BMC Veterinary Research

RESEARCH

Open Access

Effect of host breeds on gut microbiome and fecal metabolome in commercial pigs

Sui Liufu[1], Kaiming Wang[1], Bohe Chen[1], Wenwu Chen[1], Xiaolin Liu[1], Sheng Wen[1], Xintong Li[1], Dong Xu[3] and Haiming Ma[1,2,4*]

Abstract

Background Gut microbial composition and its metabolites are crucial for livestock production performance. Metabolite profiles from autopsied biospecimens provide vital information on the basic mechanisms that affect the overall health and production traits in livestock animals. However, the role of the host breed in the gut microbiome and fecal metabolome of commercial pigs remains unclear. In this work, differences in microbiota composition among three commercial pig breeds Duroc, Yorkshire, and Landrace were measured by 16S rRNA gene sequencing. Fecal metabolite compositions of the three pig breeds were detected using untargeted metabolomics.

Results There were significant differences in the gut microbiomes of the three species, indicating that host breed affects the diversity and structure of gut microbiota. Several breed-associated microorganisms were identified at different taxonomic levels. Notely, most microbial taxa were annotated as *Lactobacillacea*, *Muribaculaceae*, and *Oscillospiraceae*. Several bacteria, including *Lactobacillus*, *Subdoligranulum*, *Faecalibacterium*, *Oscillospira*, *Oscillospiraceae_UCG-002*, and *Christensenellaceae_R-7_group*, could be considered as biomarkers for improving the backfat thickness (BF) for commercial pigs. Additionally, KEGG analysis of gut microbiota further revealed that arginine biosynthesis, pyruvate metabolism, and fatty acid biosynthesis varied greatly among pig breeds. Multiple gut bacterial metabolites (e.g., spermidine, estradiol, and palmitic acid) were identified as breed-associated. Mediation analysis ultimately revealed the cross-talk among gut microbiota, metabolites, and BF thickness, proclaiming that the microbial and metabolic biomarkers identified in this study could be used as biomarkers for improving BF phenotype.

Conclusions This work provides vital insights into breed effects on gut microbiota and metabolite compositions of commercial pigs and uncovers potential biomarkers that are significant for pig breed improvement.

Keywords Host breeds, Gut microbiota, Fecal metabolites, Commercial pigs

Background

At present, the main supporting pig strains in the world are basically Duroc, Landrace, Yorkshire, and other pigs as the maternal or paternal parents, and parent-breeding pigs and commercial generation pigs are bred through crossbreeding [1, 2]. Duroc pigs were found be well suited as a crossbreeding boar owing to their fast growth rates, high feed-to-meat ratios, and excellent carcass meat quality [3]. The Landrace pigs have obvious crossbreeding advantages, which can significantly improve the growth rate, slaughter rate and leanness of crossbred progeny [4]. The Yorkshire pigs are full-bodied, distinguished by

*Correspondence:
Haiming Ma
mahaiming2000@163.com
[1] College of Animal Science and Technology, Hunan Agricultural University, Changsha, PR 410128, China
[2] Key Laboratory of Livestock and Poultry Resources (Pig) Evaluation and Utilization, Ministry of Agriculture and Rural Affairs, Changsha, PR 410128, China
[3] Department of Biological and Environmental Engineering, Yueyang Vocational Technical College, Yueyang, PR 414000, China
[4] Yuelushan Laboratory, Changsha, PR 410128, China

high leanness, fast growth and excellent feed utilization [5]. Additionally, BF thickness, as an important indicator in animal husbandry, is strongly related to economic efficiency [6, 7]. The BF thickness of animals is influenced by a combination of genetics, environment and other factors [6]. Owing to their unique intestinal flora, different pig breeds may show different nutrient absorption capabilities, which may affect feed efficiency and base fertilizer thickness [7].

Gut microbes play an important role in maintaining the host's digestion, energy metabolism, and immune function [8]. Recent studies have demonstrated that changes in the composition and function of the gut microbiota are closely related to the health status and productive performance of domestic animals [9]. Accordingly, a deeper understanding of the interaction between the intestinal microbiota and the host is crucial to better discover its functional mechanism. Both environment and host factors have been reported that could affect the community composition of gut microbiota [10, 11]. In research conducted by Pandit et al. [12], it was found that the gut microbiota of different broiler breeds contained breed-associated bacteria including *Clostridium*, *Butyrivibrio*, *Ruminococcus*, etc. Meanwhile, in recent years, there has been increasing evidence that gut microbiota-derived metabolites (e.g., fatty acids and short-chain fatty acids) were vital in regulating livestock growth performance [13]. The *Rikenellaceae_RC9_gut_group* had a positive correlation with palmitic acid in muscle and a negative correlation with stearic acid in BF. The genus *Faecalibacterium*, and family *Ruminococcaceae* were involved in the digestion of dietary fiber, affecting the production of short-chain fatty acids (SCFAs), and playing a vital role in regulating pig body weight and BF thickness [14]. In addition, it was worth noting that *Rikenellaceae and Ruminococcus* were the main genera responsible for fermenting carbohydrates and producing acetate and propionate [15].

Metabolomics discovers the types or quantities of metabolites and their patterns of change by conducting and quantifying all the small-molecule metabolites in an organism [16]. By characterizing the metabolic profile of an individual, it can fully reflect the result of a complex biological interaction of genetic and environmental factors [17]. Fecal metabolic biomarkers are critical for the production performances of farm animals because they deliver information on the net outcome of nutrient digestion and absorption [18]. Some studies have revealed breed-related metabolic molecules in different animal species. For instance, Italian Large White pigs are distinguished from Italian Duroc pigs by plasma sphingomyelin and biogenic amine levels [19]. Several serum fatty acids (e.g., oleic and linoleic acids), amino acids (e.g., glutamine and asparagine), and organic acids (e.g., citric and fumaric acids) have been suggested as breed-specific biomarkers for different beef cattle breeds [17]. However, the above studies have mainly focused on the effect of host breeds on serum metabolome rather than the fecal metabolome in farm animals. Accordingly, it is necessary to investigate the breed-associated impacts on the composition of fecal metabolites.

There is a strenuous interrelationship between the gut microbiota and fecal metabolites [20, 21]. Thus, this paper analyzed the composition of the gut microbiota and fecal metabolites of three pig breeds (Duroc, Landrace, and Large White) and explored the associations between gut bacteria, fecal metabolites, and BF thickness. The results not only help to understand the contribution of intestinal microorganisms to animal metabolism, but also reveal potential biomarkers with practical application value in promoting the health status and production performance of different pig breeds.

Materials and methods

Experimental pigs and sample collection

The Duroc, Landrace, and Yorkshire pigs were randomly selected from a large-scale commercial pig farm (Xiangtan County, Changsha City, Hunan Province, China). They were raised under the same nutritional and management conditions (160 ± 3 days of age, 5 sows and 5 boars in each group). All pigs were fed the same ration twice a day containing 14–16% crude protein, 0.25–0.60% sodium chloride, 0.60 – 1.50% calcium, 8% coarse fiber, 3,100–3,200 kJ of digestible energy, and 0.80% of lysine, and offered an ad libitum water. All pigs were healthy and were not fed probiotics, prebiotics, antibiotics, or anticoccidials throughout the trial. BF thickness in the three pig breeds was determined and recorded using B-scan ultrasonography (DAWEI, Jiangsu, China). Meanwhile, Fresh faeces from Duroc, Landrace, and Yorkshire pigs were collected directly from the rectum. All samples were kept in 2 mL freezing tubes and then immediately immersed in liquid nitrogen. The fecal samples were used for 16S rRNA gene sequencing and metabolome analysis. All animal experiments in this study were approved by the Institutional Animal Care and Use Committee of Hunan Agricultural University, Changsha, Hunan Province, China, with approval number CACAHU 20230601.

DNA extraction and 16S rRNA gene sequencing

The QIAamp fast DNA stool Mini Kit (QIAGEN, Germany) was selected for extraction of microbial DNA according to the manufacturer's guidelines. The concentration and quality of stool DNA were determined using a spectrophotometer Nanodrop 2000 (Thermo Scientific, USA) and 1.5% agarose gel electrophoresis, respectively.

The specific primers 338F (5′-ACTCCTACGGGGAGG CAGCA-3′) and 806R (5′-GGACTACHVGGGGTW TCTAAT-3′) amplified the hypervariable regions (V3-V4) of the 16S rRNA gene. The PCR amplification was performed by the following steps: an initial 94 °C denaturation step for 5 min, with subsequent 30 cycles of 95 °C for 30 s, 55 °C for 25 s, and 72 °C for 25 s, and a final prolongation step for 10 min at 72 °C. All the amplicons were sequenced on a MiSeq platform (Illumina, USA) using the paired-end method according to standard protocols. The raw data were quality first processed using QIIME (v11.0) [22] to remove the primers, barcodes, and low-quality sequences with the parameter "cutadapt". FLASH (v.1.2.11) [23] was used to merge high-quality pairwise reads into raw tags based on The SILVA database (v.138.1). To standardize the sequencing depth, the sequence library size was streamlined to 40,000 tags per sample before further analysis. Fragmentation was performed using the function "uclust" in QIIME (v11.0) to match tags into operational taxonomic units (OTUs) based on 97% sequence identity. We filtered low-quality OTUs according to the following criteria: relative abundance was less than 0.05% and OTUs appeared in 1% of the samples from test pigs. QIIME with the function "diversity" was used for the exploration of alpha and beta diversity indices, respectively. PICRUSt was used to predict KEGG metabolic pathways of gut bacterial communities [24].

Biomarker discovery in 16S rRNA data

Microbial biomarkers detection among the three groups of Duroc, Landrace and Yorkshire pigs were analyzed using linear discriminant analysis of effect sizes (LEfSe) [25] based on the standardized OTU relative abundance matrix. As a high-dimensional biomarker identification and interpretation algorithm, the LEfSe algorithm identified features that were significantly different using the non-parametric factoria Kruskal–Wallis test, and used LDA to estimate the effect size for each feature. The LDA threshold in the current study was 2.0 with a significance level of p-value smaller than 0.05.

Extraction of metabolite and LC-MS/MS profiling

The instrument platform for LC–MS analysis in this study was the UHPLC-Q Exactive HF-X system of Thermo Fisher Scientific. 50 mg solid samples were accurately weighed, and the metabolites were extracted using a 400 μL methanol: water (4:1, v/v) solution with 0.02 mg/mL L-2-chlorophenylalanin as internal standard. The mixture was allowed to settle at -10 ℃ and treated by High throughput tissue crusher Wonbio-96c (Shanghai wanbo biotechnology co., LTD) at 50 Hz for 6 min, then followed by ultrasound at 40 kHz for 30 min at 5 ℃. The samples were placed at -20 ℃ for 30 min to precipitate proteins. After centrifugation at 13,000 g at 4 ℃ for 15 min, the supernatant was carefully transferred to sample vials for LC–MS/MS analysis. As a part of the system conditioning and quality control process, a pooled quality control sample (QC) was prepared by mixing equal volumes of all samples. The QC samples were disposed of, and tested in the same manner as the analytic samples. It helped to represent the whole sample set, which would be injected at regular intervals (every 6 samples) to monitor the stability of the analysis. Simultaneously, unwanted information was dynamically removed from the MS/MS spectra.

Qualitative, quantitative and statistical analysis for metabolites

The accuracy of metabolite identification largely depended on accurate mass numbers, mass spectrometry spectra, ion fragmentation patterns, retention times, and other information. The main reference databases included MoNA (https://mona.fiehnlab.ucdavis.edu//), Metlin (https://metlin.scripps.edu/), and the self-owned standard library of Bionovogene Company (Suzhou, Jiangsu, China). To identify significantly changed metabolites (SCMs), statistical methods such as univariate and multivariate data analysis were used, and metabolites that satisfied the criteria below were considered significantly different: variable importance in projection (VIP) ≥ 1 and |fold change| (FC) ≥ 1. To probe the relationships between the metabolomic profiles of the Duroc, Landrace and Yorkshire pigs, principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were estimated using the R package "ropls".

Statistical analysis

One-way analysis of variance (ANOVA) was performed using SPSS 25.0 to analyze the differences in diversity indexes, relative abundances of bacteria, and phenotype values among the Duroc, Landrace, and Yorkshire groups. The box plots were visualized with the median and standard error. Linear discriminant analysis (LDA) was performed in R using the "LEfSe" package. Spearman correlation analysis was performed using the R package "psych" to explore the correlation between different OTUs. The major modules in the interaction network for each pig breed were calculated and visualized by using Gephi (v.0.9.2; https://gephi.org/). Mediation analysis was performed using the "mediation" package in the R software to evaluate the interactions among microbial biomarkers, key metabolites, and BF thickness. The p-value of less than 0.05 was considered statistically significant.

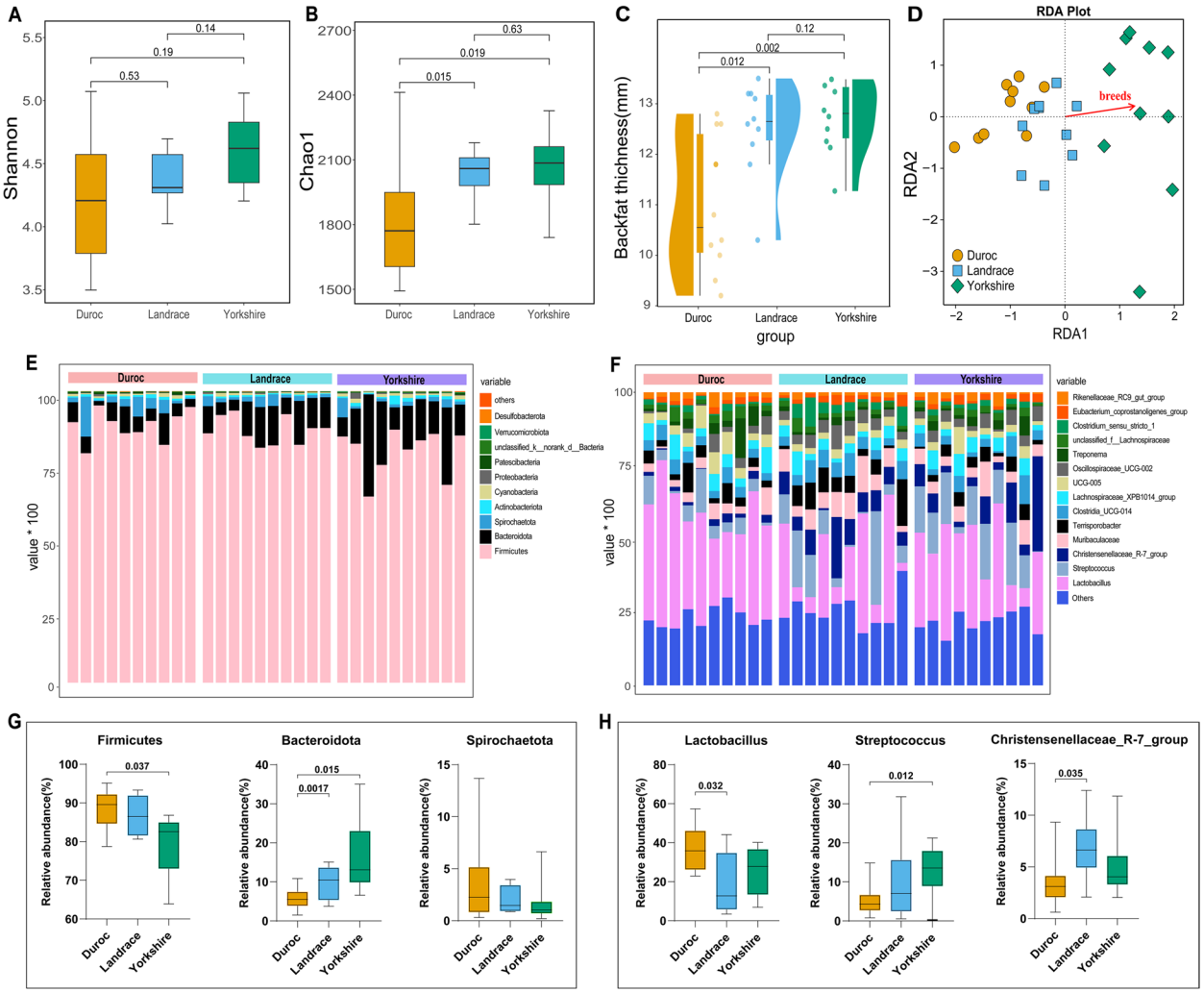

Fig. 1 Differences in diversity and structure of gut microbiota among Duroc, Landrace and Yorkshire pigs. **A**-**B** Comparison of Shannon and Chao1 indices for three groups. **C** The comparison of backfat (BF) thickness for three pig breeds. **D** Redundancy Analysis (RDA) exhibited the effect of host breeds on gut microbial community structure. **E**, **F** The gut microbial composition for Duroc, Landrace, and Yorkshire pigs in phylum (**E**) and genus (**F**) levels. **G** The difference in the relative abundance of *Firmicutes*, *Bacteroidota* and *Spirochaetota* in the phylum level among the three groups. **H** The difference in the relative abundance of *Lactobacillus*, *Streptococcus* and *Christenellaceae* in the genus level among the three groups. The box meant the interquartile range, and the box with the midline represented the median ($n = 10$ for each group)

Results

Structure and diversity of gut microbiota in Duroc, Landrace, and Yorkshire pigs

An average of 97,576.7 ± 8899.4 16S rRNA gene sequences per sample (min. = 83,542; max. = 115,270) were achieved from 30 fecal samples (Table S1). Microbial diversity indices (Shannon and Chao1) were used to assess the differences in gut microbiome across three pig breeds. As shown in Fig. 1A, the Shannon index was higher in Yorkshire than in Duroc and Landrace pigs, but there is no statistical difference ($p > 0.05$). While the Chao1 index of Duroc was significantly lower than that in Yorkshire and Landrace pigs (Fig. 1B, $p < 0.05$, Table S2).

BF thickness, as an important phenotype for commercial pig breeds, has been always the focus for breeders. The results demonstrated that BF thickness was significantly higher in Landrace and Yorkshire pigs than in Duroc pigs, respectively (Fig. 1C, $p < 0.05$). Although BF thickness was significantly higher in Yorkshire than in Landrace pigs, there was no statistical difference (Fig. 1C, $p > 0.05$). Next, to explore whether breed effects affect gut microbial community structure, the RDA analysis of each group was performed (Fig. 1D). The findings revealed that the breed exerted a significant effect on intestinal microbial communities.

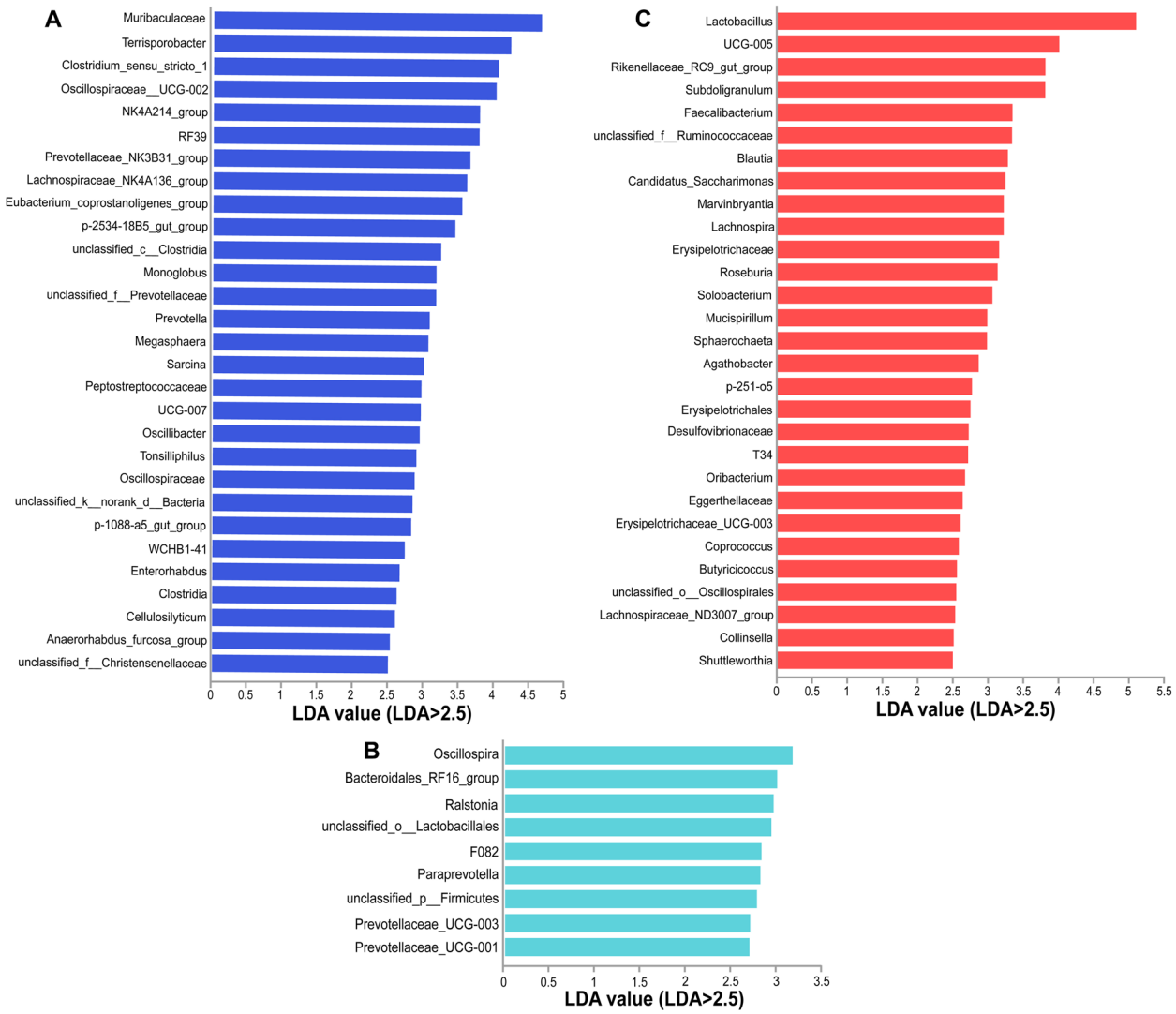

Fig. 2 The identified significantly different OTUs in Duroc, Landrace and Yorkshire pigs. **A**, the Duroc group; **B**, the Landrace group; **C**, the Yorkshire group

The results of 16S rRNA gene sequencing indicated that the intestinal flora of the three pig breeds were similar, but there were still subtle differences at the phylum and genus levels. At the phylum level (Fig. 1E), *Firmicutes*, *Bacteroidota*, and *Spirochaetota* ranked in the top three relative abundances of the three pig breeds. The abundance of *Firmicutes* was highest in Duroc pigs, and the abundance of *Bacteroidota* was highest in Yorkshire pigs (Fig. 1G, $p < 0.05$). At the genus level (Fig. 1F), *Lactobacillus*, *Streptococcus* and *Christenellaceae* were the top three genera for three breeds, with Duroc pigs having higher levels of *Lactobacillus* than Landrace and Yorkshire pigs. About the genus *Streptococcus*, it was significantly higher in Yorkshire pigs compared to Duroc pigs. *Christensenellaceae_R-7_group* was significantly higher in Landrace pig in comparison with Duroc pig (Fig. 1H, $p < 0.05$).

Microbial biomarkers for Duroc, Landrace and Yorkshire pigs

To identify the microbial biomarkers, LEfSe analysis was performed based on the relative abundance of OTUs among three pig breeds. The results showed that bacteria such as *Lactobacillus*, *Rikenellaceae_RC9_gut_group*, *UCG-005*, *Subdoligranulum*, and *Faecalibacterium* were significantly enriched in Duroc pigs (Fig. 2A). While OTUs including *Oscillospira*, *Bacteroidales_RF16_group*, and *Ralstonia* were abundant in Landrace pigs (Fig. 2B). Regarding the Yorkshire pigs, we found that *Muribaculaceae*, *Clostridium_sensu_stricto_1*, *Terrisporobacter*,

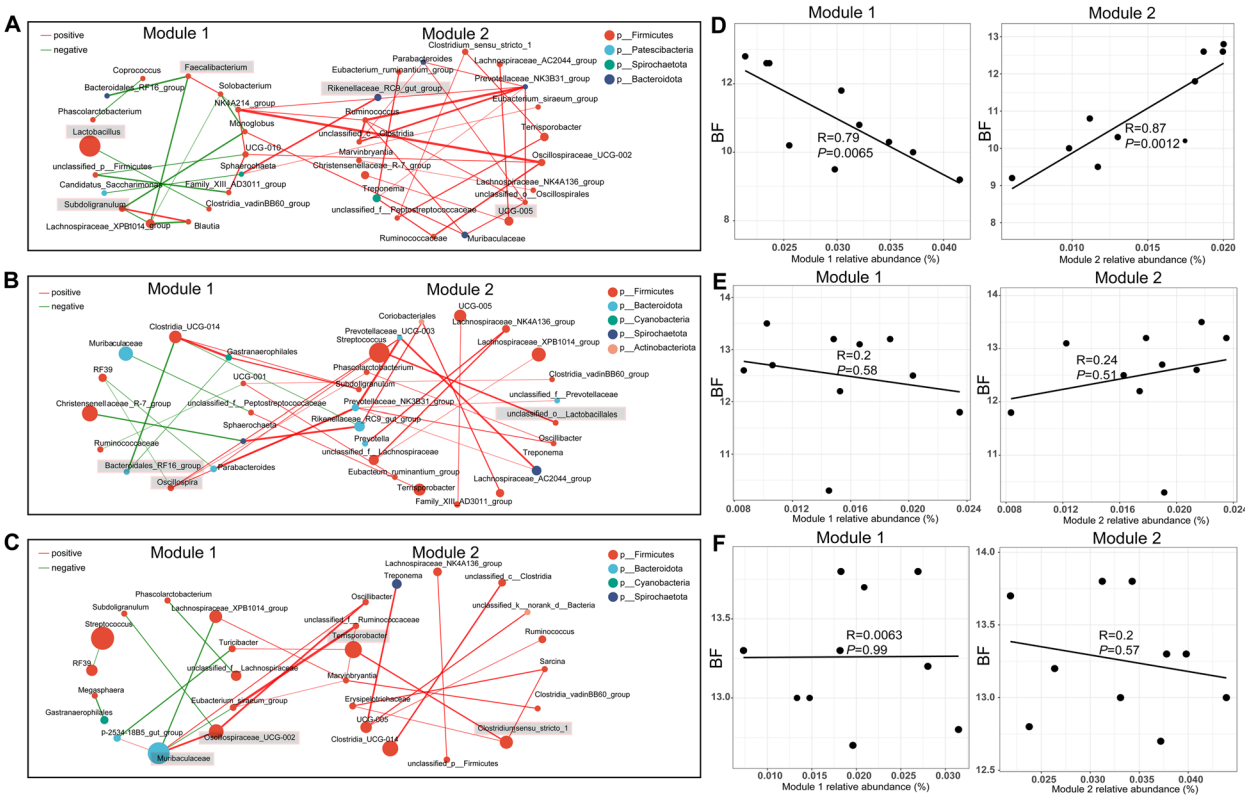

Fig. 3 Interactions among the bacteria enriched in each pig breed and their associations with BF thickness. **A**-**C** The clustering analysis for OTUs in each group; the red and green lines represented the positive and negative correlations, respectively. Each line linked to nodes represents significant correlations (FDR adjusted $p < 0.05$, $|r| > 0.80$). The node size was drawn according to the relative abundance of each OTU. **D-F** The correlation analysis between each module and BF thickness among the Duroc, Landrace, and Yorkshire pig breeds

Oscillospiraceae_UCG-002, and *NK4A214_group* were enriched in it (Fig. 2C).

Interactions of gut microbiota and their associations with BF thickness

To determine whether these significantly enriched bacteria interact and influence on intestinal wall thickness in the intestines of Duroc, Landrace and Large White pigs, the Spearman method was used to estimate the correlation coefficient between them.

The interaction network for each pig breed mainly consisted of two modules. We found that in the Duroc pigs (Fig. 3A), dominant genera such as *Lactobacillus*, *Subdoligranulum*, and *Faecalibacterium* were clustered in module 1 through negative internal interactions with other bacteria, and module 1 was negatively correlated with BF thickness ($p < 0.01$). In contrast, OTUs such as *Rikenellaceae_RC9_gut_group* and *UCG-005* aggregated in module 2 through positive internal interactions, and module 2 was positively correlated with the thickness of BF ($p < 0.01$, Fig. 3D). In Landrace pigs (Fig. 3B), dominant OTUs such as *Oscillospira*, *norank_f_Bacteroidales_RF16_group*, and *Christensenellaceae_R-7_group* were clustered in module 1 through negative internal interactions. Module 2 mainly consisted of OTUs including *unclassified_o_Lactabacillales*, *Streptococcus*, and *UCG-005* through positive internal interactions (Fig. 3E). In Yorkshire pigs (Fig. 3C), dominant bacteria such as *Muribaculaceae*, *Oscillospiraceae_UCG-002*, and *Streptococcus* were agglomerated in module 1 through negative internal interactions. While module 2 was dominated by genera such as *Terrisporobacter*, *Clostridium_sensu_stricto_1*, and *UCG-005*, which dominate through positive intra-interactions. However, in Landrace pigs and Yorkshire pigs, the interaction of each OTU in each module did not show significant associations ($p > 0.05$) with BF thickness (Fig. 3F).

Functional analysis of the gut microbiome among three pig breeds

Functional capacity of gut microbiome can reflect the metabolize status of the host at certain stages. Here, a total of 29 differentially enriched KEGG pathways were

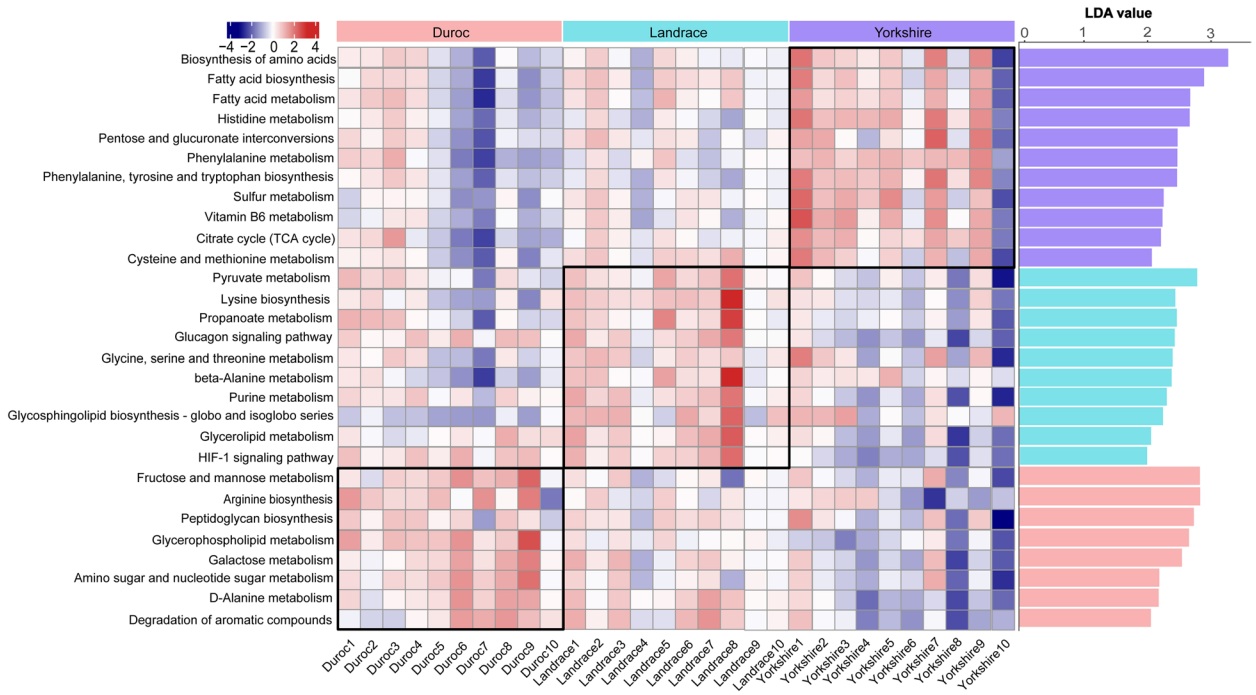

Fig. 4 The differential KEGG pathways were identified among Duroc, Landrace and Yorkshire pigs. The bar-plot (plink) represents the LDA value for KEGG pathways enriched in Duroc pigs. The bar-plot (cyan) represents LDA value for the KEGG pathways enriched in Landrace pigs. The bar-plot (violet) represents LDA value for the KEGG pathways enriched in Yorkshire pigs. LDA value > 2.0 and $p < 0.05$ was the significant threshold

detected in the gut microbiota among three pig breeds (Fig. 4). We identified that arginine biosynthesis, glycerophospholipid metabolism and D-Alanine metabolism were more active in the gut microbiome of the Duroc pigs. Simultaneously, in Landrace pigs, the gut microbiota was more capable of biosynthesis of pyruvate metabolism, lysine biosynthesis, glycerolipid metabolism and HIF-1 signaling pathway. In Yorkshire pigs, it was mainly enriched in the pathways of phenylalanine metabolism, phenylalanine, tyrosine and tryptophan biosynthesis, sulfur metabolism and vitamin B6 metabolism.

Association analysis of gut microbiota with KEGG functions

As shown in Fig. 5, we found that in Duroc pigs, *Lactobacillus* was the only genus positively associated with arginine biosynthesis. *Rikenellaceae_RC9_gut_group* had the most correlation relationships with KEGG pathways including glycerophospholipid and galactose metabolisms. ($p < 0.05$). In Landrace pigs, *Oscillospira* was negatively correlated with glycerolipid metabolism ($p < 0.05$), while *Bacteroidales_RF16_group* was positively correlated with the HIF-1 signaling pathway, purine, and glycerolipid metabolism ($p < 0.05$). In Yorkshire pigs, *Muribaculaceae* was positively correlated ($p < 0.05$) with phenylalanine metabolism and citrate cycle (TCA cycle). Importantly, we found that *Prevotellaceae_NK3B31_group*, an important genus associated with persistent inflammation in the host [26], had the greatest correlation with pathways, especially fatty acid metabolism and biosynthesis ($p < 0.05$).

Overview of metabolome data and SCMs identification

LCMS/MS was performed to investigate the differential composition of metabolites in feces from Duroc, Landrace and Yorkshire pigs. The PCA plot corresponding to the three different pig breeds showed significant differences among each pig breed (Fig. 6A). Similarly, OPLS-DA analysis demonstrated significant differences between each pig species (Fig. 6B). Overall, a total of 415 SCMs (169 up-regulated, 246 down-regulated) were filtered in Duroc vs. Landrace (Fig. 6C, Table S3); 319 SCMs (238 up-regulated, 81 down-regulated) were identified in Landrace vs. Yorkshire (Fig. 6D, Table S4); and 508 SCMs (241 up-regulated, 267 down-regulated) were screened in Duroc vs. Yorkshire (Fig. 6E, Table S5). Finally, the heatmap revealed the expression pattern of metabolites among different pig breeds (Fig. 6F). A total of 37 (Table S6), 15 (Table S7), and 33 (Table S8) SCMs were upregulated at Duroc, Landrace, and Yorkshire pigs. respectively.

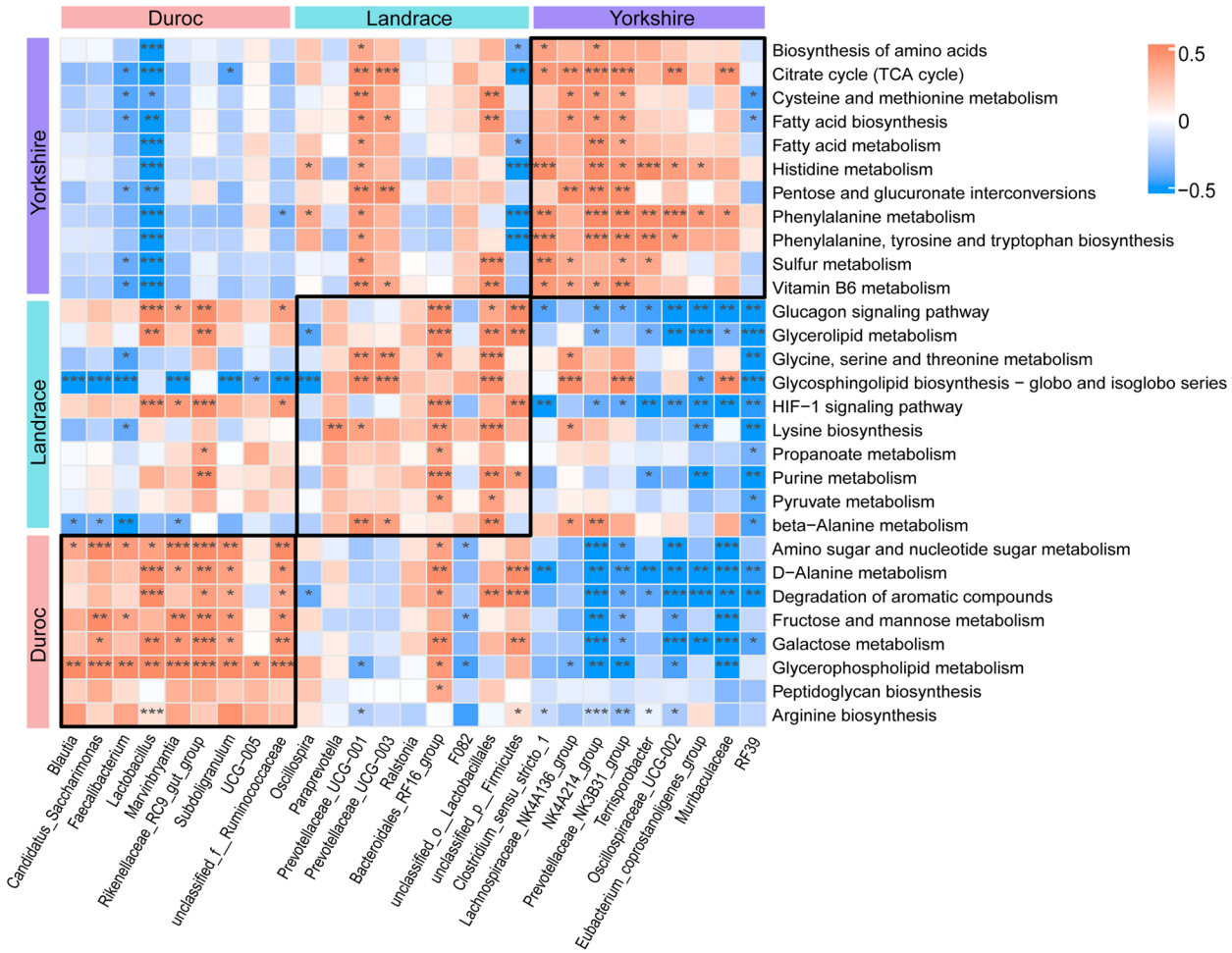

Fig. 5 Heatmap showing the correlations between breed-associated OTUs and KEGG pathways. "*" FDR adjusted $p < 0.05$, "**" FDR adjusted $p < 0.01$, and "***" FDR adjusted $p < 0.001$

KEGG enrichment analysis of SCMs

We found that among the three pig breeds, the breed-related monomers of Duroc pigs mainly belonged to fatty acyl (31.4%) and isochromoquinone (22.9%) (Fig. 7A). In Landrace pigs, were mainly annotated to organooxygen compounds (20.0%) and purine nucleosides (20.0%) (Fig. 7B). In Yorkshire pigs, it was mainly belonged to fatty acyls (30.3%), furans (12.1%), isochromanequinones (12.1%), organooxygen compounds (12.1%), and benzene and substituted derivatives (12.1%) (Fig. 7C).

Next, we performed KEGG functional enrichment analysis based on breeds-associated SCMs. In Duroc pigs, SCMs were mainly engaged in the cGMP signaling pathway, arginine and proline metabolism, and mTOR signaling pathway (Fig. 7D, $p < 0.05$). In Landrace pigs, SCMs have participated in fatty acid elongation, degradation and biosynthesis (Fig. 7E, $p > 0.05$). In Yorkshire pigs, SCMs were annotated in the estrogen signaling pathway, GnRH secretion, and prolactin signaling pathway (Fig. 7F, $p < 0.05$).

The mediation analysis for gut microbiota, fecal metabolites and BF thickness

Mediation analyses were used to assess potential associations of gut microbiota affecting BF thickness by the mediation roles of metabolites. In Duroc pigs (Fig. 8A), *Lactobacillus* and *Subdoligranulum* were associated with BF thickness via spermidine and both showed negative correlations ($p < 0.01$). In Landrace pigs (Fig. 8B), there were no significant associations between *Oscillospira* and estradiol ($p > 0.05$), but the total effect showed a positive correlation ($p < 0.05$); *Lactobacillales* significantly affected the BF thickness with the existence of estradiol, the total effect showed a positive correlation ($p < 0.01$). In Yorkshire pigs (Fig. 8C), with the presence of palmitic acid, *Muribaculaceae* highly significantly

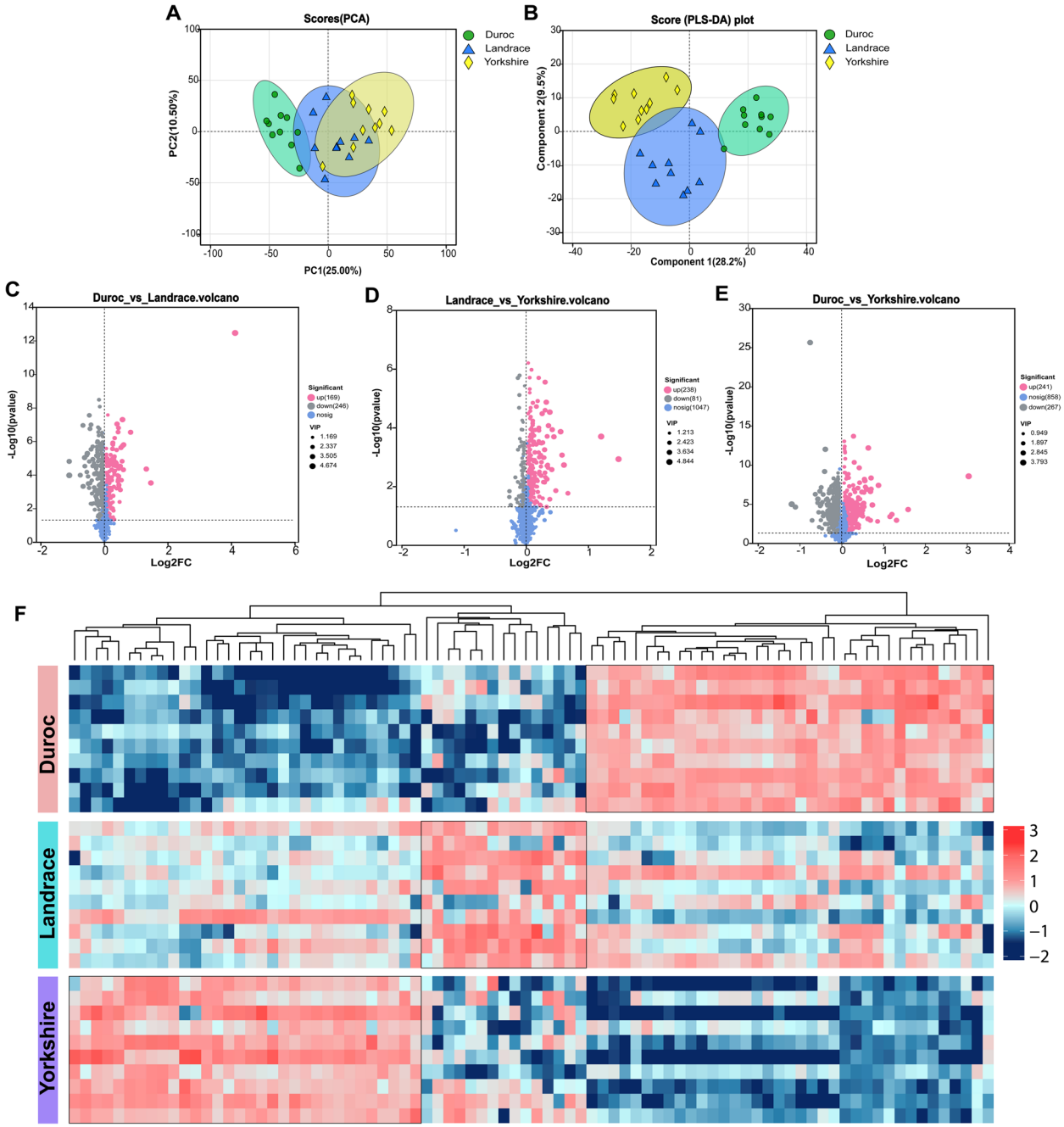

Fig. 6 The faecal metabolome analysis among three commercial pig breeds. **A**, PCA plot of metabolomic profiles among three groups (Duroc, Landrace, and Yorkshire). **B**, OPLS-DA plot for metabolomic profiles from three groups (Duroc, Landrace, and Yorkshire). **C**-**E**, Volcano plots of significantly changed metabolites (SCMs) among the three comparison groups (Duroc vs. Landrace, Landrace vs. Yorkshire, and Duroc vs. Yorkshire). **F**, Cluster heatmap of shared SCMs identified from three pig breeds. Colors with plink, cyan, and violet represent Duroc, Landrace, and Yorkshire, respectively

affected the BF thickness ($p < 0.01$). In contrast, for *Oscillospiraceae_UCG-002*, although there was no significant association between palmitic acid, the total effect showed a positive association ($p < 0.01$).

Discussion

The important roles of gut microbiota and metabolites in livestock are constantly being confirmed [27], particularly concerning energy metabolism, gut barrier function, and immune system, which are closely associated with host

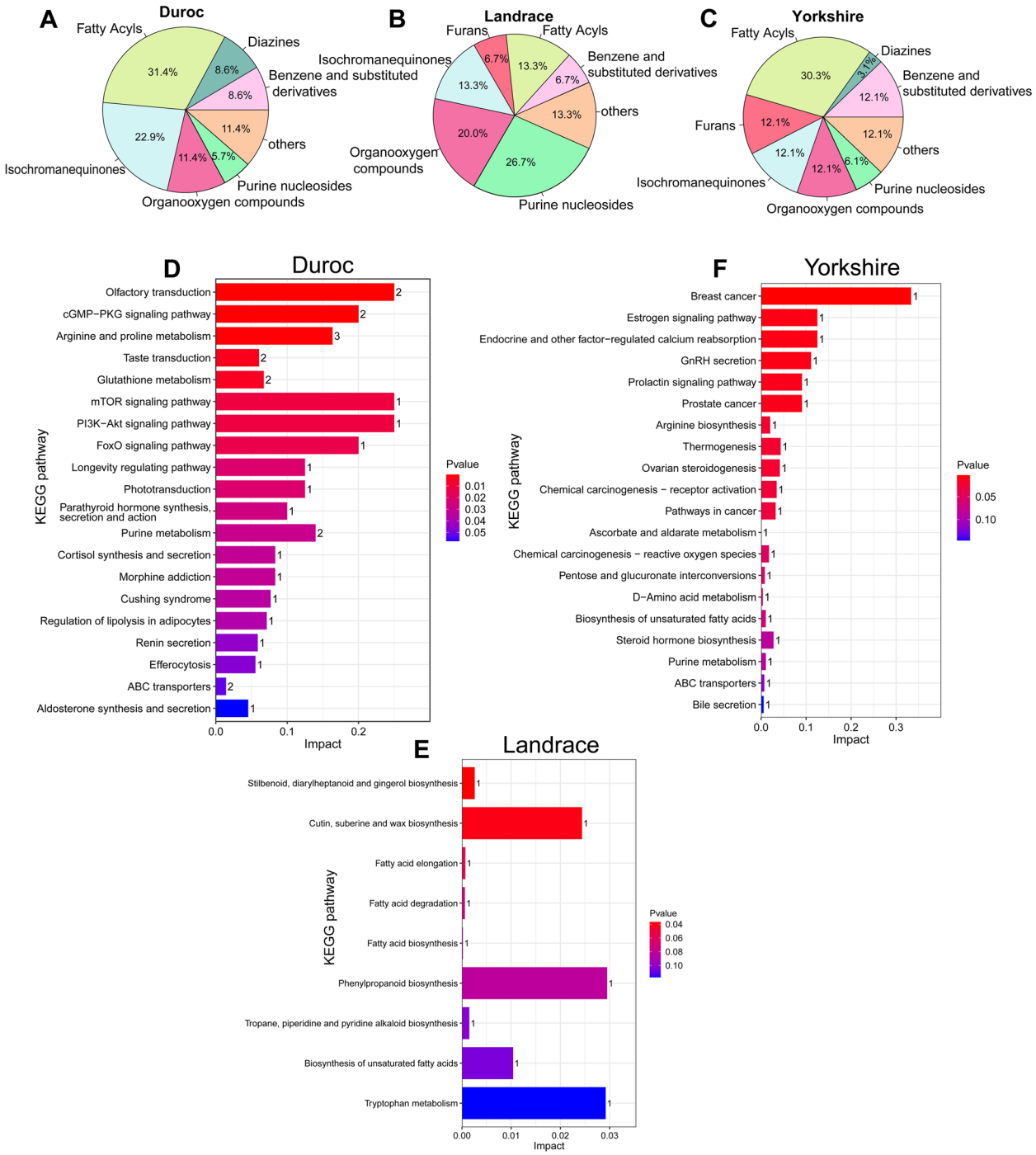

Fig. 7 The classification and functional analysis of breed-associated SCM among Duroc, Landrace and Yorkshire pigs. **A**-**C**, Overview for the classification of breed-associated SCMs in three groups (Duroc, Landrace, and Yorkshire). **D**-**F** The KEGG pathway analysis among Duroc, Landrace and Yorkshire pigs based on breed-associated SCMs

health. As globally popular pig breeds, Duroc, Landrace, and Yorkshire pigs possess different excellent phenotypic traits, particularly in growth rate, BF thickness, and litter size. Insights into breed-associated effects in the gut microbiota and metabolites might contribute to exploring the potential probiotics and improving animal health and productivity [27]. Therefore, we conducted a study of the gut microbiome and fecal metabolome in three

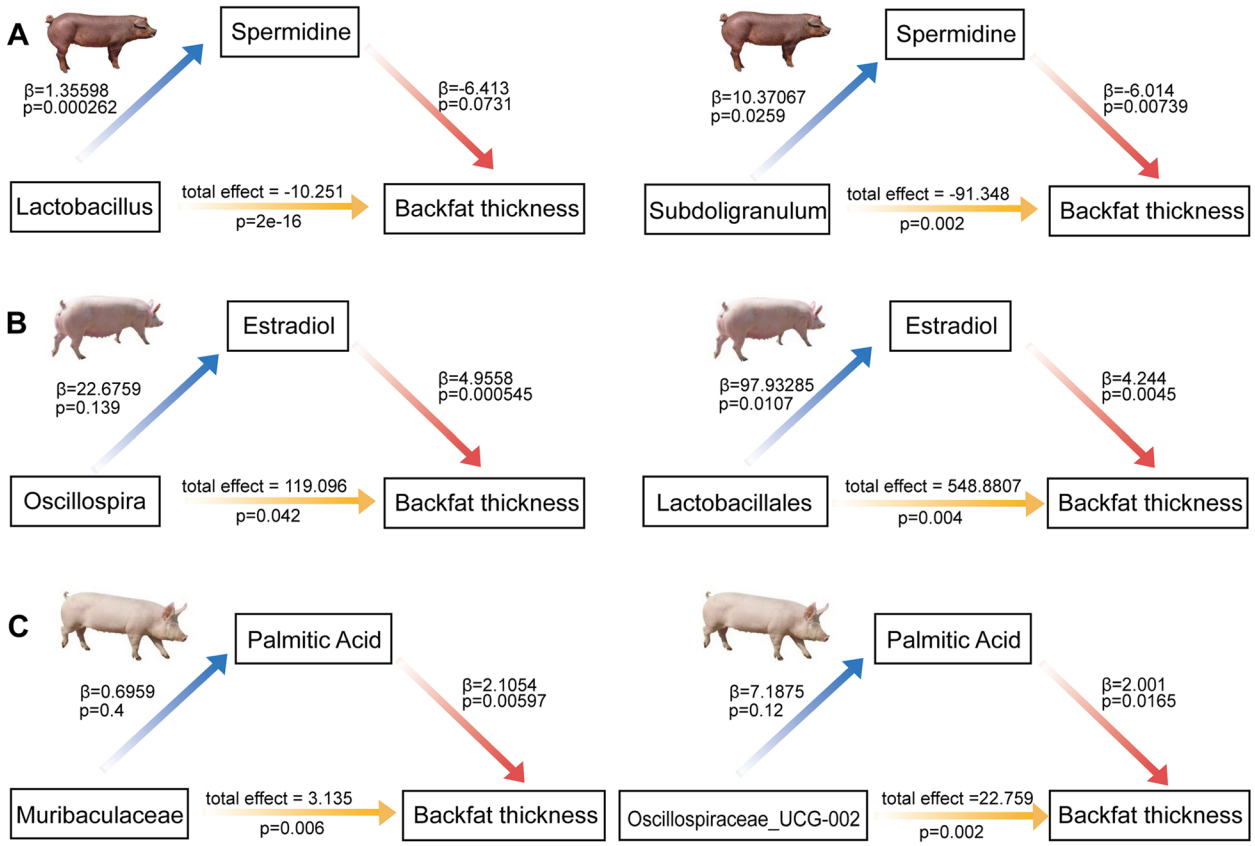

Fig. 8 The mediation analysis identifies the linkages in the microbial biomarkers, key metabolites, and BF thickness. The arrows represent the associations between the factors, and the total effect is a direct estimation of the effect on BF through gut microbiota, with Spearman coefficients and *p* values shown

breeds of Duroc, Landrace, and Yorkshire pigs to explore what gut microbial biomarkers and fecal metabolites play important roles in affecting BF thickness.

We evaluated whether breed factors would alter the gut microbiota structure, and we found that the host breed had a significant impact on the gut microbiota structure, similar to the study by Kylie et al. [28]. The Chao1 index and BF thickness of Landrace and Yorkshire pigs were significantly higher than those of Duroc pigs. Previous studies have shown significant differences in these microbial diversity indices among different chicken and horse breeds [12, 29]. Similar to a previous study, our work showed that *Lactobacillus* was the dominant biomarker in Duroc pigs compared with Landrace and Yorkshire pigs, indicating that it might play important roles in the growth and development of Duroc pigs [30]. Furthermore, this study indicated that in Duroc pigs, dominant species such as *Lactobacillus*, *Subdoligranulum*, and *Faecalibacterium* were negatively correlated with BF thickness, while *Rikenellaceae_RC9_gut_group* and *UCG-005* were positively correlated with it. A previous study showed that the proportion of *Lactobacillus* in the GI tract reflects the growth performance of pigs [31]. Some bacterial species, such as *Lactobacillus johnsonii* [32] and *Lactobacillus plantarum* [33] do belong to *Lactobacillus* indeed and are closely associated with lipid balance and body weight. Besides, *Faecalibacterium*, a butyrate-producing genus, has been reported to be crucial in improving insulin resistance and lipid metabolism disorder [34]. Interestingly, a recent study indicated that *Subdoligranulum*, a strictly anaerobic gram-negative microorganism and SCFA producer, was negatively correlated with insulin resistance, fat mass, and adipocyte diameter [35, 36]. All of these data indicated that the genera *Lactobacillus*, *Subdoligranulum*, and *Faecalibacterium* might the potential biomarkers for improving the BF thickness. As for the *Rikenellaceae RC9 gut group*, a genus related to severe intestinal inflammation, fatty acids production, and abnormal lipid metabolism [37–40]. *Oscillospiraceae_UCG-005* has been revealed that positively associated with fatty acids such as palmitic acid and pentadecanoic acid [41].

KEGG predictions analysis using PICRUSt revealed that genes involved in arginine biosynthesis metabolism

were more abundant in Duroc pig, and *Lactobacillus* were positively correlated with arginine biosynthesis metabolism. Indeed, previous studies suggested that lactic acid bacteria (e.g., *Lactobacillus plantarum* and *Lactobacillus buchneri*) were capable of producing arginine [42, 43]. Additionally, dietary L-arginine supplementation reduced white adipose tissue and abdominal fat content of pig and broiler chickens, respectively [44, 45]. Herein, our data revealed a negative correlation between *Lactobacillus* and BF thickness, and speculated that *Lactobacillus* may produce arginine which in turn affects BF thickness, nevertheless, more verification is needed. In Landrace pigs, *Oscillospira* showed a negative correlation with glycerolipid metabolism, while *Bacteroidales_RF16_group* showed a positive correlation with purine and glycerolipid metabolism. A recent study discovered that *Oscillospira* was inversely correlated with triglyceride and fasting blood glucose, indicating its potential functions in improving obesity [46]. While for *Bacteroidales_RF16_group*, it was common in a range of ruminants [47], and positively associated with propionate and acetate [48]. A recent study found that it was enriched in high-finishing weight pigs [49]. However, the mechanism of *Bacteroidales_RF16_group* metabolism is not yet clear. In Yorkshire pigs, *Muribaculaceae* was positively correlated with phenylalanine, tyrosine, and tryptophan biosynthesis. Consistent with our study, a recent study found that metabolites including l-phenylalanine, l-tyrosine, and l-tryptophan were positively correlated with *Muribaculacea* [50], which further indicated that *Muribaculacea* might play a vital role in the biosynthesis of above-related metabolites. In conclusion, there are obvious differences in the functional characteristics of the intestinal flora among the three pig breeds. This result provides important information for promoting the health and production performance of different pig breeds by clarifying the functions of the intestinal flora.

To determine the breed-related microbial metabolites and further understand the potential interaction of microbiota, metabolites, and host phenotypes. We investigated the characteristics of fecal metabolomics. We found that most breed-associated SCMs mainly belonged to fatty acids, isochromanequinones, and purine nucleosides. Similarly, the KEGG analysis of fecal metabolites showed that arginine metabolism enriched in the Duroc pig. Spermidine is an arginine metabolite [51], and *Lactobacillus* has also been proven to be one of the main producers of spermidine [52]. The mediation analysis indicated that *Lactobacillus* and *Subdoligranulum* in Duroc pigs showed a negative correlation with BF thickness with the presence of spermidine. As we discussed above, *Lactobacillus* was a main spermidine producer and exerted beneficial roles in domestic animals, especially in lipid metabolism. These results indicated that certain bacterial strains from *Lactobacillus* could be important in reducing the BF thickness. In the Landrace pigs, *Oscillospira* and *Lactobacillales* exhibited a positive correlation with BF thickness under the mediation role of estradiol. In Yorkshire pigs, *Muribaculaceae* and *Oscillospiraceae_UCG-002* could affect BF thickness with the existence of palmitic acid. Previous studies have found that estradiol is positively correlated with fat mass and BMI, suggesting that it plays a key role in promoting obesity [53]. A higher abundance of *Oscillospira* was detected in mice with gut microbial dysbiosis and was highly associated with obesity [54]. The relative abundance of *Lactobacillales* was significantly correlated with growth performance [55], and when the concentration of estradiol was high, *lactobacillus* was predominant [56, 57]. In rats, an increased abundance of intestinal *Muribaculaceae* led to a decrease in the levels of palmitic acid in the serum [58]. A decrease in the content of intestinal *Oscillospiraceae_UCG-002* promoted lipid metabolism and reduced fat deposition [59]. These results suggested that breed effects can influence the composition of gut microbiota and fecal metabolites and that interactions between gut microbiota and metabolites can influence host phenotypes. However, further studies would warrant confirming the function roles of the above bacteria such as *Lactobacillus*, *Subdoligranulum*, and *Oscillospira* in the production performance of domestic livestock.

Our study has limitations, as we only analyzed three breeds of pigs, but it provides important evidence for the influence of host breeds on the gut microbiota and fecal metabolome of pigs. It also revealed certain gut bacteria and fecal metabolites that could be considered as candidate biomarkers for pig breed improvement. In this context, further studies are needed to elucidate the potential mechanisms by which host species modulate the gut microbiota and fecal metabolome. In addition, future studies should use metagenomics and metatranscriptomics to explore more bacterial species and functional capabilities that may affect livestock growth performance.

Conclusion

In summary, this work showed that commercial pig breeds, including Duroc, Yorkshire and Landrace pigs, possessed disparate gut microbiota profiles, which might be greatly influenced by their genotype and phenotype. Similarly, further metabolomics studies also revealed the distinct metabolites profile between the three breeds, which play vital roles in regulating lipid metabolism homeostasis. The mediation analysis between microbial biomarkers, key metabolites, and BF thickness showed that there was a close relationship between the effects of microbial-derived products on host phenotypes.

Despite this study has some limitations, the findings have important enlightenments for commercial pig breed improvement.

Abbreviations

16S rRNA	16S ribosomal ribonucleic acid
OTU	Operational taxonomic unit
RDA	Redundancy analysis
LEfSe	Linear discriminant analysis effect size
KEGG	Kyoto Encyclopedia of Genes and Genomes
FDR	False discovery rate
QC	Quality control
PLS-DA	Partial least squares discriminant analysis
SCMs	Significantly changed metabolites

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-024-04308-0.

Additional file 1: Table S1. Summary of 16S rRNA gene sequencing data in faecal samples for Duroc, Landrace, and Yorkshire pigs. Table S2. Comparison of Shannon and Chao1 indices for three groups. Figure S3. Significantly changed metabolites (SCMs) identified from the comparison group Duroc vs. Landrace. Figure S4. Significantly changed metabolites (SCMs) identified from the comparison group Landrace vs. Yorkshire. Figure S5. Significantly changed metabolites (SCMs) identified from the comparison group Duroc vs. Yorkshire. Figure S6. Duroc-associated significantly changed metabolites (SCMs) identified by cluster heatmap. Figure S7. Landrace-associated significantly changed metabolites (SCMs) identified by cluster heatmap. Figure S8. Yorkshire-associated significantly changed metabolites (SCMs) identified by cluster heatmap.

Authors' contributions

Conceptualization, H.M.; data curation, S.LF.; formal analysis, K.W., B.C.; and X.L.(Xiaolin Liu).; investigation, W.C., X.L.(Xintong Li)., and S.W.; methodology, S.LF.; writing— original draft, S.LF.; writing—review and editing: D.X., H.M. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by the design and cultivation of the new breed in pigs with high-quality meat (2023ZD04046-05), Major Science and Technology Special Plan of Yunnan Province (202202AE090032 and 202102AE090046-3), Postgraduate Scientific Research Innovation Project of Hunan Province (CX20230713), Postgraduate Scientific Research Innovation Project of Hunan Agriculture University (2023XC006), and the Hunan Provincial Department of Education Scientific Research Project (23B1097).

Availability of data and materials

Amplicon sequence data in this study was deposited to China National GeneBank (CNGB) under number CNP0005692 (http://db.cngb.org/cnsa/project/CNP0005692_0b98c629/reviewlink/).

Declarations

Ethics approval and consent to participate

All animal experiments in this study were approved by the Institutional Animal Care and Use Committee of Hunan Agricultural University, Changsha, Hunan Province, China, with approval number CACAHU 20230601. Written informed consent was obtained before sample collection from the commercial farm.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 29 May 2024 Accepted: 30 September 2024
Published online: 10 October 2024

References

1. Liu Y, Zhang Y, Zhou F, Yao Z, Zhan Y, Fan Z, et al. Increased accuracy of genomic prediction using preselected SNPs from GWAS with imputed Whole-Genome Sequence data in pigs. Animals (Basel). 2023;13(24):3871.
2. Wang D, Wei G, Yang Y, Zou Y, Li X, Shi Y, et al. Identification and molecular mechanism of novel bifunctional peptides from Duroc × (Landrace × Yorkshire) pig dry-cured ham: A peptidomics and in silico analysis. Food Res Int. 2024;180:114066.
3. Font-I-Furnols M, Albano-Gaglio M, Brun A, Tejeda JF, Gispert M, Marcos B, et al. The effect of immunocastration of male and female Duroc pigs on the morphological, mechanical and compositional characteristics of pork belly. Meat Sci. 2023;204:109263.
4. Zhang J, Meng S, Wang H, Zhang C, Sun Z, Huang L, et al. Comparison of growth performance, carcass properties, fatty acid Profile, and genes involved in fat metabolism in Nanyang and Landrace Pigs. Genes (Basel). 2024;15(2):186.
5. Park J. Genome-wide association study to reveal new candidate genes using single-step approaches for productive traits of Yorkshire pig in Korea. Anim Biosci. 2024;37(3):451–60.
6. Lan Q, Liufu S, Liu X, Ai N, Xu X, Li X, et al. Comprehensive analysis of transcriptomic and metabolomic profiles uncovered the age-induced dynamic development pattern of subcutaneous fat in Ningxiang pig. Gene. 2023;880:147624.
7. Bergamaschi M, Tiezzi F, Howard J, Huang YJ, Gray KA, Schillebeeckx C, et al. Gut microbiome composition differences among breeds impact feed efficiency in swine. Microbiome. 2020;8(1):110.
8. Zmora N, Suez J, Elinav E. You are what you eat: diet, health and the gut microbiota. Nat Rev Gastroenterol Hepatol. 2019;16(1):35–56.
9. Canibe N, O'Dea M, Abraham S. Potential relevance of pig gut content transplantation for production and research. J Anim Sci Biotechnol. 2019;10:55.
10. Goodrich JK, Davenport ER, Waters JL, Clark AG, Ley RE. Cross-species comparisons of host genetic associations with the microbiome. Science. 2016;352(6285):532–5.
11. Mancin E, Maltecca C, Huang YJ, Mantovani R, Tiezzi F. A first characterization of the microbiota-resilience link in swine. Microbiome. 2024;12(1):53.
12. Pandit RJ, Hinsu AT, Patel NV, Koringa PG, Jakhesara SJ, Thakkar JR, et al. Microbial diversity and community composition of caecal microbiota in commercial and indigenous Indian chickens determined using 16s rDNA amplicon sequencing. Microbiome. 2018;6(1):115.
13. Qin J, Wei X, Cao M, Shi B. Non-12α-Hydroxylated bile acids improve piglet growth performance by improving intestinal flora, promoting intestinal development and bile acid synthesis. Animals (Basel). 2023;13(21):3380.
14. Wang J, Liu Y, Yang Y, Bao C, Cao Y. High-level expression of an acidic thermostable xylanase in Pichia pastoris and its application in weaned piglets. J Anim Sci. 2020;98(1):skz364.
15. Huang A, Cai R, Wang Q, Shi L, Li C, Yan H. Dynamic change of gut microbiota during porcine epidemic diarrhea virus infection in suckling piglets. Front Microbiol. 2019;10:322.
16. Hollywood K, Brison DR, Goodacre R. Metabolomics: current technologies and future trends. Proteomics. 2006;6(17):4716–23.
17. Liao Y, Hu R, Wang Z, Peng Q, Dong X, Zhang X, et al. Metabolomics profiling of serum and urine in three beef cattle breeds revealed different levels of tolerance to heat stress. J Agric Food Chem. 2018;66(26):6926–35.
18. Wu J, Ye Y, Quan J, Ding R, Wang X, Zhuang Z, et al. Using nontargeted LC-MS metabolomics to identify the Association of Biomarkers in pig feces with feed efficiency. Porcine Health Manag. 2021;7(1):39.
19. Bovo S, Mazzoni G, Galimberti G, Calò DG, Fanelli F, Mezzullo M, et al. Metabolomics evidences plasma and serum biomarkers differentiating two heavy pig breeds. Animal. 2016;10(10):1741–8.
20. Gasaly N, de Vos P, Hermoso MA. Impact of bacterial metabolites on gut barrier function and host immunity: A focus on bacterial metabolism and its relevance for intestinal inflammation. Front Immunol. 2021;12:658354.

21. Liu L, Deng L, Wei W, Li C, Lu Y, Bai J, et al. *Lactiplantibacillus plantarum* LPJZ-658 improves Non-Alcoholic steatohepatitis by modulating bile acid metabolism and gut microbiota in mice. Int J Mol Sci. 2023;24(18):13997.
22. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7(5):335–6.
23. Magoc T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics. 2011;27(21):2957–63.
24. Wu T, Ding J, Zhao YJ, Ding L, Zang Y, Sun HJ, et al. Microplastics shaped performance, microbial ecology and community assembly in simultaneous nitrification, denitrification and phosphorus removal process. Sci Total Environ. 2024;929:172651.
25. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12(6):R60.
26. Madjirebaye P, Peng Z, Mueed A, Huang T, Peng F, Allasra Y, et al. Promising probiotic-fermented soymilk for alleviating acute diarrhea: insights into the microbiome and metabolomics. Food Funct. 2024;15(8):4462–74.
27. Maltecca C, Bergamaschi M, Tiezzi F. The interaction between microbiome and pig efficiency: A review. J Anim Breed Genet. 2020;137(1):4–13.
28. Kylie J, Weese JS, Turner PV. Comparison of the fecal microbiota of domestic commercial meat, laboratory, companion, and shelter rabbits (Oryctolagus cuniculi). BMC Vet Res. 2018;14(1):143.
29. Li Y, Zhang K, Liu Y, Li K, Hu D, Wronski T. Community composition and diversity of intestinal microbiota in captive and reintroduced Przewalski's Horse (Equus ferus przewalskii). Front Microbiol. 2019;10:1821.
30. Wei L, Zeng B, Zhang S, Guo W, Li F, Zhao J, et al. Hybridization altered the gut microbiota of pigs. Front Microbiol. 2023;14:1177947.
31. Li XJ, Wang M, Xue Y, Duan D, Li C, Han X, et al. Identification of microflora related to growth performance in pigs based on 16S rRNA sequence analyses. AMB Express. 2020;10(1):192.
32. Wang H, Ni X, Qing X, Zeng D, Luo M, Liu L, et al. Live Probiotic Lactobacillus johnsonii BS15 Promotes growth performance and lowers fat deposition by improving lipid metabolism, intestinal development, and gut microflora in broilers. Front Microbiol. 2017;8:1073.
33. Crovesy L, Ostrowski M, Ferreira DMTP, Rosado EL, Soares-Mota M. Effect of *Lactobacillus* on body weight and body fat in overweight subjects: a systematic review of randomized controlled clinical trials. Int J Obes (Lond). 2017;41(11):1607–14.
34. Xuan W, Ou Y, Chen W, Huang L, Wen C, Huang G, et al. *Faecalibacterium prausnitzii* improves lipid metabolism disorder and insulin resistance in type 2 diabetic mice. Br J Biomed Sci. 2023;80:10794.
35. Van Hul M, Le Roy T, Prifti E, Dao MC, Paquot A, Zucker JD, et al. From correlation to causality: the case of *Subdoligranulum*. Gut Microbes. 2020;12(1):1–13.
36. Gradisteanu Pircalabioru G, Liaw J, Gundogdu O, Corcionivoschi N, Ilie I, Oprea L, et al. Effects of the lipid profile, type 2 diabetes and medication on the metabolic syndrome-associated gut microbiome. Int J Mol Sci. 2022;23(14):7509.
37. Wang Y, Li T, Chen X, Liu C, Jin X, Tan H, et al. Preliminary investigation of mixed Orchard Hays on the meat quality, fatty acid profile, and gastrointestinal microbiota in goat kids. Animals (Basel). 2022;12(6):780.
38. Wang Z, Li X, Zhang L, Wu J, Zhao S, Jiao T. Effect of oregano oil and cobalt lactate on sheep in vitro digestibility, fermentation characteristics and rumen microbial community. Animals (Basel). 2022;12(1):118.
39. Wen X, Wan F, Wu Y, Liu L, Liu Y, Zhong R, et al. Caffeic acid supplementation ameliorates intestinal injury by modulating intestinal microbiota in LPS-challenged piglets. Food Funct. 2023;14(16):7705–17.
40. Chen M, Zhao Y, Li S, Chang Z, Liu H, Zhang D, et al. Maternal malic acid may ameliorate oxidative stress and inflammation in sows through modulating gut microbiota and host metabolic profiles during late pregnancy. Antioxidants (Basel). 2024;13(2):253.
41. Wu ZL, Yang X, Zhang J, Wang W, Liu D, Hou B, et al. Effects of forage type on the rumen microbiota, growth performance, carcass traits, and meat quality in fattening goats. Front Vet Sci. 2023;10:1147685.
42. Manca de Nadra MC, Pesce de Ruiz Holgado AA, Oliver G. Arginine dihydrolase pathway in Lactobacillus buchneri: a review. Biochimie. 1988;70(3):367–74.
43. Nicoloff H, Arsène-Ploetze F, Malandain C, Kleerebezem M, Bringel F. Two arginine repressors regulate arginine biosynthesis in Lactobacillus plantarum. J Bacteriol. 2004;186(18):6059–69.
44. Tan B, Yin Y, Liu Z, Li X, Xu H, Kong X, et al. Dietary L-arginine supplementation increases muscle gain and reduces body fat mass in growing-finishing pigs. Amino Acids. 2009;37(1):169–75.
45. Fouad AM, El-Senousey HK, Yang XJ, Yao JH. Dietary L-arginine supplementation reduces abdominal fat content by modulating lipid metabolism in broiler chickens. Animal. 2013;7(8):1239–45.
46. Chen YR, Zheng HM, Zhang GX, Chen FL, Chen LD, Yang ZC. High *Oscillospira* abundance indicates constipation and low BMI in the Guangdong Gut Microbiome Project. Sci Rep. 2020;10(1):9364.
47. Gruninger RJ, Zhang XM, Smith ML, Kung L Jr, Vyas D, McGinn SM, et al. Application of 3-nitrooxypropanol and canola oil to mitigate enteric methane emissions of beef cattle results in distinctly different effects on the rumen microbial community. Anim Microbiome. 2022;4(1):35.
48. Li L, Qu J, Zhu H, Liu Y, Wu J, Shao G, et al. Effects of feeding different levels of dietary corn silage on growth performance, rumen fermentation and bacterial community of post-weaning dairy calves. Anim Biosci. 2024;37(2):261–73.
49. Lan Q, Lian Y, Peng P, Yang L, Zhao H, Huang P, et al. Association of gut microbiota and SCFAs with finishing weight of Diannan small ear pigs. Front Microbiol. 2023;14:1117965.
50. Yu Y, Zeng F, Han P, Zhang L, Yang L, Zhou F, et al. Dietary chlorogenic acid alleviates high-fat diet-induced steatotic liver disease by regulating metabolites and gut microbiota. Int J Food Sci Nutr. 2024;75(4):369–84.
51. Zhang D, Li J, Li T. Agmatine mitigates palmitate (PA)-induced mitochondrial and metabolic dysfunction in microvascular endothelial cells. Hum Exp Toxicol. 2022;41:9603271221110856.
52. Borgogna JC, Shardell MD, Grace SG, Santori EK, Americus B, Li Z, et al. Biogenic amines increase the odds of bacterial vaginosis and affect the growth of and lactic acid production by vaginal *Lactobacillus* spp. Appl Environ Microbiol. 2021;87(10):e03068–e3120.
53. Wu A, Shi Z, Martin S, Vincent A, Heilbronn L, Wittert G. Age-related changes in estradiol and longitudinal associations with fat mass in men. PLoS ONE. 2018;13(8):e0201912.
54. Yang J, Li Y, Wen Z, Liu W, Meng L, Huang H. *Oscillospira* - a candidate for the next-generation probiotics. Gut Microbes. 2021;13(1):1987783.
55. Wang C, Wei S, Xu B, Hao L, Su W, Jin M, et al. Bacillus subtilis and Enterococcus faecium co-fermented feed regulates lactating sow's performance, immune status and gut microbiota. Microb Biotechnol. 2021;14(2):614–27.
56. Mitchell CM, Ma N, Mitchell AJ, Wu MC, Valint DJ, Proll S, et al. Association between postmenopausal vulvovaginal discomfort, vaginal microbiota, and mucosal inflammation. Am J Obstet Gynecol. 2021;225(2):159.e1–159.e15.
57. Lee J, Peesh P, Quaicoe V, Tan C, Banerjee A, Mooz P, et al. Estradiol mediates colonic epithelial protection in aged mice after stroke and is associated with shifts in the gut microbiome. Gut Microbes. 2023;15(2):2271629.
58. Zhang Y, Song H, Liu Z, Ai C, Yan C, Dong X, et al. Interaction between a Sulfated Polysaccharide from Sea Cucumber and Gut Microbiota Influences the Fat Metabolism in Rats. Foods. 2023;12(24):4476.
59. Hui D, Liu L, Azami NLB, Song J, Huang Y, Xu W, et al. The spleen-strengthening and liver-draining herbal formula treatment of non-alcoholic fatty liver disease by regulation of intestinal flora in clinical trial. Front Endocrinol (Lausanne). 2023;13:1107071.

Publisher's Note