**Characteristics of the Jejunal Microbiota in 35-Day-Old Saba and Landrace Piglets**

HUAN GAO*, YUTING YANG*, ZHENHUI CAO, JINMING RAN, CHUNYONG ZHANG, YING HUANG, MINGHUA YANG, SUMEI ZHAO, QINGCONG AN* and HONGBIN PAN*

Yunnan Provincial Key Laboratory of Animal Nutrition and Feed Science, Faculty of Animal Science and Technology, Yunnan Agricultural University, Kunming, China

Submitted 18 June 2020, revised 17 August 2020, accepted 19 August 2020

**Abstract**

The balanced microbiological system is a significant hallmark of piglet health. One of the crucial factors affecting intestinal microbiota is the host's genetics. This study explored the difference in the diversity of jejunal microbiota between Saba (SB) and Landrace (LA) piglets. Nine Saba and nine Landrace piglets were fed with sow's milk until day 35. Jejunal contents were harvested for 16S rRNA sequencing. The birth weight, body weight, and average daily gain of Saba piglets were lower than those of Landrace piglets ($p < 0.01$). Firmicutes were the main phylum in Saba and Landrace piglets, and the Saba piglets had a higher ($p < 0.05$) abundance of Bacteroidetes compared with Landrace piglets. The two most abundant genera were *Lactobacilli* and *Clostridium XI* in the jejunum of Landrace and Saba piglets. Compared with Landrace piglets, the Saba piglets had significantly lower ($p < 0.05$) abundance of *Veillonella*, *Streptococcus*, and *Saccharibacteria* genera *incertae sedis*. The functional prediction showed that “$d$-glutamine and $d$-glutamate metabolism” and “one carbon pool by folate” pathways were enriched in Saba piglets, while “limonene and pinene degradation”, “tryptophan metabolism”, and “sulfur relay system” pathways were enriched in Landrace piglets. In summary, the growth performance was higher for Landrace piglets compared with Saba piglets due to their genetic characteristics. The rich diversity and fewer infection-associated taxa were observed in Saba piglets, partially accounting for their higher adaptability to environmental perturbations than Landrace piglets. Furthermore, different pig breeds may regulate their health through different metabolic pathways.

**Key words:** Saba piglets, Landrace piglets, jejunal content, the 16S rRNA gene, diversity

---

**Introduction**

The healthy creature intestine is home to microorganisms (Eckburg et al. 2005; Ley et al. 2006; Lozupone et al. 2012). Although microbiota resides in the intestines, it plays a critical role in the digestion and absorption of nutrients, maturation of immune system, anti-colonization, and stimulation of diverse host functions (Turnbaugh et al. 2006; Levy et al. 2017). The jejunum is a significant site for nutrient absorption (Martinez-Guryn et al. 2019). The jejunal microbiota is closely related to amino acid metabolism (Dai et al. 2010) and lipid deposition (Li et al. 2019).

Previous studies indicated that the host's genetics shapes the microbial repertoire (Goodrich et al. 2014; Goodrich et al. 2016). It was discovered that the intestinal microbiota in exotic pig breeds varies from Chinese indigenous pig breeds (Yang et al. 2014). To further explore this observation, two pig breeds with different host genetics (Saba and Landrace) were selected as the subjects in this study. The Saba pig is an indigenous breed in Chuxiong of Yunnan Province, China, and it is on the list of National Conservation Program for Chinese Indigenous Livestock Germplasm. Saba pigs grow slow, but this breed is characteristic of a high propensity for meat quality, ability to adapt to the environment,
and disease resistance (Jeong et al. 2014; Diao et al. 2019). In contrast, the Landrace breed was commercially selected over generations for rapid growth and enhanced carcass yield (Briggs 1983).

Before birth the intestine of newborns is believed to be free of microbes (Turnbaugh and Turnbaugh 2008). Due to contact with sows and exposure to the surrounding environment, a complex microbial community rapidly colonizes the newborn mammal (Frese et al. 2015). The balanced microbiological system (diverse intestinal microbes) is a significant hallmark of piglet health (Patil et al. 2019). Suckling piglets are an essential stage in the life of pigs, and thus more attention should be paid to the intestinal microbiota of piglets. The 35-day-old piglets easy to cause any diseases or dramatic internal environmental changes, they are about to wean; therefore, we selected 35-day-old Saba and Landrace suckling piglets as the subjects of this study. A comparison of their jejunal microbiota diversity will help comprehend the composition and functionality of gut microbiota in Chinese indigenous pigs.

**Experimental**

**Materials and Methods**

**Animals and samples collection.** All Saba and Landrace pigs were raised on a commercial farm in Chuxiong of Yunnan Province, China. Three Saba and three Landrace sows of third parity were selected for this study. They lived in six enclosures in an environmentally controlled room and were fed with the National Research Council (NRC) diet without antibiotics. After parturition, all piglets from every sow were placed in a single enclosure and fed by sow’s milk until day 35 (35 d). From each sow, three piglets were randomly selected, and their birth weight and 35 d body weight were recorded, and the average daily gain of both groups was calculated. Piglets were sacrificed, and the content from the middle of the jejunum was collected for 16S rRNA sequencing analysis.

**DNA extraction and PCR amplification.** Based on the manufacturer's instructions, the QIAamp® Fast DNA Stool Mini Kit (Qiagen, Cat No.: 19593) was used to extracted Genomic DNA from 18 samples. The V3-V4 region of the bacterial 16s ribosomal RNA genes was amplified following the method of Fadrosh and coworkers (Fadrosh et al. 2014).

**Illumina MiSeq PE250 sequencing.** The Qubit® 2.0 (Invitrogen, USA) was used to quantify DNA in the samples for library preparation. During the amplification, the barcodes were introduced by the ligated primers, which included sequencing adaptor, barcode, and sequence binding to V3-V4 region. The libraries were sequenced on the MiSeq platform (Illumina, Inc., CA, USA). All jejunal content samples from 18 piglets were subjected to 16S rRNA sequencing; however, one sample from Saba piglets and two samples from Landrace piglets failed to build a database.

**Processing of sequencing data.** The sequencing data analysis referred to the method of Li and coworkers (Li et al. 2019). Trimming of barcodes and primers was performed using Pandaseq (https://github.com/neufeld/pandaseq/releases/tag/v2.8.1), followed by the quality control (e.g., the lengths of reads and an average base quality) using Fastqc (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). 16S rDNA tags between 220bp and 500 bp, with no more than three ambiguous N, were kept, and the average Phred score of bases was no worse than 20 (Q20). The copy number of tags was enumerated, and the redundant tags were removed. Only the tags with a frequency higher than 1, which are more reliable in general, were clustered into Operational Taxonomic Units (OTUs). Each OTU had a representative tag. OTUs were clustered with a criterion of 97% similarity using the Uparse (http://drive5.com/uparse/), with chimeric sequences identified and removed using the Userach (version 7.0). Each representative tag was assigned to taxa by RDP Classifier (http://rdp.cme.msu.edu/) against the RDP database (http://rdp.cme.msu.edu/) using a confidence threshold of 0.8.

The OTU profiling table and alpha/beta diversity were also achieved by Python scripts of QIIME. Alpha diversity was the species diversity in each sample, including community abundance (Chao1 index), the diversity (Shannon and Simpson index), the phylogenetic diversity index (PD whole tree), and coverage (Good's coverage values). QIIME software was used to calculate the samples’ alpha diversity index based on the OTU results and to generate the corresponding dilution curve. The Bray-Curtis distance was calculated to estimate the dissimilarity in the community structure, which was visualized using principal coordinates analysis (PCoA). Analysis of similarities (ANOSIM) was performed in the Mothur v1.38.0. We determined the strength of these groups using multiresponse permutation procedures (MRPP). Both analyses were performed in the PC-ORD. In addition to p-values, PC-ORD generated T and A values for all comparisons in the MRPP. T was a measure of separation between groups, with more negative values indicating a stronger separation. Group homogeneity was described by A and was scaled between 0 and 1.

The linear discriminant analysis (LDA) effect size (LEfSe) method (p < 0.05, LDA > 2) was used to identify the most differentially abundant OTUs between groups, with the LDA obtained by a pair-wise computation. The Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUST) based on a closed-reference operational taxonomic unit
Jejunal microbiota of Saba and Landrace piglets

(OTU) was used to predict the abundances of functional categories in the Kyoto Encyclopedia of Genes and Genomes (KEGG) ortholog (KO). The correlation coefficients between KEGG pathways and bacterial compositions were calculated using Pearson’s correlation test in GraphPad Prism 7.

Statistical analysis. The experimental data, including growth performance and microbiota abundances, were analyzed with the SPSS 22.0 software. Grade and quantitative data were compared with the t-test between the two groups.

We used Spearman’s test to estimate the correlation between KEGG pathway and jejunal microbial composition and host growth performance. \( p < 0.05 \) was deemed to statistical significance.

Table I  
Growth performance of Saba and Landrace piglets.

| Sample name | Clean Reads | Bases (bp) | Q20 (%) | Q30 (%) | GC (%) | Average length (bp) |
|-------------|-------------|------------|---------|---------|--------|---------------------|
| LA-1        | 58278       | 24288798   | 0.9602  | 0.8873  | 0.5229 | 416                 |
| LA-2        | 55975       | 23383577   | 0.9591  | 0.8855  | 0.5188 | 417                 |
| LA-3        | 62396       | 25557801   | 0.9696  | 0.9081  | 0.527  | 409                 |
| LA-4        | 56565       | 23778906   | 0.9614  | 0.8922  | 0.5365 | 420                 |
| LA-5        | 55547       | 22929087   | 0.9671  | 0.9054  | 0.5221 | 412                 |
| LA-6        | 56336       | 23469885   | 0.9612  | 0.8928  | 0.5234 | 416                 |
| LA-7        | 57139       | 23771848   | 0.96   | 0.8902  | 0.5332 | 416                 |
| SB-1        | 63393       | 26990140   | 0.9609  | 0.8917  | 0.5154 | 425                 |
| SB-2        | 55959       | 22667528   | 0.9697  | 0.9119  | 0.5271 | 405                 |
| SB-3        | 62484       | 25749078   | 0.9661  | 0.9035  | 0.5185 | 412                 |
| SB-4        | 61527       | 26100871   | 0.9594  | 0.8867  | 0.5117 | 424                 |
| SB-5        | 56907       | 24136231   | 0.9602  | 0.8897  | 0.5123 | 424                 |
| SB-6        | 63409       | 26890638   | 0.958   | 0.8488  | 0.5496 | 424                 |
| SB-7        | 58920       | 23815087   | 0.9656  | 0.9029  | 0.5306 | 404                 |
| SB-8        | 60147       | 24637940   | 0.9626  | 0.8966  | 0.5253 | 409                 |

Results

Growth performance of Saba and Landrace piglets. The growth performance of Saba and Landrace piglets is shown in Table I. The birth weight of Landrace piglets was significantly higher than Saba piglets \( (p < 0.001) \). On day 35, the body weight and average daily gain \( (p < 0.001) \) of Landrace piglets were higher than Saba piglets \( (p < 0.001) \).

Gut microbiota DNA sequence data and quality control. Sequencing of the amplicons of the 16S rRNA gene at MiSeq generated 884,982 clean reads (mean length of 415 bp) with 368,167,415 base pairs in total, yielding an average of 58,999 clean reads (55,547–63,409), and 24,544,494 base pairs (22,667,528 bp – 26,990,140 bp) per sample (Table II). Out of the high-quality sequences, about 99.53% were between 420 and 460 bp for these two breeds.

Diversity in jejunal microbiota of Saba and Landrace piglets. We revealed that the jejunal microbiome was different in Saba and Landrace piglets. The USEARCH algorithm was used to cluster at a 0.97 similarity level, and the clustered sequences were filtered by a chimera. We obtained 489 and 365 OTUs (Fig. 1) from Saba and Landrace piglets, respectively. In total, 254 OTUs were shared by Saba and Landrace piglets. The alpha diversity index of the samples is shown in Table III. The PD whole tree in Saba piglets (17.76) was significantly higher \( (p < 0.05) \) than in Landrace piglets (13.31). The Chaol index (242.85 vs. 229.04), the observed species index (139.75 vs. 124.75), and Shannon (3.06 vs. 2.84) and Simpson (0.79 vs. 0.76) indexes
for microbiota from Saba piglets are higher than those from Landrace piglets, but the statistical significance ($p > 0.05$) was not noticed. Besides, we compared the beta diversities between all the samples (Table IV). The test statistic ($R$) of Multi Response Permutation Procedure (MRPP) was $0.031 (p = 0.102)$ on the weighted UniFrac, and $0.034 (p = 0.005)$ on the unweighted UniFrac. Also, the test statistic ($R$) of ANOSIM was $0.131 (p = 0.01; \text{Fig. 2})$ on the unweighted UniFrac. Using the unweighted UniFrac metric, the Principal Coordinates Analysis (PCoA) showed a clear separation between Saba and Landrace piglet samples (Fig. 2).

Comparison of jejunal microbiota of Saba and Landrace piglets. The jejunal bacterial taxa were diversified between Saba and Landrace piglets at the phylum level (Fig. 3A). Among these taxa, Firmicutes occurred with the highest abundance within the jejunal microbiota of Landrace piglets (95.82%), followed by Proteobacteria (1.67%), and Bacteroidetes (0.052%). Similarly, the higher relative abundance of Firmicutes (97.6%, $p > 0.05$) than Bacteroidetes (1.14%, $p < 0.05$), and a lower proportion of Proteobacteria (0.71%, $p > 0.05$) were observed in microbiota of Saba piglets.

The abundance of bacterial species within jejunal taxa is shown in Fig. 3B. The two most abundant genera

---

**Fig. 1.** Venn diagram of OTUs clustered at 97% sequence identity of microbiotas from Saba and Landrace piglets. The number of overlapping parts is the total number of OTUs between the groups, while the numbers in non-overlapping parts indicate the number of unique OTUs for each group. SB - Saba piglets, LA - Landrace piglets.

**Fig. 2.** Principal coordinate analysis (PCoA) illustrated bacterial community structures based on Bray-Curtis distances. On the PCoA plot, each color represents one group. Unweighted and weighted PCoA of $\beta$-diversity measures of all samples. PCOA1 (19.67%) and PCOA2 (13.63%).

**Unweighted Unifrac (Adonis test: $p = 0.01$ $R^2 = 0.131$)**
were *Lactobacilli* and *Clostridium XI*, accounting for 28.0% and 42.24% of the jejunal species in Landrace piglets, respectively. Compared with the microbiota of Landrace piglets, the jejunal microbial of Saba piglets had a higher abundance of *Lactobacilli* (36.81%) and a lower abundance of *Clostridium XI* (40.02%), but the
difference was not statistically significant ($p > 0.05$). Moreover, *Veillonella* (0.58% vs. 2.34%), *Streptococcus* (0.23% vs. 1.32%), and *Saccharibacteria genera incertae sedis* (0.19% vs. 1.04%) of Saba piglets were remarkably lower than in microbiota of Landrace piglets ($p < 0.05$).

**Differences of bacterial taxa between Saba and Landrace piglets.** The different number of OTUs was observed between the jejunal microbiota of Saba and Landrace piglets (Fig. 4). There was one main phylum (Firmicutes) and two genera (*Coproccocus* and *Parabacteroides*) significantly enriched in jejunal microbiota of Saba piglets. Also, multiple biomarkers were significantly enriched in jejunal microbiota of Landrace piglets, including two phyla (Candidatus Saccharibacteria and Proteobacteria), two classes (Epsilonproteobacteria and Gammaproteobacteria), two orders (Campylobacterales and Pasteurellales), eight families (Fusobacteriaceae, Leuconostocaceae, Actinomycetaceae, Enterococccaeae, Campylobacteraceae, Dermatophilaceae, Streptococcaceae, and Pasteurellaceae), thirteen genera (*Enterococcus*, *Actinomyces*, *Fusobacterium*, *Weissella*, *Pediococcus*, *Campylobacter*, *Oribacterium*, *Sharpea*, *Tonsilliphilus*, *Pasteurella*, *Saccharibacteria genera incertae sedis*, *Streptococcus*, and *Actinobacillus*). Furthermore, the increase in the abundance of the phylum Candidatus Saccharibacteria was represented by an increased abundance of the genus *Saccharibacteria genera incertae sedis* (Fig. 5).

**Correlation between microbiota and growth performance.** The correlation between jejunal microbiota and host growth performance was shown in Fig. 6. The *Coproccocus* was negatively correlated with the body weight ($p = 0.046$, $R = −0.53$), average daily gain ($p = 0.046$, $R = −0.53$), stem length ($p = 0.018$, $R = −0.61$),

| SB – Saba piglets, LA – Landrace piglets | A | Observe Delta | Expect Delta | Significance |
|-----------------------------------------|---|--------------|--------------|--------------|
| The weighted_unifrac                    | 0.0312699850241734 | 0.276682987004487 | 0.285614136784429 | 0.102 |
| The unweighted_unifrac                  | 0.0338353054352021 | 0.551581668259676 | 0.570898182641762 | 0.00  |

### Table IV

MRPP of the 16S rRNA gene between Saba and Landrace piglets.

**Fig. 4.** Alteration of the relative abundance of bacteria in the Saba and Landrace piglets using linear discriminant analysis effect size (LEfSe). Each bar represents the log 10 effect size (LDA score) for a specific taxon. A longer bar represents a higher LDA score. Only taxa meeting an LDA significant threshold of 2 are shown. These taxa showed a statistically significant difference between the Saba and Landrace piglets ($p < 0.05$ by the Wilcoxon test); each color represents one group.

$p$ – phylum, $c$ – class, $o$ – order, $f$ – family, and $g$ – genus.
height at withers \( (p=0.0063, R=-0.68) \), chest measurement \( (p=0.046, R=-0.53) \), chest depth \( (p=0.012, R=-0.64) \), abdominal girth \( (p=0.045, R=-0.53) \), and cannon circumference \( (p=0.011, R=-0.65) \). The \textit{Parabacteroides} was negatively correlated with the cannon circumference \( (p=0.048, R=-0.52) \). The \textit{Tonsilliphilus} was positively correlated with the body weight \( (p=0.0053, R=0.69) \), and cannon circumference \( (p=0.022, R=0.72) \). The \textit{Saccharibacteria genera incertae sedis} was positively correlated with the stem length \( (p=0.036, R=0.55) \), chest measurement \( (p=0.038, R=0.54) \), and abdominal girth \( (p=0.031, R=0.56) \). The \textit{Enterococcus} was positively correlated with the body weight \( (p=0.0097, R=0.65) \), average daily gain \( (p=0.015, R=0.62) \), stem length \( (p=0.049, R=0.52) \), height at withers \( (p=0.0048, R=0.70) \),

**Fig. 5.** A cladogram showed a comparison of the bacterial microbial profiles from Saba and Landrace piglets. p – phylum, c – class, o – order, f – family, and g – genus.
chest measurement \((p = 0.0066, R = 0.68)\), chest depth \((p = 0.0069, R = 0.68)\), abdominal girth \((p = 0.0070, R = 0.67)\), and cannon circumference \((p = 0.0052, R = 0.71)\). The *Pediococcus* was positively correlated with the body weight \((p = 0.023, R = 0.59)\), average daily gain \((p = 0.0060, R = 0.69)\), height at withers \((p = 0.013, R = 0.64)\), height at withers \((p = 0.0011, R = 0.66)\), chest measurement \((p = 0.0039, R = 0.70)\), chest length \((p = 0.0029, R = 0.73)\), abdominal girth \((p = 0.0078, R = 0.66)\), and cannon circumference \((p = 0.022, R = 0.62)\). The *Weissella* was positively correlated with the body weight \((p = 0.033, R = 0.56)\), average daily gain \((p = 0.033, R = 0.56)\), stem length \((p = 0.038, R = 0.54)\), height at withers \((p = 0.011, R = 0.66)\), chest measurement \((p = 0.0039, R = 0.70)\), chest depth \((p = 0.0029, R = 0.73)\), abdominal girth \((p = 0.0078, R = 0.66)\), and cannon circumference \((p = 0.022, R = 0.62)\). The *Streptococcus* was positively correlated with the body weight \((p = 0.0024, R = 0.74)\), average daily gain \((p = 0.0020, R = 0.75)\), stem length \((p = 0.0037, R = 0.71)\), height at withers \((p = 0.0073, R = 0.67)\), chest measurement \((p = 0.0011, R = 0.77)\), chest depth \((p = 0.0099, R = 0.65)\), abdominal girth \((p = 0.0097, R = 0.78)\), and cannon circumference \((p = 0.0038, R = 0.72)\). The *Sharpea* was positively correlated with the body weight \((p = 0.023, R = 0.59)\), average daily gain \((p = 0.023, R = 0.59)\), height at withers \((p = 0.011, R = 0.65)\), chest measurement \((p = 0.0011, R = 0.77)\), chest depth \((p = 0.0099, R = 0.65)\), abdominal girth \((p = 0.0013, R = 0.77)\), and cannon circumference \((p = 0.0066, R = 0.69)\).

**KEGG pathway and their correlations with microbiota.** To assess the jejunal microbiota’s metabolic potential, we performed Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) (Langille et al. 2013; Javurek et al. 2016). KEGG pathway (L3 hierarchy) analysis is shown in Fig. 7. The "\(d\)-glutamine and \(d\)-glutamate metabolism", and "one carbon pool by folate" pathway were enriched in Saba piglets. The "limonene and pinene degradation", "tryptophan metabolism", and "sulfur relay system" were enriched in Landrace piglets.
We used Spearman's correlation heatmap (Fig. 8) to study the correlation between the jejunal microbiota and the KEGG pathway. The “D-glutamine and D-glutamate metabolism” pathway was positively correlated with the presence of Firmicutes (family, $p = 0.0080$, $R = 0.67$), while negatively correlated with Fusobacteriaceae (family, $p = 0.020$, $R = -0.60$), and Fusobacterium (genus, $p = 0.017$, $R = -0.62$). The pathway of “one carbon pool by folate” was negatively correlated with Fusobacteriaceae (family, $p = 0.049$, $R = -0.52$) and Fusobacterium (genus, $p = 0.048$, $R = -0.52$). The pathway “tryptophan metabolism” was negatively correlated with Coprococcus (genus, $p = 0.023$, $R = -0.59$), but positively correlated with Gammaproteobacteria

![Fig. 8. Pearson's correlation analysis of microorganisms and signal pathways in Saba and Landrace piglets. Heatmap analysis of the correlation between microorganisms and signal pathways. Correlations with $p < 0.05$ are shown. Blue represents a significant negative correlation ($p < 0.05$), red represents a significant positive correlation ($p < 0.05$), and white represents no significant correlation ($p > 0.05$). The number represents the value of R ($p < 0.05$).]
Lactobacillus frumenti, Enterococccaeae (family, \(p = 0.024\), \(R = 0.59\)), Proteobacteria (family, \(p = 0.0089\), \(R = 0.66\)), and Enterococcus (genus, \(p = 0.031\), \(R = 0.56\)).

**Discussion**

The Landrace pig from Denmark is a typical commercial pig breed of fast growth and high carcass yield (Briggs 1983). The previous research reported that the body weight of Landrace piglets was 1.68 kg and 6.52 kg on day 1 and day 27, respectively (Li et al. 2013). In contrast, the Saba pig is an indigenous breed from China, with a relatively slow growth rate. In our study, the birth weight, body weight (day 35), and average daily gain of Landrace piglets (1.99 kg, 10.22 kg, and 0.24 kg/d, respectively) were higher than those of Saba piglets (0.76 kg, 4.69 kg, and 0.11 kg/d, respectively). The data indicated that the growth performance and intestinal microbes were different in Jinhua pigs and Landrace piglets of the same age (Xiao et al. 2018). In addition, our results show that the Coprococcus and Parabacteroides were negatively correlated with the growth performance, while the Tonsilliphilus, Enterococcus, Pedicoccus, Weissella, Streptococcus, Campylobacter, Saccharibacteria genera incertae sedis, and Sharpea were positively associated with the growth performance. The above results suggested that the composition of intestinal microbiota was significantly and closely connected with the pig breed.

It is generally believed that intestinal microorganisms have abundant metabolic profiles to maintain their basic life and have a considerable impact on host growth and health (Turnbaugh et al. 2006). The accumulating evidence suggested that diet (Pluske 2013), environment (Thompson et al. 2008), and host’s genetics (Büsing and Zeyner 2015; Hancox et al. 2015) can affect the composition of intestinal microbiota. The previous research (Yang et al. 2014) revealed that the percentages of Firmicutes and Bacteroidetes in the Chinese indigenous pig breeds (Xiaomeishan, Meishan, and Bama sows) were higher than those of exotic breeds (Landrace, Yorkshire, and Duroc sows). It is consistent with our finding that the Firmicutes, Proteobacteria, and Bacteroidetes dominated in the jejunum of both pig breeds. Furthermore, the percentage of Bacteroidetes in Saba piglets was significantly higher than in Landrace piglets. Saba piglets are obese, and Landrace piglets are lean. A previous study has shown that fat deposition is positively correlated with the presence of Bacteroidetes and Firmicutes within the intestinal microbiota (Turnbaugh et al. 2006). Nevertheless, the mechanisms between intestinal microbiota and fat deposition are still unclear, and further study is needed.

At the genus level, the two most numerous genera in the pig’s small intestine were Lactobacillus and Clostridium (Crespo-Piazuelo et al. 2018). Our study demonstrated that Clostridium XI is the most numerous bacteria in jejun of both Saba and Landrace piglets. We speculate that this was principally due to the adaptation of the microbial system for milk nutrition, as sucking lambs have a large proportion of Clostridium XI in the intestinal microbial community (Bi et al. 2019). The Lactobacilli were usually considered beneficial bacteria responsible for more effective anti-inflammation and out-competing microbiota competences (Eitold et al. 2014). Therefore, some Lactobacillus species have been used as substitutes for antibiotics for growth promotion. The average daily gain (ADG) of weaned piglets fed with Lactobacillus plantarum PFM 105 was significantly improved after three weeks (Wang et al. 2019). Again, two Lactobacillus strains (Lactobacillus frumenti and Lactobacillus gasseri LA39), when taken orally, could significantly prevent stress-induced diarrhea caused by early weaning of piglets (Hu et al. 2018). In line with that, in this study, the abundance of Lactobacillus in Saba piglets was higher than in Landrace piglets.

Furthermore, some taxa are recognized as negatively correlated with host health. Veillonella is present in piglets infected with porcine epidemic diarrhea virus (PEDV), caused by the disorder of intestinal amino acid metabolism and energy metabolism (Huang et al. 2018). Also, Veillonella and Streptococcus can increase the secretion of inflammatory cytokine and the production of anti-microbial peptides (AMP), resulting in improved mucosal thickness and epithelial barrier function in 3D-reconstructed human gingiva (Shang et al. 2018). In the present study, a higher proportion of Lactobacilli and a lower proportion of Streptococcus and Veillonella in the microbiome of Saba piglets, suggested that Saba piglets might have stronger disease resistance. Overall, our data indicated that the pig breed could influence the microbiome composition.

The gut microbiome is beneficial for pigs, contributing to improved vitamin K production, cellulose fermentation, and increased resistance to pathogens (Kim and Isaacson 2015; Stokes 2017; Yang et al. 2017). PICRUSt analysis of jejunal microbiota’s metabolic potential showed that metabolic pathways were significantly different in Saba and Landrace piglets. It is noteworthy that the different amino acid metabolism pathways were enriched in Saba and Landrace piglets. The “d-glutamine and d-glutamate metabolism” pathway was enriched in Saba piglets, while “tryptophan metabolism” pathway was enriched in Landrace piglets. The “d-glutamine and d-glutamate metabolism” pathway participates in the inhibition of lipid peroxidation and quenches free radicals during oxidative stress (Qu et al. 2020). It has been reported that tryptophan...
is related to the immune response regulation, inflammation, and oxidative stress (Anesi et al. 2019; Liu et al. 2019). Therefore, different pig breeds might have regulated their health through different metabolic pathways.

The central part of amino acids absorption is the small intestine (Wu 1998). The catabolism of arginine and lysine in the jejunum could exceed their transport into intestinal cells (Dai et al. 2010). This phenomenon may be due to the role of intestinal microorganisms. Besides, owing to the deficiency of several key enzymes, the threonine, tryptophan, histidine, lysine, and methionine cannot be metabolized by porcine intestinal cells in the presence of amino acids at physiological concentrations (Chen et al. 2007). However, the histidine, glutamate, threonine, and lysine were utilized by microbiota of the porcine small intestine (Dai et al. 2010). The above results indicated that jejunal microorganisms could participate in amino acid metabolism.

In our study, the Firmicutes enriched in the jejunal microbiome of Saba piglets were positively associated with the “D-glutamine and D-glutamate metabolism” pathway. In contrast Fusobacteriaceae and Fusobacterium that enriched the jejunal microbiome of Landrace pigs were negatively correlated with the above pathway. Finally, Coprococcus from the jejunal microbiome of Saba piglets was negatively correlated with the “tryptophan metabolism” pathway, and Enterococcus, Enterococcus, Proteobacteria, and Gammaproteobacteria from Landrace piglets were positively correlated with tryptophan metabolism. Therefore, these taxa may be related to the metabolism of amino acids in jejunum.

Conclusions

In summary, the growth performance was higher for Landrace piglets compared to Saba piglets due to their different genetic characteristics. The rich diversity and fewer infection-associated taxa were observed in Saba piglets, partially accounting for their high adaptability to environmental perturbations compared to Landrace piglets. Several taxa in the jejunum of Saba and Landrace piglets were associated with “D-glutamine and D-glutamate metabolism” and “tryptophan metabolism” respectively, suggesting that pig breeds may regulate their health through different metabolic pathways. Although the interaction between pig and microbiota needs further extensive investigations, our study would shed more light on the functional exploration and resource development of local pig intestinal microbiota in China.

Acknowledgments

This research was funded by the National Key Research and Development Program of China (2018YFD0500401), Key Program of Yunnan province Natural Science Foundation of China (2018FA021), Nature Science Foundation of China (U1802234, 31760645).

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Anesi A, Rubert J, Oluwagbemigun K, Orozco-Ruiz X, Nöthlings U, Breteler MMB, Mattivi F. Metabolic profiling of human plasma and urine, targeting tryptophan, tyrosine and branched chain amino acid pathways. Metabolites. 2019 Nov 01;9(11):261. https://doi.org/10.3390/metabo9110261
Bi Y, Cox MS, Zhang F, Suen G, Zhang N, Tu Y, Diao Q. Feeding modes shape the acquisition and structure of the initial gut microbiota in newborn lambs. Environ Microbiol. 2019 Jul;21(7):2333–2346. https://doi.org/10.1111/1462-2920.14614
Briggs HM. International pig breed encyclopedia. Indianapolis (USA): Elanco Products Company; 1983.
Büssing K, Zeyner A. Effects of oral Enterococcus faecium strain DSM 10663 NCIMB 10415 on diarrhoea patterns and performance of sucking piglets. Benef Microbes. 2015 Jan 01;6(1):41–44. https://doi.org/10.3920/BM2014.0008
Chen L, Yin YL, Jobgen WS, Jobgen SC, Knabe DA, Hu WX, Wu G. In vitro oxidation of essential amino acids by jejunal mucosal cells of growing pigs. Livest Sci. 2007 May;109(1–3):19–23. https://doi.org/10.1016/j.livsci.2007.01.027
Crespo-Piazzuelo D, Estelle J, Sevilla M, Criado-Mesas I, Ramayocaldas Y, Óvilo C, Fernández AI, Ballester M, Folch JM. Characterization of bacterial microbiota compositions along the intestinal tract in pigs and their interactions and functions. Sci Rep. 2018 Dec;8(1):12727. https://doi.org/10.1038/s41598-018-30932-6
Dai ZL, Zhang J, Wu G, Zhu WY. Utilization of amino acids by bacteria from the pig small intestine. Amino Acids. 2010 Nov; 39(5):1201–1215. https://doi.org/10.1007/s00726-010-0556-9
Diao S, Huang S, Chen Z, Teng J, Ma Y, Yuan X, Chen Z, Zhang H, Li J, Zhang Z. Genome-wide signatures of selection detection in three south China indigenous pigs. Genes (Basel). 2019 May 07; 10(5):346. https://doi.org/10.3390/genes10050346
Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA. Diversity of the human intestinal microbial flora. Science. 2005 Jun 10;308(5728):1635–1638. https://doi.org/10.1126/science.1110591
Etzold S, Kober OJ, MacKenzie DA, Tailford LE, Gunning AP, Walshaw J, Hemmings AM, Juge N. Structural basis for adaptation of lactobacilli to gastrointestinal mucus. Environ Microbiol. 2014 Mar;16(3):888–903. https://doi.org/10.1111/1462-2920.12377
Fadros W, Ma B, Gajer P, Sengamalay N, Ott S, Brodtman RM, Ravel J. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. Microbiome. 2014;2(1):6. https://doi.org/10.1186/2049-2618-2-6
Frese SA, Parker K, Calvert CC, Mills DA. Diet shapes the gut microbiome of pigs during nursing and weaning. Microbiome. 2015 Dec;3(1):28. https://doi.org/10.1186/s40168-015-0091-8
Goodrich JK, Davenport ER, Beaumont M, Jackson MA, Knight R, Ober C, Spector TD, Bell JT, Clark AG, Ley RE. Genetic determinants of the gut microbiome in UK twins. Cell Host Microbe. 2016 May;19(5):731–743. https://doi.org/10.1016/j.chom.2016.04.017

Goodrich JK, Waters JL, Poole AC, Sutter LK, Koren O, Blekhman R, Beaumont M, Van Treuren W, Knight R, Bell JT, et al. Human genetics shape the gut microbiome. Cell. 2014 Nov 15;159(4):789–799. https://doi.org/10.1016/j.cell.2014.09.053

Hancox LR, Le BM, Richards PJ, Guillou CE, Melville ME, Kefford RF. Effect of a single dose of Saccharomyces cerevisiae var. boulardii on the occurrence of porcine neonatal diarrhoea. Animal. 2015;9(11):1756–1759. https://doi.org/10.1071/AN1503287

Hu J, Ma L, Nie Y, Chen J, Zheng W, Wang X, Xie C, Zheng Z, Wang Z, Yang T, et al. A microbiota-derived bacteriocin targets the host to confer diarrhea resistance in early-weaned piglets. Cell Host Microbe. 2018 Dec;24(6):817–832.e8. https://doi.org/10.1016/j.chom.2018.11.006

Huang MZ, Wang SY, Wang H, Cui DA, Yang YJ, Liu XW, Kong XI, Li JY. Differences in the intestinal microbiota between uninfected piglets and piglets infected with porcine epidemic diarrhea virus. PLOS One. 2018 Feb 15;13(2):e0192992. https://doi.org/10.1371/journal.pone.0192992

Javurek AB, Spollen WG, Ali AMM, Johnson SA, Lubahn DB, Bivens NJ, Bromert KH, Ellersieck MR, Givan SA, Rosenfeld CS. Characterization of a novel small fimbrial microfluid and microbiome from discovery of estrogen receptor alpha genetic status. Sci Rep. 2016 Mar;6(1):23027. https://doi.org/10.1038/srep23027

Jeong HS, Kim DW, Chun SY, Sung S, Kim HJ, Cho S, Kim H, Oh SJ. Native Pig and Chicken Breeding Database: NPCDB. Asian-Australas J Anim Sci. 2014 Oct;27(10):1394–1398. https://doi.org/10.5712/ajas.2014.4.1059

Kim HB, Isaacson RE. A novel seminal fluid microbiome and influence of the immediate environment on the occurrence of porcine neonatal diarrhoea. Animal. 2015;9(11):1756–1759. https://doi.org/10.1071/AN1503287

Kong XJ, Li JY. Discovery of key metabolites during cisplatin-induced acute kidney injury using an HPLC-TOF/MS-based non-targeted urine and kidney metabolomics approach in rats. Toxicology. 2020 Feb;431:152366. https://doi.org/10.1016/j.tox.2020.152366

Ley RE, Peterson DA, Gordon JI. Human genetics shape the gut microbiome. Cell. 2014 Nov 15;159(4):789–799. https://doi.org/10.1016/j.cell.2014.09.053

Martinez-Guryn K, Leone V, Chang EB. Comparative biogeography of the gut microbiome between Jinhua and Landrace pigs. Sci Rep. 2018 Dec;8(1):16061. https://doi.org/10.1038/s41598-018-34390-y

Stokes CR. The development and role of microbial-host interactions in gut mucosal immune development. J Anim Sci Biotechnol. 2017 Dec;8(1):12. https://doi.org/10.1186/s41040-016-0138-0

Thompson CL, Wang B, Holmes AJ. The immediate environment during postnatal development has long-term impact on gut community structure in pigs. ISME J. 2008 Jul;2(7):739–748. https://doi.org/10.1038/ismej.2008.29

Turnbaugh PJ, Gordon JI. An invitation to the marriage of metagenomics and metabolomics. Cell. 2008 Sep;134(5):708–713. https://doi.org/10.1016/j.cell.2008.08.025

Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006 Dec;444(7122):1027–1031. https://doi.org/10.1038/nature05414

Wang T, Peng Y, Liu Y, Shi W, Zhang J, Dong E, Tao Y, Zhao J, Zhong J. Lactobacillus plantarum PFM 105 promotes intestinal development through modulation of gut microbiota in weaning piglets. Front Microbiol. 2019 Feb;10:5109. https://doi.org/10.3389/fmicb.2019.00090

Wu G. Intestinal mucosal amino acid catabolism. J Nutr. 1998 Aug 01;128(8):1249–1252. https://doi.org/10.1093/jn/128.8.1249

Xiao Y, Kong F, Xiang Y, Zhou W, Wang J, Yang H, Zhang G, Zhao J. Comparative biogeography of the gut microbiome between Jinhua and Landrace pigs. Sci Rep. 2018 Dec;8(1):5985. https://doi.org/10.1038/s41598-018-34390-y

Yuan H, Yang H, Fang S, Han M, Guo Y, Liu H, Yu D, Yang L, Bian G, Su Y, Zhu J, Wu Q. Examination of faecal microbial community of lantang, bama, erhuilan, meishan, xinaoishan, duroc, landrace, and yorkshire sows. Asian-Australas J Anim Sci. 2014 Jun 1;27(6):898–906. https://doi.org/10.5713/ajas.2013.13621