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Evaluation of Potential Treatments to Reduce Foaming from Swine Manures

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ABSTRACT. *Foam formation on the surface of deep pit swine manure storages poses a safety concern for both humans and animals. Although current research has provided significant insight into both the causes and mechanisms of foam formation, thus far few practical, easily implemented treatment practices are available to help mitigate manure foaming. Thus, the purpose of these experiments were to better understand the causes of foam formation, the mechanisms of foam development and stabilization, and determine the effectiveness of Narasin, Manure Magic, and other physical, chemical, and biological manure treatments to reduce foaming in deep pit swine manure storages. Our first experiment was designed to evaluate if different feed components induced foaming properties in the manure and a follow-up incubation study to evaluate if there were difference preferences for conversion to methane from carbohydrate, protein, and fats between foaming and non-foaming manures. In general, our results showed that easily degradable organic carbon induced microbial activity and results in grater foaming capacity and foam stability, but the effect was generally short lived. In terms of substrate preference or results showed that foaming manures showed high affinity for all carbon substrates tested but no difference between substrates was seen. Our second study focused on if manure additives could alter either the methane production rate, foaming capacity, or foam stability. These results gave important insights into the mechanisms of foam formation in that the inclusion of tannin instantly and significantly caused induced foam stability, while the decomposition of soybean oil induced foaming after several weeks. In particular, these results showed that fine particles that interact with proteins can induce stability within the foam and appeared to demonstrate that similar types of compounds can be synthesized by the decomposition of oils. Our last two experiments focused specifically on developing and evaluating foam mitigation strategies. These tests were conducted at the field- and lab-scale; our results failed to show significant difference in the lab-scale, presumably because microbial activity (based on methane production rates) was low for all tests. At the field-scale experiment we found that treatment with Narasin or Manure Magic reduced both methane production rate and foam stability. Our results indicated that Narasin reduced volatile solids destruction while Manure Magic increased solids destruction. Finally, microbial sequencing data indicated that our Manure Magic treatment significantly altered the microbial community away from that of our foaming control barn, but not when the manure wasn't foaming.*

Keywords. Swine manure, foaming, methane production, manure treatments

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.

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.

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 (2016) 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, protein rich particles appearing to be important in the stabilization of the foam bubble structure, c) foaming barns have lower concentrations of volatile fatty acids and higher surface tension, d) a microbial produced polyliposaccharide appears to be interacting with the foam to form the bubble stabilization structure, and e) significant microbial community differences can be seen between foaming and non-foaming manures. 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.

As methane production appears to be such a critical component of foam formation it is suggested that mitigation of this enhanced methane production may be a critical means of reducing foam formation. Many techniques have been developed to mitigate methane emission from manures including frequent manure removal from the floor or pen, the adoption of an anaerobic digestion system (Clemens et al., 2006), or covering the manure and capturing the emitted gas (Clemens and Ahlgrimm, 2001). Though different techniques have been developed, there is still a demand for new, cost-effective technologies that can aid in control of methane emissions. One approach that has received attention includes the use of dietary manipulation to increase digestibility of feedstuffs, and as a result limit organic matter in the excrement, resulting in decreased potential for conversion to methane. Another approach based on diet manipulation is the use of feed additives to inhibit methanogenesis during digestion and improve animal performance (Johnson and Johnson, 1995; Benchaar et al., 2001).

Although not verified, similar approaches might have the potential to reduce gas production from manure storage pits. One product that has been tested in the lab setting for its potential to reduce gas production from swine manure is tannins (Whitehead et al., 2012). Similarly, others have recommended the use of monensin as a pit additive to reduce foaming (Clanton, 2012). However, monensin has been reported to be toxic to pigs so its use in swine facilities is a risky proposition. Monensin is an ionophore, a lipid-soluble molecule that transports ions across cell membranes. These compounds are thought to promote animal growth by manipulating microbial flora, which can impact methane production. Another ionophore, narasin, is safe for swine and approved as a swine feed additive.

Thus, as a result, four studies where undertaken here, these included: 1) direct addition of feed products to manure to evaluate if any enhanced foaming characteristic, 2) a lab study incubation where a slew of treatments, either focused on reducing methane production via inhibition or microbial community change or alternatively focusing on reducing foam stability through either protein removal or surface tension evaluation was conducted, 3) a lab study of an inophore, a lactobacillus, and a bacillus bacteria added separately or in combination to manure, and then finally 4) a barn-scale evaluation of treatment bacillus bacteria addition and ionophores treatment to evaluate field scale effects.

Materials and Methonds

Study Desings

Feed Component Additive Study

A study was conducted at the Iowa State University Swine Research Facility to observe the effects addition of various feed components (DDGS, Wheat mids, Soybean hulls, and corn oil) had on the ability of a sample to produce foam (foaming capacity) and stabilize foam (foam stability). In this study, manure produced fed a corn diet with DDGS was collected in a single tank. Then, about 6000 grams of manure was distributed into 64 buckets and mixed with various types of feed as described in table 1. The order of additives shown in table 1 was repeated for each set of eight buckets to give eight repetitions of each mixture.

Two different grind sizes (Course - 700 µm and Fine 350 µm) were evaluated for all the fiber products evaluated. Either 200 grams of fiber product or 30 grams of the corn oil were added to eight of the buckets of each treatment. Once added all samples were mixed and allowed to ferment for varying lengths of times before being sampled. Buckets 1-24 were sampled after 7 days, buckets 25-48 were sampled after 14 days, and buckets 49-64 were sampled after 21 days. All buckets were mixed every seven days to suspend and solids that had settled in the manure. Once sampled, the manure mixtures were tested with the foaming capacity and stability apparatus.

The foaming capacity and stability apparatus used in this study, as well as the parameters used to evaluate the foaming characteristics of swine manure, were adapted from a number of other studies, including Ross et al. (1992), Bindal et al. (2002), Bamforth (2004), and Hutzler (2011). Air was passed through an in-line gas regulator (Restek Model 21666) directly into a 2-inch diameter clear PVC column. The flow rate of air through the column was measured and controlled with a variable area flow meter (Dwyer RMA-SSV). For the purposes of this experiment, it was determined that a flow rate of 200 cubic centimeters per minute (0.0033 L/s) was appropriate based on preliminary trials. In order to conduct the foaming capacity experiment, a sample volume of approximately 300 mL was poured into the column and the initial level was recorded based on measuring tape placed on the columns. The sample was then aerated through a cylindrical air stone at 0.0033 L/s until a steady state height was reached or the foam layer reached the maximum height of the column. The time of aeration was recorded along with the height of foam produced and the level of the foam-liquid interface. A foaming capacity index was calculated as the height of foam produced divided by the initial level and multiplied by a factor of 100.

Table 1. Additive types with corresponding labels used for each of the three trials.

Bucket	Additive	Amount Added to Bucket
1	None (Control)	-
2	Fine Ground DDGS	200 g
3	Course Ground DDGS	200 g
4	Fine Ground Wheat Midds	200 g
5	Course Ground Wheat Midds	200 g
6	Fine Ground Soybean Hulls	200 g
7	Course Ground Soybean Hulls	200 g
8	Corn Oil	30 g

Once all samples were mixed into the 5-gallon buckets, they were allowed to ferment for varying lengths of times before being sampled. Buckets 1-24 were sampled after 7 days, buckets 25-48 were sampled after 14 days, and buckets 49-64 were sampled after 21 days. Once sampled, the manure mixtures were tested with the foaming capacity and stability apparatus. Statistical analysis was performed using JMP Pro 10. Fixed factors were established according to the diet type associated with each sample and the trial period. For the bucket additive study, fixed factors included the mixture type as well as the fermentation time in days.

In a similar follow-up study manure was obtained from five commercial production facilities, three of which were foaming and two which weren't during manure sampling collection. Approximately 100-ml of manure from each of the was placed into six glass containers following the MPR procedure discussed in detail in the evaluation methods. To give of these bottles different substrates were added (estimated to be approximately 1 g available COD) was added. Substrate added included sugar, soluble fiber, protein, soybean hulls, and corn oil; this was an addition of 1, 0.9, 0.83, 2.51, and 0.37 grams of sugar, fiber, protein, soy hulls, and corn oil respectively which based on measured COD values was 1.2, 1.0, 1.1, 1.8, and 0.77 grams of COD added). This test was conducted to determine if foaming microbial communities responded differently than non-foaming microbial communities. Data was assessed as cumulative methane production per gram of COD added and a comparison between foaming and non-foaming manures for every day methane was measured. Methane production measurements were recorded on days 3, 7, 11, 16, 20, 25, 30, 37, 42, 47, 54, 65, 72, and 80 of the incubation.

Methane production was corrected for the residual amount of methane made by the control without additive amendment at each point in time so the amount of methane produced represents the volume of methane produced per added gram of COD. Statistical analysis was conducted by evaluating cumulative methane production with factors of surface texture (foaming vs. non-foaming) and additive type (Fiber, oil, protein, sugar, soybean hulls) with the analysis run by measurement date to handle the repeated measures aspect of the design.

Manure Foaming Product Evaluation

As producers are interested in potential cures for foaming an assortment of potential treatments were evaluated, these included a control, tannins (0.5%), course biochar (1%), humic substances (1%), foam buster [soybean oil] (1%), free fatty acids (1%), acetic acid (3 mL/L), ammonium hydroxide (33 mL/L), sodium hydroxide (1%), Narasin (1.5 mg/L), spent hops (2 g/L), diluted control (added 33 mL/L DI water), fine biochar (0.5%), and soluable fiber (0.5%).acetic acid, addition was evaluated. Each treatment was added to 9 vials of manure from two different sites. These treatments were selected with the intention of providing information on specific mechanisms either related to foam or the prevention of foam. As shown in Van Weelden et al. (2016) methane production appears to be important to contributing to the formation of foam. Thus eight of the treatments focused on evaluating how altering this parameter impacted the methane production rate and foaming capacity and stability of the manure. Three of the treatments, Narasin, tannins, and the spent hops were added with the intention of inhibiting or discouraging methane production. Four of the treatments (foam buster, free fatty acids, acetic acid, and humic substances) were added with the intention of initially inhibiting methane production but with the understanding that these could serve eventually serve as substrates. Finally, one treatment, the soluble fiber, was added with the intention of encouraging methane production.

Similarly, several treatments were targeted at modifying pH, these included addition of acetic acid, ammonia hydroxide, and sodium hydroxide. According to Van Weelden et al. (2016) the pH of foaming manure was slightly more basic than its non-foaming counterpart and this was mainly due to acetic acid accumulation. Although two treatments to make the manure more basic might seem counter intuitive, further investigation had shown that proteins were important for stabilizing the foam and it is believed that the pH treatment has the potential to denature the proteins and thus reduce foam stability. Finally, two biochars of fine and course particle size were added as we had seen that fine particles were causing foam stabilization and provided a particle which could be added to evaluate this effect.

Finally, one treatment was a simple water addition to serve as a negative control to treatments where water was added, but also to evaluate if it was possible that knocking down foam with sprinkler water, while removing foam in the short term, actually encouraged grater foam production.

Lab Incuation of Specific Foam Mitigation Products

Based on the previous study and increased analysis of microbial data and its link to manure characteristics, we proceeded with an additional study focused on four potential treatments: Manure Magic addition, lactobacillus addition, Narasin treatment, Narasin treatment with Manure Magic, and Narasin treatment with the lactobacillus addition, as compared to the control. Given the results of the first additive study, a previous Narasin treatment study reported by Reagen et al. (2015), an analysis of microbial community structure by Yang et al. (2017), a decision was made to do a more focused study on just three additives, Narasin, ManureMagic, lactobacillus, and then combinations of Narasin and ManureMagic and Narasin and lactobacillus. These treatments were choses to evaluate three different mechanisms. Narasin was added to treat methane production, ManureMagic was added to help break down proteins, lactobacillus was chosen it was one of the key microbial differences between foaming and non-foaming manure microbial communities. In this study nine different vials of manure were treated with each of the products. Every two weeks the amount of methane produced by the vial was measured and three of the vials destructively sampled to evaluate the foaming capacity and stability of the manure.

Field Evaluation of Foam Mitigation Products

As a compliment to these lab-scale investigations a field-scale experiment was also conducted. This experiment was conducted at a 1200-head swine finishing facility in North Central Iowa. The barn is uses a single feed system and is operated as an all-in, all-out facility so pigs and diet are consistent across the barn. However, the pit is divided into four parts allowing separate treatment of the manure inside.

As a part of this study pit 1 and 2 were left as controls, pit 3 was treated with Narasin, and pit 4 was treated with Manure Magic. Pit 3 was treated with 7.0 kg narasin (3.5kg in each side) on 5/16/16 and 4.0kg narasin (2 kg in each side) on 12/04/16. Pit 4 was treated with 1.4kg manure magic (0.7kg in each side) on 5/16/16 and 6kg manure magic (3kg in each side) on 12/04/16. The initial treatment occurred after manure pump-out in the spring with the second treatment occurring several months after fall manure removal.

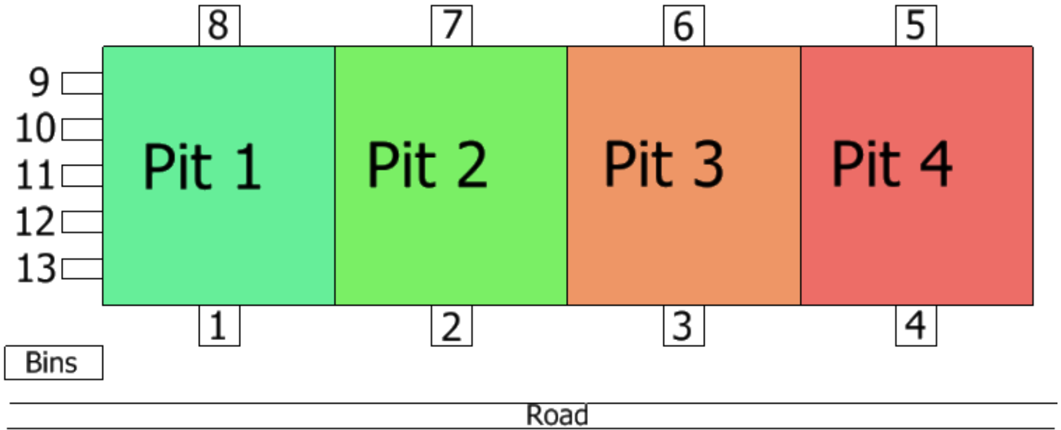


Figure 1. Deep pit swine barn in north east Iowa where the manure samples for this study were taken. This facility is a deep pit swine finishing facility with two pump puts on each of the 4 pits with five tunnel fans on the west side.

Manure samples were taken from each pit on the following dates: 5/16/16, 5/31/16, 6/14/16, 6/27/16, 7/11/16, 7/25/16, 8/8/2016, 8/21/2016, 9/3/2016, 9/14/2016, 10/1/2016, 10/16/2016, 11/12/2016, 12/4/2016, 12/28/2016, 2/3/2017, 2/18/2017, 3/29/2017, 4/12/2017, 4/26/2017, 5/12/2017. These manure samples were tested for Total and Volatile Solids, methane production rate, foaming capacity, and foam stability after every collection. In addition, 30 mL from each sample were placed in centrifuge tube and then placed in a freezer for future DNA extraction and microbial analysis. During every site visit NH_3 and H_2S concentrations in the pit exhaust air were monitored using a Draiger meter. The amount of foam on the surface of the manure during each site visit was also recorded.

Evaluation Methods

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, 2000). Approximately 30 mL of a manure sample was poured into a pre-weighed porcelain dish after thorough mixing. After obtaining the weight of the full crucible, the sample was dried in a 104°C oven for approximately 24 hours. After drying the sample was weighed again. The percent of total solids was determined by equation 1 below.

$$\%\text{total solids} = \frac{\text{weight of dried sample and dish} - \text{weight of crucible}}{\text{weight of wet sample and dish} - \text{weight of cricible}} * 100$$

After obtaining the dried weight of the sample, the crucible with the dried contents was placed in a muffle furnace at 550°C for approximately 8 hours. Once cooled, the final weight of the ash and crucible was obtained, and the volatile solids content was determined by equation 2.

$$\%\text{volatile solids} = \frac{\text{weight of dried sample and dish} - \text{weight of ash and dish}}{\text{weight of wet sample and dish} - \text{weight of cricible}} * 100$$

Methane Production Rate

The methane production rate test was loosely inspired by the biochemical methane production potential tests of Moody et al. (2011a) and Owen et al. (1979), the anaerobic toxicity assay of Moody et al. (2011b), and its development and use is fully detailed in Andersen et al. (2015). Briefly, 100 grams of manure was poured into a clear, 250 mL graduated glass serum bottle (Wheaton Science Products No.:223950). The exact volume of these serum bottles was recorded using a water displacement method; based on measurements in the AWML this volume was approximately 283.33 mL. The exact mass of manure added to the bottle was recorded (difference in mass between the empty bottle and the mass of the manure and the bottle, graduated marks on the bottle side are used to estimate 100 mL while adding the manure). A sleeve stopper septum (Sigma-Aldrich Z564729) was then placed on top of the bottle to seal it from the atmosphere. The bottle was placed on a laboratory counter and incubated at room temperature. Biogas production was measured periodically by inserting the needle of a glass, gas-tight syringe (Micro-Mate interchangeable hypodermic Syringe 50cc Lock Tip, Popper & Sons, Inc. New Hyde Park, New York) into the sleeve septum. When inserted, pressure in the bottle displaced the wetted barrel of the syringe. The volume of biogas extracted was read from graduated markings on the syringe body. The volume and time at which the sample was collected was then recorded and the biogas injected into an infrared gas analyzer (NDIR-CH4 Gasanalyzer University Kiel, Germany) to obtain the methane content.

These bottles were weighed before and after the 100 mL samples were added. After weighing the bottles the second time, septa (Sigma-Aldrich Z564729) were placed on the bottles to make them air tight. These bottles were then set aside out of the sun for about two weeks. After about two weeks the volume of biogas produced was measured using a syringe (Micro-Mate interchangeable hypodermic Syringe 50cc Lock Tip, Popper & Sons, Inc. New Hyde Park, New York) and the concentration of methane in the biogas was also tested using a gas analyzer (NDIR-CH4 Gasanalyzer University Kiel, Germany).

$$\text{MPR} = \frac{\text{methane percent}(\%)}{100\%} * \frac{\text{biogas produced}(\text{mL}) + \text{head space}(\text{mL}) * \rho_{\text{manure}}\left(\frac{\text{g}}{\text{mL}}\right)}{\text{time}(\text{days}) * \text{mass manure sample}(\text{g})}$$

Foaming Capacity and Stability

The foaming capacity and stability apparatus used in this study, as well as the parameters used to evaluate the foaming characteristics of swine manure, were adapted from several other studies, including Ross et al. (1992), Bindal et al. (2002), Bamforth (2004), and Hutzler (2011) and a complete description of how this test was implemented is available in Van Weelen et al. (2013). Briefly, air was passed through an in-line gas regulator (Restek Model 21666) directly into a 2-inch diameter clear PVC column. The flow rate of air through the column was measured and controlled with a variable area flow meter (Dwyer RMA-SSV). For the purposes of this experiment, it was determined that a flow rate of 200 cubic centimeters per minute (0.0033 L/s) was appropriate based on preliminary trials. In order to conduct the foaming capacity experiment, a sample volume of approximately 300 mL was poured into the column and the initial level was recorded based on measuring tape placed on the columns. The sample was then aerated through a cylindrical air stone at 0.0033 L/s until a steady state height was reached or the foam layer reached the maximum height of the column. The time of aeration was recorded along with the height of foam produced and the level of the foam-liquid interface. A foaming capacity index was calculated as the height of foam produced divided by the initial manure level and multiplied by a factor of 100. The foam stability measurement occurred immediately after the foaming capacity was determined. Once aeration ceased, the final height of foam became the initial level recorded at time zero. Once this level was established, the descending height of the foam was recorded at expanding time intervals. Simultaneously, the ascending level of the foam-liquid interface was recorded at the same time intervals. The descending height of foam 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. The half-life of the foam was determined with equation 5 as a measure of the foam stability.

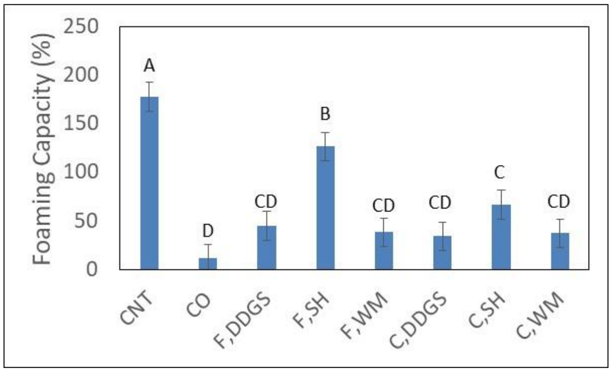
$$\text{foaming capacity} = \frac{\text{steady state height} - \text{initial height}}{\text{initial height}} * 100\%$$

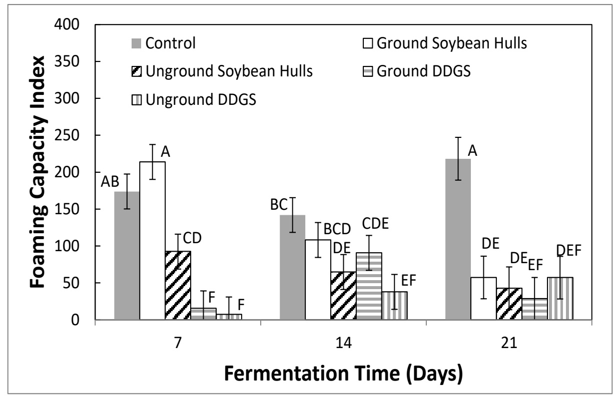
$$t_{\frac{1}{2}} = \frac{\ln(2)}{\text{decay coefficient k}} \tag{1}$$

Results and Discussion

Feed Component Additive Study

One of the consistent hypothesis suggested for causes for the formation of foam has been to suggest that changes in the way pigs are fed has led to this issue, either expecting a direct feed component to be the cause or a microbial bi-product produced via its decomposition. To more strongly evaluate this possibility four feed components were evaluated, with different grind sizes used to test the fiber sources (DDGS, soy hulls, and wheat mids) and then corn oil as the 4th options. As an effort to generate foam differing carbohydrate sources and particle sizes were added directly to the manure to provide readily available carbon and to evaluate if the decomposition of these substances caused formation of a surfactant that could lead to foam formation or stabilization. The data on foaming capacity indicated that incubation length was not significant ($p = 0.5779$), but that both the additive ($p < 0.0001$), and incubation length by additive interaction were ($p = 0.0179$). In this case the interaction was mostly due to the soybean fiber (both fine and course ground) which showed a decreasing foaming capacity with time, and the DDGS additives with had greater foaming capacities in the 2nd and 3rd weeks of incubation, as compared to the controls and other additives which maintained relatively consistent foaming capacities throughout. As a results this data is summarized in two ways, first the overall means for foaming capacity are provided in figure 1a; however, due to the interaction, the impact on incubation time on foaming capacity is shown for several select additives in figure 1b. The main effects indicated that the control, or no additive, had the largest foaming capacity with both soybean hull treatments also having the next highest foaming capacities. The interaction by time graph indicated ground soybean hulls initially had the highest foaming capacity but with longer incubations times this capacity decreased. A contrast statement testing differences between finely ground and coarsely ground additions indicated no difference in foaming capacity ($p = 0.2765$); however, in the case of soyhulls the finer grinding resulting in a larger response to foaming capacity. It is believed in large part these results were driven by production of long-chain free fatty acids, their destruction SCFA, and their eventual decomposition as soyhulls are the most available of the added substrates. In this study, corn oil resulted in reduced foaming capacity throughout the incubation period used.





(a) (b)

Figure 2. Impact of carbohydrate source and particle size on additive (a) and the interaction of carbohydrate source and particle size with incubation time (b). Error bars represent the standard error of the mean. Means not connected with the same letter are different at α = 0.05. CNT-Control, CO-Corn Oil, F-DDGS – Finely ground DDGS, F

Finally, analysis of foam stability indicated that incubation length ($p = 0.0004$), additive ($p < 0.0001$), and the incubation length by additive factor ($p < 0.0001$) were all significant. In generally the results can be summarized into two groups: 1) foam stability was greatest in the finely ground soybean hull diet at day 7 at 33 minutes followed by the course ground soybean hulls at 24 minutes, all other incubation lengths by additive combinations had similar half-lives of 0-5 minutes.

The differences in the behavior of the control mixture, the soybean hull mixture, and other samples provoke some hypotheses with respect to a foaming mechanism. In particular, the efficiency of fermentation of the different additives, or lack thereof, may have directly affected the foaming characteristics of the sample. In the case of the soybean hull mixture, the initial spike of the foaming capacity followed by a gradual decline by week may reflect the relatively high cellulose composition of soybean hulls, which are more readily digested by the microbial population. In fact, this elevated microbial response to the additive addition could have led to the accumulation of some "biosurfactant" as described by Ganidi et al. (2009). This latter hypothesis may also support the increasing foaming trend in the control diet as the weeks progressed if the control diet was less quickly utilized by the bacteria in the bucket.

The second portion of this study took a more detailed look at the impact different potential feed components had on methane production, comparing foaming and non-foaming manures to evaluate if they responded differently to different ingredients. In this study, no impact of the additive or additive x surface interactions were seen. Major differences were seen between foaming and non-foaming manures, but this followed similar trends for all of the components tested, thus it wouldn't appear that foaming manure microbial communities show an affinity for substrates that non-foaming manures do not, but rather the foaming community is more adapt at converting all of the added components into methane. The foaming manures generated approximately 350 mL CH4 per gram of COD added while the non-foaming manures only generated approximately 40 mL CH4 per gram of COD (figure 3). In theory, approximately 350 mL of methane should have been generated per gram of COD destroyed, thus it would appear that over the 80-day hydraulic retention time used in this study foaming manures were capable of converting almost all of the potential COD added into methane.

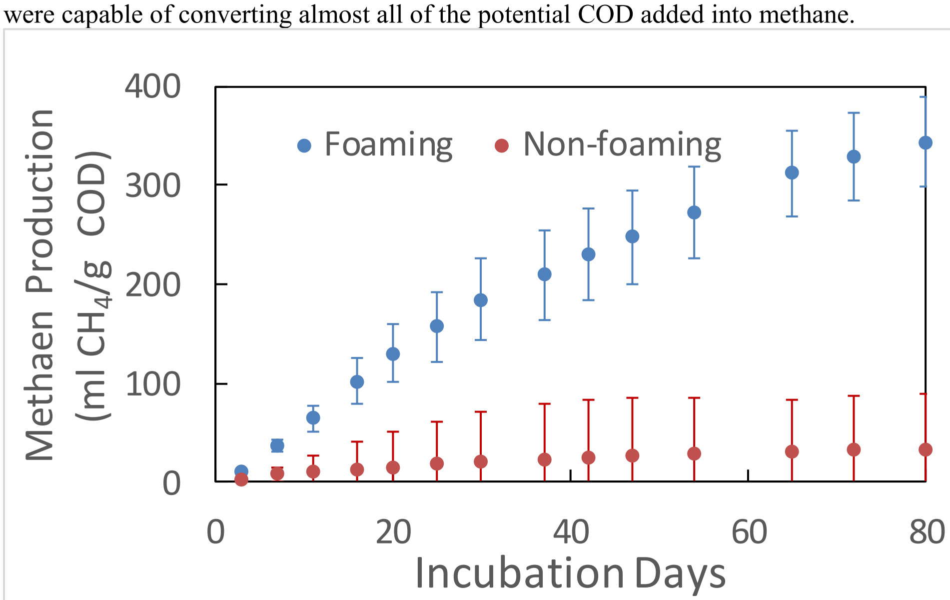


Figure 3. Methane production from foaming and non-foaming manure per gram of chemical oxygen demand added.

Manure Foaming Product Evaluation

Foaming capacity and foam half-life measurements were almost always low with the exception of a few treatments. The addition of tannins caused stable foam to form, this was at first surprising and unexpected; however, tannins have been shown to interact with proteins and it is believed that the addition of tannins provided something of the appropriate particle size that could interact with proteins in the manure to form a stable, merengue like structure. The initial foam stability then slowly decreased over the next four weeks of incubation. As a contrast to this, the foam buster (soybean oil) and free fatty acid both gained foam stability over the course of the incubation. As a whole this would seem to indicate that something about the decomposition of oils leads foam stability, supporting the results from the first feed component experiment. However, based on the substrate addition experiment, if this is microbial exudate it may not be dependent on the type of carbon source as no preference was seen between different carbon sources.

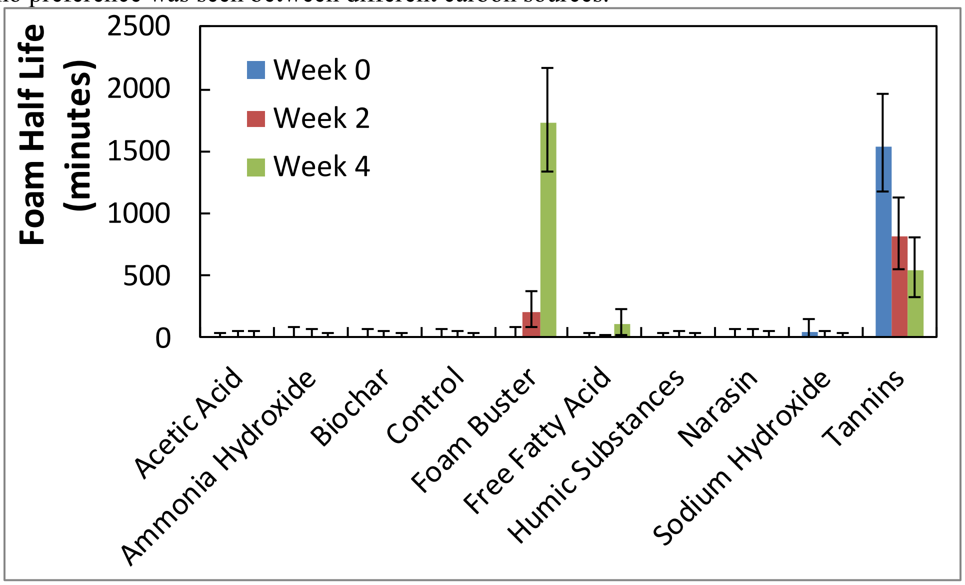


Figure 4. Foam-half life as a function of product addition and incubation time. Error bars represent a standard error of the mean.

In terms methane production rate, six of the treatments caused reduced methane production rates as compared to the controls upon treatment. These were the addition of water, sodium hydroxoid, inophore (Narasin), then the addition of acetate, foam buster, and humic substances. After two weeks of incubation only four products showed reduced methane production rates these were tannins, humic substances, and foam buster. Thus only the oil products appeared to inhibit methane production consistently. However, over the course of the incubation

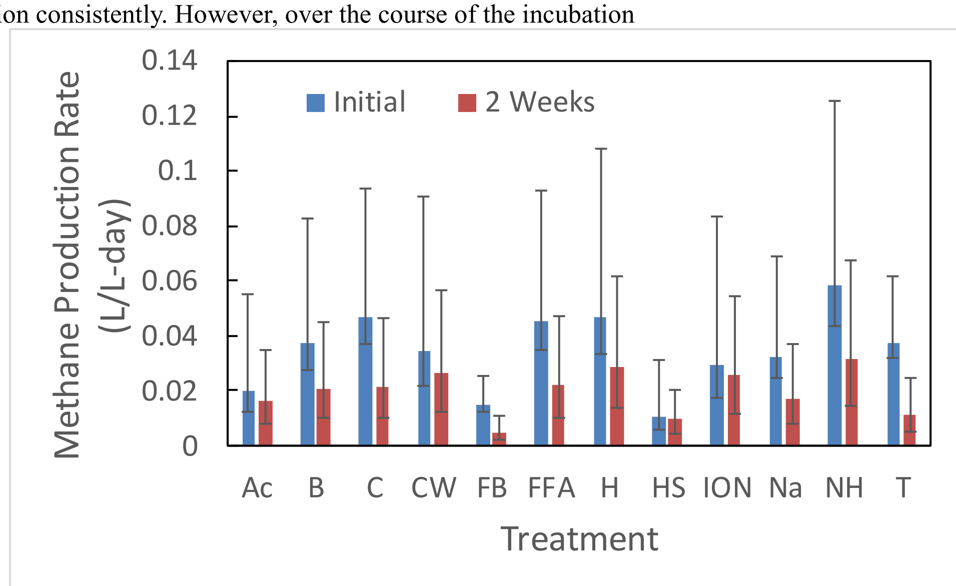
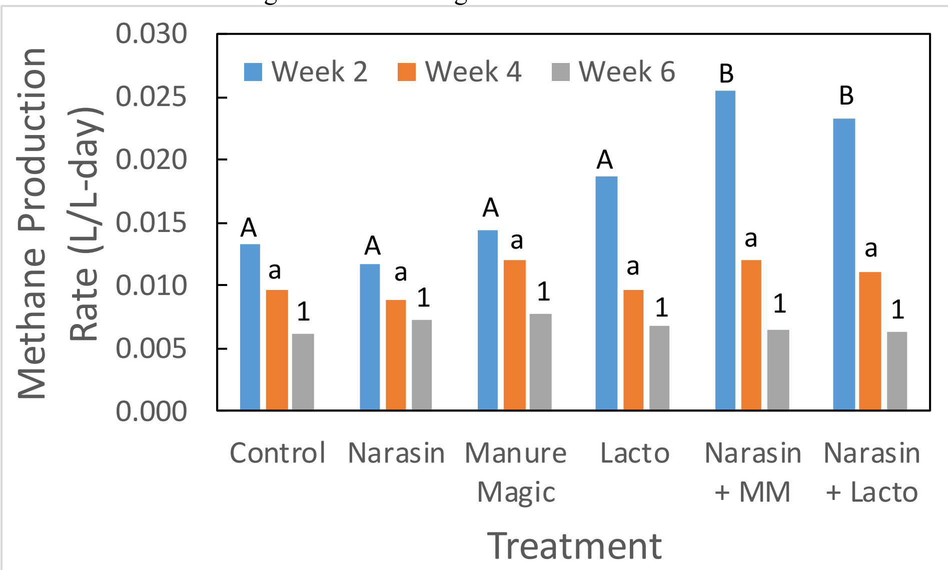
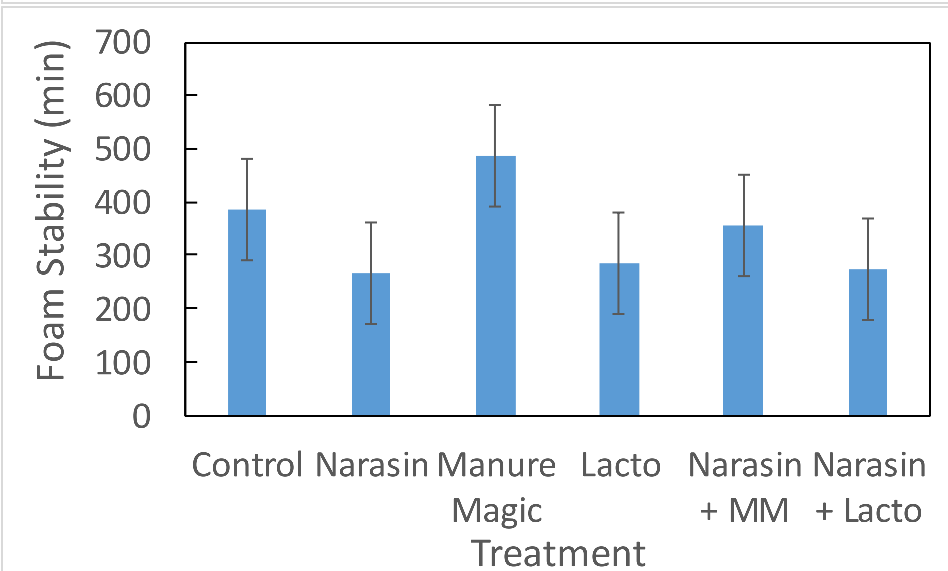


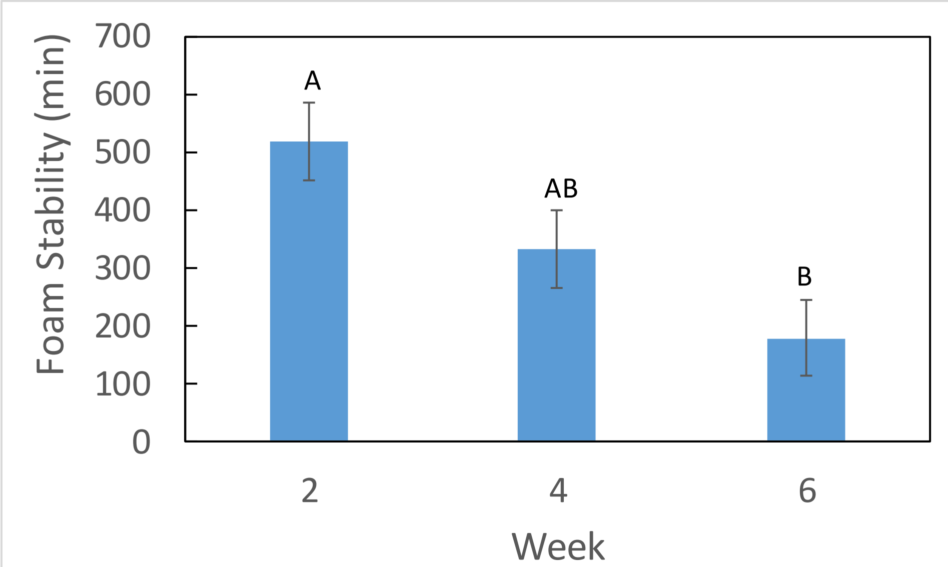
Figure 5. Methane production rate response to treatment, error bars represent the standard error of the mean.

Lab Incuation of Specific Foam Mitigation Products

Given the results of the first additive study, a previous Narasin treatment study reported by Reagen et al. (2015), an analysis of microbial community structure by Yang et al. (2017), a decision was made to do a more focused study on just three additives, Narasin, ManureMagic, lactobacillus, and then combinations of Narasin and ManureMagic and Narasin and lactobacillus. These treatments were choses to evaluate three different mechanisms. Narasin was added to treat methane production, ManureMagic was added to help break down proteins, lactobacillus was chosen it was one of the key microbial differences between foaming and non-foaming manure microbial communities.







Field Evaluation of Foam Mitigation Products

This data was taken from a facility that had a history of foaming, but at the beginning of the experiment there was no foam in the facility. We began collecting and testing samples on May 5th 2016 and continued taking samples about once every two weeks till May 12th 2017. We first witnessed an accumulation of foam on February 3rd 2017. From that time on we were able to see that the control pits definitely had a greater accumulation of foam than the treated pits with a maximum of 41cm on both of the control pits and a maximum of 10cm on both treated pits. Thus would seem to indicate that while the treatments were not able to completely eliminate the occurrence of foam, both reduced its severity (figure 8).

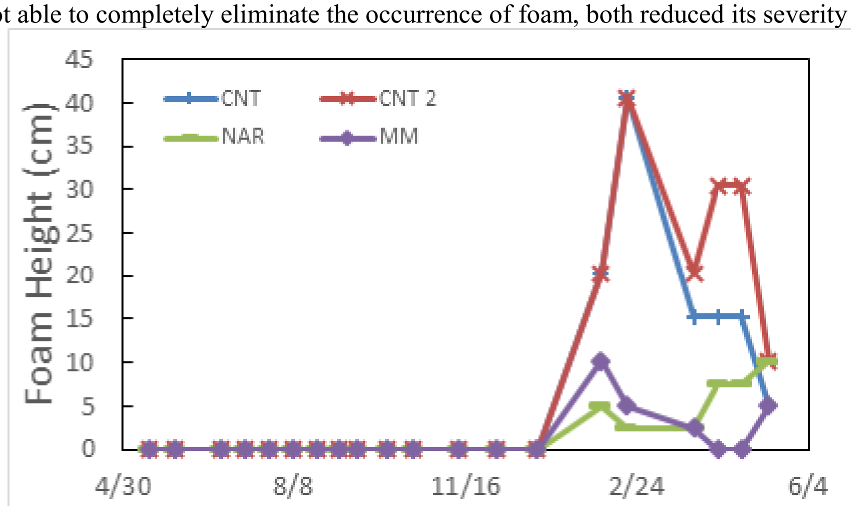
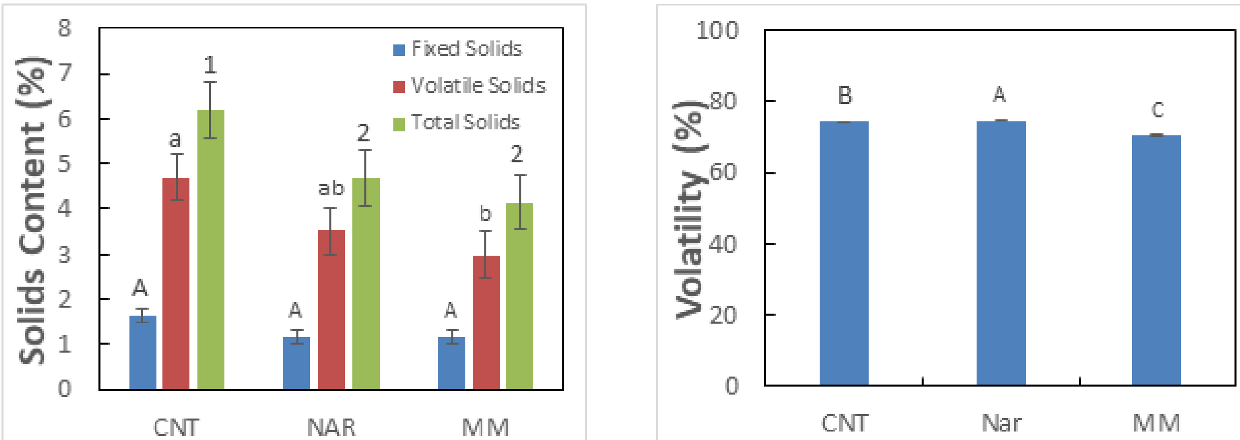


Figure 8. Foam height in the barn for control (CNT), Manure Magic (MM), and Narasin (NAR).

A statistical difference between total solids content of control pit (CNT=6.19%) and the two treated pits (NAR=4.69%, MM=4.15%) with no significant difference between the two treated pits was found. Data from Van Weelden et al. (2015) had previously indicated that foaming barns (at the surface) had higher solid content than non-foaming barns; however, they were unable to state whether this was caused by the foam suspending particles to the surface similar to what occurs in a dissolved air floatation system or the higher solids content was a driver of foam formation. Again we have a similar issue here but our data would suggest that the higher solids content of the control pits occurred prior to the occurrence of foam. As we had limited replication in this study (only control pit replicates) it is possible that the higher solids content was due to some factor other than treatment. To help better evaluate if this was the case we also looked at other solids characteristics such as the volatile solids (primarily organic matter) and fixed solids (primarily dissolved salts). The control pits had a significantly higher volatile solids content (CNT=4.70%) than the pit treated with Manure Magic (MM=2.98%) while the pit treated with Narasin (NAR=3.51%) was not statistically different than either pit. Although it was not statistically different we saw a higher percentage of fixed in the control pit (CNT=1.65%, NAR=1.18%, MM=1.17%). This difference could indicate that there was more dilution water entering the treated pits, but the lack of statistical significance would seem to indicate that the solids differences is primarily due to the treatment effects. That is, if these fixed solids are treated as a tracer of the manure the lack of differences between pits would suggest that differences in volatile solids and total solids, which can both be impacted by treatment, is presumably due to the actual treatments applied. If the fixed solids are used as a co-variate in the statistical analysis, as an effort to account for different dilution water entering each pit, the volatile solids contents of the manure would have been CNT=4.7%, NAR=4.9%, MM=4.2%. This trend would be more towards what was expected as the work of Reagen et al. (2014) suggested that treatment with Narasin reduced volatile solids destruction while the product Manure Magic is sold as an additive to remove volatile solids from the manure. Another way of visualizing this effect is to plot the volatility (percent of solids that are volatile). When viewed this way we find that the CNT = 74.11%, NAR= 74.53%, MM=70.44%) with all three being statistically different from each other. This would seem to indicate that Narasin reduced the destruction of volatile solids, while Manure Magic enhanced solids destruction.



(a) (b)

Figure 9. (a) Total Solids (TS) Volatile Solids (VS) and Fixed Solids, for control (CNT), Manure Magic (MM), and Narasin (NAR). (b) Volatility for control (CNT), Manure Magic (MM), and Narasin (NAR). Error bars represent the standard error of the mean (SEM). Different letters represent a statistical difference of α > 0.05.

The methane production rate (MPR) of the control pits (CNT = 0.10 L CH_4/L-day) was significantly higher than both treated pits (NAR = 0.075 L CH_4/L-day, MM = 0.0697 L CH_4/L-day). According to Van Weelden et al. (2015), the methane production rate was one of the biggest differences between foaming and non-foaming manures, with foaming manures averaging around 0.15 L CH_4/L-day and non-foaming manures around 0.05 L CH_4/L-day. In the case of Narasin treatment, the reduction was anticipated as that is what Reagen et al. (2014) found when she dosed Narasin into manure in a lab setting. The methane reduction when the manure was treated with Manure Magic was unexpected as this product is designed to enhance solids breakdown which presumably would mean conversion to carbon dioxide and methane for the destruction of organic carbon compounds, however, despite the fact that we found lower solids content in the manure we recorded lower methane production rates from this manure. In either case, it would appear that both treatments were effective in reducing the amount of methane produced by the manure.

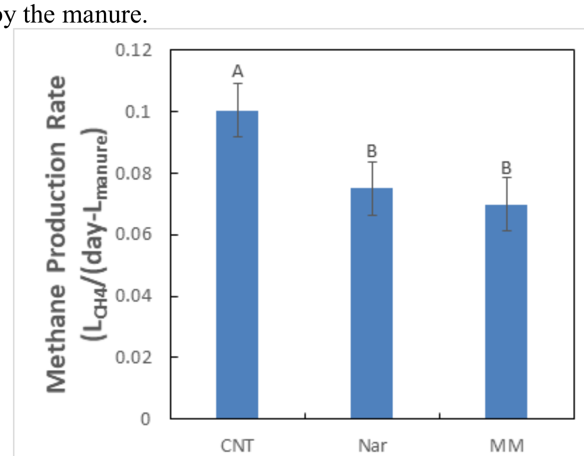
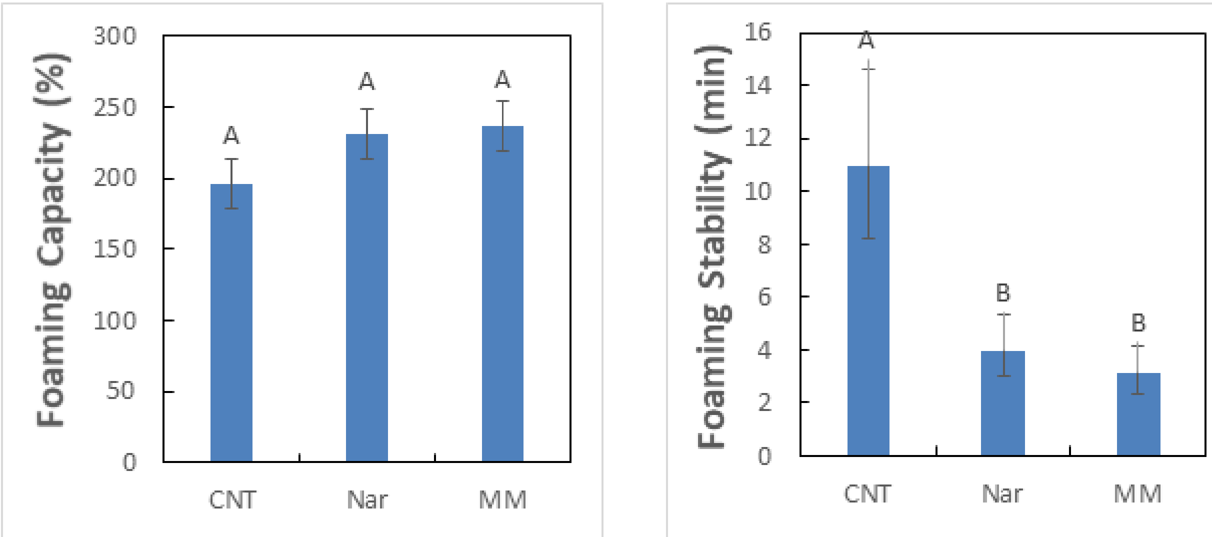


Figure 10. Methane Production Rate (MPR) for control (CNT), Manure Magic (MM), and Narasin (NAR). Error bars represent the standard error of the mean (SEM). Different letters represent a statistical difference of α > 0.05.

In terms of foaming capacity and stability, while Van Weelden et al. (2015) found differences between these parameters in foaming and non-foaming barns, they noted a clearer distinction for foam stability. Our results here would mirror that as no difference in foaming capacity of the control barn was found as compared to the treated barns. Moreover, the control manures trended lower (CNT=195.9%) than the two treated barns (NAR=231.0%, MM=236.5).

However, the control pits did have a significantly higher foaming stability (CNT=11.0min) than both treated pits (NAR=4.0min, MM=3.1min) however the Narasin treated pit and the Manure Magic treated pit did not have a statistical difference. It should be noted that while the stability shown in figure 11 b is relatively low at only 11 minutes, samples prior to foaming exhibited no stability, greatly reducing the overall average.



(a) (b)

Figure 11. (a) Foaming Capacity for control (CNT), Manure Magic (MM), and Narasin (NAR). (b) Foaming Stability for control (CNT), Manure Magic (MM), and Narasin (NAR). Error bars represent the standard error of the mean (SEM). Different letters represent a statistical difference of α > 0.05.

A correlation analysis was also used to evaluate if any of the parameters we measured were correlated. In this case, the methane production rate was not correlated to both the total or volatile solids content, but was correlated to higher foaming capacities. However, we did find that foam stability was correlated with higher solids content (both total and volatile solids) which supports the mechanism proposed in our introduction that some solid particles, presumably find solids are helping stabilize the foam.

Table 2. Correlation matrix for Foaming Capacity (FC), Foaming Stability (FS), Methane Production Rate (MPR), Total Solids (TS), and Volatile Solids (VS). Bold indicates a statistical correlation of p≤±0.05.

	FC	FS	MPR	TS	VS
FC		**-0.4058**	**-0.3894**	**-0.4311**	**-0.4237**
FS			0.1117	**0.4398**	**0.4018**
MPR				0.0849	0.0933
TS					**0.988**
VS					

As mentioned, microbial communities were also measured. At this time, we have measured microbial communities from the pits treated with Manure Magic and one of the control pits. Microbial communities were similar from the beginning of the study through October when the manure was agitated and pumped out. At that time, we saw a microbial community change, but the two pits remained similar. More product was added to the treated pit in December, at which point the microbial communities diverged (Figure 12), with the treated pit not foaming and the control pit foaming. Overall, this data meshes with the chemical and physical data we measured that showed differences in solids content, methane production rate, and foam stability between the treated and control manure pits.

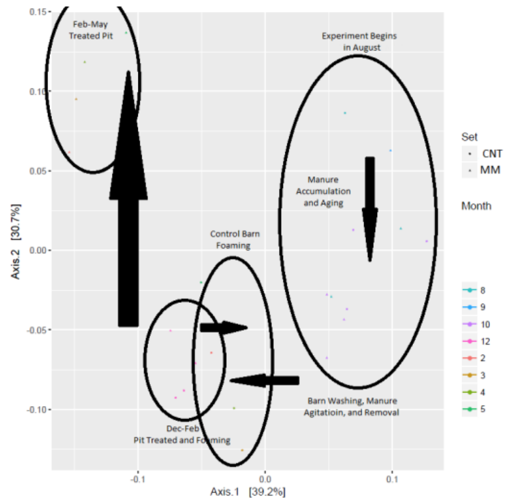
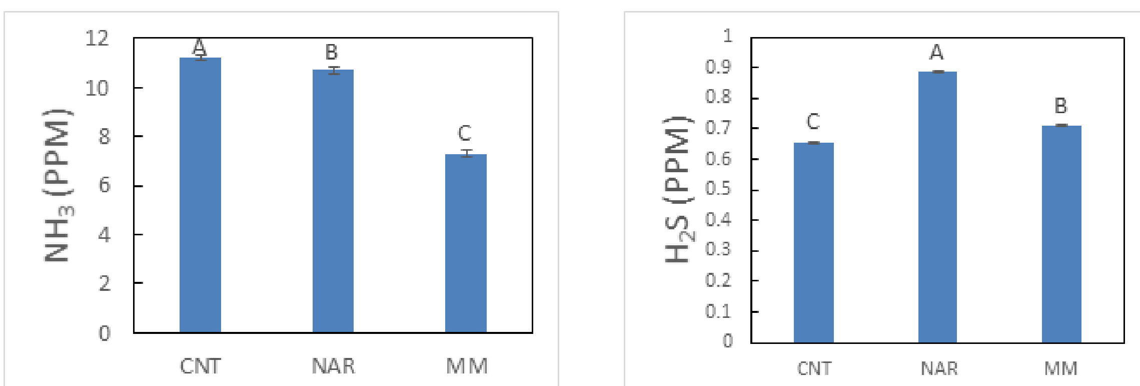


Figure 12. (a), Ammonia readings for control (CNT), Manure Magic (MM), and Narasin (NAR). (b) Hydrogen Sulfide readings for control (CNT), Manure Magic (MM), and Narasin (NAR). Error bars represent the standard error of the mean (SEM). Different letters represent a statistical difference of $p < 0.05$.

In addition to these foaming properties, we also monitored both ammonia and hydrogen sulfide concentrations in the air exhausted from the pit fans. In-barn air quality and barn emissions are of interest for animal health and barn odor. Air quality data was taken from the pit fans for the duration of the experiment (Fig. 13). This data shows that each pit was statistically different NH_3 concentration in the barn air (CNT=11.2PPM, NAR=10.7PPM, and MM=7.3PPM) and statistically different in H_2S concentration (CNT=0.65PPM, NAR=0.89PPM, MM=0.71PPM). In this case the hydrogen sulfide and ammonia concentrations are inverses of each other, which would seem to indicate these differences are most likely due to a pH effect, presumably that the control barns are more basic than either the Narasin or Manure Magic treated barns.



(a) **(b)**

Figure 13. (a), Ammonia readings for control (CNT), Manure Magic (MM), and Narasin (NAR). (b) Hydrogen Sulfide readings for control (CNT), Manure Magic (MM), and Narasin (NAR). Error bars represent the standard error of the mean (SEM). Different letters represent a statistical difference of $p < 0.05$.

Conclusions

Overall the results of these studies support the work of Van Weelden et al. (2015, 2016) on difference between foaming and non-foaming manures. The treatment practices evaluated here showed high variability in the lab setting, often giving some signs of potential foam reduction either via reduced foam stability or methane production rate, but typically never giving clear statistical differences, generally do to the low number of statistical replicates often used as well as the variability among replicates. Overall, we found that adding any type of foodstuff to foaming manure increased methane production rates and the added COD was quickly converted into methane, but we did not find any evidence for preference to a specific substrate. In our second study our wide assortment of treatment practices we failed to find a substance that consistently removed both methane production, foaming capacity, and foam stability. However, we did identify several substances that led to enhanced foam stability. Specifically, our work demonstrated that tannins instantly increased foam stability, presumably because these tannins were able to interact with proteins in the manure and were of the correct particle size to provide a stabilizing backbone to the foam structure, again fitting with what Van Weelden et al. (2015, 2015) had shown about foam. We also saw that the decomposition of oils could result in a similar natural phenomena of foam stabilization. In our final studies on Narasin, Manure Magic, and Lactobacillius, we saw indications of positive impacts of treatment reducing foaming characteristics at the lab scale, but it wasn't until we attempted this study at the field-scale that statically significant differences occurred. We found that treatment with Manure Magic reduced solids content, methane production rate, foam stability, and resulted in an altered microbial community, Treatment with Narasin resulted in greater solids content, but also reduced methane production rate, foam stability, and reduced the actual occurrence of foam on the manure.

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