Effects of Low-Protein Level Diets on Growth Performance and Gut Microbiota in Growing-Finishing Pigs (Postprint)

Authors: Wang Jing, Wang Sixin, Liu Hui, Zhang Wei, Zhang Dongyan, Wang Yamin, Ji Haifeng

Date: 2018-12-25T00:00:00+00:00

Abstract

This study aimed to investigate the effects of low-protein-level diets on growth performance and intestinal microbiota in growing-finishing pigs. One hundred and forty crossbred growing-finishing pigs (Landrace × Large White) with an initial body weight of (45.5±3.64) kg were selected and randomly divided into 2 groups, with 5 replicates per group and 14 pigs per replicate. The control group was fed a normal-protein-level diet with a protein content of 15.05%, while the experimental group was fed a low-protein-level diet with a protein content of 12.97%. The pre-trial period lasted for 5 days, and the formal trial period lasted for 30 days. The results showed that: 1) The low-protein-level diet had no significant effect on the growth performance of growing-finishing pigs ($P>0.05$). 2) The low-protein-level diet could increase the richness and diversity of intestinal microbiota in growing-finishing pigs. The fecal microbiota composition of the two groups of experimental pigs showed minor differences at the phylum level, but substantial differences at the genus level. Significance analysis of species differences revealed that bacterial relative abundance was significantly different between the two groups at all taxonomic levels except the phylum and class levels ($P<0.05$). The low-protein-level diet significantly increased the relative abundance of beneficial bacteria such as Lachnospiraceae, Ruminococcaceae, Butyrivibrio, and Pseudobutyrivibrio, which can utilize carbohydrates and fiber degradation to produce butyrate ($P<0.05$), and significantly decreased the relative abundance of bacteria such as Paraeggerthella and Delftia that can easily cause infection in the host ($P<0.05$). Furthermore, analysis of community composition differences revealed that pigs in the low-protein-level diet group had a distinct gut microbial flora compared with those in the normal-protein-level diet group. Therefore, a 2% reduction in dietary protein level had no adverse effects on the growth performance of growing-finishing pigs, but could improve intestinal microbiota balance and benefit host health.

Full Text

Effects of Low-Protein Diets on Growth Performance and Intestinal Microbiota of Growing-Finishing Pigs

WANG Jing, WANG Sixin, LIU Hui, ZHANG Wei, ZHANG Dongyan, WANG Yamin, JI Haifeng*

Institute of Animal Husbandry and Veterinary Medicine, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China
*Corresponding author: JI Haifeng, professor, E-mail: jhf207@126.com

Abstract

This study investigated the effects of low-protein diets on growth performance and intestinal microbiota of growing-finishing pigs. A total of 140 "Landrace × Large White" crossbred growing-finishing pigs with an initial body weight of (45.5±3.64) kg were randomly allocated into 2 groups, each consisting of 5 replicates with 14 pigs per replicate. The control group was fed a normal-protein diet containing 15.05% crude protein, while the experimental group received a low-protein diet containing 12.97% crude protein. The pre-experimental period lasted 5 days, followed by a 30-day formal experimental period. The results showed that: (1) the low-protein diet had no significant effect on growth performance of growing-finishing pigs ($P>0.05$); (2) the low-protein diet increased the richness and diversity of intestinal microbiota. The fecal microbiota composition differed little between groups at the phylum level but showed substantial differences at the genus level. Species difference analysis revealed significant differences in bacterial relative abundance between the two groups at all taxonomic levels except phylum and class ($P<0.05$). The low-protein diet significantly increased the relative abundance of beneficial bacteria such as *Lachnospiraceae*, *Ruminococcaceae*, *Butyrivibrio*, and *Pseudobutyrivibrio* that can utilize carbohydrates and fiber to produce butyrate ($P<0.05$), while significantly decreasing the relative abundance of potentially pathogenic bacteria such as *Paraeggerthella* and *Delftia* ($P<0.05$). Additionally, community composition analysis demonstrated that pigs fed the low-protein diet had distinct intestinal microbial communities compared to those fed the normal-protein diet. These findings indicate that reducing dietary protein level by approximately 2% has no adverse effects on growth performance of growing-finishing pigs and can improve intestinal microbiota balance, thereby benefiting host health.

Keywords: low-protein diet; growing-finishing pigs; growth performance; intestinal microbiota

Introduction

Research has shown that using the ideal protein model to reduce dietary protein levels by 2%-4% below current recommended standards for growing-finishing pigs, while supplementing with synthetic amino acids to meet amino acid requirements, does not affect animal growth performance or health status [1-2]. Low-protein diets formulated in this manner can save costs on protein feed ingredients and reduce nitrogen excretion in animal manure. Application in swine production practice can improve feed utilization efficiency and decrease environmental pollution from excreta. The porcine intestine harbors a vast and diverse microbial community that plays a crucial role in nutrient digestion and absorption, immune function, and maintenance of host health through material exchange and information transfer with the host [3-4]. Studies have demonstrated that different nitrogen levels and patterns create substantial differences in intestinal microbial structure and composition, thereby affecting host nutrient absorption and utilization [5]. Current research on low-protein diets in growing-finishing pigs has primarily focused on growth performance, meat quality, and blood biochemical parameters, while reports on the effects of low-protein diets on porcine intestinal microbiota remain scarce. This study investigated the effects of reducing dietary protein level by approximately 2% below the standards recommended in the *Feeding Standard of Swine* (2004) while supplementing essential amino acids on growth performance and intestinal microbial communities of growing-finishing pigs under commercial production conditions, providing a theoretical basis for further improvement of the ideal amino acid model and rational formulation of low-protein diets.

1. Materials and Methods

1.1 Experimental Animals and Design

A single-factor randomized design was employed. A total of 140 "Landrace × Large White" crossbred growing-finishing pigs with identical genetic background and initial body weight of (45.5±3.64) kg were randomly divided into 2 groups according to similar body weight and consistent sex ratio. Each group contained 5 replicates with 14 pigs per replicate. The control group was fed a normal-protein diet containing 15.05% crude protein, while the experimental group received a low-protein diet containing 12.97% crude protein. Synthetic amino acids were supplemented in the low-protein diet to maintain identical standardized ileal digestibility (SID) amino acid levels between the two diets. Diet formulation for growing-finishing pigs referenced the nutrient requirements recommended in the *Feeding Standard of Swine* (NY/T 65-2004). The composition and nutrient levels of experimental diets are presented in Table 1 .

Table 1 Composition and nutrient levels of experimental diets (DM basis) %

Items	Groups	
	Control group	Experimental group
Ingredients		
Corn		
Wheat bran		
Soybean meal		
Lys · HCl		
Met		
Thr		
Trp		
Premix[1])		
Total		
Nutrient levels[2])		
DE/(MJ/kg)		
Crude protein	15.05	12.97
Total phosphorus		
Lys		
Met		
Thr		
Trp		
Val		
Ile		
Leu		
Phe		
His		
Arg		
Asp		
Ser		
Glu		
Gly		
Ala		
Cys		
Tyr		
Pro		

[1]) The premix provided the following per kg of diet: VA 5.6 kIU, VD 1.6 kIU, VE 18 IU, VK 0.8 mg, VB 0.8 mg, VB 4 mg, VB 0.6 mg, VB 0.012 mg, niacin 15.6 mg, pantothenic acid 3.6 mg, folic acid 0.68 mg, biotin 0.005 mg, choline chloride 300 mg, Cu 150 mg, Fe 140 mg, Mn 52 mg, Zn 120 mg, I 1.8 mg, Se 0.32 mg.
[2]) Crude protein, calcium, total phosphorus, and amino acids were measured values, while DE was a calculated value.

1.2 Animal Management

The experiment was conducted at Xiqing Minfeng Farm in Fangshan District, Beijing. The pre-experimental period lasted 5 days, followed by a 30-day formal experimental period. Experimental pigs were housed in pens by replicate with ad libitum access to feed and water. During the trial, cleaning and disinfection followed the farm' s routine procedures to maintain good environmental hygiene. Dedicated personnel managed the experiment according to the design. Daily observations and records were maintained regarding pig health status, with documentation of diarrhea, morbidity, and mortality.

1.3 Growth Performance Measurement

Individual body weights were measured at the beginning and end of the experiment, with feed intake recorded throughout the trial. Growth performance evaluation indices included initial body weight, final body weight, average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G).

1.4 Sample Collection

On the day before the experiment concluded, fresh fecal samples were collected from 6 randomly selected pigs per replicate. Samples from every 3 pigs were pooled in equal amounts, immediately placed in sterile centrifuge tubes, stored on dry ice, transported to the laboratory, and preserved at -80°C for subsequent bacterial DNA extraction.

1.5 DNA Extraction and Sequencing

Total DNA from fecal samples was extracted using the E.Z.N.A. Stool DNA Kit (Omega Bio-tek, USA) following the manufacturer' s instructions. Extracted total DNA was purified and its concentration and purity assessed by agarose gel electrophoresis. The V3–V4 conserved region of 16S rRNA was amplified using universal bacterial primers 338F (5 -ACTCCTACGGGAGGCAGCA-3) and 806R (5 -GGACTACHVGGGTWTCTAAT-3). The amplification system and program are shown in Table 2 . PCR products were detected by 2% agarose gel electrophoresis, and PCR products were recovered by gel extraction using the AxyPrep DNA Gel Extraction Kit (Axygen, USA). Amplicons were sequenced on the Illumina MiSeq platform using paired-end sequencing (Shanghai Majorbio Bio-pharm Technology Co., Ltd., Shanghai). The PCR reaction program was: 95°C for 3 min; 27 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 45 s; and final extension at 72°C for 10 min.

Table 2 PCR reaction system and program

Ingredients	Dose
5×FastPfu Buffer	4.0 μL
2.5 mmol/L dNTPs	2.0 μL

Ingredients	Dose
5 μmol/L Primer F	0.8 μL
5 μmol/L Primer R	0.8 μL
FastPfu DNA Polymerase	0.4 μL
DNA template	10.0 ng
RNase-Free ddH O	To 20.0 μL

1.6 Data Processing and Statistical Analysis

Growth performance data were analyzed by one-way ANOVA using the SPSS 17.0 software package. If significant differences were detected among groups, Duncan' s multiple comparison test was performed, with $P<0.05$ as the significance criterion.

For fecal microbiota sequencing, raw reads were first assembled based on overlap relationships, then filtered and trimmed using Trimmomatic and Qiime v.1.5.0, and chimeras were removed using the Uchime algorithm v.4.2.40. Optimized high-quality sequences were clustered into operational taxonomic units (OTUs) at 97% similarity level based on the Silva 16S sequence database, followed by taxonomic classification using the RDP classifier and statistical analysis of microbiota composition at each taxonomic level for each sample. Alpha diversity analysis was performed using Qiime software, including diversity indices (Shannon and Simpson indices) and richness indices (ACE and Chao1 indices). Principal coordinates analysis (PCoA) based on Bray-Curtis distance was used to analyze inter-group distances in microbial composition among fecal samples. The LEfSe algorithm was employed to analyze differential species at various taxonomic levels.

2. Results

2.1 Effects of Dietary Protein Level on Growth Performance of Growing-Finishing Pigs

As shown in Table 3 , no significant differences were observed in growth performance indices including ADG, ADFI, and F/G between the experimental and control groups ($P>0.05$). However, ADG was slightly higher while ADFI and F/G were slightly lower in the experimental group compared to the control group. These results indicate that under the conditions of this experiment, reducing dietary protein level by approximately 2% while supplementing synthetic amino acids did not affect growth performance of growing-finishing pigs.

Table 3 Effects of dietary protein level on growth performance of growing-finishing pigs

Items	Control group	Experimental group	P-value
Initial body weight/kg	45.6±4.0	45.4±3.7	
Final body weight/kg	70.7±4.3	71.2±3.6	
Average daily gain/(g/d)	784±56	805±58	
Average daily feed intake/(g/d)	2,000±85	1,985±135	
Feed/gain	2.55±0.07	2.50±0.23	

2.2 Effects of Dietary Protein Level on Intestinal Microbiota

2.2.1 Sequence and OTU Statistics High-throughput sequencing was used to analyze microbial community diversity in feces of finishing pigs from control and experimental groups. A total of 1,230,704 valid sequences were obtained from 20 samples, with an average sequence length of 436.20 bp. The vast majority of sequence lengths (99.94%) ranged from 421 to 460 bp. OTU clustering of the 20 valid samples yielded an average of 1,098 OTUs per sample. Rarefaction curves were generated by random sampling to simulate the relationship between input sequence number and OTU output. As shown in Figure 1 [Figure 1: see original paper], the rarefaction curves for all samples tended to plateau, indicating that the sequencing depth was adequate to reflect the majority of microbial information in the samples. Figures A and B represent the OTU rank-abundance curve and dilution curve, respectively.

2.2.2 Effects of Dietary Protein Level on Intestinal Microbiota Diversity As shown in Table 4 , although no significant differences were observed in diversity and richness indices between the two groups (P>0.05), the Shannon, ACE, and Chao1 indices were higher while the Simpson index was lower in the low-protein diet group compared to the normal-protein diet group. These results suggest that feeding a low-protein diet enhanced bacterial diversity and richness in feces of finishing pigs.

Table 4 Effects of dietary protein level on intestinal microbiota diversity of growing-finishing pigs

Items	Control group	Experimental group	P-value
Diversity indexes			
Shannon index	4.06±0.35	4.25±0.26	
Simpson index	0.07±0.02	0.06±0.02	
Richness indexes			
ACE index	869.55±36.76	897.52±51.81	
Chao 1 index	880.69±38.90	909.72±52.78	

2.2.3 Effects of Dietary Protein Level on Intestinal Microbiota Composition At the phylum level (Figure 2-A), the dominant fecal microbiota in

both experimental and control groups consisted primarily of Firmicutes and Bacteroidetes, with Firmicutes accounting for nearly 80% and Bacteroidetes approximately 15%. Other phyla including Spirochaetae, Tenericutes, and Cyanobacteria collectively represented 5.69% on average. The proportions of Firmicutes and Bacteroidetes were very similar between experimental and control groups (80.07% vs. 79.64% and 14.10% vs. 14.82%, respectively), with minimal intergroup differences. Other phyla showed slight variations between groups.

At the genus level (Figure 2-B), substantial differences in microbiota composition were observed between the two groups. A total of 205 genera were identified, with 36 genera having relative abundance >1% and collectively accounting for approximately 82.98% of total abundance. The genera with higher relative abundance in the experimental group were *Streptococcus* (17.95%), *Clostridium sensu stricto 1* (13.22%), *Lactobacillus* (6.52%), and norank_f_Bacteroidales S24-7 (6.24%), while those in the control group were *Clostridium sensu stricto 1* (18.38%), *Streptococcus* (15.90%), *Lactobacillus* (7.81%), and *Terrisporobacter* (6.50%). Additionally, three genera were unique to the experimental group: *Anaerofustis*, *Beta proteobacteria*, and *Streptococcaceae*, though all had low relative abundance.

2.2.4 Species Difference Significance Analysis LEfSe-based multilevel species difference discriminant analysis (Figure 3 [Figure 3: see original paper]) revealed no significantly differential bacteria at phylum and class levels between experimental and control groups. However, differential bacteria were identified at other taxonomic levels. At the genus level, the relative abundances of *Lachnospira*, *Pseudobutyrivibrio*, *Butyrivibrio*, and *Oscillibacter* were significantly higher in the experimental group ($P<0.05$), while *Paraeggerthella* and *Clostridium sensu stricto 6* were significantly higher in the control group ($P<0.05$).

2.2.5 Inter-Group Community Difference Comparison Figure 4 [Figure 4: see original paper] shows PCoA clustering and partial least squares discriminant analysis (PLS-DA) of fecal microbial community composition from the two groups. The results demonstrate that microbiota from the experimental group clustered distinctly and could be clearly separated from the control group, indicating that pigs fed the low-protein diet had different intestinal microbial communities compared to those fed the normal-protein diet.

3. Discussion

3.1 Effects of Low-Protein Diet on Growth Performance of Growing-Finishing Pigs

Numerous studies have demonstrated that reducing dietary protein level by 2%–3% while supplementing essential amino acids to meet nutritional requirements or conform to ideal amino acid ratios does not significantly affect growth performance of growing-finishing pigs [2,6-7]. However, when dietary protein level

is reduced by 3% or more, reported effects on growth performance vary, with responses depending on the degree of protein reduction and the types and levels of supplemented essential amino acids [8-10]. When dietary protein level is reduced beyond a certain threshold, growth performance cannot be maintained regardless of amino acid supplementation and balancing. Furthermore, with advancing synthetic amino acid technology, the cost of formulating low-protein diets does not increase markedly and may actually decrease due to reduced inclusion of soybean meal and other protein ingredients. The present study demonstrated that reducing dietary protein level by approximately 2% during the growing-finishing phase had no significant effect on pig growth performance. ADG and ADFI were slightly higher while F/G was slightly lower in the experimental group compared to the control group, though differences were not significant. Based on feed ingredient prices at the time, actual feed cost per pig was reduced, and improved growth performance enhanced production efficiency. These results are consistent with previous findings, confirming that reducing dietary protein level by approximately 2% with appropriate essential amino acid supplementation does not affect growth performance of growing-finishing pigs and can improve production efficiency.

3.2 Effects of Low-Protein Diet on Diversity and Richness of Intestinal Microbiota

The quantity and structure of intestinal microbiota play important roles in host digestion and health [4,11]. Zhang et al. [12] reported that dietary factors accounted for 57% of changes in intestinal microbiota, indicating that dietary composition significantly affects microbial quantity and composition. Shannon and Simpson indices typically reflect intestinal microbiota diversity, which is generally positively correlated with microbial community stability and resistance to pathogen infection [13]. In this study, the low-protein diet increased fecal microbiota diversity in growing-finishing pigs, suggesting beneficial effects on intestinal health. Additionally, fecal microbiota richness was higher in the low-protein diet group compared to the normal-protein diet group, consistent with findings by Cao [14] who reported that low-protein diets could increase intestinal microbiota richness and diversity in growing-finishing pigs. Fan et al. [15] demonstrated that when dietary protein level decreased from 16% to 13%, ileal microbiota richness and diversity increased, with higher proportions of beneficial *Lactobacillus* in the ileum and *Megasphaera* in the colon. However, when dietary protein level was reduced to 10%, microbiota diversity in both ileum and colon decreased and pig growth performance declined. These findings indicate that moderate reduction in dietary protein level can promote and regulate intestinal microbiota diversity, whereas excessive reduction decreases diversity and impairs growth performance.

3.3 Effects of Low-Protein Diet on Intestinal Microbiota Composition

Intestinal microbiota is primarily composed of Firmicutes (35%–80%) and Bacteroidetes (17%–60%) at the phylum level [16]. In this study, Firmicutes and Bacteroidetes were the most abundant microbial phyla in all groups, with no significant inter-group differences, indicating that reducing protein level did not substantially affect the dominant microbiota structure at the phylum level. Previous studies [17] and earlier research [8] have shown that moderately reducing dietary protein level while supplementing synthetic amino acids can improve apparent biological value of nitrogen and benefit nitrogen metabolism balance in finishing pigs. This improved nitrogen utilization efficiency is partly related to changes in intestinal microbiota induced by low-protein diets. At the genus level, *Streptococcus* was the dominant genus in both groups, with increased relative abundance in feces of pigs fed the low-protein diet. This genus primarily utilizes amino acids to synthesize bacterial protein [18], and its increase may result from supplemented limiting amino acids in the low-protein diet providing more substrates for *Streptococcus* metabolism, thereby affecting nitrogen metabolism and utilization efficiency through increased bacterial protein synthesis. *Clostridium sensu stricto 1*, another dominant genus, was markedly reduced in feces of pigs fed the low-protein diet. Fan et al. [15] also reported that *Clostridium sensu stricto 1* decreased significantly in pig ileum with reduced dietary protein level, likely due to insufficient nitrogen substrate for fermentation caused by lower dietary protein. The low-protein diet significantly increased populations of *Lachnospiraceae* [19], *Ruminococcaceae* [19], *Butyrivibrio* [20], and *Pseudobutyrivibrio* [20], which can metabolize dietary carbohydrates and fiber to produce butyrate. Butyrate regulates energy metabolism and immune responses in intestinal epithelial cells [21] and stimulates intestinal cells to produce antimicrobial peptides, helping defend against pathogen invasion, suppress intestinal inflammation, and protect intestinal health [22]. The increase in these bacterial taxa may be attributed to significantly increased proportions of carbohydrates and starch in the low-protein diet, providing sufficient metabolic substrates. *Paraeggerthella* abundance was significantly higher in the normal-protein diet group compared to the low-protein diet group. This genus has been shown to cause invasive infections in immunocompromised individuals and can induce bacteremia through damaged intestinal epithelium [23]. *Delftia*, another opportunistic pathogen with higher abundance in the normal-protein diet group, suggests that appropriate dietary protein reduction may help decrease infection risk from harmful bacteria. Wellock et al. [24] and Bhandari et al. [25] also found that reducing dietary protein level in weaned piglets significantly decreased populations of pathogenic bacteria such as *Escherichia coli* in the intestine and feces. The reduction in *E. coli* with low-protein diets is thought to result from decreased substrates available for proliferation. Additionally, *Oscillibacter* abundance was significantly higher in the low-protein diet group. *Oscillibacter* is a major valerate-producing anaerobe [26], and excessive valerate can irritate the intestinal mucosa, potentially exerting regulatory effects on the intestine. Cao [14] reported that feeding finishing pigs a low-protein

diet with cottonseed meal and corn germ meal replacing 50% of soybean meal nitrogen significantly increased *Oscillibacter* relative abundance in rectal feces. This study also showed that reducing dietary protein level significantly affected some unclassified bacterial taxa, whose functional characteristics remain unclear. Therefore, the reasons for increased or decreased abundance of these unclassified bacteria under low-protein diet conditions require further investigation.

3.4 Effects of Low-Protein Diet on Intestinal Microbiota Community Structure

Inter-group and species difference analyses clearly separated the intestinal microbial communities of the two groups into distinct clusters, with microbiota from the low-protein diet group showing tighter clustering. These results demonstrate that low-protein diets significantly affect intestinal microbial community structure in growing-finishing pigs. Analysis of microbiota composition and species differences revealed that the low-protein diet increased populations of protein- and amino acid-degrading bacteria as well as butyrate-producing beneficial bacteria while decreasing harmful bacterial taxa, indicating that appropriate dietary protein reduction shifts intestinal microbiota toward a healthier and more balanced state, thereby promoting healthy growth of finishing pigs. Luo et al. [27] also reported distinct differences in intestinal microbiota between low- and normal-protein diet groups, with microbiota from low-protein diet groups clustering separately from normal-protein diet groups, confirming that dietary protein level changes modulate intestinal microbial community structure.

4. Conclusion

Under the conditions of this experiment, reducing dietary protein level by approximately 2% below normal levels while supplementing appropriate synthetic amino acids did not negatively affect growth performance or health status of growing-finishing pigs. Moreover, this dietary modification altered intestinal microbial composition and community structure in a direction beneficial to pig health.

References

[1] Kerr BJ, Southern LL, Bidner TD, et al. Influence of dietary protein level, amino acid supplementation, and dietary energy levels on growing-finishing pig performance and carcass composition. *Journal of Animal Science*. 2003;81(12):3075-3087.

[2] Kerr BJ, McKeith FK, Easter RA. Effect on performance and carcass characteristics of nursery and finisher pigs fed reduced crude protein, amino acid-supplemented diets. *Journal of Animal Science*. 1995;73(2):433-440.

[3] Isaacson R, Kim HB. The intestinal microbiome of the pig. *Animal Health Research Reviews*. 2012;13(1):100-109.

[4] Yang YX, Dai ZL, Zhu WY. Important impacts of intestinal bacteria on utilization of dietary amino acids in pigs. *Amino Acids*. 2014. doi:10.1007/s00726-014-1807-y.

[5] Fan P. Effects of low-protein diets on intestinal microbiota of weaned piglets and finishing pigs [Master' s thesis]. Beijing: China Agricultural University; 2016.

[6] Zervas S, Zijlstra RT. Effects of dietary protein and fermentable fiber on nitrogen excretion patterns and plasma ammonia in grower pigs. *Journal of Animal Science*. 2002;80(12):3247-3256.

[7] Prandini A, Sigolo S, Morlacchini M, et al. Microencapsulated lysine and low-protein diets: effects on performance, carcass characteristics and nitrogen excretion in heavy growing-finishing pigs. *Journal of Animal Science*. 2013;91(9):4226-4234.

[8] Deng D. Study on nutritional and physiological effects of low-protein diets supplemented with essential amino acids in pigs [PhD thesis]. Changsha: Institute of Subtropical Agriculture, Chinese Academy of Sciences; 2007.

[9] Tuitoek K, Young LG, de Lange CFM, et al. The effect of reducing excess dietary amino acids on growing-finishing pig performance: an evaluation of the ideal protein concept. *Journal of Animal Science*. 1997;75(6):1575-1583.

[10] Figueroa JL, Lewis AJ, Miller PS, et al. Nitrogen metabolism and growth performance of gilts fed standard corn-soybean meal diets or low-crude protein, amino acid-supplemented diets. *Journal of Animal Science*. 2002;80(11):2911-2919.

[11] Kim HB, Isaacson RE. The pig gut microbial diversity: understanding the pig gut microbial ecology through the next generation high throughput sequencing. *Veterinary Microbiology*. 2015;177(3/4):242-251.

[12] Zhang C, Zhang M, Wang S, et al. Interactions between gut microbiota, host genetics and diet relevant to development of metabolic syndromes in mice. *The ISME Journal*. 2009;4(2):232-241.

[13] Konstantinov SR, Favier CF, Zhu WY, et al. Microbial diversity studies of the porcine gastrointestinal ecosystem during weaning transition. *Animal Research*. 2004;53(4):317-324.

[14] Cao K. Effects of different nitrogen sources on porcine intestinal microbial diversity and major protease-producing bacterial strains [Master' s thesis]. Changchun: Jilin Agricultural University; 2016.

[15] Fan PX, Liu P, Song PX, et al. Moderate dietary protein restriction alters the composition of gut microbiota and improves ileal barrier function in adult pig model. *Scientific Reports*. 2017;7:43412.

[16] Shoaie S, Karlsson F, Mardinoglu A, et al. Understanding the interactions between gut bacteria and human through metabolic modeling. *Scientific Re-*

ports. 2013;3:2532.

[17] Zeng Y, Wang J, Ji H, et al. Effects of low-protein diets on production performance, nitrogen metabolism and blood biochemical parameters of finishing pigs. *Journal of Domestic Animal Ecology*. 2017;38(6):30-36.

[18] Dai ZL, Li XL, Xi PB, et al. Metabolism of select amino acids in bacteria from the pig small intestine. *Amino Acids*. 2012;42(5):1597-1608.

[19] Barrios C, Beaumont M, Pallister T, et al. Gut-microbiota-metabolite axis in early renal function decline. *PLoS One*. 2015;10(8):e0134311.

[20] Deng YF, Liu YY, Zhang YT, et al. Efficacy and role of inulin in mitigation of sulfur-containing compounds in pigs. *Journal of the Science of Food and Agriculture*. 2017;97(8):2382-2391.

[21] Donohoe DR, Garge N, Zhang XX, et al. The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metabolism*. 2011;13(5):517-526.

[22] Dou XJ, Han JL, Song WT, et al. Sodium butyrate improves porcine host defense peptide expression and relieves the inflammatory response upon toll-like receptor 2 activation through histone deacetylase inhibition in porcine kidney cells. *Oncotarget*. 2017;8(16):26532-26551.

[23] Lee MR, Huang YT, Liao CH, et al. Clinical and microbiological characteristics of bacteremia caused by *Eggerthella*, *Paraeggerthella*, and *Eubacterium* species at a university hospital in Taiwan from 2001 to 2010. *Journal of Clinical Microbiology*. 2012;50(6):2053-2055.

[24] Wellock IJ, Fortomaris PD, Houdijk JGM, et al. The effect of dietary protein supply on the performance and risk of post-weaning enteric disorders in newly weaned pigs. *Animal Science*. 2006;82(3):327-335.

[25] Bhandari SK, Opaepju FO, Krause DO, et al. Dietary protein level and probiotic supplementation effects on piglet response to *Escherichia coli* K88 challenge: performance and gut microbial population. *Livestock Science*. 2010;133(1/2/3):185-188.

[26] Iino T, Mori K, Tanaka K, et al. *Oscillibacter valericigenes* gen. nov., sp. nov., a valerate-producing anaerobic bacterium isolated from the alimentary canal of a Japanese clam. *International Journal of Systematic and Evolutionary Microbiology*. 2007;57(8):1840-1845.

[27] Luo Z, Cheng Y, Zhu W. Effects of low-protein diets on cecal metabolites and microbiota of finishing pigs. *Animal Husbandry and Veterinary Medicine*. 2015;47(10):5-9.

Note: Figure translations are in progress. See original paper for figures.