Amino Acid and Mineral Manipulation in Pig Diets to Reduce Nitrogen and Odors in Pig Manure

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Introduction

Recent 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 a major focus of regulations, recent public concerns and lawsuits have revolved around odors from pork operations. Most research has focused on measuring odor and gas intensity and occurrence with proposed attempts to reduce or mask odors (Miner, 1995). The first line of defense against any emitted aerial contaminant is source control. In the case of swine odor, the obvious source control technique is diet manipulation.

Odorous compounds result from the anaerobic microbial degradation of proteins and carbohydrates (Drochner, 1987). Ammonia, short-chain volatile fatty acids, amines, skatole, indole, p-cresol, H_2S and other sulfur-containing compounds have been identified in air samples over manure pits (Hobbs et al., 1995). Significant levels of sulfide-containing compounds (dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, hydrogen sulfide, carbon disulfide, and dimethyl sulfoxide) were measured in previous studies (Sutton et al., 1995). Primary sulfur sources are derived from the amino acids methionine and cystine, and/or from trace minerals in the diet. The objectives of this research were to determine the effects of dietary methionine and cystine level and sulfur-containing mineral additions to the diet on the production of odorous sulfide compounds and other odorous compounds from cecal contents, fresh manure and stored manure.

Materials and Methods

Research studies were conducted in two phases: 1) swine metabolism studies and 2) *in vitro* incubation studies. Swine metabolism studies determined the effects of dietary treatments on odorous compound production in the pig intestinal system. The *in vitro* studies evaluated longer-term treatment effects on fresh manure decomposition and odorous compound evolution using model slurry pit systems.

Twelve (12) growing gilts, averaging approximately 120 lb, in three groups of four each, were cecally cannulated using standard procedures. Each group of pigs came from the same litter. Each pig was fed each of four diets in a 4x4 Latin square experimental design. Dietary treatments were:

1. Diet I: a standard 13% crude protein (CP) corn-soy diet (0.54% methionine+cystine; 0.6% lysine; 0.16% tryptophan; 0.53% threonine) with 225 ppm ferrous sulfate and 250 ppm copper sulfate;

2. Diet II: an 8% CP corn-soy diet with 0.43% crystalline L-lysine (0.6% total lysine), 0.20% DL-methionine (0.36% total methionine), 0.02% L-tryptophan (0.11% total tryptophan) and 0.075 % L-threonine (0.41% total threonine), with 355 ppm ferric chloride and 80 ppm copper oxide;
3. Diet III: Diet II with 225 ppm ferrous sulfate and 250 ppm copper sulfate; and
4. Diet IV: Diet I with 355 ppm ferric chloride and 80 ppm copper oxide.

Pigs were fed ad libitum with constant access to water and were housed in the Purdue Animal Sciences controlled environment building. Cecal contents, feces and urine were sampled after a 10-day diet adaptation. 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. Flasks of cecal contents and a mixture of feces and urine were incubated for 18 hours, and gas samples from the headspace of the flasks were collected for analysis. Liquid matrix samples were analyzed for ammonia, total nitrogen, pH, volatile fatty acids (VFA) and dry matter.

Long-term (90-day) incubations were conducted to mimic microbial odor production in anaerobic pits. Fresh manure (fecal and urine mixtures) from pigs on the same diets was used for the incubation trial and was added three times each week to simulate manure excretion into the pit. There were three replicate anaerobic model pits per dietary treatment. Gas samples were collected for analysis. Ammonium and total nitrogen, dry matter and pH were measured. Volatile fatty acid concentrations of the acidified samples were determined.

Results

There was no significant effect of diet on the pH, dry matter, nitrogen components or VFA of the cecal contents of the pigs (data not shown here). Table 1 shows the composition of gases in the headspace of cecal contents; the dietary changes altered some individual volatile organic compounds (VOC) in cecal contents. The pH, ammonia nitrogen and total nitrogen in fresh manure was significantly lower from pigs fed the 8% CP amino acid supplemented diets as compared to the 13% CP standard diets (Table 2). Dry matter of the fresh manure tended to be higher for the low CP diets. On a dry matter basis, ammonium nitrogen was reduced 45%, total nitrogen was reduced 44%, and the pH of the manure was reduced by 1.1 to 1.3 units by manipulating the nitrogen in the diet. Fresh manure from pigs fed the 8% CP diet supplemented with amino acids and with ferrous sulfate and copper sulfate had the lowest concentrations of nitrogenous compounds and pH as compared to the 13% CP diets and the 8% CP diet with ferric chloride and copper oxide mineral sources. Reducing the CP in the diet and supplementing with synthetic amino acids reduced the pH and total nitrogen concentrations of urine (Table 2). This can probably be attributed to improved balance and higher availability of amino acids, resulting in a more efficient utilization of dietary nitrogen. Lower urea concentration in urine leads to a lower pH. Compared to the other diets, nitrogen (ammonium and total) in feces was reduced by feeding the pigs the 8% CP synthetic amino acid diet with mineral sulfate sources (Table 2). The pH and dry matter of feces were not affected by diet. There was a trend towards reduced total VFA concentrations with the amino acid supplemented 8% CP diet with mineral sulfates compared to the other diets (Table 3). There was no clear effect of diet on the VOC in fresh manure headspace air (Table 4).

Tables 5 to 7 summarize data on the long-term storage of manure in model anaerobic pits from the pigs fed the experimental diets. Lower ammonium and total nitrogen concentrations were observed in anaerobically stored manure from pigs fed the 8% CP amino acid supplemented diets (Table 5). Pigs fed the low CP amino acid supplemented diet with ferrous sulfate and copper sulfate had the lowest pH, ammonium and total nitrogen concentrations. Ammonium nitrogen and total nitrogen reduction with this diet were 48% and 40%, respectively, compared to the 13% CP standard diets. The source of mineral did not affect pH and nitrogen composition. Concentrations of acetate, isovalerate, and total VFA were reduced in the stored manure from pigs fed the low CP diets with supplemental amino acids compared to the other diets (Table 6). Total VFA were reduced from 55 to 61% with the reduced CP diets. In addition, stored manure from pigs fed the 8% CP diet with ferric chloride and copper oxide had higher concentrations of acetate, isovalerate and total VFA than the 8% CP diet with ferrous sulfate and copper sulfate.

Highest VFA concentrations were found in manure from pigs fed the 13% standard diets. Table 7 lists the 12 most predominant VOC in the headspace air of the manure pits with long term anaerobically stored manure. Although not consistent with all VOC, reducing the CP content and supplementing with amino acids tended to reduce most VOC. On average, the odorous sulfur-containing VOC were reduced 63% by reducing the CP and adding synthetic amino acids, and were reduced 49% by reducing the level of mineral sulfates (ferrous and copper) in the diet.

Summary

Research trials were conducted with growing-finishing crossbred gilts to determine the effects of amino acid and mineral supplements on nitrogen excretion and volatile organic compounds, especially sulfides, in cecal contents, fresh manure and anaerobically stored manure. Reducing the CP from 13% to 8% and supplementing with crystalline amino acids reduced pH in manure from 8.2 to 7.0. Ammonium nitrogen in fresh manure was reduced 45% by nitrogen manipulation. The lower pH and ammonium nitrogen in the manure were due to lower pH and urea nitrogen contents of urine. Ammonium and total nitrogen were reduced (48% and 40%, respectively) in stored manures with the lower CP diets. Total VFA in stored manures were reduced 55 to 61% with the reduced CP diets. Odorous sulfur-containing volatile organic compounds were reduced by 63% by reducing the dietary CP with synthetic amino acid additions, and were reduced by 49% by reducing the level of mineral sulfates in the diet.

Implications

Results from this research show that reducing the crude protein in typical corn-soybean meal diet from 13% to 8% and supplementing with limiting amino acids (lysine-HCl, threonine, methionine and tryptophan) will reduce the pH, total nitrogen and ammonium nitrogen in freshly excreted manure. This was due to reducing the amount of urea nitrogen excreted in urine and due to reducing the pH of urine. There was little effect of mineral sulfate levels on nitrogenous or volatile organic compounds in air samples from fresh manure or on the chemical composition of the manure. Diet manipulation reduced volatile fatty acid concentration (57%) and sulfur-containing volatile organic compounds (63%) in long term anaerobically stored manure from pigs fed lower crude protein diets and supplemental amino acids.

It is apparent that manipulating the nitrogen components and, to a lesser extent, the minerals sources of the diet will affect nutrient composition and odors from stored manures. Reducing nitrogen excretion will decrease land requirements for manure application. Reducing volatile fatty acids and odorous sulfur compounds will lessen the impact of odors on neighboring residences throughout the year.

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Table 1. Effect of diet on volatile organic compounds in the headspace air from cecal contents.

	Diet (% CP)[a]			
Gas Compounds	I: Standard (13)	II: Syn. AA (8+AA)	III: Syn. AA (8+AA)	IV: Standard (13)
	gm per gram of material per L of air			
Ethanol	7.63	8.60	11.55	8.39
1-Propanol	4.61	4.73	3.90	4.69
Methyl phenol	0.27	0.04	0.06	0.02
Skatole	0.16	0.00	0.11	0.00
Propanoic acid,	0.62	1.28	0.97	1.19
Propanoic acid, propyl	1.23	0.87	0.94	0.91
Propanoic acid, methyl	0.07	0.00	0.00	0.37
Propanoic acid, ethyl	0.77	0.79	0.76	0.32
Ethanethioic acid	1.15	1.17	0.83	1.76
Benzene	4.43	3.78	4.35	3.10
2,2-Dimethyl hexane	0.33	0.52	0.33	0.35
Hexane	0.30	0.37	0.28	0.09
Dimethyl sulfide	0.16	0.04	0.12	0.22
Dimethyl disulfide	3.51	3.54	3.43	4.21
Dimethyl trisulfide	0.12	0.10	0.03	0.11
Dimethyl sulfoxide	0.04	0.03	0.06	0.06
Ethyl acetate	2.14	2.00	1.31	0.79
n-Propyl acetate	1.67	1.29	1.31	1.85
Methylene chloride	0.41	0.47	0.45	0.27
1-Butanol	0.83	0.83	0.30	0.70
Butanoic acid	1.31	1.90	1.49	2.01
Butanoic acid, ethyl	1.03	1.24	0.98	1.15
Butanoic acid, 3-methyl	0.24	0.32	0.25	0.41
Butanoic acid, propyl	1.03	0.67	0.44	0.67
Pentanoic acids	1.19	1.55	2.42	1.42
Acetone	3.04	2.43	1.18	0.61
Propanethioate, s-methyl	0.59	0.39	0.49	0.60

[a] Diet I: 13% CP with ferrous sulfate and copper sulfate;
Diet II: 8% CP with synthetic amino acids and ferric chloride and copper oxide;
Diet III: 8% CP with synthetic amino acids and ferrous sulfate and copper sulfate;
Diet IV: 13% CP with ferric chloride and copper oxide.

Table 2. Effect of diet on ammonium nitrogen (NH_4-N), total nitrogen (TKN), dry matter (DM) and pH in fresh manure, urine and feces.

Diet (% CP)[a]	pH	DM %	NH_4-N mg/L	NH_4-N %DM	TKN mg/L	TKN %DM
Fresh Manure						
I: Standard (13)	8.2[b]	11.2	5912	6.08[b]	1.26	13.08[b]
II: Syn. AA (8+AA)	7.1[c]	15.4	4813	3.37[c]	1.17	8.02[c]
III: Syn. AA (8+AA)	6.9[c]	15.8	4411	2.94[c]	1.07	7.20[c]
IV: Standard (13)	8.2[b]	10.7	5397	5.47[b]	1.32	14.27[b]
SEM	0.18	1.5	522	0.56	0.10	0.62
Urine						
I: Standard (13)	7.6[b]	5.2	2125	5.78	1.33	27.20[b]
II: Syn. AA (8+AA)	5.6[c]	6.7	2805	4.24	1.20	17.63[c]
III: Syn. AA (8+AA)	5.5[c]	6.6	2899	4.50	1.07	16.20[c]
IV: Standard (13)	7.5[b]	5.3	1803	3.50	1.31	25.45[b]
SEM	0.14	0.6	334	1.60	0.10	1.12
Feces						
I: Standard (13)	6.9	38.8	2559	6.89[b]	1.27	3.31[c]
II: Syn. AA (8+AA)	6.8	37.9	2045	5.51[bc]	1.20	3.20[c]
III: Syn. AA (8+AA)	6.8	39.5	1909	4.93[c]	1.19	3.03[d]
IV: Standard (13)	7.0	38.4	2553	6.69[b]	1.32	3.48[b]
SEM	0.1	0.9	140	0.34	0.43	0.49

[a] Diet I: 13% CP with ferrous sulfate and copper sulfate;
Diet II: 8% CP with synthetic amino acids and ferric chloride and copper oxide;
Diet III: 8% CP with synthetic amino acids and ferrous sulfate and copper sulfate;
Diet IV: 13% CP with ferric chloride and copper oxide.
[b,c,d] Different letter superscripts in a column indicate means are significantly different ($P<.05$).

Table 3. Effect of diet on volatile fatty acids[a] (VFA) in fresh manure.

	VFA, mmol/L						
Diet (% CP)[b]	Ac	Pr	iB	B	iV	V	Total
I: Standard (13)	63	21	2.6	10	2.4	4.2	102.8
II: Syn. AA (8+AA)	51	22	2.4	11	2.5	4.9	94.0
III: Syn. AA (8+AA)	49	20	2.1	9	2.3	4.1	86.9
IV: Standard (13)	57	18	2.4	11	2.6	3.7	94.4
SEM	4	3	0.2	1	0.3	0.8	8.2

[a] VFA: Ac = acetic; Pr = propionic; iB = isobutyric; B = butyric; iV = isovaleric; V = valeric.
[b] Diet I: 13% CP with ferrous sulfate and copper sulfate;
Diet II: 8% CP with synthetic amino acids and ferric chloride and copper oxide;
Diet III: 8% CP with synthetic amino acids and ferrous sulfate and copper sulfate;
Diet IV: 13% CP with ferric chloride and copper oxide.

Table 4. Effect of diet on volatile organic compounds in the headspace air from fresh manure.

	Diet (% CP)[a]			
Gas Compounds	I:Standard (13)	II: Syn. AA (8+AA)	III:Syn. AA (8+AA)	IV:Standard (13)
	gm per gram of material per L of air			
Ethanol	.70	.57	.78	.72
1-Propanol	.15	.11	.13	.13
Methyl phenol	.10	.18	.15	.09
Ethanethioic acid	.02	.02	.03	.01
Benzene	.04	.05	.65	.48
2,2-Dimethyl hexane	.07	.09	.09	.04
Dimethyl sulfide	.08	.12	.16	.11
Dimethyl disulfide	.45	.57	.47	.45
Dimethyl trisulfide	.04	.10	.04	.03
Dimethyl sulfoxide	.05	.05	.05	.02
Carbon disulfide	.01	.04	.03	.01
Ethyl acetate	.09	.06	.10	.09
Acetone	1.58	3.37	1.75	2.79
Hexane	.04	.05	.04	.04
1-Butanol	.01	.01	.00	.02
Butanoic acid	.00	.01	.00	.00
Skatole	.01	.00	.00	.00

[a] Diet I: 13% CP with ferrous sulfate and copper sulfate;
Diet II: 8% CP with synthetic amino acids and ferric chloride and copper oxide;
Diet III: 8% CP with synthetic amino acids and ferrous sulfate and copper sulfate;
Diet IV: 13% CP with ferric chloride and copper oxide.

Table 5. Effect of diet on ammonium nitrogen (NH_4-N), total nitrogen (TKN), dry matter (DM) and pH of anaerobically stored manure.

Diet (% CP)[a]	pH	DM %	NH_4-N mg/L	NH_4-N %DM	TKN mg/L	TKN %DM
I: Standard (13)	8.0	6.0	6196[b]	9.08[b]	8653[b]	12.7[b]
II: Syn. AA (8+AA)	7.9	6.0	3771[c]	6.40[bc]	5736[c]	9.6[bc]
III: Syn. AA (8+AA)	7.3	6.0	3203[c]	4.85[c]	5223[c]	8.0[c]
IV: Standard (13)	7.9	5.4	6161[b]	9.97[b]	8822[b]	14.0[b]
SEM	0.2	0.3	655	1.27	808	1.5

[a] Diet I: 13% CP with ferrous sulfate and copper sulfate;
Diet II: 8% CP with synthetic amino acids and ferric chloride and copper oxide;
Diet III: 8% CP with synthetic amino acids and ferrous sulfate and copper sulfate;
Diet IV: 13% CP with ferric chloride and copper oxide.
[b,c] Different letter superscripts in a column indicate means are significantly different (P<.05).

Table 6. Effect of diet on volatile fatty acids[a] (VFA) in anaerobically stored manure.

	VFA, mmol/L						
Diet (% CP)[b]	Ac	Pr	iB	B	iV	V	Total
I: Standard (13)	143[c]	9	3.3	10.4	5.0[cd]	1.6	173.2[cd]
II: Syn. AA (8+AA)	81[d]	4	2.7	5.8	4.0[d]	2.0	99.4[d]
III: Syn. AA (8+AA)	43[e]	10	2.1	6.9	2.5[e]	2.4	67.6[e]
IV: Standard (13)	182[c]	13	4.1	10.8	6.9[c]	1.4	218.4[c]
SEM	24	2	0.6	3.1	1.0	1.0	28.4

[a] VFA: Ac = acetic; Pr = propionic; iB = isobutyric; B = butyric; iV = isovaleric; V = valeric.
[b] Diet I: 13% CP with ferrous sulfate and copper sulfate;
Diet II: 8% CP with synthetic amino acids and ferric chloride and copper oxide;
Diet III: 8% CP with synthetic amino acids and ferrous sulfate and copper sulfate;
Diet IV: 13% CP with ferric chloride and copper oxide.
[c,d,e] Different letter superscripts in a column indicate means are significantly different (P<.05).

Table 7. Effect of diet on volatile organic compounds in the headspace air from anaerobically stored manure.

	Diet (%CP)[a]			
Gas compounds	I: Standard (13)	II: Syn. AA (8+AA)	III: Syn. AA (8+AA)	IV: Standard (13)
	gm per gram of manure per L of air			
Ethanol	.175	.169	.183	.177
Propanoic acid	.058	.000	.003	.000
Benzene	.203	.258	.322	.300
Dimethyl sulfide	.008	.000	.000	.000
Dimethyl disulfide	.142	.033	.052	.067
Dimethyl trisulfide	.001	.000	.000	.001
Dimethyl sulfoxide	.008	.000	.003	.003
Carbon disulfide	.092	.005	.056	.075
Ethyl acetate	.000	.005	.005	.011
Acetone	.011	.006	.023	.006
2,2 Dimethyl Hexane	.022	.028	.025	.028
Hexane	.000	.031	.019	.011

[a] Diet I: 13% CP with ferrous sulfate and copper sulfate;
Diet II: 8% CP with synthetic amino acids and ferric chloride and copper oxide;
Diet III: 8% CP with synthetic amino acids and ferrous sulfate and copper sulfate;
Diet IV: 13% CP with ferric chloride and copper oxide.