

Gut Microbiota Related to Fiber Digestibility were Identified by Variation of Apparent Fiber Digestibility in Chinese Suhuai pig

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Research article

Keywords: Suhuai Pig, Gut microbiota, Apparent NDF digestibility, Apparent ADF digestibility, 16S rRNA gene sequencing, Microbial function

Posted Date: January 6th, 2020

DOI: <https://doi.org/10.21203/rs.2.20110/v1>

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Abstract

Background: Dietary fiber plays an important role in porcine gut health and welfare. Fiber mainly degraded by the gut microbiota, whereas most gut microbiota related to fiber digestibility of pigs are still unidentified. To reveal gut microbiota associated with apparent digestibility of neutral detergent fiber (NDF) and acid detergent fiber (ADF), apparent NDF, ADF digestibility of 274 Suhuai female finishing pigs at the age of 160 days were measured. The gut microbiota of Suhuai pigs were analyzed through 16S rRNA gene sequencing, respectively. Results: Large phenotypic variations in apparent NDF and ADF digestibility were separately found among Suhuai pigs. The coefficient of variation of NDF and ADF digestibility was 12.08% and 18.08%, respectively. The mean values of digestibility of H-NDF and H-ADF groups were 30.20% and 33.76% more than those of the L-NDF and L-ADF groups ($P \leq 0.01$), respectively. A total of 927 and 935 operational taxonomic units (OTUs) were confirmed from two types of fecal samples, respectively. There were 14 phyla in all samples and the abundances of Spirochaetae, Bacteroidetes and unclassified_k__norank were significantly different between H-NDF and L-NDF groups ($P \leq 0.05$) and the abundances of Spirochaetae, Verrucomicrobia, unclassified_k__norank and Fibrobactere were significantly different between H-ADF and L-ADF group ($P \leq 0.05$). A total of 188, 183, 188 and 185 genera were separately identified in H-NDF, L-NDF, H-ADF and L-ADF groups, while 6, 1, 5 and 2 genera were separately specific to H-NDF, L-NDF, H-ADF and L-ADF groups. The microbiota of H-NDF and H-ADF clustered separately from the microbiota of the L-NDF and L-ADF along principal coordinate 1, respectively. Compared with L-NDF group, 29 genera were found to be potential biomarkers in H-NDF group. Compared with L-ADF group, 23 genera were found to be potential biomarkers in H-ADF group. The most important functions and metabolic pathways of the above potential biomarkers included carbohydrate transport and metabolism. Conclusions: Microbial community structures were significantly different between high and low fiber digestibility groups. Twenty nine and 23 genera were found to be potential biomarkers in H-NDF and H-ADF group, respectively. The biomarkers may be the key functional microbiota related to apparent fiber digestibility.

Background

In order to improve performance, save cost and reducing pollutants of pig production, efforts have been made to formulate diets over the last 25 years [1]. Fiber has the capacity to reduce ammonia emission and to improve gut health and pig welfare, and that was the reason why fiber was studied in diet of pig. Dietary fiber was indicated the non-digestible constituents of the plant cell wall in 1953, and now defining dietary fiber as non-digestible carbohydrates and lignin in plants [2]. Several fiber analysis procedures are available including apparent digestibility of crude fiber (CF), neutral detergent fiber (NDF) and acid detergent fiber (ADF). The ADF and NDF methods as developed by Van Soest and Wine [3] were often used for fiber analysis. Cellulose, hemicellulose, and lignin are the main components of NDF and ADF includes hemicellulose, and lignin as the major components.

All animals are associated with a diverse microbial community that is mainly composed of bacteria [4]. Bacteria are essential for the breakdown of cellulose as animal digestive enzymes cannot digest most

complex carbohydrates and plant polysaccharides [5]. These carbohydrates and plant polysaccharides are metabolized to short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate by microbes [6, 7]. Five to twenty percent of pig total energy is provided by bacterial fermentation end products in the colon [8–10]. The extent of absorption and utilisation of the volatile fatty acids produced in large intestine of pig determine the acceptability of fiber [11]. In our previous study [12], Collinsella and Sutterella were correlated with the fermentation of NDF and Clostridium, Collinsella, Robinsoniella^{and} Turicibacter were correlated with the fermentation of ADF. Because the gut bacteria related to fiber digestibility are difficult to be isolated and cultured, these bacteria communities need to further verification and more microbiota associated with NDF and ADF digestibility need to further .discovery.

Modern commercial pigs show a relatively poor capacity to digest dietary fiber, while Chinese indigenous pigs might own a stronger capacity to digest fiber than modern commercial pigs [13]. Suhuai pig, a synthetic Chinese breed that was derived from Huai pig (Chinese indigenous pig, 25%) and Large White (75%), was chosen as an experimental animal model in this study. We hypothesized that there were variations in fiber digestibility within Suhuai pig breed, which were mainly caused by differences in the gut microbiota related to carbohydrate transport and metabolism between groups of high and low fiber digestibility in Suhuai pig. This study aimed to detect phenotypic variation of fiber digestibility within Suhuai pig breed and reveal gut microbiota associated with apparent NDF and ADF digestibility by 16S rRNA gene sequencing of Suhuai pigs with extreme apparent NDF and ADF digestibility, respectively.

Results

Variation of apparent NDF and ADF digestibility within Suhuai pigs

Data regarding the apparent NDF and ADF digestibility of the 274 Suhuai pigs were shown in **table 1**. The coefficient of variation (CV) of apparent NDF and ADF digestibility was 12.08% and 18.08%, respectively.

Table 1 Data regarding the apparent NDF and ADF digestibility

Apparent digestibility	N	Range, %	Mean \pm SE	CV, %
NDF	274	44.57-88.34	70.39 \pm 0.64	12.08
ADF	274	29.98-83.09	62.89 \pm 1.19	18.80

Comparison of apparent NDF and ADF digestibility between high and low groups of Suhuai pigs

Apparent digestibility of NDF and ADF were both significantly different between high and low groups. The mean values of H-NDF and H-ADF groups were 30.20% and 33.76% more than those of L-NDF and L-ADF groups, respectively ($P < 0.01$, **Table 2**).

Table 2 Comparison of apparent NDF and ADF digestibility between high and low groups

Group	High		Low	
	N	Mean ± SE	N	Mean ± SE
Apparent NDF digestibility	6	83.30±5.22 ^A	6	53.10±5.07 ^B
Apparent ADF digestibility	6	75.69±4.75 ^A	6	41.93±8.47 ^B

^{AB} The mean difference is significant at a level of 0.01.

DNA sequence data and bacterial community structure of NDF and ADF samples between high and low groups of Suhuai pigs

More than one million sequences were obtained from all samples, and there were 38,973 high quality sequences per sample and a range of 29,641 to 49,819. The average sequence length was 240bp. A total of 927 OTUs were identified from H-NDF and L-NDF groups, Core OTUs comprised approximately 94% of the total OTUs (**Supplementary Figure 1A**) in NAD group, while 45 and 11 OTUs were characteristically showed in H-NDF and L-NDF groups, respectively. At the same time, core OTUs comprised approximately 92% of the total OTUs (**Supplementary Figure 1B**) in ADF group, while 49 and 26 OTUs were characteristically showed in H-ADF and L-ADF groups, respectively.

Shannon and simpson indexes were significantly different between the two types of samples in NDF groups ($P < 0.05$, Supplementary Table 2). There were no significantly different between the two types of samples in ADF groups ($P > 0.05$, Supplementary Table 2). The microbiota of H-NDF and H-ADF clustered separately from the microbiota of the L-NDF and L-ADF along principal coordinate 1, respectively (Figure 1A, B). Microbial composition had a strong difference between high and low digestibility groups of NDF and ADF, respectively (Adonis/PERMANOVA, $P < 0.01$, Supplementary Figure 2A, B).

Fourteen phyla were identified from the four groups (**Supplementary Figure 3A**): *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Tenericutes*, *Spirochaetae*, *Verrucomicrobia*, *Proteobacteria*, *Planctomycetes*, *unclassified_k_norank*, *Saccharibacteria*, *Cyanobacteria*, *Chlamydiae*, *Fibrobacteres* and *Lentisphaerae*. *Firmicutes* and *Bacteroidetes*, comprised more than 91% of the total sequences, were the most predominant phyla in all samples. The abundances of *Spirochaetae*, *Bacteroidetes* and *unclassified_k_norank* were significantly different between H-NDF group and L-NDF group ($P < 0.05$). In the meantime, the abundances of *Spirochaetae*, *Verrucomicrobia*, *unclassified_k_norank* and *Fibrobactere* were significantly different between H-ADF group and L-ADF group ($P < 0.05$).

At genus level, 189 genera were identified from NDF samples, and 182 of those existing defined as core genera while 6 and 1 genera were uniquely identified in H- NDF and L- NDF, respectively (**FigureS4 A**). Meanwhile, 190 genera were distinguished from ADF samples, and 183 of those existing defined as core genera while 5 and 2 genera were uniquely identified in H-ADF and L-ADF, respectively (**FigureS4 B**). The 2 most dominant genera were *Lactobacillus* and *Streptococcus* belong to the phylum *Firmicutes*, comprised more than 25% and 47% of the total sequences in H-NDF group and L-NDF group, respectively (**FigureS3 B**). The 2 most predominant genera in H-ADF group and L-ADF group, separately containing

about 44% and 35% of the total sequences, were *Lachnospira* and *Streptococcus* also belong to the phylum *Firmicutes* (**FigureS3 D**).

A total of 30 genera were found to be potential biomarkers between H-NDF group and L-NDF group; 29 genera were unique to H-NDF group and 1 WAS unique to L-NDF group (**Figure3 A**). At the same time, a total of 29 genera were found to be potential biomarkers between H-ADF group and L-ADF group; 23 genera were unique to H-ADF and 6 were unique to L-ADF (**Figure3 B**). *Eubacterium coprostanoligenes* group, *Candidatus Soleaferrea*, *dgA 11 gut_group*, *Family XIII AD3011 group*, *norank f p 2534 18B5 gut group*, *norank f Porphyromonadaceae*, *Oscillibacter*, *Ruminococcaceae NK4A214 group*, *Succinivibrio*, *Treponema 2*, *unclassified f Ruminococcaceae* and *unclassified k norank* were found to be potential biomarkers between H-NDF/H-ADF group and L-NDF/L-ADF group ($H-NDF \cap H-ADF$, **Figure3 A, B**). *Anaeroplasma*, *Anaerovibrio*, *Erysipelotrichaceae UCG 003*, *Lachnospiraceae ND3007 group*, *Lachnospiraceae NK4A136 group*, *Lachnospiraceae NK4B4 group*, *norank f Bacteroidales S24 7 group*, *norank f Mitochondria*, *Prevotella 1*, *Ruminiclostridium 1*, *Ruminococcaceae UCG 004*, *Ruminococcaceae UCG 005*, *Ruminococcus 1*, *Staphylococcus*, *unclassified f Lachnospiraceae*, *unclassified o Bacteroidales* and *unclassified p Bacteroidetes* were found to be potential biomarkers only between H-NDF group and L-NDF group ($H-NDF$, **Figure3 A**). *Christensenellaceae R 7 group*, *Fibrobacter*, *norank c WCHB1 41*, *norank f Clostridiales vadinBB60 group*, *norank o Bradymonadales*, *Prevotella 2*, *Prevotellaceae UCG 001*, *Quinella*, *Ruminococcaceae UCG 002*, *Schwartzia* and *unclassified o Clostridiales* were found to be potential biomarkers only between H-ADF group and L-ADF group ($H-ADF$, **Figure3 B**).

Prediction function of microbial metabolism

Sixty four functions were predicted in the present study. General function prediction only (8.56%), Carbohydrate transport and metabolism (8.25%), Amino acid transport and metabolism (8.23%), Replication (8.16%), Translation (7.85%), Transcription (7.50%), Cell wall/membrane/envelope biogenesis (6.47%), Energy production and conversion (5.51%), Inorganic ion transport and metabolism (5.08%) and Signal transduction mechanisms (4.49%) were the most enrichment functions. At the same time, 39 metabolic pathways were predicted. Membrane Transport (12.97%), Carbohydrate Metabolism (10.42%), Replication and Repair (9.69%), Amino Acid Metabolism (9.16%), Translation (6.47%), Energy Metabolism (5.54%), Poorly Characterized (4.81%), Nucleotide Metabolism (4.45%) and Metabolism of Cofactors and Vitamins (4.04%) were the most enrichment pathways.

According to the Clusters of Orthologous Groups of proteins (COG) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases, the top 10 in abundance predictive functions of potential biomarkers in H-NDF group and H-ADF group were shown in **table 3**, respectively. And the top 10 in abundance microbial metabolic pathways of potential biomarkers in H-NDF group and H-ADF group were shown in **table 4**, respectively. The most important functions and metabolic pathways of the above different potential biomarkers included carbohydrate transport and metabolism and Carbohydrate Metabolism, respectively.

Table 3 The proportion of each group in the top 10 function abundance (%)

Functions	H-NDF	H-ADF	H-NDF	H-ADF
Carbohydrate transport and metabolism	8.41	10.68	5.53	
General function prediction only	8.10	8.42	8.45	
Amino acid transport and metabolism	7.53	7.85	8.39	
Cell wall/membrane/envelope biogenesis	7.49	6.35	6.85	
Translation	7.32	6.21	6.35	
Transcription	7.32	8.97	6.59	
Replication	7.19	6.75	7.38	
Energy production and conversion	5.73	5.25	6.82	
Signal transduction mechanisms	5.43	6.00	6.70	
Inorganic ion transport and metabolism	5.00	5.26	4.95	

Table 4 The proportion of each group in the top 10 metabolic pathways abundance (%)

Pathways	H-NDF	H-ADF	H-NDF	H-ADF
Membrane Transport	13.89	15.25	11.33	
Carbohydrate Metabolism	9.64	10.71	9.31	
Amino Acid Metabolism	8.90	9.21	10.12	
Replication and Repair	8.64	8.50	8.48	
Translation	6.27	5.38	5.43	
Cell Motility	5.90	-	4.62	
Energy Metabolism	5.16	5.52	5.74	
Poorly Characterized	5.10	4.63	4.78	
Nucleotide Metabolism	3.98	3.82	3.78	
Metabolism of Cofactors and Vitamins	3.48	3.88	4.44	
Cellular Processes and Signaling	-	3.86	-	

Discussion

Chinese indigenous pig showed a better character about fiber tolerance compared with foreign varieties [13]. Here Suhuai pig, one of synthetic Chinese pig breeds, were chosen to identify microbes associated with fiber degradation. We hypothesized that there was variation in fiber digestibility in Suhuai pig breed and we found that coefficients of variation of apparent NDF and ADF digestibility in Suhuai Pigs were 12.08 and 18.80%, respectively. This demonstrated that a large phenotypic variation in apparent NDF and ADF digestibility among Suhuai pigs, respectively. To reveal gut microbiota associated with fiber digestibility, a comparative analysis of gut microbiota community structure as conducted on high and low groups of NDF and ADF, respectively. The average apparent NDF and ADF digestibility of the high groups were separately 83% and 76%, whereas those of the low groups were separately only 53% and 42%, a difference of 30% and 34%, respectively.

A variety of commensal bacteria exist in animal large intestine who participate many physiological processes beneficial to the host [14]. In present study, the data showed a large microbial community in the Suhuai pigs. More than one million sequences were obtained from all samples, and there were 38,973 high quality sequences per sample and a range of 29,641 to 49,819. A total of 927 and 935 OTUs were

identified from NDF and ADF fecal samples, respectively. At the phylum level, Firmicutes and Bacteroidetes were the most predominant phyla, comprised more than 90% of the total sequences, which was consistent with previous researches [12, 15–17]. At the genus level, the 2 most predominant genera were *Lactobacillus* and *Streptococcus* belong to the phylum Firmicutes in NDF group. And the 2 most predominant genera in ADF group were *Lachnospira* and *Streptococcus* also belong to the phylum Firmicutes. In our previous study, *Lactobacillus*, comprised 15% of total sequences, was the most dominant genera [12]. Regardless of the breed, *Prevotella*, *Blautia*, *Oscillibacter*, and *Clostridium* were generally prevalent in pig gastrointestinal tract [17].

The data between the two types of samples had statistical significance was analyzed by Adonis/PERMANOVA [18]. Microbial composition had a strong difference between high and low groups of NDF and ADF (adonis/PERMANOVA $P < 0.01$). The result illustrated that the gut microbiota between the two types of samples in NDF group and ADF group were statistically significant and all data comparisons made between different groups in this study were meaningful.

Although chao and ace indexes had no different between the two types of samples of this study, shannon and simpson indexes were significantly different between the two types of samples in NDF groups. The PCA of UniFrac distance matrices showed that the variation between H-group and L-group was primarily explained by the apparent NDF or ADF digestibility, respectively. This suggested that the differences of microbial community structure between high and low groups were related to apparent NDF and ADF digestibility. However, these diversity indexes only showed the overall situation of microbiota in each group, as the objective of the present study was to reveal gut microbiota associated with apparent NDF and ADF digestibility, we needed to know the microbiota with higher abundance in the H-group and then predicted their microbes functions. A total of 29 and 23 genera were found to be potential biomarkers in H-NDF group and H-ADF group, respectively. Twelve of these genera were uniquely enriched in both H-NDF and H-ADF groups compared with L-NDF and L-ADF groups, respectively. Seventeen and eleven of these genera were uniquely enriched only in H-NDF group and H-ADF group, respectively. The two types of samples in NDF and ADF groups showed different microbial community structure. In Heinritz research [19], the abundances of *Lactobacilli*, *Bifidobacteria* and *Faecalibacterium prausnitzii* were higher and the abundance of *Enterobacteriaceae* was lower in the low-fat/high-fiber pig group. In the study of Tan et al [20], the relative abundances of *Campylobacter* and *Butyrivibrio* were higher in cecum, and *Coprococcus* were higher in colon. Genera of *Fibrobacter intestinalis*, *Ruminococcus flavefaciens*, *Ruminococcus albus* and *Butyrivibrio* spp. are highly active cellulolytic bacterial species in pig gut, which are the dominant cellulolytic bacteria in the rumen [21]. *Prevotella*, *Bacteroides ruminicola*, *F. sugiginogenes*, *R. flavefaciens* and *Butyrivibrio* spp. are related with hemicellulose fermentation process. Chen [22] illuminated that different fiber sources resulted in inconsistent microbiota composition in pigs gut. As expected, a number of the predicted functions of these potential biomarkers in H-NDF group and H-ADF group were associated with microbial cell metabolism. Carbohydrate transport and metabolism was a very important microbial function of these potential biomarkers which was high abundance in high fiber digestion groups. In previous study, they indicated that other abundant proteins from distal pig intestines have high sequence homology with the recognized carbohydrate membrane

transport protein [23]. In their results, the most abundant SEED subsystem (MG-RAST annotation pipeline) was carbohydrate metabolism, represented 13% of both pig fecal metagenomes. The above results showed that the utilization of various carbohydrates was closely related to gut microbes.

Conclusions

A large phenotypic variation in apparent NDF and ADF digestibility among Suhuai pigs, respectively. Microbial community structures were significantly different between high and low fiber digestibility groups. Twenty nine and 23 genera were found to be potential biomarkers in H-NDF group and H-ADF group, respectively. The most important functions and metabolic pathways of the above different potential biomarkers included carbohydrate transport and metabolism. The biomarkers may be the key functional microbiota related to apparent fiber digestibility.

Methods

Sample Collection in Animal

A total of 274 healthy Suhuai pigs were selected to collect fecal and diet samples (no disease or diarrhea happened one week before sampling) at age of 160 days were collected from Huaiyin Pig Breeding Farm, Huaian, China, under same husbandry conditions. All pigs were selected according to a unified breed standard and fed with an antibiotic-free corn-soybean diet (**Supplementary Table 1**). One month before sampling, antibiotics in the feed or for any therapeutic purposes were not provided for pig.

Premix: VA (KIU/kg): 500-700; VD3 (KIU/kg): 100-200; VE (IU/kg): ≥ 2000 ; VK3 (mg/kg): 75-800; VB1 (mg/kg): ≥ 75 ; VB2 (mg/kg): ≥ 400 ; VB6 (mg/kg): ≥ 100 ; VB12 (mg/kg): ≥ 2.5 ; Niacin (mg/kg): ≥ 3000 ; Pantothenic acid (mg/kg): ≥ 1000 ; Folic acid (mg/kg): ≥ 50 ; Choline (g/kg): ≥ 10 ; Iron (g/kg): 5-10; Copper (g/kg): 0.6-1.2; Manganese(g/kg): 2.5-5.0; Zinc(g/kg):6-12; Iodine(mg/kg): 60-120; Selenium (mg/kg): 20-40; Water (%): ≤ 10 .

Diet samples and approximately 200 g of each fecal sample were collected in plastic bags and fecal samples were mixing with 15 ml 10% sulfuric acid to be fixed on site. These samples were used for analyzing apparent nutrient digestibility. Each sample was individually collected in 2 ml centrifuge tubes without any treatment for 16S rRNA gene sequencing. All samples were kept in ice box for preservation and transportation and then stored at -80°C in the laboratory [24].

Chemical Analysis

Fecal samples from Suhuai pigs were dried at 65°C to a constant weight. Determination of NDF and ADF contents was using ANKOM A200 filter bag technique (AOAC 962.09) [25]. The following equation was used to calculate the digestibility of each sample [25].

$$CAD_D(\%) = 100 \times \left(1 - \frac{DC_F \times AIA_D}{DC_D \times AIA_F} \right)$$

Where CAD_D represents the apparent dietary components digestibility; DC_F represents the dietary component in feces; AIA_D represents the AIA concentration in diet; DC_D represents the dietary component in diet; AIA_F represents the AIA concentration in feces.

16S rRNA Sequencing and Bioinformatics Analysis

The gut microbiota population in the Suhuai pigs with extreme high apparent digestibility of NDF (n=6) and ADF (n=6), low apparent digestibility of NDF (n=6) and ADF (n=6) were analyzed, respectively. Fecal microbial DNA was isolated with Soil DNA Kit (Omega, D5625-01). Afterwards, DNA concentration was measured by UV spectrophotometer (Eppendorf, Bio Photometer). Hypervariable V4 region of 16S rRNA gene with the length of approximately 280bp was targeted for sequencing [26]. PCR amplification was performed with gene specific primers 520F(5'-GCACCTAAYTGGGYDTAAAGNG-3') and 802R (5'-TACNVGGGTATCTAATCC-3') on following conditions: 98°C, 5 min (initial denaturation); 98°C, 10 s; 50°C, 30 s; 72°C, 30 s (for 25 cycles); 72°C, 5 min. Q5 high-fidelity DNA polymerase was used to PCR (NEB, M0491L) at 25µl final volume with 20ng template DNA. AxyPrep DNA Gel Extraction Kit (Axygen, AP-GX-250) was used to amplicons. Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, P7589) were used to quantified the purified amplicons and pooled together.

Merged fastq files exported to the Quantitative Insights into Microbial Ecology (QIIME) software [27]. Chimera identification and removal were using UCHIME [28] in mother [29]. The non-repeat sequences were extracted from the optimized sequences to reduce the redundant computation in the analysis of the intermediate process (<http://drive5.com/usearch/manual/dereplication.html>). Remove single sequences that do not repeat (<http://drive5.com/usearch/manual/singletons.html>). Similar sequences were clustered into OTUs using the seed-based uclust algorithm 24 at a 97% identity threshold [29]. Taxonomic identification was assigned using an RDP classifier [30, 31]. Taxonomy was assigned using the Silva (Release128 <http://www.arb-silva.de>). Venn diagrams, and rank abundance distribution curve were performed by using Mothur.

Statistical Analysis

The apparent fiber digestibility was calculated using SAS 9.4 software [32]. Alpha diversity was calculated using Mothur [29]. Linear discriminate analysis effect size (LEfSe) was used to identify the bacteria enriched [33]. Pair-wise phylogenetic distance was measure by weighted UniFrac [34] to compare community compositions across samples. Principal co-ordinates analysis (PCoA) were used compress dimensionality into 2D principal coordinate analysis plots [35], enabling visualization of sample

relationships. PICRUSt was used to explore the functional composition of that bacterial community data might convey [36].

List Of Abbreviations

NDF	neutral detergent fiber
ADF	acid detergent fiber
H-NDF	high apparent digestibility of NDF
L-NDF	low apparent digestibility of NDF
H-ADF	high apparent digestibility of ADF
L-ADF	low apparent digestibility of ADF
OTUs	operational taxonomic units
CF	crude fiber
SCFAs	short-chain fatty acids
LEfSe	Linear discriminate analysis effect size
PCoA	Principal co-ordinates analysis
CV	coefficient of variation

Declarations

Ethics approval and consent to participate

All experimental animals were raised according to Guidelines for the Care and Use of Laboratory Animals prepared by the Institutional Animal Welfare and Ethics Committee of Nanjing Agricultural University, Nanjing, China. All experimental protocols were approved by the Nanjing Agricultural University Animal Care and Use Committee (Certification No.: SYXK (Su) 2017-0007). Informed consent about all procedures and the use of animals was verbally obtained from all participants, including veterinary officers and farmers.

Consent for publication

Not applicable.

Availability of data and materials

The 16s rRNA gene sequencing raw data of manuscript has been successfully registered with the BioProject database. All data generated or analyzed during this study are included in this published article and its supplementary information files, will be freely available to any scientist wishing to use them for non-commercial purposes upon request via e-mail with the corresponding author.

Competing interests

The authors declare no conflicts of interest.

Funding

This research was financially supported by the National Natural Science Foundation (31872318), the Fundamental Research Funds for the Central Universities(KJQN201704), the National Science and Technology Program for the Twelfth Five Year Plan for Rural Development (2015BAD03B02-4), the Sanxin Agricultural Engineering Project of Jiangsu Province (JATS (2018) 275), and Key Project for Jiangsu Agricultural New Variety Innovation (PZCZ201732). The funding bodies had no role in the design of the study, collection, analysis, and interpretation of data and in writing the manuscript.

Authors' contributions

Conceived and designed the experiments, PHL and RHH. Performed the experiments, QN, XM, TRD, TXL, GP, CXL, CG, HW and LJF. Analyzed the data, QN and XM. Contributed reagents/materials/analysis tools, PHL RHH PPN ZPZ and QL. Contributed to writing of the manuscript, QN PHL RHH and SWK.

Acknowledgements

We thank Huaiyin pig breeding farm in Jiangsu Province, China for help with samples.

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Figures

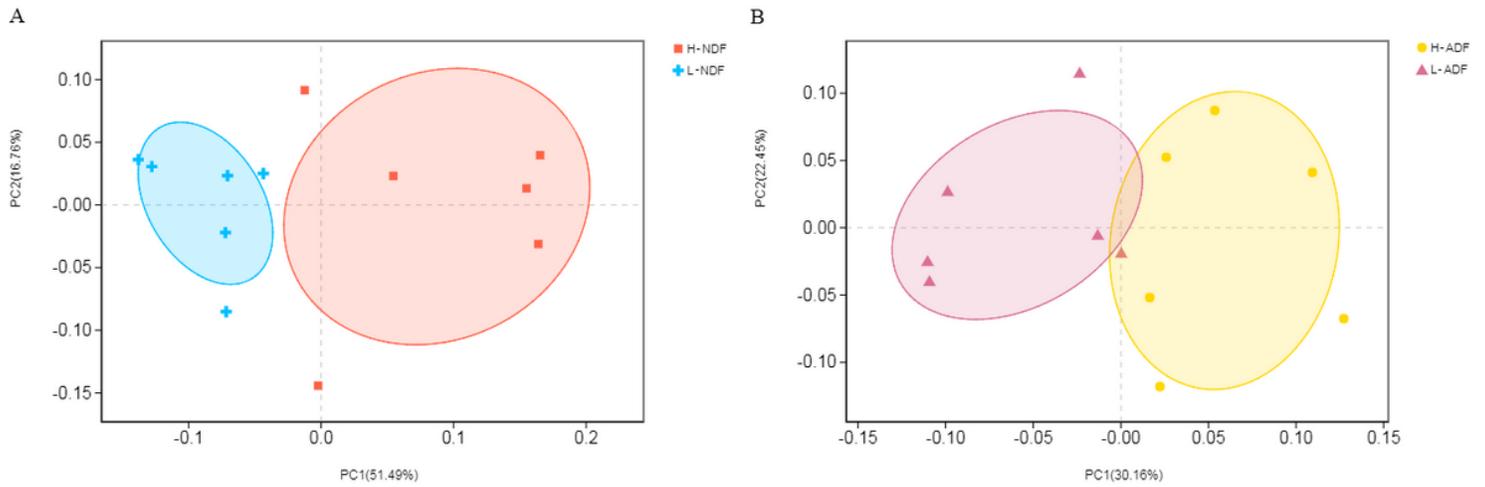


Figure 1

PCoA of high and low groups of NDF and ADF, respectively. PCoA was generated by using weighted UniFrac distance between the two types in NDF and ADF groups. The first principal coordinate separated the two types of samples in NDF group, explained 51.49% of sample variation (A). The first principal coordinate, explained 30.16% of sample variation, separated the two types of samples in ADF group (B). H-NDF and H-ADF represent high apparent digestibility of NDF and ADF, L-NDF and L-ADF represent low apparent digestibility of NDF and ADF, respectively.

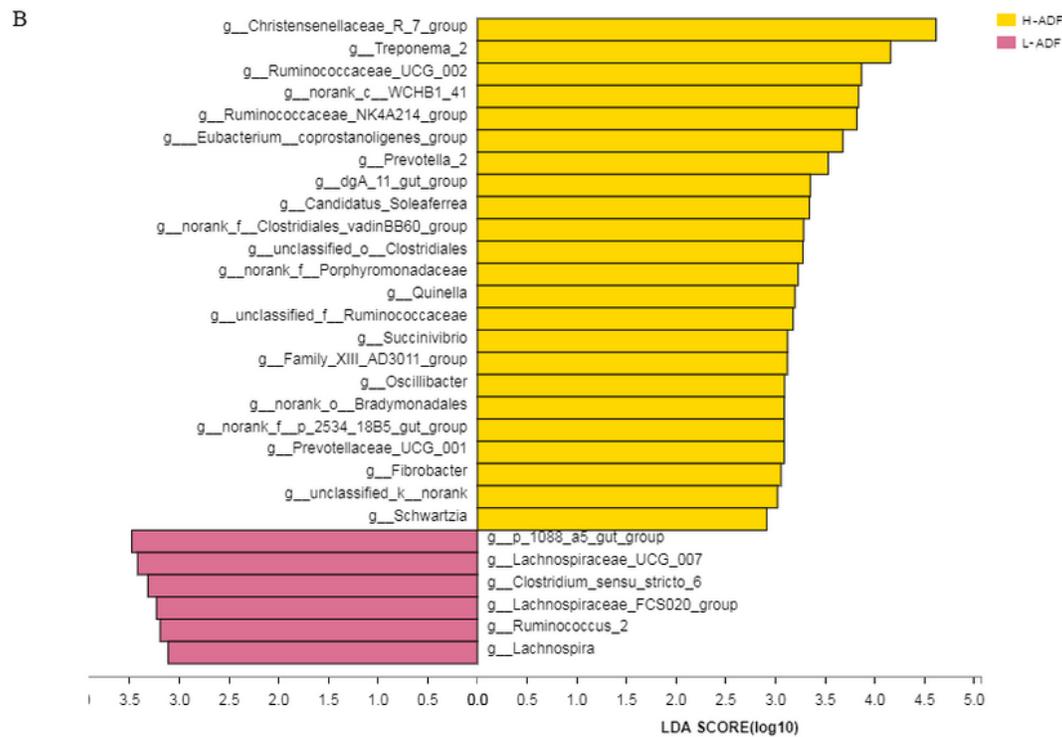
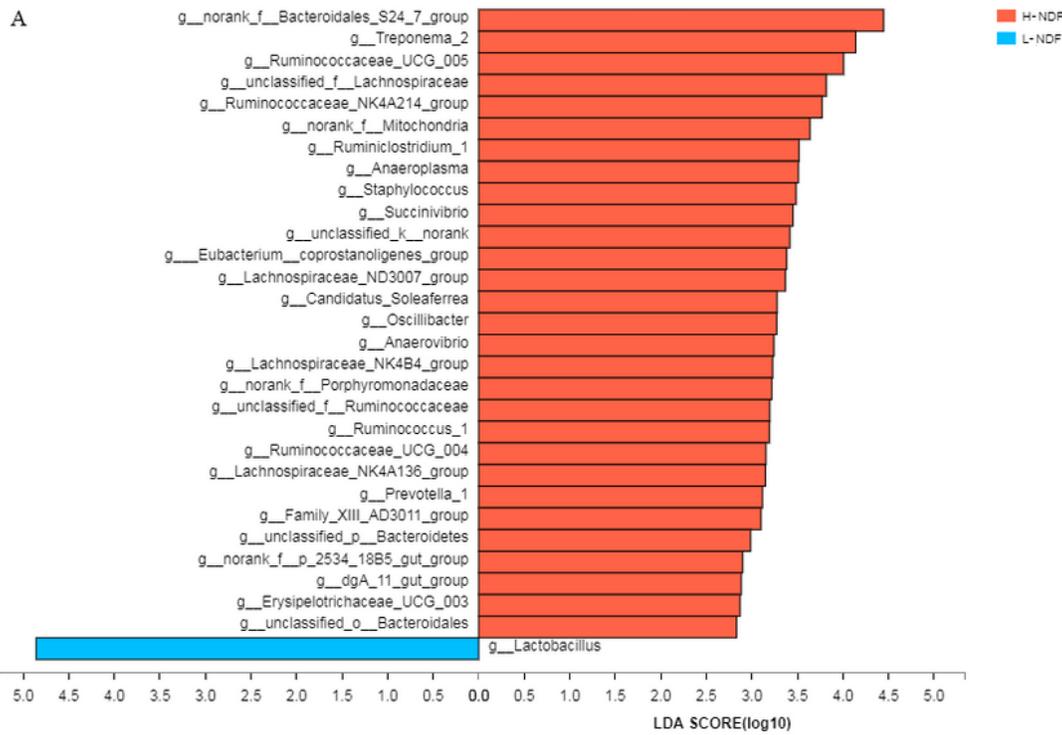


Figure 2

Missing; Figure3 A, B Linear discriminant analysis (LDA) effect size (LEfSe) analysis. Genera LDA scores above 2 are shown. H-NDF and H-ADF represent high apparent digestibility of NDF and ADF, L-NDF and L-ADF represent low apparent digestibility of NDF and ADF, respectively.

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