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Comparison of early-life jejunal microbiota diversity in two pig breeds

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Abstract: The pig intestinal microbiota perform multiple physiological functions during the lifespan of the host. Host genetics is considered a major factor shaping the intestinal microbiota. This study explored the jejunal microbial diversity and potential functional differences between Dahe (DH) and Diannan small-ear (DS) pigs, two important native Chinese breeds with different growth characteristics. Nine piglets of each breed (DH and DS) were fed sow milk until the age of 35 days. Jejunal chyme samples were collected for 16S rRNA sequencing. The birth weight, final body weight at day 35, and average daily gain tended to increase more in DH piglets than in DS piglets. The jejunal microbial Shannon index was also higher in DH piglets than in DS piglets ($P < 0.05$). At the genus level, the relative abundances of *Clostridium XI*, *Clostridium sensu stricto*, *Turicibacter*, *Megasphaera*, *Veillonella*, *Mitsuokella*, and *Selenomonas* in DH pigs were higher than those in DS individuals, whereas *Streptophyta*, *Enterococcus*, *Lactococcus*, and *Weissella* were lower in DH pigs than in DS pigs ($P < 0.05$). Furthermore, linear discriminant effect size analyses revealed 25 differential bacterial taxa between DH and DS piglets. Spearman's analysis found that *Enterococcus* was negatively correlated with final body weight ($P = 0.025$, $r = -0.56$) and average daily gain ($P = 0.034$, $r = -0.53$), while average daily gain was positively correlated with *Clostridium XI* ($P = 0.01$, $r = 0.63$) and *Mitsuokella* ($P = 0.007$, $r = 0.64$). Kyoto Encyclopedia of Genes and Genomes annotation identified eight functional categories related to amino acids and energy metabolism in DH piglets, while three categories were related to lipid metabolism in DS piglets. Our findings suggest that DH pigs have higher microbial diversity, while DS pigs may have higher fat deposition ability.

Keywords: 16S rRNA; Dahe pig; Diannan small-ear pig; jejunum; microbial composition

The mammalian gut is colonized by complex and abundant microorganisms (Kamada et al. 2013), the composition and colonization of the gut microbiota in newborn animals are shaped by complicated factors, such as dietary change, administration of probiotics and prebiotics, and supplementation of in-feed antibiotics (Bian et al. 2016). More importantly, host genetics has been considered an indispensable factor in shaping the intestinal microbiota of mammals (Lynch and Hsiao 2019).

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The gut microbiota provide animals with many essential functions, including maintenance of normal functions of the intestinal villi, regulation of immune responses, enhanced resistance to pathogens, production of volatile fatty acids and vitamins, and increased energy harvesting capacity (Buffie and Pamer 2013). The small intestine is the first place of contact between the intestine and the diet, providing carbon sources and other nutrients to the microbiota before passing into the large intestine (Piccolo et al. 2017). Although the small intestine harbours microbes with lower diversity and abundance ($\approx 10^3$–10^5 microbial cells/g) than the colon ($\approx 10^{10}$–10^{12} cells/g) (Macpherson and Harris 2004), the microbes in the small intestine are critical transducers of dietary signals that allow the host to adapt to changes in lipid digestion and absorption (Martinez-Guryn et al. 2018). Furthermore, the jejunum has a unique immediate role in responding to the luminal microbe-diet interplay, and the microbiota in the jejunum strongly influence glucose and energy metabolism (El Aidy et al. 2013). Therefore, the jejunal microbiota exert a profound impact on the host.

The breed can influence early-life microbiota colonization in pigs (Mu et al. 2019). Indigenous Chinese pigs exhibit high adaptability to environmental changes and resistance to diseases, along with expressing ultra-fine muscle fibres, strong meat cutability, high intramuscular fat, and flavourful meat (Hao et al. 2011; Zhang et al. 2018). The Dahe (DH) and Diannan small-ear (DS) pigs are indigenous breeds found in Dahe-Yingshang town, Qujing city, and Xishuangbanna city in the Yunnan province of China. Both breeds have been labelled as national protected breeds of livestock and poultry genetic resources by the National Agriculture Ministry of China (Dong et al. 2014). Little is known about the gut microbiota of indigenous Chinese piglets in terms of colonization and function; therefore, DH and DS sucking piglets at 35 days of age were selected to explore jejunal microbiota diversity and the inferred functions of the jejunal microbiota.

MATERIAL AND METHODS

Experimental animal management and sampling

Three DH sows (Dahe Breeding Pig Farm, Yunnan Province, China) and three DS sows (Bangge Animal Husbandry Technology Co., Ltd., Yunnan Province, China) at the third parity born on the same day were chosen for insemination by the same boar, in order to confer the piglets from each breed with the same genetic background. The six sows were reared in separate pens in a house on an experimental farm at Yunnan Agricultural University and fed the same National Research Council (NRC 2012) antibiotic-free diet. All piglets from the six sows were fed breast milk from birth until day 35. Birth weight (BW) and final body weight (FBW) on day 35 were recorded to calculate the average daily gain (ADG) to evaluate growth performance. On day 35, for each pig breed, nine piglets from three sows (three piglets per sow) were randomly selected (random number generator) and euthanized within 30 min by overdose with a pentobarbital solution (100 mg/kg, Xinyu Biological Technology Co., Ltd., Shanghai, China) delivered intravenously into the *vena cava*. The chyme of the middle section of the jejunum was collected and immediately preserved in liquid nitrogen for subsequent DNA extraction and 16S rRNA amplicon sequencing, as previously described (Li et al. 2019). All pig experimental procedures were approved by the Institutional Animal Care and Use Committee of the Yunnan Agricultural University (YNAU20190416). The care and use of animals fully complied with local animal welfare laws, guidelines and policies.

Microbial DNA extraction and 16S rRNA amplification

Total genomic DNA of jejunal chyme samples was extracted with the Fast DNA Stool Mini kit (Cat No. 19593; Qiagen, Düsseldorf, Germany), following the manufacturer's recommendations. The V3–V4 region of the 16S rRNA gene was amplified via PCR using the universal primer pair 341F (5'-CCTACGGGRSGCAGCAG-3') and 806R (5'-GGACTACVVGGGTATCTAATC-3'). The amplification was initially denatured at 95 °C for 3 min, followed by 30 cycles of 98 °C for 20 s, 58 °C for 15 s, 72 °C for 20 s and a final extension at 72 °C for 5 minutes. All the amplicon pooled libraries were sequenced on the Illumina MiSeq PE250 (Illumina, San Diego, CA, USA) at Realbio Technology Co., Ltd. (Shanghai, China). The datasets generated for this study were deposited as raw

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fastq files in Sequence Read Archive with accession number PRJNA681410 (https://www.ncbi.nlm.nih.gov/bioproject/?term=prjna681410).

Bioinformatics analyses

Bioinformatics analysis was performed according to the recommendations of a previous study (Li et al. 2019). Briefly, paired-end reads in the FASTA files were assembled using PANDAseq software v2.8.1 (https://github.com/neufeld/pandaseq/releases/tag/v2.8.1). Quality control of sequencing data and the filtering process were conducted as the methods described by predecessors (Caporaso et al. 2010). Each representative read was assigned to a taxon by the ribosomal database project (RDP) database (http://rdp.cme.msu.edu/) with a threshold value of 0.8–1. The operational taxonomic units (OTUs)-based analysis with a 97% similarity was performed using UPARSE (http://drive5.com/uparse/).

The bacterial annotation and taxonomic information for each OTU were determined to estimate the richness of the microbial species of each sample. Alpha diversity of Chao1, Shannon, Simpson, Good's coverage, observed species, PD whole tree, and beta diversity of principal component analysis and analysis of similarities based on unweighted UniFrac distance metrics were determined using QIIME software v1.9.1 (Caporaso et al. 2010). A linear discriminant effect size analysis was conducted to identify the biomarkers among groups (Segata et al. 2011). Based on high-quality reads, functional categories of the Kyoto Encyclopaedia of Genes and Genomes (KEGG) orthology were predicted by phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) (Langille et al. 2013).

Statistical analysis

The growth performance data were analysed using the analysis of covariances with SPSS v22.0 software (IBM Corp., Armonk, NY, USA). The growth performance data are expressed as the mean ± standard error of the mean (SEM). GraphPad Prism v7.0 Spearman's analysis was used to estimate the correlation between host growth performance and jejunal microbiota. Statistical significance was set at $P < 0.05$.

RESULTS

Growth performance of DH and DS piglets

As shown in Table 1, birth weight (BW), final body weight at day 35 (FBW) and average daily gain (ADG) tended to increase more in DH piglets than in DS piglets.

Taxonomic distribution of jejunal microbiota in DH and DS piglets

All jejunal chyme samples from the 18 piglets were subjected to 16S rRNA sequencing. One sample from each group was excluded from further analysis because of the low quality of DNA. After quality control, a total of 958 142 clean reads were generated, with an average of 53 524 clean reads (44 615–61 124) per sample and an average clean read length of 419 bp [Table S1 in electronic supplementary material (ESM); for the supplementary material see the electronic version]. Based on 97% sequence similarity, we obtained 639 OTUs in the DH piglets and 523 in the DS piglets (Figure 1). To minimize the effect of the sequencing depth on the measurement of microbial composition, the

Table 1. Growth performance of Dahe (DH) and Diannan small-ear (DS) piglets

Items	DH	DS	*P*-value
Birth weight (kg)	0.85 ± 0.09[a]	0.68 ± 0.1[b]	0.017
Final body weight (kg)	5.34 ± 0.42	4.51 ± 0.77	0.084
Average daily gain (kg)	0.13 ± 0.01	0.11 ± 0.02	0.084

[a,b]Means in the same row with different superscripts are significantly different ($P < 0.05$)

Data are presented as mean ± SEM, $n = 9$ per treatment group

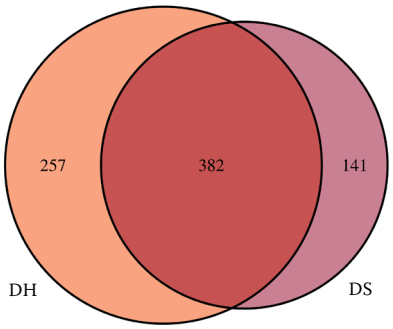

Figure 1. Operational taxonomic unit overlap of Dahe (DH) and Diannan small-ear (DS) pigs

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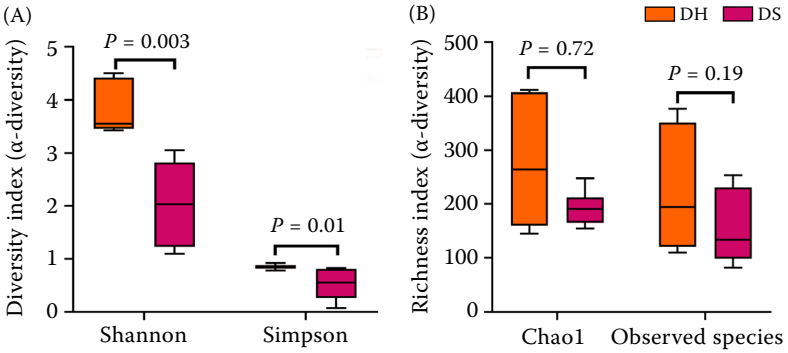

Figure 2. Boxplot of alpha diversity indices between groups
(A) Analysis of bacterial community diversity (Shannon and Simpson) of jejunal microbiota. (B) Analysis of bacterial community richness indices (Chao1 and observed species) of jejunal microbiota

library size was rarefied to 35 316 reads per sample using the QIIME pipeline.

Bacterial community richness, diversity, and sequencing depth were characterized by calculating the alpha diversity indices based on the OTUs of each sample. Good's coverage estimator indices of the samples ranged from 0.997 2 to 0.999 0 (Table S2 in ESM), suggesting that the sequencing results likely represent the bacterial diversity of the entire sampled population. The Shannon and Simpson indices were used to determine the bacterial community diversity, which was greater in DH piglets (Shannon: DH – 3.60, DS – 2.03, $P = 0.009$) than in DS piglets (Simpson: DH – 0.80, DS – 0.52, $P = 0.044$) (Figure 2A). There was no significant difference between DH and DS piglets in the Chao1 and observed species indices used to determine the bacterial community richness (Chao1: DH – 279.89 vs DS – 230.17, $P = 0.356$; observed species: DH – 225.37 vs DH – 153.13, $P = 0.145$) (Figure 2B). We then compared the two groups in terms of beta diversity using principal component analysis, which returned that the microbial OTUs were clearly divided into two groups (Figure 3A). We also calculated analysis of similarities based on unweighted UniFrac distances between DH and DS piglets. When all samples were analysed, the microbiota compositions of the two breeds were significantly different ($R = 0.484$; $P = 0.002$) (Figure 3B).

Bacterial community structure in DH and DS piglets

We conducted a taxonomic analysis using the RDP classifier to define the composition of the jejunal microbiota. At the phylum level, the three most dominant phyla detected in both groups were Firmicutes (79.08% in DH and 60.81% in DS), Proteobacteria (13.81% in DH and 33.27% in DS), and Bacteroidetes (4.14% in DH and 0.89% in DS) (Figure 4A). Meanwhile, the ratio of Firmicutes/Bacteroidetes was 69.46 in DH and 286.77 in DS pigs.

At the genus level, the predominated genera for both breeds were *Lactobacillus*, *Clostridium XI*, *Veillonella*, *Clostridium sensu stricto* and *Escherichia/Shigella* (Figure 4B). The relative abundances of *Clostridium XI*, *Clostridium sensu stricto*, *Veillonella*, *Turicibacter*, *Megasphaera*, *Mitsuokella*,

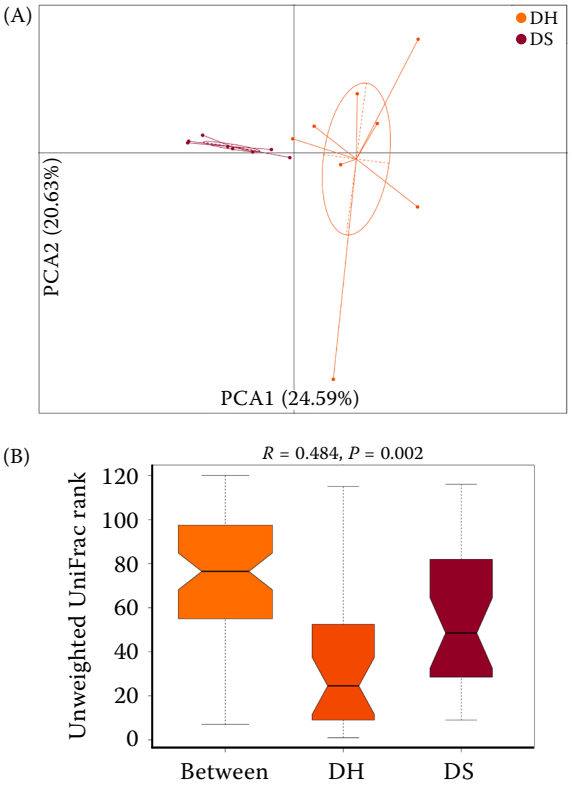

Figure 3. Beta diversity of jejunal 16S rRNA sequencing data
(A) Principal component analysis based on 16S rDNA profiling of jejunal chyme. Principal components (PCs) 1 and 2 explain 24.59% and 20.63% of the variance, respectively. (B) Analysis of similarities of unweighted UniFrac distances of jejunal microbiota between Dahe (DH) and Diannan small-ear (DS) pigs

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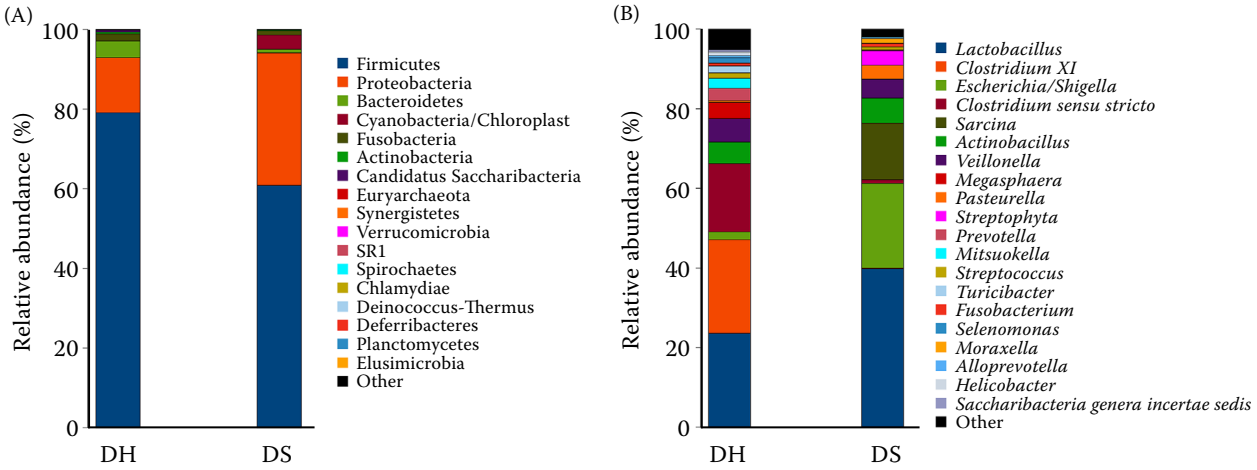

Figure 4. Distribution of the taxonomic composition of samples at the phylum and genus level
(A) Relative jejunal microbial abundance at the phylum level between Dahe (DH) and Diannan small-ear (DS) piglets. (B) Relative jejunal microbial abundance at the genus level between DH and DS piglets. The ratio of each taxon in certain samples is directly displayed

Helicobacter, *Acinetobacter*, *Clostridium XlVb*, *Methanobrevibacter*, *Howardella*, *Sharpea* and *Rhizobium* in DH piglets were significantly higher than those of DS piglets ($P < 0.05$); whereas the relative abundances of *Streptophyta*, *Enterococcus*, *Lactococcus* and *Weissella* in DH pigs were significantly lower than those of DS pigs ($P < 0.05$) (Figure 5). In addition, the relative abundances of the genera *Lactobacillus* and *Escherichia/Shigella* showed no statistically significant difference between the two pig breeds (*Lactobacillus*: DH – 23.57% vs DS – 39.80%, $P = 0.35$; *Escherichia/Shigella*: DH – 1.92% vs DS – 21.29%, $P = 0.16$).

Correlation between growth performance and differential genera

The correlation between the jejunal differential microbiota and host growth performance is shown

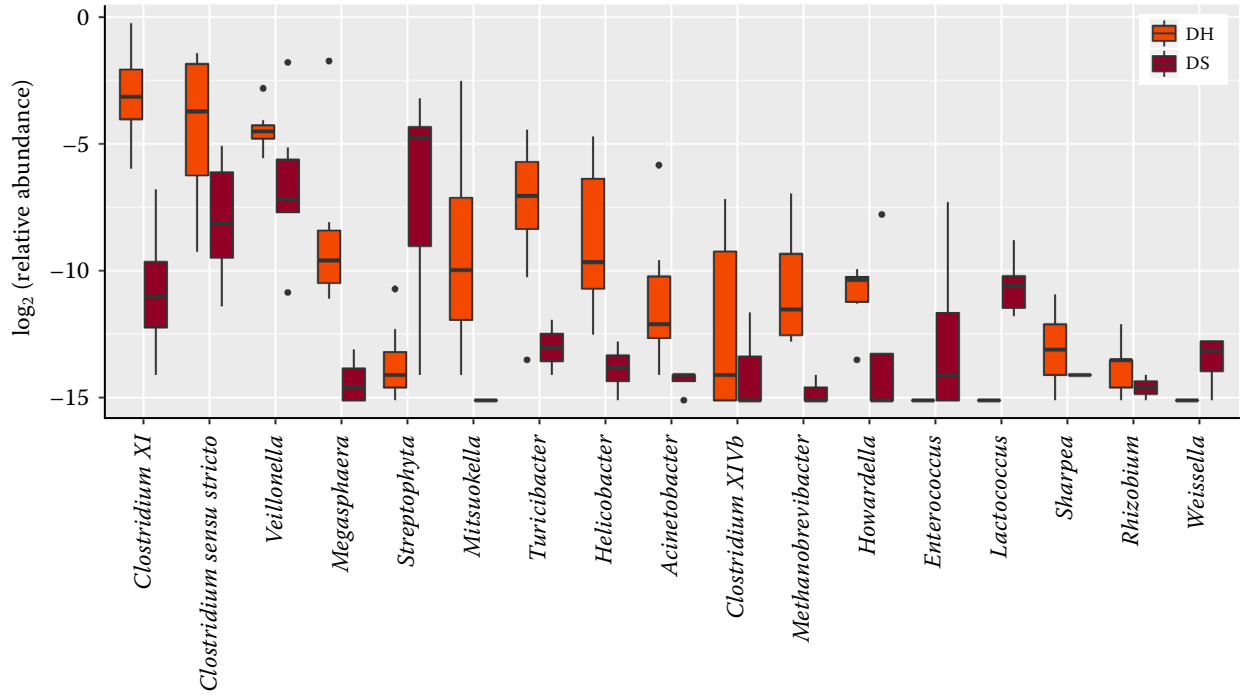

Figure 5. Boxplot of the differentially abundant genera in the Dahe (DH) and Diannan small-ear (DS) pig groups

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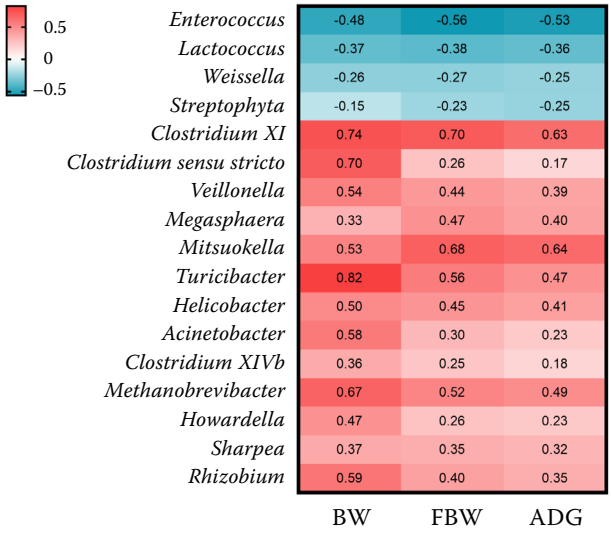

Figure 6. Spearman's correlation analysis of differential genera and growth performance in Dahe (DH) and Diannan small-ear (DS) pigs

Blue represents a significant negative correlation ($P < 0.05$), red represents a significant positive correlation ($P < 0.05$). The number represents the value of r

in Figure 6. *Enterococcus* was negatively correlated with FBW ($P = 0.025$, $r = -0.56$), and ADG ($P = 0.034$, $r = -0.53$). BW was positively correlated with *Clostridium XI* ($P = 0.001$, $r = 0.74$), *Clostridium sensu stricto* ($P = 0.003$, $r = 0.70$), *Veillonella* ($P = 0.032$, $r = 0.54$), *Mitsuokella* ($P = 0.036$, $r = 0.53$), *Turicibacter* ($P < 0.01$, $r = 0.82$), *Acinetobacter* ($P = 0.022$, $r = 0.58$), *Methanobrevibacter* ($P = 0.006$, $r = 0.67$), and *Rhizobium* ($P = 0.018$, $r = 0.59$). FBW was positively correlated with *Clostridium XI* ($P = 0.003$, $r = 0.070$), *Mitsuokella* ($P = 0.004$, $r = 0.68$), *Turicibacter* ($P = 0.024$, $r = 0.56$), and *Methanobrevibacter* ($P = 0.006$, $r = 0.52$). ADG was positively correlated with *Clostridium XI* ($P = 0.01$, $r = 0.63$) and *Mitsuokella* ($P = 0.007$, $r = 0.64$).

Structures and functions of the jejunal microbiota in DH and DS piglets

To uncover the specific bacterial taxa associated with the distinct pig breeds, linear discriminant effect size analysis was performed to compare the jejunal microbial composition between the DH and DS piglets. The most differentially abundant phylotypes in the two pig breeds are shown in Figure 7. The phylotypes enriched in the DH piglets comprised one phylum (Euryarchaeota), six classes (Alphaproteobacteria, Methanobacteria, Epsilonproteobacteria, Erysipelotrichia, Negativicutes and Clostridia), six orders (Methanomassilicoccales, Rhizobiales, Campylobacterales, Erysipelotrichales, Selenomonadales and Clostridiales), 10 families (Sanguibacteraceae, Hyphomicrobiaceae, Methanobacteriaceae, Phyllobacteriaceae, Rhizobiaceae, Acidaminococcaceae, Helicobacteraceae, Lachnospiraceae, Erysipelotrichaceae, Veillonellaceae and Peptostreptococcaceae), and 21 genera (*Sanguibacter*, *Howardella*, *Blautia*, *Cellulosilyticum*, *Clostridium XlVb*, *Devosia*, *Acinetobacter*, *Methanobrevibacter*, *Allisonella*, *Sharpea*, *Luteolibacter*, *Rhizobium*, *Acidaminococcus*, *Helicobacter*, *Selenomonas*, *Turicibacter*, *Mitsuokella*, *Veillonella*, *Megasphaera*, *Clostridium sensu stricto* and *Clostridium XI*). The phylotypes enriched in the DS piglets included two families (Leuconostocaceae and Streptomycetaceae) and four genera (*Weissella*, *Lactococcus*, *Enterococcus*, and *Kitasatospora*).

According to the PICRUSt-based functional prediction, 54 KEGG pathways in the jejunal chyme microbiota were significantly different between the two pig breeds (linear discriminant analysis, LDA > 2) (Figure 8). The pathways enriched in the DH piglets were mainly related to amino acid metabolism (including phosphonate and phosphinate, cyanoamino acid, histidine, glycine, serine, threonine, alanine, aspartate, glutamate, arginine, proline, cysteine, methionine, and lysine biosynthesis), and energy metabolism (carbon fixation pathways in prokaryotes and methane metabolism). The pathways enriched in the DS piglets were mainly related to lipid metabolism (such as alpha-linolenic acid, lipoic acid, and fatty acid).

DISCUSSION

Previous reports have shown that breed differences contribute to varying growth traits in pigs (White et al. 1995). China has a long history of raising pigs and has nurtured 88 indigenous breeds (Yu et al. 2013) that generally have a lower growth performance than European commercial pig breeds, such as Duroc, Landrace, and Yorkshire. However, many Chinese indigenous pigs, including DH and DS, have the unique characteristics of consuming coarse grain feed and conferring good meat qual-

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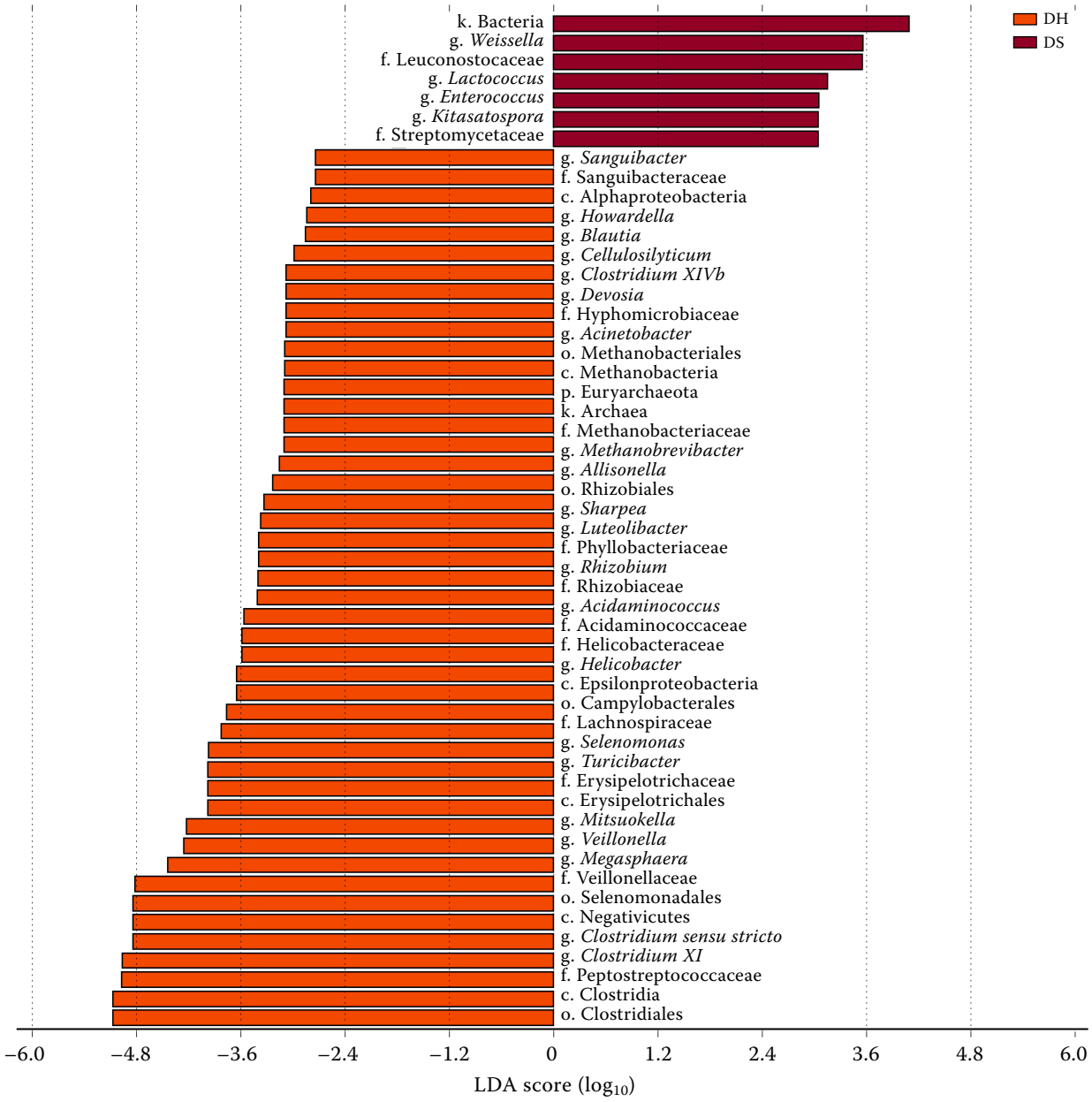

Figure 7. Bacterial taxa that differed significantly between Dahe (DH) and Diannan small-ear (DS) pig groups
Only genera meeting an LDA (linear discriminant analysis) significant threshold > 2 are shown

ity (Dai et al. 2010). In the present study, the BW, FBW, and ADG of DH piglets tended to be greater than in DS piglets, suggesting that DH pigs may have a higher growth potential.

The gut microbiota is involved in host physiological functions during the host's entire lifespan (Milani et al. 2017). Microbiota colonization is initiated at birth and shaped by various factors, including host genetics (Ursell et al. 2012). Gut microbes are transmissible through generations, and host genetics may influence gut bacterial composition and biodiversity in animals and humans (Lu et al. 2018). Previous studies have indicated that genetically related individuals tend to have more similar microbial communities than unrelated individuals (Tims et al. 2013), and the intestinal microbiota in monozygotic twins are more similar than in dizygotic twins (Hansen et al. 2011).

In terms of pigs, the faecal microbiota composition of Duroc boars, Yorkshire, Landrace, and Hampshire pigs were related to the breed (Xiao et al. 2017). The bacterial communities were more diverse in the duodenum, jejunum, and caecum of Jinhua pigs than in Landrace pigs (Xiao et al.

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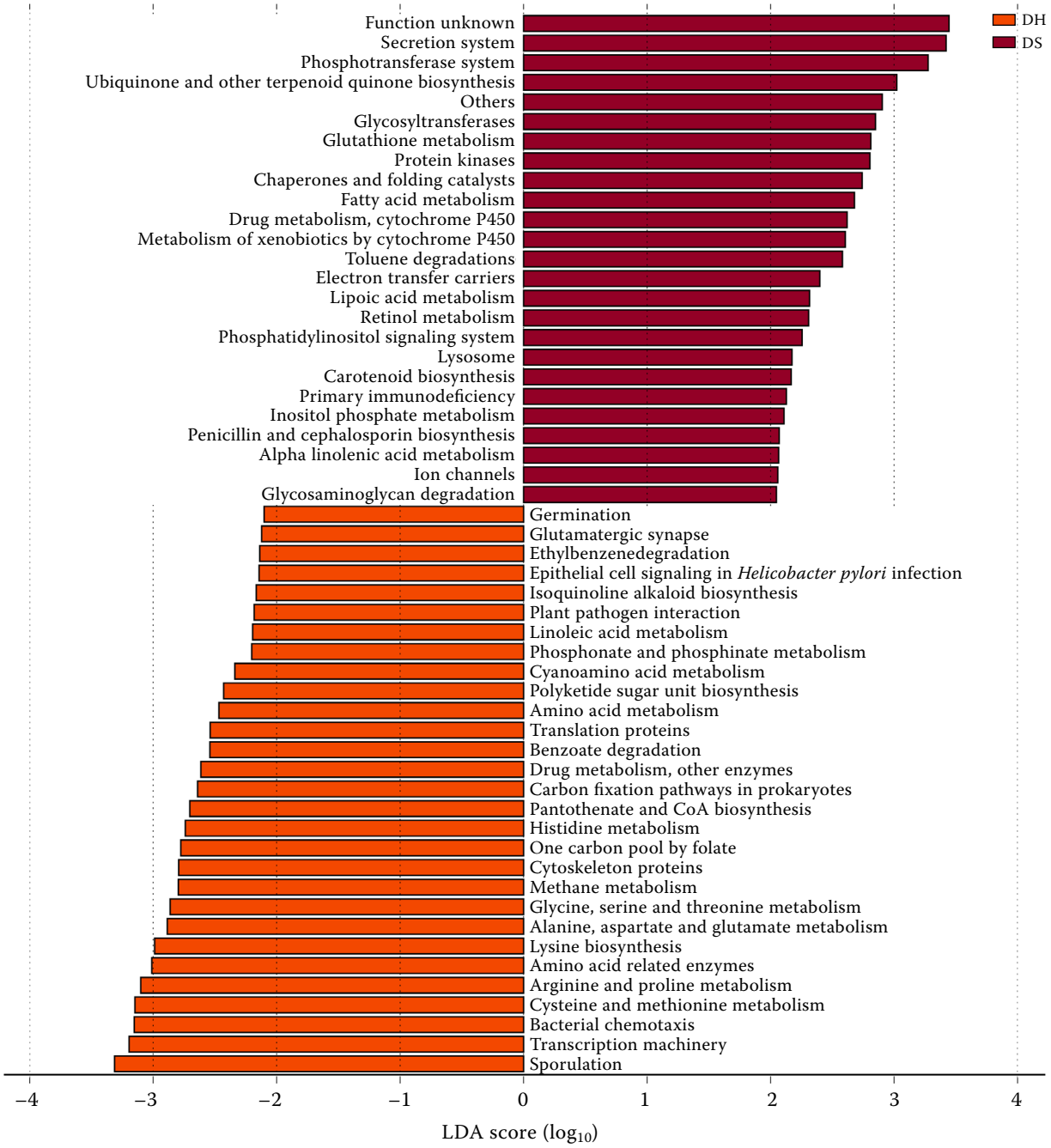

Figure 8. KEGG analysis of enriched pathways at L3 hierarchy in the Dahe (DH) and Diannan small-ear (DS) pig groups (linear discriminant analysis – LDA > 2)

2018), which is why the samples were collected from the jejunum in our study. We demonstrated that the Shannon and Simpson indices in DH piglets were higher than in DS pigs, indicating that DH piglets have greater jejunal microbial diversity. The difference in microbial diversity between the two breeds may partly be attributed to the difference in the genetic background of the host.

Breastfeeding may enrich vaginally acquired lactic acid-producing bacteria in the intestine of the offspring and constrain the growth of several strains of *E. coli* (Chen et al. 2018). *Lactobacillus* is a major player in disease prevention, while strains from *Escherichia-Shigella* have been proven potential pathogens that could induce dysentery and diarrhoea (Fairbrother et al. 2005). The abundance of *Lactobacillus* in Meishan piglets

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was higher than in Yorkshire piglets whereas the abundance of *Escherichia-Shigella* in Meishan piglets was lower (Bian et al. 2016). In the present study, the abundance of *Lactobacillus* was higher in DH piglets than in DS piglets, whereas that of *Escherichia-Shigella* was lower, suggesting that the growth of *Escherichia-Shigella* was restricted by *Lactobacillus*.

Lactococcus produces L (+) lactate from glucose (Ringo et al. 2018). The genus *Weissella* are obligate heterofermentative bacteria producing CO_2 from carbohydrate metabolism with either D (–) lactic acid or a mixture of D (–)- and L (+) lactic acid and acetic acid as the major end products of sugar metabolism (Ringo et al. 2018). The relative abundances of *Lactococcus* and *Weissella* in DH pigs were lower than in DS pigs, implying that DS piglets may have higher lactate production ability.

The relative abundances of *Veillonella*, *Turicibacter*, *Megasphaera*, *Mitsuokella*, and *Selenomonas* in DH piglets were greater than in DS piglets. Previous research revealed that members of the genera *Veillonella*, *Megasphaera*, *Mitsuokella,* and *Selenomonas* are capable of utilizing lactate and/or succinate to produce short-chain fatty acids (SCFAs) (Duncan et al. 2004; Bhute et al. 2016; Zhang et al. 2019), thus playing an important role in improving intestinal health, restraining intestinal inflammation, and stimulating the growth and proliferation of small intestine cells in pigs (Liu 2015). Furthermore, SCFAs can lower the gut pH and subsequently provide added value for probiotic microbiota such as *Turicibacter*, which thrive at lower pH (Getachew et al. 2018). In addition, the majority of SCFAs can be utilized as an energy source by colonocytes (Bergman 1990). Correlation analysis revealed that *Veillonella* and *Mitsuokella* were positively correlated with BW, while *Mitsuokella* was positively correlated with FBW and ADG in the present study. Therefore, these lactate-utilizing bacteria in DH piglets may produce abundant SCFAs and further promote their growth.

In the present study, the jejunal microbiota of the DH and DS piglets were dominated by Firmicutes, Bacteroidetes, and Proteobacteria in agreement with published data (Pajarillo et al. 2014). The ratio of Firmicutes/Bacteroidetes in DH pigs was lower than in DS pigs and the predicted functions in DS piglets are mainly related to fat deposition, which may suggest that the DS pigs have higher fat deposition ability, corroborating previous findings that this ratio was positively related to fat deposition in humans (Mariat et al. 2009).

CONCLUSIONS

In summary, DH pigs have higher microbial diversity, while DS pigs may have higher fat deposition ability. Our findings reveal information on the intestinal microbiota of DH and DS piglets and may suggest a strategy for improving growth performance in the indigenous pig industry.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Bergman EN. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiol Rev. 1990 Apr;70(2):567-90.

Bhute S, Pande P, Shetty SA, Shelar R, Mane S, Kumbhare SV, Gawali A, Makhani H, Navandar M, Dhotre D, Lubree H, Agarwal D, Patil R, Ozarkar S, Ghaskadbi S, Yajnik C, Juvekar S, Makharia GK, Shouche YS. Molecular characterization and meta-analysis of gut microbial communities illustrate enrichment of Prevotella and Megasphaera in Indian subjects. Front Microbiol. 2016 May 9;7: 14 p.

Bian G, Ma S, Zhu Z, Su Y, Zoetendal EG, Mackie R, Liu J, Mu C, Huang R, Smidt H, Zhu W. Age, introduction of solid feed and weaning are more important determinants of gut bacterial succession in piglets than breed and nursing mother as revealed by a reciprocal cross-fostering model. Environ Microbiol. 2016 May;18(5):1566-77.

Buffie CG, Pamer EG. Microbiota-mediated colonization resistance against intestinal pathogens. Nat Rev Immunol. 2013 Nov;13(11):790-801.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010 May;7(5):335-6.

Chen X, Xu J, Ren E, Su Y, Zhu W. Co-occurrence of early gut colonization in neonatal piglets with microbiota

https://doi.org/10.17221/63/2021-CJAS

in the maternal and surrounding delivery environments. Anaerobe. 2018 Feb;49:30-40.

Dai FW, Feng DY, Cao QY, Ye H, Zhang CM, Xia WG, Zuo JJ. Developmental differences in carcass, meat quality and muscle fibre characteristics between the Landrace and a Chinese native pig. S Afr J Anim Sci. 2010;39(4):267-73.

Dong K, Yao N, Pu Y, He X, Zhao Q, Luan Y, Guan W, Rao S, Ma Y. Genomic scan reveals loci under altitude adaptation in Tibetan and Dahe pigs. PLoS One. 2014 Oct 17;9(10): 11 p.

Duncan SH, Louis P, Flint HJ. Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. Appl Environ Microbiol. 2004 Oct;70(10):5810-7.

El Aidy S, Merrifield CA, Derrien M, van Baarlen P, Hooiveld G, Levenez F, Dore J, Dekker J, Holmes E, Claus SP, Reijngoud DJ, Kleerebezem M. The gut microbiota elicits a profound metabolic reorientation in the mouse jejunal mucosa during conventionalisation. Gut. 2013 Sep;62(9):1306-14.

Fairbrother JM, Nadeau E, Gyles CL. Escherichia coli in postweaning diarrhea in pigs: An update on bacterial types, pathogenesis, and prevention strategies. Anim Health Res Rev. 2005 Jun;6(1):17-39.

Getachew B, Aubee JI, Schottenfeld RS, Csoka AB, Thompson KM, Tizabi Y. Ketamine interactions with gut-microbiota in rats: relevance to its antidepressant and anti-inflammatory properties. BMC Microbiol. 2018 Dec 22;18(1): 10 p.

Hansen EE, Lozupone CA, Rey FE, Wu M, Guruge JL, Narra A, Goodfellow J, Zaneveld JR, McDonald DT, Goodrich JA, Heath AC, Knight R, Gordon JI. Pan-genome of the dominant human gut-associated archaeon, Methanobrevibacter smithii, studied in twins. Proc Natl Acad Sci USA. 2011 Mar 15;108 Suppl 1:4599-606.

Hao LL, Yu H, Zhang Y, Sun SC, Liu SC, Zeng YZ, Ai YX, Jiang HZ. Single nucleotide polymorphism analysis of exons 3 and 4 of IGF-1 gene in pigs. Genet Mol Res. 2011 Aug 16;10(3):1689-95.

Kamada N, Seo SU, Chen GY, Nunez G. Role of the gut microbiota in immunity and inflammatory disease. Nat Rev Immunol. 2013 May;13(5):321-35.

Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Vega Thurber RL, Knight R, Beiko RG, Huttenhower C. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol. 2013 Sep;31(9):814-21.

Li X, Cao Z, Yang Y, Chen L, Liu J, Lin Q, Qiao Y, Zhao Z, An Q, Zhang C, Li Q, Ji Q, Zhang H, Pan H. Correlation between jejunal microbial diversity and muscle fatty acids deposition in broilers reared at different ambient temperatures. Sci Rep. 2019 Jul 30;9(1): 12 p.

Liu Y. Fatty acids, inflammation and intestinal health in pigs. J Anim Sci Biotechnol. 2015 Dec 9;6(1): 9 p.

Lu D, Tiezzi F, Schillebeeckx C, McNulty NP, Schwab C, Shull C, Maltecca C. Host contributes to longitudinal diversity of fecal microbiota in swine selected for lean growth. Microbiome. 2018 Jan 4;6(1): 5 p.

Lynch JB, Hsiao EY. Microbiomes as sources of emergent host phenotypes. Science. 2019 Sep 27;365(6460):1405-9.

Macpherson AJ, Harris NL. Interactions between commensal intestinal bacteria and the immune system. Nat Rev Immunol. 2004 Jun;4(6):478-85.

Mariat D, Firmesse O, Levenez F, Guimaraes V, Sokol H, Dore J, Corthier G, Furet JP. The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. BMC Microbiol. 2009 Jun 9;9(1): 6 p.

Martinez-Guryn K, Hubert N, Frazier K, Urlass S, Musch MW, Ojeda P, Pierre JF, Miyoshi J, Sontag TJ, Cham CM, Reardon CA, Leone V, Chang EB. Small intestine microbiota regulate host digestive and absorptive adaptive responses to dietary lipids. Cell Host Microbe. 2018 Apr 11;23(4):458-69.

Milani C, Duranti S, Bottacini F, Casey E, Turroni F, Mahony J, Belzer C, Delgado Palacio S, Arboleya Montes S, Mancabelli L, Lugli GA, Rodriguez JM, Bode L, de Vos W, Gueimonde M, Margolles A, van Sinderen D, Ventura M. The first microbial colonizers of the human gut: Composition, activities, and health implications of the infant gut microbiota. Microbiol Mol Biol Rev. 2017 Nov 8;81(4): 67 p.

Mu C, Bian G, Su Y, Zhu W. Differential effects of breed and nursing on early-life colonic microbiota and immune status as revealed in a cross-fostering piglet model. Appl Environ Microbiol. 2019 Apr 18;85(9): 12 p.

NRC – National Research Council. Nutrient requirements of swine. 11th ed. Washington: National Academies Press; 2012. 420 p.

Pajarillo EA, Chae JP, Balolong MP, Kim HB, Seo KS, Kang DK. Pyrosequencing-based analysis of fecal microbial communities in three purebred pig lines. J Microbiol. 2014 Aug 1;52(8):646-51.

Piccolo BD, Mercer KE, Bhattacharyya S, Bowlin AK, Saraf MK, Pack L, Chintapalli SV, Shankar K, Adams SH, Badger TM, Yeruva L. Early postnatal diets affect the bioregional small intestine microbiome and ileal metabolome in neonatal pigs. J Nutr. 2017 Aug 1;147(8):1499-509.

Ringo E, Hoseinifar SH, Ghosh K, Doan HV, Beck BR, Song SK. Lactic acid bacteria in finfish-an update. Front Microbiol. 2018 Aug 10;9: 37 p.

Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C. Metagenomic biomarker

https://doi.org/10.17221/63/2021-CJAS

discovery and explanation. Genome Biol. 2011 Jun 24;12(6): 18 p.

Tims S, Derom C, Jonkers DM, Vlietinck R, Saris WH, Kleerebezem M, de Vos WM, Zoetendal EG. Microbiota conservation and BMI signatures in adult monozygotic twins. ISME J. 2013 Apr;7(4):707-17.

Ursell LK, Clemente JC, Rideout JR, Gevers D, Caporaso JG, Knight R. The interpersonal and intrapersonal diversity of human-associated microbiota in key body sites. J Allergy Clin Immunol. 2012 May;129(5):1204-8.

White BR, Lan YH, McKeith FK, Novakofski J, Wheeler MB, McLaren DG. Growth and body composition of Meishan and Yorkshire barrows and gilts. J Anim Sci. 1995 Mar;73(3):738-49.

Xiao Y, Li K, Xiang Y, Zhou W, Gui G, Yang H. The fecal microbiota composition of boar Duroc, Yorkshire, Landrace and Hampshire pigs. Asian-Australas J Anim Sci. 2017 Oct;30(10):1456-63.

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 Apr 13;8(1): 10 p.

Yu G, Xiang H, Wang J, Zhao X. The phylogenetic status of typical Chinese native pigs: Analyzed by Asian and European pig mitochondrial genome sequences. J Anim Sci Biotechnol. 2013 Mar 8;4(1): 7 p.

Zhang Z, Xiao Q, Zhang QQ, Sun H, Chen JC, Li ZC, Xue M, Ma PP, Yang HJ, Xu NY, Wang QS, Pan YC. Genomic analysis reveals genes affecting distinct phenotypes among different Chinese and western pig breeds. Sci Rep. 2018 Sep 6;8(1): 12 p.

Zhang D, Liu H, Wang S, Zhang W, Wang J, Tian H, Wang Y, Ji H. Fecal microbiota and its correlation with fatty acids and free amino acids metabolism in piglets after a Lactobacillus strain oral administration. Front Microbiol. 2019 Apr 16;10: 13 p.

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