Dietary Protein and Cellulose Effects on Chemical and Microbial Characteristics of Swine Feces and Stored Manure

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The objectives of this study were to investigate the effects of dietary crude protein (14.5 or 12.0%) and cellulose (8.7 or 2.5%) levels on composition of feces and manure after 8 wk of diet feeding and storage. Pigs were fed twice daily; after each feeding, urine and feces were collected and added to manure storage containers. On weeks 2 and 8 after initiation of the experiment, fresh fecal and manure samples were obtained. On Week 8, increased dietary cellulose resulted in significantly higher levels of volatile fatty acids (VFA) and phenols in feces compare to other diets. In contrast, dietary protein had the greatest effect on manure chemical composition; lower protein decreased sulfur content, ammonia, and phenolic compound concentrations. High levels of either dietary cellulose or protein tended to increase microbial community similarity in fecal samples, but only high protein increased similarity among manure sample microbial communities. Fecal and manure samples from Week 8 differed from samples taken in Week 2 both in chemical and microbiological composition. Week 2 samples had lower concentrations of many of chemical compounds and microbial diversity than samples from Week 8. The fecal results indicate that after 2 wk of feeding experimental diets the animals were not fully adapted to the diets. More importantly, after only 2 wk of urine and fecal collection, manure was not representative of stored manure, limiting its usefulness in developing standards and recommendations for on-farm management practices.

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Dietary formulation has the potential to lower odor through reducing excretion of odorous compounds in feces and manure of swine. Nutritionists can manipulate sources of carbon, nitrogen, and sulfur, but they must balance the needs and performance of the animal with the cost of the formulation. To date, the only areas that have been studied are reduction in crude protein (CP), sources of carbohydrates, and fiber content. Decreasing dietary protein in swine diets has been shown to reduce ammonia emissions from manure (Canh et al., 1998; Otto et al., 2003; Portejoie et al., 2004; Panetta et al., 2006; Leek et al., 2007). Carbohydrate sources, other than corn, have been shown to reduce ammonia emissions in manure (Mroz et al., 2000), while increasing fiber has resulted in lower levels of ammonia in manure (Shriver et al., 2003). Not all studies show a positive relationship between diet and reduced emission. Clark et al. (2005) found that increasing non-starch polysaccharides reduced total and ammonia nitrogen concentration in manure with decreased carbon dioxide but no change in methane emissions. Consequently, more work is needed to understand how diet is related to the formation of malodor compounds in manure.

Microorganisms are considered the primary agents producing malodorous compounds through the fermentation of dietary waste material. Yet, studies investigating the effects of microorganisms on odor production typically analyzed either fresh manure, 7 d old or less, or manure for longer times without addition of feces and urine (Canh et al., 1998; Gralapp et al., 2002; Miller and Varel, 2003; Otto et al., 2003; Portejoie et al., 2004; Clark et al., 2005; Miller et al., 2006; Panetta et al., 2006; Le et al., 2007; Leek et al., 2007). Few have characterized the changing nature of manure composition over time and its effects on microbial composition and odorous compound production. We have found no studies showing how diet affects the microbial community in manure. Two groups have characterized microbial diversity in swine manure (Whitehead and Cotta, 2001; Cotta et al., 2003; Snell-Castro et al., 2005), but both groups used samples collected at one time from a single manure storage pit with the dominant bacterial groups reported as the low G+C Gram-positives, *Bacillus-Lactobacillus-Streptococus*, and *Flexibacter-Cytophaga-Bacteroides* (Whitehead and Cotta, 2001; Cotta et al., 2003; Snell-Castro et al., 2005).

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The purpose of the present investigation was to monitor changes in the microbial community and malodorous chemical composition in both fresh feces and manure. We have designed a 700 L manure storage system to study the effects of dietary treatments on manure composition and microbial community structure. These tanks emulate on-farm manure storage in that feces and urine are added continually throughout the experiment and tank capacity allows for long term manure collection. Other advantages of this design are that use of multiple tanks allows replication of experimental treatments under controlled environmental conditions and homogenization of tank contents for manure sampling. We used these tanks together with swine metabolism crates to study the effects that dietary crude protein and cellulose levels have on the microbial community and malodorous chemical composition of fresh feces and stored manure. Samples were taken after 2 wk of manure storage to compare fecal and manure composition with that after 8 wk.

Materials and Methods

Animals and Diets

All procedures involving animal handling and testing were reviewed and approved by the Iowa State University Committee on Animal Care (approval #7-30-5494-S). 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% crude protein in combination with either 2.5 or 8.7% cellulose. Briefly, the decrease in protein was achieved by inclusion of crystalline amino acids and the increase in cellulose by inclusion of soybean hulls (12.1% CP, 2.1% fat, 46% cellulose) (NRC, 1989). All diets were formulated to 3400 kcal ME kg^{-1} and 0.70% true ileal digestible Lys content. Other nutrients were fed to meet animal requirements according to the NRC (1998). Pigs were moved to individual stainless steel metabolism crates (1.2 × 2.4 m), adapted for 1 wk, and then fed approximately 3% of their body weight of their respective experimental diets. The control diet (14.5% CP and 2.5% cellulose) was fed twice daily during the adaptation to metabolism crates. A detailed diet composition is given in Kerr et al. (2006). Water was supplied ad libitum through nipple waters. Metabolism crates were designed to allow for separate collection of feces and urine.

Experimental diets were fed twice daily (0700 and 1900). After each feeding, feces and urine from each metabolism crate were collected and added to its assigned manure storage container. Each stainless steel manure storage container measured 122 cm high and was 96.5 cm in diameter. The lid on each container was fitted with an apparatus which pulled a constant stream of air over the manure (7 L min^{-1}) and allowed the addition of daily fecal and urine collections and the collection of manure samples. Manure tanks were designed to have a similar surface area as pigs maintained in growing-finishing barns with deep pit manure storage systems. Room temperature was maintained at 21.5°C and manure temperature averaged 18.5°C (± 2.0°C).

Samples were obtained at the end of Week 2 and Week 8 for subsequent analysis. Freshly voided feces were collected in sterile plastic specimen cups. Manure samples were taken after mixing each tank with a 15 cm stainless steel propeller for 3 min at a speed of 850 rpm, with the propeller positioned to avoid introduction of oxygen. For this paper "feces" indicates freshly defecated material and "manure" indicates feces + urine.

Bacterial Analyses

Fecal and manure samples were diluted serially (from 10^0 through 10^{-8}) in ½ strength peptone water (all microbiological media were obtained from Fisher Scientific, Pittsburgh, PA). Total anaerobes were determined by counting colonies from 10^{-8} to 10^{-5} dilutions on Wilken-Chalgren agar after 5 d at 37°C with anaerobic atmosphere and total aerobes by counting colonies from 10^{-6} to 10^{-3} dilutions on Nutrient agar after 2 d at 37°C.

Denaturing gradient gel electrophoresis (DGGE) was used to obtain a profile of dominant bacteria in the fresh fecal and manure samples. Nucleic acids were extracted using QIAgen Stool Kit (QIAgen, Valencia, CA) (Li et al., 2003), with three replicate extractions pooled. The PCR primers for DGGE DNA amplification targeted the variable part of the 16S ribosomal RNA gene (V3 region) 357f-gc (CCT ACG GGA GGC AGC AG– CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG G) and 519r ATT (ACC GCG GCK GCT GG) (Yu and Morrison, 2004). For amplification each 50 µL reaction mix contained final concentrations of 80 ng μL^{-1} DNA template, 1 µmol L^{-1} of each primer with 1.25 U FailSafe PCR Enzyme Mix plus 25 µL FailSafe PCR PreMix D (Epicenter, Madison, WI). After an initial denaturing step for 5 min at 94°C, 10 cycles of touchdown PCR ran 30 s at 94°C, 30 s annealing with 0.5°C/cycle temperature decrease (61 to 56°C) and extension for 1 min at 72°C followed 25 cycles of 30 s at 94°C, 30 s at 56°C, and 1 min at 72°C followed by 7 min at 72°C for final elongation using a Dyad Thermal Cycler (MJ Research Inc., Watertown, MA) (Yu and Morrison, 2004).

A model 475 Gradient Delivery System (BioRad, Hercules, CA) was used to pour DGGE gels with 35 to 50% urea-formamide denaturing gradients (100% denaturant is defined as 7 mol L^{-1} urea and 40% formamide). Gels were 10% polyacrylamide (37.5:1 acrylamide-bisacrylamide) in 0.5 × Tris-acetate-EDTA buffer (pH 8.0). Gels were allowed to polymerize at least 4 h before use. Denaturing gradient gel electrophoresis was performed using the BioRad Dcode Universal Mutation Detection System (BioRad) with 10-µL aliquots of cleaned PCR products (plus 10 µL loading buffer) electrophoresed at 100 V with 400 mA for 16 h in 0.5 × TAE buffer at a constant temperature of 60°C. Gels were stained with SYBR Gold (Invitrogen, Carlsbad, CA) for 40 min according to the manufacturer's instructions and photographed with a Kodak Image Station 4000MM Pro (Carestream Health, Inc, New Haven, CT).

Final gel image processing, band numbers per sample, and cluster analysis of DGGE banding patterns was performed using BioNumerics software (Applied Maths, Austin, TX). Bands were counted if they were at least 2% of the strongest band in each lane after normalization of gels. Constructed trees (using Dice similarity coefficients and unweighted pair group method with arithmetic means) show groupings of samples by similarity in banding patterns. Diversity indices were calculated: species richness (S) was determined by the number of bands, the Shannon-Wiener index (H') as $H' = -\Sigma P_i \ln P_i$ with P_i equal to n_i/N, where n_i is the peak height of a band and N is the sum of all peak heights in the densitometric curve, and evenness (E) as $E = H'/H'_{max}$, where H'_{max} =

ln *S* (Yu and Morrison, 2004). Species richness represents the total number of different species present in the sample; *H'* accounts for species richness and the proportion of each species within a sample and *E* measures how similar the abundances of different species are in the sample (an evenness of one occurs when the proportions of all subspecies are the same).

Chemical Analysis

Methods for chemical component analysis are described in detail in Kerr et al. (2006) and briefly outlined here. To compare samples, chemical components were calculated on a dry matter basis for both fresh feces and manure. Fresh fecal and manure pH were measured using a pH meter (Corning Model 530 with Corning probe #476436, Corning Inc., Corning, NY) and dry matter (DM) determined by 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) while C, N, and S were analyzed using a VarioMAX CNS analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Volatile fatty acids (VFA), phenols, and indoles were analyzed using gas chromatography on an Agilent 6890 gas chromatograph (GC) equipped with a flame ionization detector and polyethylene glycol polymer column analyzing the free fatty acid phase (Aglient Technologies, Wilmington, DE).

Statistical Analysis

Statistical analyses on chemical characteristics were performed using the GLM procedure of SAS statistical software (SAS Institute, 1990). Data were analyzed as a factorial arrangement of treatments within a randomized complete block design with the individual pig or manure tank as the experimental unit. Effects of dietary treatment were analyzed using results from Week 8. There were six observations per treatment, except for pigs fed the low CP, AA-supplemented diets containing a standard level of dietary cellulose where there were only four observations per treatment. Data, on a DM basis, are presented as least square means (LSM), for dietary effects (Week 8) the treatment LSM are presented, and for storage time (Week 2 vs. Week 8) effects main effect LSM are presented. Differences were considered significant if $P \leq 0.10$.

Results and Discussion

Average pig initial and final body weights were 105 and 153 kg, respectively, over the 8 wk feeding period. Average daily fecal outputs as well as manure volume at Weeks 2 and 8 are presented in Table 1.

Effects of Dietary Treatment, Week 8

Increased dietary cellulose increased most VFA compounds (except butanoic acid) and 4-methylphenol concentration levels (Table 2). Increases in VFA concentrations in feces with high dietary cellulose treatments were expected as a result of microbial fermentation in the large intestine (Macfarlane and Gibson, 1994). Changes in dietary protein had no effect on any of the measured values in fecal material except to increase total nitrogen content. Significant interactions of protein and cellulose level were observed for fecal DM, total nitrogen, and sulfur content (Table 2). The interactive effect on fecal DM content was due to increased cellulose decreasing fecal DM to a greater extent when fed in combination with low protein. Two other significant interactions (nitrogen and sulfur) resulted from increased cellulose increasing fecal content when fed with high protein but decreasing content when low protein was fed.

There were no effects of dietary treatment for total anaerobic and aerobic bacteria in feces, (Table 2). Diversity of bacteria in fecal samples, represented by species richness (*S*), increased when high cellulose was fed. Feeding whole crop rice to pigs increased species richness in DGGE analysis of feces (Wang et al., 2007). These results are in contrast to results where increased insoluble non-structural polysaccharides in pig diets resulted in decreased species richness (Högberg et al., 2004; Castillo et al., 2007). Other diversity indices, evenness (*E*) and Shannon Wiener index (*H'*), did not differ due to either protein or cellulose level. Similarity in banding patterns, as demonstrated by cluster analysis, was more pronounced for protein level in the diet than for cellulose, based on similarity values of >50% (Fig. 1). Lack of discrete clustering of DGGE patterns by treatment for individual pigs is often seen (Zhu et al., 2003; Wang et al., 2007); environmental factors are thought to have less of an effect on the composition of intestinal bacteria than host factors (Zoetendal et al., 1998; Simpson et al., 2000; McCracken et al., 2001).

Reduced dietary protein significantly increased the percentage of DM and significantly decreased ammonia, 3-methylbutanoic acid, phenol, 4-methylphenol, and 4-ethylphenol concentrations in manure (Table 3). Higher dietary cellulose increased the percentage of DM and decreased ammonia concentrations in manure. As previously reported by Portejoie et al. (2004) we found that manure had markedly increased nitrogen compared to that in feces, presumably because of the addition of urine. When high cellulose was fed with the high protein diet, manure ammonia level was reduced to almost half the concentration of that in the low cellulose and high protein diet. This reduction was likely due to two factors, ammonia volatilization and microbial incorporation of ammonia into microbial biomass during cellulose fermentation. Ammonia volatilization is confirmed by the concurrent decrease in nitrogen; however, decreased manure nitrogen only accounts for part of the decrease in manure ammonia. Conversion of ammonia into microbial biomass is likely as ammonia is the preferred nitrogen source for a number of anaerobic bacteria

Table 1. Fecal output and manure volume from pigs fed diets with different protein and cellulose content for 8 wk.

	Dietary treatments (Protein x Cellulose)†				
Component	High x Low	High x High	Low x Low	Low x High	SEM
As-is basis					
Fecal output, g d^{-1}‡	626	917	544	788	389
Manure volume, L					
Week 2	263	260	257	266	22
Week 8	474	474	496	514	83

† Level of dietary protein: High = 14.5% and Low = 12.0% and level of dietary cellulose: High = 8.7% and Low = 2.5%.

‡ Significant effects ($P \leq 0.10$) for protein and cellulose.

Table 2. Fecal characteristics as affected by dietary protein and cellulose content at Week 8. Means within rows that do not share the same letter(s) differ ($P \leq 0.10$).

	Dietary treatments (Protein x Cellulose)†					Source of variation ($P \leq$)		
Component	High x Low	High x High	Low x Low	Low x High	SEM	Protein	Cellulose	Interaction‡
As-is basis								
DM, %	34.0ab	30.6b	37.57a	31.5b	1.8	0.24	0.58	0.02
pH	6.72ab	6.35b	6.47b	7.0a	0.20	0.18	0.01	0.40
Anaerobes, $\times 10^{10}$ CFU§ mL^{-1}	6.25a	4.03ab	3.05ab	2.7b	1.64	0.14	0.39	0.53
Aerobes, $\times 10^{5}$ CFU mL^{-1}	9.51a	8.28ab	5.54ab	4.0b	3.05	0.12	0.50	0.90
Dry matter basis								
NH_4–N, μmol g^{-1}	116b	158a	118b	106b	17.4	0.12	0.35	0.08
Nitrogen, %	3.0b	3.8a	3.0b	3.0b	0.2	0.02	0.02	0.01
Carbon, %	41.0b	45.7a	41.6b	44.2a	1.0	0.60	0.01	0.20
Sulfur, %	0.4b	0.4a	0.4ab	0.4b	0.02	0.35	0.32	0.03
Acetic acid, μmol g^{-1}	202ab	234a	170b	252a	28.7	0.77	0.03	0.32
Propanoic acid, μmol g^{-1}	74.7ab	95.1a	51.6b	82.9a	13.3	0.14	0.04	0.64
2-methylpropanoic acid, μmol g^{-1}	11.2bc	15.2a	9.7c	14.1ab	1.6	0.35	0.01	0.91
Butanoic acid, μmol g^{-1}	38.8b	55.4a	35.3b	39.8ab	8.6	0.21	0.17	0.42
3-methylbutanoic acid, μmol g^{-1}	13.9b	21.4a	13.1b	17.8ab	3.0	0.40	0.03	0.61
Pentanoic acid, μmol g^{-1}	8.0b	12.1a	7.7b	9.5ab	1.6	0.29	0.04	0.42
Hexanoic acid, μmol g^{-1}	0.8b	1.9a	0.7b	1.0b	0.4	0.12	0.04	0.29
Total VFA¶, μmol g^{-1}	350ab	435a	288b	417a	50.6	0.37	0.02	0.46
4-methylphenol, μmol g^{-1}	1.9b	4.6a	2.6b	3.1ab	0.8	0.53	0.03	0.23
3-methylindole, μmol g^{-1}	0.4	0.6	0.5	0.4	0.2	0.91	0.67	0.23
Total aromatics#, μmol g^{-1}	2.5b	5.3a	3.5ab	3.7ab	1.0	0.75	0.09	0.13
Microbial diversity indices								
Richness	4.5ab	5.3ab	3.5b	5.8a	0.82	0.78	0.09	0.40
Evenness	0.80ab	0.74b	0.82ab	0.87a	0.05	0.17	0.90	0.30
Shannon-Wiener index	1.3ab	1.2b	1.0ab	1.5a	0.2	0.82	0.17	0.11

† Level of dietary protein: High = 14.5% and Low = 12.0% and level of dietary cellulose: High = 8.7% and Low = 2.5%.

‡ Interaction = statistical interaction of protein and cellulose.

§ CFU = colony forming units.

¶ Total VFA is total volatile fatty acids = acetic + propanoic + 2-methylpropanoic + butanoic + 3-methylbutanoic + pentaonic +hexanoic + 4-methylpentaonic acids acids.

Total Phenolics = phenol + 4-methylphenol + 4-ethylphenol + indole + 3-methylindole.

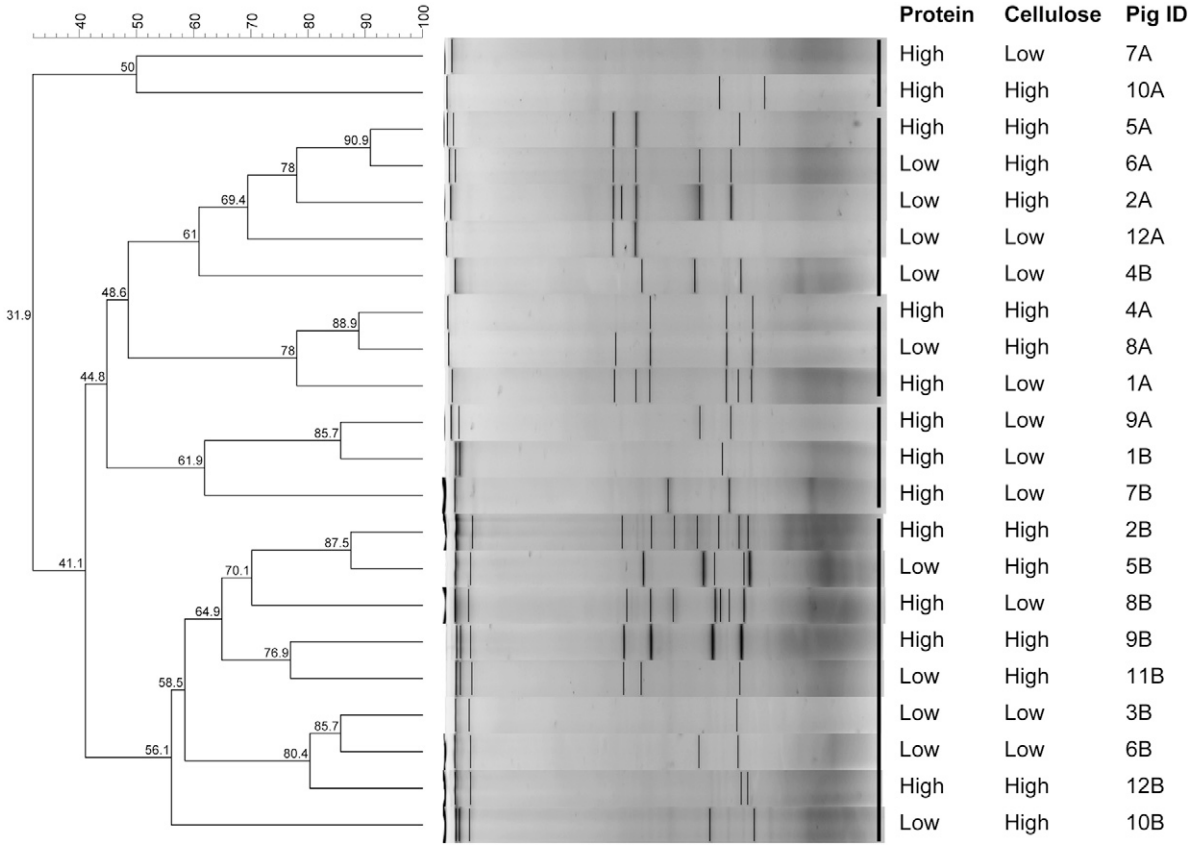

Fig. 1. Cluster analysis, with similarity values (%) and DGGE banding patterns for bacteria in fecal samples from pigs fed diets varying in protein (High = 14.5 and Low = 12.2%) and cellulose (High = 8.7 and Low = 2.5%) at Week 8. Samples are coded by animal number, run (A or B), and week of sampling. Lines at the end of the DGGE gel image represent groupings by similarity >50%. The bar at the top is a similarity continuum.

Table 3. Manure characteristics as affected by dietary protein and cellulose content at Week 8. Means within rows that do not share the same letter(s) differ ($P \leq 0.10$).

	Dietary treatments (Protein x Cellulose)†					Source of variation ($P \leq$)		
Component	High x Low	High x High	Low x Low	Low x High	SEM	Protein	Cellulose	Interaction‡
As-is basis								
DM, %	2.0b	3.0ab	2.8ab	3.7a	0.8	0.01	0.01	0.88
pH	8.34a	7.66b	7.56b	6.82c	0.22	0.26	0.16	0.95
Anaerobes, $\times 10^{10}$ CFU§ mL^{-1}	1.57b	4.27a	2.39b	5.46a	0.77	0.14	0.01	0.78
Aerobes, $\times 10^{5}$ CFU mL^{-1}	1.15b	1.81ab	2.02ab	2.23a	0.56	0.19	0.38	0.65
Dry matter basis								
NH_4–N, μmol g^{-1}	10670a	5988b	5034b	3738b	1230	0.01	0.01	0.12
Nitrogen, %	9.6a	6.3b	5.8b	6.3b	1.3	0.12	0.24	0.10
Carbon, %	51.8	43. 8	33.3	45.7	9.2	0.30	0.78	0.21
Sulfur, %	1.6a	1.1b	1.0b	1.0	0.2	0.08	0.13	0.17
Acetic acid, μmol g^{-1}	2738a	1899ab	1473b	1960ab	512	0.19	0.69	0.15
Propanoic acid, μmol g^{-1}	446.5	377.4	292.8	412. 5	100.6	0.51	0.78	0.29
2-methylpropanoic acid, μmol g^{-1}	86.1	65.1	54.6	53.1	17.1	0.15	0.45	0.51
Butanoic acid, μmol g^{-1}	195	169	124	192	55.9	0.62	0.67	0.34
3-methylbutanoic acid, μmol g^{-1}	96.7a	70.4ab	51.9b	54.9b	16.9	0.05	0.43	0.32
Pentanoic acid, μmol g^{-1}	19.8	21.7	8.3	21.8	9.2	0.48	0.34	0.47
Hexanoic acid, μmol g^{-1}	16.6	12.9	12.6	10.2	4.6	0.41	0.45	0.86
Total VFA¶, μmol g^{-1}	3599a	2616ab	2019b	2704ab	697	0.23	0.81	0.18
Phenol, μmol g^{-1}	10.21a	6.6b	5.2b	4.3b	1.7	0.02	0.13	0.37
4-methylphenol, μmol g^{-1}	37.1	37.1	23.4	23.8	9.0	0.09	0.98	0.98
4-ethylphenol, μmol g^{-1}	4.7a	3.0ab	0.7c	2.1bc	1.0	0.01	0.87	0.08
3-methylindole, μmol g^{-1}	1.4	1.6	0.3	1.3	0.8	0.33	0.40	0.54
Total aromatics#, μmol g^{-1}	53.7a	48.3ab	29.6b	31.6b	11.3	0.05	0.86	0.70
Microbial diversity indices								
Richness	7.2	8.2	7.8	8.3	0.9	0.65	0.64	0.80
Evenness	0.84b	0.84b	0.91a	0.79a	0.03	0.70	0.03	0.04
Shannon-Wiener index	1.7	1.7	1.8	1.7	0.1	0.61	0.69	0.30

† Level of dietary protein: High = 14.5% and Low = 12.0% and level of dietary cellulose: High = 8.7% and Low = 2.5%.

‡ Interaction = interaction of protein and cellulose.

§ CFU = colony forming units.

¶ Total VFA = acetic + propanoic + 2-methylpropanoic + butanoic + 3-methylbutanoic + pentaonic +hexanoic + 4-methylpentaonic acids.

Total Aromatics = phenol + 4-methylphenol + 4-ethylphenol + indole + 3-methylindole.

including cellulolytic species (Cotta and Russell, 1997). Increased manure phenolic concentrations that we observed may have resulted from microbial fermentation of aromatic amino acids (tyrosine, phenylalanine, and tryptophan) when high protein diets were fed (Macfarlane and Gibson, 1994). Similar to fermentation patterns in the gastrointestinal tract, results in manure demonstrated the importance of coordinating nitrogen and structural carbohydrate metabolism for manure microbial populations. When cellulose was low and nitrogen in excess there was conversion of nitrogen to ammonia. Implications of this are fully discussed in Kerr et al. (2006).

One of the most interesting observations of this study was that in feces dietary cellulose level appeared to be the primary driving factor for differences in chemical composition (main effect of cellulose was significant for 12 characteristics as compared to two interactions) while in manure the primary driving factor appeared to be dietary protein level (main effect of protein was significant for eight characteristics compared to three for cellulose and two interactions).

Dietary treatments did not affect total aerobic bacteria numbers in the manure; increased cellulose increased total anaerobic bacteria (Table 3). Dietary treatment did not affect *S* or *H'* but *E* was significant for the effect of cellulose and the interaction of cellulose and protein. The diet with low protein and cellulose concentrations had the greatest *E,* indicating similar proportions of all species present in the DGGE analysis (Fig. 2), while all other dietary treatments were the same. Similarity among DGGE banding patterns was high; 21 of 22 samples had 50% or greater similarity to each other. At a similarity level of 70%, DGGE patterns strongly clustered by level of protein in the diet (Fig. 2). Interestingly, even though each manure tank received the feces from the same animal, differences due to dietary treatments in the manure did not follow those in the feces. This is likely due to the high level of nitrogen and higher pH in manure due to the addition of urine and was reflected in the higher *S* in manure when compared to feces (Tables 2 and 3).

Changes in Composition and Microbiology, Week 2 versus Week 8

There were no significant interactions between week and dietary treatment. The main effects of dietary protein and cellulose in feces and manure for Weeks 2 and 8 are presented in Tables 4 and 5. Fecal dry matter nitrogen, carbon, and sulfur contents, along with pH, and bacterial counts were not significantly different between Weeks 2 and 8. In general, VFA concentrations decreased from Week 2 to Week 8 while aromatic compound concentrations increased. Typically, swine nutrition research uses an experimental period of about 2 wk for digestibility and/or availability studies. The changes we observed in chemical composition of the feces between Weeks 2 and 8 indicate that the animals were not fully adapted to the experimental diets. This is supported by the differences in DGGE banding patterns and diversity indices demonstrating differences in bacterial communities between Week 8 and week 2 (see results below).

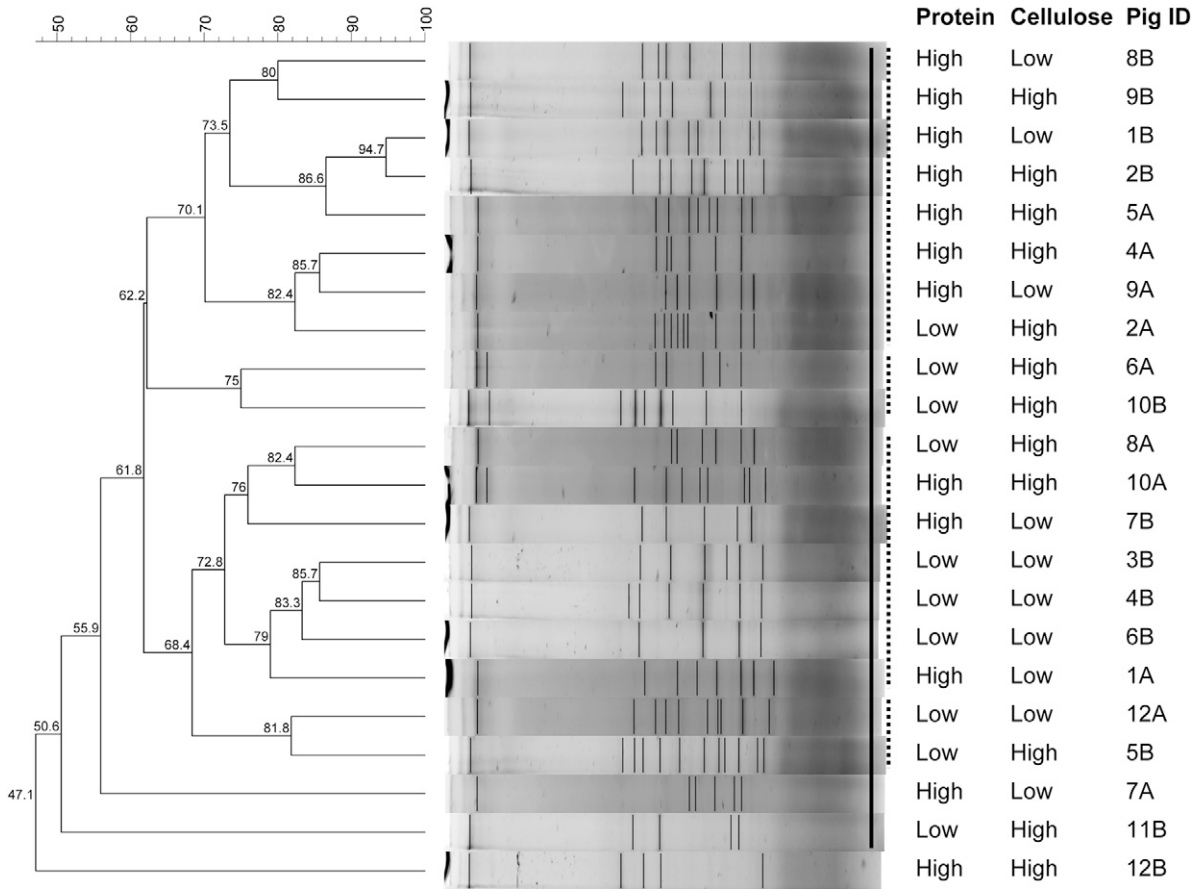

Fig. 2. Cluster analysis, with similarity values (%) and DGGE banding patterns for bacteria in manure samples from storage tanks containing waste from pigs fed diets varying in protein (High = 14.5 and Low = 12.2%) and cellulose (High = 8.7 and Low = 2.5%) at Week 8. Samples are coded by animal number, run (A or B), and week of sampling. Lines at the end of the DGGE gel image represent groupings by similarity, with the solid line >50% and dashed line >70% similarity. The bar at the top is a similarity continuum.

Although bacterial counts did not differ, diversity indices *S* and *E* were significantly different between Weeks 2 and 8. Species richness (*S*) was lower in Week 8 compared to Week 2 while *E* was increased (Table 4). Högberg et al. (2004) demonstrated differences in coliform diversity between Day 0, 9, and 17 when pigs were fed different non-structural carbohydrates. Shannon-Wiener index did not differ between weeks. Similarity among DGGE patterns was high at Week 2 without clusters by treatment (Fig. 3). Because all pigs were fed the same diet before the experiment began, Week 2 DGGE banding patterns may be more reflective of differences among individuals. Banding patterns for samples taken at Week 8 show a greater degree of clustering due to dietary treatment indicating adaptation of the microbial community to the different diets.

In manure, ammonia concentration and pH were decreased in Week 8 compared to Week 2. Nitrogen, carbon, and sulfur contents of manure were not significantly different between weeks. Concentrations of VFA and phenolics were higher in Week 8 than in Week 2. Data from Week 2 underestimated the load of VFA in the manure by about half (average total VFA concentration was 1445 μmol g^{-1} in Week 2 and 2734 μmol g^{-1} in Week 8) and aromatic compounds by almost three times (average total aromatic compound concentration was 14.8 μmol g^{-1} in Week 2 and 40.8 μmol g^{-1} in Week 8). In contrast, ammonia concentrations at Week 8 were lower than at in Week 2. This is likely related to adaptation of the bacterial populations; the storage tanks were closed to keep ammonia volatilization to a minimum. This study highlights the need for longer periods for manure storage with continuous addition of feces to manure because the short term manure storage (2 wk) resulted in underestimates of VFA and phenolic and over estimations of ammonia concentrations.

As with feces, bacterial counts in manure samples did not differ between weeks (Table 5). Microbial diversity indices *E* and *H'* were higher in Week 8 compared to Week 2 and there was a tendency toward increased *S*. Similar to observations in feces, Week 2 DGGE banding patterns were highly similar among samples and clustering by treatment was not evident (Fig. 4). Banding patterns and diversity indices indicate the bacterial communities in manure developed over time and that the level of dietary protein influenced which bacteria were present in the manure.

Conclusions

The results of this study demonstrate that manure composition is affected by microbial fermentation of nitrogen and carbohydrate. In this study, nitrogen was the major factor in manure fermentation; however, additional dietary cellulose was able to lower the concentrations of many of the main odorous compound responses

Table 4. Comparison of protein and cellulose main effects on fecal composition between Week 2 and 8.

	Main effects								
	Week 2				Week 8				
	Protein†		Cellulose‡		Protein		Cellulose		Week
Component	High	Low	Low	High	High	Low	Low	High	($P \leq$)
As-is basis									
DM, %	31.3	31.2	32.8	29.8	32.3	34.5	35.8	31.1	0.13
pH	6.39	6.74	6.48	6.65	6.54	6.75	6.60	6.69	0.50
Dry matter basis									
NH_4–N, μmol g^{-1}	311	236	234	313	137	112	117	132	0.07
Nitrogen, %	3.3	2.9	2.8	3.4	3.4	3.0	3.0	3.4	0.36
Carbon, %	43.6	44.3	42.1	45.8	43.3	42.9	41.3	44.9	0.07
Sulfur, %	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.81
Acetic acid, μmol g^{-1}	339	332	308	363	218	211	186	243	0.01
Propanoic acid, μmol g^{-1}	134	113	107	140	85	67	63	89	0.01
2-methylpropanoic acid, μmol g^{-1}	19.3	16.7	15.1	26.9	13.2	11.9	10.5	14.7	0.01
Butanoic acid, μmol g^{-1}	66.3	49.0	57.8	57.5	47.1	37.5	37.0	47.6	0.01
3-methylbutanoic acid, μmol g^{-1}	23.0	18.8	17.8	24.1	17.7	15.5	13.1	19.6	0.01
Pentanoic acid, μmol g^{-1}	12.2	9.3	11.7	9.8	10.1	8.6	7.8	10.8	0.57
Hexanoic acid, μmol g^{-1}	5.1	5.0	4.6	5.4	1.4	0.8	0.7	1.5	0.01
Total VFA§, μmol g^{-1}	590	544	522	621	392	352	319	426	0.01
Phenol, μmol g^{-1}	0.7	0.7	0.6	0.7	0.7	0.7	0.2	0.1	0.01
4-methylphenol, μmol g^{-1}	2.1	1.7	1.5	2.4	1.7	2.0	2.8	3.9	0.01
3-methylindole, μmol g^{-1}	2.3	2.11	1.9	2.6	2.1	2.6	0.5	0.5	0.01
Total aromatics¶, μmol g^{-1}	5.2	4.7	4.1	5.7	3.49	3.6	3.0	4.5	0.02
Microbial diversity indices									
Richness	6.5	5.1	4.8	6.8	4.9	4.7	4.0	5.6	0.07
Evenness	0.73	0.68	0.73	0.68	0.77	0.84	0.81	0.81	0.01
Shannon-Wiener index	1.3	1.0	1.1	1.3	1.3	1.2	1.1	1.4	0.60

† Level of dietary protein: High = 14.5% and Low = 12.0%.

‡ Level of dietary cellulose: High = 8.7% and Low = 2.5%.

§ Total VFA = acetic + propanoic + 2-methylpropanoic + butanoic + 3-methylbutanoic + pentaonic +hexanoic + 4-methylpentaonic acids.

¶ Total aromatics = phenol + 4-methylphenol + 4-ethylphenol + indole + 3-methylindole.

Table 5. Comparison of protein and cellulose main effects on manure composition between Week 2 and 8.

	Main effects								
	Week 2				Week 8				
	Protein†		Cellulose‡		Protein		Cellulose		Week
Component	High	Low	Low	High	High	Low	Low	High	($P \leq$)
As-is basis									
DM, %	1.4	1.5	1.2	1.7	2.5	3.2	2.4	3.3	0.01
pH	8.33	7.96	8.37	7.92	8.00	7.19	7.95	7.24	0.01
Dry matter basis									
NH_4–N, μmol g^{-1}	9874	8014	12294	5593	8329	4387	7853	4863	0.04
Nitrogen, %	6.5	6.4	8.1	4.8	7.9	6.1	7.7	6.3	0.36
Carbon, %	37.6	47.6	47.2	38.1	47.8	39.5	42.5	44.7	0.49
Sulfur, %	1.1	1.9	1.4	1.8	1.3	1.0	1.3	1.0	0.51
Acetic acid, μmol g^{-1}	1013	1133	1206	940	2319	1716	2105	1929	0.03
Propanoic acid, μmol g^{-1}	210	150	229	180	412	354	371	395	0.01
2-methylpropanoic acid, μmol g^{-1}	26.5	18.3	21.7	23.2	75.6	53.8	70.3	59.1	0.01
Butanoic acid, μmol g^{-1}	70.9	47.9	55.1	63.8	182	158	160	180	0.01
3-methylbutanoic acid, μmol g^{-1}	43.3	33.2	38.2	38.38	83.6	53.4	74.3	62.7	0.05
Pentanoic acid, μmol g^{-1}	7.1	3.7	3.1	7.7	20.7	15.1	14.1	21.7	0.01
Hexanoic acid, μmol g^{-1}	37.7	45.7	48.3	35.0	14.7	11.4	14.6	11.5	0.01
Total VFA§, μmol g^{-1}	1409	1482	1602	1288	3107	2361	2809	2660	0.01
Phenol, μmol g^{-1}	0.8	0.2	0.3	0.63	8.4	4.8	7.7	5.5	0.01
4-methylphenol, μmol g^{-1}	1.8	2.2	2.3	1.7	37.1	23.6	30.3	30.5	0.01
4-ethylphenol, μmol g^{-1}	0.4	0.4	0.4	0.4	3.9	1.4	2.7	2.6	0.01
3-methylindole, μmol g^{-1}	12.1	11.7	16.6	7. 2	0.1	0.0	0.1	0.0	0.01
Total aromatics¶, μmol g^{-1}	15.0	14.5	19.6	10.0	51.0	30.6	41.7	39.9	0.01
Microbial diversity indices									
Richness	6.8	6.9	6.1	7.6	7.7	8.0	7.5	8.3	0.19
Evenness	0.79	0.76	0.77	0.78	0.84	0.85	0.88	0.82	0.05
Shannon-Wiener index	1.5	1.4	1.3	1.6	1.7	1.8	1.8	1.7	0.03

† Level of dietary protein: High = 14.5% and Low = 12.0%.

‡ Level of dietary cellulose: High = 8.7% and Low = 2.5%.

§ Total VFA = acetic + propanoic + 2-methylpropanoic + butanoic + 3-methylbutanoic + pentaonic +hexanoic + 4-methylpentaonic acids.

¶ Total aromatics = phenol + 4-methylphenol + 4-ethylphenol + indole + 3-methylindole.

to nitrogen. Continual addition of urine and feces and long-term storage in manure storage models more realistically mimic the dynamic biology of conventional pit storage systems. Short-term manure storage (2 wk or less) is not recommended because it will likely underestimate VFA and aromatic compound concentrations, while potentially over estimating ammonia concentrations.

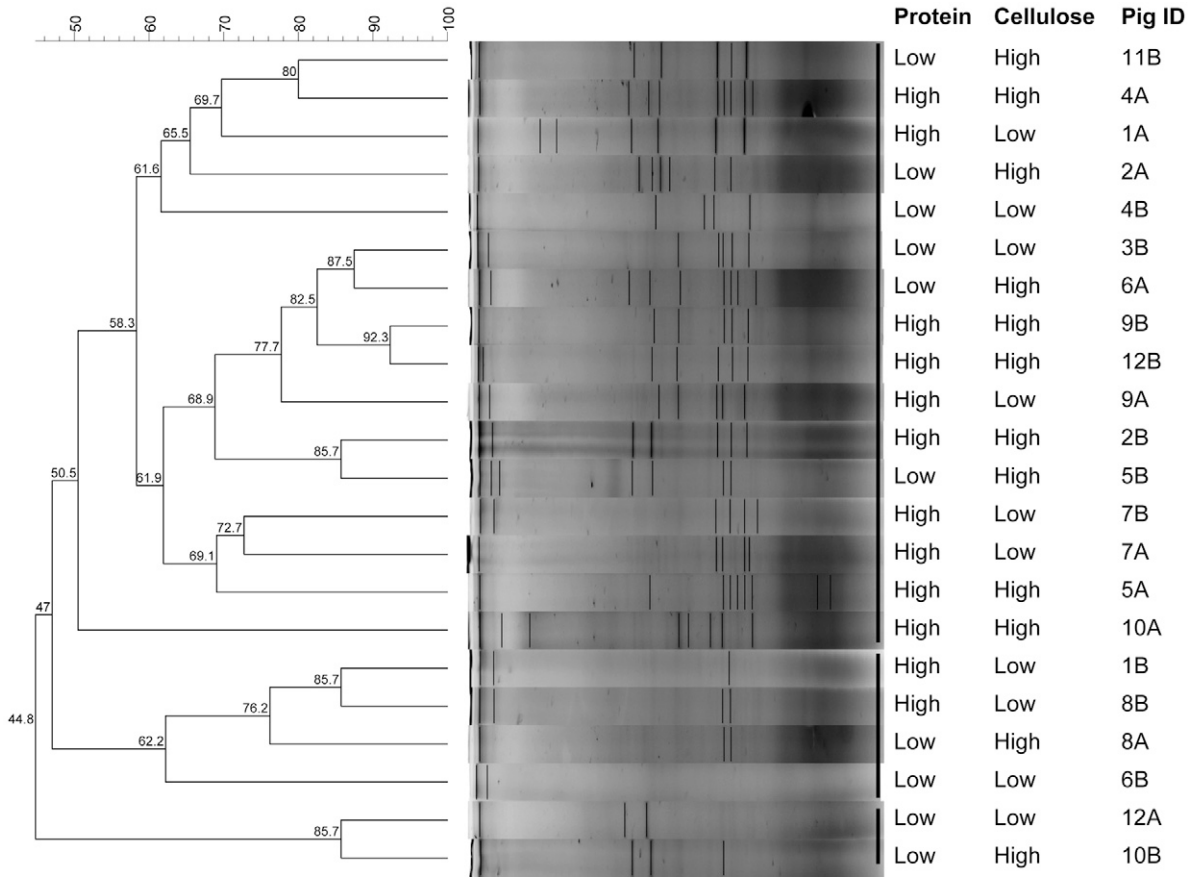

Fig. 3. Cluster analysis, with similarity values (%) and DGGE banding patterns for bacteria in fecal samples from pigs fed diets varying in protein (High = 14.5 and Low = 12.2%) and cellulose (High = 8.7 and Low = 2.5%) at Week 2. Samples are coded by animal number, run (A or B), and week of sampling. Lines at the end of the DGGE gel image represent groupings by similarity >50%. The bar at the top is a similarity continuum.

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References

Canh, T.T., A.J.A. Aarnink, J.B. Schutte, A. Sutton, D.J. Langhout, and M.W.A. Verstegen. 1998. Dietary protein affects nitrogen excretion and ammonia emission from slurry of growing-finishing pigs. Livest. Prod. Sci. 56:181–191.

Castillo, M., G. Skene, M. Roca, M. Anguita, I. Badiola, S.H. Duncan, H.J. Flint, and S.M. Martine-Orúe. 2007. Application of 16S rRNA gene-targeted fluorescence in situ hybridization and restriction fragment length polymorphism to study porcine microbiota along the gastrointestinal tract in response to different sources of dietary fibre. FEMS Microbiol. Ecol. 59:138–146.

Chaney, A.L., and E.P. Marbach. 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8:130–132.

Clark, O.G., S. Moehn, I. Edeogu, J. Price, and J. Leonard. 2005. Manipulation of dietary protein and nonstarch polysaccharide to control swine manure emissions. J. Environ. Qual. 34:1461–1466.

Cotta, M.A., and J.B. Russell. 1997. Digestion of nitrogen in the rumen: A model for metabolism of nitrogen compounds in gastrointestinal environments. p. 380–423. *In* R.I. Mackie and B.A. White (ed.) Gastrointestinal microbiology. Vol. 1. Gastrointestinal ecosystems and fermentations. Chapman and Hall, New York.

Cotta, M.A., T.R. Whitehead, and R.L. Zeltwanger. 2003. Isolation, characterization, and comparison of bacteria from swine faeces and manure storage pits. Environ. Microbiol. 5:737–745.

Gralapp, A.K., W.J. Powers, M.A. Faust, and D.S. Bundy. 2002. Effects of dietary ingredients on manure characteristics and odorous emissions from swine. J. Anim. Sci. 80:1512–1519.

Högberg, A., J.E. Lindberg, T. Leser, and P. Wallgren. 2004. Influence of cereal non-starch polysaccharides on ileo-caecal and rectal microbial populations in growing pigs. Acta Vet. Scand. 45:87–98.

Kerr, B.J., C.J. Ziemer, S.L. Trabue, J.D. Crouse, and T.B. Parkin. 2006. Manure composition of swine as affected by dietary protein and cellulose concentrations. J. Anim. Sci. 84:1584–1592.

Le, P.D., A.J.A. Aarnink, A.W. Jongbloed, C.M.C. van der Peet Schwering, N.W.M. Ogink, and M.W.A. Verstegen. 2007. Effects of crystalline amino acid supplementation to the diet on odor from pig manure. J. Anim. Sci. 85:791–801.

Leek, A.B.G., E.T. Hayes, T.P. Curran, J.J. Callan, V.E. Beattie, V.A. Dodd, and J.V. O'Doherty. 2007. The influence of manure composition on emissions of odour and ammonia from finishing pigs fed different concentrations of dietary crude protein. Bioresour. Technol. 98:3431–3439.

Li, M., J. Gong, M. Cottrill, H. Yu, C. de Lange, J. Burton, and E. Topp. 2003. Evaluation of QIAamp DNA Stool Mini Kit fro ecological studies of gut microbiota. J. Microbiol. Methods 54:13–20.

Macfarlane, G.T., and G.R. Gibson. 1994. Metabolic activities of the normal colonic flora. p. 17–52. *In* S.A.W. Gibson (ed.) Human health: The contributions of microorganisms. Springer-Verlag, New York.

McCracken, V.J., J.M. Simpson, R.I. Mackie, and H.R. Gaskins. 2001. Molecular ecological analysis of dietary and antibiotic-induced alterations of the mouse intestinal microbiota. J. Nutr. 131:1862–1870.

Miller, D.N., E.D. Berry, J.E. Wells, C.L. Ferrell, S.L. Archibeque, and H.C. Freetly. 2006. Influence of geneotype and diet on steer performance,

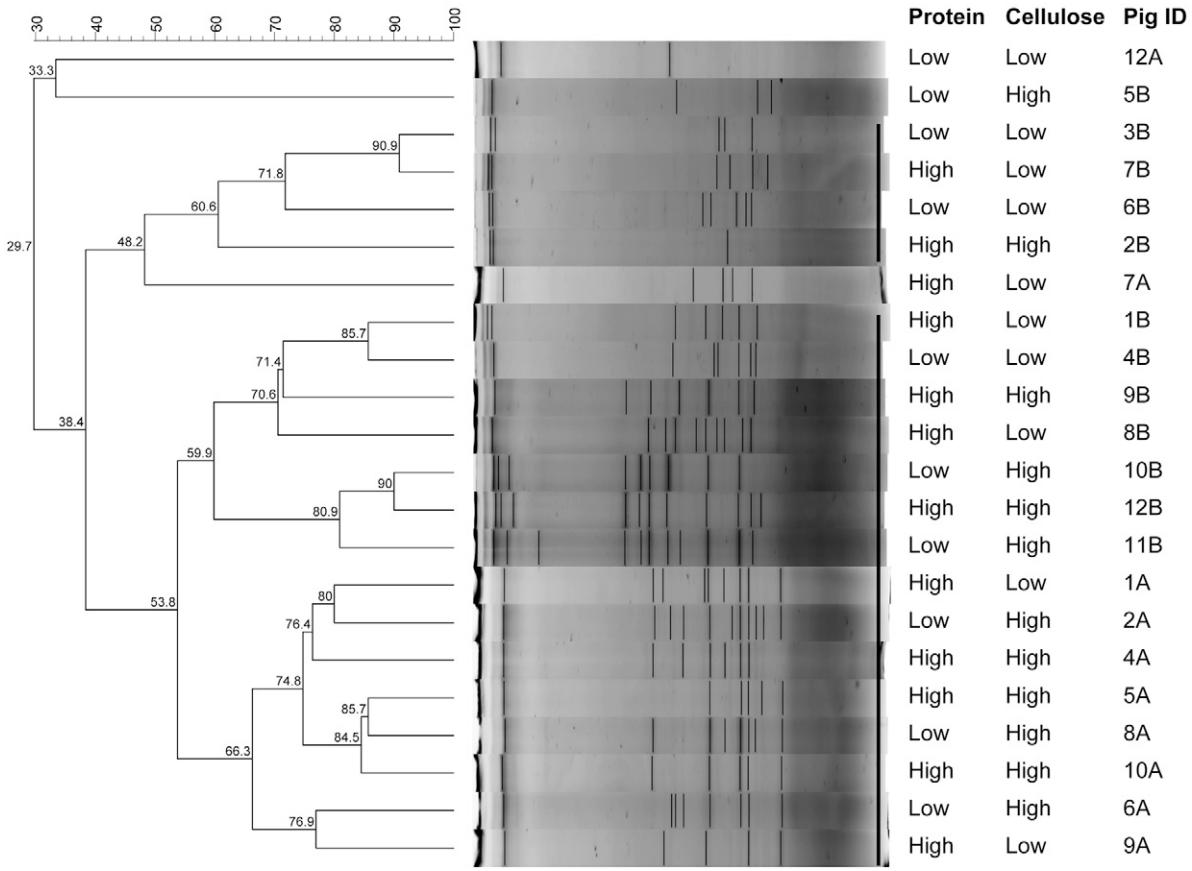

Fig. 4. Cluster analysis, with similarity values (%) and DGGE banding patterns for bacteria in manure samples from storage tanks containing waste from pigs fed diets varying in protein (High = 14.5 and Low = 12.2%) and cellulose (High = 8.7 and Low = 2.5%) at Week 2. Samples are coded by animal number, run (A or B), and week of sampling. Lines at the end of the DGGE gel image represent groupings by similarity >50%. The bar at the top is a similarity continuum.

manure odor, and carriage of pathogenic and other fecal bacteria: III. Odorous compound production. J. Anim. Sci. 84:2533–2545.

Miller, D.N., and V.H. Varel. 2003. Swine manure composition affects the biochemical origins, composition, and accumulation of odorous compounds. J. Anim. Sci. 81:2131–2138.

Mroz, Z., A.J. Moeser, K. Vreman, J.T.M. von Diepen, T. van Kempen, T.T. Canh, and A.W. Jongbloed. 2000. Effects of dietary carbohydrates and buffering capacity on nutrient digestibility and manure characteristics in finishing pigs. J. Anim. Sci. 78:3096–3106.

NRC. 1998. Nutrient requirements of swine. 9th rev. ed. Natl. Acad. Press, Washington, DC.

NRC. 1989. Nutrient requirements of dairy cattle. 6th rev. ed. Natl. Acad. Press, Washington, DC.

Otto, E.R., M. Yokoyama, S. Hengemuehle, R.D. von Bermuth, T. van Kempen, and N.L. Trottier. 2003. Ammonia, volatile fatty acids, phenolics, and odor offensiveness in manure from growing pigs fed diets reduced in protein concentration. J. Anim. Sci. 81:1754–1763.

Panetta, D.M., W.J. Powers, H. Xin, B.J. Kerr, and K.J. Stalder. 2006. Nitrogen excretion and ammonia emissions from pigs fed modified diets. J. Environ. Qual. 35:1297–1308.

Portejoie, S., J.Y. Dourmand, J. Martinez, and Y. Lebreton. 2004. Effect of lowering dietary crude protein on nitrogen excretion, manure composition, and ammonia emissions from fattening pigs. Livest. Prod. Sci. 58:45–55.

SAS Institute. 1990. SAS user's guide: Statistics. SAS Inst., Cary, NC.

Shriver, J.A., S.D. Carter, A.L. Sutton, B.T. Richert, B.W. Senne, and L.A. Pettey. 2003. Effects of adding fiber sources to reduced crude protein, amino-acid supplemented diets on nitrogen excretion, growth performance, and carcass traits of finishing pigs. J. Anim. Sci. 81:492–502.

Simpson, J.M., V.J. McCracken, H.R. Gaskins, and R.I. Mackie. 2000. Denaturing gradient gel electrophoresis analysis of 16S ribosomal DNA amplicons to monitor changes in fecal bacterial populations of weaning pigs after introduction of *Lactobacillus reuteri* strain MM53. Appl. Environ. Microbiol. 66:4705–4714.

Snell-Castro, R., J.-J. Godon, J.-P. Delgenès, and P. Dabert. 2005. Characterisation of the microbial diversity in a pig manure storage pit using small subunit rDNA sequence analysis. FEMS Microbiol. Ecol. 52:229–242.

Wang, H.-F., W.-Y. Zhy, W. Yao, and J.-X. Liu. 2007. DGGE and 16S rDNA sequencing analysis of bacterial communities in colon content and feces of pigs fed whole crop rice. Anaerobe 13:127–133.

Whitehead, T.R., and M.A. Cotta. 2001. Characterisation and comparison of microbial populations in swine faeces and manure storage pits by 16S rDNA gene sequence analyses. Anaerobe 7:181–187.

Yu, Z., and M. Morrison. 2004. Comparisons of different hypervariable regions of *rrs* genes for use in fingerprinting of microbial communities by PCR-denaturing gradient gel electrophoresis. Appl. Environ. Microbiol. 70:4800–4806.

Zhu, W.-Y., B.A. Williams, S.R. Konstantinov, S. Tamminga, W.M. De Vos, and A.D.L. Akkermans. 2003. Analysis of 16S rDNA reveals bacterial shift during in vitro fermentation of fermentable carbohydrate using piglet faeces as inoculum. Anaerobe 9:175–180.

Zoetendal, E.G., A.D. Akkermans, and W.M. De Vos. 1998. Temperature gradient gel electrophoresis analysis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. Appl. Environ. Microbiol. 64:3854–3859.