Manure composition of swine as affected by dietary protein and cellulose concentrations[1]

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ABSTRACT: An experiment was conducted to investigate the effects of reducing dietary CP and increasing dietary cellulose concentrations on manure DM, C, N, S, VFA, indole, and phenol concentrations. Twenty-two pigs (105 kg initial BW) were fed diets containing either 14.5 or 12.0% CP, in combination with either 2.5 or 8.7% cellulose. Pigs were fed twice daily over the 56-d study, with feed intake averaging 2.74 kg/d. Feces and urine were collected after each feeding and added to the manure storage containers. Manure storage containers were designed to provide a similar unit area per animal as found in industry (7,393 cm^2). Before sampling on d 56, the manure was gently stirred to obtain a representative sample for subsequent analyses. An interaction of dietary CP and cellulose was observed for manure acetic acid concentration, in that decreasing CP lowered acetic acid in pigs fed standard levels of cellulose but increased acetic acid in pigs fed greater levels of cellulose ($P = 0.03$). No other interactions were noted. Decreasing dietary CP reduced manure pH ($P = 0.01$), NH_4 ($P = 0.01$), isovaleric acid ($P = 0.06$), phenol ($P = 0.05$), and 4-ethyl phenol ($P = 0.02$) concentrations. Increasing dietary cellulose decreased pH ($P = 0.01$) and NH_4 ($P = 0.07$) concentration but increased manure C ($P = 0.03$), propionic acid ($P = 0.01$), butyric acid ($P = 0.03$), and cresol ($P = 0.09$) concentrations in the manure. Increasing dietary cellulose also increased manure DM ($P = 0.11$), N ($P = 0.11$), and C ($P = 0.02$) contents as a percentage of nutrient intake. Neither cellulose nor CP level of the diet affected manure S composition or output as a percentage of S intake. Headspace N_2O concentration was increased by decreasing dietary CP ($P = 0.03$) or by increasing dietary cellulose ($P = 0.05$). Neither dietary CP nor cellulose affected headspace concentration of CH_4. This study demonstrates that diets differing in CP and cellulose content can significantly impact manure composition and concentrations of VFA, phenol, and indole, and headspace concentrations of N_2O, which may thereby affect the environmental impact of livestock production on soil, air, and water.

Key words: cellulose, composition, manure, odor, protein, swine

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INTRODUCTION

Swine production has undergone extensive changes during the last 3 decades resulting in larger numbers of swine produced on increasingly smaller areas of land. This has led to increased awareness by the general public and regulatory agencies about issues concerning pollution of air, soil, and water from swine production facilities (Hobbs et al., 1997; Mackie et al., 1998; Schiffman et al., 2005). Because whole-body retention of N, P, and S in swine is only about 50% of total dietary intake (Shurson et al., 1998; Sands et al., 2001; van Kempen et al., 2003), excess nutrients can be released into the environment via excretions from animals. Recent air monitoring studies have shown that livestock production facilities have the potential to affect air quality through release of odorous compounds, such as hydrogen sulfide, ammonia, and volatile organic compounds into the environment (Schiffman et al., 2001; Zahn et al., 2001a,b).

The composition of the manure and potential release of nutrients and volatile emissions into the environment from livestock operations and land-applied manure is partially controlled by dietary inputs (Miller and Varel, 2003). Two approaches to changing dietary composition are controlling dietary CP and fiber content

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Table 1. Composition of experimental diets, as-fed basis[1]

Ingredient, % — Crude protein, % / Cellulose[2]	14.5 Standard	12.0 Standard	14.5 High	12.0 High
Corn	78.95	85.70	60.63	69.85
Soybean meal	16.31	9.07	15.36	8.24
Soybean hulls	0.00	0.00	17.27	14.69
Fat blend[3]	2.00	2.00	4.25	4.16
Dicalcium phosphate	0.93	0.98	0.93	0.98
Limestone	0.88	0.91	0.60	0.67
Sodium chloride	0.30	0.30	0.30	0.30
Vitamin-mineral mix[4]	0.40	0.40	0.40	0.40
Choline chloride, 60%	0.07	0.07	0.07	0.07
Selenium premix[5]	0.05	0.05	0.05	0.05
L-Lysine·HCl	0.11	0.35	0.11	0.35
L-Threonine	0.00	0.10	0.02	0.12
L-Tryptophan	0.00	0.03	0.00	0.03
DL-Methionine	0.00	0.04	0.01	0.07
L-Isoleucine	0.00	0.00	0.00	0.01
L-Valine	0.00	0.00	0.00	0.01
Calculated composition, %				
Nitrogen	2.33	1.92	2.33	1.92
Crude protein	14.5	12.0	14.5	12.0
Cellulose	2.67	2.27	9.35	7.95
Analyzed composition, %				
Carbon	40.4	40.2	40.9	40.8
Nitrogen	2.17	1.77	2.18	1.78
Sulfur	1.82	1.62	1.77	1.69

[1]All diets were formulated to 3,400 kcal ME/kg, 0.70% true digestible lysine, 0.62 SAA:Lys, 0.175 Trp:Lys, 0.655 Thr:Lys, 0.58 Ile:Lys, 0.68 Val:Lys, 0.55% Ca, and 0.22% available P.

[2]Dietary cellulose was increased by 2.5-fold to 7.95 or 9.35% (relative to 2.27% in the 12% CP diet and 2.67% in the 14.5% CP diet, respectively) by addition of soybean hulls.

[3]Rendered animal fat, 70%; and used restaurant grease, 30%.

[4]Provided the following (per kg of diet): 17.5 mg of Cu, 175 mg of Fe, 60 mg of Mn, 2 mg of I, 150 mg of Zn, 6,600 IU of vitamin A, 1,650 IU of vitamin D_3, 33 IU of vitamin E, 33 μg of vitamin B_{12}, 9.9 mg of riboflavin, 49.5 mg of niacin, and 26.4 mg of pantothenic acid.

[5]Provided 0.3 mg per kg of diet.

(Sutton et al., 1999). Numerous experiments have shown that a reduction in dietary CP and supplementation of diets with crystalline AA can have a profound impact on N excretion (Kerr, 1995). However, there are limited data on the impact of supplemental fiber into swine diets on nutrient excretion, manure composition, and odor generation (Shriver et al., 2003).

Therefore, an experiment was conducted to evaluate potential interactive effects of altering dietary CP and fiber level on nutrient (C, N, and S) excretion, manure composition (pH, VFA, NH_4, cresols, phenols, and indoles), and headspace N_2O and CH_4 from finishing pigs using a dynamic experimental manure storage system.

MATERIALS AND METHODS

All procedures involving animal handling and testing were reviewed and approved by the Iowa State University Committee on Animal Care. Twenty-two PIC (Pig Improvement Corporation, Lexington, KY) finishing pigs were used to establish the effect of feeding diets containing 14.5 or 12.0% CP in combination with standard or elevated (a calculated 2.5-fold increase) cellulose (Table 1). A standard diet was formulated with 14.5% CP and 2.67% cellulose. Crude protein was reduced to 12.0% by decreasing soybean meal and supplementing crystalline AA to meet AA requirements. Dietary cellulose was increased by 2.5-fold to 7.95 or 9.35% (relative to 2.27% in the 12% CP diet and 2.67% in the 14.5% CP diet, respectively) by addition of soybean hulls. All diets were formulated to 3,400 kcal of ME/kg and 0.70% true ileal digestible Lys content. Other nutrients were fed to meet animal requirements according to the NRC (1998).

Ambient temperature in the metabolism room was maintained at approximately 21°C, and lighting was provided continuously. Pigs were moved to individual stainless steel metabolism crates (1.2 × 2.4 m) and fed their treatment diets at approximately 3% of their BW. Average initial and final BW were 104.6 and 153.3 kg, respectively, over the 56-d feeding and collection period. Diets were fed twice daily at 0700 and 1900 with manure collection beginning on d 1. Feed intake was recorded daily with orts subtracted from total feed intake. Total nutrient intake was calculated from actual feed consumption and the analyzed diet composition. Water was supplied ad libitum through nipple waterers.

After each feeding, feces and urine from each metabolism crate were collected and added to a manure storage container for each individual crate. Each stainless steel manure storage container measured 122 cm high and 96.5 cm in diameter. The lid on each container was fitted with threaded couplers to accommodate fittings and tubing with which to pull a constant stream of air over the manure (7 L/min), add daily fecal and urine collections, and take manure samples. Manure tanks were designed to have a similar surface area as used for pigs maintained in growing-finishing barns with deep pit manure storage systems. Manure volume was obtained by measuring the depth of each manure container at the end of the experiment. Manure samples for analysis were obtained after mixing each tank with a 15-cm stainless steel propeller for 3 min at a speed of 850 rpm.

Manure temperature was measured using a thermocouple thermometer (Fluke 51-Series II, Fluke Corp., Everett, WA), pH using a pH meter (Corning Model 530 with Corning probe #476436, Corning Inc., Corning, NY), bulk density by weighing 7 mL of well-mixed manure in a 10-mL graduated cylinder, and DM by 24-h freeze drying (Virtis Benchtop K Series, SP Industries, Gardiner, NY).

Ammonia was analyzed colorimetrically (Chaney and Marbach, 1962) using a Varian Cary 50 Spectrophotometer (Varian Analytical Instruments, Walnut Creek, CA). Briefly, approximately 2 g of mixed manure was pipetted into a 15-mL centrifuge tube, 6 mL of 0.1 *N* HCl was added, the tube was vortexed, and the sample was filtered to remove large particles. Subsequently, two 1-mL aliquots of the filtered samples were pipetted into microcentrifuge tubes and centrifuged at 20,000 × *g* for 20 min at 4°C. The supernatant was additionally filtered through a 0.2-μm syringe filter and frozen at

–20°C until analyzed. As a result of acidification, all results are reported as ammonium-N.

Carbon, N, and S were analyzed using a VarioMAX CNS analyzer (Elementar Analysensysteme GmbH, Hanau, Germany), which uses catalytic tube combustion to volatilize the sample. Resultant gases are cleaned up to remove unwanted substances, and the target gases are converted to N_2, CO_2, and SO_2, separated from each other using adsorption columns, and after heating are measured using a thermal conductivity detector.

Volatile fatty acids, phenols, and indoles were analyzed using gas chromatography. Briefly, approximately 4 g of mixed manure were pipetted into a tared 15-mL polypropylene centrifuge tube, 1 mL of HPLC grade water and 5 mL of HPLC grade acetone were added, and each tube was sonicated for 15 s using a Misonix XL-2020 sonicator (Misonix Incorporated, Farmingdale, NY). After sonication, 100 μL of o-phosphoric acid was added, and the tube was vortexed. Tubes were then centrifuged at 21,000 × *g* for 23 min at 4°C. The supernatant was filtered through a 0.2-μm syringe filter and then analyzed on an Agilent 6890 gas chromatograph equipped with a flame ionization detector and DB-FFAP column (30 m × 0.25 mm × 0.25 μm; Aglient Technologies, Wilmington, DE). The gas chromatograph parameters were the following: split mode, 20:1; inlet temperature, 220°C; initial inlet pressure, 168 kPa; injection volume, 1 μL; constant column flow, 1.4 mL/min (helium); and detector temperature, 250°C. The oven temperature program was: initial temperature, 35°C, 0.5 min hold; ramp of 10°C/min to 90°C, 2.0 min hold; ramp of 12°C/min to final temperature of 230°C, hold for 6 min.

At 3 time points during the last week of the experiment, samples of headspace gas in the manure tanks were collected and analyzed for the trace gases, N_2O and CH_4. Gas samples were collected with a 10-mL polypropylene syringe through rubber stoppers in the tops of the tanks. Gas samples were injected into evacuated glass vials (6 mL) fitted with butyl rubber stoppers. Nitrous oxide and CH_4 concentrations in the samples were determined with a Shimadzu gas chromatography (Model GC17A, Shimadzu Corporation, Columbia, MD) equipped with a ^{63}Ni, electron capture detector and a flame ionization detector. Separation was achieved using stainless steel columns (3.2 mm in diameter × 1.8 m long) with Porapak Q (80 to 100 mesh). Samples were introduced into the gas chromatograph using an autosampler, as described by Arnold et al. (2001).

Statistical analyses were performed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Data were analyzed as a factorial arrangement of treatments within a randomized complete block design, with the individual pig or manure container as the experimental unit. There were 6 observations per treatment, except for the pigs fed the low CP, AA-supplemented diets containing a standard level of dietary cellulose, in which there were only 4 observations per treatment due to the loss of 2 pigs. In addition, principle component analysis using a correlation matrix was performed on different dietary treatment groups using concentrations of odorous compounds in the manure as the dependent variable for treatment separations.

RESULTS AND DISCUSSION

Feed intake was controlled; consequently ADFI did not differ between dietary treatments (Table 2). At these equalized feed intakes, reduction in dietary CP had only a slight impact on ADG ($P = 0.10$) and GF ($P = 0.11$). This was expected; many studies have shown that pig performance is similar when fed standard and moderately low CP diets, provided limiting AA requirements are met by supplementing crystalline AA (Kerr and Easter, 1995; Tuitoek et al., 1997; Kerr et al., 2003a,b). There was no impact of additional cellulose on pig performance. Although performance values are not typically reported in balance trials, performance data are presented to demonstrate that animal nutrition supported positive BW gains over the 56-d experiment and was not far removed from levels expected from research animals provided ad libitum access to feed.

As expected, reducing soybean meal and replacing limiting AA with crystalline AA greatly reduced N intake ($P = 0.01$, Table 2). The reduction in S intake in pigs fed the low CP diets ($P = 0.05$) was also expected because of the replacement of soybean meal (0.45% S) with corn (0.10% S). It was unclear as to why S intake was not reduced in pigs fed low CP diets with high cellulose (CP × cellulose interaction, $P = 0.07$), but this may be due to variation in determining the S content of the diets. Dietary treatment had no impact on total C intake.

Reduction in dietary CP lowered manure pH ($P = 0.01$), which is supported by the decreased manure NH_4 concentration ($P = 0.01$, Table 3). Manure NH_4 concentration is proportional to urinary urea excretion, and typically, pigs fed low CP, AA-supplemented diets have less urea excreted in urine (Kerr and Easter, 1995). Others (Cahn et al., 1998; Shriver et al., 2003; Velthof et al., 2005) also reported a lower manure pH due to feeding reduced CP, AA-supplemented diets. In addition, Miller and Varel (2003) reported that supplementing manure with protein (casein) increased manure pH compared with nontreated manure. In our experiments, the lower manure pH from animals fed low CP diets may be attributed to a reduction in the buffering capacity of NH_4-N from these diets. We did not see a reduction in manure total N concentration (Table 3) due to feeding the lower CP diet as has been noted by others (Sutton et al., 1999; Crocker and Robison, 2002; Shriver et al., 2003). This lack of significant difference may be due to the high variability in our total N values (CV~25%). This variability may be a result of volatilization of NH_3 in the pigs fed greater CP diets because pigs fed greater CP diets have greater rates of

Table 2. Pig performance and total nutrient intake as affected by dietary protein and cellulose content[1]

		Pig performance			Total nutrient intake			
Dietary treatment[2]	Pigs	ADG, g	ADFI, g	G:F	DM, kg	N, kg	C, kg	S, g
12.0% CP, standard cellulose	4	936	2,792	0.336	138.4	2.78	62.9	254
14.5% CP, standard cellulose	6	834	2,764	0.303	137.8	3.35	62.5	282
12.0% CP, high cellulose	6	897	2,765	0.326	136.8	2.76	63.1	262
14.5% CP, high cellulose	6	825	2,650	0.312	130.9	3.24	60.7	263
Standard deviation		116.4	164.5	0.0328	8.14	0.188	3.76	16.0
Main effect								
12.0% CP	10	916	2,778	0.331	137.6	2.77	63.0	258
14.5% CP	12	829	2,706	0.307	134.4	3.29	61.6	272
Standard cellulose	10	884	2,778	0.320	138.1	3.06	62.7	268
High cellulose	12	861	2,707	0.319	133.9	3.00	61.9	262
Source of variation, *P*-value								
Protein level		0.10	0.33	0.11	0.37	0.01	0.41	0.05
Cellulose level		0.65	0.34	0.95	0.26	0.45	0.63	0.44
Protein × cellulose		0.77	0.55	0.52	0.47	0.57	0.55	0.07

[1]Average initial and final BW were 104.6 (SD = 5.24) and 153.3 (SD = 9.60) kg, respectively, over the 56-d trial. Initial age was approximately 165 d.

[2]Dietary cellulose was increased by 2.5-fold to 7.95 or 9.35% (relative to 2.27% in the 12% CP diet and 2.67% in the 14.5% CP diet, respectively) by addition of soybean hulls.

NH_3 emissions (von Pfeiffer, 1993; Sutton et al., 1999; Otto et al., 2003). The increased rate of NH_3 emission may have reduced our ability to measure significant differences between CP levels of the different diets. Because we did not account for the potentially large N losses during urine and fecal collection (van Kempen et al., 2003) or from the manure storage containers, we cannot discern the mechanism that resulted in the lack of a CP protein effect on total N in the manure. However, it should be pointed out that animals fed a low CP diet did have numerically lower N contents in their manure. We did not see a difference in manure S concentration as a function of the lowered S intake in pigs fed the low CP diets (Table 3). Similar to losses in NH_3 emissions, we cannot account for any volatile S losses that may have occurred from either our fecal and urine collection methods or from our manure storage systems.

Increasing dietary cellulose resulted in a reduction in manure pH ($P = 0.01$), which is supported by the lower NH_4 ($P = 0.07$, Table 3) and greater total VFA ($P = 0.01$) concentrations in the manure, both of which control manure pH (Sommer and Husted, 1995). This contrasts work by Sutton et al. (1999), Mroz et al. (2000), and Shriver et al. (2003), who reported no significant change in the manure pH as a result of supplementing additional fiber to the diet. In the current experiment, dietary fiber had no effect on total manure N concentration, which is consistent with Gralapp et

Table 3. Manure characteristics as affected by dietary protein and cellulose content[1]

					Composition, as-is				
Dietary treatment[2]	Vol., L	BD, g/mL	pH	T, °C	DM, %	NH_4-N, μmol/g	N, g/L	C, g/L	S, g/L
12.0% CP, standard cellulose	474	0.970	7.56	19.8	2.80	118	1.36	8.15	0.239
14.5% CP, standard cellulose	496	0.975	8.34	19.3	2.01	189	1.71	9.56	0.293
12.0% CP, high cellulose	474	0.971	6.82	19.2	3.68	103	1.84	13.60	0.274
14.5% CP, high cellulose	514	0.976	7.66	19.3	3.00	145	1.68	11.60	0.269
Standard deviation	83.1	0.007	0.432	0.58	1.46	34.3	0.406	3.512	0.0541
Main effect									
12.0% CP	474	0.971	7.19	19.0	3.24	110	1.60	10.88	0.257
14.5% CP	504	0.976	8.00	19.3	2.51	167	1.69	10.58	0.281
Standard cellulose	485	0.972	7.95	19.3	2.41	153	1.53	8.86	0.266
High cellulose	494	0.974	7.24	19.2	3.34	124	1.76	12.60	0.272
Source of variation, *P*-value									
Protein level	0.41	0.13	0.01	0.36	0.27	0.01	0.61	0.85	0.32
Cellulose level	0.82	0.64	0.01	0.51	0.16	0.07	0.22	0.03	0.81
Protein × cellulose	0.80	0.94	0.88	0.51	0.93	0.35	0.17	0.28	0.23

[1]Vol. = volume; BD = bulk density; and T = temperature.

[2]Dietary cellulose was increased by 2.5-fold to 7.95 or 9.35% (relative to 2.27% in the 12% CP diet and 2.67% in the 14.5% CP diet, respectively) by addition of soybean hulls.

Table 4. Total manure nutrients as affected by dietary protein and cellulose content

	Nutrient				Nutrient, % of intake[1]			
Dietary treatment[2]	DM, kg	N, g	C, g	S, g	DM	N	C	S
12.0% CP, standard cellulose	12.62	637	3,870	112	9.1	22.8	6.1	44.1
14.5% CP, standard cellulose	9.16	824	4,417	138	6.6	24.5	7.1	48.9
12.0% CP, high cellulose	17.04	886	6,602	132	12.3	31.8	10.3	49.9
14.5% CP, high cellulose	15.48	886	6,133	140	11.8	27.0	10.0	52.8
Standard deviation	7.914	266.1	2,193.4	30.8	5.59	7.92	3.17	10.04
Main effect								
12.0% CP	14.83	762	5,236	122	10.7	27.3	8.2	47.0
14.5% CP	12.32	855	5,275	139	9.2	25.8	8.5	50.9
Standard cellulose	10.89	731	4,144	125	7.9	23.6	6.6	46.5
High cellulose	16.26	886	6,368	136	12.0	29.4	10.1	51.4
Source of variation, *P*-value								
Protein level	0.47	0.43	0.96	0.23	0.55	0.67	0.81	0.39
Cellulose level	0.14	0.20	0.03	0.43	0.11	0.11	0.02	0.28
Protein × cellulose	0.78	0.43	0.60	0.51	0.69	0.37	0.65	0.84

[1]Calculated as nutrient content in manure divided by the total nutrient intake of the pig × 100.

[2]Dietary cellulose was increased by 2.5-fold to 7.95 or 9.35% (relative to 2.27% in the 12% CP diet and 2.67% in the 14.5% CP diet, respectively) by addition of soybean hulls.

al. (2002) and Mroz et al. (2000) who reported no significant changes in total manure N concentration with dietary fiber treatments. In contrast, Sutton et al. (1999) reported that the addition of 5% cellulose to the diet reduced total manure N concentration. It should be noted, however, that the Sutton et al. (1999) work was with fresh and not stored manure, which may account for some of the discrepancies between that study and the current study. The design of our system makes direct comparison to other data difficult because our system is dynamic in that manure is continuously added and purged with air, whereas many experimental manure evaluation systems are static (i.e., an initial manure sample followed over time with no manure addition). Increasing dietary cellulose increased the C concentration of the manure ($P = 0.03$, Table 3), indicating incomplete intestinal cellulose digestion in these pigs. This is reflected in the increase manure DM, although this effect was not significant. We are not aware of any other data evaluating C balance (intake and manure content) in swine.

Total nutrients in the manure (manure volume × bulk density × manure composition) differed little due to dietary treatment (Table 4). Only in pigs fed the high cellulose diets was there an increase in the C mass ($P = 0.03$) relative to pigs fed the standard cellulose diet. This is supported by the numerical increase in total DM mass, but this effect was not significant. As a percentage of nutrient intake of pigs fed these diets, CP level had no impact on DM, N, C, or S mass in the manure. This is surprising given that pigs fed low CP diets retain a greater percentage of their N intake relative to pigs fed greater CP diets (Kephart and Sherritt, 1990; Lopez et al., 1994; Kerr and Easter, 1995; Shriver et al., 2003). The limited data available from stored manure makes it difficult to assess whether our results are typical. Increasing dietary cellulose had no effect on S mass in the manure as a percentage of the nutrient intake of pigs but did appear to increase manure DM ($P = 0.11$), N ($P = 0.11$), and C ($P = 0.02$) mass as a percentage of nutrient intake. The increase in N mass in the manure due to increased dietary cellulose content may be attributed to both N in the form of bacterial proteins and other nonvolatile nitrogenous compounds (von Pfeiffer, 1993; Sutton et al., 1999) and lower manure pH. von Pfeiffer (1993) and Sutton et al. (1999) report lower NH_3 emissions with increased fiber diets.

Undigested dietary polysaccharides and oligosaccharides, proteins, and endogenous proteins and peptides are fermented by manure microbial communities to VFA (mainly acetic, propionic and butyric acids, with smaller proportions of valeric, caproic, isobutyric, isovaleric, isocaproic, and heptanoic acids; Mackie et al., 1998; Miller and Varel, 2003). In our experiment, dietary CP level had no major impact on VFA concentrations in manure, with the only difference being a decrease in isovaleric acid concentration ($P = 0.06$); pigs fed the low CP diets had lower concentrations compared with pigs fed the standard CP diets (Table 5). Miller and Varel (2003) also reported an increase in branched-chain VFA (valeric, isobutyric, and isovaleric) with addition of protein (casein) utilizing an in vitro incubation system. However, casein addition was designed to increase protein substrate concentration by 50%, well above a situation found in practice. Hobbs et al. (1996) reported conflicting VFA results due to increasing dietary CP. Acetic and propionic acid concentrations were reduced in the manure slurry from growing pigs but not from finishing pigs, whereas butyric acid concentration decreased in finishing pig manure but not that of growing pigs. Using feather meal to increase dietary CP, van Heugten and van Kempen (2002) reported increased fecal butyric, valeric, and isovaleric acids but no change in fecal acetic or propionic acid concentrations. In contrast, Otto et al. (2003) reported that reducing dietary CP increased fecal VFA concentrations (in-

Table 5. Major manure volatile fatty acid concentrations as affected by dietary protein and cellulose content[1]

	Fatty acid, μmol/g wet wt							
Dietary treatment[2]	Ac	Pr	iBu	Bu	iVal	Val	Cap	tVFA
12.0% CP, standard cellulose	36.54[b]	7.28	1.32	2.99	1.27	0.23	0.32	49.96[b]
14.5% CP, standard cellulose	48.08[ab]	7.76	1.49	3.32	1.70	0.32	0.27	62.95[ab]
12.0% CP, high cellulose	55.34[a]	11.51	1.59	5.73	1.60	0.59	0.27	76.62[a]
14.5% CP, high cellulose	48.45[ab]	9.84	1.75	4.37	1.83	0.47	0.32	67.07[a]
Standard deviation	9.028	1.791	0.477	1.775	0.384	0.366	0.108	12.273
Main effect								
12.0% CP	45.94	9.39	1.45	4.36	1.43	0.41	0.29	63.29
14.5% CP	48.27	8.80	1.62	3.85	1.77	0.39	0.30	65.01
Standard cellulose	42.31	7.52	1.41	3.15	1.49	0.28	0.29	56.45
High cellulose	51.90	10.67	1.67	5.05	1.71	0.53	0.30	71.85
Source of variation, *P*-value								
Protein level	0.56	0.46	0.42	0.52	0.06	0.93	0.97	0.75
Cellulose level	0.03	0.01	0.22	0.03	0.20	0.14	0.95	0.01
Protein × cellulose	0.03	0.18	0.99	0.29	0.57	0.51	0.30	0.05

[a,b]Means within columns without a common superscript letter differ ($P < 0.05$).

[1]Abbreviations: Ac, acetic acid; Pr, propionic acid; iBu, isobutyric acid; Bu, butyric acid; iVal, isovaleric acid; Val, n-valeric acid; Cap, n-caproic acid; and tVFA, total volatile fatty acids.

[2]Dietary cellulose was increased by 2.5-fold to 7.95% or 9.35% (relative to 2.27% in the 12% CP diet and 2.67% in the 14.5% CP diet, respectively) by addition of soybean hulls.

cluding propionic, butyric, isobutyric, isovaleric, and valeric acid). Production of VFA by microbial fermentation depends on many factors in addition to CP levels, including microbial community structure, gut epithelial cell turnover, pH, and carbohydrate composition. It is likely that these other factors contribute to the variability in VFA concentrations reported.

Increasing dietary cellulose increased total VFA production ($P = 0.01$) because of increased acetic ($P = 0.03$), propionic ($P = 0.01$), and butyric ($P = 0.03$) acids (Table 5). No other volatile fatty acids had significant concentration increases. Sutton et al. (1999) also reported an increase in VFA production in fresh manure with the addition of 5% cellulose to growing-finishing pigs. In contrast, Miller and Varel (2003) did not report an increase in VFA accumulation using microcrystalline cellulose using an in vitro incubation system. We noted an interaction between dietary CP and cellulose for acetic acid concentration in the manure ($P = 0.03$); lowering dietary CP decreased acetic acid concentration in pigs fed standard levels of dietary cellulose but increased acetic acid concentration in manure from pigs fed greater levels of dietary cellulose. This suggests a less efficient microbial fermentation in manure from pigs fed low dietary CP and standard cellulose but more efficient fermentation when these pigs were fed low dietary CP but high dietary cellulose. The differences in total VFA concentration (CP × cellulose interaction, $P = 0.05$) primarily reflect acetic acid concentration changes (Table 5).

Microbial production of indoles and phenols results from AA metabolism with phenol, p-cresol, and 4-ethyl phenol proposed as the main products of tyrosine fermentation, whereas indole and 3-methyl indole are products of tryptophan metabolism (Mackie et al., 1998). By lowering dietary CP and supplementation with limiting AA, excesses of these 2 AA would be reduced, which may have a subsequent impact on indole and phenol concentrations in manure. In our experiment decreasing dietary CP reduced manure phenol concentration ($P = 0.05$). Decreasing dietary CP also reduced 4-ethyl phenol but only in pigs fed the standard level of cellulose (CP × cellulose interaction, $P = 0.09$, Table 6). Cresol (p-cresol) was reduced, but only numerically ($P = 0.27$). Indole concentration was below our detection limit. Our data are supported by Hobbs et al. (1996), who reported reductions in 4-ethyl phenol and indole concentrations in fresh manure from growing pigs, and phenol and 4-ethyl phenol in fresh manure from finishing pigs, as dietary CP levels were reduced and replaced with crystalline AA. In contrast, van Heugten and van Kempen (2002) reported a reduction in fecal indole concentration by increasing dietary CP using feather meal, whereas 3-methyl indole was unaffected. Phenols and indoles differed little due to the level of dietary cellulose fed to finishing pigs as p-cresol was only numerically reduced ($P = 0.09$).

Principle component analysis was also used to assist in understanding dietary effects on manure composition; however, a simple bivariate scatter plot between NH_4 and pH revealed diet groups with no formal clustering considered (Figure 1). Pigs fed the reduced CP supplemented with AA with high cellulose diet generally had the lowest pH and NH_4 concentration, whereas pigs feed the standard diet generally had the greatest manure pH and NH_4 concentration. Figure 1 demonstrates that the ammonia ion is the dominant ion buffering swine manure pH, whereas the VFA content of the manure had little effect on the manure pH. Sommer and Husted (1995) have shown that manure pH is

Table 6. Major manure phenol and indole concentrations as affected by dietary protein and cellulose content

	Odorant, μmol/g of wet wt			
Dietary treatment[1]	Phenol	p-Cresol	4-ethyl phenol	3-methyl indole
12.0% CP, standard cellulose	0.12	0.59	0.02	0.01
14.5% CP, standard cellulose	0.19	0.64	0.09	0.03
12.0% CP, high cellulose	0.13	0.73	0.06	0.04
14.5% CP, high cellulose	0.17	0.96	0.07	0.04
Standard deviation	0.064	0.293	0.033	0.031
Main effect				
12.0% CP	0.12	0.66	0.04	0.02
14.5% CP	0.18	0.80	0.08	0.03
Standard cellulose	0.16	0.61	0.05	0.02
High cellulose	0.15	0.84	0.07	0.04
Source of variation, *P*-value				
Protein level	0.05	0.27	0.02	0.50
Cellulose level	0.67	0.09	0.34	0.12
Protein × cellulose	0.66	0.51	0.09	0.55

[1]Dietary cellulose was increased by 2.5-fold to 7.95% or 9.35% (relative to 2.27% in the 12% CP diet and 2.67% in the 14.5% CP diet, respectively) by addition of soybean hulls.

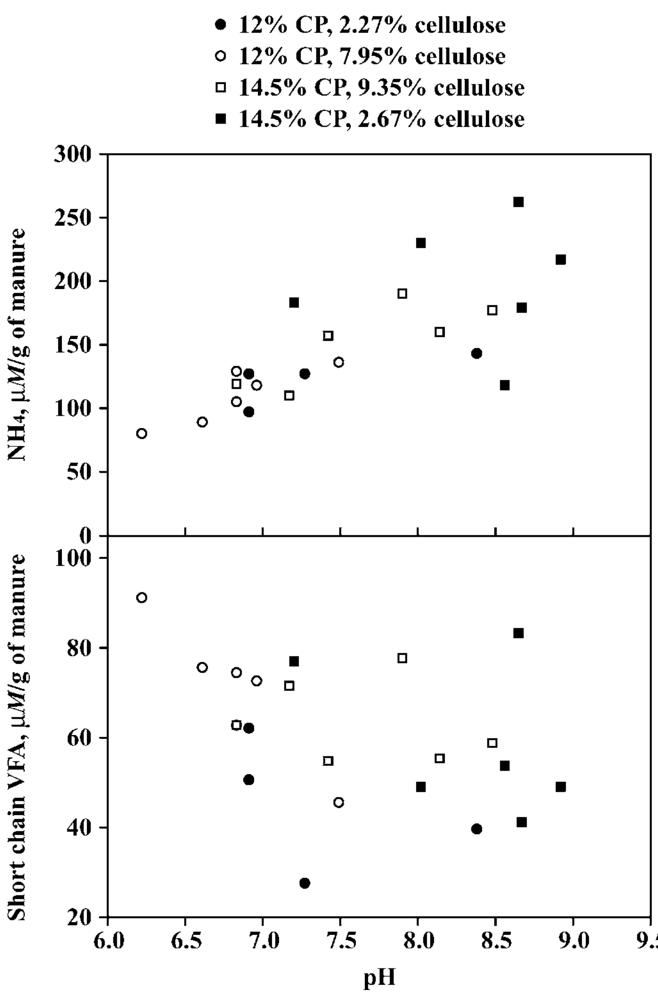

Figure 1. Relationship of short-chain fatty acid and ammonium-N concentrations and manure pH.

strongly controlled by the levels of ammonia ($NH4^+/NH_3$), carbonate ($CO_2/HCO_3^-/CO_3^{-2}$), and acetate (CH_3COOH/CH_3COO^-) ions in the manure. In this study, the ratio of NH_4 ions to VFA ions was 3:1 in the control diet, whereas in the high fiber and low CP diets that ratio dropped down to 2:1, and in the low CP with high fiber diet that ratio further dropped to 1.8:1. Miller and Varel (2003) also observed swine manure pH to be dominated by the ammonium ion.

Livestock operations are also considered important sources of thermally active gases (CH_4 and N_2O) that contribute to global warming (IPCC, 1997). It has been estimated that CH_4 and N_2O emissions from animal manures accounted for 41 and 16.7 Tg CO_2 equivalence, respectively, in the United States in 2000 (EPA, 2002). However, trace gas emissions from swine lagoon systems can be highly variable. In a recent study, Harper et al. (2000) observed CH_4 fluxes ranging from 1.4 to 125.8 kg of $CH_4 \cdot ha^{-1} \cdot d^{-1}$ from a 4-stage swine waste lagoon system in Georgia. Nitrous oxide emissions from the same lagoon system were lower and ranged from 0 to 3.1 kg of $N_2O \cdot ha^{-1} \cdot d^{-1}$. In another study, Desutter and Ham (2005) determined the average daily CH_4 flux from a swine manure lagoon to be 118 kg of $CH_4 \cdot ha^{-1} \cdot d^{-1}$. One factor that may contribute to the variability associated with CH_4 and N_2O production in swine manure is diet. This effect, however, has been poorly studied. Velthof et al. (2005) observed that swine diets had a significant impact on CH_4 emissions from stored manure, with CH_4 emission being positively correlated to manure DM, C, and VFA. Whereas N_2O production from stored manure was not measured, these investigators did monitor N_2O emissions from soils receiving the manure and concluded that there were no straightforward effects of diet composition on N_2O emissions from manure applied to soil. In our experiment, we observed no significant effect of diet on CH_4 concentration in the

Table 7. Headspace nitrous oxide and methane concentrations in swine manure as affected by dietary protein and cellulose content

Dietary treatment[1]	Concentration, ppm	
	Nitrous oxide	Methane
12.0% CP, standard cellulose	3.72	167.2
14.5% CP, standard cellulose	1.33	247.6
12.0% CP, high cellulose	5.15	132.8
14.5% CP, high cellulose	3.51	132.5
Standard deviation	2.646	159.46
Main effect		
12.0% CP	4.44	150.0
14.5% CP	2.42	190.0
Standard cellulose	2.53	207.4
High cellulose	4.33	132.6
Source of variation, *P*-value		
Protein level	0.03	0.46
Cellulose level	0.05	0.18
Protein × cellulose	0.68	0.46

[1]Dietary cellulose was increased by 2.5-fold to 7.95 or 9.35% (relative to 2.27% in the 12% CP diet and 2.67% in the 14.5% CP diet, respectively) by addition of soybean hulls.

headspace above stored manure (Table 7). Under the conditions of this research study, Pearson Product Moment analysis of manure properties with CH_4 concentration revealed that CH_4 concentration was correlated ($P = 0.08$) to C:N ratio of the manure (r = –0.923), and that a negative correlation also existed between CH_4 concentration and N_2O concentration (r = –0.9, $P = 0.10$). Nitrous oxide concentration exhibited significant correlations with NH_4 concentration (r = –0.968, $P = 0.032$), pH (r = –0.977, $P = 0.023$), and manure C:N ratio (r = 0.903, $P = 0.097$). Typically, N_2O production in stored manure is not expected, primarily because it is a good electron acceptor. However, N_2O production has been observed in lagoon storage systems (Harper et al., 2000) where there was an inverse relationship between N_2O flux and CH_4 flux. Clearly, additional investigations are necessary to assess the complex relationships between diet, manure properties, and N_2O and CH_4 production.

In summary, using a dynamic manure collection allowing for continual addition of feces and urine, we found that a reduction of dietary CP with crystalline AA supplementation decreased manure pH, NH_4, isovaleric acid, phenol, and 4-ethyl phenol concentrations. Increased dietary cellulose resulted in increased manure C content, p-cresol, acetic, propionic, and butyric acid concentrations but decreased pH and NH_4 concentrations. The lack of effect of lower CP diets on manure N content was not expected but may result from differences in N volatilization in our system.

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