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Narasin as a Manure Additive to Reduce Methane Production from Swine Manure

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Abstract. *Animal production systems are an important source of anthropogenic methane emissions. Production of methane results from microbial activity by anaerobic bacteria populations within the stored manure that breaks down organic material and converts it to biogas. Swine manures obtained from three deep pit storages in Central Iowa were dosed with Narasin, an ionophore, to evaluate its inhibitory effects on methane and biogas production. Four Narasin dosing rates were evaluated, these included 0 (Control), 7.5, 15, and 30 mg Narasin/kg of manure. Overall, the results indicated that Narasin had an inhibitory effect on methane and biogas production, with greater inhibition being seen at higher dosing rates. The inhibitory effect weakened with time such that after 120 days of incubation there was no statistical difference in cumulative methane production between samples dosed with Narasin and the control. Two additional treatments, based on the addition of an easily available carbohydrate, sugar, were also evaluated. Sugar (10 g per kg of manure) was added to manure both with (15 mg Narasin/kg) and without (0 mg Narasin/kg manure) Narasin amendment. The addition of sugar was performed to evaluate the impact an easily available substrate had on the inhibitory effects of Narasin. The results suggested that methane production was initially increased by the addition of sugar, but that the increased methane production lasted for less than 6 days, at which point cumulative methane production was similar to the control. When treated with both Narasin and sugar the inhibitory effect did not impact the gas production during the sugar digestion phase, but did result in reduced methane and biogas production thereafter. Overall the results indicated that Narasin can be an effective pit additive but further study is needed to recommend dosing frequency and to evaluate how the continuous addition of manure impacts Narasin effectiveness. Thus, this paper will describe a scaled up lab experiment that will be used to evaluate the effect of dosing frequency of Narasin to determine how producers could most effectively use it at the farm scale.*

Keywords. *Manure production, manure management, methane, biogas, swine production, Narasin.*

Introduction

An increased demand for animal protein has increased farm sizes (more acres per farm or animals per farm) as operations looked to improve their economy of scale, and an increased use of animal confinement facilities instead of pasture based production. These same trends have held true for the swine industry. Along with these improvements, there have been increased society demands for more sustainable production, especially

with regards to greenhouse gas mitigation. Traditionally, swine manure has been returned to the land as a fertilizer to support crop growth (Hatfield et al., 1998). In most instances, this practice continues to this day; however, due to the prevalence of corn and soybean production throughout the Midwestern United States, land application windows are often limited to either the spring prior to planting or after harvest in the fall. Thus, long-term storage (6-12 months) of manure is typically required on swine farms. In Iowa, swine finishing operations typically utilize deep-pits to store the manure between land application events. These deep-pits are located within the swine production building, beneath a slatted floor on which the pigs are raised. This allows the manure to fall through to the storage pit below, where it remains until field conditions are appropriate for manure application. Storing the manure within the building limits the opportunity for rainwater dilution of the manure, minimizing manure volumes and maintaining the fertilizer value. However, this long term storage can lead to ammonia and greenhouse gas emissions from the manure.

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 and capturing the emitted gas (Clemens and Ahlgrimm, 2001). Though different techniques have been developed for the mitigation of methane, there is still a demand for new, cost-effective technology which can aid in control. One approach that has received attention includes the use of dietary manipulation to increase digestibility of feedstuff, and as a result limit organic matter in the excrement, resulting in decreased potential for conversion to methane. Another approach based on diet manipulation, mostly tested in rumens, is the use of feed additives to decrease the fermentation of organic matter and inhibit methanogenesis (Johnson and Johnson, 1995; Benchaar et al., 2001).

Although not verified, similar approaches might have the potential to reduce gas production by adding a product directly to the 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 Rumensin as a pit additive to reduce foaming (Clanton, 2012). However, Rumensin has been reported to be toxic to pigs if consumed (lethal dose of approximately 16 mg/kg) so this is a risky proposition. Another ionosphere, Narasin, is safe for swine. Thus, the objective of this study was to evaluate if addition of Narasin to swine manure would reduce methane and biogas production from the manure and if so, what dosing rate of Narasin would be required to achieve reduction.

Material and Methods

Treatment Design

Manure samples were collected from three deep-pit swine finishing facilities in Central Iowa. At each site, a manure pump-out port was selected for sample collection. A manure sample was collected from the midpoint of the liquid manure level using a vacuum pump to collect the sample. Manure samples were brought back to the Agricultural Waste Management lab and tested for total solids, volatile solids, and pH. The manure from each site was then divided into eighteen portions of 100 grams each that were randomly assigned to six treatments. Three replicates of each manure by treatment combination used. Of the six treatments, four were utilized to evaluate the impact of Narasin dose level on methane and biogas production from the manure. The Narasin dosing rates were 0, 7.5, 15, and 30 mg Narasin per kg of manure. These dosing rates were selected based on using Narasin as a feed additive for swine; the labeled dosing rate in feed would equate to roughly 30 mg Narasin per kg of manure, but only about ½ of the Narasin activity is estimated to remain after digestion in the animal, giving an approximate activity in the manure of 15 mg Narasin per kg of manure. Doses of doubles and half this rate were also selected to provide a range of treatments for comparison.

Two additional treatments were also utilized; these were a sugar addition of ten grams sugar per kg of manure and a sugar addition of one gram sugar per kg of manure with a dose of 15 mg Narasin per kg of manure. This level of sugar addition was chosen to add approximately 20% more COD to the manure and to evaluate how Narasin effectiveness was impacted. The addition of these two treatments provides a factorial treatment structure when a subset of four of the six treatments are considered. In the factorial design, the treatments were Narasin rate (0, 15 mg/kg manure) and sugar addition (0, 10 g/kg manure), fully crossed with each other for each of the three manure types. These additional treatments were chosen to better elucidate the mechanism of Narasin in limiting methane production and to evaluate if Narasin was still effective when easily degradable substrate was present.

Measurement of Biogas and Methane Production

The 100 grams of manure was poured into a clear, 250 mL graduated serum bottle (Wheaton Science Products No.:223950) and then dosed with narasin and sugar as appropriate for that treatment. 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 septa (Sigma-Aldrich Z564729) was then placed on top of the bottle to seal it from the atmosphere and the time the bottle sealed recorded. The bottle was placed on a laboratory counter and incubated at room temperature for 130 days. 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. Biogas and methane production were assayed on days 3, 6, 10, 14, 19, 25, 32, 40, 47, 51, 63, 73, 83, 89, 96, 103, 109, 114, 121, and 130.

The biogas production in liters of gas generated per liter of manure is the amount of biogas produced, equal to the volume of gas measured, corrected to standard ambient temperature and pressure (25°C, 0.986 atm), from the syringe displacement method divided by the volume of manure used in the assay. Methane production was calculated similarly, but in this case the total mass of methane produced was corrected by adding the amount of biogas measured to the volume of the headspace in the bottle minus the methane left in the headspace of the bottle at the previous measurement. This correction was required as all of the methane produced during the incubation isn't extracted, i.e., some remains in the head space of the bottle. This value is multiplied by the methane content of the biogas and then divided by the volume of manure used in the incubation. At the conclusion of the incubation the manure was again assayed for pH, total solids, and volatile solids content so that changes, i.e., solids destruction, could be calculated.

Statistical Analysis

Statistical analysis was performed on cumulative methane and biogas production for days when biogas samples were collected. Analysis was performed in JMP Pro 10. The statistical model consisted of two terms: manure source which was utilized as a random variable and treatment which was treated as a fixed factor. Fisher's least significant difference was utilized as a means separation procedure. In addition contrast statements were utilized to provide a more detailed analysis of the results. Three contrast statements were utilized to analyze the factorial subset of treatments (narasin x sugar addition). The first contrast statement evaluated the impact of sugar by comparing the control and 15 mg Narasin/kg manure addition against the sugar addition and the sugar addition with 15 mg narasin/kg of manure. The second contrast statement evaluated the impact of narasin by comparing the control and sugar addition treatments to the 15 mg Narasin/kg manure addition and the sugar addition with 15 mg Narasin/kg of manure. Finally, the third contrast statement evaluated the impact of the Narasin x sugar addition interaction. This contrast compared the control and sugar addition with 15 mg Narasin/kg of manure against the sugar addition and the 15 mg Narasin/kg of manure addition. Similar statistical analysis was performed on the total solids, volatile solids, and pH of the manure.

Results and Discussion

Narasin Dosing Rate

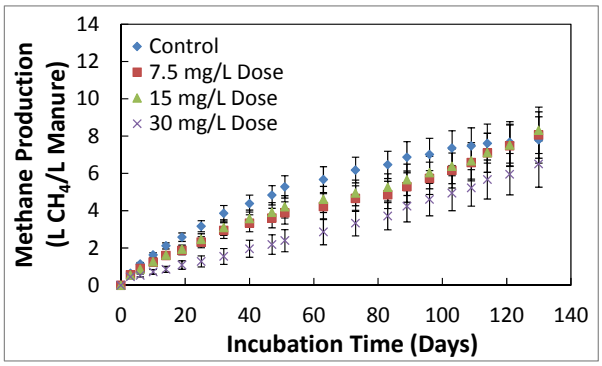
The manure samples collected represented a range of different manure conditions with farm A, B, and C having initial total (and volatile) solids concentrations of 8.36% (6.67%), 6.73% (5.27%), and 6.49% (5.25%) respectively. Based on our recent sampling of over 60 swine manure deep pits on production operations in Iowa, these solids concentrations are representative of deep-pit systems within Iowa (Van Weelden et al., 2013). The initial pH's of the samples were 7.86, 7.59, and 7.00 for farm A, B, and C respectively. At the time of collection, between 80 and 110 cm of manure were present and the manure temperature ranged between 12.6° and 15°C. Of these barns both barn A and B had foam present at the time of sample collection (20 cm and 33 cm respectively), while barn C had no foam present, but instead 0.6 cm of crust on the surface. However, all three barns did experience various degrees of foaming within the calendar year.

Despite the initial similarities to the manures all three produced significantly different amounts of methane over the 130 days of incubation. Farm A's manure produced 7.9 L CH_4/L of manure, farm B's manure produced 9.3 L CH_4/L manure, and farm C's manure generated 5.0 L CH_4/L of manure. This resulted in methane production of 117, 161, and 129 L CH_4 per kg of volatile solids for Farm A, B, and C respectively. These levels of methane

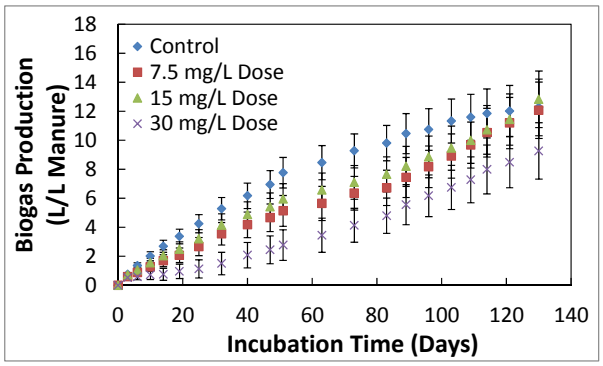
production are similar to the ultimate methane production potentials reported by Van Weelden et al. (2013) for similar manure samples based on their use of a biochemical methane potential assay and those reported by Moody et al. (2011) for swine manure obtained from a deep-pit storage system. This would seem to indicate that the majority of the methane generation potential of these samples had been exhausted during this incubation. Visual observation of the data (figure 1) would tend to support this for Farms A and C, as the methane production rates of the manures were slowing greatly near the end of the incubation; however, farm B had not yet shown an indication that methane production was slowing.

Biogas generation among the three manures was more similar with farm A and B not being statistically different from each other (11.9 and 13.7 L biogas/L manure respectively); however, farm C's manure had lower statistically lower biogas generation, producing 6.7 L biogas per L of manure. Temporal trends noted for methane held true for biogas as both farm A's and C's manure exhibited slowing rates, while farm B's manure again exhibited a linear pattern.

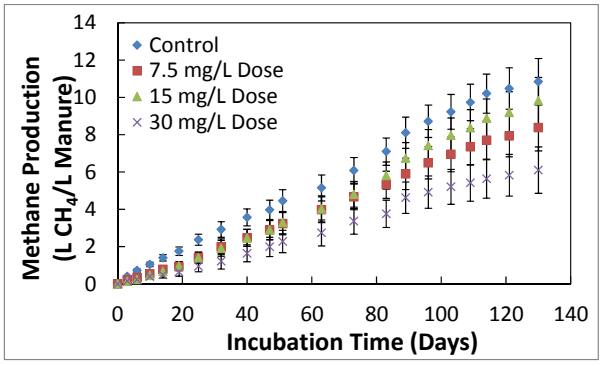
For all three manures, methane and biogas production were greatest for the control treatment, and reduced for all three Narasin dosing rates. The impact of Narasin was most pronounced at the beginning of the study, immediately impacting both methane and biogas production. As the study proceeded samples treated with Narasin began to produce methane at a rate faster than the control so that cumulative methane totals per L of manure became more similar near the end of the study. Similar trends were seen for biogas production. As all three manures had different methane production potential characteristics a method was needed to normalize the data so comparisons between sites could be performed. This was done by dividing the cumulative methane production of a sample treated with a Narasin dose by the average gas production of the control samples for that manure, effectively indicating what percent of the controls gas production the treatment sample had produced. When evaluated in this manure it was apparent that the rate of both methane and biogas production was impacted immediately with the addition of Narasin. The amount of this reduction appeared to be proportional to the dosing rate as the highest dosing rate exhibited the greatest reduction. Visually, the Narasin appeared to be very effective for approximately 25 days, at which point the rate of biogas production in the samples treated with the Narasin became greater than that of the control. Even though methane and biogas production rates started to recover in comparison to the control at around day 25, the reduction in cumulative methane and biogas remained significant until the end of the 130 day incubation period. Differences between Narasin dosing rates was less dramatic for biogas production as no significant difference between rates were detected at any point in the study.



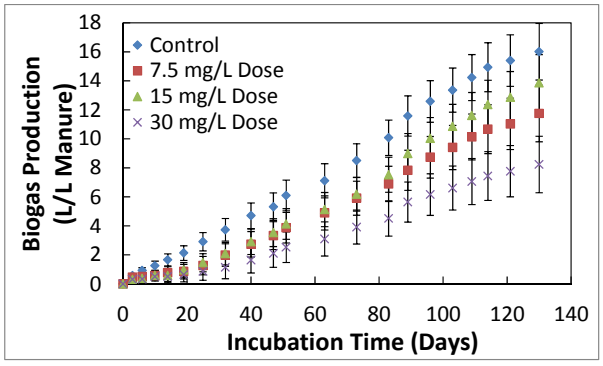
(a) Farm A – Methane



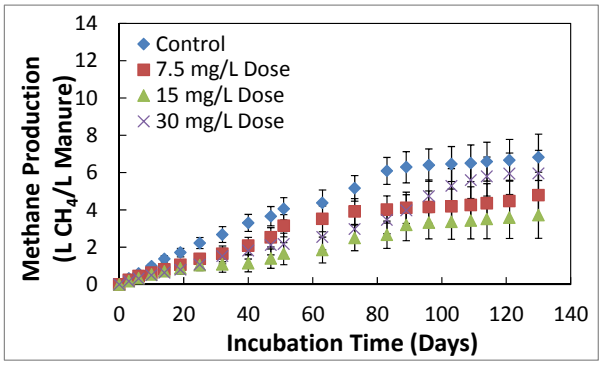
(b) Farm A - Biogas



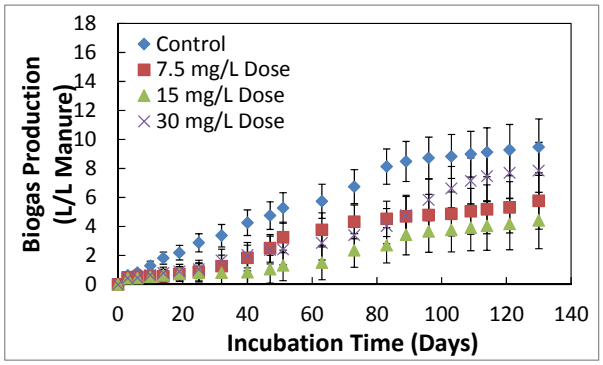
(c) Farm B - Methane



(d) Farm B - Biogas

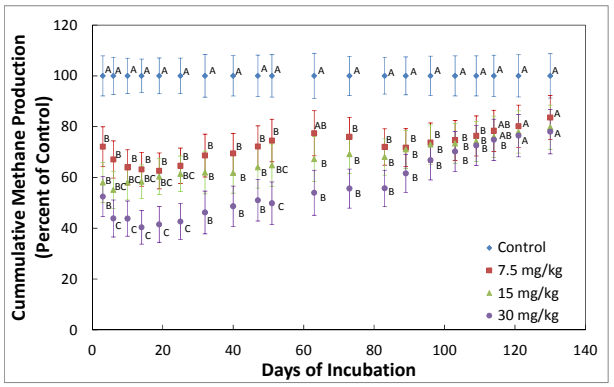


(e) Farm C - Methane

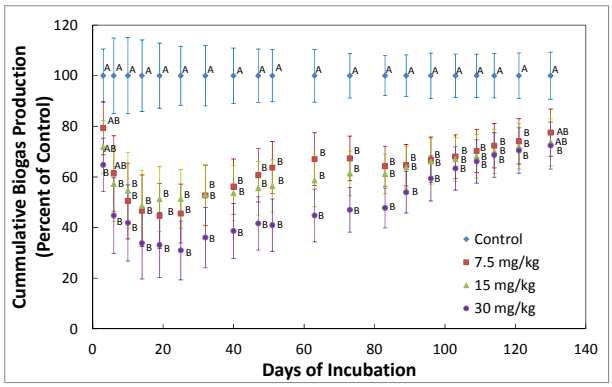


(f) Farm C -Biogas

Figure 1. Methane and biogas production of manure obtained from Farms A, B, and C and dosed with either no, 7.5, 15, and 30 mg/L of Narasin per kg of manure.



Normalized Methane



Normalized Biogas

Figure 2. Normalized methane and biogas production with different Narasin dosing rates.

Another way of looking at this data is as inhibition graphs similar to those that would be generated in an anaerobic toxicity assay (ATA). The ATA methodology was developed by Owen et al. (1979) to evaluate potential substrate toxicity. During the assay, toxicity is evaluated in terms of the ability of a given substance to inhibit methane production (ISO, 2003). The general concept of an ATA is to introduce increasing levels of a potential toxicant to multiple assays containing a standard amount of inoculum and a standard feedstock. The percent inhibition is then plotted against the concentration of the potential toxicant included in the incubation. In a typical ATA methodology this is done at one point-in-time, typically within the first two to four days after initiating the incubation and methane production rate (Moody et al., 2011). This is done as the methane production rate during this period is approximately constant as methane generation is substrate limited. This indicates how the microbial organisms are impacted by potential toxicants, but not their ability to overcome the inhibition. However, by repeating the analysis at various time intervals it may be possible to evaluate if the degree of inhibition is decreasing.

As the treatment structure utilized here is similar to that of an ATA, the data was analyzed in a manner similar to what would have been done for an ATA. The results indicated that at the early and middle stages of the incubation, Day 0 through Day 51, the amount of inhibition was related to the Narasin inclusion rate. Inhibition was approximately linearly related to Narasin dose over the range of concentrations evaluated. At the later stages of the incubation, Day 103 through Day 130, the samples dosed with higher levels of Narasin had overcome their inhibition to the point where there was no relationship between dosing rate and Narasin inclusion and all levels resulted in similar inhibition in comparison to the control. It should be noted that despite no longer showing a relationship between Narasin dose and the amount of inhibition, the Narasin treated samples had not yet recovered to such an extent that they produced as much methane as the control. Days 51 through 103 represented the time period of transition in this study, where the samples at higher dosing rates were overcoming their inhibition. This can be seen in figure 3 by decreases in the slope of the best fit line and by decreased levels of inhibition.

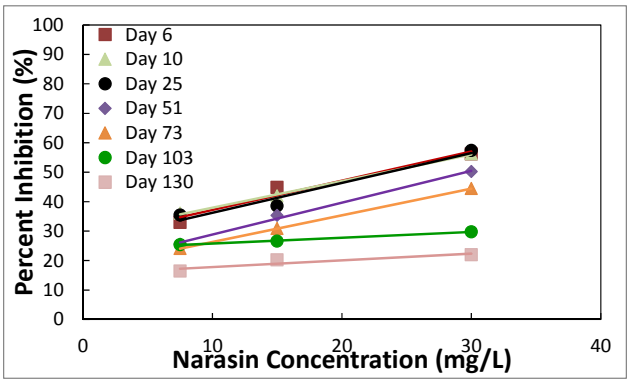
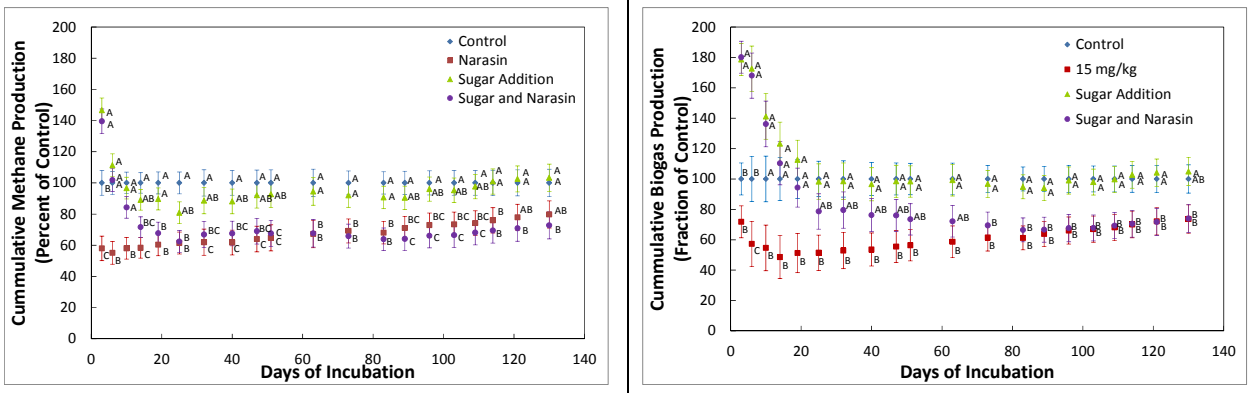


Figure 3. Percent inhibition as a function of Narasin inclusion at various times throughout the incubation.

Impact of Narasin and Sugar Addition

Now we are going to think about the four treatments that make up our factorial treatment structure. There were two factors being considered; sugar addition, Narasin addition, and the sugar x Narasin interaction. In general, sugar enhanced methane and biogas production at the beginning (before about 6 days for methane and 19 days for biogas) of the incubation, but the effect quickly disappeared, with cumulative methane production actually lagging behind the control and Narasin only treatments during the middle portion of the incubation. This lag had disappeared by the end of the 130 day incubation. Sugar addition caused a greater increase in biogas production than in methane production at the beginning of the incubation; this result was expected as due to its chemical composition sugar will result in biogas that is 50% methane and 50% carbon dioxide which is a lower methane content than the biogas generated from decomposition of organic material within the swine manure.

The results indicated that Narasin was effective at reducing both methane and biogas production from the manures; however, in this case one of the most interesting aspects of the results was that at the beginning of the experiment (through day 10) a significant ($p < 0.05$) interaction between sugar and Narasin additions was noted. This interaction was caused by Narasin being unable to reduce methane or biogas production during the sugar degradation portion of the incubation, but being effective when applied to the manure without sugar addition. After the impact of the sugar addition on methane and biogas production had subsided, the impact of Narasin quickly became effective on cumulative methane and biogas production. These results would seem to indicate that Narasin, which is an anti-microbial agent, does not impact microbial populations that control biogas and methane production from sugar, but interferes with microbial pathways involved in the conversion of organic material within the manure into methane.



(a) Normalized Methane | **(b) Normalized Biogas**

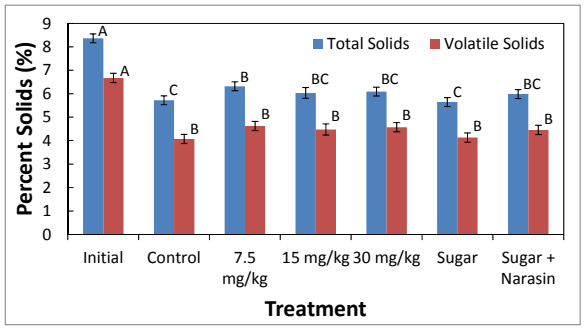
Figure 4.Normalized methane (a) and biogas (b) production from manures receiving the sugar and Narasin additions.

Impact on Total and Volatile Solids Destruction

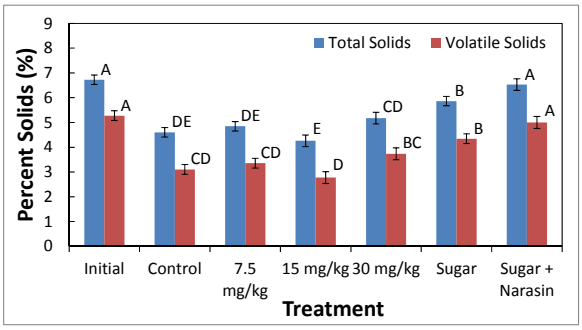
The impact that Narasin addition had on total and volatile solids concentrations was evaluated in two ways. The first was to compare total and volatiles solids concentration of the various treatments after the manure had been incubated for 130 days and the prior to incubation concentrations. In general, concentrations of both total and volatile solids were significantly reduced during the incubation (figure 5), but relatively little difference was seen between treatments. In general all treatments were similar, but the sugar with Narasin addition did tend to be slightly higher, significantly so in the case of Farm B. In general, differences between treatments were most pronounced in this manure. This might have been due to the fact that it had not yet begun to exhibit a plateau to its methane production rate, indicating the greatest amount of methane production potential remaining. The other manures didn't exhibit these differences in a nearly as pronounced manner possibly due to the greater overall decomposition of the other manure samples. This could indicate that these differences between treatments were transient, and that given sufficient incubation time differences between treatments would have been decrease as methane and biogas production became more similar. Frequent sampling throughout the incubation for solids concentrations would be required to verify if these transient differences exist.

An alternative way of evaluating the impact of Narasin addition on solids is by calculating solids destruction (both total and volatile) over the course of the assay. In making this comparison the change the change in solids concentration was divided by the initial solids concentration to determine destruction. In the case when sugar was added, initial solids content was adjusted to account for the amount of sugar added. This amount

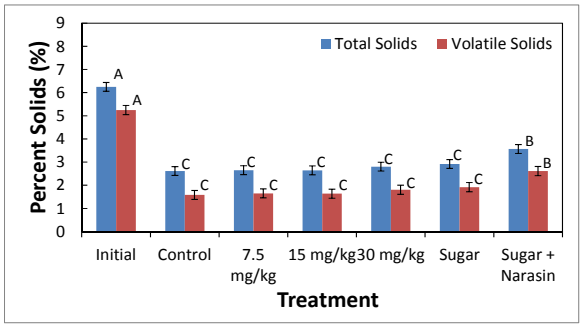
was also added to the amount destroyed as only the amount in the manure originally and what remained post incubation was assayed. Solids destruction was then analyzed by blocking by site and comparing treatments. In general, the results indicated solids destruction was greater in samples receiving sugar addition (figure 6). This would be expected as the added sugar is an easily degradable substrate and as such should be broken down during the incubation. The impact or Narasin was less clear, but in general solids destruction decreased with increasing rate of Narasin addition. To better evaluate these effects total and volatile solids destruction was regressed against Narasin dosing rate. The results of this indicated that total solids destruction decreased by 0.19% for every additional mg of Narasin added to a kg of manure. The result was slightly more dramatic for volatile solids as its destruction decreased by 0.26% for ever additional mg of Narasin added to a kilogram of manure. These changes are actually relatively small as they indicate that on average volatile solids destruction would be reduced by about 8% when dosing manure at 30 mg Narasin/kg of manure and while total solids would destruction would be reduced by about 6%. In practice this deference would result in less than about 1% difference in solids concentration differences between manure treated with and not treated with Narasin.



(a) F104



(b) F107



(c) F237

Figure 5. Total and volatile solids concentrations prior to and at the conclusion of the 130 incubation with various treatments for (a) Farm A, (b) Farm B, and (c) Farm C. Treatments within a manure source not sharing the same letter were significantly different at α = 0.05.

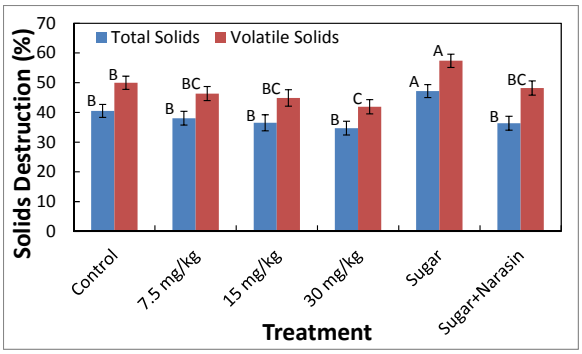


Figure 6. Total and volatile solids destruction for each of the six treatments evaluated.

Future Work: Determining Narasin Addition Frequency with Deep Pit Simulator

Since it was shown in the previous experiment that Narasin can be effective at reducing methane production when added to manure, it became evident that a larger scale study was necessary to determine how producers could use it in an actual deep pit. The previous study indicated that Narasin lost effectiveness after 45 days, so more frequent dosing could be beneficial in reducing methane production. Thus, an experiment will be performed by using lab-scale deep-pit simulators with a semi-continuous input of swine manure to evaluate the impact of Narasin on methane and biogas production from the manure. Manure will be added to a sealed PVC tube, under anaerobic conditions, and gas production will be continuously measured using an inverted tipping bucket, similar to the set up shown in Figure 7. Methane analysis of the biogas will be performed weekly. All of the simulators will start with about ¼ of the total simulator volume filled with manure and then manure will be added twice per week at a rate that is consistent with actual swine production, proportional to the volume of the simulator. Different rates and dosing frequencies of Narasin will be used in the simulators and compared to a control treatment to evaluate if Narasin is effective at slowing the rate of methane production. Narasin treatments will be designed so that the amount of Narasin added will equal 15 mg Narasin per L manure when the simulator is at its' final volume of 4 L. Three replications of four treatments will be present: control (no Narasin added); 60 mg Narasin added at the beginning of the trial; 30 mg Narasin added at the beginning of the trial then a second dose of 30 mg Narasin when the manure volume reaches 2 L;, and a volume of Narasin added with manure weekly so that the total amount of Narasin when the simulator is full is 60 mg. Each trial will last longer than, in order to see if Narasin loses its effectiveness after that time, as was shown in the previous experiment. Biogas production volume will be analyzed from each treatment to determine if Narasin dosing causes a difference in gas production. Also, initial and final total and volatile solids will be measured. The results will help producers in determining if Narasin can be effective at reducing foaming and provide recommendations on the most effective dosing frequency needed to reduce methane emissions.

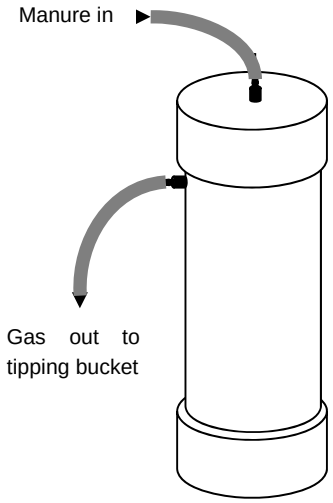


Figure 7. Diagram of deep pit simulator set up.

Conclusions

The results indicated that Narasin had an inhibitory effect on methane and biogas production, with greater

inhibition being seen at higher dosing rates. The inhibitory effect weakened with time such that after 120 days of incubation there was no statistical difference in cumulative methane production between samples dosed with Narasin and the control. Two additional treatments, based on the addition of an easily available carbohydrate, sugar, were also evaluated. Sugar (10 g per kg of manure) was added to manure both with (15 mg Narasin/kg) and without (0 mg Narasin/kg manure) Narasin amendment. The addition of sugar was performed to evaluate the impact an easily available substrate had on the inhibitory effects of Narasin. The results suggested that methane production was initially increased by the addition of sugar, but that the increased methane production lasted for less than 6 days, at which point cumulative methane production was similar to the control. When treated with both Narasin and sugar, the inhibitory effect did not impact the gas production during the sugar digestion phase, but did result in reduced methane and biogas production thereafter. Results also indicated that treating manures with Narasin resulted in reduced total and volatile solids destruction, with total and volatile solids destruction decreasing by 0.19% and 0.26% respectively for every incremental increase in Narasin dosing rate (mg Narasin/kg of manure). Overall the results indicated that Narasin can be an effective pit additive but further study is needed to recommend dosing frequency and to evaluate how the continuous addition of manure impacts Narasin effectiveness.

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