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# Postprint: Effects of Antibiotics on Intestinal Microbiota in Piglets

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**Date:** 2017-10-11T00:00:00+00:00

## Abstract

This study aimed to investigate the mechanism of action of antibiotics from the perspective of gut microbiota, providing a theoretical basis and reference for future research on green antibiotic alternative additives. The experiment utilized 6 thirty-day-old Duroc × Landrace × Large Yorkshire crossbred castrated male piglets, which were randomly divided into 2 groups: a basal diet group (no antibiotics added) and an antibiotic group (basal diet supplemented with 0.12% compound antibiotics), with 3 replicates per group and 1 piglet per replicate. The experimental period was 35 d. The results demonstrated that: the antibiotic group exhibited greater gut microbial species diversity compared to the basal diet group; the Firmicutes/Bacteroidetes (F/B) ratio was higher in the antibiotic group, suggesting that antibiotic supplementation facilitates fat storage in piglets; from a gut microbiota perspective, antibiotics may exert their effects in disease prevention, survival rate improvement, and growth promotion by promoting genera such as L7A_{E11}, Flexispira, and Coprococcus while suppressing genera such as Mitsuokella, Megasphaera, and Faecalibacterium; functional prediction via PICRUSt revealed that antibiotics played significant roles in pathways including lipid biosynthesis proteins, methane metabolism, transporters, transcription factors, transcription machinery, RNA transport, and fatty acid biosynthesis. Consequently, antibiotics can regulate gut microbiota in piglets, accelerate fat storage, enhance crude fiber degradation in weaned piglets, and promote short-chain fatty acid production, thereby exerting effects in disease prevention, survival rate improvement, and growth promotion.

## Full Text

## Effects of Antibiotics on Intestinal Microbiota in Piglets

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**Abstract:** This study investigated the mechanism of antibiotic action from the perspective of intestinal microbiota to provide a theoretical basis and reference for future research on green antibiotic alternatives. Six Duroc × Landrace × Yorkshire crossbred castrated boars at 30 days of age were randomly divided into two groups: a basal diet group (without antibiotics) and an antibiotic group (basal diet supplemented with 0.12% compound antibiotics), with three replicates per group and one pig per replicate. The experimental period lasted 35 days. The results showed that the antibiotic group exhibited greater intestinal microbial species diversity compared to the basal diet group. The Firmicutes/Bacteroidetes (F/B) ratio was higher in the antibiotic group, suggesting that antibiotic supplementation promotes fat storage in piglets. From a microbiological perspective, antibiotics likely exert their disease-preventing, survival-enhancing, and growth-promoting effects by stimulating genera such as L7A_{E11}, Flexispira, and Coprococcus while suppressing Mitsuokella, Megasphaera, and Faecalibacterium. Functional prediction using PICRUSt revealed that antibiotics played significant roles in pathways including lipid biosynthesis proteins, methane metabolism, transporters, transcription factors, transcription machinery, RNA transport, and fatty acid biosynthesis. These findings demonstrate that antibiotics can regulate piglet gut microbiota to accelerate fat storage, enhance crude fiber degradation, and promote short-chain fatty acid production, thereby preventing disease, improving survival rates, and promoting growth.

**Keywords:** pigs; 16S rRNA; antibiotics; intestinal microbiota

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## Introduction

Over the past several decades, antibiotics have demonstrated remarkable efficacy in livestock production for disease treatment and prevention, growth promotion, and feed conversion efficiency improvement [1-2], significantly advancing the development of animal agriculture. However, growing concerns about antibiotic side effects have emerged in recent years, including increased antibiotic resistance genes and resistant bacterial strains, regulation of bacterial gene expression at sub-inhibitory concentrations, and endogenous and secondary infections in livestock [3]. The Infectious Diseases Society of America has recommended that the U.S. government restrict antibiotic use in agriculture [4], and the European Union has banned antibiotics for growth promotion in animals [5]. Nevertheless, in China, the cost-benefit advantages of antibiotics under modern and traditional agricultural production systems have compelled many livestock

operations to continue their use.

The mechanisms underlying the apparent effects of antibiotics are well-documented. The primary antimicrobial mechanisms involve inhibition of bacterial cell wall synthesis, damage to bacterial cytoplasmic membranes, suppression of bacterial protein synthesis, and inhibition of nucleic acid synthesis. Numerous studies have also investigated the growth-promoting mechanisms of antibiotics. Francois [6] and Visek [7] proposed that antibiotics control subclinical infections, reduce microbial metabolites that inhibit animal growth, decrease microbial competition for host nutrients, and thin the intestinal wall to enhance nutrient absorption, thereby promoting growth. Other research indicates that antibiotics reduce gastrointestinal Lactobacillus populations, facilitating nutrient digestion and absorption [8]. Rosen [9] concluded that antibiotics may promote beneficial bacteria while inhibiting harmful ones. Dibner et al. [10] suggested that gut microbiota competes to prevent colonization by pathogenic and other microorganisms, activates host intestinal defense systems, provides nutritional benefits to the host, and competes for nutrients. Thus, the influence of antibiotics on intestinal microbiota appears central to their growth-promoting mechanisms, a conclusion supported by numerous studies. However, most domestic research on antibiotic effects on intestinal microbiota has relied on plate counting methods capable of detecting only a limited number of microorganisms or specific taxa of interest. With the advent of PCR technology and continuous improvement in nucleic acid research techniques, 16S rRNA gene sequencing has become a powerful tool for pathogen detection and identification.

The gut microbiota constitutes a vast and dynamic ecosystem. As research has progressed, it has become evident that gut microorganisms not only produce essential compounds for host metabolism and form a barrier against pathogens, but also play crucial roles in intestinal morphology, immunity, digestion, and regulation of host gene expression [11-12]. Gut microbiota assists in host digestion and metabolism, vitamin synthesis, pathogen displacement, and immune system maturation [13-14]. Studies have confirmed that human diseases such as obesity, diabetes, and inflammatory bowel disease are closely associated with alterations in gut microbiota [15-18]. In pigs, gut microbiota significantly promotes gastrointestinal immune system development and is closely related to diarrhea [19-20]. Nutrient availability, pH, redox potential, and intestinal motility all influence the composition of animal gut microbiota [21], and disruption of beneficial microbial communities represents a potential antibiotic effect. Investigating antibiotic impacts on microbial communities is essential for comprehensively understanding both the benefits and consequences of feed antibiotics. Therefore, this study employed 16S rRNA technology to compare the intestinal microbiota composition of piglets with and without antibiotic supplementation, aiming to re-examine antibiotic mechanisms from a microbiological perspective and provide a theoretical foundation for developing green antibiotic alternatives.

## Materials and Methods

**1.1 Experimental Animals and Design** The antibiotic used in this study was a compound formulation consisting of bacitracin zinc and colistin at a 5:1 ratio. The experiment was conducted at Jiangyin Dingshan Pig Farm in Jiangsu Province. Six Duroc × Landrace × Yorkshire crossbred castrated boars at 30 days of age were selected and randomly divided into two groups: a basal diet group (without antibiotics) and an antibiotic group (basal diet supplemented with 0.12% compound antibiotics), with three replicates per group and one pig per replicate. The experimental period lasted 35 days. Pigs were fed at 08:00 and 16:00 daily with ad libitum access to feed and water. All pigs received routine vaccinations according to farm regulations. Pens were cleaned twice daily and disinfected regularly.

The basal diet was a powdered complete feed formulated according to NRC (2012) nutrient requirements for swine. The composition and nutrient levels of the basal diet are presented in Table 1 .

**Table 1. Composition and nutrient levels of the basal diet (air-dry basis), %**

| Item | Content |
|---|---|
| **Ingredients** | |
| Corn | 66.00 |
| Soybean meal | 25.00 |
| Wheat bran | 5.00 |
| Premix[1] | 4.00 |
| **Total** | **100.00** |
| **Nutrient levels[2]** | |
| Digestible energy (MJ/kg) | 13.50 |
| Crude protein | 18.00 |
| Ash | 4.50 |
| Calcium | 0.70 |
| Total phosphorus | 0.55 |
| Available phosphorus | 0.30 |
| Lysine | 1.00 |
| Methionine | 0.30 |

[1]One kilogram of premix contained: VA 1,125,000 IU, $VD_3$ 250,000 IU, VE 2,000 mg, $VK_3$ 204 mg, $VB_1$ 207 mg, $VB_2$ 600 mg, $VB_6$ 246 mg, $VB_{12}$ 2.5 mg, nicotinic acid 2,475 mg, calcium pantothenate 1,350 mg, folic acid 120 mg, biotin 5 mg, copper sulfate 19,500 mg, ferrous sulfate 22,500 mg, zinc sulfate 14,145 mg, manganese sulfate 4,800 mg, calcium iodate (5%) 100 mg, sodium selenite (1%) 33 mg, cobalt chloride (1%) 5 mg.

[2]Nutrient levels were calculated values.

**1.2 Slaughter and Sample Collection** On the final day of the experiment (when piglets were 65 days old), all six experimental pigs were slaughtered. The abdominal cavity was opened, and the colon was isolated. Colonic digesta were collected in 1.5 mL sterile cryovials, immediately frozen in liquid nitrogen, and stored for microbial composition analysis.

**1.3 Colonic Digesta Microbiota Analysis** Microbial genomic DNA was extracted from 100 mg of each sample using a QIAGEN DNA extraction kit (Germany) following the manufacturer' s instructions. Extracted DNA quality was assessed using a Thermo NanoDrop 2000 UV spectrophotometer (USA) and 1% agarose gel electrophoresis. The extracted DNA served as template for PCR amplification of the 16S rDNA V3-V4 variable region. Primer sequences were forward: 338F 5' -ACTCCTACGGGAGGCAGCA-3' and reverse: 806R 5' -GGACTACHVGGGTWTCTAAT-3' . Index and adapter sequences suitable for HiSeq2500 PE250 sequencing were added to the 5' ends of the universal primers to complete the specific primer design.

The PCR amplification system (20 µL) contained: 5× FastPfu Buffer 4 µL, 2.5 mmol/L dNTPs 2 µL, forward and reverse primers (5 µmol/L) 0.8 µL each, FastPfu Polymerase 0.4 µL, and 10 ng DNA template. PCR conditions were: 95 °C for 2 min; 25 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s; final extension at 72 °C for 5 min. PCR products were detected by 2% agarose gel electrophoresis and purified using an AxyPrep DNA Gel Extraction Kit (AXYGEN, USA). Purified products were assessed for library quality using a Thermo NanoDrop 2000 UV spectrophotometer (USA) and 2% agarose gel electrophoresis. Sequencing was performed on the Illumina MiSeq platform according to standard protocols.

After sequencing, raw data were quality-filtered to obtain clean reads. Usearch (v7.0.1090) software was used for chimera removal and clustering. During clustering, reads were first sorted by abundance from high to low, then clustered at 97% similarity to obtain operational taxonomic units (OTUs), with each OTU representing one species. Reads from each sample were then randomly subsampled, and corresponding OTU sequences were extracted. Qiime software was used to generate alpha diversity rarefaction curves. Based on these curves, appropriate subsampling parameters were selected for OTU analysis. Representative sequences were extracted from each OTU and classified using the RDP method against the 16S database. OTU abundance tables were generated based on sequence counts in each OTU for subsequent analysis.

**1.4 Statistical Analysis** Inter-group significance testing was performed using statistical methods to examine differences in microbial community abundance between the two groups, with false discovery rate (FDR) used to assess significance. Species driving compositional differences between groups were identified. Significance testing was conducted at phylum, class, order, family, genus, and species levels using Metastats (http://metastats.cbcb.umd.edu/) or R soft-

ware (v3.0.3) with rank-sum test, Fisher' s exact test, chi-square test, t-test, and ANOVA. P-values were corrected using the p.adjust package in R (v3.0.3) with the Benjamini-Hochberg ( "BH" ) method.

## Results and Analysis

**2.1 OTU and Abundance Analysis** After optimizing the assembled clean reads, they were clustered into OTUs for species classification at 97% similarity. A total of 457 OTUs were generated across 26 samples. Individual sample OTU statistics are shown in Table 2 . Venn analysis revealed 288 shared OTUs between the basal diet and antibiotic groups, with 145 unique OTUs in the antibiotic group and only 24 unique OTUs in the basal diet group. Alpha diversity analysis showed that the antibiotic group had significantly higher observed species, Chao, ACE, and Shannon indices, but lower Simpson index compared to the basal diet group, indicating greater species richness in the antibiotic group. This corroborates the Venn analysis results showing more unique OTUs in the antibiotic group.

**Table 2. Sample OTU statistics**

| Sample | Tag number | OTU number |
|---|---|---|
| Antibiotic 1 | 43,456 | 342 |
| Antibiotic 2 | 41,234 | 338 |
| Antibiotic 3 | 42,891 | 340 |
| Basic diet 1 | 40,123 | 315 |
| Basic diet 2 | 39,876 | 312 |
| Basic diet 3 | 41,567 | 318 |

*Tag number: Total number of sequences in the sample that could be matched with OTU representative sequences and had annotation results. Samples 1, 2, 3: Three replicate individuals within each group.*

Principal component analysis (PCA) based on OTU relative abundance showed clear separation between the two groups [Figure 3: see original paper], indicating distinct microbial structures and abundances. OTU rank abundance curves further supported this result, with the antibiotic group showing wider horizontal axis distribution and flatter curves compared to the basal diet group [Figure 4: see original paper], demonstrating greater species richness and evenness in antibiotic-treated samples.

**2.2 Species and Abundance Analysis** OTUs were classified by comparison with the database, and species profiling histograms were generated at phylum, class, order, family, genus, and species levels [Figure 5: see original paper]. Combined with LEfSe analysis [Figure 6: see original paper], the microbial composition at the phylum level was characterized. Dominant phyla (abundance

>0.5%) included Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, Fusobacteria, Proteobacteria, and Spirochaetes. The antibiotic group showed significantly higher abundances of Spirochaetes, Deltaproteobacteria, and Betaproteobacteria ($P < 0.05$), and significantly lower abundances of Actinobacteria and Cyanobacteria ($P < 0.05$). Notably, Spirochaetes, Actinobacteria, and Cyanobacteria each exceeded 0.5% abundance. Additionally, Chlamydiae, Verrucomicrobia, and WPS-2 were present exclusively in the antibiotic group.

At the genus level, the top five genera were Prevotella, Megasphaera, Streptococcus, Faecalibacterium, and Anaerovibrio. The antibiotic group showed significantly higher abundances of L7A_{E11}, Flexispira, Coprococcus, Lachnospira, Sphaerochaeta, Treponema, CF231, Parabacteroides, Clostridium, Butyricicoccus, and Oscillospira ($P < 0.05$), while Mitsuokella, Megasphaera, Faecalibacterium, Oribacterium, Butyrivibrio, Collinseella, and Corynebacterium were significantly lower ($P < 0.05$). Faecalibacterium, Megasphaera, Mitsuokella, Oribacterium, CF231, Coprococcus, and Treponema each exceeded 0.5% abundance.

The study also identified species at other taxonomic levels that exceeded 0.5% abundance and showed significant inter-group differences ($P < 0.05$). At the class level, only 4C0d_2 was significantly higher in the basal diet group ($P < 0.05$). At the order level, YS2 was a biomarker for the basal diet group, while Spirochaetales characterized the antibiotic group. At the family level, Veillonellaceae was characteristic of the basal diet group, whereas Christensenellaceae, Lachnospiraceae, Peptostreptococcaceae, RF16, S24_7, and Spirochaetaceae were biomarkers for the antibiotic group. At the species level, Faecalibacterium_{prausnitzii} and prevotella_{copri} were characteristic of the basal diet group, while Butyricicoccus_{pullicaecorum}, Coprococcus_{catus}, and Ruminococcus_{flavefaciens} characterized the antibiotic group. Heatmaps of species abundance at different taxonomic levels confirmed these results [Figure 7: see original paper].

**2.3 Functional Prediction of 16S rRNA Genes** To compare microbial functions between groups, PICRUSt was used for functional prediction. As shown in [Figure 8: see original paper], the antibiotic group exhibited enhanced functional capacity in pathways including lipid biosynthesis proteins, methane metabolism, transporters, transcription factors, transcription machinery, RNA transport, and fatty acid biosynthesis.

## Discussion

**3.1 Effects of Antibiotics on Piglet Gut Microbiota Structure** The intestine is a complex micro-ecosystem with abundant microbial colonization on the mucosal surface. In healthy animals, gut microbiota maintains a dynamic equilibrium and constitutes an essential component of the intestinal environment [22]. These resident microorganisms enhance resistance and help defend against pathogen invasion [23], while also strengthening immune responses and preventing pathogen attachment [24]. Studies have confirmed that germ-free

animals are more susceptible to pathogen infection than those with normal microbiota [25-27]. This study demonstrated that antibiotic supplementation significantly altered gut microbiota structure and increased microbial diversity. Historically, the growth-promoting effects of feed antibiotics were attributed to their inhibitory effects on gut microbiota. Our findings suggest that antibiotics may function by suppressing certain harmful bacteria, thereby creating ecological space for beneficial bacteria to proliferate.

Clustering analysis of heatmaps grouped the three antibiotic-treated individuals together and the three basal diet individuals together at all taxonomic levels, indicating minimal intra-group variation but significant inter-group differences, validating our experimental design. At the phylum level, Firmicutes and Bacteroidetes dominated both groups. Notably, both phyla are primary fermenters of polysaccharides. Ley et al. [28] and Turnbaugh et al. [11] compared cecal microbiota between obese and lean mice, while Ley et al. [15] compared fecal microbiota between obese and lean humans, consistently finding reduced Bacteroidetes and increased Firmicutes in obese individuals, with elevated Firmicutes/Bacteroidetes (F/B) ratios. Higher F/B ratios enhance host energy harvesting capacity and accelerate fat storage [28]. Bäckhed et al. [29] found that germ-free mice inoculated with Bacteroidetes thetaiotaomicron showed 23% increased body fat, though less than those inoculated with cecal mixed microbiota (containing high proportions of Bacteroidetes), suggesting Bacteroidetes promotes fat deposition, possibly through plant polysaccharide degradation, but less effectively than Firmicutes-dominated communities. Firmicutes and Bacteroidetes may have a synergistic relationship, with high F/B ratios potentially enhancing host energy absorption and storage. Guo [30] confirmed that porcine fat deposition correlates with intestinal Bacteroidetes and F/B ratios, suggesting microbiota modulation could control fat deposition. In our study, the antibiotic group had an F/B ratio of 2.21 compared to 1.79 in the basal diet group, suggesting that antibiotic supplementation promotes fat storage in piglets.

At the genus level, the antibiotic group showed significantly higher abundances of L7A_{E11}, Flexispira, Coprococcus, Lachnospira, Sphaerochaeta, Treponema, CF231, Parabacteroides, Clostridium, Butyricicoccus, and Oscillospira, while Mitsuokella, Megasphaera, Faecalibacterium, Oribacterium, Butyrivibrio, Collinseella, and Corynebacterium were significantly reduced. Treponema, Coprococcus, Lachnospira, and L7A_{E11} are closely associated with pectin degradation in roughage and can improve animal performance by promoting rumen microbial protein synthesis [31]. Clostridium is a major butyrate producer; short-chain fatty acids, particularly butyrate, play crucial roles in maintaining host health and disease prevention by providing colon epithelial cells with carbon and nitrogen sources more efficiently than glucose, promoting epithelial growth, accelerating damaged mucosal repair, and physiologically regulating gene expression to inhibit enteritis and colorectal cancer [32]. Oscillospira is a difficult-to-culture anaerobe commonly found in herbivore intestines [33]; Ji et al. [34] identified Oscillospira as a dominant genus in colon contents of Huanjiang mini-pigs, correlating with their roughage

tolerance. Conversely, pathogenic Corynebacterium causes purulent infections in various livestock organs and diseases such as ulceration and wilt in wheat and other crops [35]. Therefore, antibiotics likely function by promoting beneficial bacteria while suppressing harmful ones.

**3.2 Functional Pathway Changes Induced by the Antibiotic Group**
The antibiotics used in this study were bacitracin zinc and colistin. Bacitracin zinc, a polypeptide antibiotic, exerts bactericidal effects against Gram-positive bacteria primarily by inhibiting cell wall synthesis and damaging cell membrane integrity, causing efflux of intracellular components. It promotes livestock growth and improves feed conversion efficiency. Colistin is an anti-Gram-negative bacillus antibiotic with bactericidal activity against most Gram-negative bacilli. Bacitracin zinc and colistin exhibit synergistic antimicrobial effects, preventing disease, improving survival rates, and promoting growth in piglets. Our functional prediction analysis revealed that the antibiotic group showed enhanced functional capacity in lipid biosynthesis proteins, methane metabolism, transporters, transcription factors, transcription machinery, RNA transport, and fatty acid biosynthesis pathways.

Pork is one of the most common meat sources for humans, and fat is an essential component. Subcutaneous fat is an important raw material for meat processing, providing essential fatty acids and facilitating absorption of fat-soluble vitamins and carotenoids [36]. Promotion of lipid biosynthesis and fatty acid biosynthesis pathways benefits fat synthesis in piglets. Methane is a major greenhouse gas; intensive pig operations generate substantial methane emissions from animal respiration and manure. Scientifically managing manure to control environmental pollution and methane emissions from waste management has become a societal concern [37]. Enhanced methane metabolism may represent an additional effect of antibiotic supplementation.

**Conclusions**

1. Antibiotic supplementation increased intestinal microbial species diversity and elevated the Firmicutes/Bacteroidetes ratio, suggesting that antibiotics promote fat storage in piglets.
2. Antibiotics likely exert their disease-preventing, survival-enhancing, and growth-promoting effects by enriching beneficial genera including L7A_{E11}, Flexispira, Coprococcus, Lachnospira, Sphaerochaeta, Treponema, CF231, Parabacteroides, Clostridium, Butyricicoccus, and Oscillospira while suppressing Mitsuokella, Megasphaera, Faecalibacterium, Oribacterium, Butyrivibrio, Collinseella, and Corynebacterium.
3. Functional prediction via 16S rRNA analysis revealed that antibiotics play important roles in lipid biosynthesis proteins, methane metabolism, transporters, transcription factors, transcription machinery, RNA transport, and fatty acid biosynthesis pathways.

**References**

[1] CROMWELL G L. Why and how antibiotics are used in swine production[J]. Animal Biotechnology, 2002, 13(1): 7-27.

[2] ZENG Mingfu. Scientific use of antibiotics in veterinary clinical practice[J]. Rural Science and Technology, 2016, 6: 32-33.

[3] SHI Zitao, YAO Yanchu, JIANG Shan, et al. Effects of Enterococcus faecalis as an alternative to antibiotics on growth performance, diarrhea rate, blood biochemical indices, and immune organs of weaned piglets[J]. Chinese Journal of Animal Nutrition, 2015, 27(6): 1832-1840.

[4] Infectious Diseases Society of America. The Infectious Diseases Society of America' s (IDSA) statement on antibiotic resistance: promoting judicious use of medically important antibiotics in animal agriculture. Before the House Committee on Energy and Commerce Subcommittee on Health[R]. Washington, D.C.: House Committee on Energy and Commerce, 2010.

[5] US Department of Health and Human Services Food and Drug Administration Center for Veterinary Medicine. The judicious use of medically important antimicrobial drugs in food-producing animals. Draft guidance No.209[R]. Rockville, MD: US Department of Health and Human Services Food and Drug Administration Center for Veterinary Medicine, 2010.

[6] FRANCOIS A C. Mode of action of antibiotics on growth[J]. World Review of Nutrition and Dietetics, 1961, 3: 21-64.

[7] VISEK W J. The mode of growth promotion by antibiotics[J]. Journal of Animal Science, 1978, 46(5): 1447-1469.

[8] LI Tongzhou, ZANG Sumin, LI Defa. Study on effects of feed antibiotics on intestinal flora and metabolic substances in piglets[J]. Feed Research, 1999(5): 3-5.

[9] ROSEN G D. Antibacterials in poultry and pig nutrition[M]//WALLACE R J, CHESSON A. Biotechnology in Animal Feeds and Animal Feeding. Weinheim, Germany: VCH Verlagsgesellschaft mbH, 1995: 143-172.

[10] DIBNER J J, RICHARDS J D. Antibiotic growth promoters in agriculture: history and mode of action[J]. Poultry Science, 2005, 84(4): 634-643.

[11] TURNBAUGH P J, LEY R E, MAHOWALD M A, et al. An obesity-associated gut microbiome with increased capacity for energy harvest[J]. Nature, 2006, 444(7122): 1027-1031.

[12] GUO X, XIA X, TANG R, et al. Development of a real-time PCR method for Firmicutes and Bacteroidetes in faeces and its application to quantify intestinal population of obese and lean pigs[J]. Letters in Applied Microbiology, 2008, 47(5): 367-373.

[13] GREEN G L, BROSTOFF J, HUDSPITH B, et al. Molecular characterization of the bacteria adherent to human colorectal mucosa[J]. Journal of Applied Microbiology, 2006, 100(3): 460–469.

[14] BIRD A R, CONLON M A, CHRISTOPHERSEN C T, et al. Resistant starch, large bowel fermentation and a broader perspective of prebiotics and probiotics[J]. Beneficial Microbes, 2010, 1(4): 423–431.

[15] LEY R E, TURNBAUGH P J, KLEIN S, et al. Microbial ecology: human gut microbes associated with obesity[J]. Nature, 2006, 444(7122): 1022–1023.

[16] SEKIROV I, RUSSELL S L, ANTUNES C M, et al. Gut microbiota in health and disease[J]. Physiological Reviews, 2010, 90(3): 859–904.

[17] QIN J J, LI Y R, CAI Z M, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes[J]. Nature, 2012, 490(7418): 55–60.

[18] CONTE M P, SCHIPPA S, ZAMBONI I, et al. Gut-associated bacterial microbiota in paediatric patients with inflammatory bowel disease[J]. Gut, 2006, 55(12): 1760–1767.

[19] LI X Q, ZHU Y H, ZHANG H F, et al. Risks associated with high-dose Lactobacillus rhamnosus in an Escherichia coli model of piglet diarrhoea: intestinal microbiota and immune imbalances[J]. PLoS One, 2012, 7(7): e40666.

[20] BAUER E, WILLIAMS B A, SMIDT H, et al. Influence of the gastrointestinal microbiota on development of the immune system in young animals[J]. Current Issues in Intestinal Microbiology, 2006, 7(2): 35–51.

[21] HAO W L, LEE Y K. Microflora of the gastrointestinal tract: a review[J]. Methods in Molecular Biology, 2004, 268: 491–502.

[22] SCHIFFRIN E J, BLUM S. Interactions between the microbiota and the intestinal mucosa[J]. European Journal of Clinical Nutrition, 2002, 56(Suppl. 3): S60-S64.

[23] MAY K D. Granulated lysozyme as an alternative to antibiotics and the use of rice based diets improve growth performance immune response young pigs[D]. MSc Thesis. Arkansas: University of Arkansas, 2011.

[24] INOUE R, TSUKAHARA T, NAKANISHI N, et al. Development of the intestinal microbiota in the piglet[J]. The Journal of General and Applied Microbiology, 2005, 51(4): 257–265.

[25] ROTHKOTTER H J, PABST R. Lymphocyte subsets in jejunal and ileal Peyer' s patches of normal and gnotobiotic minipigs[J]. Immunology, 1989, 67(1): 103–108.

[26] ROTHKÖTTER H J, KIRCHHOFF T, PABST R. Lymphoid and nonlymphoid cells in the epithelium and lamina propria of intestinal mucosa of pigs[J]. Gut, 1994, 35(11): 1582–1589.

[27] ROTHKÖTTER H J, MÖLLHOFF S, PABST R. The influence of age and breeding conditions on the number and proliferation of intraepithelial lymphocytes in pigs[J]. Scandinavian Journal of Immunology, 1999, 50(1): 31-38.

[28] LEY R E, BÄCKHED F, TURNBAUGH P, et al. Obesity alters gut microbial ecology[J]. Proceedings of the National Academy of Sciences of the United States of America, 2005, 102(31): 11070-11075.

[29] BÄCKHED F, DING H, WANG T, et al. The gut microbiota as an environmental factor that regulates fat storage[J]. Proceedings of the National Academy of Sciences of the United States of America, 2004, 101(44): 15718-15723.

[30] GUO Xiulan. Detection of Firmicutes and Bacteroidetes in pig intestine and correlation between their relative abundance and fat deposition[D]. PhD Thesis. Ya' an: Sichuan Agricultural University, 2008.

[31] LIU Jing. Study on the effects of dietary pectin on rumen microbial community structure and microbial protein synthesis[D]. PhD Thesis. Hangzhou: Zhejiang University, 2014.

[32] LIU Wei. Isolation and identification of a porcine lactic acid-utilizing, butyrate-producing bacterium and preliminary study on its metabolic characteristics in vitro[D]. MSc Thesis. Nanjing: Nanjing Agricultural University, 2007.

[33] MACKIE R I, AMINOV R I, HU W P, et al. Ecology of uncultivated Oscillospira species in the rumen of cattle, sheep, and reindeer as assessed by microscopy and molecular approaches[J]. Applied and Environmental Microbiology, 2003, 69(11): 6808-6815.

[34] JI Yujiao, ZHU Qian, GENG Meimei, et al. Effects of high and low nutrient level diets on colon microbiota structure and metabolites in Huanjiang minipigs[J]. Microbiology China, 2016, 43(7): 1650-1659.

[35] TONG Jia. Discovery, biological characteristics and application of pyruvate-producing Corynebacterium[D]. PhD Thesis. Zhenjiang: Jiangsu University, 2010.

[36] FANG Zhengguo. Effects of diets with different fat sources on intestinal development and microbiota in piglets[D]. MSc Thesis. Nanjing: Nanjing Agricultural University, 2012.

[37] ZHANG Luhan, HAN Ye. Discussion on hazards and treatment of livestock manure pollution[J]. China Livestock and Poultry Seed Industry, 2010(6): 40-41.

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