Reduction of Odorous Sulfide and Phenolic Compounds in Pig Manure Through Diet Modification

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Introduction

Increased public concern, legislation and environmental regulations have focused on pollution and have created a major threat to the viability and growth of the pork industry. Even though water pollution control has been the major focus of regulations and concern, recent public concerns and law suits have revolved around odors from pork operations. Several communities are creating zoning ordinances in an attempt to keep the pork industry from expanding.

Most research has focused on measuring odor and gas intensity and occurrence (primarily ammonia, hydrogen sulfide, skatole, indole, aliphatic aldehydes, mercaptans, and amines) with proposed attempts to reduce or mask odors. The first line of defense against high concentrations of any emitted aerial contaminant is source control. In the case of swine odor, the obvious source control technique is diet manipulation. However, little research has been conducted on dietary modification or pre-excretion control to alter fresh and stored swine manure odors, yet fresh manure from pigs is an initial source of odors. Consequently, determination of the biological creation of gaseous compounds, reduction of precursor compounds creating odors, and development of effective biological control is needed for efficient and long lasting odor control. Research was initiated to develop ways to significantly reduce odors, especially sulfide-containing odors, from pork operations with diet modification while sustaining efficient pork production. The objective of this study was to determine the effects of dietary methionine and cystine level, and cellulose and anthraquinone additions to the diet on production of odorous sulfide compounds and other odorous compounds from cecal contents, fresh manure and stored manure.

Materials and Methods

Three groups of four growing gilts (12 total), averaging 120 pounds, were surgically cannulated in the cecum using standard procedures. Each group of pigs came from the same litter. Each pig was fed each of four diets in a 4 x 4 Latin square experimental design. Dietary treatments were:

(1) Diet I: a standard 13% crude protein corn-soy diet with total methionine+cystine, 0.47%; total lysine, 0.61%; total tryptophan, 0.12%; threonine, 0.39%;
(2) Diet II: a 10% crude protein corn-soy diet with crystalline 0.36% lysine (0.6% total lysine), 0.0% methionine (0.40% total methionine+cystine), 0.02% tryptophan (0.10% total tryptophan), and 0.1% threonine (0.40% total threonine);
(3) Diet III: Diet II with 5% cellulose; and
(4) Diet IV: Diet I with anthraquinone (10 ppm).

Anthraquinone was tested because it has been proposed to suppress sulfide formation in the digestive system during microbial degradation of organic matter. Cellulose was tested as a specific fiber source that might refer to other common fiber sources, such as soybean hulls, wheat bran and sugar beet pulp, for positive odor and nutrient excretion responses. Pigs were fed *ad libitum* with constant access to water and were housed in the Purdue Animal Sciences controlled environment building in stainless steel metabolism crates for the separate collection of urine and feces. Cecal contents, feces and urine were sampled after diet adaptation. Total collections of feces and urine were blended. Cecal samples were directly collected from the cannula for nutrient analyses. Another subsample was centrifuged and the liquid supernatant was analyzed for volatile fatty acids (VFA). Three daily collections of cecal contents, feces and urine were obtained for each group of pigs and period of the trial for each dietary treatment. Long term (90 day) incubations were conducted to mimic microbial odor production in anaerobic pits and lagoons. Mixtures of fecal and urine collections were initially used for inoculum in the incubation trial, and fresh manure mixtures from pigs on the same diets were added every other day. Liquid matrix samples were analyzed for pH, dry matter, ammonia, total nitrogen, volatile fatty acids, and sulfides. Gas matrix samples were analyzed for the presence and quantification of 40 compounds.

Results

Tables 1 to 8 summarize the data from the three replicate 4 x 4 Latin square studies. There was no significant effect of diet on the pH (Table 1) or the volatile fatty acids concentrations (Table 2) in the cecal contents of the pigs. Cecal ammonium and total nitrogen were decreased by at least 24% and the dry matter content of cecal contents was higher from pigs fed the low crude protein (10% CP) diet and amino acids with 5% cellulose compared to the standard diets (13% CP). Table 3 lists the 26 most prominent volatile organic compounds (VOC) found in the headspace gas of cecal contents from pigs fed the experimental diets. Reducing the crude protein (CP) content of the diet gave variable results with no clear effect on VOC. However, the addition of cellulose to the low CP diet reduced dimethyl sulfide and dimethyl disulfide, methylene chloride, and sulfur dioxide, but increased other VOC. Anthraquinone addition to the standard diet reduced methyl phenol and the sulfur-containing VOC.

In contrast to responses in the cecum, the pH and total nitrogen in fresh manure was significantly lower from pigs fed the 10% CP amino acid supplemented diets as compared to the 13% CP standard diets (Table 4). Ammonia nitrogen was significantly lower for the 10% CP diet with cellulose when compared to the standard diet. Dry matter of the fresh manure tended to be higher for the low CP diets, especially with cellulose added. On a dry matter basis, ammonium nitrogen was reduced 33% and total nitrogen was reduced 48% in fresh manure when the 10% CP diet was fed with cellulose. The pH of fresh manure was reduced 0.9 units by manipulating the nitrogen and adding cellulose to the diet. There was an increase in total VFA concentrations when the 10% CP amino acid diet was supplemented with cellulose (Table 5). Acetate and propionate were the primary acids that were increased.

Urine (Table 6) and feces (Table 7) were collected separately and analyzed for pH, dry matter and nitrogen components to further explain diet effects on ammonium concentrations in the manure and VOC concentrations in air samples. The pH and total nitrogen concentrations of urine were reduced by reducing the CP in the diet and supplementing with synthetic amino acids.

This was due to providing the pig with the correct balance and availability of amino acids, resulting in a more efficient utilization of dietary protein and less urinary N excretion. Lower urea concentration in urine results in a lower pH. Compared to the other diets, total N in feces was reduced by feeding pigs the 10% CP synthetic amino acid diet. In addition, ammonium and total N were reduced 30% and 25% on a dry matter basis, respectively, in feces of pigs fed the 10% CP synthetic amino acid diet with added cellulose. The pH and dry matter content of feces were not affected by diet.

The 10 most predominant VOC in fresh manure headspace air are listed in Table 8. Reducing the protein content, supplementing with synthetic amino acids, and adding cellulose tended to reduce dimethyl disulfide, methyl phenol and other phenols in fresh manure. Addition of anthraquinone to a standard diet reduced phenolic compounds, especially methyl phenol, in freshly excreted manure compared to manure from pigs fed the control diet. Based upon changes in the nutrient concentrations of the fresh manure and lower concentrations of the VOC in fresh manure compared to cecal contents, it appears that the longer residence time and enhanced microbial activity in the large colon has provided the precursors for effective changes when the manure is anaerobically stored in pits.

Similar results were observed in anaerobically stored manure (Table 9) as with fresh manure. Pigs fed the 10% CP amino acid supplemented diets had lower ammonium and total nitrogen concentrations (by at least 49%) in the manure as compared to manure from pigs fed the standard diets. Pigs fed the low CP amino acid supplemented diet with cellulose had the lowest pH, ammonium and total nitrogen concentrations. Ammonium nitrogen and total nitrogen reduction with this diet were 70% and 52% on a dry matter basis, respectively, compared to the 13% CP standard diets.

Similar responses were noted with reduced concentrations of acetate, isovalerate, and total VFA in stored manure from pigs fed the low CP diets with supplemental amino acids compared to the 13% CP diet (Table 10). Total VFA were reduced from 41 to 83% with the reduced CP diets. It is not known why VFA were reduced by the cellulose addition. Typically, one would expect an increase in VFA production. Methane production was not measured in this study, and potentially more methane production proceeded with the cellulose diet, which would result in less detectable VFA concentrations.

Table 11 lists the 10 most predominant VOC in the headspace air of the manure pits with long term anaerobically stored manure. Total VOC concentration was similar for all diets; however, the concentrations of specific VOC were different. In stored manure, addition of cellulose to a low crude protein diet tended to reduce dimethyl sulfide, dimethyl disulfide, carbon disulfide and phenolic compounds when compared to a standard diet. Cellulose added to a low crude protein diet supplemented with amino acids altered odors significantly in manure. Addition of anthraquinone to a standard diet reduced carbon disulfide, hexane, and 2-butanone in stored manure compared to manure from pigs fed the control diet. Although there is evidence that anthraquinone might reduce certain VOC, additional work is needed to establish whether additional anthraquinone is effective in controlling sulfur-containing odors from manure.

Implications

Results from this research show that reducing the crude protein in typical corn-soybean meal diet from 13% to 10% and supplementing with limiting amino acids (lysine-HCl, threonine, methionine, tryptophan) will reduce the pH 0.5 to 0.8 units, reduce total nitrogen 33 to 48%, and with the addition of cellulose, reduce ammonium nitrogen 33% in freshly excreted manure. These reductions are a result of the metabolically reduced amount of urea nitrogen excreted in urine and the reduced pH of urine. However, the greatest impact of diet manipulation was a 41 to 83% reduction in volatile fatty acid concentration in long term anaerobically stored pit manure with the lower crude protein diets and supplemental amino acids. The diet reducing the nitrogen components in stored manure the most was the 10% crude protein synthetic amino acid diet with additional cellulose.

Reducing nitrogen excretion means almost 50% less land needed for manure application. By adding cellulose, producers can reduce volatile fatty acids (83%) and other VOC, including odorous sulfur compounds, and reduce the impact of odors on neighboring residences and communities.

Table 1. Effect of diet on ammonium nitrogen (NH_4-N), total nitrogen (TKN), dry matter (DM), and pH in cecal contents.

Diet (% CP)*	pH	DM %	NH_4-N mg/L	NH_4-N %DM	TKN mg/L	TKN %
I: Standard (13%)	5.4	10.9[b]	344[ab]	0.33[a]	3061[ab]	2.92[a]
II: Syn AA (10%)	5.5	12.3[ab]	366[a]	0.31[a]	3313[a]	2.80[a]
III: AA + Cellulose	5.5	13.0[a]	319[b]	0.25[b]	2721[b]	2.10[b]
IV: Std + Anth	5.4	11.9[ab]	375[a]	0.34[a]	3199[a]	2.79[a]
SEM	0.05	0.45	14	0.02	131	0.11

* Diet I: Standard (Std) 13% CP; Diet II: 10% CP with synthetic amino acids (AA);
Diet III: Diet II with 5% cellulose; Diet IV: Diet I with 10 ppm anthraquinone (Anth).
[a,b] Different letter superscripts in a column are significantly different (P<.05).

Table 2. Effect of diet on volatile fatty acid** (VFA) in cecal contents.

Diet (% CP)*	Ac	Pr	iB	B	iV	V	Total
	---	---	---	mmol/L	---	---	---
I: Standard (13%)	66	36	0.14	13	0.02	2.8	118.1
II: Syn AA (10%)	63	36	0.15	12	0.51	3.3	114.9
III: AA + Cellulose	68	32	0.14	12	0.0	2.4	115.1
IV: Std + Anth	69	36	0.19	14	0.0	3.0	121.6
SEM	1.9	1.2	0.05	0.76	0.2	0.5	5.0

* Diet I: Standard (Std) 13% CP; Diet II: 10% CP with synthetic amino acids (AA);
Diet III: Diet II with 5% cellulose; Diet IV: Diet I with 10 ppm anthraquinone (Anth).
** Volatile fatty acids: acetic (Ac), propionic (Pr), isobutyric (iB), butyric (B), isovaleric (iV), valeric (V).

Table 3. Effect of diet on volatile organic compounds in the headspace air from cecal contents.

Gas compounds	Diet*			
	I: Standard (13%)	II: Syn. AA (10%)	III: AA + Cellulose	IV: Std + Anth
	------------- gm per gram of material per L of air -----------------			
Ethanol	3.65	3.17	1.76	1.59
Methyl phenol	2.52	1.38	2.99	1.28
Propanoic acid,	0.70	0.29	0.89	0.29
Propanoic acid, propyl	0.79	0.32	0.70	0.39
Propanoic acid, methyl	0.56	0.00	0.59	1.00
Propanoic acid, ethyl	1.03	0.10	0.40	0.00
Ethanethioic acid	1.31	0.29	2.20	0.76
Benzene	3.28	2.96	0.99	2.26
2,2-Dimethyl hexane	7.87	12.43	11.03	6.10
Hexane	2.09	2.50	2.01	3.08
Dimethyl sulfide	0.00	0.40	0.00	0.00
Dimethyl disulfide	2.91	5.06	1.60	1.58
Dimethyl trisulfide	0.02	0.30	0.01	0.02
Carbon disulfide	0.00	0.20	0.00	0.00
n-Propyl acetate	0.62	0.39	0.70	0.69
Methylene chloride	0.67	3.30	0.00	0.00
1-Butanol	0.29	0.00	0.72	0.00
Butanoic acid	0.92	0.39	2.61	0.90
Butanoic acid, ethyl	0.93	0.39	0.55	0.00
Pentanoic acids	0.92	0.20	2.41	0.43
1-Hexanol, 2 ethyl	0.08	0.10	0.00	0.00
Acetic acid, butyl	0.15	0.10	0.10	0.20
Methyl pentane	1.06	1.70	1.29	2.65
Heptane, 3-methylene	0.24	0.47	0.29	2.83
Sulfur dioxide	0.06	0.07	0.01	0.00
Propanethioate, s-methyl	0.96	0.00	1.10	0.99

* Diet I: Standard (Std) 13% CP; Diet II: 10% CP with synthetic amino acids (AA);
Diet III: Diet II with 5% cellulose; Diet IV: Diet I with 10 ppm anthraquinone (Anth).

Table 4. Effect of diet on ammonium nitrogen (NH_4-N), total nitrogen (TKN), dry matter (DM) and pH in fresh manure.

Diet (% CP)*	pH	DM %	NH_4-N mg/L	NH_4-N %DM	TKN g/L	TKN %DM
I: Standard (13%)	8.4[a]	10.1[b]	3463	3.61[a]	12.0	13.77[a]
II: Syn AA (10%)	7.9[b]	13.1[b]	4067	3.45[a]	10.4	9.26[b]
III: AA + Cellulose	7.5[c]	16.9[a]	3747	2.42[b]	10.1	7.10[c]
IV: Std + Anth	8.2[ab]	11.1[b]	3699	3.66[a]	10.1	10.63[b]
SEM	0.12	1.0	384	0.28	0.06	0.47

* Diet I: Standard (Std) 13% CP; Diet II: 10% CP with synthetic amino acids (AA);
Diet III: Diet II with 5% cellulose; Diet IV: Diet I with 10 ppm anthraquinone (Anth).
[a,b,c] Different letter superscripts in a column are significantly different (P<.05).

Table 5. Effect of diet on volatile fatty acid** (VFA) in fresh manure.

Diet (% CP)*	Ac	Pr	iB	B	iV	V	Total
	mmol/L						
I: Standard (13%)	28[b]	11[b]	0.7	6[b]	1.0	2.0	48.1[b]
II: Syn AA (10%)	32[b]	13[ab]	0.9	8[ab]	1.2	2.4	57.3[b]
III: AA + Cellulose	40[a]	16[a]	1.0	10[a]	0.7	3.5	71.5[a]
IV: Std + Anth	32[b]	12[b]	0.8	7[b]	1.1	2.3	55.0[b]
SEM	2	1	0.2	0.9	0.3	0.5	4.7

* Diet I: Standard (Std) 13% CP; Diet II: 10% CP with synthetic amino acids (AA);
Diet III: Diet II with 5% cellulose; Diet IV: Diet I with 10 ppm anthraquinone (Anth).
** Volatile fatty acids: acetic (Ac), propionic (Pr), isobutyric (iB), butyric (B), isovaleric (iV), valeric (V).
[a,b] Different letter superscripts in a column are significantly different (P<.05).

Table 6. Effect of diet of pH, dry matter (DM) and nitrogen components of urine.

Diet (% CP)*	pH	DM %	NH_4-N mg/L	NH_4-N %DM	TKN g/L	TKN %DM
I: Standard (13%)	7.3[a]	5.6	1076[b]	2.21[bc]	12.0	21.79[a]
II: Syn AA (10%)	6.4[b]	5.6	1464[a]	2.59[ab]	9.8	17.18[b]
III: AA + Cellulose	6.5[b]	6.0	1569[a]	2.78[a]	9.9	16.95[b]
IV: Std + Anth	7.3[a]	5.5	1102[b]	2.02[c]	10.9	19.91[a]
SEM	0.11	0.3	90	1.47	0.07	0.86

* Diet I: Standard (Std) 13% CP; Diet II: 10% CP with synthetic amino acids (AA);
Diet III: Diet II with 5% cellulose; Diet IV: Diet I with 10 ppm anthraquinone (Anth).
[a,b] Different letter superscripts in a column are significantly different (P<.05).

Table 7. Effect of diet of pH, dry matter (DM) and nitrogen components of feces.

Diet (% CP)*	pH	DM %	NH_4-N mg/L	NH_4-N %DM	TKN g/L	TKN %DM
I: Standard (13%)	6.4	41.6	2470[a]	5.93[a]	14.4	3.46[a]
II: Syn AA (10%)	6.4	42.1	2336[a]	5.51[a]	13.3	3.06[b]
III: AA + Cellulose	6.2	46.4	1911[b]	4.15[b]	12.0	2.60[c]
IV: Std + Anth	6.4	42.7	2637[a]	6.17[a]	13.7	3.22[ab]
SEM	0.1	1.5	140	0.03	0.06	0.09

* Diet I: Standard (Std) 13% CP; Diet II: 10% CP with synthetic amino acids (AA);
Diet III: Diet II with 5% cellulose; Diet IV: Diet I with 10 ppm anthraquinone (Anth).
[a,b,c] Different letter superscripts in a column are significantly different (P<.05).

Table 8. Effect of diet on volatile organic compounds in the headspace air from fresh manure.

Gas compounds	Diet*			
	I: Standard (13%)	II: Syn. AA (10%)	III: AA + Cellulose	IV: Std + Anth
	------------- gm per gram of material per L of air -----------------			
Ethanol	.30	.29	.03	.28
1-Propanol	.17	.14	.00	.00
Ethanethioic acid	.01	.05	.01	.00
2,2-Dimethyl hexane	.84	1.18	.90	.90
Dimethyl disulfide	.31	.60	.19	.28
1-Hexanol, 2 ethyl	.05	.00	.01	.00
Ethyl acetate	.00	.18	.00	.00
Hexane	.26	.12	.57	.33
Methylene chloride	.67	3.33	2.50	.67
Methyl phenol	.06	.03	.01	.02

* Diet I: Standard (Std) 13% CP; Diet II: 10% CP with synthetic amino acids (AA);
Diet III: Diet II with 5% cellulose; Diet IV: Diet I with 10 ppm anthraquinone (Anth).

Table 9. Effect of diet on ammonium nitrogen (NH_4-N), total nitrogen (TKN), dry matter (DM) and pH of stored manure.

Diet (% CP)*	pH	DM %	NH_4-N mg/L	NH_4-N %DM	TKN mg/L	TKN %DM
I: Standard (13%)	8.2^a	6.9^a	7938^a	11.5^a	9301^a	13.5^a
II: Syn AA (10%)	7.6^b	4.7^c	3069^b	6.5^{ab}	4749^b	10.1^{ab}
III: AA + Cellulose	7.4^b	5.8^b	2014^b	3.5^c	3751^b	6.5^b
IV: Std + Anth	8.5^a	6.2^{ab}	9109^a	14.6^a	10985^a	17.6^a
SEM	0.1	0.3	464	0.83	638	1.0

* Diet I: Standard (Std) 13% CP; Diet II: 10% CP with synthetic amino acids (AA);
Diet III: Diet II with 5% cellulose; Diet IV: Diet I with 10 ppm anthraquinone (Anth).
^{a,b,c} Different letter superscripts in a column are significantly different (P<.05).

Table 10. Effect of diet on volatile fatty acid** (VFA) in stored manure.

Diet (% CP)*	Ac	Pr	iB	B	iV	V	Total
	mmol/L						
I: Standard (13%)	136[a]	13	1.1	8.6	8.3[ab]	0.0	167.5[a]
II: Syn AA (10%)	63[b]	14	2.2	8.8	4.9[b]	4.1	99.4[b]
III: AA + Cellulose	19[c]	5	2.1	2.2	0.0[c]	0.0	29.0[c]
IV: Std + Anth	130[a]	6	2.2	1.8	4.0[a]	0.0	144.1[a]
SEM	9	2	0.4	1.8	0.06	0.09	12.8

* Diet I: Standard (Std) 13% CP; Diet II: 10% CP with synthetic amino acids (AA); Diet III: Diet II with 5% cellulose; Diet IV: Diet I with 10 ppm anthraquinone (Anth).

**Volatile fatty acids: acetic (Ac), propionic (Pr), isobutyric (iB), butyric (B), isovaleric (iV), valeric (V).

[a,b,c] Different letter superscripts in a column are significantly different (P<.05).

Table 11. Effect of diet on volatile organic compounds in the headspace air from stored manure.

Gas compounds	Diet*			
	I: Standard (13%)	II: Syn. AA (10%)	III: AA + Cellulose	IV: Std + Anth
	gm per gram of material per L of air			
Ethanol	.010	.012	.000	.011
Benzene	.038	.046	.108	.050
Dimethyl sulfide	.038	.188	.000	.162
Dimethyl disulfide	.188	.104	.062	.103
Dimethyl trisulfide	.000	.000	.000	.008
Carbon disulfide	.033	.000	.000	.000
2-Butanone	.054	.017	.000	.000
Pentane, methyl	.021	.000	.000	.004
2,2-Dimethyl hexane	.125	.346	.442	.292
Hexane	.021	.117	.188	.000

* Diet I: Standard (Std) 13% CP; Diet II: 10% CP with synthetic amino acids (AA); Diet III: Diet II with 5% cellulose; Diet IV: Diet I with 10 ppm anthraquinone (Anth).