pressurized biogas to displace the plunger on the syringe to provide a volume measurement. The biogas was injected into a non-dispersive infrared methane analyzer (NDIR-CH_4 Gasanalyzer University Kiel, Germany) to obtain the percent of methane present in the gas sample. Results were evaluated based on methane produced per gram of whole sample and methane production per gram of volatile solids added (Moody et al., 2011).

2.5. Methane production rate

A methane production rate (MPR) assay (7 d) was used to measure the intrinsic rate at which bacteria within a sample produce methane. It is different from the BMP because it is conducted over a shorter time (ensuring sample is not substrate limiting), not inoculated nor diluted, is conducted at room temperature, and the samples are kept stationary rather than agitated. Details of the procedure are found in Andersen et al. (2015). In brief, 100 mL of well-mixed sample was added to 250 mL serum bottle sealed with septa and incubated at room temperature (approximately 23 °C) for 7 d. Following incubation period, biogas production was checked by removing biogas from the headspace of the sample as previously discussed in BMP section and the infrared methane analyzer used to determine methane content. The rate of methane production was calculated using Eq. (1).

$$\mathrm{MPR} = \frac{1440C_{methane}(V_{biogas} + V_{headspace})\rho_{manure}}{100M_m t_{incubation}} \quad (1)$$

2.6. Surface tension

Surface tension was measured to evaluate the impact surface-active agents present in solution without identifying or quantify any specific compound. Surface tension was measured using a CSC Precision Ring Tensiometer (CSC Scientific Company, Inc., Fairfax, VA). In brief, 40 mL of samples was brought to room temperature, agitated vigorously, and once mixed placed in a sample tray with a duNouy ring used to determine the force needed to break the liquid interface. The instrument recorded value directly as dyne/cm (equivalent to mN/m), and reported in N/m.

2.7. Foaming capacity and stability testing

Details of the apparatus used to measure foaming capacity and stability have been previously reported (Van Weelden, 2015). In brief, 300 mL of sample was placed into the bottom of a 5.1-cm diameter clear PVC column and air passed through at 200 mL min^{-1} until either a steady-state height of foam was achieved or the foam layer reached the maximum height of the column (approximately 33 cm above the liquid level). The time of aeration was recorded along with the height of foam produced and the level of the foam–liquid interface. The foaming capacity index was calculated as the height of foam produced divided by the initial manure level and multiplied by 100.

Following aeration (foam layer reaching its maximum height in the PVC tube), air to the foaming apparatus was turned off (time zero) and foam height loss was monitored over predetermined time intervals. The descending height of foam layer was normalized to percent of initial foam height and plotted as a function of time. A first-order exponential decay model fit the data well in most cases and was used to estimate the half-life of the foam (Eq. (2)), where $t_{1/2}$ is the foam half-life in minutes and C_d is the decay coefficient from the first-order exponential model fitting (min^{-1}).

$$t_{1/2} = \frac{\ln 2}{C_d} \quad (2)$$

2.8. Bacterial community composition

To determine the microbial community composition in the manure, genomics DNA was extracted from 200 mg of manure samples using the FastDNA SPIN Kit for Soil (MP Biomedical). Bacterial community composition was assessed using automated ribosomal intergenic spacer analysis (ARISA) as described previously (Kent et al., 2007; Yannarell and Triplett, 2005). 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, which can then 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 version 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, UK). Analysis of similarity (ANOSIM) was used to evaluate patterns of microbial community similarity among groups of samples (Clarke and Green, 1988). ANOSIM generates a test statistics, R, whose magnitude indicates the degree of difference between groups of samples, with a score of 1 indicating completely different assemblages among samples, and 0 indicating no distinction in composition among samples.

2.9. Statistical analysis

The statistical analysis of the diet study was performed using JMP Pro 10 and the Standard Least Squares procedure, with differences tested at $\alpha = 0.05$. Data were analyzed as a complete factorial design with manure inoculation, feed particle size, and fiber source as fixed factors. Interactions of manure inoculation × feed particle size, manure inoculation × fiber source, feed particle size × fiber source, and manure inoculation × feed particle size × fiber source were also considered. If interactions were not significant at $p < 0.25$ level they were pooled to error.

3. Results and discussion

3.1. Total and volatile solids

Statistical analysis of the samples from the diet study showed that manure inoculation, grind size, fiber source were significantly different ($p < 0.05$), and there was interactions between grind size × fiber source and manure inoculation × grind size factors ($p < 0.05$). The effect of manure inoculation on manure solids content was unexpected because the older manure was hypothesized to have the higher solids content because the settled solids generated during the first feeding trial were used to inoculate the tanks for the second feeding trial. The difference in solids content between feeding trials was likely due to total volume of manure produced between the two trials, 182 L of manure in trial 1 and 212 L of manure in trial 2. As the same amount of feed was consumed in both trials, the higher manure production rate of the pigs was due to their increased water consumption (data not shown). That is, the difference in solids content of the manure from trial 1 and trial 2 could be explained by differences in water use, the total mass of solids within the manure storage tank was the same for both trials at 12,500 g per pig during trial 1 and 12,400 g per pig during trial 2.

After correcting to account for differences in water consumption during the feeding trials, only grind size, fiber source, and the grind size × fiber source interaction were significant. As expected, animals fed the coarse ground feeds produced manures with greater total solids content than manures produced from animal fed fine ground diets, presumably due to lower digestibility of a coarser ground feed (NRC, 2012). Higher fiber diets tended to have higher solids contents when feed was finely ground (0.0876% TS/%NDF in the diet, $r = 0.98$), but when coarsely ground this relationship wasn't clear (Fig. 1). Manure from pigs fed the finer ground C–DDGS diet had a greater reduction in solids content (40%) than pigs fed the finer ground C–SH and CSBM diets (18% and 23% reduction respectively), leading to a significant grind × fiber source interaction. This would suggest finer grinding had a larger impact on DDGS than on soybean hulls, potentially indicating that finer grinding is more beneficial in improving hemicellulose digestion than cellulose digestion due to the solubility of these particular fibers.

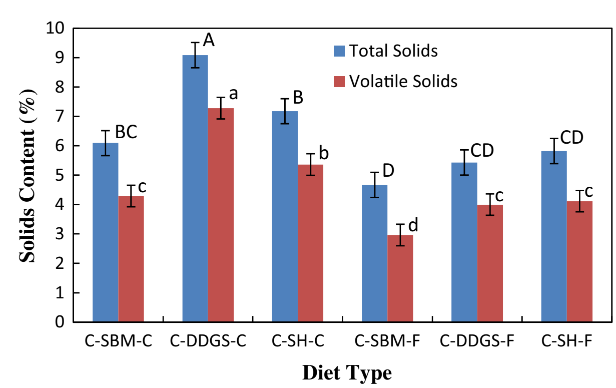

Fig. 1. Total and volatile solids concentrations for C–SBM–C (Corn–Soybean Meal–Coarse), C–DDGS–C (Corn–DDGS–Coarse), C–SH–C (Corn–Soybean Hulls–Coarse), C–SBM–F (Corn–Soybean Meal–Fine), C–DDGS–F (Corn–DDGS–Fine), C–SH–F (Corn–Soybean Hulls–Fine). Error bars represent the standard error of the mean. Capital letters indicate differences ($\alpha = 0.05$) among total solids concentrations and lower case letters represent differences among volatile solids concentrations.

Field research from commercial swine facilities have demonstrated that manures from foaming barns tend to have higher solids content, especially when these solids accumulate in the upper layers of the manure (Van Weelden et al., 2015). Implications of this research are that commercial growers who rely on high fibrous feeds may be at risk for foaming issues, but finely grinding of feed may help mitigate the negative consequences of fibrous feeds, especially finer grinding of DDGS.

Volatile solids was strongly correlated to total solids ($R^2 = 0.986$), so results were expected to be similar to total solids. Before analyzing the results of this feeding trial, however, the concentrations of volatile solids content of the manure were corrected to account for differences in water consumption between trial 1 (fresh manure) and trial 2 (inoculated manure). This analysis indicated that grind size, fiber source, and the fiber source × grind size all significant ($p < 0.05$) affected volatile solids. These results mirrored those of the total solids content (Fig. 1).

3.2. Particle sizes

Previous work by Masse et al. (2002) and Marcato et al. (2007) showed that swine manure typically has a bimodal particle size distribution, with this being true of both raw and anaerobically digested swine manures. The results found here exhibited a similar pattern (Fig. 2). Following terminology used by Rodriguez and Lomas (2002), particles in the 1–100 μm range are "supracolloids" and include bacterial floc, single cells, and organic residues while the larger particles 100–2500 μm are often residual fed particles. In evaluating the particle size distributions, the following hypotheses were used: (1) the differences in the amount of small particles (<100 μm) would be driven by the production of microbial by-products, (2) differences in large particles (>100 μm) would be driven by the grinding of the fed. Based on this, the data was evaluated in multiple ways. The first was to look at differences in the average particle size of material in the manure, the second was to look at differences in the percent of particles in different size classes, and the third was to determine the total mass of particles in each size class present in the manure. Particle size classifications followed those used on soils and were: clay size (<2 μm), fine silt (2–25 μm), coarse silt (25–50 μm), very fine sand (50–100 μm), fine sand (100–250 μm), medium sand sized particles (250–500 μm), coarse sand (500–1000 μm), and very coarse sand (1000–2500 μm).

The average particle sizes of material within the manure was only significantly impacted by the grind size of the feed, with particles in the manures from pigs fed the coarsely ground diets having an average particle size of 238 μm and particles in manures from pigs fed the finely ground diets having an average particle size of 138 μm. As a point of comparison, average particle size in the coarsely ground feed was 631 μm. This indicates that the average particle size in the manure was 38% the size it was in the feed. Similarly, the average particle size in the finely ground feed was 374 μm; in this case the average particle size in the manure was 37% of what it was in the feed. This difference in particle size was as expected as it was presumed that the majority of the material in the manure was residual feed material that was not digested by the pig.

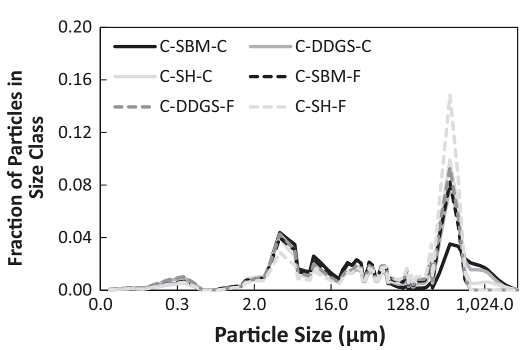

Fig. 2. Particle sizes of the manure from pigs fed the different diets C–SBM–C (Corn–Soybean Meal–Coarse), C–DDGS–C (Corn–DDGS–Coarse), C–SH–C (Corn–Soybean Hulls–Coarse), C–SBM–F (Corn–Soybean Meal–Fine), C–DDGS–F (Corn–DDGS–Fine), C–SH–F (Corn–Soybean Hulls–Fine).

To get a greater understanding of the data, differences in the size classes as described above were also analyzed. No difference was seen in the percent of particles in the clay size range, but the fine silt category (2–25 μm) was significantly impacted by manure inoculation, fiber source, and the manure inoculation × fiber source interaction ($p < 0.05$), while feed grind size showed a trend ($p = 0.12$). These particles were of particular interest because previous research (Andersen, unpublished data) showed that the foam layer in foaming manures was significantly enriched in particles within this size range. Moreover, previous results suggested that these particles were being separated from the manure and accumulating on the surface, presumably by increased biogas flux rates (Van Weelden et al., 2015; Andersen et al., 2015). Thus, identifying dietary factors that contribute to greater amounts of particles within this size range could lead to identifying a root cause of foaming. The results indicate that the inoculated manure was enriched in these fine silt sized particles in comparison to the non-inoculated manure (40% of solids volume as compared to 35% of solids volume respectively). Similarly, manure from pigs fed the coarse ground diets had a greater percent of its solids in the fine silt particle size range than manure from pigs fed the fine ground diets (40% vs 36%), though in this case the effect was not significant ($p = 0.12$). Finally, the fiber source of the animal diet also had a significant impact on manure from the C–SH diet by lowering significantly the percent of particle in the fine silt size fraction (31%) compared to either the C–DDGS diet (38%) or the C–SBM diet (44%).

The coarse silt fraction (25–50 μm) was significantly impacted by the fiber source, with animals fed the CSBM diet having a manure with a greater percent of its particles in this fraction (12%), than in manure from pigs fed the C–DDGS or C–SH based diets (9%) (Table 3). For larger particle size classes, grind size effects dominated differences presumably via digestibility effects judging by differences in pigs fed C–SBM as compared to pigs fed C–SH and C–DDGS based diets. For example, in the very fine sand fraction, manure from pigs fed the finely ground feed had 10% of its particles in this class while manure from pigs the coarse ground fraction only had 7% of its particles in this class. In the fine sand fraction both grind size, fiber source, and the grind size × fiber source interaction were all significant with manure from pigs fed the fine grind diets having 14% of its particles in this size class as compared to 8% in the manures from pigs fed the coarse grind diets. Fiber source also had an impact, with manure from pigs fed C–SH having 15% of its particles in this class while manure from pigs fed C–DDGS had 11%, and manure from pigs fed C–SBM had 7% of its particles in this class. In the case of medium sand, the grind size was also important, with manure from pigs fed the finely ground diets having more of their particles in this range than in manure from pigs fed the coarsely ground diets. Similarly, the fiber level was an important parameter leading to more particles within this size range.

Another method of evaluating this data is as total mass of particles in each size range, rather than as a percent of particles in each size (Table 3). To obtain this value, the total mass of solid particles in the manure was multiplied by the fraction of particles in each size classification. It was assumed that particle density was constant across different particle sizes because the Cilas 1190 gives

Table 3
Particles size distributions (mass of particles in different particles sizes) classes as impacted by diet, grind size, and manure inoculation. Values not connected by the same letter within a group are significantly different at $\alpha = 0.05$.

	Particle size [% of particles] mass of particles [g/L]															
	Clay		Fine silt		Course silt		VF sand		Fine sand		medium sand		Course sand		VC sand	
	%	g/L	%	g/L	%	g/L	%	g/L	%	g/L	%	g/L	%	g/L	%	g/L
Fine grind	5.5	288b	36	1866b	10.6	553b	10.3a	549	14.3a	772a	22.3a	1222	23	1279b	0b	0b
Coarse grind	5.9	430a	40	2860a	9.9	708a	7.4b	548	8.0b	615b	15.4b	1194	23	1796a	6a	494a
Not inoculated	5.5	377	35a	2423	9.8	665	8.7	586	11.2	740	20.2a	1370a	26a	1810a	4	301
Inoculated	5.9	340	40b	2304	10.6	595	9	511	11.2	646	17.5b	1045b	20b	1265b	2	192
DDGS	6.1	434a	38a	2712a	9.4b	655	8.9	609a	11.3b	765b	18.4b	1323b	23ab	1771a	3	311
Soybean meal	5.8	303b	44a	2318ab	12.0a	627	8.4	602a	7.4c	367c	12.7c	1625a	18b	1046b	4	285
Soybean hulls	5.2	340b	31b	2059b	9.3b	609	9.3	434b	14.8a	949a	25.5a	675c	28a	1796a	2	145
Coarse, DDGS	6.0	535a	38ab	3353a	8.4c	749	7.3bc	650a	8.7c	774abc	17c	1555a	25ab	2401a	6a	623a
Coarse, Soybean meal	5.8	338bc	46a	2969ab	11.7ab	688	6.6c	392c	2.9b	178d	8.1d	532c	18b	1235bc	8a	570a
Coarse, Soybean hulls	5.8	415b	35bc	2534bc	9.5bc	686	8.3b	603ab	12.4b	893ab	20.9b	1494a	25ab	1752ab	4a	290a
Fine, DDGS	6.2	333bc	38ab	2072bcd	10.3abc	568	10.5a	567ab	13.9b	755bc	19.7bc	1090b	21b	1141bc	0b	0b
Fine, Soybean meal	5.8	267c	42ab	1944cd	12.2a	561	10.2a	476bc	11.8d	556c	17.3c	818bc	18b	857c	0b	0b
Fine, Soybean hulls	4.6	264c	27c	1583d	9.1c	529	10.3a	602ab	17.2a	1004a	30.1a	1757a	31a	1839ab	0b	0b

Clay: 0–2 μm; Fine silt: 2–25 μm; Course silt: 25–50 μm; VF sand: 50–100 μm.
Fine sand: 100–250 μm; Medium sand: 250–500 μm; Course sand: 500–1000 μm; VC sand: 1000–2500 μm.

fraction by volume. Based on the characteristics of particles sizes in foaming and non-foaming manures, the fine silt fraction was most important as it is enriched in the foam. Thus, manures that tend to have more of these particles would be assumed to have the greatest potential to form stabilized foam. The results suggested that both grind size and fiber source affected the mass of these particles present, with manures from pigs fed coarse ground diets having 28.6 ± 1.5 g/L of particles in this size range while manures from pigs fed finely ground diets having 23.0 ± 1.5 g/L of these particles. Thus, by decreasing grind size, a producer could reduce the potential to generate these particles by 20%. The results also indicated that fiber source was important, with manures from the pigs fed the C–DDGS diet (27.1 ± 1.8 g/L) having the most of these particles with manures from pigs fed the C–SBM or the C–SH diets having lower amounts (23.2 and 20.6 g/L respectively).

3.3. Methane production characteristics

Manures from pigs fed the coarse ground diets had significantly higher MPR (0.020 L CH_4/L-day) than manure from pigs fed the finely ground diets (0.012 L CH_4/L-day) (Fig. 3). Similarly, manure from pigs feed the C–SH diets had higher MPR (0.024 L CH_4/L-day) than in manure from pigs fed either the C–DDGS diet (0.014 L CH_4/L-day) or C–SBM diet (0.010 L CH_4/L-day) (Fig. 3a). This indicates that MPR is at least in part driven by the amount of consumable carbon (volatile solids) entering the manure storage. To get a better approximation of this the NDF% in the diet was regressed against the MPR of the manure for both course and fine grind diets (grind sizes were regressed separately as grind size had a significant impact on MPR). In both cases the MPR was strongly positively correlated with NDF% in the diet (0.0015 L CH_4/L-day, $r = 0.985$ for coarse grind diets; 0.0006 L CH_4/L-day, $r = 0.908$ for fine grind diets). This would appears to suggest that fiber in the diet plays a key role in the rate of methane production.

There was significant ($p < 0.01$) effect of diet type and grind size on manure BMP (Fig. 3b). On average, manure from pigs fed the coarsely ground diets showed greater BMP than manure from pigs fed finely ground diets, which reflects the digestibility of the different grind sizes in the gastrointestinal tract of the pig (NRC, 2012). That is, since finely ground feeds have greater digestibility than coarse ground feeds more of the energy in them is retained in the pig for growth and not available for methane production during digestion of the manure. This is reflected in a lower volatile

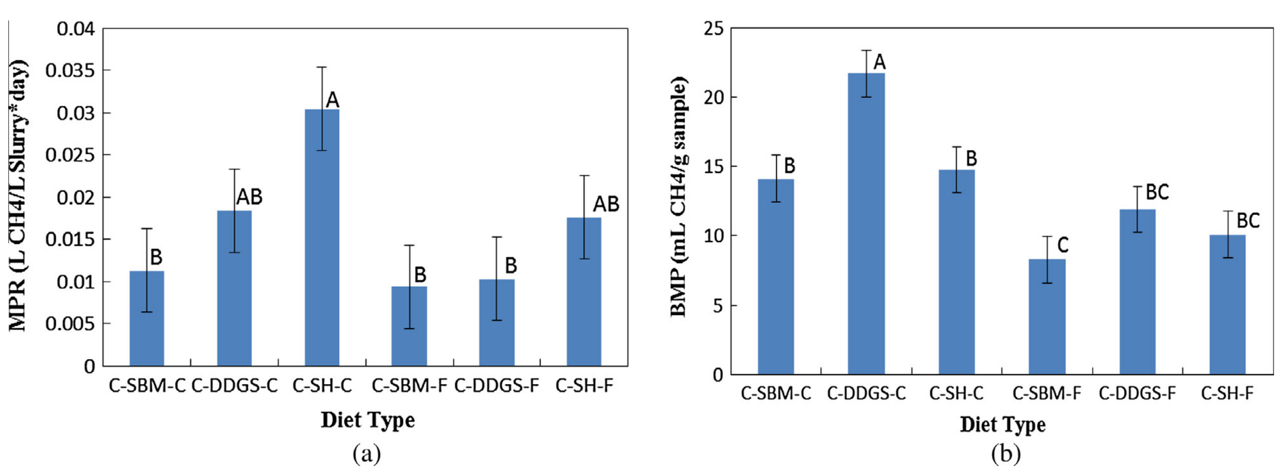

Fig. 3. Average methane production rates per volume sample (a) and (b) biochemical methane production potential (b) C–SBM–C (Corn–Soybean Meal–Coarse), C–DDGS–C (Corn–DDGS–Coarse), C–SH–C (Corn–Soybean Hulls–Coarse), C–SBM–F (Corn–Soybean Meal–Fine), C–DDGS–F (Corn–DDGS–Fine), C–SH–F (Corn–Soybean Hulls–Fine). Error bars represent the standard error of the mean and capital letters indicate differences ($\alpha = 0.05$).

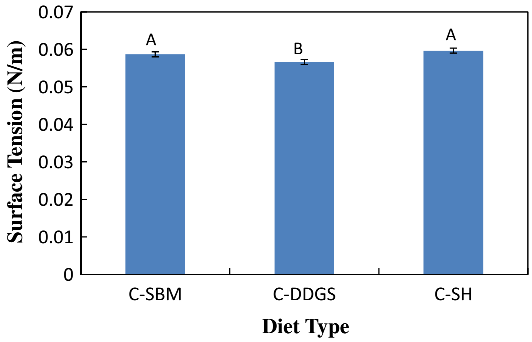

Fig. 4. Average surface tension of the manure samples obtained from different diet fiber sources C–SBM (Corn–Soybean Meal), C–DDGS (Corn–DDGS), C–SH (Corn–Soybean Hulls). Error bars represent the standard error of the mean and capital letters indicate differences ($\alpha = 0.05$).

solids content in the manure of animals fed the finely ground feeds. With respect to fiber source, manure from pigs fed the C–DDGS diet had the highest cumulative BMP. This was true regardless of dietary particle size. Although, not conclusive, higher BMP in manure from animals fed C–DDGS could be reflective of the foaming manure characteristics because feeding C–DDGS could eventually lead from higher BMP to higher field MPR, and higher field MPR are strongly associated with foaming manures (Van Weelden et al., 2015).

If BMP results were normalized to the volatile solids content of the manure, there were no significant differences due to grind size, diet source, or manure inoculation. Although not significant, the results did indicate that manure from the pigs feed coarse ground diets tended to have higher BMP (306 ± 16 mL/g VS) than manures from pigs fed finely ground diets (272 ± 16 mL/g VS). Moreover, the results indicated that as NDF% of the diet increased the BMP of the manure decreased by 3.3 (mL CH_4/g VS) for every additional 1% NDF in the diet ($r = 0.958$).

3.4. Surface tension, foaming capacity, and foam stability

The results of the surface tension measurements are shown below in Fig. 4. In this case, dietary fiber source and manure inoculation were found to be significant ($p < 0.01$), but no other terms where. In this case, the inoculated manure had a lower surface tension (0.05652 N/m) than the un-inoculated manure (0.05946 N/m). The surface tension for the manure from pigs fed the C–DDGS diet (0.0556 N/m) was lower than the manure from pigs fed the other two diets (0.0597 for CSBM and 0.0587 for CSBM–SH). It has been speculated that oil in the diet that is passing through the animal (i.e., undigested) could be contributing to the lowering the surface tension (Yan et al., 2014). At first, the data in this study would seem to indicate this isn't the case as DDGS and SH had similar ether extract fat contents, but DDGS had lower surface tension. However, intact fats are less digestible than added fats so these results may be supportive of this theory. Alternatively, the lower surface tension may be due to microbial compounds being produced during the breakdown of volatile solids in the manure. In this study, total VFA concentrations were higher in manures from animals fed higher fiber levels (14,300 and 15,200 μg/g for manures from DDGS and soybean hulls diets respectively, as compared to only 8800 μg/g in the manures from the soybean meal diet). The presence of significantly higher VFA content of manures from the DDGS and soybean hull diets suggests their surface tension should be lower than the SBM diet, but again offers no explanation why surface tension in the DDGS diet would be lower than the SH diet. Similarly, no difference in VFA content was found between the un-inoculated manure and inoculated manure, failing to explain why the inoculated manure had lower surface tension. In terms of surface tension for different diets, the concentration of volatile solids in the manure was the most strongly correlated ($r = 0.5931$), suggesting that increasing VS content reduced ST.

Both manure inoculation and grind size affected ($p < 0.05$) foaming capacity, while fiber source had no impact. The un-inoculated manure had a higher foaming capacity than the inoculated manure. This is opposite of what would be expected based on a three-phase foaming model (Davenport et al., 2008), where the liquid phase is expected to be a surfactant that lowers the surface tension of the manure. However, these results align with field foaming manures in that commercial barns that tended to foam had higher surface tensions and greater foaming capacity (Van Weelden et al., 2015). Similar to the Van Weelden et al. (2015) where foaming manure had higher total solids contents, the results from this study showed a strong positive correlation between foaming capacity and total solids content (13% FC/1% TS, $r = 0.884$). However, the results also strongly indicated that this was correlated (0.8822) to the presence of fine particles (sum of clay, fine silt, course silt). As previously mentioned, manure from animals fed course grind diets had more of these particles, and as a result exhibited greater foaming capacity than manure from animals fed finer ground diets (Fig. 5). No correlation between the larger particle size amount and foaming capacity was found.

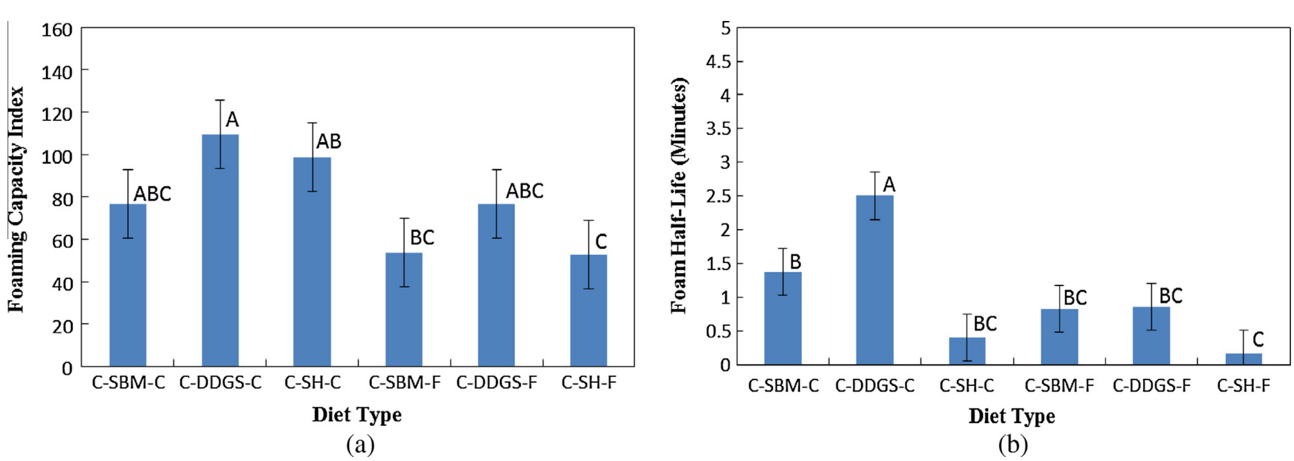

Fig. 5. Average foaming capacity (a) and foam stability (b) for C–SBM–C (Corn–Soybean Meal–Coarse), C–DDGS–C (Corn–DDGS–Coarse), C–SH–C (Corn–Soybean Hulls–Coarse), C–SBM–F (Corn–Soybean Meal–Fine), C–DDGS–F (Corn–DDGS–Fine), C–SH–F (Corn–Soybean Hulls–Fine). Error bars represent the standard error of the mean and capital letters indicate differences ($\alpha = 0.05$).

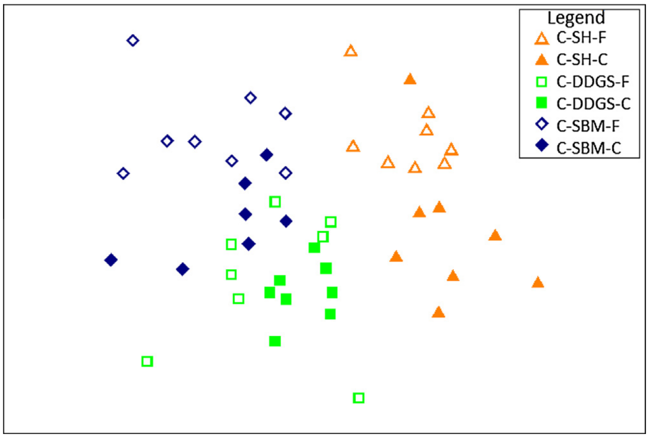

Fig. 6. Non-metric multidimensional scaling plot based on the ARISA analysis (S17 Bray Curtis similarity) to illustrate differences in microbial community as a result of fiber source and grind size. FSBM – fine ground soybean meal, CSH – coarse ground soybean hulls, CDDGS – coarse ground DDGS, FSH – fine ground soybean hulls, FDDGS – fine ground DDGS, and CSBM – coarse ground soybean meal.

Foam stability was significantly influenced by manure inoculation, grind size, and fiber source ($\alpha < 0.05$), but no interactions were found to be significant. The un-inoculated manure had a higher foam stability than the inoculated manure, as did manure obtained from pigs fed the coarsely ground diets. This is again reflective of the overall solids content of the manure, but was only correlated to the presence of fine particle (clay, fine silt, course silt). The foam half-life increased by 0.0008 min/g of particles in this size class, $r = 0.8609$. Large particles showed either no correlation or negative correlation to foam stability.

Information in this study would suggest that diets rich in fiber and coarsely ground will produce manures which have a greater propensity to foam. This is based on their higher total and volatile solids contents, MPR and BMP; all suggesting that given time, increased dietary fiber levels and coarsely ground feed will increase the biogas production of manure. Foam capacity and stability trends were also driven by diet and grind size, with diets that had the most particles in the fine sized categories generating manure with the largest foam capacity and stability. The presence of the particles was most closely related to the amount of organic material in the manure, but the source of fiber was also important with DDGS generating more of these particles than other fiber sources.

3.5. Microbial community

In addition to the physical and chemical properties, the microbial community in these manures was also assessed. Others (Gates, 2013; Pepple et al., 2012) have shown that different microbial assemblages are present in foaming and non-foaming manures, but as of yet haven't provided specific microbial species enriched within foaming barns. In this feeding trial, distinct microbial community structures developed between the treatments. The strongest distinction occurred among fiber sources ($R = 0.599$); still grind size ($R = 0.373$) impacts were also significant. In terms of fiber source, manure from pigs fed the C–SH diet resulted in the most distinct microbial community structure ($R = 0.702$, $R = 0.715$ when compared to CSBM and C–DDGS respectively). This difference was presumably caused by difference in fiber within the manure. Soybean hulls consist primarily of cellulose, while corn fiber and DDGS fiber consist of hemicellulose. In general, hemicellulose has sugar side chains, while cellulose is found in tightly bound aggregates (Kerr and Shurson, 2013).

In addition, manure from pigs fed the CSBM diet was also distinct from manure obtained from pigs fed the C–DDGS diet ($R = 0.542$). This was presumably caused by a carbon gradient in the manure, similar to what was seen for grind size. Even though grind size of the feed had a smaller impact on microbial community, its impact within diet was still evident (Fig. 6) and similar on all fiber sources (R-statistics of 0.316, 0.433, and 0.364 between the coarse and fine grind sizes for the pigs fed the C–SBM, C–SH, and C–DDGS diets, respectively). In fact, this the difference between CSBM and C–DDGS is directionally similar to that of grind size, with lower carbon loading resulting in the upper left and higher carbon loading in the lower right.

4. Conclusions

Foaming properties of the manure were impacted by feed grind size and fiber source. Factors that reduced digestibility (bigger grind size, more neutral detergent fiber) resulted in increased foaming properties. The data suggests this is due to greater amount of carbon in the manure which increased methane production potential, solids content, and fine silt sized particles in the manure. Aging the manure resulted in more fine silt sized particles (2–25 μm), which play an essential role in stabilizing foam bubbles in this system; however, it did little to alter other foaming properties.

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