Final Grant # 98-004 Report
National Pork Producer
09/30/2000

I. Project title

Effect of dietary protein on endogenous amino acid contribution to microbial production of odorous metabolites in the intestinal tract of the growing pig**- Project identification number** 98-004.

Principle Investigator: Nathalie L. Trottier/ Melvin T. Yokoyama

Institution: Michigan State University

II Abstract

Growing pigs were used to test whether decreasing dietary crude protein concentration could reduce nitrogen excretion, ammonia emission, volatile organic compounds (VOC) and odor, and to determine the gut endogenous contribution to these components. Another goal was to determine the amino acid digestibility as affected by dietary crude protein reduction. Six diets were fed in a latin square design. The diets consisted of 15, 12, 9 and 6% CP corn-soybean meal (CSBM) based diets, a 15% CP casein based diet and a protein-free diet. The protein-free diet and the casein diet were used to determine endogenous protein contribution to fecal nitrogen, ammonia, and VOC. The 12, 9 and 6% CP CSBM diets were obtained by diluting the 15% CP CSBM diet with cornstarch in order to maintain equal AA profile arising from intact protein sources, i.e., corn and soybean meal. In order to meet the amino acid requirements of the growing pig, diet formulation was based on the NRC (1998) ideal apparent amino acid digestibility pattern. Crystalline amino acids were added to the 12, 9 and 6% CP CSBM diets. Among them, glutamic acid was added to balance the essential amino acid (EAA) to non-essential amino acid (NEAA) ratio. Pigs were housed in metabolism cages to allow fecal and urinary collection. Reducing dietary crude protein resulted in a linear decrease in total nitrogen excretion ($P<.05$) and a quadratic decrease in urinary excretion ($P < .05$). This was accompanied by a reduction in ammonia emission ($P < .05$). Reduction of CP beyond 9% did not yield significant reduction in N excretion and ammonia emission, which were similar to that of pigs fed protein free diet. Thus, in pigs fed diets containing 9 and 6% CP, nitrogen excretion and ammonia emission were mainly of endogenous origin. Reducing crude protein and including amino acids improved nitrogen utilization linearly ($P < .05$) and amino acid digestibility ($P < .05$). Volatile fatty acids concentration from slurry increased with decreasing crude protein concentration ($P < .05$). The phenolic compounds were not detected to significant levels in any of the dietary treatment groups. The odor panel showed an increase in odor offensiveness as intact CP decreased. Comparing reduced intact CP diets to the 15% CP CSBM diet, only the 9 and 6% CP CSBM diets were found to be more offensive ($P<0.05$) with qualitative rankings of "moderately" offensive. The order of offensiveness (least to most) for the remaining treatments are as follows: protein-free, 12% CP CSBM , 15% CP casein, 2.70, 2.77, and 2.81, respectively, with qualitative rankings equivalent to "mild-moderately" offensive. In conclusion, reducing

dietary crude protein to 9% was sufficient to minimize nitrogen excretion and ammonia emission while maintaining nitrogen retention similar to pigs fed a standard 15% corn-soybean meal based diet. However, the reduction in dietary crude protein was accompanied by an increase in volatile fatty acids and odor offensiveness.

III Introduction

Nitrogen (N), ammonium (NH_4^+) and volatile organic compounds (VOC) are major components of pig manure that have negative effects on the environment. Environmental pollution results from N contamination of surface and ground water, and emissions of noxious odors, ammonia (NH_3), and VOC into the air. People exposed to noxious odors and NH_3 experience eye and respiratory irritations, headache and drowsiness. Elevated NH_3 concentrations in swine houses decreases productivity of reproductive females and increases incidence of respiratory illness. Odorous compounds in feces and urine originate from fermentation or metabolism of undigested and(or) unabsorbed proteins and amino acids not utilized. Ammonium originates from oxidative deamination of amino acids through removal of " -amino groups. Toxic NH_4^+ is converted to urea in the liver and excreted in the urine. Dietary manipulation, such as decreasing dietary crude protein (CP) concentrations may be of immediate benefit to swine producers. Appropriate level of crude protein remains to be determined. Therefore, this study focused on three major aspects. First, we determined the minimum dietary CP needed to attain N excretion rate, NH_3 emission, odor offensiveness score and VOC concentrations equivalent to that contributed by endogenous proteins alone. Second, we quantified the endogenous proteins contribution to N excretion rate. Third, we tested whether N retention could be maintained in pigs fed reduced CP diets containing 80, 60 and 40% of the Control diet's intact protein concentration.

IV Objectives

The **main objective** of this project was to quantify the malodorous metabolites (e.g. volatile fatty acids, volatile phenolic, and aromatic metabolites) produced by amino acids originating from dietary, endogenous, and microbial sources in growing pigs fed different dietary protein levels and quality. The quality was tested using an intact protein based diet formulated with casein. The endogenous source was determined using a protein free diet.

Specific Objectives:

A. To determine the amount of amino acids in the endogenous secretions, feces, and urine of growing pigs fed different dietary protein levels and quality.
B. To determine the amount of odorous volatile fatty acids and volatile phenolic and indolic metabolites in feces and urine of growing pigs fed different dietary protein levels and quality.
C. To measure fecal, apparent, and true amino acid digestibility in growing pigs fed different dietary protein levels and quality.
D. To conduct an olfactory panel evaluation of the manure odor produced form feeding the different dietary protein levels and quality.

V Procedures

1) Objectives B and D

Animals, Experimental design and Diets

The experiment was approved by the Michigan State University All University Committee on Animal Use and Care (approval number #:03/97-0). Six crossbred ((Yorkshire x Landrace) x Duroc) barrows, with an initial BW of 44.67 kg ± 1.85 kg were allocated to six dietary treatments in a 6 x 6 Latin square design. Pigs were penned individually in stainless steel metabolism cages (1.2 m x 0.75 m) equipped with low-pressure water nipples providing free access to water. Pigs were housed in an environmentally controlled room maintained at 21° C.

The six diets consisted of 15, 12, 9 and 6% CP corn-soybean meal (CSBM) based diets, a 15% CP casein based diet and a protein-free diet. The protein-free diet and the casein diet were used to determine endogenous protein contribution to fecal nitrogen, ammonia, and VOC. Diet ingredient and nutrient composition are provided in the following tables (Tables 1, 2, and 3).

The 12, 9 and 6% CP CSBM diets were obtained by diluting the 15% CP CSBM diet with cornstarch in order to maintain equal AA profile arising from intact protein sources, i.e., corn and soybean meal. In order to meet the amino acid requirements of the growing pig, diet formulation was based on the NRC (1998) ideal apparent amino acid digestibility pattern. Because we formulated our diets on an apparent digestibility basis, the dietary protein concentrations were decreased from what was originally proposed. Hence, instead of using 18% CP as our control diet, we used 15% CP. Crystalline amino acids were added to the 12, 9 and 6% CP CSBM diets. Among them, glutamic acid was added to balance the essential amino acid (EAA) to non-essential amino acid (NEAA) ratio. Feed was provided at 3.5% BW and divided into three equivalent meals per day (8:00, 12:00 and 16:00). To reduce feed wastage, water was added to the meal (approximately 100 mL/300 g) and mixed to form a mash. Body weights were measured on d 1 of each period.

Table 1: Ingredient composition of experimental diets (as fed)

Ingredient, %	15%	12%	9%	6%
Corn	74.56	59.65	44.74	29.82
Soybean meal, 44% CP	20.00	16.00	12.00	8.00
Corn starch[a]	-	12.57	29.87	46.33
Sucrose	-	5.00	5.00	5.00
Corn oil	2.44	3.00	3.00	3.00
Solka floc[b]	-	0.60	1.20	1.75
Dicalcium Phosphate	0.70	0.70	0.70	0.70
Limestone	0.70	0.70	0.70	0.70
Vitamin premix[c]	0.60	0.60	0.60	0.60
Mineral premix[d]	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50
Sowpac vitamin premix[e]	-	-	0.03	0.05
Amino Acids[f]				
L-Lysine-HCl, 78.8%	-	0.149	0.296	0.444
L-Threonine	-	0.035	0.119	0.202
L-Valine	-	-	0.072	0.185
L-Isoleucine	-	-	0.041	0.140
L-Leucine	-	-	-	0.150
L-Phenylalanine	-	-	-	0.125
DL-Methionine	-	-	0.042	0.084
L-Tryptophan	-	-	0.024	0.049
L-Histidine	-	-	-	0.063
L-Glutamate	-	-	0.572	1.609

[a] Argo Foods, CPC International, Inc., Englewood Cliffs, NJ 07632-9976.

[b] Harland Teklad, Madison, WI 53744-4220.

[c] Provided per kg of premix: vit. A 918583 IU, vit. D_3 91858 IU, vit. E 11023 IU, vit. K 735 mg, riboflavin 735 mg, pantothenic acid 2939 mg, niacin 4409 mg, vitamin B_{12} 5512 mcg, thiamin 184 mg, pyridoxine 165 mg.

[d] Provided per kg premix: Cu 2000 mg, Fe 20000 mg, Zn 20000, Mg 2000 mg, I 30 mg, Se 60 mg.

[e] Provided per kg premix: vit. A 918583 IU, biotin 73487 mcg, choline 128602 mg, folic acid 551 mg.

Table 2. Nutrient composition of experimental diets (as fed)

Item	Dietary treatment			
	15%	12%	9%	6%
Composition, calculated[a]				
Crude protein, %	14.95	12.10	10.23	9.10
ME, kcal/kg	3365	3414	3415	3385
IAA:DAA	45:55	45:55	45:55	45:55
Amino acids, %				
Arginine	0.93	0.75	0.56	0.37
Histidine	0.38	0.31	0.23	0.22
Isoleucine	0.60	0.48	0.40	0.38
Leucine	1.43	1.14	0.86	0.72
Lysine	0.74	0.71	0.67	0.64
Methionine	0.25	0.20	0.19	0.18
Methionine + cystine	0.53	0.42	0.36	0.30
Phenylalanine	0.72	0.58	0.43	0.41
Phenylalanine + tyrosine	1.28	1.03	0.77	0.63
Threonine	0.56	0.48	0.46	0.42
Tryptophan	0.17	0.13	0.12	0.12
Valine	0.70	0.56	0.49	0.46
Composition, analyzed[b]				
Dry Matter, %	90.40	91.74	92.02	92.09
Crude protein, %	14.64	12.25	11.03	9.85
Amino acids, %				
Indispensable				
Arginine	0.89	0.81	0.65	0.53
Histidine	0.37	0.33	0.27	0.26
Isoleucine	0.56	0.51	0.46	0.49
Leucine	1.39	1.26	1.07	0.95
Lysine	0.70	0.69	0.68	0.65
Methionine	0.28	0.26	0.28	0.31
Methionine + cystine	0.47	0.43	0.43	0.43
Phenylalanine	0.70	0.59	0.51	0.56
Phenylalanine + tyrosine	1.23	1.04	0.89	0.85
Threonine	0.50	0.46	0.44	0.44
Valine	0.64	0.58	0.56	0.62
Dispensable				
Alanine	0.88	0.76	0.66	0.54
Aspartate + asparagine	1.34	1.14	0.95	0.73
Cystine	0.19	0.17	0.15	0.12
Glutamate + glutamine	2.73	2.33	2.39	2.86
Glycine	0.58	0.49	0.41	0.33
Proline	0.97	0.84	0.73	0.58
Serine	0.71	0.60	0.51	0.41
Tyrosine	0.53	0.45	0.38	0.30

Table 2 continued

[a] Total dietary amino acid concentration calculated using analyzed amino acid concentration in corn and NRC (1998) amino acid concentration in soybean meal. Lysine concentration calculated using both corn and soybean meal analyzed value.

[b] Values represent averages across three batches of diets.

Table 3: Ingredient composition of semi-purified experimental diets (as fed)

Ingredient, %	Casein	Protein-free
Corn starch[a]	64.52	81.52
Casein[b]	17.00	-
Sucrose	5.40	5.40
Corn oil	4.00	4.00
Solka floc[b]	4.00	4.00
Dicalcium phosphate	2.70	2.70
Vitamin premix[c]	0.60	0.60
Mineral premix[d]	0.50	0.50
Potassium chloride[e]	0.40	0.40
Limestone	0.30	0.30
Salt	0.25	0.25
Sowpac vitamin premix[f]	0.25	0.25
Magnesium oxide[g]	0.08	0.08

[a] Argo Foods, CPC International, Inc., Englewood Cliffs, NJ 07632-9976

[b] Harland Teklad, Madison, WI 53744-4220

[c] Provided per kg of premix: vit. A 918583 IU, vit. D_3 91858 IU, vit. E 11023 IU, vit. K 735 mg, riboflavin 735 mg, pantothenic acid 2939 mg, niacin 4409 mg, vitamin B_{12} 5512 mcg, thiamin 184 mg, pyridoxine 165 mg.

[d] Provided per kg premix: Cu 2000 mg, Fe 20000 mg, Zn 20000, Mg 2000 mg, I 30 mg, Se 60 mg.

[f] Provided per kg premix: vit. A 918583 IU, biotin 73487 mcg, choline 128602 mg, folic acid 551 mg.

Sample Collection

The experiment consisted of six collection periods. Each period lasted 10 d. Feces and urine were collected for a duration of 5 d from each pig following a 5-d adaptation period to the diets. Ferric oxide was used as an indigestible marker to indicate the initiation and termination of collection for fecal matter. Each pig received 5 g of ferric oxide added to 100 g of respective dietary treatment, mixed thoroughly with 125 mL of water at the first meal on d 1 and d 6. The remaining meal allotment was fed after the ferric oxide-feed mixture was consumed completely. Total daily fecal samples were collected, weighed and stored at 4 /C for 5 d. Fecal samples were homogenized for 15 min using a Hobart mixer (Model A-200, Hobart Manufacturing, Troy, Ohio). Sub-samples were collected (500 g) and stored in 490-mL plastic containers and frozen at -20 /C. For urine collection, 10 mL of 6 N HCl was added daily to each of the 10-L collection vessels to reduce the pH of the urine and prevent volatilization of NH_4^+. Urine was initially filtered through glass wool as it was collected. The urine was filtered again through four layers of cheese cloth into a clean bucket. Approximately 20% of daily urine volume was stored in 4-L plastic bottles throughout the collection period at 4 /C. At the end of each collection period, the urine was homogenized, and a sub-sample of pooled urine was frozen at -20 /C.

Sample Analysis

For chemical analysis, fecal samples were freeze-dried (VirTis Model 25-SRC, VirTis Co., Gardiner, NY). Fecal and feed samples were finely ground using a cyclone mill (Cyclotec Sample Mill 1093, Sweden) with a 1-mm screen. Nitrogen content in feces and feed was analyzed using the LECO nitrogen analyzer (LECO® FP-2000, LECO Co., St. Joseph, MI, AOAC #990.03). Dry matter in feces and feed was determined by drying 2-g samples overnight at 60/C using a vacuum oven (Model 583/Full View, National Appliance Co., Portland, Oregon). Urine was filtered using Whatman filter paper and nitrogen concentration measured using the same procedure as described for feed and feces. Amino acid analysis was performed on feed samples using the Pico"Tag ® method (Waters Co., Milford, MA) following a 24-h acid hydrolysis in 6 N HCl at 105 /C and 121 mm Hg. Samples were brought up to volume (40 mL) and filtered using Whatman filter paper. The amino acid filtrate was sub-sampled and dried using vacuum centrifugation. The amino acid hydrosylate was reconstituted, dried again, derivatized with PITC® and separated using a Waters high pressure liquid chromatographer (Waters Co., Milford, MA) fitted with a 15 cm hydrosylate column.

Ammonia Emission and Odor Panel

Approval for use of human subjects was granted by University Committee on Research Involving Human Subjects (Approval IRB# 99284, Category 1-C). At the conclusion of each collection period, stock slurries were prepared for NH_3 emission and odor panel. A sub-sample (200 g) of fresh homogenized feces were added to 1000 mL of fresh homogenized urine for each pig. The stock slurries were stored in 3.8 L plastic containers at room temperature (21/C) and fermented 30 d. After the fermentation period, slurries were shaken vigorously and 25 mL of each slurry was placed into a 100-mL glass beaker in duplicate, covered tightly with aluminum foil and allowed to ferment an additional 24 h for the NH_3 emission test. Using Gastec ammonia detector tubes (Gastec Corp., Gastec Detector Tube No.3M, Japan) the foil seal was punctured and head space air was sampled approximately 2.5 cm above the slurry surface at a rate of 100 mL@min^{-1}. After air sampling, each slurry sample was agitated and covered with aluminum foil and the procedure was repeated at 48 and 72 h. Ammonia was recorded as ppm per 100 mL air per min. For the odor panel, six sub-samples of slurry per diet (10-mL each) were prepared from the stock slurries. Each sub-sample was poured into a plastic vial containing a cotton ball and capped. Every sample set contained all diets per collection period. The six sets of vials were allowed to rest for 1 h and double randomized prior to panelist evaluation. Volunteers were asked to sniff each sample individually and rest a minimum three minutes before sniffing the next sample. Individuals were asked to classify the severity of odor offensiveness. Offensiveness was classified on severity of 1 to 5 scale: (1) none, (2) mildly, (3) moderately, (4) strongly, or (5) extremely offensive. Responses were marked following the sniffing of each sample.

Volatile Organic Compounds

Volatile Fatty Acids. Volatile fatty acids (VFA) in feces were determined for each pig, period and diet. Fecal samples were thawed and a 2-g sample was taken. Each sample was diluted with 8 mL of distilled water and 2 drops of concentrated HCl in a centrifuge tube, mixed using a vortex and centrifuged at 17,400 g for 10 min. The supernatant was filtered using .22 µm filter (Millipore Co., Bedford, MA 01730) and pipetted into sterile 2.0 mL gas chromatograph (GC) vials (SUPELCO, 2-mL, Cat. No.

27265, Supelco Park, Bellefonte, PA 16823-0048). The VFA concentrations were determined using a gas chromatograph (Varian Model 3700 FID, Varian, Inc., Walnut Grove, California, 94598) with a 1.85 m x 32 mm column (15% SP1220 @ 1% phosphoric acid on 80-100 Chromosorb). Nitrogen was used as a carrier gas and flow rate was set at 25 mL @ min^{-1}. Air and hydrogen gas was used for combustion and flow rates were set at 30 mL @ min^{-1}. Injection temperature was 110°C and held for 5 min, then temperature was ramped at 3°C @ min^{-1} until final temperature of 127 °C was reached and held for 3 min. A standard solution containing 10 mmol @ mL^{-1} of each of the following VFA: acetate, propionate, isobutyrate, butyrate, isovalerate and valerate was injected (2 µL) and a standard curve determined. Experimental samples (2 µL) were manually injected.

Phenolic Compounds. Urine samples were thawed and centrifuged at 27,200 g for 25 min. Supernatant was filtered through Whatman paper (size 4) and 1 mL samples were pipetted in duplicate into 2-mL GC crimp top vials (SUPELCO, 2-mL, Cat. No. 27058, Supelco Park, Bellefonte, PA 16823-0048) containing 0.5 mL salt solution (3 ppm NaCl). The samples were analyzed using a gas chromatograph-mass spectrophotometer (Varian Gas Chromatograph CP-3800, Varian Mass Spectrophotometer Saturn 2000, Varian, Inc.,Walnut Creek, California 94598). Two different polar solvent absorption fibers were used. A polyacylate solvent absorption fiber for samples from protein-free, 6% and 9% CP CSBM diets. A polydimethylsiloxane (PDMS) solvent absorption fiber was used for samples from 15% CP casein,12 and 15% CP CSBM diets.

Statistical Analysis

Nitrogen balance data were analyzed using the Mixed procedures of SAS (1999). The dependent variables nitrogen intake (NI), fecal nitrogen output (FN), urinary nitrogen output (UN), and total nitrogen output (TNO) were analyzed for a 6 x 6 Latin square. The main effects of pig, period, protein source (CSBM or casein) and diet were included in the statistical model. Differences between least squares means (LSM) values for diet were made using Adjusted Tukey-Kramer Honestly Significantly Difference for the dependant variables NI, FN, UN, TNO . Residual plots of 15% CP casein and protein-free diets revealed high variance as compared with other diets and were therefore removed prior to analysis of the dependant variables. Dependant variables nitrogen retained (NR), nitrogen absorbed (NA), nitrogen digestibility (ND), nitrogen retained as percent of intake (NPI), and nitrogen retained as percent of absorbed (NPA) for a 4 x 6 Latin rectangle including main effects of pig, period and diet. Differences between LSM for dependant variables NR, NA, ND, NPI and NPA were separated using the Bonferroni Least Significant Difference (P value = 0.05 was divided by 6 and significance was estimated at $P<0.01$). Nitrogen intake, TNO, NR, ND, NPI and NPA were regressed against dietary protein concentration (15, 12, 9, and 6% CP CSBM) to determine linear and(or) quadratic relationship using Proc Mixed of SAS (1999). The main effects included in the regression model were pig, period, and diet.

Statistical analysis of the dependant variables NH_3, VFA's, i.e., acetate (ACE), propionate (PROP), isobutyrate (ISOB), butyrate (BUTY), isovalerate (ISOV), and valerate (VAL) and phenolics, i.e., p-Cresol and p-Ethylphenol were performed using the proc Mixed procedure of SAS (1999) in 6x6 Latin square. The model included the main effects of pig, period, and diet. Least square mean differences for NH_3, VFA's, total VFA's and individual VFA as percent of total VFA's, and phenolics were separated by Tukey's multiple comparison test and difference established at $P<0.05$. Isobutyrate was not detected in feces of the 15% CP CSBM diet, therefore to determine LSM estimates for total VFA and individual VFA as percent of total VFA, a value of zero replaced the missing values. Regression of NH_3 emission

to determine linear and(or) quadratic relationship with the main effects of pig, period, and diet was performed using proc Mixed of SAS (1999).

The odor panel results were analyzed using the GENMOD procedure of SAS (2000) which allows assessment of differences in responses when the responses are designated as qualitative values. Estimates with significant differences ($P<0.05$) between diets were reported as log odds ratios. The statistical model included the main effects of person, gender, randomized order, pig, period, and diet to test the dependant variable of odor offensiveness. Only the main effects of pig and diet were found to be significant ($P<0.05$). The significant difference between diets was determined at $P<0.05$. The log odds ratio comparisons of LSM were transformed to an index to better understand the differences in odor. Odor of offensiveness was compared to 15% CP CSBM (index value = 100). The indices for 15% CP casein, protein-free, 6, 9, and 12% CP CSBM were derived by multiplying the appropriate log odds ratio (i.e., 15% CP CSBM vs. 15% CP casein, 15% CP CSBM v. protein-free, 15% CP CSBM vs 6 etc.) by the 15% CP CSBM index value.

2) Objectives A and C

Animals, Diets and Experimental Design

The experiment was approved by the Michigan State University All University Committee on Animal Use and Care (approval number 03/97-0). Six crossbred barrows ((Landrace x Yorkshire) x Duroc), with an initial BW 35.52 ± 0.98 kg were surgically fitted with stainless steel T-cannula at the terminal ileum according to the procedures of Stein et al. (1998). Pigs were treated with preventative antibiotic and anti-inflammatory medication 3 days post-surgery. Pigs were allowed to recover for three weeks in individual pens (1.5 m x .75m) with smooth sided panels made of polyvinyl chloride. Each pen was equipped with a suspended water line fitted with a low-pressure nipple and wire flooring. The environmental temperature was maintained at 21/ C.

Pigs were randomly allocated to six dietary treatments in a 6 x 6 Latin square design. The average BW at the beginning of experimental period was 53.13 ± 1.78 kg. Six dietary treatments consisted of 15% , 12%, 9% and 6% CP corn-soybean meal based diets, a 15% CP casein based and a protein free diets as previously described for Objectives B and D. Feed was provided in three equivalent meals per day (800, 1200 and 1600 h) at 5 x maintenance ($BW^{.75}$, 106 kcal/kg ME). Adjustments to feed intake were made prior to each collection period. Feed allotment for each period was estimated based on weight gains from experiment 1. Body weights were measured at the initiation of the first period and at the termination of the sixth period.

Sample Collection and Analysis

The experiment consisted of six collection periods. Chromic oxide (0.25% of diet) was used as an indigestible marker for determination of digestibility of amino acids. Digesta was collected over two consecutive days for 12 h each day from each pig following a 5-day adaptation period to the diets. Samples were collected in 250-mL disposable baby bottle bags (Playtex) fastened to the cannula by a cable tie. Digesta was kept on ice throughout the sampling period, pooled into 4-L plastic bottles, and stored at 4/C until sampling period was completed. Pooled digesta was homogenized for each pig stored in plastic bottles and frozen. For chemical analysis, digesta samples were freeze dried (Tri-Philizer MP, FTS Systems, Stone Ridge, New York). Digesta and feed samples were finely ground using a cyclone mill

(Cyclotec Sample Mill 1093, Sweden) with a 1 mm screen. Dry matter and amino acid analysis of digesta and feed was determined as described previously.

Statistical Analysis

Digestibility coefficients of each amino acid were analyzed by using the GLM procedures of SAS (2000), in a Latin square design. Least square means were separated by Bonferroni's (Dunn) test. The main effects of pig, period and diet were included in the model.

VI Results

Objective B:

Determine the amount of odorous volatile fatty acids and volatile phenolic and indolic metabolites in feces and urine of growing pigs fed different dietary protein levels and quality.

The primary aim of this study was to determine the effect of CP reduction on N excretion, NH_3 emissions and volatile organic compounds. We had hypothesized that decreasing CP while ensuring an ideal AA balance would maintain N balance, yet reduce N excretion, NH_3 emissions, and VOC to similar levels contributed by endogenous protein. The following table (Table 4) shows the nitrogen excretion results.

Throughout the experiment all pigs remained healthy with an average daily dry matter intake of 1.857 kg ± 0.15 and an average daily gain of 860 g ± 0.09 while consuming one of the five protein containing diets. Feed intake (g/d) was similar for all diets but was lower ($P<0.05$) for 15% CP CSBM and protein-free diets. Fecal matter excretion (g/d, dry matter basis) was found to decrease ($P<0.05$) as intact CP was reduced.

Table 4. Nitrogen excretion in pigs fed diets reduced in dietary protein concentration[a]

Item	15%	12%	9%	6%	Prot-free	Casein	SEM[b]
n	6	6	6	6	6	6	
DM intake, g/d	1780 ± 43	1957	2035	1828	1563	1912 ± 43	41
N Intake, g/d	42.47^{vw}	39.36^{wx}	33.37^{xy}	26.33^{y}	0^{z}	48.81^{v}	1.56
N Output[c], g/d	18.76^{w}	14.79^{x}	11.91^{x}	6.89^{y}	4.04^{y}	14.62^{x}	0.82
N Feces, g/d	8.46^{w}	7.45^{w}	6.66^{w}	3.85^{x}	1.63^{y}	2.02^{xy}	0.48
N Urine, g/d	10.30^{wx}	7.34^{xy}	5.25^{yz}	3.04^{z}	2.41^{z}	12.60^{w}	0.84
N Absorbed[d], g/d	34.02^{w}	31.91^{wx}	26.71^{xy}	22.48^{y}	-1.63^{z}	46.79^{v}	1.49
Fecal DM, g/d	266.38^{w}	245.03^{w}	192.93^{x}	140.40^{y}	110.77^{y}	106.46^{y}	11.12

[a] Least squares means across rows with different superscript are different $P<0.05$.

[b] Standard error of mean

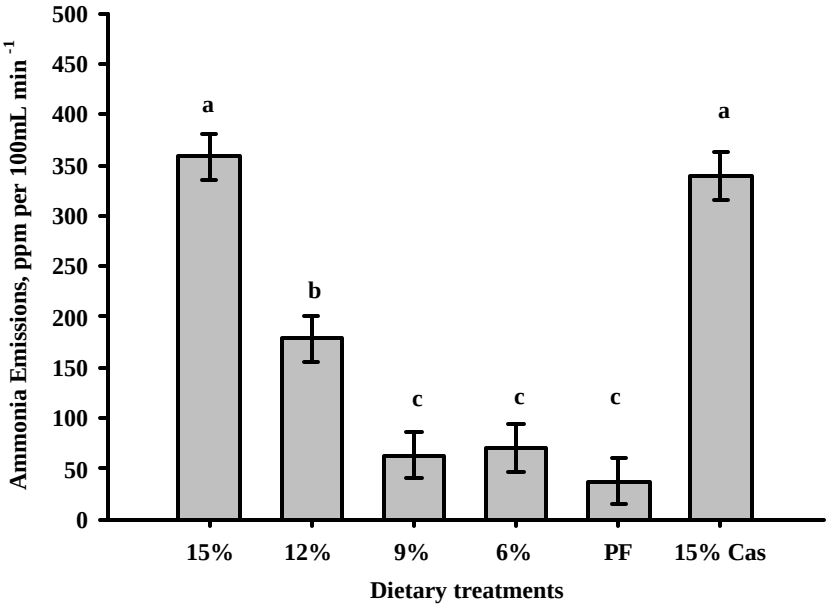
[c] N output = N fecal + N urine

[d] N absorbed = N intake - N fecal

Reducing dietary CP from 15% to 6% CP resulted in a linear decrease of 63% in TNO. Of all the diets fed, only the 6% CP CSBM and the protein-free diets had equivalent TNO indicating that TNO was mainly of endogenous origin when pigs were fed the 6% CP CSBM diet. Because FN was relatively constant when CP was reduced from 15% to 9% in this study, the contribution of FN to TNO reduction was not significant. Fecal N output in this study resulted from the fact that the intact protein is less digestible than CAA. The undigested intact protein was the primary source of FN. While FN did not contribute significantly to TNO, decreasing UN output was significant in reducing total N excretion. Urinary nitrogen was dramatically reduced by 50% when dietary CP was reduced from 15 to 9%. In this study, reducing dietary CP from 15 to 9% was sufficient to minimized UN output. No further significant reduction in UN was observed beyond feeding the 9% CP CSBM diet.

Evaluation of casein, a high quality protein, showed that TNO was not reduced to the same extent as feeding the 6% CP CSBM and protein-free diets. Fecal nitrogen was similar between the 15% CP casein and the 6% CP CSBM and protein-free diets, meaning that endogenous N losses were similar at the gut level and the casein was nearly 100% digested and absorbed. However, the TNO from feeding the 15% CP casein diet increased due to the dramatic increase in UN output. The higher TNO of 15% CP casein can be explained by the reduced efficiency of N utilization resulting from casein's amino acid balance (Officer et al., 1997). Comparing casein's amino acid pattern (NRC, 1998) to the ideal digestible amino acid pattern required by growing pigs (NRC, 1998) showed that many of casein's amino acids were found in excess of the ideal pattern. This imbalance of amino acids led to poorer N utilization. The excess amino acids were catabolized which increased UN excretion.

Results of this study show that decreasing the concentration of UN can significantly lessen the total N contamination to the environment and therefore reduce NH_3 emissions. Ammonia is produced by the bacterial and enzymatic degradation of urea and other nitrogenous components found in urine (Hartung and Phillips, 1994). Attempts have been made to separate manure solids from the liquid because bacterial urease present in feces hydrolyzes the urea in the urine into NH_4^+, which de-ionizes then volatilizes as NH_3 (Spoelstra, 1980). The following figure (Figure 1) shows the effect of reducing CP concentration and feeding a high quality feed ingredient on ammonia emission from manure slurry. Ammonia emissions decreased 80% by reducing CP from 15 to 9%.



The increased UN output dramatically impacted the NH_3 emissions despite casein's excellent protein quality. Similar N concentrations in urine from feeding both the 15% CP CSBM and 15% CP casein diets resulted in equivalent NH_3 emissions being released.

Summary: Overall, feeding diets of reduced crude protein concentration with crystalline amino acid supplementation dramatically decreased total nitrogen output and ammonia emission. This decrease was mainly due to a decrease in urinary nitrogen output indicating improved nitrogen utilization. Reducing dietary crude protein to 9% is sufficient to minimize urinary and fecal nitrogen excretion and to minimize ammonia emission. Reduction beyond 9% crude protein therefore does not result in significant nitrogen output and ammonia emission reduction. Despite the superior protein quality found in casein, ammonia emission was as high as found when feeding a 15% crude protein diet. Thus reduction in urinary nitrogen excretion through improvement in nitrogen utilization is of main importance.

This study demonstrated that N utilization, i.e. NPI (nitrogen retained as a percent of nitrogen intake) and NPA (nitrogen retained as a percent of nitrogen absorbed), was improved while reducing intact CP from 15 to 6%. This is shown in the following table (Table 5). This improvement resulted from the inclusion of amino acids. The highest N utilization was achieved when the 6 and 9% CP CSBM diets were fed.

Table 5: Nitrogen utilization and digestibility of experimental diets[a]

Item	15%	12%	9%	6%	SEM[b]	P-value	
						Linear	Quadratic
n	6	6	6	6			
DM intake, g/d	1787^{y}	1957^{x}	2035^{x}	1828^{y}	21.50		
N intake, g/d	42.47^{x}	39.36^{x}	33.37^{y}	26.33^{z}	0.68	***	**
N output[c], g/d	18.76^{w}	14.79^{x}	11.91^{y}	6.89^{z}	0.55	***	NS
N absorbed[d], g/d	34.02^{x}	31.91^{x}	26.71^{y}	22.48^{z}	0.53	***	†
N retained[e], g/d	23.71^{xy}	24.58^{x}	21.46^{yz}	19.44^{z}	0.62	***	*
N digestibility[f],%	80.28^{y}	81.09^{xy}	80.13^{y}	85.45^{x}	1.03	**	*
NPI, %	55.90^{z}	62.38^{y}	64.69^{y}	73.80^{x}	1.28	***	NS
NPR, %	69.68^{z}	76.90^{y}	80.57^{xy}	86.28^{x}	1.41	***	NS

[a]Least squares means across rows with different superscripts are different P<0.01. † P<0.10
* P<0.05, ** P< 0.01, *** P<0.001
[b]Standard error the of mean
[c]N output = N fecal + N urine; [d]N absorbed = N intake - N fecal; [e]N retained = N intake - N output;
[f]N digestibility = N absorbed / N intake

We also determined whether N balance could be maintained in pigs fed reduced CP diets formulated to meet both the requirements for indispensable amino acids based on ideal digestible amino acid profile (NRC, 1998), and for non-specific N through addition of the amino acid, glutamate. We found that nitrogen retention (NR) of pigs fed the 12 and 9% CP CSBM diets was equal to that of pigs fed the 15% CP CSBM diet. However, further reduction of CP beyond 9% decreased NR. Nitrogen digestibility improved in the 6% CP CSBM compared to the 15% CP CSBM diet. This may be a result of reduced endogenous N secretions when feeding the 6% CP CSBM diet as compared to feeding the 9, 12 and 15%

CP CSBM diets. Recovery of endogenous N influences digestibility of feed stuffs and it has been documented that endogenous secretions increase when dietary CP concentrations are increased

Summary: Nitrogen utilization was significantly improved when reducing crude protein concentration from 15% to 6%. However, because nitrogen retention was lower in pigs fed the 6% crude protein diet, we concluded that reducing to 9% crude protein would be of greatest benefits in terms of nitrogen retention and environmental impact. It is nonetheless interesting to mention that despite the fact that amino acids were added to meet the nutritional requirements (both on an ideal digestible and a nitrogen basis), reducing crude protein concentration beyond a certain level does not allow the animal to thrive to level that would be of economical benefit.

Volatile fatty acids and phenolics are known to be major constituents of odor offensiveness This project addressed the question whether reducing CP would also reduce VOC such as volatile fatty acids (VFA) and phenolics, and odor offensiveness to levels similar to those found when protein-free or casein based diets are fed to growing pigs. Results on VOC are shown in the tables below (Tables 7 and 8).

Table 7: Volatile organic compounds in feces and urine from feeding experimental diets[a]

VOC	15%	12%	9%	6%	Prot-free	15% Casein
Volatile fatty acid, mM						
Fecal Total VFA[b]	$42.73^{y} \pm 19.97$	$130.21^{x} \pm 17.24$	$140.25^{x} \pm 17.34$	$138.34^{x} \pm 13.82$	$56.28^{y} \pm 22.48$	$25.83^{y} \pm 22.47$
Acetate	17.94 ± 7.46	17.57 ± 7.46	23.08 ± 7.46	27.35 ± 7.46	33.07 ± 8.56	35.10 ± 11.80
Propionate	$23.19^{y} \pm 2.29$	$26.98^{xy} \pm 2.29$	$35.04^{x} \pm 2.29$	$27.38^{x} \pm 2.29$	$1.13^{z} \pm 3.05$	$0.00^{z} \pm 3.63$
Isobutyrate	ND	$16.00^{x} \pm 2.44$	$21.89^{x} \pm 2.12$	$22.09^{x} \pm 2.12$	$1.89^{y} \pm 2.82$	$0.00^{y} \pm 2.82$
Butyrate	$5.43^{y} \pm 1.99$	$26.13^{x} \pm 1.99$	$29.25^{x} \pm 1.99$	$25.64^{x} \pm 1.99$	$0.00^{y} \pm 2.65$	$0.00^{y} \pm 3.14$
Isovalerate	$0.21^{z} \pm 3.07$	$18.79^{y} \pm 2.28$	$27.72^{x} \pm 2.28$	$22.07^{xy} \pm 2.28$	$1.69^{z} \pm 3.12$	$0.83^{z} \pm 3.12$
Valerate	$0.38^{z} \pm 2.63$	$21.42^{x} \pm 2.26$	$14.21^{xy} \pm 2.65$	$13.82^{y} \pm 2.26$	$1.05^{z} \pm 3.04$	$0.00^{z} \pm 3.64$
Urine Phenolics, ppm						
p-Cresol	0.53 ± 3.01	0.08 ± 1.26	ND	5.35 ± 1.64	0.00 ± 4.78	2.15 ± 1.99
p-Ethylphenol	0.76 ± 0.03	0.05 ± 0.03	0.61 ± 0.07	0.64 ± 0.10	0.55 ± 0.09	0.00 ± 0.05

Table 8: Proportion of individual volatile fatty acids to total volatile fatty acids

Volatile fatty acid, % total	15%	12%	9%	6%	Prot-free	15% Casein
Acetate	$37.82^{y} \pm 4.49$	$15.33^{z} \pm 3.89$	$16.59^{z} \pm 3.90$	$20.25^{z} \pm 3.12$	$92.58^{x} \pm 5.06$	$81.26^{x} \pm 5.06$
Propionate	$47.81^{x} \pm 1.58$	$19.92^{y} \pm 1.36$	$22.16^{y} \pm 1.37$	$19.92^{y} \pm 1.09$	$3.51^{z} \pm 1.78$	$4.37^{z} \pm 1.78$
Isobutyrate	$0.00^{z} \pm 0.84$	$11.48^{y} \pm 0.72$	$13.42^{y} \pm 0.73$	$15.66^{x} \pm 0.58$	$1.03^{z} \pm 0.94$	$1.61^{z} \pm 0.94$
Butyrate	$12.01^{y} \pm 2.07$	$21.51^{x} \pm 1.79$	$20.14^{x} \pm 1.80$	$18.61^{x} \pm 1.43$	$1.22^{z} \pm 2.34$	$9.71^{y} \pm 2.34$
Isovalerate	$1.58^{z} \pm 1.08$	$15.20^{y} \pm 0.93$	$17.96^{x} \pm 0.94$	$15.87^{xy} \pm 0.75$	$1.95^{z} \pm 1.22$	$2.95^{z} \pm 1.22$
Valerate	$1.26^{z} \pm 1.33$	$16.55^{x} \pm 0.54$	$9.72^{y} \pm 1.15$	$9.69^{y} \pm 0.92$	$0.00^{z} \pm 1.50$	$0.09^{z} \pm 1.50$

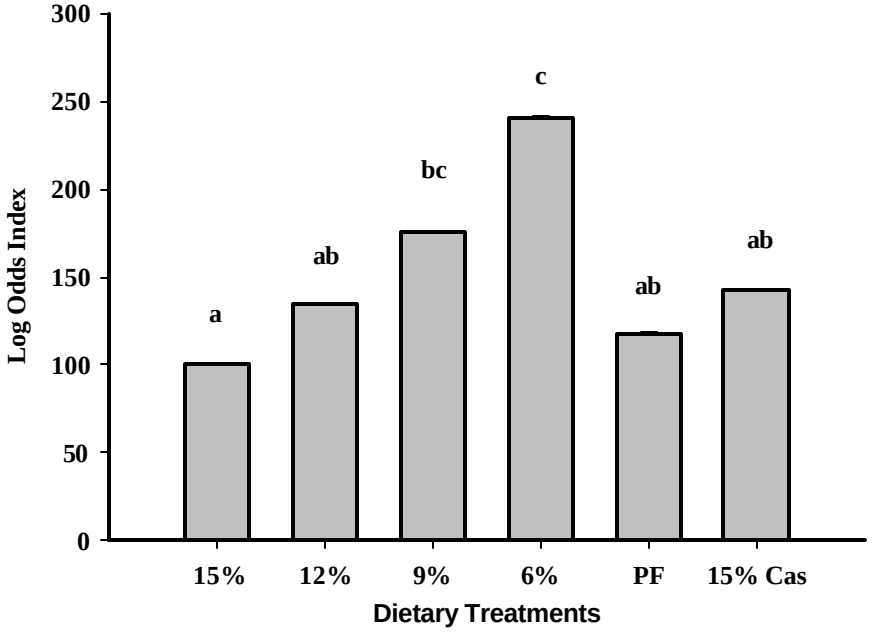
This study found that total VFA concentrations in feces dramatically increased when pigs were fed the 12, 9 and 6% CP CSBM diets compared to being fed the 15% CP CSBM, 15% CP casein and protein-free diets. The increase in total VFA concentrations reported in the present experiment were consistent with findings in the literature. In our study, ACE concentrations in feces were similar across all dietary treatments. However PROP, ISOB, BUTY, ISOV and VAL were all found in higher concentrations in feces when the 12, 9, and 6% CP CSBM diets were fed compared to feeding pigs the 15% CP CSBM, 15% CP casein and protein-free diets. Both para-Cresol and para-Ethylphenol were detected in very small concentrations in the urine, and no differences were found.

Objective D:
Conduct an olfactory panel evaluation of the manure odor produced from feeding the different dietary protein levels and quality.

Response indices of odor offensiveness are provided in Figure 2.

The results of the odor panel showed an increase in odor offensiveness as intact CP decreased. The 15% CP CSBM diet was the least offensive manure slurry with LSM equal to 2.58. This LSM value is equivalent to the qualitative ranking of "mild-moderately" offensive. Comparing reduced intact CP diets to the 15% CP CSBM diet, only the 9 and 6% CP CSBM diets were found to be more offensive ($P<0.05$) with qualitative rankings of "moderately" offensive. The order of offensiveness (least to most) for the remaining treatments are as follows: protein-free, 12% CP CSBM , 15% CP casein, 2.70, 2.77, and 2.81, respectively, with qualitative rankings equivalent to "mild-moderately" offensive.

Summary: *The odor panel responses revealed that manures resulting from feeding the 9 and 6% CP corn-soybean meal based diets were more offensive compared to the 15% CP corn-soybean meal based diet. Relating the results of the VOC to the odor panel, it was determined that the increased concentrations of isobutyrate, butyrate, isovalerate, and valerate in the reduced crude protein diets may have contributed to the increased odor offensiveness. The role of phenolics in odor offensiveness of this study may have been less significant because only two compounds could be identified in urine. To reduce the crude protein concentration of a typical corn-soybean meal based diet, it is necessary to change in feed ingredients ratio, i.e. increasing the amount of corn or corn-starch to dilute the protein contained in soybean meal. Whether this would have contributed to increased the VOC will require further research. Increase in total VFA's may be due to changes in preference in fermentation substrates and(or) changes in the hind-gut microbial populations. Other work in our lab has shown that corn starch digestion is not 100% complete, and that approximately 10 to 15% is available to the hind gut, thus providing substrates to the micro flora for fermentation. Endogenous protein contributed minimally to the VOC and odor, as seen from the protein free and casein diets.*



Objective A & C:

Determine the amount of amino acids in the endogenous secretions, feces, and urine, and measure fecal, apparent, and true amino acid digestibility in growing pigs fed different dietary protein levels and quality.

Results of amino acids recovered in the endogenous secretions are shown in the following table (Table 9). The branched-chain amino acids (isoleucine, leucine, valine) were found to be the greatest contributor to endogenous secretions. However, the amount of amino acids measured in the ileal digesta were not significantly affected by the protein levels fed, as shown in Table 9.

Table 9: Endogenous losses feeding protein-free and casein based diets, g/kg DMI

	Prot-free	Casein	SEM
Amino Acid			
Essential			
Arginine	0.3258[x]	0.1635[y]	0.0189
Histidine	0.0938	0.0715	0.0174
Isoleucine	0.2307	0.2148	0.0092
Leucine	0.3932	0.2835	0.2300
Lysine	0.2341	0.1614	0.0210
Methionine	0.0763	0.0673	0.0130
Phenylalanine	0.2065	0.1329	0.0135
Threonine	0.3772	0.3645	0.0219
Valine	0.3158	0.2652	0.0177

Table 10. Amino acid concentration in digesta of pigs fed experimental diets[a], g/kg DM

Diets	15%	12%	9%	6%
Essential Amino Acids				
Arginine	3.72	3.96	4.05	4.77
Histidine	1.83	1.83	1.83	1.87
Isoleucine	3.36	3.30	3.19	3.38
Leucine	8.62	8.49	7.76	7.52
Lysine	3.88	3.75	3.37	3.99
Methionine	1.67	1.59	1.67	1.71
Phenylalanine	3.81	3.79	3.50	3.57
Threonine	4.46	3.94	4.55	4.56
Valine	4.52	4.38	4.32	4.51

[a] Diets fed were 15, 12, 9 and 6% CP corn-soybean meal based diets. Crystalline amino acids were included in the 6, 9 and 12% CP CSBM diets to meet digestible amino acid requirements.

Amino acid digestibility of the diets were improved by reducing the dietary crude protein concentration and including amino acids (Tables 11 and 12). When correcting for endogenous losses, digestibility mainly increased for the 15 and 12% CP diets, as shown in Table 12. Feeding diets containing 9% and 6% CP yielded digestibility values of 90% on an apparent basis and up to 95% on a standardized basis.

Summary: Reducing crude protein to 9% is sufficient to maximize amino acid digestibility and minimize endogenous losses.

Table 11: Apparent ileal amino acid digestibility of experimental diets (dry matter basis)[a]

Amino acid	15%	12%	9%	6%	SEM[b]
Arginine	88.54^{xy}	87.56^{y}	91.20^{x}	86.77^{y}	0.73
Histidine	85.88^{y}	85.39^{y}	90.12^{x}	89.38^{x}	0.57
Isoleucine	83.10^{y}	83.11^{y}	90.18^{x}	90.09^{x}	0.71
Leucine	82.48^{y}	82.22^{y}	89.49^{x}	88.82^{x}	0.73
Lysine	81.26^{y}	84.98^{y}	92.22^{x}	91.63^{x}	0.86
Methionine	81.39^{y}	82.10^{y}	89.76^{x}	90.05^{x}	0.82
Phenylalanine	84.25^{y}	83.92^{y}	90.14^{x}	90.40^{x}	0.68
Threonine	75.32^{z}	79.49^{y}	86.01^{x}	86.19^{x}	0.88
Valine	79.94^{y}	80.31^{y}	88.81^{x}	89.14^{x}	0.86

Table 12: Standardized amino acid ileal digestibility using protein free diet to estimate endogenous amino acid losses (dry matter basis)[a]

Amino acid	15%	12%	9%	6%	SEM[b]
Arginine	91.70^{y}	91.44^{y}	95.93^{x}	92.92^{xy}	0.73
Histidine	88.14^{y}	88.23^{y}	93.52^{x}	93.02^{x}	0.57
Isoleucine	86.73^{y}	87.59^{y}	94.98^{x}	94.69^{x}	0.71
Leucine	84.98^{y}	85.35^{y}	93.09^{x}	92.79^{x}	0.73
Lysine	84.77^{y}	88.52^{y}	95.86^{x}	94.97^{x}	0.86
Methionine	84.06^{y}	85.36^{y}	92.93^{x}	93.07^{x}	0.82
Phenylalanine	86.91^{y}	87.24^{y}	94.07^{x}	94.18^{x}	0.68
Threonine	81.85^{z}	86.89^{y}	93.78^{x}	93.98^{x}	0.88
Valine	84.32^{y}	85.68^{y}	94.34^{x}	94.32^{x}	0.86

[a]Least squares means within row with different superscripts are different P<0.01.
[b]Standard error of mean

Overall summary:

Feeding diets of reduced crude protein concentration with crystalline amino acid supplementation dramatically decreased total nitrogen output and ammonia emission. This decrease was mainly due to a decrease in urinary nitrogen output indicating improved nitrogen utilization. Reducing dietary crude protein to 9% is sufficient to minimize urinary and fecal nitrogen excretion and to minimize ammonia emission. Reduction beyond 9% crude protein therefore does not result in significant nitrogen output and ammonia emission reduction. Despite the superior protein quality found in casein, ammonia emission was as high as found when feeding a 15% crude protein diet. Thus reduction in urinary nitrogen excretion through improvement in nitrogen utilization is of main importance. Reducing crude protein to 9% is sufficient to maximize amino acid digestibility and minimize endogenous losses.

Nitrogen utilization was significantly improved when reducing crude protein concentration from 15% to 6%. However, because nitrogen retention was lower in pigs fed the 6% crude protein diet, we concluded that reducing to 9% crude protein would be of greatest benefits in terms of nitrogen retention and environmental impact. It is nonetheless interesting to mention that despite the fact that amino acids were added to meet the nutritional requirements (both on an ideal digestible and a nitrogen basis), reducing crude protein concentration beyond a certain level does not allow the animal to thrive to level that would be of economical benefit.

The odor panel responses revealed that manures resulting from feeding the 9 and 6% CP corn-soybean meal based diets were more offensive compared to the 15% CP corn-soybean meal based diet. Relating the results of the VOC to the odor panel, it was determined that the increased concentrations of isobutyrate, butyrate, isovalerate, and valerate in the reduced crude protein diets may have contributed to the increased odor offensiveness. The role of phenolics in odor offensiveness of this study may have been less significant because only two compounds could be identified in urine. To reduce the crude protein concentration of a typical corn-soybean meal based diet, it is necessary to change in feed ingredients ratio, i.e. increasing the amount of corn or corn-starch to dilute the protein contained in soybean meal. Whether this would have contributed to increased the VOC will require further research. Increase in total VFA's may be due to changes in preference in fermentation substrates and(or) changes in the hind-gut microbial populations. Other work in our lab has shown that corn starch digestion is not 100% complete, and that approximately 10 to 15% is available to the hind gut, thus providing substrates to the micro flora for fermentation. Endogenous protein contributed minimally to the VOC and odor, as seen from the protein free and casein diets.