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Variation of Meat Quality and Relationship to Gut Microbiota Among Different Pig Breeds

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ABSTRACT

Meat production is of great importance to the world's food supply and economic development, and meat quality determines the purchasing desire of consumers. Recent studies show intestinal microorganisms are involved in several physiological functions of the host and therefore are likely to regulate meat quality. This study aimed to compare the carcass performance, meat quality traits and serum parameters of three different pig breeds, Jinhua (JH) pigs ($n=8$), Duroc×Berkshire×Jiaxinghei (DBJ) pigs ($n=8$), Duroc×Landrace×Yorkshire (DLY) pigs ($n=8$) and to investigate a possible relationship between gut microbiota composition and these traits. Meat quality results showed that compared with DLY pigs, JH pigs had lower water loss and shear force in the longissimus dorsi muscle, and higher intramuscular fat content and inosine monophosphate content were observed in JH pigs. Serum biochemical indicators showed the content of nonesterified fatty acid in JH pigs was lowest. Furthermore, the gut microbiota analysis indicated that JH pigs harboured more abundant *Lachnospiraceae*, *Prevotellaceae NK3B31* and *Marvinbryantia*. Spearman correlation analysis showed that *unidentified genus of family Lachnospiraceae*, *genus Prevotella* and *genus Alloprevotella* were positively correlated with IMF content and marbling score in the longissimus dorsi muscle of pigs. In conclusion, our results indicated the quality of JH pork was superior to DBJ and DLY pigs, and the difference in meat qualities was related to the abundance of fibre-degrading bacteria. Our study provides insight into further understanding of the relationship between microbiota and meat quality, nutrient metabolism and fat deposition, which is critical to the pork industry and swine intestinal health.

1 | Introduction

Meat is one of the most important sources of protein in the human diet and an indispensable food in many cultures; therefore, the nutritional value of meat products has received much attention (Chen et al. 2022a). In a narrow sense, the evaluation of pork quality includes various traits such as meat colour, marbling, pH value, drip loss and so on (Thorslund et al. 2016). Among them,

Abbreviations: ASV, Amplicon sequence variant; DBJ, Duroc×Berkshire×Jiaxinghei pigs; DLY, Duroc×Landrace×Yorkshire pigs; Glob, Globulin; HDL, High-density lipoprotein; HPLC, High-performance liquid chromatography; IMF, Intramuscular fat; IMP, Inosine monophosphate acid; JH, Jinhua pigs; KEGG, Kyoto Encyclopedia of Genes and Genomes databases; LDL, Low-density lipoprotein; LEFSe, LDA Effect Size analysis; NEFA, Nonesterified fatty acid; PCoA, Principal Coordinate Analysis.

pH is essential for the technological and sensory quality of pork (Martins et al. 2020), the marbling score reflects the deposition of adipose tissue within muscle (Bazile et al. 2019) and higher intramuscular fat (IMF) is a key factor influencing pork quality, which affects the tenderness, flavour and juiciness of the meat, directly determining the consumer's desire to buy (Xie et al. 2021).

Several studies indicate that meat quality is regulated by various internal and external factors such as pig breed, diet and growth environment. Pigs of different genotypes have different growth performance and meat quality potential. Compared with European breeds, Chinese indigenous pig breeds have higher IMF and better meat quality. As a famous local breed in China, Jinhua (JH) pig, also known as Jinhua two-headed black pig, has early sexual maturity, good meat quality and strong reproductive performance (Li et al. 2016). Comparing the developmental changes in carcass composition and meat quality traits between JH pigs and Landrace pigs, the researchers found IMF content at 125 days of age was 31.25% greater in JH pigs than in Landrace pigs, and marbling score at 35, 80 and 125 days of age was 50.48%, 50.00% and 28.92% higher in JH pigs than in Landrace pigs, respectively(Miao et al. 2009).

On the other hand, intestinal microorganisms are complex microbial communities that colonise the digestive tract of animals, with significant individual variability, and their composition and distribution have strong spatiotemporal specificity(Fan et al. 2021). Host genetics and the lean/obese nature of a particular pig breed play an important role in the gut microbiome profiles, and the number of specific microbes varies among different pig breeds(Patil et al. 2020). Studies revealed that high-fat pigs had a richer abundance of Archaeal species with methanogenesis functions, leading to more efficient fat deposition (Zhao et al. 2022). In addition, animal gut microbial communities play an important role in the absorption and utilisation of nutrients; studies showed that intestinal microorganisms can cause fat deposition or obesity and are involved in the regulation of muscle fibre development(Bäckhed et al. 2007; le Roy et al. 2013), which has potential value in the regulation of meat quality.

However, the differences in meat quality and microbial community among different pig breeds are still unclear, and further systematic research is needed. In this study, three different breeds of pigs were compared, including JH pigs (purebred Chinese indigenous swine), Duroc × Berkshire × Jiaxinghei (DBJ) pigs (crossbred between Western swine and indigenous swine) and Duroc × Landrace × Yorkshire (DLY) pigs (Western commercial hybrid swine), and the purpose of this study was to investigate if the regulatory role of the intestinal microorganisms may play in regulating meat quality and fat deposition, and search for the biomarkers associated with excellent meat quality traits.

2 | Materials and Methods

2.1 | Animal Ethics, Slaughter Procedure and Sample Collection

All the animal procedures applied in this study were approved by the Institutional Animal Care and Use Committee at Zhejiang University (Hangzhou, China). All the animal procedures involving live swine handling, management and slaughter applied in this study were approved by the Committee on Animal Care and Use and Committee on the Ethics of Animal Experiments of Zhejiang University (ZJU20001).

The experiment was conducted at the slaughterhouse of Qinglian Food Co (Zhejiang, China). Eight 180-day-old JH pigs, DBJ pigs and DLY pigs were randomly selected for slaughter and sampled on 1 day. All pigs were raised in the same environment and had *ad libitum* access to the same feed and water. The formulation and chemical composition of the pig diet are presented in Table 1. After euthanasia by electric shock, carcass weight was measured and the three-point backfat thickness was measured with callipers, and the mean was calculated. The longissimus dorsi muscle was collected and stored at 4°C and −20°C for subsequent meat quality measurement and amino

TABLE 1 | Ingredients and chemical composition of diet offered (%, as-fed basis).

Items	Values
Ingredients, %	
Maize	47.6
Soybean meal	8.5
Alfalfa, dehydrated	1.5
Wheat, soft	8.0
Barley	6.0
Wheat feed flour	4.0
Rice bran, oil > 5%	16.0
Rice, polished, broken	5.0
Limestone	0.9
Premix	2.5
Chemical composition	
DM (%)	87.3
CP (%)	16.2
CF (%)	4.3
EE (%)	5.7
Ash (%)	5.0
NDF (%)	13.0
ADF (%)	4.7
Ca (%)	0.7
P (%)	0.8
DE (Kcal/kg)	3358.2
ME (Kcal/kg)	3226.1
GE (Kcal/kg)	3892.0

Abbreviations: ADF, acid detergent Fibre; Ca, total calcium; CF, crude fibre; CP, crude protein; DBJ, Duroc × Berkshire × Jiaxinghei pigs; DE, digestible energy; DLY, Duroc × Landrace × Yorkshire pigs; DM, dry matter; EE, ether extract; GE, gross energy; JH: Jinhua pigs; ME, metabolic energy; NDF, neutral detergent fibre; P, total phosphorus.

acid and fatty acid content determination, respectively. Blood was sampled and centrifuged to extract serum for subsequent serum biochemical tests. The digesta of the pig colon were collected, rapidly frozen in liquid nitrogen, and then subsequently transferred to a −80°C refrigerator for microbiological analysis.

2.2 | Chemical Analyses

The basal diet meets the NRC (2012) requirements for finishing pigs as shown in Table 1. The values of dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE), Ash, total Ca and total P were measured according to the National Standard of the People's Republic of China (GB/T 6435–2014, GB/T 6432–2018, GB/T 6434–2022, GB/T 6433–2006, GB/T 6438–2007, GB/T 6436–2018, GB/T 6437–2018). While the values of gross energy (GE), metabolic energy (ME), neutral detergent fibre (NDF) and acid detergent fibre (ADF) were calculated based on the tables of feed ingredients from INRA-CIRAD-AFZ feed tables (Bontems et al. 2004).

2.3 | Meat Quality

The meat characteristics were analysed included pH_{24h}, drip loss, marbling score, meat colour score, brightness (L*), redness (a*), yellowness (b*), colour difference value (CIE ΔE*), chroma (C*), IMF content and inosine monophosphate (IMP) content, using longissimus muscle from the left side of each carcass.

After 24 h of slaughter, the pH meter (Matthäus pH Star, Germany) was calibrated in standard phosphate buffers (pH = 4.00, 7.00 and 9.00); then the glass electrode was inserted in the centre of the cross section and read after the value stabilised according to the instrument's operational requirements.

Drip loss was examined to assess water holding capacity. The longest dorsal muscle was cut into a 2 × 2 × 2 cm square along the direction of the muscle fibres and weighed (W1); then muscle fibres were brought vertically downward and threaded through one end of the meat strip with a thin wire, suspended in a plastic bag (the meat sample could not touch the plastic bag wall). The bag was sealed with a rubber band and hung in a refrigerator at 4°C for 48 h. Then, the meat column was taken out and dried with the residual liquid on the surface and weighed (W2). The same meat sample was measured in four replicates, and the results were expressed as the average value.

At 24 h post mortem, meat colour and marbling scores were evaluated based on official colour and marbling quality standards (National Pork Board, Des Moines, IA USA). Meat lightness (CIE L*), redness (CIE a*) and yellowness (CIE b*) were subsequently measured with a colour difference meter (colorFlex, HunterLab, USA), and the colour difference value (CIE ΔE*) was calculated.

2.4 | Intramuscular Fat and Inosine Monophosphate Acid

The IMF content was calculated following the Soxhlet extraction method by weighing (W1) around 2 g of meat samples (excluding fascia), wrapping them in filter paper and drying to constant weight (W2), extracting with ether for 6 h, drying again to constant weight (W3) and calculating the IMF content according to the following formula(Huang et al. 2020; Latimer Jr. 2023; Zhang et al. 2019). Each sample was repeated three times and the average value was calculated.

$$X = (W2 - W3) / W1 \times 100$$

The analysis of IMP was modified from the method described by Tikk(Tikk et al. 2006). Pork samples (50 mg) and 5% perchloric acid (5 mL) were added to the centrifuge tube, homogenised for 10 s and centrifuged at 1699*g* for 5 min at 4°C. The samples were left on an ice bath for 15 min before 5 mL of perchloric acid was added, then centrifuged at 1699*g* for 5 min at 4°C, repeated twice. Finally, the supernatant was adjusted to pH 6.5 with 0.5 M NaOH before being transferred to a Waters tube. The sample supernatant (20 μL) was injected onto the column (Waters Xbridge C18, USA) and quantified by high-performance liquid chromatography (HPLC) (Alliance HPLC system Waters2695, USA) using UV detection (248 nm). To obtain optimal separation, the eluents used were (A) 5 mM tetrabutylammonium hydrogen sulphate and 25 mM KH2PO4 (pH 5.5) and (B) acetonitrile, with a flow rate of 0.8 mL/min.

2.5 | Fatty Acid Composition and Amino Acid Composition

Sixty milligrams of sample was weighed and 2.0 mL of undecylenic acid triglyceride internal standard solution was added. The methyl ester was prepared using isooctane and potassium hydroxide methanol solution. The single fatty acid methyl ester standard solution and the mixed fatty acid methyl ester standard solution were injected separately into the gas chromatograph, and the peaks were characterised following the method of Ma (Ma et al. 2018). The composition of amino acid was determined using liquid chromatography–mass spectrometry.

2.6 | Serum Biochemical Parameters

Serum was obtained by centrifugation at 956*g* for 10 min at 4°C, snap frozen in liquid nitrogen and stored at −80°C for subsequent analysis. Serum biochemical parameters were determined using an automated biochemical analyser (Beckman, Miami, FL) following the instructions.

2.7 | 16S Sequencing and Bioinformatics Analyses

Bacterial DNA was isolated and purified from the contents of various types of porcine colon using the QIAamp DNA Stool Mini Kit following the instructions, followed by amplification of the V3-V4 hypervariable region of the 16S rRNA gene. PCR products from the same samples were mixed and detected by 2% agarose gel electrophoresis using the AxyPrep DNA Gel Recovery Kit (AXYGEN) to recover PCR products and Tris–HCl to elute; 2% agarose electrophoresis was used to detect PCR products.

After sample splitting of PE reads obtained from MiSeq sequencing, the double-ended reads were first quality controlled and filtered according to the sequencing quality, while splicing was performed according to the overlap relationship between the double-ended reads to obtain optimised data after quality-controlled splicing (Illumina, San Diego, USA). Then, the optimised data were processed using the sequence noise reduction method (DADA2) to obtain amplicon sequence variant (ASV) representative sequence and abundance information. Raw reads were deposited in the NCBI Sequence Read Archive database (accession number: PRJNA973856).

R-4.2.0 was utilised for bioinformatic analysis, the 'vegan' R package was used to calculate the Bray–Curtis distance for different types of microorganisms in the pig colon. The 'psych' package was used to calculate the correlation coefficient and significance, and then the false discovery rate was used to adjust the significance values. The 'ggplot2' and 'pheatmap' packages were used for visualisation. Bacterial taxa differentially represented in pig breeds were identified by LEFSe with an LDA threshold > 3.5 or 4.0 online (http://www.bic.ac.cn). PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) was used for functional prediction of sequencing data, aligned the representative sequences with the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and further analysed their differences with the STAMP software.

2.8 | Data Analysis

Statistical analyses were performed using SPSS software (SAS, Chicago, IL, USA) with one-way ANOVA and Student–Newman–Keuls test to compare statistical differences. Differences between the three groups were defined as statistically significant at $p < 0.05$.

3 | Results

3.1 | Half Carcass Weight and Backfat Thickness

The half carcass weight and back fat thickness of JH pig, DBJ pig and DLY pig are shown in Figure 1. The half carcass weight of JH pig was significantly lower than that of DBJ pig ($p < 0.001$) and DLY pig ($p < 0.001$), and the back fat thickness was significantly elevated compared to that of DLY pig ($p < 0.001$).

3.2 | Effect of Breed on the Meat Quality

Meat quality was affected by the breeds of pigs; JH pigs exhibited superior quality (Figure 2). Comparing the quality of the longissimus dorsi muscle of the three breeds of pigs, the marbling of JH pigs was significantly elevated compared to DLY pigs and DBJ pigs ($p < 0.05$). The drip loss of JH pigs was significantly lower than that of the other two pig breeds ($p < 0.05$), ($p < 0.001$). Furthermore, the drip loss of DBJ pigs was significantly elevated compared to DLY pigs ($p < 0.05$).

To evaluate the differences in flavour substance among the three breeds, we analysed the IMF and IMP content of longissimus dorsi muscle of the pigs (Figure 3). Consistent with the trend in meat quality, JH pigs had a significantly greater advantage in IMF content and flavour substance content, with JH pigs showing significantly higher IMF content than DBJ ($p < 0.05$) and DLY pigs ($p < 0.001$); similarly, JH pigs showed significantly higher IMP content than DBJ ($p < 0.01$) and DLY pigs ($p < 0.001$).

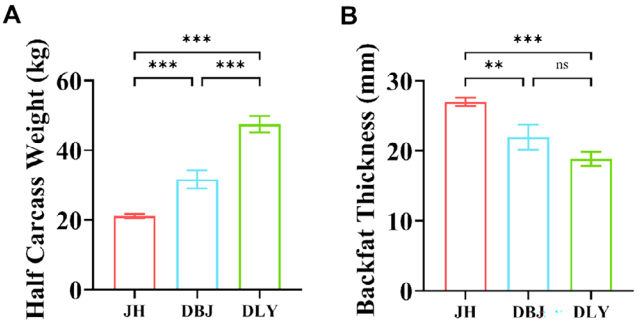

FIGURE 1 | Differences in half carcass weight (A) and backfat thickness (B) between 180-day-old JH, DBJ and DLY pigs. Data are expressed as mean ± SEM ($n = 8$). *, ** and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively. JH: Jinhua pigs; DBJ: Duroc × Berkshire × Jiaxinghei pigs; DLY: Duroc × Landrace × Yorkshire pigs.

3.3 | Effect of Breed on the Serum Parameters

The serum parameters of JH, DBJ and DLY pigs are shown in Table 2. The levels of alanine transaminase, glutathione transaminase and Glob (globulin) in serum were not significantly different among the three pig breeds. The free fatty acid content of JH pigs was significantly lower than that of DBJ and DLY pigs. The serum triglyceride, HDL (high-density lipoprotein), LDL (low-density lipoprotein), total protein and urea contents of the three pig breeds were not significantly different. The Glob, Glu and HDL contents of JH pigs were more abundant than those of other breeds of pigs but not significantly. The LDL and TP levels of JH pigs were less than those of DLY pigs. Furthermore, we found that the nonesterified fatty acid (NEFA) content in the serum of JH pigs was significantly lower than that of DBJ and DLY pigs.

3.4 | Fatty Acid Composition and Amino Acid Composition of Longissimus Dorsi Muscle in Different Breeds of Pigs

DBJ pigs exhibited the largest content of total unsaturated fatty acids and three types of unsaturated fatty acids in the longissimus dorsi muscle, containing C14, C16 and C18 (Table 3).

Furthermore, we analysed a variety of unsaturated fatty acids, which were higher in the longest dorsal muscle of JH pigs than in DLY pigs, whereas the content of C20:1 cis11 in JH pigs was significantly higher than that in DLY pigs ($p < 0.05$). Regarding C18:2, the level was significantly higher in JH pigs than in DBJ pigs ($p < 0.01$).

The total amount of protein in longissimus dorsi muscle of JH pigs is lower than that of DBJ pigs and DLY pigs ($p > 0.05$,

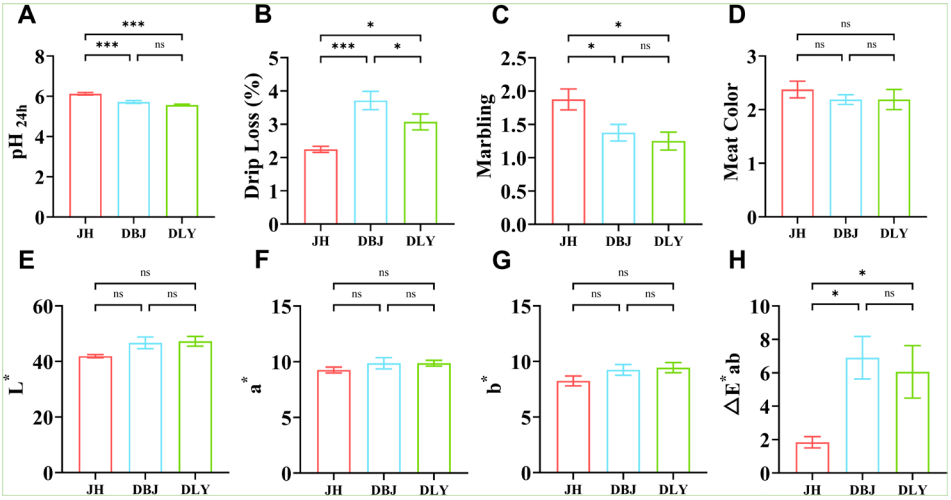

FIGURE 2 | Differences in meat quality traits from three breeds of pig. (A) The meat pH value 24 h after slaughter. (B) Drip loss. (C) The subjective value of meat Marbling as measured by the meat marbling scoring method. (D) The subjective value of meat colour as measured by the meat colour scoring method. (E) Lighthening (CIE L*). (F) Redness (CIE a*): Red-green. (G) Yellowness (CIE b*): Yellow-blue. (H) The colour difference value (CIE ΔE*). Data are expressed as mean ± SEM ($n = 8$). *, ** and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively. JH: Jinhua pigs; DBJ: Duroc × Berkshire × Jiaxinghei pigs; DLY: Duroc × Landrace × Yorkshire pigs. $\Delta E^*ab = \sqrt{(\Delta L^*)\hat{}2 + (\Delta a^*)\hat{}2 + (\Delta b^*)\hat{}2}$

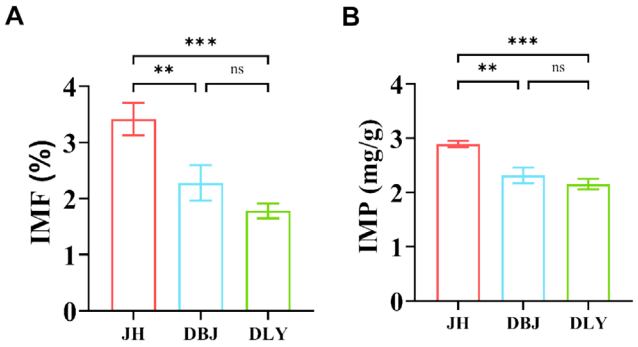

FIGURE 3 | Intramuscular fat and Inosine monophosphate content in the longissimus dorsi muscle of different breeds of pigs. (A) IMF, Intramuscular fat; (B) IMP, Inosine monophosphate. Data are expressed as mean ± SEM ($n = 8$). *, ** and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively. JH: Jinhua pigs; DBJ: Duroc × Berkshire × Jiaxinghei pigs; DLY: Duroc × Landrace × Yorkshire pigs.

$p < 0.01$) (Table 4). In addition, the proline content of JH pigs was significantly higher than that of DLY pigs ($p < 0.01$), while the trend of arginine content was consistent but not significantly different. Regarding the umami-tasting amino acids, DBJ pigs had the highest aspartic acid, glutamic acid, glycine, alanine, tyrosine and phenylalanine concentrations, with DBJ pigs having significantly higher phenylalanine concentrations than JH pigs. Besides, the alanine and phenylalanine content of JH pigs showed an upward trend compared with DLY pigs ($p > 0.05$).

3.5 | Gut Microbiome Composition and LDA Effect Size Analysis (LEFSe) of Different Breeds of Pigs

In addition, to reveal the differences in gut microbes among different breeds of pigs, we collected fresh colonic digesta from 180-day-old JH, DBJ and DLY pigs and performed 16S rRNA gene sequencing on the bacterial DNA isolated from it. A total of 667,521 high-quality reads were generated from 4272 features (ASVs), with an average of 27,813 reads per sample. We used the Shannon index (Figure 4A), Sobs (Figure 4B) and Ace index (Figure 4C) to evaluate the α-diversity of colonic microorganisms in different breeds of pigs, and we found that all three diversity indices were significantly higher in JH pigs than in DBJ and DLY pigs ($p < 0.0001$, $p < 0.01$), while DBJ pigs showed the lowest α-diversity index. To assess the diversity of gut microbiota structure among pig breeds, we performed the principal coordinate analysis (PCoA) plots based on Bray–Curtis distance and Jaccard distance (Figure 4D,E), which revealed that the microbial community structures were dramatically diverse among different pig breeds. Anosim analysis further indicated the differences among groups were greater than the differences within groups and the gut microbiota diversity among pig breeds was significant ($R = 0.798$, $p = 0.001$).

Comparing the colonic chyme microbial communities of JH, DBJ and DLY pigs, at the genus level, the top 36 most abundant genera were displayed on stacked bar charts. We found that *Lactobacilli* were the most dominant genus in JH and DBJ pigs (8.38%, 21.62%), while *Clostridium sensu stricto 1* was the most abundant in DLY pigs (12.26%) (Figures 4Fand S1). Notably, *Lachnospiraceae* dominated in the colonic microbial communities of JH pigs similarly to *Lactobacillus*, representing around 7.81%, whereas it was only 2.38% and 3.43% in the colon of DBJ and DLY pigs respectively (Figures 4F and S1). To clearly compare the differences in bacterial composition, we selected the top 20 genera and visualised them in the form of a heatmap, which showed that *Lachnospiraceae*, *Lactobacillus*, *Prevotella*, *Alloprevotella* and other genera were enriched in JH pigs (Figures 4H and S2). Venn plot showed the number of ASVs specific to JH, DBJ and DLY pigs was 847, 517 and 682, respectively, whereas the number of ASVs common to the three breeds was 515 (Figure 4I). Moreover, LEFSe analysis was applied to evaluate the specific microbial enrichment among different breeds of pigs (Figure 4J,K). At LDA threshold > 3.5, a total of 23 genera were significantly enriched in JH pigs, including

TABLE 2 | Serum parameters of three different pig breeds.

	JH		DBJ		DLY		
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	***p***
A/G	0.75	0.041	0.78	0.049	0.91	0.050	0.057
ALT(U/L)	61.50	3.737	53.75	5.580	98.38	17.065	0.016
AST(U/L)	79.88	11.560	87.88	14.660	64.88	3.843	0.343
Alb(g/L)	31.11	1.221	31.61	1.562	35.06	1.211	0.100
Glob(g/L)	41.80	1.107	41.01	1.200	39.16	2.117	0.476
Glu(mmol/L)	7.26	0.724	5.58	0.604	6.46	0.420	0.162
HDL-C(mmol/L)	0.90	0.058	0.79	0.070	0.78	0.051	0.278
LDL-C(mmol/L)	1.39	0.076	1.37	0.068	1.51	0.107	0.475
NEFA(mmol/L)	0.08^{b}	0.013	0.23^{a}	0.039	0.25^{a}	0.038	0.002
TChol(mmol/L)	2.42	0.141	2.22	0.141	2.32	0.123	0.598
TG(mmol/L)	0.68	0.080	0.58	0.105	0.46	0.040	0.172
TP(g/L)	72.91	1.319	72.63	1.670	74.23	2.827	0.841
Urea(mmol/L)	4.57	0.436	5.60	0.388	5.36	0.433	0.217

Note: Data are expressed as mean ($n = 8$). ^{a,b}Mean values in a row sharing no common superscript are significantly different ($p < 0.05$).
Abbreviations: A/G, albumin/globulin; Alb, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DBJ, Duroc × Berkshire × Jiaxinghei pigs; DLY, Duroc × Landrace × Yorkshire pigs; Glob, Globulin; Glu, Glucose; HDL-C, high-density lipoprotein; JH, Jinhua pigs; LDL-C, low-density lipoprotein; NEFA, Nonestesterified fatty acid; TChol, total cholesterol; TG, triglycerides; TP, total protein.

TABLE 3 | Fatty acid (FA) composition (g/100 g) of longissimus dorsi muscle from pigs of different breeds.

	JH		DBJ		DLY		
	Mean	**SEM**	**Mean**	**SEM**	**Mean**	**SEM**	***p***
Fat	2.34	0.347	2.43	0.397	1.83	0.173	0.376
C14:0	0.04	0.004	0.05	0.005	0.03	0.002	0.105
C16:0	0.74	0.070	0.80	0.090	0.60	0.021	0.129
C18:0	0.38	0.036	0.46	0.054	0.33	0.012	0.086
∑ SFA	1.16	0.108	1.30	0.148	0.96	0.033	0.105
C16:1 *cis-9*	0.09	0.011	0.09	0.013	0.08	0.002	0.552
C18:1 *cis-9*	1.12	0.116	1.21	0.151	0.98	0.025	0.335
C20:1 *cis-11*	0.03^{a}	0.003	0.02^{ab}	0.003	0.02^{b}	0.001	0.017
C18:2	0.18^{ab}	0.018	0.23^{a}	0.022	0.15^{b}	0.017	0.023
C20:4 *cis-5,8,11,14*	0.03	0.002	0.06	0.034	0.03	0.001	0.430
C22:2 *cis-13,16*	0.09	0.010	0.06	0.005	0.07	0.001	0.015
C18:1 *trans-9*	0.01	0.001	0.01	0.000	0.01	0.000	0.094
∑ UFA	1.49	0.151	1.68	0.173	1.28	0.026	0.134
∑ FA	2.66	0.255	2.98	0.316	2.25	0.053	0.112

Note: Data are expressed as mean ($n = 8$). ^{a,b}Mean values in a row sharing no common superscript are significantly different ($p < 0.05$).
Abbreviations: ∑FA, total fatty acids; JH, Jinhua pigs; DBJ, Duroc × Berkshire × Jiaxinghei pigs; DLY, Duroc × Landrace × Yorkshire pigs; ∑SFA, Saturated fatty acids, mainly including the C14:0, C16:0 and C18:0; ∑UFA, Unsaturated fatty acids, mainly including the C18:1 cis-9, C18:2, C22:2 cis-13,16 and C16:1 cis-9.

Lachnospiraceae, *Prevotella*, *Alloprevotella* and so on. At LDA threshold > 4.0, we further compared the differences in specific bacterial taxa enrichment between JH pigs and the two other pigs. Compared with DBJ or DLY pigs, *Lachnospiraceae*, *Prevotella*, *Alloprevotella* and *Faecalibacterium* were enriched in JH pigs. In addition, JH pigs showed a greater enrichment

TABLE 4 | Amino acid (AA) composition (g/100 g) of longissimus dorsi muscle from pigs of different breeds.

	JH		DBJ		DLY		
	Mean	SEM	Mean	SEM	Mean	SEM	*p*
Nutritionally EAA							
Threonine	1.02	0.006	1.04	0.020	1.03	0.008	0.360
Valine	1.12	0.006	1.15	0.024	1.14	0.009	0.445
Methionine	0.57	0.009	0.59	0.023	0.58	0.014	0.752
Isoleucine	1.11	0.006	1.12	0.024	1.10	0.007	0.639
Leucine	1.88	0.012	1.93	0.037	1.88	0.013	0.334
Phenylalanine	0.91^{b}	0.006	1.02^{a}	0.029	0.86^{ab}	0.059	0.020
Lysine	2.02	0.013	2.05	0.039	2.04	0.017	0.722
Histidine	1.00	0.017	1.01	0.045	1.02	0.017	0.900
∑ EAA	9.62	0.069	9.81	0.131	9.65	0.080	0.345
Nutritionally NEAA							
Serine	0.87	0.006	0.88	0.018	0.88	0.008	0.796
Glutamic acid	3.37	0.026	3.42	0.080	3.41	0.032	0.802
Glycine	0.91^{b}	0.009	0.97^{a,b}	0.026	0.95^{a}	0.008	0.062
Alanine	1.30	0.008	1.32	0.031	1.30	0.006	0.539
Tyrosine	0.84	0.005	0.84	0.017	0.84	0.007	0.936
Arginine	1.47	0.010	1.47	0.033	1.45	0.011	0.657
Proline	0.81^{a}	0.007	0.82^{a,b}	0.036	0.73^{b}	0.017	0.038
Aspartic acid	2.04	0.013	2.07	0.039	2.05	0.015	0.590
∑ NEAA	11.60	0.075	11.79	0.275	0.06	11.593	0.655
∑ AA	21.24	0.148	21.69	0.466	21.25	0.121	0.472
TP	23.05^{b}	0.169	23.28^{b}	0.194	24.03^{a}	0.317	0.021

Note: Data are expressed as mean ($n=8$) ^{a,b}Mean values in a row sharing no common superscript are significantly different ($p<0.05$).
Abbreviations: ∑AA, total amino acids; DBJ, Duroc × Berkshire × Jiaxinghei pigs; DLY, Duroc × Landrace × Yorkshire pigs; JH, Jinhua pigs; TP, total protein.

of *Prevotellaceae NK3B31* than DBJ pigs, and a more abundant *Lactobacilli* than DLY pigs.

3.6 | Correlation Analysis Between Gut Microbiota and Pig Phenotypes

Furthermore, to identify potential microbial biomarkers associated with rich fat deposition in JH pigs, Spearman's rank correlation coefficient between the top 20 genera and meat quality, meat AA, meat FA and serum traits was performed and visualised in clustered heat maps.

In terms of meat quality, *Prevotella* ($p_{adj}<0.05$) showed a positive correlation with IMF content in longissimus dorsi muscle of pigs. *Alloprevotella* and *Lachnospiraceae* showed the same trend, while the difference was not significant. *Christensenellaceae_R-7* ($p_{adj}<0.05$) and *ucg_002* ($p_{adj}<0.05$) were negatively correlated with IMP content. Clostridium_sensu_stricto_1 ($p_{adj}<0.05$) was negatively correlated with marbling score (Figure 5A). Regarding the fatty acid profile of pork, *Christensenellaceae_R-7* was negatively correlated with C18:1 trans-9, while the difference was not significant (Figure 5D). Regarding serum parameters, serum NEFA was positively correlated with *Clostrdium_sensu_stricto_1* ($p_{adj}<0.05$) *and was* negatively correlated with *Lachnospiraceae* ($p_{adj}<0.05$). *Alloprevotella* and *Prevotella* showed the same trend, while the difference was not significant (Figure 5C).

To identify the correlation of microbiota, we generated a heatmap of Spearman's rank correlation coefficient between the top 20 taxa (Figure 5E). Four main clusters according to the dendrogram on the left-hand side of the heatmap, indicated by the red lines. Furthermore, we correlated distance-corrected dissimilarities of these four clusters and IMF-related meat quality indicators and serum indicators (Figure 5F). Genus of cluster 1 was positively related to each other within the groups in heatmap. Interestingly, cluster 4 contained eight of the top 20 taxa, includes *Prevotella*, and this cluster owned the strongest positive correlation with IMF in Mantal test (Mantal's $R=0.256$). Next,

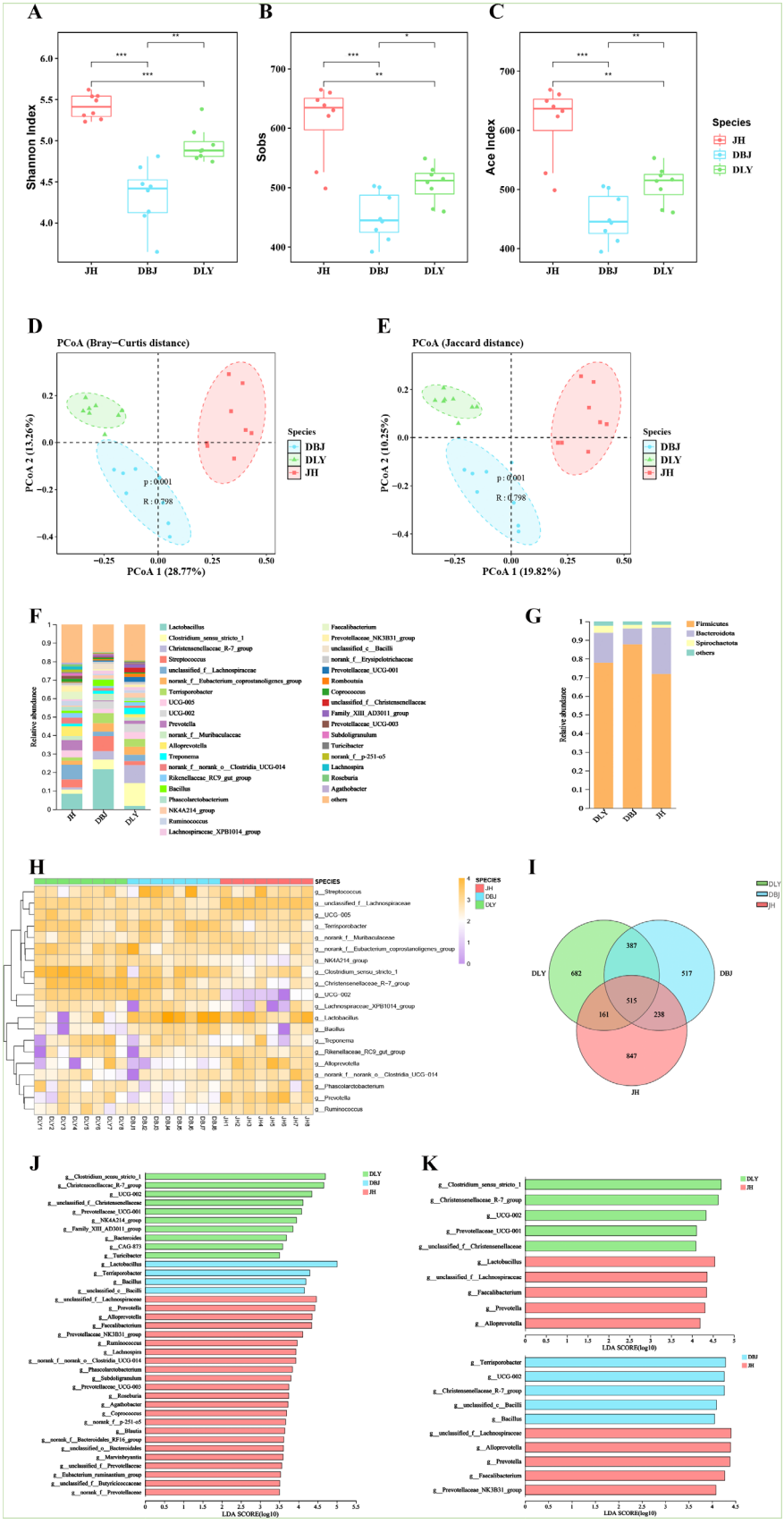

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FIGURE 4 | Microbial community composition and LDA Effect Size analysis diversity of JH, DBJ and DLY pigs. (A–C) The α-diversity of colon microbial diversity of three different pig breeds. (A) Shannon index. (B)Sobs. (C)Ace index. Data are expressed as minimum to maximum ($n = 8$). *, ** and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. (D–E) The β-diversity of colon microbial diversity of three different pig breeds. (D) PCoA, the principal coordinate analysis based on the Bray-Curtis distance. (E) PCoA, the principal coordinate analysis based on the Jaccard distance. (F–I) Community composition of colonic microbiota from JH, DBJ and DLY pigs. Stacked bar charts show differences in microbial community composition in the colon of different pig breeds, at the genus level (F) and phylum level (G), respectively. (H) Heat map shows the top 20 genera ranked by abundance among the three types of porcine gut microbiota. (I) Venn diagram reveals the number of common and specific ASVs in the colon digesta of different pig breeds. (J–K) Histograms of LDA scores reveal the most differentially abundant taxa among different pig breeds. (J) Bacterial taxa differentially represented in pig breeds identified by LEFSe with LDA threshold > 3.5. (K) Bacterial taxa differentially represented in pig breeds identified by LEFSe with LDA threshold > 4.0. JH: Jinhua pigs; DBJ: Duroc × Berkshire × Jiaxinghei pigs; DLY: Duroc × Landrace × Yorkshire pigs. f: Family; g: Genus.

we selected seven indicators related to lipid deposition and the RDA analysis was conducted to identify the factors related to microbiota; the results are shown in Figure 5G. IMF ($r^2 = 0.669$), NEFA ($r^2 = 0.559$) and marbling ($r^2 = 0.441$) were the three most important factors; among them, NEFA showed a negative correlation with the other two indicators. Similarly, the network showed a positive correlation between *Prevotella*, *Alloprevotella* and other genera with IMF and marbling, while showing a negative correlation with NEFA (Figure 5H).

3.7 | Difference in Functional Potential of Microbial Community in Different Breeds of Pig

The PICRUSt analysis was performed to predict how enriched bacteria might contribute to variation in host phenotype between breeds, specifically growth and adipogenesis, by mapping the 16S rRNA sequencing data to the KEGG modules. Compared with DLY pigs, metabolic pathways, biosynthesis of secondary metabolites, other glycan degradation, sphingolipid metabolism and alanine, aspartate and glutamate metabolism were predicted to be enriched in JH pigs. Whereas, we observed 17 KEGG pathways with significantly increased abundance in the colonic microbiomes of JH pigs compared to DBJ pigs, including metabolic pathways, biosynthesis of secondary metabolites, phenylalanine, tyrosine and tryptophan biosynthesis, lipopolysaccharide biosynthesis and oxidative phosphorylation (Figure 6).

4 | Discussion

In recent years, with the improvement of people's living standards and the pursuit of precise nutrition, the livestock and poultry industry has been striving for improved product quality and more distinctive products, while consumers have been seeking enjoyable, safe, healthy and high-quality food products(Henchion et al. 2014; Huang et al. 2020). While genetic factors are known to be key determinants of meat quality variation, recent studies suggest that gut microbiota may also influence meat quality characteristics, such as meat colour; however, the mechanism involved is unclear, and few studies have investigated the relationship between microbes and meat quality in pig breeds(Bergamaschi et al. 2020; Liang et al. 2024; Yang et al. 2025). In our study, we compared meat qualities, serum parameters and colonic microbial composition of three different breeds of pigs and investigated a potential relationship between gut microbial composition and pig phenotypes.

As a traditional local Chinese pig breed, JH pigs are characterised by excellent meat quality, slow growth and stronger fatty deposition(Miao et al. 2009; Li et al. 2016). Our study showed JH pigs had the highest IMF content and marbling scores in the longissimus dorsi muscle, and these results revealed the strong fat deposition ability of JH pigs as fatty pigs. Drip loss is related to the IMF content and distribution, since higher IMF values are associated with a decrease in the moisture diffusivity coefficient, the amount of exudate or moisture on the cut surface of the loin(Abdullah et al. 2014; Huang et al. 2020; Martins et al. 2020). In our study, the drip loss of JH pigs is higher than that of DBJ and DLY pigs, which is consistent with the higher IMF content, marbling score and pH_{24h} observed in JH pigs. Flavour constitutes an essential group of sensory attributes for the consumption quality of pork; among them, IMP is considered an essential ribonucleotide in meat flavour perception with its umami taste characteristics(Meinert et al. 2009; Reina et al. 2014; Tikk et al. 2006). In our study, we found the IMP content of JH pigs was approximately 2.89 mg/g, significantly higher than that of DBJ and DLY pigs, which partly explains the superior flavour characteristics of JH pork. We found JH pigs contained the greatest amount of C20:1 cis-11, C22:2 cis-13,16 and C18:1 trans-9 in the longissimus dorsi muscle compared to DBJ pigs and DLY pigs. To common knowledge, C18:1 trans-9 might be pork flavour precursors, which are significant for one or more of the volatile compounds and sensory variables of pork flavours(Song et al. 2017).

Furthermore, serum lipid-related indicators revealed the high fat deposition capacity of JH pigs. Among the three pig breeds, JH pigs showed the most elevated level of serum triglyceride and cholesterol, which is consistent with previously reported results(McNeel et al. 2000). In addition, we found that the increased serum triglyceride and cholesterol levels in JH pigs may induce greater HDL levels and reduced LDL levels. Triglycerides are hydrolysed by lipoprotein lipase after entering the bloodstream, leading to the release of excess surface shell components, such as unesterified cholesterol and exchangeable apolipoproteins, phospholipids and their transfer to circulating lipoproteins, especially circulating HDL. Meanwhile, LDL is the major carrier of cholesterol, which is removed from the circulation by LDL receptor (LDLR)-mediated endocytosis(Rigotti et al. 2003).

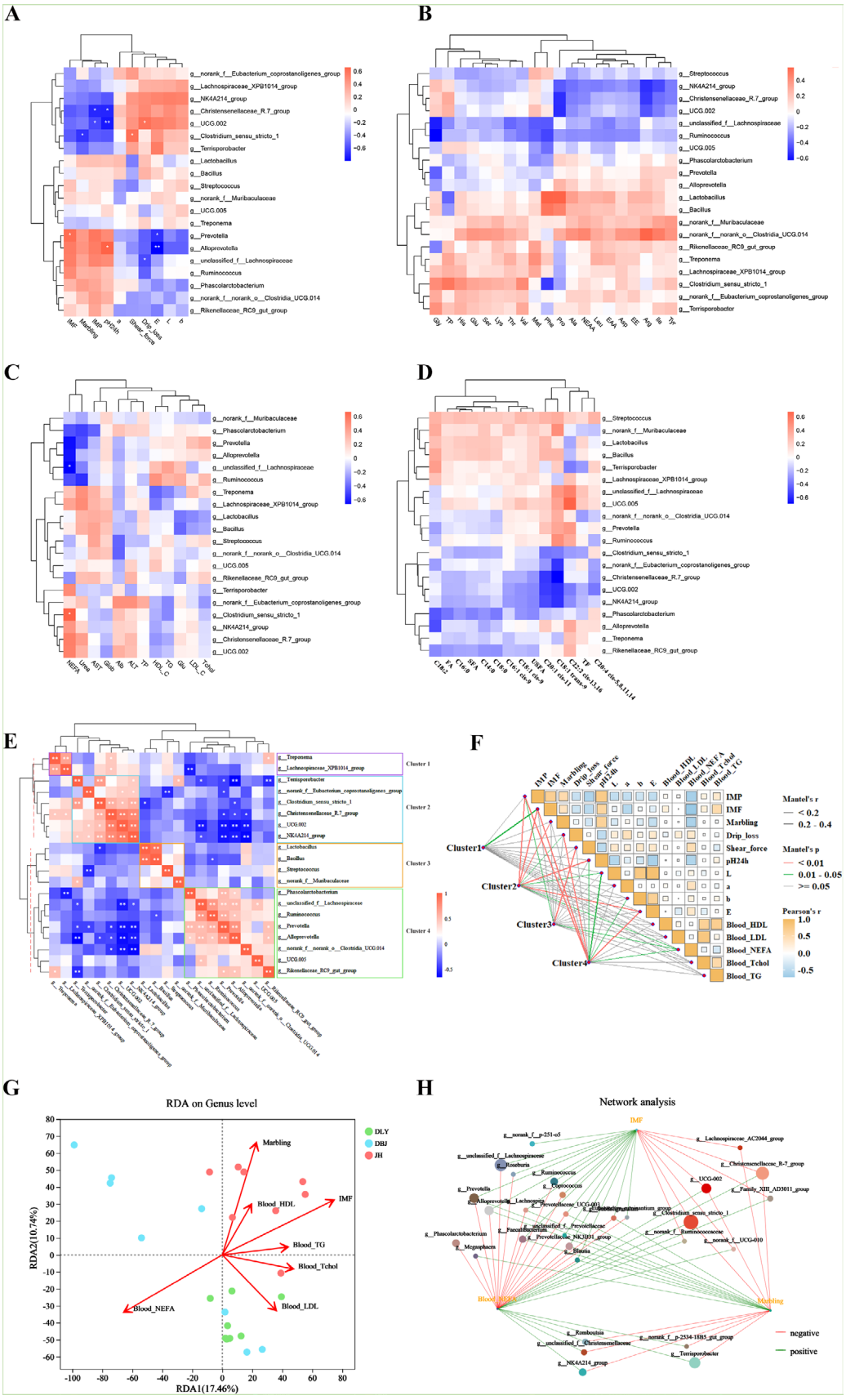

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FIGURE 5 | Correlation analysis. (A–D) Heatmaps showing Spearman's rank correlation coefficient and significant test between the top 20 genera and meat quality traits (A), amino acid compositions (B), serum indices (C) and fatty acid compositions (D) respectively. * and ** indicate $p_{adj} < 0.05$ and $p_{adj} < 0.01$. (E) Heatmap of top 20 genera by spearman's rank. The purple, blue, yellow and green boxes indicated the most dominant cluster of microbiota. * and ** indicate $p < 0.05$ and $p < 0.01$, respectively. (F) The four most clusters are related to meat quality and serum quality by Mantal tests. The correlation between meat quality and serum quality is shown by Spearman's rank correlation coefficient. (G) The redundancy analysis. The arrow length represents the strength of the correlation between the microbiota and indicators related to lipid deposition. (H) The correlation network between the microbiota and three indicators related to lipid deposition. The red lines denote a negative association and the blue lines denote a positive association. JH: Jinhua pigs; DBJ: Duroc × Berkshire × Jiaxinghei pigs; DLY: Duroc × Landrace × Yorkshire pigs. f: Family; g: Genus.

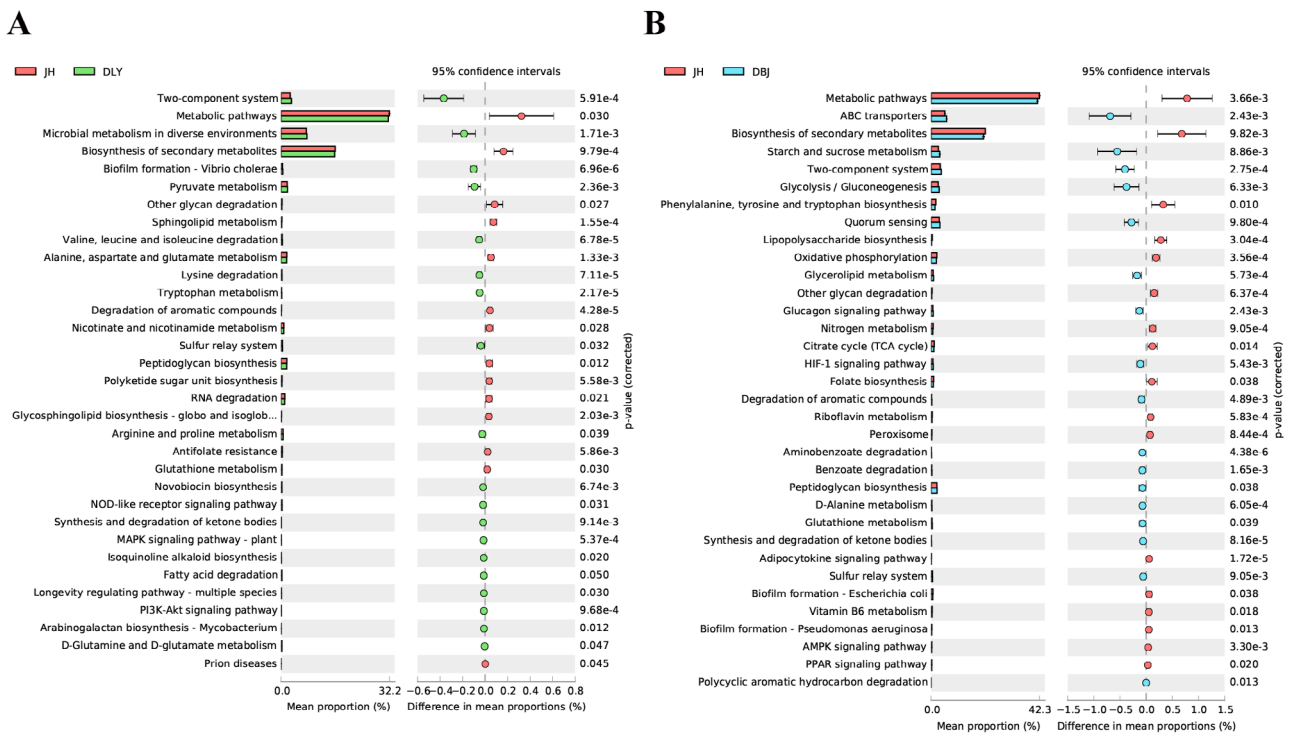

FIGURE 6 | (A) Post hoc plot showed KEGG pathways between JH pigs and DLY pigs. (B) Post hoc plot showed KEGG pathways between JH pigs and DBJ pigs. Statistical significance difference among different pig breeds based on Welch's *t*-test ($p < 0.05$) in STAMP. The coloured circles represent 95% confidence intervals calculated using Welch's inverted method.

In our study, JH pigs showed increased α-diversity and a distinct separation in bacterial community structure compared to the other pig breeds, which may explain the phenotypes of meat quality and serum index. The most abundant phylum in the colonic chyme microbiota of JH pigs was Firmicutes, with *Lactobacillus* as the dominant genus, consistent with previous research(Song et al. 2022; Xiao et al. 2018). We further note an *unidentified genus of family Lachnospiraceae* dominated in JH pigs (7.81%), the same as *Lactobacillus* (8.38%). Lachnospiraceae are widely recognised as a group of short-chain fatty acid-producing bacteria that produce acetate and butyrate, which may underlie the strong fat deposition capacity of JH pigs(Sorbara et al. 2020).

It is worth pointing out that most of the genera significantly enriched in JH pigs, such as *Lachnospiraceae*, *Prevotellaceae NK3B31* and *Marvinbryantia*, were capable of degrading polysaccharides or fibres to produce short-chain fatty acids, which were a direct energy source for colonic epithelial cells and closely associated with intestinal barrier function(Cuevas-Sierra et al. 2020; Huang et al. 2021). SCFAs produced by the gut microbiota have been shown to derive from microbial glycolysis of complex resistant carbohydrates and may be involved in the regulation of several biological processes, including host metabolism and immune function(Maglie et al. 2024). SCFAs infusion through the ileum improves meat quality and reduces drip loss through the regulation of lipid metabolism(Jiao et al. 2021). Consistent with *Lachnospiraceae*, *Marvinbryantia* was positively correlated with butyrate production. Gao's research revealed 50% alfalfa supplementation could improve *Marvinbryantia* level, and associated with the high SCFAs concentration in the colon of Tibetan pigs(Gao et al. 2022). *Prevotellaceae NK3B31*, *Prevotella* and *Alloprevotella* are all members of the family Prevotellaceae, common acetate and succinate producing bacteria whose abundance is closely correlated with BMI, obesity and gut health, although their contribution to host–microbiota cross-talk are unclear, with conflicting reports of effects on human health, particularly on glucose homeostasis(Chen et al. 2022b; Tett et al. 2021; Trautmann et al. 2022). As the most predominant features of pig gut microbioyta, *Prevotella* were associated

with plant food-based diet and fibre digestion(Tett et al. 2021; Wang et al. 2019). Chen's research proposed high abundance of *Prevotella* may be associated with excessive energy uptake and increased fat accumulation of pigs(Chen et al. 2021). Other studies suggested that *Prevotella*-enriched gut microbiota may induce improved feed intake, growth performance and feed efficiency in pigs, while some studies indicated the negative association between *Prevotella* and production traits(Amat et al. 2020). All this evidence supported the colonisation of high-abundance fibre-degrading bacteria may be attributed to the excessive fat deposition capacity of JH pigs, since their fibre fermentation produces short-chain fatty acids involved in carbon metabolism, energy intake and circulatory levels(Amat et al. 2020; Li et al. 2020).

In this study, *Prevotella* were shown to be the potential genera to respond to IMF, although the mechanisms of their interactions need to be further investigated. Gavage of *P. copri* has been shown to improve pork colour and drip loss, and high abundance of *P. copri* is strongly associated with fat deposition in pigs(Chen et al. 2021; Liang et al. 2024). Our study also found that *Lachnospiraceae*, *Prevotella* and *Alloprevotella* were positively correlated with IMF content of pigs. Notably, *Prevotella* exhibited a negative correlation with serum NEFA, which was associated with fat deposition. Previous studies have demonstrated that alterations in the composition of the intestinal microbiota led to a change in SCFA production, which is associated with an increase in serum NEFA levels(Rodríguez-Carrio et al. 2017). Consistent with these findings, our experiments revealed a lower serum NEFA in JH pigs, which may be linked to the unique intestinal microbial composition in JH pigs that are involved in the production of unsaturated fatty acids. The cluster 4 encompassing eight of the top 20 taxa includes *Prevotella*, and this cluster owned the strongest positive correlation with IMF in the Mantal test (Mantal's $R = 0.256$). Consistently, the correlation network revealed a strong positive correlation between *Prevotella* and IMF. Further, in the RDA analysis, we found that IMF exhibited the highest explained variance for microbial community, which implied that gut microbes interacted with the meat quality. Based on these results, we provisionally concluded that the high abundance of fibre-degrading bacteria in the colonic microbes of JH pigs was closely related to lipid deposition and the formation of superior meat quality.

Diverse gut microbiota existed in different pig breeds and had specialised metabolic functions and activities. In our study, *Prevotella* and other fibre-degrading bacteria were enriched in JH pigs. Consistently, PICRUSt analysis further revealed an enrichment in glycan biosynthesis and metabolism pathways in the JH breed, such as other glycan degradation, peptidoglycan biosynthesis, glycosphingolipid biosynthesis and so on. With respect to fatty acid metabolism, sphingolipid metabolism was enriched in JH pigs. To the best of our knowledge, sphingolipids comprise one of the major categories of eukaryotic lipids which play structural and signalling roles in cells, especially playing an essential role in the onset of muscle insulin resistance and myogenic differentiation. Compared with DBJ pigs, the TCA cycle, oxidative phosphorylation and nitrogen metabolism were enriched in JH pigs, which were associated with carbohydrate metabolism and energy metabolism respectively.

5 | Conclusion

In our study, we characterised differences in meat qualities, serum indicators, fatty acid composition and amino acid composition of three different breeds of pigs. Furthermore, correlations between different caecal microbiota and these traits were established, indicating a high abundance of fibre-degrading bacteria was enriched in JH pigs, and these bacteria were correlated with superior meat quality. Collectively, these correlation results inspired us to pursue further understanding of the relationship between microbiota and nutrient metabolism, energy harvest and fat deposition, which is essential for the pork industry and intestinal health.

Author Contributions

Qifan Zhang: conceptualization, methodology, investigation, writing – original draft. **Man Du:** investigation, visualization. **Siyu Wei:** formal analysis, visualization. **Luoyi Zhu:** visualization. **Rong Yan:** methodology. **Mingliang Jin:** writing – review and editing. **Yizhen Wang:** project administration, resources, supervision.

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Ethics Statement

All the animal procedures applied in this study were approved by the Committee on Animal Care and Use and the Committee on the Ethics of Animal Experiments of Zhejiang University (ZJU20001).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in NCBI Sequence Read Archive (SRA) database at https://dataview.ncbi.nlm.nih.gov/, reference number PRJNA973856.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.