Impact of narasin on manure composition, microbial ecology, and gas emissions from finishing pigs fed either a corn-soybean meal or a corn-soybean meal-dried distillers grains with solubles diets

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ABSTRACT: An experiment was conducted to determine the effect of feeding finishing pigs a corn-soybean (CSBM) diet or a CSBM diet supplemented with 30% dried distillers grains with solubles (DDGS), in combination with or without a growth-promoting ionophore (0 or 30 mg narasin/kg of diet), has on manure composition, microbial ecology, and gas emissions. Two separate groups of 24 gilts (initial BW = 145.1 kg, SD = 7.8 kg) were allotted to individual metabolism crates that allowed for total but separate collection of feces and urine during the 48-d collection period. After each of the twice-daily feedings, feces and urine from each crate was collected and added to its assigned enclosed manure storage tank. Each tank contained an individual fan system that pulled a constant stream of air over the manure surface for 2 wk prior to air (day 52) and manure sampling (day 53). After manure sampling, the manure in the tanks was dumped and the tanks cleaned for the second group of pigs. Except for total manure Ca and P output as a percent of intake and for manure methane product rate and biochemical methane potential ($P \leq 0.08$), there were no interactions between diet composition and narasin supplementation. Narasin supplementation resulted in increased

manure C ($P = 0.05$), increased manure DM, C, S, Ca, and phosphorus as a percent of animal intake ($P \leq 0.07$), and increased manure volatile solids and foaming capacity ($P \leq 0.09$). No effect of narasin supplementation was noted on manure VFA concentrations or any of the gas emission parameters measured ($P \geq 0.29$). In contrast, feeding finishing pigs a diet containing DDGS dramatically affected manure composition as indicated by increased concentration of DM, C, ammonia, N, and total and volatile solids ($P = 0.01$), increased manure DM, N, and C as a percent of animal intake ($P = 0.01$), increased manure total VFA and phenols ($P \leq 0.05$), decreased gas emissions of ammonia and volatile sulfur compounds (VSC; $P = 0.01$), increased emissions of phenols and indoles ($P \leq 0.06$), decreased methane production rate ($P = 0.01$), and slight differences in microbial ecology ($R \leq 0.47$). In conclusion, feeding a diet which contains an elevated level of indigestible fiber (i.e., DDGS) resulted in more fiber in the manure which therefore dramatically affected manure composition, gas emissions, and microbial ecology, while narasin supplementation to the diet did not exhibit a significant effect on any of these parameters in the resultant swine manure.

Key words: gas emissions, ionophore, manure composition, microbial ecology, pigs

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INTRODUCTION

Ionophores have been shown to improve growth performance in pigs fed corn-soybean meal-based or fiber-rich diets (Lindemann et al., 1985; Merrill

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et al., 1995; Arkfeld et al., 2015). Ionophores have a higher affinity toward Gram-positive bacteria, fungi, and coccidia, bacteria which tend to produce lactate, butyrate, and formate in the hind gut (Miyazaki et al., 1974; Schelling, 1984; Russell, 1987). Thus feeding an ionophore may result in increased propionate or succinate as fermentation products resulting in a greater energetic efficiency in the animal (Weuthrich et al., 1998). Indeed, ionophores have been shown to affect hind gut bacterial fermentation (Holtzgraefe et al., 1985a, 1985b; Marounek et al., 1997) and N digestibility (Thacker et al., 1992; Wuethrich et al., 1998). If ionophores are not fully digested or degraded in the hind gut of the pig, they may also have residual effects on bacterial populations and fermentative processes in swine manure, resulting in a reduction in methane production. With a renewed interest in swine manure composition and gas production due to the incidence of pit foaming and flash fires (Andersen et al., 2015; Van Weelden et al., 2015, 2016a), there is interest on how diet composition (Le et al., 2005; Kerr et al., 2006; Ziemer et al., 2009; Van Weelden et al., 2016b) and feed additives may reduce incidences of these occurrences. While there are no known specific microorganisms solely responsible for pit gas production and foaming, adding ionophores to deep pit manure has been shown in vitro to modulate both microbial ecology and methane production (Cotta et al., 2003; Whitehead and Cotta, 2007; Andersen and Regan, 2014). The objectives of this study were to evaluate the potential interactive effect of diet type and narasin, an approved ionophore for swine growth promotion, on manure composition, microbial ecology, and gas emission.

MATERIALS AND METHODS

The experiment was approved by the Iowa State University Animal Care and Use Committee.

Diets and Experimental Design

An experiment was conducted using 2 groups of 24 gilts (Cam borough 22 sows $\times$ L337 boars, Pig Improvement Company, Hendersonville, TN; initial BW = 145.1 kg, SD = 7.8 kg) which were randomly allotted to individual metabolism crates that allowed for total but separate 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

21 °C, and lighting was provided continuously. Gilts were fed twice daily (0700 and 1900 h) an amount of feed that approximated 3% of their BW, which is considered near full feed, with actual feed intake (feed offered less feed not consumed) utilized for all calculations.

Gilts were fed treatment diets in a 2×2 factorial arrangement, with the 2 diet types consisting of either a low fiber, corn-soybean meal (CSBM) or a high fiber diet containing 30.34% dried distillers grains with solubles (DDGS), each formulated to meet or exceed the AA and mineral needs according to the NRC (2012) recommendations; in combination without (0 g/t) or with 27.2 g/t of narasin (Skycis 100, Elanco Animal Health, Indianapolis, IN), Table 1. Each group of gilts was fed for a 48-d period. Daily, after each of the twice-daily feedings, feces and urine from each metabolism crate was collected and added to its assigned enclosed manure storage tank (1 crate assigned to its corresponding storage tank). Each stainless steel manure storage tank measured 61 cm high and was 96.5 cm in diameter which resulted in a surface area of 0.73 m² which is approximately the allotted amount of surface area for 1 pig in a swine finishing barn. The lid on each tank was fitted with threaded couplers to accommodate fittings and piping from which to add manure, allow air flow (room air inlet, air fan, and PVC exhaust air outlet), and take air samples. Each tank contained a fan that pulled a constant stream of room air over the manure surface (approximately 2.95 m³/min) and exhausted this air outside the building for 2 wk prior to when air samples were obtained (day 52), and prior to manure sampling (day 53) so as not to agitate the manure and inflate air emissions simulating what would occur from manure stored at swine production facilities. At the end of the animal feeding and feces and urine collection time period, animals were removed from the room, the room cleaned with water, and the shallow pit under the slats in the room drained (from room washing water) to reduce background gases from these sources within the room. The tank manure was then allowed to sit for 2 d (no manure added and no agitation) before manure-tank air sampling. Following air sampling, manure samples for analysis were obtained after mixing each tank with a polyvinyl paddle to obtain a homogenous manure sample. After manure sampling, the manure in the tanks was dumped and the tanks cleaned for the second group of pigs. Additional detail on the effects of diet and narasin on energy, nutrient, mineral, and fiber digestibility are presented elsewhere (Kerr et al., 2017).

Table 1. Experimental diet formulation, as-is basis

Ingredient, %	CSBM ^a	DDGS ^a
Corn	82.02	66.70
Soybean meal, 46.5% CP	15.60	–
DDGS	–	30.34
Soybean oil	–	0.32
Monocalcium phosphate	0.74	0.38
Limestone	0.83	1.09
Sodium chloride	0.35	0.35
Vitamin mix ^b	0.15	0.15
Trace mineral mix ^c	0.20	0.20
L-lysine·HCl	0.11	0.46
L-threonine	–	0.07
L-tryptophan	–	0.04
Calculated composition, %		
NE, kcal/kg	2,516	2,516
CP, %	14.3	14.3
sidLys, % ^d	0.65	0.65
Ca, %	0.48	0.48
P, %	0.45	0.41
sttdP, %	0.23	0.23
S, %	0.17	0.30
NDF, %	8.7	17.5
Analyzed composition, %		
CP, %	13.8	13.8
C, %	38.9	40.5
Ca, %	0.54	0.50
P, %	0.46	0.47
S, %	0.19	0.26
NDF, %	6.7	12.8

^aCSBM, corn-soybean meal-based diet; DDGS, dried distillers grains with solubles. Narasin treatment diets (30 mg/kg) were created by adding 0.03% Skycis-100 premix (Elanco Animal Health, Indianapolis, IN) to diets at the expense of corn.

^bProvided per kilogram of complete diet: 4,594 IU of vitamin A; 525 IU of vitamin D; 37.5 IU of vitamin E; 2.3 mg of vitamin K; 42 mg of niacin; 20.3 mg of pantothenic acid; 8.3 mg of riboflavin; 0.038 mg of vitamin B₁₂.

^cProvided per kilogram of complete diet: Zn, 220 mg as ZnSO₄; Fe, 220 mg as FeSO₄; Mn, 52 mg as MnSO₄; Cu, 22 mg as CuSO₄; I, 0.4 mg as Ca(IO₃)₂; and Se, 0.3 mg as Na₂SeO₃.

^dDiets formulated to a minimum of 0.58 TSAA:Lys, 0.65 Thr:Lys, 0.175 Trp:Lys, 0.53 Ile:Lys, 0.68 Val:Lys.

Manure Analyses

Manure volume was measured using the depth of manure in each tank at the time of sampling, manure temperature was measured in situ prior to agitation using a thermocouple thermometer (Fluke 51-Series II, Fluke Corp., Everett, WA), and manure pH from the stirred manure sample using a pH meter (Corning Model 530, probe #476436, Corning Inc., Corning, NY). Manure total ammoniacal nitrogen (TAN) was analyzed using an ammonium probe (Thermo Orion Meter 290A+, probe #9512) that was previously described in

Trabue and Kerr (2014). In brief, 3 g of manure was weighed into a 100-mL beaker, after which 99 mL of nanopure water and a stir bar were added. While mixing, 2 mL of ionic strength adjuster solution (Orion 951211, Thermo Fisher Scientific Inc.) were added. The pH of the mixture was maintained at ≥11 in order for the probe to accurately measure TAN. The probe was inserted into the beaker, and the concentration was recorded. For manure sulfide, an S²⁻ probe (Thermo Orion Meter 290A+, probe #9616) was used to quantify sulfide levels as previously described in Trabue and Kerr (2014). In brief, 2 g manure was weighed into a 100-mL beaker after which 38 mL of deaerated nanopure water, 40 mL of SAOB solution (Thermo Fisher Scientific Inc.), and a stir bar were added. The calibrated probe was inserted into each beaker and the concentration recorded. Manure DM was obtained by collecting 5 liters of manure and drying at 75 °C in a forced air oven and dried to a constant dryness. Manure C, N, and S were analyzed by thermocombustion (VarioMax, Elementar Analysensysteme GmbH, Hanau, Germany) while manure Ca and phosphorus (PHOS) were analyzed by digesting the sample with concentrated nitric acid following method (II)A (Analytical Methods Committee, 1960), with the residue dissolved in 1 N HCl followed by inductively coupled plasma spectrometry (Optima 5300DV, PerkinElmer, Shelton, CT).

Manure VFA (acetate, propionate, butyrate, isobutyrate, isovalerate, valerate, isocaproic, caproic, and heptanoic), phenols (phenol, cresol, ethylphenol, and propylphenol), and indoles (indole and skatol) were analyzed as previously described in Weber et al. (2010). In brief, 4 g of manure were placed into a 15-mL polypropylene centrifuge tube and centrifuged. One milliliter of supernatant was removed and added to a 20-mL headspace vial in which it was acidified (145 mL of *o*-phosphoric acid, target pH of 2.0 to 2.5) and salted (0.3 g of NaCl) before being sealed with a screw-on septum cap. Extracts were analyzed as previously described in Trabue et al. (2016b). In brief, extracts were stirred and incubated at 70 °C for 15 min on a robotic autosampler (MPS2, Gerstel, Inc.). After the incubation period, headspace gas concentrations were sampled using solid phase microextraction fibers (Millipore Sigma, St. Louis, MO) exposed to vial headspace contents for 5 min. Fibers were desorbed at 230 °C for 300 s in the GC system inlet (Model 7980, Agilent Technologies, Inc.) equipped with a flame ionization detector and a FFAP column (30 m × 0.25 mm × 0.25 mm; Agilent Technologies, Inc.). The gas chromatography parameters were as follows: splitless mode; inlet

temperature, 230 °C; inlet pressure, 24.56 psi; septum purge flow, 30 mL/min; constant column flow 1 mL/min (helium); and detector temperature, 300 °C. The gas chromatography oven temperature program was initial temperature C/min, 100 °C, 2 min hold; ramp of 10 °C min⁻¹ to the final temperature of 240 °C, hold for 2 min.

Air Analyses

Concentrations of NH₃, CH₄, CO₂, N₂O, and H₂S were measured from room air (ambient concentrations) and from exhaust air to obtain a net gas concentration for each manure storage tank (24 total) using a photoacoustic multigas analyzer (Model 1312, INNOVA AirTech Instruments A/S) and an H₂S analyzer (API Model 101E, Teledyne Technologies, Inc.), respectively. Over a 3-d period each tank was sampled once a day for 15 to 30 min depending on stability of NH₃ gas measurement. Background room measurements were taken at the beginning, middle, and end of each day. Tank gas concentrations were corrected for background room concentrations by subtracting tank concentration from room air. Instrument operation details were previously discussed in [Trabue and Kerr \(2014\)](#).

Gas concentrations of odorous volatile organic compounds (VOC) were taken from room air (ambient concentrations) and exhaust air to obtain a net gas concentration from each manure storage tank (24 total). Air samples were collected on sorbent tubes for thermal desorption (TDS) gas chromatography analysis as detailed in [Trabue et al. \(2010\)](#). In brief, air samples were collected on sorbent tubes at 100 mL/min for approximately 12 liters using individual personal gas samplers (Models 220 or AirCheck 2000, SKC, Inc.). Sorbent tubes were analyzed by TDS (model TDSA, Gerstel, Inc.) using a gas chromatography system (model 6890N GC, Agilent Technologies, Inc.) equipped with a mass spectrometer detector (5973N Inert MSD, Agilent Technologies, Inc.). The TDS/gas chromatograph/mass spectrometer system was equipped with a programmed temperature vaporizer inlet (CIS 4, Gerstel, Inc.) and 30 m × 0.25 mm × 0.25 mm FFA phase column (J&W Scientific, Inc.). The TDS, programmed temperature vaporizer inlet, and gas chromatograph oven program were previously described in [Trabue et al. \(2010\)](#). The mass spectrometer was operated in selective ion monitoring/scan mode. Compounds were identified using mass spectra and retention times of reference standards. External standard curves were used for quantitation of samples.

Volatile sulfur compounds concentrations were taken from room air (ambient concentrations) and exhaust air to obtain a net gas concentration from each manure storage tank (24 total). Air samples were collected as grab samples (less than 10 min) in glass evacuated canisters with analytical details reported in [Trabue et al. \(2008, 2010\)](#). Analysis of canisters was performed using a canister system (Entech Instrument, Inc., Simi Valley, CA) that was coupled to a GC system (Agilent 6890N, Agilent Technologies, Inc.). The GC was equipped with gas separation column (30 m × 0.32 mm × 0.25 µm) (GS-Gaspro, Agilent Technologies, Inc.) using helium gas at 0.7 mL/min constant flow, and equipped with a mass spec detector (5973 Inert MSD, Agilent Technologies, Inc.). For determining gas concentrations of the target VSC the following selected ions were monitored: 34 (hydrogen sulfide); 48 (methanethiol); 60 (carbonyl sulfide); 76 (dimethyl sulfide); 62 (dimethyl sulfide); and 94 (dimethyl disulfide). External standard curves were used for quantitation of samples. Odor strength was determined by an odor activity value (OAV, defined as the concentration of odorant in air/odor threshold of the odorant) based on compound or class of compounds rather than concentrations alone ([Hales et al., 2012](#); [Parker et al., 2012](#); [Wu et al., 2015](#)).

Measurement of Biogas and Methane Production

Total and volatile solids (TS and VS, respectively) 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, approximately 30 mL of well-mixed sample was poured into a pre-weighed porcelain dish and mass recorded. The crucible was oven dried at 104 °C for 24 h, cooled in a desiccator, and weighed again for dry weight. The sample was subsequently heated in a muffle furnace at 550 °C for 12 h, cooling in a desiccator, and weighed to determine the ash content. Total and volatile solids were reported as a percentage of original mass.

Methane production rate (MPR) was measured to provide a short-term gas production rate which indicates a rate at which bacteria already present in the manure can produce methane. Biochemical methane potential (BMP) of the manure was also measured to estimate the total volume of methane that microbes in the manure were able to produce to mimic long-term incubation of manure that occurs in a manure storage facility. Both of these measures

are an indication of the ability of the microbial community in the manure to convert manure-substrates into methane. To measure MPR, 100 mL of well-mixed sample was added to a 250-mL serum bottle, similar to that used for the BMP assay, and upon the sealing of the sample with a sleeve stopper septa, the exact time was recorded along with the mass of sample added to the bottle. The sample was incubated at room temperature (approximately 23 °C). An incubation period of approximately 7 d was selected based on preliminary trials to achieve measureable quantities of biogas and methane. Once the 7-d incubation period was over, the sample was checked for biogas production with the gas-tight syringe and analyzed for methane content. The rate of methane was calculated as follows:

$$\begin{aligned} \text{MPR (L CH}_4\text{•L manure}^{-1}\text{•d}^{-1}) = & \\ ((\text{methane \%}/100) (\text{biogas produced (mL)} & \\ +V_{\text{headspace}}) & \\ \times \rho_{\text{manure (g/mL)}}) / (\text{mass of sample (g)} & \\ \times \text{incubation period (min)}) \times (1,440 \text{ min}) / \text{d.} & \end{aligned}$$

Upon collection, the biogas from each sample was injected into a nondispersive infrared methane analyzer (NDIR-CH₄ Gasanalyzer, University of Kiel, Germany) to obtain the percent of methane present. To measure BMP, 100 mL of manure was added to 250-mL serum bottles, the bottles were sealed and incubated, with biogas and methane production measured weekly by inserting a gas-tight syringe through the septum toper until gas production ceased (i.e., <10 mL CH₄ per week). The biogas was then extracted, volume measured, and tested for methane on a regular basis. Results were evaluated based on methane production per gram of VS added (Moody et al., 2011). Both BMP and MPR were recorded and reported on a volume (per liter) or weight (per gram of VS) basis.

Foaming Capacity and Stability Testing

The foaming capacity and stability apparatus used in this study was adapted from similar studies (Ross and Ellis, 1992; Bindal et al., 2002; Hutzler et al., 2011). This test was selected to evaluate the foaminess, or foaming tendency of the liquid as well as the stability of any foam generated. These tests specifically evaluated whether the manure had the physical characteristics to foam. In brief, air was passed through an in-line gas regulator into a 5.1-cm diameter clear PVC column, and the flow rate of air through the column was measured and

controlled with a variable area flow meter. A sample volume of approximately 300 mL was poured into the column and the initial level was recorded. The sample was then aerated through a cylindrical air stone at 0.2 L/min until a steady state height of foam was reached or the foam layer reached the maximum height of the column (approximately 33 cm above the initial 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 was calculated as the height of foam produced divided by the initial manure level and multiplied by 100 (based on our apparatus, maximum measureable foaming capacity was approximately 250). Foam stability was performed immediately after the foaming capacity was determined. Once aeration ceased, the 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 fits the data well in most cases, and was used to estimate the half-life of the foam from the time constant as follows:

$$t_{(1/2)} (\text{min}) = (\ln(2)) / (\text{decay coefficient } k)$$

Bacterial Community Composition

To determine the microbial community composition in the manure, DNA was extracted from 200 mg of manure (FastDNA SPIN Kit for Soil, MP Biomedical). Bacterial community composition was assessed using automated ribosomal intergenic spacer analysis (ARISA) as described previously (Yannarell and Triplett, 2005; Kent et al., 2007). The ARISA method uses PCR to amplify the internal transcribed spacer region of the bacterial rRNA operon. Different lengths of this intergenic spacer region represent different bacterial populations, and can be used to develop a DNA fingerprint of the microbial community that is analogous to a census of microbial populations. Determination of DNA fragment sizes was carried out using commercial software (GeneMarker version 1.95, SoftGenetics, State College, PA). Patterns of similarity among bacterial communities were assessed using Bray–Curtis similarity and nonmetric multidimensional scaling (NMDS) analysis was implemented using

commercial software (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). Analysis of similarity generates a test statistic, *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. Canoco ordination plots were used to determine correlation between microbial community composition and physical foaming characteristics.

Statistical Methods

Data were analyzed as a randomized complete block design with the group used as the block and the individual pig (tank) as the experimental unit, resulting in 12 observations per dietary treatment, 48 pigs (tanks) across 4 dietary treatments. Dietary treatments were arranged in a 2×2 factorial design with the main effects being diet type (CSBM or DDGS) and narasin addition (0 or 27.2 g/t). All data were subjected to ANOVA using Proc GLM (SAS Inst. Inc., Cary, NC) with treatment means reported as LSMEANS. Unless otherwise noted, there were no interactions noted between diet type and narasin addition ($P \geq 0.10$), such that the interaction term was omitted from the statistical model and only the main effects of diet or narasin reported along with the SEM for the main effect means. Differences among means were considered significant at $P \leq 0.10$. Microbial data were analyzed using ANOSIM which generates an *R* value whose magnitude indicates the

degree of difference between groups of samples, as described previously.

RESULTS AND DISCUSSION

Manure Characteristics and Total Manure Nutrient Output

Except for total manure Ca and PHOS output as a percent of animal Ca and PHOS intake, there were no interactions noted between diet type and narasin addition ($P \geq 0.10$) on any other manure characteristic or manure nutrient output parameter measured. Pigs fed diets containing DDGS produced manure which had lower pH and greater $\text{NH}_4\text{-N}$, S^{2-} , DM, N, C, and S concentrations ($P \leq 0.01$); but slightly less Ca and PHOS concentrations ($P \leq 0.11$) than pigs fed diets not containing DDGS (Table 2). On a percent of animal intake basis, total manure DM, N, C, and S were greater ($P \leq 0.01$) for pigs fed the DDGS-based diets compared to pigs fed the CSBM diet (Table 3). Narasin only increased manure C concentration ($P \leq 0.05$), Table 2, but as a percent of animal intake, narasin supplementation increased manure DM, C, and S output ($P \leq 0.03$) compared to pigs fed the CSBM diet, Table 3. There was an interaction for manure Ca and PHOS output as a percent of animal Ca and PHOS intake ($P \leq 0.08$), where narasin supplementation resulted in an increase in manure output of Ca and PHOS in pigs fed the CSBM diet, but not in pigs fed the DDGS diets (Table 4); averaged across pigs fed the CSBM and DDGS diets, narasin increased manure Ca and PHOS output ($P \leq 0.07$), Table 3.

The effects of feeding pigs diets containing DDGS compared to CSBM diet on energy and nutrient digestibility has been discussed extensively elsewhere (Stein and Shurson, 2009; Cromwell et al.,

Table 2. Manure characteristics as affected by diet composition and narasin supplementation^a

Diet	Narasin	Volume, liters	T, °C	pH	μM/g manure		Composition, g/liter					
					NH ₄ -N	S ⁻²	DM, %	N	C	S	Ca	PHOS
CSBM		175	19.1	8.06	219	0.63	5.33	3.32	23.31	0.62	1.58	1.64
DDGS		182	19.6	7.66	258	1.16	6.93	4.15	30.90	0.96	1.40	1.54
Diet <i>P</i> value		0.01	0.12	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.11
	No	177	19.18	7.83	227	0.84	5.93	3.71	25.97	0.76	1.44	1.55
	Yes	180	19.43	7.89	249	0.96	6.33	3.76	28.24	0.81	1.54	1.64
Narasin <i>P</i> value		0.18	0.43	0.51	0.11	0.40	0.11	0.78	0.05	0.15	0.18	0.14
SEM		1.5	0.22	0.04	10	0.11	0.17	0.13	0.95	0.03	0.08	0.07

T = temperature.

^aInitial BW = 121.0 kg, SD = 11.5 kg; final BW = 151.0 kg, SD = 7.9 kg. Each of the 2 trials consisted of a 48-d feces and urine collection into 1 tank per pig, with 12 individually penned gilts per treatment. Diets with DDGS contained 30.34% DDGS while the narasin diets contained 30 mg narasin/kg diet.

Table 3. Total manure nutrient output as affected by diet composition and narasin supplementation^a

Diet	Narasin	Manure nutrient, % of animal intake					
		DM	N	C	S	Ca	PHOS
CSBM		8.40	21.70	8.90	48.07	44.30	53.42
DDGS		11.65	29.32	12.10	57.21	44.03	52.53
Diet <i>P</i> value		0.01	0.01	0.01	0.01	0.91	0.60
	No	9.64	24.98	9.94	50.34	42.10	50.91
	Yes	10.41	26.03	11.07	54.93	46.23	55.04
Narasin <i>P</i> value		0.03	0.25	0.01	0.01	0.07	0.02
SEM		0.25	0.75	0.36	1.47	2.23	2.05

^aInitial BW = 121.0 kg, SD = 11.5 kg; final BW = 151.0 kg, SD = 7.9 kg. Each of the 2 trials consisted of a 48-d feces and urine collection into 1 tank per pig, with 12 individually penned gilts per treatment. Diets with DDGS contained 30.34% DDGS while the narasin diets contained 30 mg narasin/kg diet.

2011; Gutierrez et al., 2013, 2014a, 2014b; Kerr et al., 2013, 2015a, 2015b, 2017; Wu et al., 2016a, 2016b, 2016c) and was not an objective of the current experiment. That said, a change in energy or nutrient digestibility (represented by a change in fiber digestibility in this experiment), can have (Kerr et al., 2006; Ziemer et al., 2009; Trabue and Kerr, 2014; Trabue et al., 2016b; Van Weelden et al., 2016a, 2016b), and data obtained from this experiment are no exception. In contrast, the information on the impact of supplementing antibiotics or ionophores into the diet and subsequent effects on energy and nutrient digestibility is variable; where feeding antibiotics (Derick et al., 1986; Roth and Kirchgessner, 1993; Pilcher et al., 2015) or ionophores (Holzgraeffe et al., 1985a, 1985b; Moore et al., 1986; Wuethrich et al., 1998) have had variable effects on N digestibility and balance. The impact of feeding antibiotics or ionophores to finishing pigs on subsequent manure composition is nonexistent. This is not surprising given that characterization of manure bacteria and composition in a complex matrix (Cotta et al., 2003; Trabue et al. 2016b) and the selection of the

manure storage tank and length of storage (Trabue et al. 2016a) can have profound effects on the data obtained making interpretation of the results challenging. In the current experiment, narasin had minimal effect on manure composition (Table 2) and this is supported by Kerr et al. (2017) who, using the same pigs, reported that energy or nutrient digestibility in finishing pigs was unaffected by narasin supplementation. The greater amount of manure nutrients as a percentage of animal intakes due to narasin supplementation (Table 3) is best understood from the perspective that narasin inhibits gross biomass functions as seen in lower degradation rates of total and volatile solids of manure treated with and without narasin (Andersen and Regan, 2014), but some more specific functions are unaffected and this difference may be due in part to the level of functional redundancy for that particular function. However, physical and chemical parameters associated with emissions (i.e., manure crust formation) may obscure true effects (Trabue and Kerr, 2014). Manure from pigs fed the DDGS diets had an observable crust formation which is similar to what was reported previously (Trabue and Kerr, 2014); however, the crust coverage was not quantitatively measured in the current study because the focus of this paper was more on the narasin treatment and its potential interaction with diet type, and not on the CSBM vs. DDGS comparison. Crusts associated with DM in manure, via the feeding of a fibrous diet, has also been reported by Wood et al. (2012). As a consequence, narasin has an effect on the microbial community function, but our emission study did not reveal how narasin was impacting those functions given the complexity of the manure matrix.

Table 4. Total manure Ca and P output as affected by diet composition and narasin supplementation^a

Diet	Narasin	Manure nutrient, % of animal intake ^b	
		Ca	PHOS
CSBM	No	40.16 ^y	49.83 ^y
CSBM	Yes	48.43 ^x	57.02 ^x
DDGS	No	44.03 ^{xy}	51.99 ^y
DDGS	Yes	44.03 ^{xy}	53.06 ^{xy}
Diet × narasin, <i>P</i> value		0.07	0.08
SEM		2.22	1.70

^aInitial BW = 121.0 kg, SD = 11.5 kg; final BW = 151.0 kg, SD = 7.9 kg. Each of the 2 trials consisted of a 48-d feces and urine collection into 1 tank per pig, with 12 individually penned gilts per treatment. Diets with DDGS contained 30.34% DDGS while the narasin diets contained 30 mg narasin/kg diet.

^bSuperscripts reflect treatment differences (xy, $P \leq 0.10$).

Manure VOC and Gas Emissions

There were no interactions noted between diet type and narasin addition ($P \geq 0.10$) on major manure

VOC (Table 5), major gas emissions (Table 6), or greenhouse gas (GHG) emissions (Table 7). Pigs fed diets containing DDGS resulted in manure having increased ($P \leq 0.09$) concentrations of acetic, propionic, total VFA, and total phenolics; while narasin supplementation was without effect ($P \geq 0.29$) (Table 5). Pigs fed diets containing DDGS resulted in manure with decreased ($P \leq 0.01$) emissions of NH_3 and VSC, but increased ($P \leq 0.06$) emissions of phenols and indoles (Table 6). Narasin supplementation was without effect ($P \geq 0.46$) (Table 6). Neither diet type or narasin supplementation affected manure GHG or GHG-equivalence emissions ($P \geq 0.45$), and the only effect on manure C, N, or S emissions per unit of intake was a reduction in g N emissions per kg N intake for pigs fed the DDGS diet compared to pigs fed the CSBM diet ($P \leq 0.01$), Table 7.

The effect of feeding DDGS to pigs on the subsequent manure VOC, gas emissions, or GHG emissions was expected and has been discussed in depth in previous work from this laboratory (Kerr et al., 2006; Ziemer et al., 2009; Trabue et al., 2014, 2016b; Van Weelden et al., 2016a, 2016b). Supplementation of antibiotics or ionophores have been shown to affect intestinal or fecal concentrations of fatty acids, indoles, or phenolics (Yokoyama et al., 1982; Hawe et al., 1992; Weuthrich et al., 1998) and ionophores have been shown to affect manure microbial ecology and in vitro emissions from swine manure (Cotta et al., 2001; Whitehead and Cotta, 2007; Andersen and Regan, 2014). However, no data could be found reporting the effect of feeding an ionophore to pigs on subsequent manure VOC or gas emissions. These results show that gas emissions of odorants were not affected by narasin treatment even through the general biomass was impacted by the ionophore supplementation. This was expected

for the odorous compounds because manure concentrations of VOC, sulfide, and ammonia were not significantly different between narasin treatments (Tables 2 and 5). The lack of significant difference in any volatile emissions is surprising, but it may indicate that manure biomass function does not track well with gas emissions. It should also be noted that gas emissions variability was high so small differences in means may be hard to detect. Part of the high variability is found in the manure matrix for the solids content, pH, and crust formation, which will all have impact on gas emissions. Consequently, the physical chemical environment may play a major role in obscuring volatile gas emission differences.

Engineering-Based Biochemical Manure Parameters

Pigs fed diets containing DDGS produced manure which had greater ($P \leq 0.01$) TS and VS compared to manure from pigs fed diets not containing DDGS (Table 8). The addition of narasin to diets resulted in a small increase in VS ($P \leq 0.09$), but exhibited no effect on TS content. There was an interaction between diet type and narasin addition on MPR when evaluated per gram of VS ($P \leq 0.05$), which was due to narasin having no effect on MPR when included in the CSBM diet (0.89 vs. 0.78, respectively), but increased MPR when included in the DDGS diet (0.33 vs. 0.55, respectively), Table 9. There was also an interaction between diet type and narasin addition on BMP when evaluated per gram of manure ($P \leq 0.01$), which was due to narasin reducing BMP when included in the CSBM diet (11.41 vs. 7.87, respectively), but increased BMP when included in the DDGS diet (9.24 vs. 13.33, respectively), Table 9. Because we have no logical explanation for these interactive differences, we

Table 5. Major manure volatile compounds as affected by diet composition and narasin supplementation^a

Dietary treatment		Fatty acid, mmol/g wet wt				$\mu\text{mol/g}$ of wet wt	
Diet	Narasin	Acetic	Propionic	Butyric	Total ^b	Phenolics ^b	Indoles ^b
CSBM		64.7	9.4	6.5	87.5	1,147	21.2
DDGS		77.1	13.5	7.4	105.1	1,323	22.0
Diet <i>P</i> value		0.09	0.01	0.15	0.04	0.05	0.88
	No	68.6	11.1	7.3	94.0	1,266	22.8
	Yes	73.3	11.9	6.6	98.6	1,204	20.4
Narasin <i>P</i> value		0.51	0.39	0.29	0.58	0.49	0.66
SEM		5.0	0.7	0.5	5.9	62.9	3.91

^aInitial BW = 121.0 kg, SD = 11.5 kg; final BW = 151.0 kg, SD = 7.9 kg. Each of the 2 trials consisted of a 48-d feces and urine collection into 1 tank per pig, with 12 individually penned gilts per treatment. Diets with DDGS contained 30.34% DDGS while the narasin diets contained 30 mg narasin/kg diet.

^bVolatile fatty acids (acetate, propionate, butyrate, isobutyrate, isovalerate, valerate, isocaproic, caproic, and heptanoic), phenols (phenol, cresol, ethylphenol, and propylphenol), and indoles (indole and skatol).

Table 6. Major gas emissions from manure as affected by pigs fed different diet composition and narasin supplementation^a

Dietary treatment		Gas emissions, g d ⁻¹ AU ⁻¹							
Diet	Narasin	NH ₃	H ₂ S	VSC ^b	VFA ^c	Phenols ^c	Indoles ^c	VOC ^d	OAV ^e
CSBM		76.9	1.52	0.70	7.35	0.14	0.002	9.33	485
DDGS		53.4	2.12	0.25	8.44	0.23	0.007	10.81	585
Diet <i>P</i> value		0.01	0.25	0.01	0.64	0.06	0.01	0.53	0.28
	No	65.3	1.66	0.43	7.09	0.18	0.005	9.31	509
	Yes	65.1	1.96	0.52	8.70	0.19	0.004	10.85	562
Narasin <i>P</i> value		0.97	0.58	0.49	0.49	0.90	0.46	0.51	0.57
SEM		4.8	0.36	0.09	1.63	0.03	0.001	1.65	65

^aInitial BW = 121.0 kg, SD = 11.5 kg; final BW = 151.0 kg, SD = 7.9 kg. Each of the 2 trials consisted of a 48-d feces and urine collection into 1 tank per pig, with 12 individually penned gilts per treatment. Diets with DDGS contained 30.34% DDGS while the narasin diets contained 30 mg narasin/kg diet.

^bVSC, volatile sulfur compounds; sum of hydrogen sulfide, methanethiol, carbonyl sulfide, dimethyl sulfide, dimethyl sulfoxide, dimethyl disulfide.

^cVolatile fatty acids (acetate, propionate, butyrate, isobutyrate, isovalerate, valerate, isocaproic, caproic, and heptanoic), phenols (phenol, cresol, ethylphenol, and propylphenol), and indoles (indole and skatol).

^dVOC, volatile organic compounds; sum of VFA, phenols, and indoles.

^eOAV, odor activity value; calculated from the odorant concentrations in the air to published odor threshold value of the odorant (Hales et al., 2012; Parker et al., 2012; Wu et al., 2015).

have elected to discuss the main effect of diet type and narasin addition only. In general, pigs fed diets containing DDGS had a lower MPR, regardless if evaluated on a VS or a volume basis ($P \leq 0.01$) compared to pigs fed the CSBM diets. While MPR was greater in pigs fed the CSBM diets, BMP was not found to be affected by diet type ($P \geq 0.10$), regardless if measured on a VS or volume basis. No effect of narasin supplementation was noted on MPR or BMP, regardless of being measured on a VS or volume basis ($P \geq 0.50$).

There were no interactions between diet type and narasin supplementation for foaming capacity or foam stability ($P \geq 0.10$), and although there were numerical increases noted for foaming capacity and stability due to feeding pigs the DDGS diet, these

were not found to be significant ($P \geq 0.16$), Table 8. There was a small increase ($P \leq 0.08$) in foaming capacity due to narasin supplementation, with no effect on foaming stability ($P \geq 0.17$). This small increase in foaming capacity may be related to compounds associated with the volatile solids that increase in narasin supplemented diets. In addition, compounds such as proteins also enhance foam capacity and stability.

There were small differences noted in the microbial communities in the manure samples as affected by the diet type or narasin supplementation. In general, the difference in the manure microbial community due to diet type was more pronounced when narasin was included in the diet (CSBM with narasin vs. DDGS with narasin, $R = 0.47$) than

Table 7. Greenhouse gas and nutrient emissions from manure as affected by pigs fed different diet composition and narasin supplementation^a

Dietary treatment		Gas emissions						
Diet	Narasin	CH ₄ , g d ⁻¹ AU ⁻¹	N ₂ O, g d ⁻¹ AU ⁻¹	CO ₂ , g d ⁻¹ AU ⁻¹	GHG-eq ^b , g d ⁻¹ AU ⁻¹	C, g/kg C intake	N, g/kg N intake	S, g/kg S intake
CSBM		17.0	0.18	1,602	2,080	108	257	90
DDGS		16.9	0.21	1,828	2,312	119	187	83
Diet <i>P</i> value		0.96	0.45	0.63	0.63	0.71	0.01	0.75
	No	16.5	0.19	1,883	2,349	122	220	76
	Yes	17.4	0.20	1,575	2,043	105	224	97
Narasin <i>P</i> value		0.71	0.69	0.48	0.53	0.52	0.86	0.30
SEM		1.8	0.03	333	340	19	17	14

^aInitial BW = 121.0 kg, SD = 11.5 kg; final BW = 151.0 kg, SD = 7.9 kg. Each of the 2 trials consisted of a 48-d feces and urine collection into 1 tank per pig, with 12 individually penned gilts per treatment. Diets with DDGS contained 30.34% DDGS while the narasin diets contained 30 mg narasin/kg diet.

^bGHG-eq, greenhouse-equivalence; = CO₂ + (CH₄ × 25) + (N₂O × 298).

Table 8. Engineering-based biochemical data parameters of manure as affected by pigs fed different diet composition and narasin supplementation^a

Dietary treatment		Manure biochemical measurement							
Diet	Narasin	TS, %	VS, %	MPR, L CH ₄ ·kg VS ⁻¹ ·d ⁻¹	MPR, L CH ₄ ·L manure ⁻¹ ·d ⁻¹	BMP, L CH ₄ /kg VS	BMP, L CH ₄ /L manure	FM-CAP, %	FM-STAB, min
CSBM		5.55	3.77	0.84	0.033	0.261	9.6	90	2.1
DDGS		7.65	6.12	0.44	0.019	0.226	11.3	112	2.4
Diet <i>P</i> value		0.01	0.01	0.01	0.01	0.24	0.16	0.16	0.58
	No	6.31	4.69	0.61	0.026	0.251	10.3	87	1.8
	Yes	6.89	5.17	0.67	0.026	0.236	10.6	115	2.6
Narasin <i>P</i> value		0.15	0.09	0.50	0.89	0.60	0.81	0.08	0.17
SEM		0.39	0.28	0.08	0.003	0.028	1.2	15.2	0.6

FM-CAP = foaming capacity; FM-STAB = foaming stability.

^aInitial BW = 121.0 kg, SD = 11.5 kg; final BW = 151.0 kg, SD = 7.9 kg. Each of the 2 trials consisted of a 48-d feces and urine collection into 1 tank per pig, with 12 individually penned gilts per treatment. Diets with DDGS contained 30.34% DDGS while the narasin diets contained 30 mg narasin/kg diet.

when narasin was not included in the diet (CSBM vs. DDGS, $R = 0.14$), Table 10. It appeared that narasin had a greater impact on the microbial community of manure of pigs fed the DDGS diet ($R = 0.16$) compared to manure derived from pigs fed the CSBM ($R = 0.01$) diet, Table 10.

The differences noted in the engineering-based biochemical data (Table 8) and ANOSIM microbial community data (Table 10) as affected by the supplementation of DDGS in the current experiments are supported by others (Van Weelden et al., 2015, 2016a, 2016b) who evaluated the effects of feeding DDGS or other fibrous feedstuffs on these same parameters. While the ANOSIM microbial community data (Table 10) suggest some microbial ecology differences due to narasin supplementation. However, the lack of a consistent effect of narasin on manure compositional changes (Table 2), manure VOC (Table 5), emissions of major gases (Table 6)

or GHG (Table 7), and on engineering-based biochemical parameters (Table 8) suggests that narasin affect is not targeted to a particular functional property of the manure nor to a particular population in the manure, but rather its impact is more broadly affected at the gross biomass level that is seen in lower degradation of volatile solids and increase in manure nutrients (Andersen and Regan, 2014).

In conclusion, the study described herein provides supporting information indicating that feeding a diet containing elevated levels of indigestible fiber results in more fiber in the manure which therefore dramatically affect manure composition, gas emissions, and microbial ecology. In contrast, narasin addition did not exhibit a significant effect on any of these parameters in the resultant swine manure, suggesting narasin degradation or reduced activity during the digestion process in the animal.

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Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee

Table 9. Manure MPR and BMP as affected by diet composition and narasin supplementation^a

Diet	Narasin	Manure nutrient, % of animal intake ^b	
		MPR, L CH ₄ ·kg VS ⁻¹ ·d ⁻¹	BMP, L CH ₄ /L manure
CSBM	No	0.89 ^a	11.41 ^{ab}
CSBM	Yes	0.78 ^{ab}	7.87 ^c
DDGS	No	0.33 ^c	9.24 ^{bc}
DDGS	Yes	0.55 ^b	13.33 ^a
Diet × narasin		0.05	0.01
SEM		0.09	1.16

^aInitial BW = 121.0 kg, SD = 11.5 kg; final BW = 151.0 kg, SD = 7.9 kg. Each of the 2 trials consisted of a 48-d feces and urine collection into 1 tank per pig, with 12 individually penned gilts per treatment. Diets with DDGS contained 30.34% DDGS while the narasin diets contained 30 mg narasin/kg diet.

^bSuperscripts reflect treatment differences (abc, $P \leq 0.05$).

Table 10. ANOSIM microbial community comparisons for the protein and carbohydrate studies

Comparison	<i>R</i> statistic ^a
CSBM vs. DDGS	0.14
CSBM vs. CSBM with narasin	0.01
DDGS vs. DDGS with narasin	0.16
CSBM with narasin vs. DDGS with narasin	0.47

^aAnalysis of similarity was used to evaluate patterns of microbial community similarity among groups of samples (Clarke and Green, 1988). The ANOSIM test statistic, R , 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.

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