

## **Addition of Carbohydrates to Low Crude Protein Pig Diets to Reduce Manure Nitrogen Excretion and Odors**

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### **Introduction**

The threat of odors emitted from pork production operations in some areas of Indiana has restricted growth of the industry and has created negative relations with residential neighbors. Odors from manure result from microbial decomposition of the organic matter and nutrients in undigested feed, and normal exogenous end products of cellular metabolism. Reducing nutrient excretions and controlling compounds creating the odors in manure are needed to significantly reduce odors emitted from pork production units. Minimizing nutrient excesses in diets, improving nutrient digestibility and utilization, and balancing nutrient levels to meet the needs of the pig and of the microflora in the digestive system are approaches needing evaluation as potential methods of odor control. Reducing the dietary crude protein level and supplementing with synthetic amino acids has reduced nitrogen excretion from pigs, with a 25 to 30% reduction reported; theoretically, a nitrogen reduction of 40 to 50% is possible. In a previous research study, total nitrogen excretion from pigs was reduced 28% by supplementing four essential synthetic amino acids (lysine, threonine, tryptophan, methionine) in a 10% crude protein corn-soy diet as compared to a standard 13% crude protein corn-soy diet. Altering the ratio of nitrogen excretion in urine and feces is a potential means for reducing ammonia emissions. By reducing the nitrogen excretion in urine as urea, which is the primary precursor for ammonia volatilization, and shifting the nitrogen excretion into the feces, which is primarily in the form of bacterial protein, ammonia volatilization is reduced. Changing the carbohydrate structure in the diet to increase bacterial utilization of nitrogen in the cecum and colon results in a significant reduction of nitrogen excretion in urine. Recent research in human and poultry nutrition suggests that the addition of small amounts of certain dietary fiber or oligosaccharides changes the bacterial populations in the lower gastrointestinal tract and reduces the amount and form of nitrogen excretion. The objectives of this study were to determine the sources and concentrations of odorous compounds in cecal contents, fresh manure and anaerobically stored manure from swine, and to determine the effects of dietary nitrogen and carbohydrate manipulation on production of odorous compounds.

### **Materials and Methods**

Three groups of four crossbred gilts (12 total), averaging 120 lb, were surgically cannulated in the cecum using standard procedures. The pigs were fed each of four diets in 4x4 Latin Square designed experiment with three replicate trials. Dietary treatments were:

1. 13% crude protein diet, standard commercial diet;

2. 10% crude protein with four synthetic essential amino acids to meet the NRC requirements of the pigs (lysine, threonine, tryptophan and methionine), amino acid supplemented diet;
3. the amino acid supplemented diet with 2% sucrose thermal oligosaccharide caramel (STOC); and
4. the amino acid supplemented diet with 5% cellulose.

All diets were corn-soy based, fortified with a vitamin and mineral premix and tylan. After a 2-week adaptation to each diet and placement in digestibility crates, cecal samples were collected on three separate days, four hours after feeding. Manure and urine samples were collected over a 24-hour period on three separate days. The manure and urine were mixed and initial manure samples were obtained; subsamples were used for 90-day anaerobic incubations. During the incubations, fresh manure was added three times per week, using typical manure production values for the flask volume. Separate gas samples were collected from the daily cecal and fresh manure samples. Gas samples were collected from anaerobic incubation flasks at 60 and 90 days after initiation of the study. Liquid matrix subsamples were acidified and analyzed for short chained volatile fatty acids (VFA), and ammonia, total nitrogen, dry matter content, and pH were determined.

## Results and Discussion

The use of synthetic amino acids while reducing levels of natural protein sources in the diet and inclusion of 2% STOC or 5% cellulose significantly affected nitrogenous compounds and pH in fresh manure and manure stored in anaerobic incubations. Other odorous compounds in the headspace from cecal, fresh manure and anaerobic manure varied in composition and concentrations between diets.

Results for cecal contents are given in Table 1. Ammonium nitrogen concentrations (on a dry matter basis) were reduced ( $P < .05$ ) in pigs fed the addition of 5% cellulose to the diet as compared to the standard diet. The ammonium nitrogen levels of the amino acid supplemented diet without additional carbohydrate sources and the STOC-containing diet were in between. Total nitrogen, dry matter, pH and VFAs were not different in pigs fed the different diets.

Results for fresh manure are given in Table 2. All amino acid supplemented diets significantly reduced ( $P < .05$ ) pH, ammonium nitrogen and total nitrogen compared to the standard diet. However, the addition of 5% cellulose further reduced ammonium nitrogen concentrations and total nitrogen (on a dry matter basis) as compared to the rest of the diets. Dry matter level was higher from pigs fed the 5% cellulose diet compared to the other diets. The pH was reduced 0.74 units by the addition of synthetic amino acids to the diet, 0.59 units with STOC addition, and 1.27 units with 5% cellulose added ( $P < .01$ ). The pH of 6.4 in fresh manure from pigs fed the cellulose-containing diet significantly reduced ammonia evolution in the air. The pH of urine was significantly reduced from 7.3 to 5.4 by the addition of 5% cellulose in the diet (data not shown). Ammonium nitrogen excretion was reduced 34%, 37% and 68% ( $P < .01$ ) on a dry matter basis with the addition of synthetic amino acids to the diet, addition of 2% STOC and addition of 5% cellulose, respectively. A similar trend was noted with total nitrogen concentrations.

The only changes in volatile fatty acid concentrations in fresh manure were higher acetic, butyric and total VFA concentrations from pigs fed the diet containing cellulose ( $P < .05$ ). Propionic acid tended

to be increased with the 5% cellulose diet, however not significantly. The increased VFA production gives evidence that the additional cellulose enhanced microbial activity in the colon, which was expected. With the reduction in nitrogen excretion, it appears that greater nitrogen in the colon was utilized in bacterial protein synthesis, which the additional cellulose stimulated. Because of residence time in the digestive system, treatment differences were noted more in the colon as compared to the cecum.

Similar responses to the diets were evident during the anaerobic incubation of the manures (Table 3). The pH of manure from pigs fed the amino acid supplemented diet with and without STOC and cellulose were lower ( $P < .05$ ) than the standard diet. The pH in stored manure was reduced 1.02, 1.00 and 1.40 pH units from pigs fed the synthetic amino acid supplemented diet, the STOC-containing diet and the cellulose-containing diet, respectively, as compared to the standard diet. Ammonium and total nitrogen concentrations in stored manure had similar dietary treatment effects as fresh manure. Ammonium nitrogen concentrations on a wet basis were reduced by 54%, 62% and 73% ( $P < .05$ ) from pigs fed the amino acid supplemented diet, the STOC-containing diet and the cellulose-containing diet, respectively, compared to the standard diet. Similarly, total nitrogen levels were reduced by 47%, 55% and 35% ( $P < .05$ ) with the amino acid supplemented diet, the STOC-containing diet and the cellulose-containing diet, respectively, as compared to the standard diet.

Acetic acid was the predominant VFA in the stored manure, especially with the standard diet. Total VFA concentrations were highest for both the standard and amino acid supplemented diets, with the diets containing cellulose or STOC having lower VFA contents. The highest molar percent of propionate was observed with stored manure from pigs fed the cellulose or STOC diets. The elevated butyric acid concentrations were a negative response which could cause an odor concern. Further work is needed to effectively manipulate microbial decomposition to shift the microbes from degradation reactions creating butyrate. Possibly there is still an imbalance in C:N ratio or insufficient specific carbohydrate source to promote enhanced microbial decomposition. The lower concentrations of VFAs in manure from pigs fed the 2% STOC and 5% cellulose diets are advantageous; this needs further study because of the lower volatile organics in the air.

Analysis of gas collected from cecal contents has identified several predominant compounds, as listed in Table 4. Phenols, alcohols, sulfur-containing compounds and organic acids were present. Some of the same compounds were identified in gas collected from fresh manure (Table 5) at relatively lower concentrations. There was a reduction in volatile organic compound concentrations between the cecum and fresh manure samples. Addition of 2% STOC or 5% cellulose was variable in influencing concentrations of specific volatile organic compounds found in cecal contents, fresh and stored manures. Prevalent concentrations of dimethyl sulfide, dimethyl disulfide, carbon disulfide, 2,2-dimethyl hexane, 2 ethyl-1-hexanol and benzene were observed in gas collected from anaerobically stored manures (Table 6). It was noted that there were significantly less prevalent numbers of volatile organic compounds identified from fresh manure compared to cecal content and especially from anaerobically stored manure compared to fresh manure. The predominant compounds remaining in the anaerobically stored manures were sulfur-containing compounds, and probably resulted from excess levels or imbalances of sulfur-containing amino acids in the pig's diet.

## Summary

Based upon this study, reducing the crude protein level of pig diets and supplementing with essential amino acids to meet the pig's lean tissue production significantly reduced total nitrogen excretion and ammonium nitrogen concentrations, and altered the concentrations and ratios of selected volatile fatty acids and other odorous compounds identified in fresh manure. In addition, low level addition of an oligosaccharide (sucrose thermal oligosaccharide caramel) and cellulose further reduced total nitrogen and especially ammonium nitrogen in fresh manure, indicating a stimulation of microbial fermentation in the colon. Similar more pronounced results were observed with anaerobically stored manures. A significant added benefit with cellulose in the diet was the reduction of pH of fresh and stored manure, which would control ammonia volatilization. Ammonium and total nitrogen in fresh manure (wet basis) was the lowest from pigs fed the 5% cellulose diet (49% and 33% reduction), with moderate levels in fresh manure from the amino acid supplemented diet (27% and 23% reduction) and 2% STOC diet (24% and 17% reduction), and the highest levels excreted in the standard diet. Acetic, butyric and total VFA were higher in fresh manure from the pigs fed the 5% cellulose diet compared to other diets. Ammonium and total nitrogen in anaerobically stored manure were reduced from pigs fed the 5% cellulose diet (73% and 35%) and 2% STOC diet (62% and 55%) as compared to the standard diet. The pH was reduced in fresh manure and anaerobically stored manure from pigs fed the 5% cellulose diet (1.27 and 1.40 units), 2% STOC diet (.59 and 1.00 units), and the amino acid supplemented diet (.74 and 1.02 units), with the 5% cellulose diet causing the most pH reduction. Sulfide compounds such as dimethyl sulfide and dimethyl disulfide, along with various organic acids and phenols, were additional predominant compounds emitted from swine manures and cecal contents.

## Implications

Reducing the crude protein levels of a typical corn-soy based diet and balancing with essential amino acids resulted in more efficient nitrogen utilization by the pig, and less excretion of nitrogenous compounds and odors in manures. Similarly, using the two different types of carbohydrates in this study resulted in reduced excretion of nitrogen and some odorous compounds. The relative costs of these diets and pig performance studies need to be determined for implementation into production units.

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Table 1. Effect of diet on pH, nitrogen components and volatile fatty acids in cecal contents.<sup>1</sup>

Diet (% CP)	pH	DM %	NH <sub>3</sub> -N %DM	TKN <sup>2</sup> %DM	Volatile Fatty Acids <sup>3</sup> , mmole/L						
					Ac	Pro	iBut	But	iVal	Val	Total
Standard (13)	5.28	12.8	0.348 <sup>a</sup>	2.96	107.1	61.6	0.69	24.9	1.31	8.15	203.8
Suppl. (10+AA)	5.25	13.3	0.325 <sup>ab</sup>	3.01	99.7	58.8	0.60	21.8	0.76	7.84	189.5
STOC (10+AA)	5.17	12.9	0.330 <sup>ab</sup>	3.06	96.1	59.4	0.51	23.6	0.76	9.94	190.3
Cellulose (10+AA)	5.32	13.5	0.288 <sup>b</sup>	2.62	105.3	57.7	0.96	20.2	0.97	6.48	191.6

<sup>1</sup> Different letter superscripts within a column indicate means are significantly different (P<.05).

<sup>2</sup> TKN = Total Kjeldahl nitrogen.

<sup>3</sup> Volatile fatty acids: acetic (Ac), propionic (Pro), isobutyric (iBut), butyric (But), isovaleric (iVal), valeric (Val).

Table 2. Effect of diet on pH, nitrogen components and volatile fatty acids in fresh manure.<sup>1</sup>

Diet (% CP)	pH	DM %	NH <sub>3</sub> -N %DM	TKN <sup>2</sup> %DM	Volatile Fatty Acids <sup>3</sup> , mmole/L						
					Ac	Pro	iBut	But	iVal	Val	Total
Standard (13)	7.67 <sup>a</sup>	12.6 <sup>b</sup>	5.33 <sup>a</sup>	10.91 <sup>a</sup>	60.3 <sup>b</sup>	23.9	1.89	11.9 <sup>b</sup>	3.33	4.83	106.0 <sup>b</sup>
Suppl. (10+AA)	6.93 <sup>b</sup>	14.7 <sup>b</sup>	3.54 <sup>b</sup>	7.02 <sup>b</sup>	56.8 <sup>b</sup>	24.5	1.64	12.2 <sup>b</sup>	3.58	4.88	103.5 <sup>b</sup>
STOC (10+AA)	7.08 <sup>b</sup>	15.3 <sup>b</sup>	3.36 <sup>b</sup>	7.14 <sup>b</sup>	58.2 <sup>b</sup>	26.4	2.15	14.5 <sup>b</sup>	3.76	5.85	110.5 <sup>b</sup>
Cellulose (10+AA)	6.40 <sup>b</sup>	18.9 <sup>a</sup>	1.73 <sup>c</sup>	4.33 <sup>c</sup>	69.0 <sup>a</sup>	30.7	2.07	18.5 <sup>a</sup>	3.96	6.88	131.1 <sup>a</sup>

<sup>1</sup> Different letter superscripts within a column indicate means are significantly different (P<.05).

<sup>2</sup> Total Kjeldahl nitrogen.

<sup>3</sup> Volatile fatty acids: acetic (Ac), propionic (Pro), isobutyric (iBut), butyric (But), isovaleric (iVal), valeric (Val).

Table 3. Effect of diet on pH, nitrogen components and volatile fatty acids in anaerobically stored manure.<sup>1</sup>

Diet (% CP)	pH	DM %	NH <sub>3</sub> -N mg/L	TKN <sup>2</sup> mg/L	Volatile Fatty Acids <sup>3</sup> , mmole/L						
					Ac	Pro	iBut	But	iVal	Val	Total
Standard (13)	8.32 <sup>a</sup>	9.12 <sup>a</sup>	7432 <sup>a</sup>	10322	300	21.1	6.06	15.99	18.40	3.84 <sup>b</sup>	365.2
Suppl. (10+AA)	7.30 <sup>b</sup>	6.56 <sup>b</sup>	3454 <sup>b</sup>	5488	238	31.8	7.30	47.47	14.20	14.59 <sup>a</sup>	353.7
STOC (10+AA)	7.32 <sup>b</sup>	6.02 <sup>b</sup>	2813 <sup>b</sup>	4640	152	25.0	7.42	33.53	14.42	15.85 <sup>a</sup>	248.2
Cellulose (10+AA)	6.92 <sup>b</sup>	6.42 <sup>b</sup>	2010 <sup>b</sup>	6751	163	24.9	5.36	34.53	11.59	15.54 <sup>a</sup>	254.8

<sup>1</sup> Different letter superscripts within a column indicate means are significantly different ( $P < .05$ ).

<sup>2</sup> Total Kjeldahl nitrogen.

<sup>3</sup> Volatile fatty acids: acetic (Ac), propionic (Pro), isobutyric (iBut), butyric (But), isovaleric (iVal), valeric (Val).

Table 4. Effect of diet on relative organic compound concentrations in headspace air from cecal contents.

Compound	Diet (% CP)			
	Standard (13)	Suppl. (10+AA)	STOC (10+AA)	Cellulose (10+AA)
	ml/gm of cecal contents/L of gas			
Ethanol	6.88	5.12	5.91	6.00
1-Propanol	4.01	3.06	4.00	2.77
1-Butanol	0.72	0.56	0.22	0.40
Methyl phenol	0.26	0.23	0.28	0.21
n-Propyl acetate	2.03	2.04	1.77	1.32
Propanoic acid	0.96	0.43	0.51	0.95
Propanoic acid, ethyl ester	0.21	0.50	0.55	0.86
Propanoic acid, methyl ester	0.30	0.12	0.14	0.09
Propanoic acid, butyl ester	0.29	0.17	0.16	0.15
Propanoic acid, propyl ester	0.64	0.79	0.89	0.71
Propanoic acid, pentyl ester	0.11	0.11	0.11	0.00
Butanoic acid	1.00	1.26	1.07	1.45
Butanoic acid, ethyl ester	0.71	0.79	0.88	0.90
Pentanoic acid	0.94	1.33	0.74	0.99
Pentanoic acid, propyl ester	0.20	0.20	0.14	0.20
Pentanoic acid, ethyl ester	0.26	0.27	0.41	0.26
Hexane	0.17	0.11	0.00	0.03
2,2-Dimethyl hexane	2.96	2.70	3.33	2.78
Benzene	1.96	1.56	2.68	2.30
Ethanethioic acid, s-methyl ester	2.64	2.40	2.30	1.58
Butanethioic acid, s-methyl ester	0.18	0.11	0.24	0.07
3-Methyl-butanoic acid	0.26	0.25	0.31	0.11
Butanoic acid, propyl ester	0.37	0.46	0.53	0.34
Dimethyl sulfide	0.08	0.62	0.16	0.50
Dimethyl disulfide	3.90	3.23	3.67	3.84
Dimethyl trisulfide	0.04	0.00	0.02	0.00
S-methyl propanethionate	1.97	1.80	1.49	1.30
Dimethyl sulfoxide	0.07	0.23	0.19	0.23
Ethyl acetate	1.55	2.38	1.39	1.58
2-Butanone	2.00	3.00	0.00	0.00
Acetone	0.64	0.31	0.48	0.38
Acetic acid	0.13	0.64	0.33	0.21
Acetic acid, butyl ester	0.33	0.26	0.26	0.15

Table 5. Effect of diet on relative organic compound concentrations in headspace air from fresh manure.

Compound	Diet (% CP)			
	Standard (13)	Suppl. (10+AA)	STOC (10+AA)	Cellulose (10+AA)
	ml/gm of manure/L of gas			
Ethanol	0.44	0.29	0.28	0.35
1-Propanol	0.12	0.10	0.06	0.10
Methyl phenol	0.14	0.15	0.20	0.24
n-Propyl acetate	0.01	0.00	0.00	0.02
Propanoic acid, ethyl ester	0.02	0.07	0.07	0.03
Butanoic acid	0.00	0.01	0.00	0.00
Butanoic acid, ethyl ester	0.00	0.00	0.01	0.01
Benzene	0.14	0.21	0.19	0.34
2,2-Dimethyl hexane	0.37	0.40	0.24	0.39
Ethanethioic acid, s-methyl ester	0.02	0.08	0.07	0.05
Dimethyl sulfide	0.09	0.30	0.33	0.27
Dimethyl disulfide	0.50	0.68	0.77	0.65
Dimethyl trisulfide	0.04	0.10	0.11	0.06
S-methyl propanethionate	0.00	0.02	0.01	0.00
Dimethyl sulfoxide	0.06	0.04	0.10	0.05
Carbon disulfide	0.02	0.07	0.10	0.09
Ethyl acetate	0.03	0.06	0.02	0.03
2-Butanone	0.00	0.01	0.04	0.06
2-Pentanone	0.02	0.01	0.02	0.03
3-Pentanone	0.04	0.02	0.03	0.01
Acetone	0.14	0.12	0.08	0.08



Table 6. Effect of diet on relative organic compound concentrations in headspace air from anaerobically stored manure.

Compound	Diet (% CP)			
	Standard (13)	Suppl. (10+AA)	STOC (10+AA)	Cellulose (10+AA)
	ml/gm of manure/L of gas			
Ethanol	0.01	0.01	0.00	0.00
Acetone	0.00	0.00	0.01	0.01
Ethyl acetate	0.01	0.02	0.00	0.02
2,2 Dimethyl hexane	0.11	0.09	0.05	0.14
Benzene	0.07	0.13	0.04	0.04
Dimethyl sulfide	0.20	0.08	0.16	0.05
Dimethyl disulfide	0.16	0.23	0.15	0.14
Dimethyl sulfoxide	0.01	0.02	0.02	0.01
Carbon disulfide	0.01	0.05	0.07	0.14
Dimethyl trisulfide	0.00	0.01	0.01	0.01
2-Ethyl-1-hexanol	0.28	0.30	0.24	0.30