Lipid Metabolism and Microbial Profiles Between Shaziling and Yorkshire Pigs

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Research

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Abstract

Background: Shaziling pig, a Chinese indigenous breed, has been classified as a fatty pig model, while the lipid metabolism and gut microbial development in Shaziling pigs were rarely reported.

Methods: Here, we compared the metabolic and microbial profiles at 30, 60, 90, 120, 150, 210, and 300 days of age between Shaziling and Yorkshire pigs.

Results: Predictably, there were marked difference in the liver lipids (i.e., cholesterol, glucose, and low density lipoprotein) and the expressions (i.e., SREBP1/2, LXRα/β, DGAT1/2, and FABP1-3) between Shaziling and Yorkshire pigs. Bacteria in the ileal digesta and mucosa were further analyzed, and the results showed that Shaziling pigs had a higher α-diversity and the abundances of probiotics, such as Lactobacillus_johnsonii, Lactobacillus_amylovorus, and Clostridium_butyricum. 35 differentiated metabolites were identified in the mucosa between Shaziling and Yorkshire pigs, which were enriched in the carbohydrate, protein, glucose and amino acid metabolism and bile acid biosynthesis. Furthermore, 7 differentiated microbial species were markedly correlated with 35 metabolites, indicating the role of gut microbiota in host metabolism.

Conclusions: In conclusion, Shaziling pigs exhibited different metabolic and microbial profiles compared with the Yorkshire pigs, which might contribute to the diverse metabolic phenotypes.

Background

The Shaziling pig, a famous indigenous breed in China, is characterized by higher intramuscular fat and meat quality compared with the imported pig breeds. Previous studies mainly discussed the differences in genetics, involving epigenetics, quantitative genetics, and metagenomics between Shaziling and introduced pig breeds[1, 2]. However, there are little studies on the regulation of Shailing pork quality from the perspective of nutrition, which hinders the breeding of Shailing pigs and the promotion of meat products. Meanwhile, indigenous breeds are rich in microbial resources, which play an important role in host metabolism[3, 4], meat quality[5], feed efficiency[6, 7], and gut development[8–10] in farming animals. Thus, analyzing and screening gut microbiota in Shaziling pigs may enrich the probiotic library and guide a more health pig industry[11].

In recent years, with the development of germ-free animal and microbiome (16S rDNA sequencing, metagenomics), it has been widely confirmed that gut microbiota are involved in the regulation of host lipid metabolism[4, 12–14]. In addition, fecal microbial transplantation and probiotics treatment further confirmed that remodeling gut microbial compositions improve host lipid metabolism[15, 16], which may further target the fat deposition in the muscle. However, microbial compositions have not been analyzed in Shaziling pigs, thus the current study aimed to compare the microbial and metabolic differences between Shaziling and Yorkshire pigs (an imported lean pig breed) to provide a microbial perspective for the research on meat quality of indigenous pig breeds in China.
Methods

Animals and diets

Shaziling pig (a local breed in Hunan province, China) was selected as the fatty pig model, and Yorkshire pig as lean animal model. All pigs were free to basic diets (satisfying the nutritional requirements) and drinking water and slaughtered at 30, 60, 90, 120, 150, 210, 300 days of age. Samples of liver, ileal chyme and mucosa, and muscle were collected and stored at -80°C (n=6).

Liver lipid analysis

0.1g of the liver samples were homogenized in a centrifuge tube with 0.9 mL normal saline, then the supernatant was extracted and centrifuged at 1500g, 4° for 10 min. Triglyceride (TG), total cholesterol (TC), total bile acid (TBA), glucose (GLU), high density lipoprotein (HDL), and low density lipoprotein (LDL) were detected by an automatic biochemical instrument (KHB 450, Shanghai Kehua bio-engineering co.,ltd).

Reverse transcription-PCR

Ileal mucosa and liver samples were frozen in liquid nitrogen and ground, and total RNA was isolated by using TRizol reagent (Invitrogen, USA) and then treated with DNase I (Invitrogen, USA). Reverse transcription was conducted at 37°C for 15 min at 95°C for 5 s. The primers used in this study were designed according to the pig sequence (table 1). PCR cycling and relative expression determination were performed according to previous studies[12].

Bacterial profiling

Total genome DNA from ileal chyme and mucosa was extracted using CTAB method, DNA concentration and purity was monitored on 1% agarose gels. According to the concentration, DNA was diluted to 1ng/µL using sterile water. 16S rDNA genes of distinct regions (16S V3-V4) were amplified used specific primer (515F-806R) with the barcode. All PCR reactions were carried out with 15 µL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs); 2 µM of forward and reverse primers, and about 10 ng template DNA. Sequencing libraries were generated usingTruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) following manufacturer's recommendations and index codes were added according to our previous study[17]. Microbial communities were investigated by iTag sequencing of 16S rDNA genes[18].

Mucosal metabolomics analysis

The metabolome was detected and analyzed using the LC-MS system, 30mg ileal mucosa was transferred to 1.5 mL tube. 20µL of L-2-chlorophenylalanine (0.3 mg / mL) dissolved in methanol as internal standard and 0.4 mL mixture of methanol and water (4/1, vol/vol) were added to each sample. Samples were grinded and then extracted by ultrasonic for 10 min in ice-water bath, stored at -20 °C for 30 min. The extract was centrifuged at 4°C (13,000 rpm) for 10 min. 300µL of supernatant in a glass vial
was dried in a freeze concentration centrifugal dryer. 300μL mixture of methanol and water (1/4, vol/vol) were added to each sample, samples vortexed for 30 s, extracted by ultrasonic for 3 min in ice-water bath, then placed at -20°C for 2 h. Samples were centrifuged at 4°C (13,000 rpm) for 10 min. The supernatants (150μL) from each tube were collected using crystal syringes, filtered through 0.22 μm microfilters and transferred to LC vials. Then the samples were detected by LC-MS system.

**Statistical analysis**

All statistical analyses were performed using T-test and Pearson correlation analysis in SPSS 20.0 software (SPSS Inc., Chicago, Illinois, USA). The data are expressed as the means±standard errors of the means (SEMs). P value of 0.05 was considered significant. All figures in this study were drawn by using GraphPad Prism 8.0.

**Results**

**Lipid metabolism between Shaziling and Yorkshire pigs**

Exogenous intake and free lipids are assembled in the liver and distributed to the tissues of the body[19]. Therefore, the lipid levels were analyzed between Shaziling and Yorkshire pigs and the concentrations of TC and LDL were distinctly higher in Shaziling pig at 30, 60 and 300 days, GLU was notably increased in Shaziling pig at 30, 60 and 150 days, whereas TG was lowered in Shaziling pig at 90 and 150 days (Fig 1).

The absorption and transport of lipids in the gut and the assembly and metabolism in the liver are important components of lipid metabolism[20]. Then, we analyzed the expression of several genes associated with lipid metabolism in the mucosa (i.e., MOGAT2, DGAT1/2, CD36, and FABP1-4) and liver (i.e., ACC, PPARα/γ, SREBP1/2, and LXRα/β). Compared with the Yorkshire pigs, the expressions of SREBP1, LXRα, and SREBP2 were higher at 90 days and 300 days in Shaziling pigs (Fig 2A), while LXRβ mRNA abundance was lower at 30 days (Fig 2A). In the mucosa, DGAT1, FABP1, FABP2, and FABP3 expressions were lower at 90 days in Shaziling pigs (Fig 2B), while DGAT2 was higher at 30 days (Fig 2B).

Summary, lipid metabolism and absorption related genes were differentiated between Shaziling and Yorkshire pigs, which might be by explained by the microbial and genetic difference.

Together, Shaziling pigs showed a marked difference in lipid metabolism compared with the Yorkshire pigs, the mechanisms might be associated with the genetic and environmental factors, especially for gut microbiota.

**Bacterial development in the ileal cavity**

To understand the relationship between gut microbes and lipid metabolism, we firstly aimed to compare bacterial alterations at different ages between Shaziling and Yorkshire pigs. The results showed that the alpha diversity (observed_species, shannon, chao1, ACE, and PD_whole_tree) of Shaziling pig was significantly higher at day 90 and 150 compared with the lean subjects (Fig 3A). Next, we analyzed the
bacterial changes at the phylum level and found that 14 out of 15 phyla were altered and exhibited a time-dependent pattern, including Firmicutes, Chlamydiae, Bacteroidota, Fusobacteriota, Actinobacteriota, Campilobacterota, Verrucomicrobiota, Cyanobacteria, unidentified_Bacteria, Desulfbacterota, Chororflexi, Spirochaetota, Acidobacteriota, and Planctomycetes (Fig 3B), and 17 out of 20 species were identified to be differentiated between two datasets, such as Lactobacillus_amylovorus, Chlamydia_suis, Escherichia_coli, Turicibacter_sp_H121, Romboutsia_ilealis, Streptococcus_gallolyticus, Fusobacterium_mortiferum, Lactobacillus_delbrueckii, Limnobacter_thiooxidans, Campylobacter_jejuni, Brevundimonas_vesicularis, Trueperella_pyogenes, Acinetobacter_wuhouensis, Rothia_endophytica, Lactobacillus_teuteri, Prevotella_stercorea, and Alloprevotella_tannerae (supplementary fig 1A).

Commensal bacteria profiles between Shaziling and Yorkshire pigs

Previous study indicated that gut barrier function and host metabolism are mainly govern by mucosal commensal bacteria[21], which has not been well studied compared with the chymous and fecal microbiota Thus, we further explored the commensal bacteria in the mucosa between Shaziling and Yorkshire pigs. Similarly, the results showed that the α-diversity of Shaziling pig was significantly higher at 90 and 150 days (Fig 4A) and principal component was markedly differentiated between Shaziling and Yorkshire pigs (Fig 4A). Next, we analyzed the top 10 phyla, which were markedly altered, including Firmicutes, Proteobacteria, Chlamydiae, Fusobacteriota, Bacteroidota, Actinobacteriota, Campilobacterota, Verrucomicrobiota, Cyanobacteria, and unidentified_Bacteria (Fig 4B). At the species level, we found that 13 out of 20 species were markedly changed at entire stage, including Lactobacillus_johnsonii, Lactobacillus_amylovorus, Chlamydia_suis, Escherichia_coli, Romboutsia_ilealis, Turicibacter_sp_H121, Fusobacterium_mortiferum, Lactobacillus_delbrueckii, Actinobacillus_porcitonsillarum, Limnobacter_thiooxidans, Clostridium_butyricum, Campylobacter_jejuni, and Acinetobacter_wuhouensis (supplementary fig 1B). At 300 days, 7 species out of 15 species were markedly altered, including Lactobacillus_johnsonii, Lactobacillus_amylovorus, Chlamydia_suis, Turicibacter_sp_H121, Fusobacterium_mortiferum, Clostridium_butyricum and Campylobacter_jejuni (Fig 4C). Together, there was a marked difference in gut microbiota between Shaziling and Yorkshire pigs, which might contribute to the different metabolic phenotypes.

Ileal metabolic profiles between Shaziling and Yorkshire pigs

Microbial metabolites are one of the important factors affecting host lipid metabolism [22]. Thus, we further analyzed the metabolic profiles of the mucosa at 300 days of age between Shaziling and Yorkshire pigs (Fig 5). First, we screened 86 different metabolites using VIP>1, P<0.05 as the standard (supplementary table 1), and then we further identified 35 metabolites using VIP>2, P<0.05, including naringenin, levan, vaccenic acid, 4-Hydroxytamoxifen, ricinoleic acid, deoxyguanosine LysoPA(16:0/0:0), 13S-hydroxyoctadecadienoic acid, 11Z-eicosenoic acid, deoxyinosine, glucosamine, sphingosine, propionic acid, pseudouridine, alloporinol riboside, N-acetylmannosamine, pectic acid, D-glucose, D-galactose, ferulic acid, 4-sulfate, L-norleucine, L-proline, 4-hydroxybenzaldehyde, m-coumaric acid, 2-
phenylacetamide, phenylacetic acid, trans-cinnamic acid, L-histidine, indoleacrylic acid, asymmetric dimethylarginine, 4-hydroxycinnamic acid, sulfolithocholic acid, 3beta,7alpha-dihydroxy-5-cholestenoate, L-urobilin, 24-hydroxycholesterol (Fig 5). KEGG metabolic pathway enrichment showed that these differentiated metabolites were mainly related to carbohydrates, protein digestion and absorption, glucose and amino acid metabolism, and bile acid biosynthesis (supplementary table 2).

Pearson correlation analysis showed that the significant positive correlations were identified between Lactobacillus johnsonii and LsyoPA(16:0/0:0), Lactobacillus amylovorus and LsyoPA(16:0/0:0), Chlamydia suis and levan, deoxyinosine, Turicibacter oxi_H121 and levan, 4-hydroxytam deoxyguanosine, deoxyinosine, allopurinol riboside, Fusobacterium mortiferum and L-norleucine, trans-cinnamic acid, indoleacrylic acid, asymmetric dimethylarginine, 3beta,7alpha-dihydroxy-5-cholestenoate, L-urobilin, 24-hydroxydium sool, Clostridium butyricum and LsyoPA(16:0/0:0), Campylobacter jejuni and L-proline. The negative correlations were noticed between Turicibacter sp_H121 and 4-hydroxybenzaldehyde, m-coumaric acid, 2-phenylacetamide, phenylacetic acid, trans-cinnamic acid, asymmetric dimethylarginine, Fusobacterium mortiferum and propionic acid and D-galactose (Fig 5).

Summary, the current data indicated that gut microbiota were highly associated with metabolic profiles, which further targeted the different metabolic phenotypes.

Discussion

Chinese indigenous breeds such as Shaziling pig, have many excellent traits, such as strong stress resistance and high meat quality. However, there is few research on the mechanism of regulating meat quality by nutritional methods. In this trial, we focused on the relationship between the differences in microbes at different stages and the lipid metabolism of the host, in order to explore new ideas for nutritional regulation of lipid metabolism in Shaziling pigs.

The liver is one of the important organs for host lipid metabolism, including lipid assembly and transportation[23]. Meanwhile, the absorption and transport of lipids in the small intestine also determines the fate of lipid metabolism[19], and a two-way effect occurs with intestinal microbes and metabolites[24, 25]. Thus, the expression of genes related to lipid absorption and transport in the small intestine and genes related to liver lipid metabolism may reveal the molecular mechanism of lipid metabolism. Germ-free mice demonstrated that the changes of gut microbiota community including decreased relative abundance of Lachnospiraceae, enhanced occurrence of Desulfovibrionaceae, Clostridium lactatifermants and Flintibacter butyricus was associated with impaired glucose metabolism, lower counts of enteroendocrine cells, fatty liver, and elevated amounts of hepatic triglycerides, cholesteryl esters, and monounsaturated fatty acids[26]. Furthermore, using metagenomics and metabolomics to screen an uncultivated microorganism, the microbial cholesterol dehydrogenase that contributes to the formation of fecal alcohol, and it played an important role in reducing the concentration of gut and serum cholesterol[27]. Interestingly, there may be a bridge between gut microbes and metabolites and lipid levels, and several relevant genes regulated the concentration of lipids. Recent studies have found that theabrownin reduced liver cholesterol and decreased lipogenesis by the gut
microbe-bile acid-FXR-FGF15 signaling pathway[25]. In diet-induced obesity, the deletion of the *MyD88* gene in hepatocytes altered the gut microbes and their metabolites, the host phenotype was attributed to the gene expression and transcription factor activity (*PPARα, FXR, LXR* and *ATAT3*) and bile acid profiles involved in glucose, lipid metabolism and inflammation[28]. In this trial, we found that the variational tendency of lipid concentration was consistent with the gene expression of relevant lipid metabolism in the liver, and they were higher than control group. While the tendency of the gene expression associated with lipid absorption and transport was different in the mucosa, Shaziling gene expression was lower than the control group before 150 days of age, and reached or exceeded the control group after 150 days. This result indicated that there were temporal and spatial differences in lipid metabolism, which is worth further exploration.

Recently, the relationship between gut microbes and host lipid metabolism has been extensively studied[19, 29, 30]. Although Shaziling pigs are fatty model, they are well known for their high meat quality. Further, two distinct types of pigs have been studied that their microbial variations of Yorkshire and Shaziling pig at different stages, which helps us understand and grasp the relationship between microbes and lipid metabolism. The organism establishes a huge microbiota from birth to coordinate the health of the body. The abundance of microorganisms also changes plays different roles at different stages[31, 32]. A study investigated the human microbiota in the first 1,000 days and found that the concurrent assembly of the microbiota and endocrine, immune and metabolic pathways indicate tight regulatory interdependence and between microbiota and host underlying growth and development, and it is intergenerational, thereby perpetuating growth impairments into successive generations[32]. In addition, piglets are prone to weaning emergency syndrome due to factors such as weaning, environment, drugs and feed, resulting in intestinal microorganism disturbance[33]. Furthermore, one of the important roles of intestinal microbes in adults and the elderly is to participate in the body's lipid metabolism[34, 35]. In this trial, we investigated the development of microorganisms in Yorkshire and Shaziling pig's chyme at 30, 60, 90, 120, 150, 210 and 300 days. Simultaneously, we found that the abundance of gut microbes in Shaziling pigs was higher than Yorkshire pigs, we further analyzed that there are significant differences including 14 phyla (such as *Firmicutes, Chlamydiae, Bacteroidota*, etc), and 17 species (such as *Lactobacillus_amylovorus, Chlamydia_suis, Escherichia_coli, Turicibacter_sp_H121*, etc).

Additionally, we have analyzed that the microbial abundance in the mucosa was higher than in the chyme. Therefore, we further explored the commensal microorganisms of Shaziling pig in the mucosa. Importantly, several studies have reported that the commensal microorganisms in the mucosa and the mucus layer together maintain the gut barrier function[21]. Thoroughly studies have found that the molecular mechanisms of commensal bacteria regulating host lipid metabolism were different, *Lactobacillus paracasei* promoted lipid storage through the L-lactic acid-malonyl-CoA-lipid β oxidation pathway; in contrast, *E. coli* improved lipids oxidation by the acetic acid-acetyl-CoA/AMP-AMPK/PGC-1α/PPARα pathway [36]. In this experiment, we screened several discrepant bacteria, including 10 phyla (*Firmicutes, Proteobacteria, Chlamydiae*, etc.) and 13 species (*Lactobacillus_johnsonii, Lactobacillus_amylovorus, Chlamydia_suis*, etc.).
Metabolomics is extensively studied for the relationship between microbial metabolites and host metabolism[37]. Most experiments revealed that gut microbes governed host lipid metabolism[38, 39]. However, there are manifold intestinal microbes and complex interactions between microorganisms, so the mechanism by which microbes regulate host lipid metabolism is ambiguity. Microbial metabolites may be a bridge to reveal the relationship between microbes and host metabolism[40, 41], thus we investigated mucosal metabolites by non-targeted metabolomics, and then analyzed the correlation between mucosal microbes. KEGG pathway analysis showed that metabolites were mainly involved in the protein and carbohydrates digestion and absorption, glucose metabolism and primary bile acids biosynthesis. In addition, Module–trait associations indicated that microorganisms were related to plasma metabolites, including Clostridales, Tenericutes, Methanobrevibacter and Christensenellaceae was positively correlated with acetate, glutamine, and polyunsaturated fatty acids, whereas Blautia was negatively correlated with TG, MUFA, pyruvate, glycerol, alanine, isoleucine, leucine, and glyca[42]. Further research found that microbiota-derived inositol phosphate regulates histone deacetylase 3 (HDAC3) activity to promote epithelial repair[41]. In this experiment, we revealed that Lactobacillus johnsonii, Lactobacillus amylovorus, Chlamydia suis, Turicibacter oxi_H121, Fusobacterium mortiferum, Clostridium butyricum and Campylobacter jejuni were markedly correlated with host lipid metabolism, and suggested that gut microbes and metabolites are involved in lipid metabolism. However, the function of microorganisms and the mechanism of regulating lipid metabolism need further study.

Conclusion

In conclusion, our results indicated that the gut microbes of Shaziling pigs influenced its metabolic phenotype. The gut microbial abundance, lipid level and gene expression of Shailing pigs were altered at different stages, and the abundance was higher than the control group. Correlation analysis showed that microorganisms and metabolites were involved in host lipid metabolism, but the functions of gut microbiota and metabolites at entire stages and the specific mechanisms that regulating host lipid metabolism required us to continue to explore.

Abbreviations

TC, total cholesterol; TG, total triglycerides; TBA, total bile acid; HDL, high density lipoprotein; LDL, low-density lipoprotein; ACC, acetyl-CoA carboxylase; PPARα, peroxisome proliferator activated receptor α; PPARγ, peroxisome proliferator activated receptor γ; SREBP1, cholesterol regulatory element binding protein 1; SREBP2, cholesterol regulatory element binding protein 1; LXRα, liver X receptor α; LXRβ, liver X receptor β; MOGAT2, monoacylglycerol-O-acyltransferase 2; DGAT1, diacylglycerol Acyltransferase 1; DGAT2, diacylglycerol Acyltransferase 2; FABP1, fatty acid-binding protein 1; FABP2, fatty acid-binding protein 2; FABP3, fatty acid-binding protein 3; FABP4, fatty acid-binding protein 4.

Declarations

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**Availability of data and materials**

All data generated or analyzed during this study can be made available by the corresponding author upon reasonable request.

**Ethics approval**

The animal model and experimental procedures used in this experiment were approved by the Hunan Agricultural University Institutional Animal Care and Use Committee (202005).

**Authors’ contributions**

Jie Ma is the primary investigator in this study. Yehui Duan participated in the animal experiments. Xiaoxiao Liang performed statistical data analysis. Tiejun Li and Xingguo Huang participated in sample analysis. Yulong Yin designed this study and Jie Yin examined the manuscript as corresponding author.

**Conflicts of interest**

The authors declare that they have no conflict of interest.

**Consent for publication**

Not applicable.

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Tables

Table 1. Primers used for gene expression analysis by real-time PCR
| Gene    | Forward sequence (5'-3')                  | Reverse sequence (5'-3')                  |
|---------|---------------------------------|---------------------------------|
| β-actin | CTACGCCAACACGGTGCTGTC            | CTTCCTGCTTGCTGAAGCCATCTCG       |
| GAPDH   | TCGAGTGACGGAGATTTGGC             | TGACAGGGCTTACCCTCTCC            |
| ACC     | CGGAATATCCAGAGGGCAGGA           | CCAGTCAGTGCTTGTGAAGCCA          |
| PPARα   | CAGCATAAACCCGCCTTTTG            | TCTCGTGCTTGCTGAAGCGC            |
| PPARγ   | GCAAGGACAGAGAAGAGGTG            | GCCAGGTCGCTGCTATCTAATTCC        |
| SREBP1  | CAGGAGGCGAAGCTGAATA             | CTGTTGCTGCTGCTGAAGGA           |
| SREBP2  | TGGCTTTGCTGCTGCTGCTACATCTCG    | CGCTGCTTGGCTTGCATCC             |
| LXRα    | GACAAGGACTGACACCATCC            | CCTCAACCACAAAGGACAT            |
| LXRβ    | GAACAAGGGGACGAAAAAGCAG          | TGAAGGGGACCATTACCA             |
| MOGAT2  | GGCGCTCTGAGGGCATAA              | AAGGGGACCGAAACCAC             |
| DGAT1   | AACCTGACCTACCCGCGATCT           | GGAAGCGGGAAGAAGTTGAGC          |
| DGAT2   | GCGGGAGATACCTGATGCTTCTG         | ACCAGGTCGCTGCTCG              |
| CD36    | GACACGACACCTACACCACAGGC         | CGAGGCCAGAGATTGAACCACATC       |
| FABP1   | ATCACTACGGGTCCAAGGT             | CAACCTAACCACGCTGCTTGACC        |
| FABP2   | CGGAAGTGAACCTCACTGGGAA          | CTGGACCATTTCATCCCGA            |
| FABP3   | GATGACGGAGGATGGTCAAGCTCA        | TAAGTGCAGTGCAAACTGC           |
| FABP4   | AAGAAGTGAGGGAGGCTTTCG          | ATTCTTGTAGCGGTGACACCTTTC       |

**Figures**

**Fig 1**

**Lipids in the liver**

![Graphs showing lipids in the liver over days](image-url)
Lipids in the liver. Lipids in the liver (A-F) were detected using T-test between Cont and SZL group in SPSS 20.0, and the figures were drawn in GraphPad Prism 8.0. Values are presented as the means ± SEMs. Differences were denoted as follows: *, P<0.05, **, P<0.01, ***, P<0.001.

Fig 2

A Relative expression in the liver

B Relative expression in the mucosa

Figure 2

Gene expression in the liver and mucosa. Relative expression in the liver (A) and relative expression in the mucosa (B) were detected using T-test between Cont and SZL group in SPSS 20.0, and the figures were drawn in GraphPad Prism 8.0. Values are presented as the means ± SEMs. Differences were denoted as follows: *, P<0.05, **, P<0.01, ***, P<0.001.

Fig 3

A α-Diversity in the chyme

B Phylum in the chyme
Figure 3

Microbial development in the chyme. α-Diversity in the chyme(A), phylum in the chyme(B). The datas were assessed using T-test between Cont and SZL group in SPSS 20.0, and the figures were drawn in GraphPad Prism 8.0. Values are presented as the means ± SEMs. Differences were denoted as follows: *, P<0.05, **, P<0.01, ***, P<0.001.

Figure 4

Commensal bacteria in the mucosa. Diversity in the mucosa(A), phylum in the mucosa(B), and the datas were assessed using one-way ANOVA by comparing the different stages of Shaziling pigs in SPSS 20.0. Species were altered in Shaziling pig(C), the datas were assessed using T-test by comparing the Shaziling and Yorkshire pigs at 300 days in SPSS 20.0, all the figures were drawn in GraphPad Prism 8.0. Values are presented as the means ± SEMs. Differences were denoted as follows: *, P<0.05, **, P<0.01, ***, P<0.001.
Figure 5

Metabolic profile and correlation analysis. We screened 35 metabolites with VIP>2 and P<0.05 as standards, and then pearson correlation analysis was performed with 7 different bacteria in the mucosa at 300 days. Differences were denoted as follows: *, P<0.05, **, P<0.01.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFiles.docx