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Multi-omics integration analyses reveal microbiome and metabolome features in pregnant sow diarrhea induced by porcine epidemic diarrhea virus

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Abstract

Gut microbial dysbiosis and its derived-metabolites changes have been evidenced to participant in diarrhea piglets; little is known underlying the crosstalk between gut microbiota and metabolites in pregnant sow diarrhea induced with PEDV. In this study, we performed fecal metagenomic and metabolomic profiling in diarrheic pregnant sows infected with PEDV to evaluate the functional characteristics of gut microbiota and metabolites. Microbiome analysis revealed the alterations in composition and diversity of gut microbiota in diarrheic pregnant sows compared with non-diarrheic. The relative abundances of the genera *Prevotella, Treponema* and *Bacteroides* were significantly lower and the abundant of *Lactobacillus* and *Ruminococcus* were increased in diarrheic pregnant sows. In addition, we found that the increase of *Ruminococcus_sp_CAG563*, *Mycoplasma_sp_CAG472*, *Prevotella_sp_CAG520*, *Candidatus_Melainabacteria_bacterium* and *Eubacterium_coprostanoligenes* was the important characteristics in diarrheic pregnant sows. In addition, metabolomic analysis showed a distinct metabolic profile in diarrheic pregnant sows infected with PEDV and the differential metabolites were associated with secondary bile acid biosynthesis, protein digestion and absorption, amino acid biosynthesis. Moreover, our multi-omics data integration analysis indicated that the significant dominant bacteria in diarrheic pregnant sows were positively correlated with 5-aminovaleric acid, pantothenate, 8,4-oxyneolignan-4-xyloside and xanthine, while the predominant coexistence of *Treponema, Bacteroides,* and *Fibrobacter* promoted the production of dodecanedioic acid, sesamol and sebacic acid in non-diarrheic pregnant sows infected with PEDV. Taken together, our findings revealed the dynamic changes in the microbiota and metabolites of diarrheic pregnant sows during PEDV infection, identifying microbiota-derived metabolites associated with host resistance, providing novel insight into the host–gut microbiota interaction.

Keywords Pregnant sow diarrhea, PEDV, Gut microbiota, Metagenomics, Metabolomic

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Introduction

Porcine epidemic diarrhea virus (PEDV) infection not only causes diarrhea and death in piglets but also leads to the diarrhea and abortion in pregnant sow, which may reduce nutrient intake, impair piglet development and severely compromise reproductive performance [1]. PEDV is a single-stranded positive-sense and enveloped RNA alphacoronavirus with a genome characterized by a high mutation rate and recombination frequency, which can enhance its pathogenicity and increase the difficulty of prevention and control [2]. The classic G1 strain, G2a, G2b, G2c sublineages, and S-INDEL strain of PEDV have been successfully isolated, and these strains exhibit the distinct pathogenicity, leading to the restrictive efficacy of current vaccines and therapeutic measures [3–5]. PEDV infection cause intestine walls transparency and thinning as well as intestinal epithelial cells necrosis [6], indicating that the maintenance of intestinal health is crucial for resisting PEDV infection.

Accumulating evidence has demonstrated that gut microbiota and its derived metabolites play pivotal roles in maintaining intestinal homeostasis and regulating host immune response. The replication of herpes simplex virus type 1 (HSV-1) is significantly increased in antibiotic-treated mice with the reduced gut bacterial community and the downregulated expression of gut microbiota-derived interferon-stimulated genes (ISGs), suggesting that gut microbiota is essential for mediating antiviral innate immunity [7]. Based on fecal metagenomic sequencing, chikungunya virus (CHIKV) infection decreased the diversity and richness of gut microbiota and upregulated the levels of bile acid to promote systemic inflammatory [8]. The abundance of *Faecalibacterium prausnitzii* (*F. prausnitzii*) is significantly reduced in Crohn disease (CD) patients, and *F. prausnitzii* supplementary can reduced IL-1β-induced IL-8 secretion, block NF-κB activation and increase IL-10 secretion to exhibit anti-inflammatory effects against Crohn disease [9]. Oral administration of *Akkermansia muciniphila* displayed a protective effect against influenza virus infection with more mild lung pathological damage through improving innate immune response [10]. *Clostridium butyricum* acted as a novel immunobiotic that maintains intestinal health and exerts broad-spectrum antiviral activity against influenza A virus (PR8), newcastle disease virus (NDV), and herpes simplex virus (HSV) infection via increasing the expression of anti-inflammatory cytokines [11].

In addition, during virus infection, host interacts with gut microbiota to produce a variety of metabolites such as short-chain fatty acids (SCFAs), tryptophan and bile acid (BA) metabolites, which influence host immunity and inflammatory responses [12]. H9N2 infection altered gut microbial diversity profiles in mice, and treatment of *Prevotella copri* inhibited viral infection by increasing the production of isovaleric acid and isobutyric acid [13]. Commensal bacteria-derived short-chain fatty acids (SCFAs) can downregulate the expression of the SARS-CoV-2 receptor ACE2 in the intestine and lung via GPR41/GPR43-mediated adaptive immunity, thereby resisting SARS-CoV-2 infection [14]. Gut microbiota-derived butyrate interacted with HDAC to inhibit RIG-I-induced type I IFN signalling, leading to the enhanced transmissible gastroenteritis virus (TGEV) infection in the late stage of viral infection [15]. Outer membrane vesicles derived from *Faecalibacterium prausnitzii* increased glycerophospholipids metabolites to change the distribution of intestinal cell subpopulation and reduce PEDV-induced necroptosis,thus protecting piglets against PEDV infection [16]. *Lactobacillus reuteri and Lactobacillus amylovorus* promoted bile acids metabolism to increase the production of deoxycholic acid and lithocholic acid, which can enhance the intestinal expression of SLA-I and recruit CD8 + CTLs in piglets [17]. These findings collectively suggest that gut microbiota and its derived metabolites are closely associated with virus infection and disease procession.

Currently, research on the changes and functions of gut microbiota induced by PEDV mainly focuses on piglets, whereas few studies have investigated the changes of gut microbiota in sow with diarrhea and their impact on piglet growth and development. Hence, in this study, we performed metagenomic and metabolomic analyses to explore the correlation between gut microbiota and PEDV-induced diarrhea in pregnant sow and identified gut microbiota-derived metabolite involved in host resistance to diarrhea. This study provides novel insight into host-gut microbiota interaction and offers new therapeutic strategies against PEDV infection.

Materials and methods

Experimental animals and design

Fifty-day pregnant hybrid sows (Landrace × Yorkshire) in the same batch with an average parity of 3.2 ± 0.6 were housed at Hubei Agricultural Development Group Co., Ltd (Hubei, China) and fed a standard gestation diet. When sows exhibited diarrhea and anorexia symptoms, pathogen detection was immediately conducted, which confirmed infection with PEDV 2C strain with the absence of other pathogenic microorganisms (Fig. 1). After being confirmed virus-positive for ten days, some of pregnant sows developed severe diarrhea (Dia group), while others remained no clinical symptoms (Con group). Therefore, fresh fecal samples were collected from 10 sows in the Con group and 9 sows from the Dia group suffering from diarrhea, immediately frozen in liquid nitrogen and then transferred to – 80 °C for subsequent gut metabolomic and metabolomics profiling analysis.

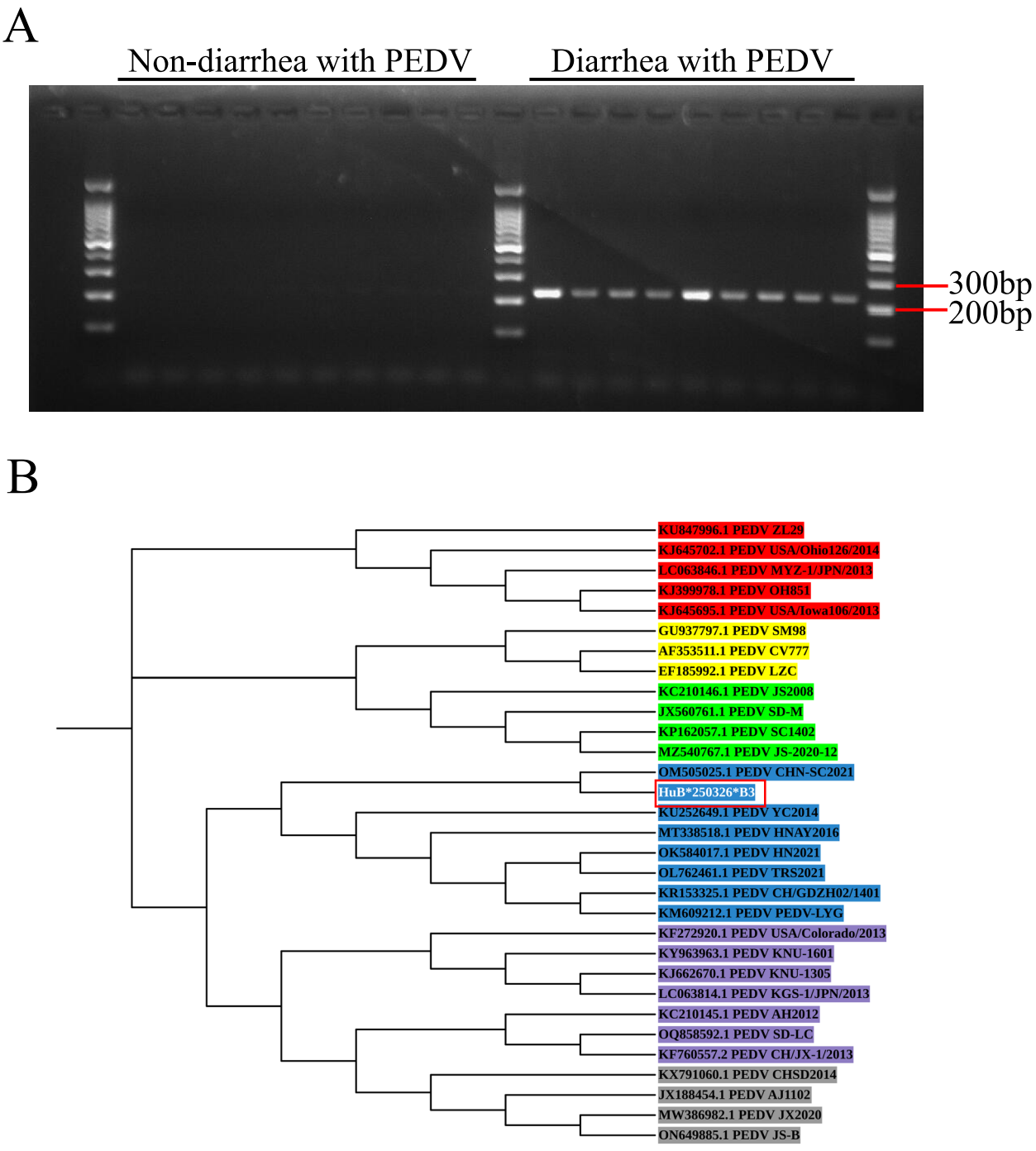

Fig. 1 Pregnant sows naturally infected with PEDV. **A** PCR results of samples from non-diarrheic and diarrheic pregnant sows with PEDV. **B** The phylogenetic tree of PEDV strain isolated in this research by using the neighbor-joining method in MEGA11 software. Red: S-INDEL, Yellow: G1a, Green:G1b, Blue:G2c, Purple:G2a, Grey:G2b

All experimental procedures in this study were carried out in accordance with guidelines for animal studies issued by the Animal Care and Use Committee of Wuhan polytechnic University (Wuhan, China).

Metagenome and bioinformatics analysis

Fecal samples of non-diarrheic and diarrheic pregnant sows naturally infected with PEDV were collected and immediately frozen in liquid nitrogen. Total microbial DNA was extracted using the CretMagTM Power Soil DNA Kit (CretBiotech,China) according to manufacturer's instructions and then purified and quantified for

shotgun metagenome sequencing. Genomic DNA was fragmented to an average size of about 400 bp using Covaris M220 (Gene Company Limited, China) following the paired-end library construction using NEXTflexTM Rapid DNA-Seq (Bioo Scientific, Austin, TX, USA). The DNBSEQ-T7 platform was used to sequence with DNBSEQ-T7RS Reagent kit (FCL PE150) V2.0 according to the manufacturer's instructions. After sequencing, the low-quality reads were filtered, and clean reads were obtained using fastp (v0.23.2) software [18]. After removing host originated reads, the high-quality reads were assembled to contigs for taxonomic profiling using the MEGAHIT software [19]. Prodigal software [20] was used to identify the open reading frames (ORFs) in contigs. Linear discriminant analysis of effect size (LEfSe) [21] was used to quantitate differential species and functional pathway abundance was calculated using the KEGG orthology database and eggNOG database.

Metabolomic analysis

Fifty milligram fecal samples of non-diarrheic and diarrheic pregnant sows naturally infected with PEDV were pulverised and added to extraction buffer including methanol, acetonitrile and water for metabolite extraction. After centrifugation (20 min, 12,000 rpm, 4℃), the supernatant was collected and injected into the liquid chromatography-tandem mass spectrometry (LC–MS/MS) system for untargeted metabolomic analyses. The sample extracts were analysed using an UPLC-Orbitrap-MS system (UPLC, Vanquish; MS, HFX). The raw LC–MS/MS data were acquired on the Q-Exactive using Xcalibur 4.1 (Thermo Scientific) and were used for peak detection, quantification and alignment by using TMBQ software [22], and metabolomic compounds were annotated using the KEGG and HMDB databases. Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) were performed to assess the change of metabolomic profile, and the differentially expression metabolites were screened according to the following criteria: variable importance value (VIP) > 1, *P*-value < 0.05, and fold change (FC) > 1.5 or < 0.667. VIP values were extracted from OPLS-DA result. Functional enrichment analysis of differentially expression metabolites was performed using the KEGG database.

Metagenomics and metabolomics correlation analysis

To reveal the function of gut microbiota and their derived metabolites in diarrheic pregnant sow infected with PEDV, the correlation analysis between differential metagenomics and metabolomics landscape was performed.

Statistical analysis

All statistical analyses were constructed with R software (version 4.2.3), and all data were presented as mean ± SD. Two-sided wilcoxon rank-sum test was used to assess the phenotypic difference between two groups. Differential taxa and metabolites were identified using a combination of Wilcoxon rank-sum test and linear discriminant analysis effect size (LEfSe) for species.The Student's t test was used to determine the significance and a p-value < 0.05 was considered as statistically significant in this study.

Results

Gut microbiome composition and diversity change in pregnant sow infected with PEDV

PEDV infection causes diarrhea and affects the composition and functional activity of the gut microbiota in piglets, but its impact on pregnant sows has not been investigated. To address this knowledge gap, fecal samples from non-diarrheic and diarrheic pregnant sows naturally infected with PEDV were processed for metagenomics analysis. Shotgun metagenomic sequencing obtained 8.5-12G raw data in each sample with an average N50 contigs length of 1448 bp (Table 1). The number of non-redundant genes in non-diarrheic and diarrheic groups was visualized with Venn diagram, and 15,610 clusters were shared (Fig. 2A). In addition, based on the genus data, we found no significant differences in species diversity between health and diarrheic groups by analysing the alpha diversity with three different indices (shannon, simpson and invsimposn index) (Fig. 2B-D), but the beta diversities analysis of bacterial communities with principal coordinate analysis (PCoA) using the Bray–Curtis distance revealed significant differences between health and diarrheic groups (Fig. 2E).

Furthermore, the beta diversity analysis using the NMDS indicated that gut bacteria in diarrheic pregnant sows clustered separately from non-diarrheic (stress = 0.1398) (Fig. 2F). Subsequently, the relative abundance of the gut microbiota at multiple levels was quantified. At the genus level, *Prevotella, Treponema, Bacteroides* were the most abundant taxa and the abundant of *Lactobacillus* and *Ruminococcus* were decreased in non-diarrheic groups (Fig. 2G). Compared to non-diarrheic groups at the species level, the diarrheic pregnant sows had higher relative abundances of *Lactobacillus_johnsonii and Limosilactobacillus_reuteri, and* a decrease in *Treponema_bryantii* and *Firmucutes_bacterium_CAG555* (Fig. 2H).

To illustrate the overall species profiles, a cladogram was constructed (Fig. 3A), and the ANOSIM analysis of the overall microbial communities showed significantly different between non-diarrheic and diarrheic groups infected with PEDV at all taxa level (Fig. 3B). Furthermore, the results of linear discriminant analysis

Table 1 Data quality in metagenome

Samples	Total_Reads	Raw bases(GB)	Removed_low_quality_Reads	Removed Low Qualitybases(GB)	Removed_host_Reads	Clean_bases(GB)	GC_Content	Q20	Q30
Dia_8	########	9.7568	63,935,972(98.29)	9.2586	62,886,972(96.68)	9.1108	43.98%	98.59%	95.89%
Dia_9	########	10.8893	71,455,490(98.43)	10.4958	70,295,612(96.83)	10.3278	43.16%	98.73%	96.24%
Dia_2	########	10.0721	65,973,066(98.25)	9.7337	63,975,844(95.28)	9.4455	44.30%	98.51%	95.57%
Dia_3	########	9.1437	59,913,956(98.29)	8.8631	58,588,754(96.11)	8.6697	45.43%	98.56%	95.75%
Dia_1	########	11.3837	74,472,508(98.13)	10.9245	72,359,668(95.35)	10.6199	44.29%	98.44%	95.46%
Dia_6	########	12.1546	79,677,902(98.33)	11.6499	79,291,262(97.85)	11.5961	42.76%	98.65%	96.01%
Dia_7	########	10.6089	69,477,600(98.23)	10.2599	69,047,726(97.63)	10.1982	43.94%	98.46%	95.51%
Dia_4	########	10.1916	66,703,300(98.17)	9.854	66,013,548(97.16)	9.7575	41.41%	98.53%	95.71%
Dia_5	########	10.9716	72,020,762(98.46)	10.3783	71,382,210(97.59)	10.2922	42.17%	98.83%	96.56%
Con_4	########	9.9402	65,036,346(98.14)	9.4334	62,241,068(93.92)	9.0317	44.14%	98.57%	95.91%
Con_5	########	12.1583	79,836,724(98.5)	11.6469	72,606,734(89.58)	10.6168	43.57%	98.87%	96.64%
Con_6	########	10.7923	70,748,708(98.33)	10.3036	70,168,942(97.53)	10.2209	46.30%	98.77%	96.39%
Con_7	########	11.6452	76,385,558(98.39)	11.1975	75,051,658(96.67)	11.0044	44.24%	98.80%	96.43%
Con_1	########	10.1735	66,584,854(98.17)	9.7053	65,756,642(96.95)	9.5863	44.08%	98.79%	96.44%
Con_2	########	10.7784	70,654,456(98.33)	10.1086	66,140,816(92.05)	9.4922	43.98%	98.77%	96.41%
Con_3	########	9.4235	61,700,000(98.21)	9.0346	61,423,694(97.77)	8.9962	42.53%	98.56%	95.79%
Con_8	########	10.9973	72,008,172(98.22)	10.4821	68,058,820(92.83)	9.9043	46.03%	98.79%	96.46%
Con_9	########	12.4567	81,488,986(98.13)	11.5255	78,689,854(94.76)	11.1429	42.94%	98.76%	96.43%
Con_10	########	8.5424	55,932,170(98.21)	8.2199	54,231,584(95.23)	7.9729	45.75%	98.50%	95.60%

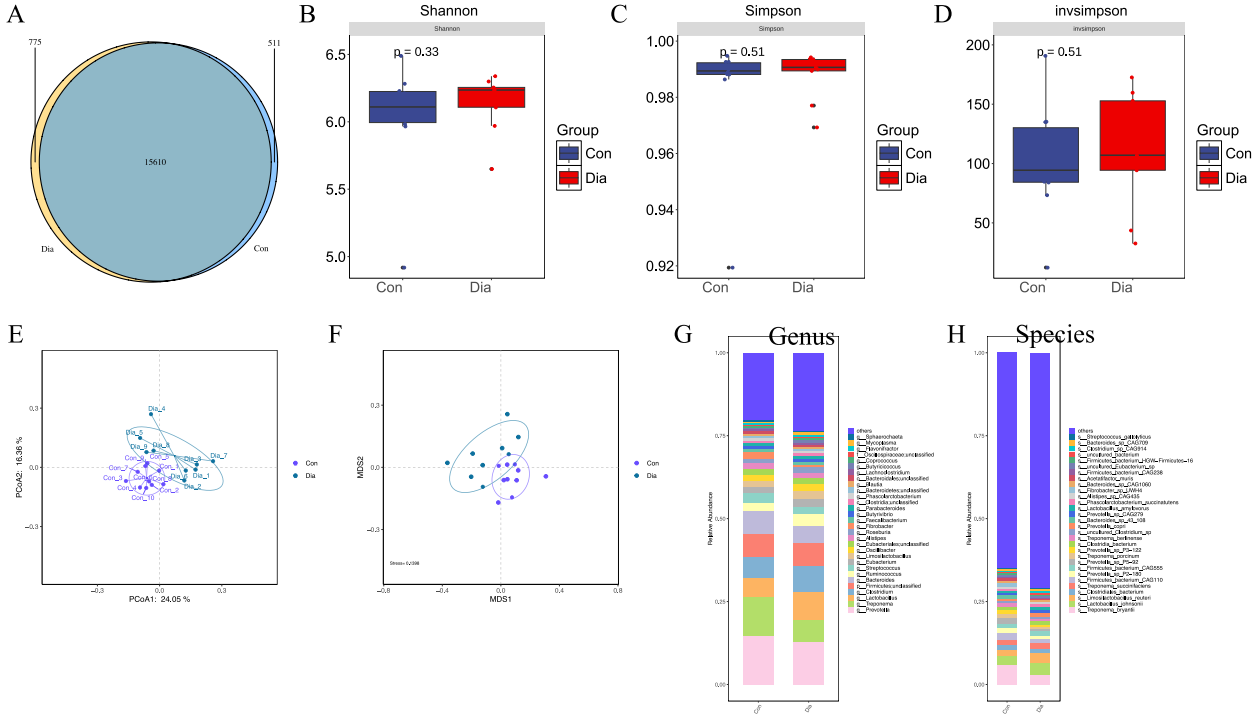

Fig. 2 Analysis of intestinal microbiome signatures in non-diarrheic and diarrheic groups infected with PEDV. **A** The Venn diagram displays overlap between non-diarrheic and diarrheic groups infected with PEDV. **B**-**D** Alpha diversity indexes (shannon, simpson and invimpson) were used to assess the microbial diversity in diarrheic pregnant sows compared to non-diarrheic sows. $n = 10$ (Con), $n = 9$ (Dia). P-values from Wilcoxon rank-sum test. **E**–**F** Beta diversity measured by principal coordinates analysis (PCoA) ($p < 0.01$) (**E**) and on-metric multi-dimensional scaling (NMDS) analysis (stress = 0.1398) (**F**). **G** The gut microbial composition between non-diarrheic and diarrheic groups at the genus level. **H** The gut microbial composition between non-diarrheic and diarrheic groups at the species level. Con: non-diarrheic group, Dia:diarrheic group

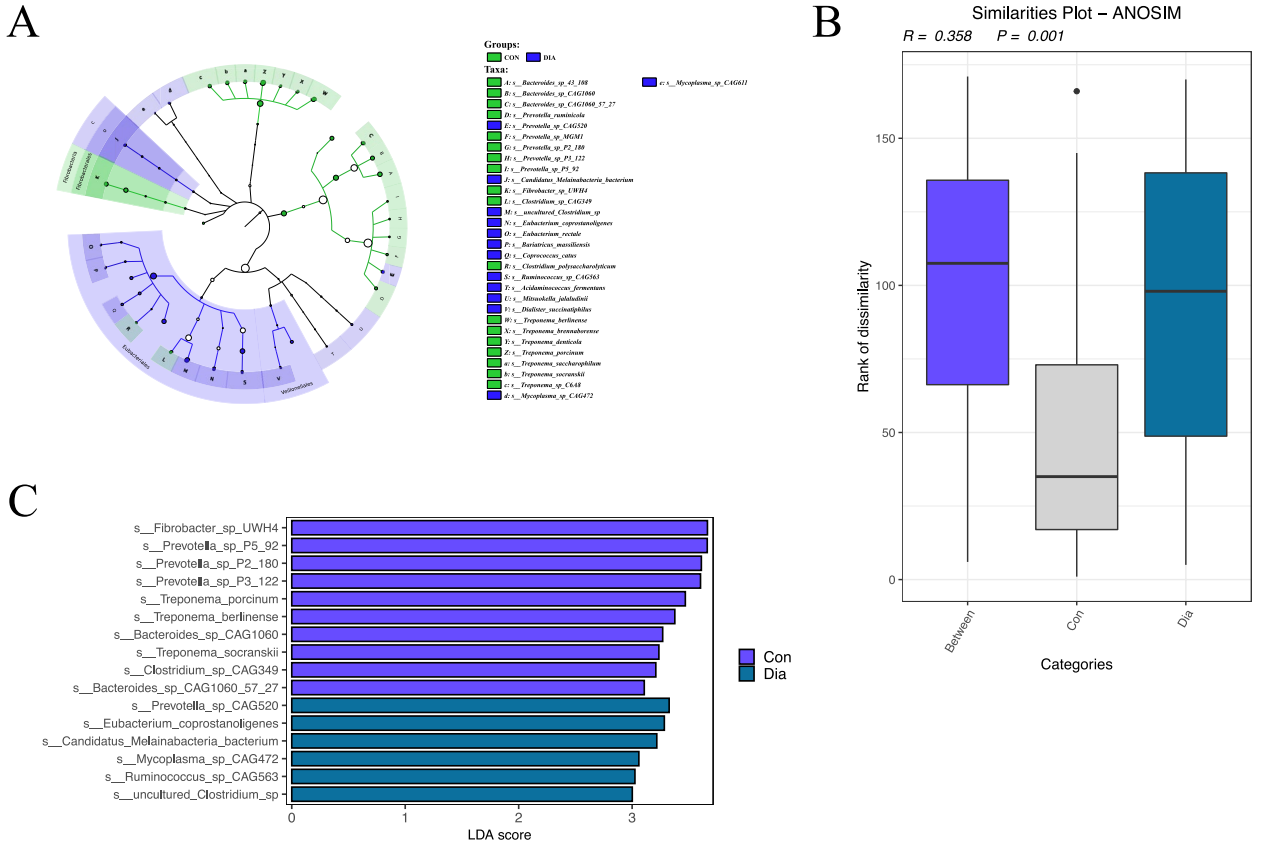

Fig. 3 Analysis of differential intestinal microbes between non-diarrheic and diarrheic groups. **A** The evolutionary branch diagram for microbiota distribution. **B** The ANOSIM analysis of the overall microbial communities between non-diarrheic and diarrheic groups infected with PEDV at all taxa level. **C** Linear discriminant analysis (LDA) score determined by linear discriminant analysis effect size (LEfSe) to screen the main microbiota species

effect size (LEfSe) analysis showed that *Fibrobacter_sp_UWH4, Prevotella_sp_P5_92, Prevotella_sp_P2_180, Prevotella_sp_P3_122, Treponema_porcinum, Treponema_berlinense, Bacteroides_sp_CAG1060, Treponema_socranskii, Clostridium_sp_CAG349* and *Bacteroides_sp_CAG1060_57_27* were dominated in the non-diarrheic pregnant sows, while *Prevotella_sp_CAG520, Eubacterium_coprostanoligenes, Candidatus_Melainabacteria_bacterium, Mycoplasma_sp_CAG472, Ruminococcus_sp_CAG563* and *uncultured_Clostridium_sp* were enriched in the diarrheic pregnant sows (all LDA > 3, $P < 0.05$, Fig. 2C). To explore the distinct biological functions of the differential microbiota, KEGG, eggNOG and CAZy databases were used. The results showed that most abundant pathways for diarrheic pregnant sows were metabolism of terpenoids and polyketides and amino acid metabolism (Fig. 4A-C). Taken together, these results are consistent with the hypothesis that non-diarrheic pregnant sows may maintain resistance to PEDV-induced diarrhea in association with distinct gut microbial patterns and their derived metabolites (Table 1).

Alteration of metabolomic profiling in non-diarrheic and diarrheic pregnant sows in PEDV infection

Given that gut dysbiosis could regulate the production of metabolite to affect the host environment, we further performed metabonomic analysis to investigate the associations of microbial function with metabolites in non-diarrheic pregnant sows. Orthogonal Projections to Latent Structures-Discriminant Analysis (OPLS-DA) showed significant difference between non-diarrheic (Con) and diarrheic (Dia) group ($R^2Y = 0.89$, $Q^2 = 0.859$) (Fig. 5A). 100 upregulated and 163 downregulated metabolites were identified using the criteria of variable importance in projection (VIP) > 1, fold change > 1.5 or fold change < 0.667, and *P*-values < 0.05, respectively (Fig. 5B and Supplementary data 1). These differential metabolites were classified into the categories of carboxylic acids and derivatives, fatty acyls, prenol lipids, steroids and steroid derivatives (Fig. 5C). Furthermore, the enrichment analysis of the identified differential metabolites revealed that secondary bile acid biosynthesis, biosynthesis of amino acids, protein digestion and absorption, ABC transporters, 2-Oxocarboxylic acid metabolism and phenylalanine metabolism (Fig. 5D). Representatively differential metabolites are present,

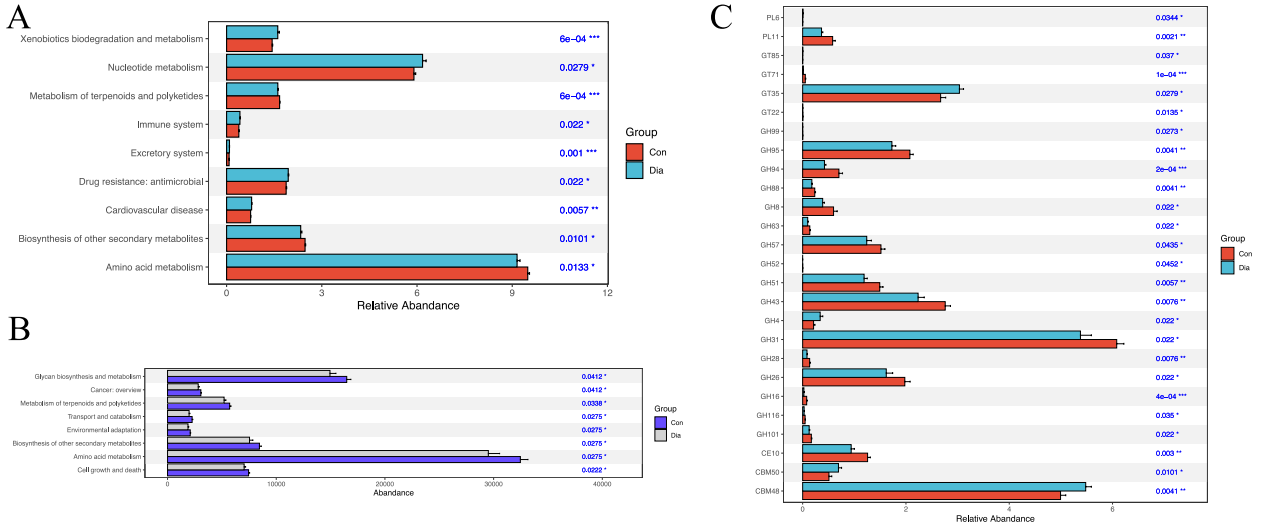

Fig. 4 Analysis of the distinct biological functions of the differential microbiota. **A** KEGG pathways analysis showed the relative enrichment of the differential microbiota. **B** eggnog databases showed the relative enrichment of the differential microbiota. **C** Comparative analysis of microbial functional genes based on CAZy databases

such as pantothenate (pantothenic acid), xanthine,5-aminovaleric acid, 3-hydroxyoctadecanoic acid and 3-sulfanyhexyl pentanoate were significantly increased, while the lower levels of dodecanedioic acid, sebacic acid, sesamol was fund in diarrheic pregnant sows (Fig. 6).

Correlation analysis of differential gut microbiota and metabolites

To further explore the functional correlation of gut bacteria with differential metabolites between non-diarrheic and diarrheic pregnant sows, the joint analysis of metagenomics and metabolomics data was performed. The results showed that 15 differentially enriched genera were significantly associated with the 20 metabolic (Fig. 7A). The dominated microbial taxa, *including Fibrobacter_sp_UWH4, Prevotella_sp_P5_92, Prevotella_sp_P2_180, Prevotella_sp_P3_122, Treponema_porcinum, Treponema_berlinense, Bacteroides_sp_CAG1060, Treponema_socranskii, Clostridium_sp_CAG349* and *Bacteroides_sp_CAG1060_57_27* in the non-diarrheic pregnant sows showed positive correlation with differentially accumulated metabolites, including dodecanedioic acid, sesamol and sebacic acid, while their abundances were predominantly negatively correlated with 5-aminovaleric acid, pantothenate, 8,4-oxyneolignan-4-xyloside and xanthine (Fig. 7B).

Discussion

Porcine epidemic diarrhea virus (PEDV) has become one of the most important pathogens of highly infectious diseases in the pig industry, causing acute diarrhea. Pregnant sows are the core of swine production, and the diarrheic pregnant sow caused by PEDV infection not only affects their own nutrient absorption but also may lead to miscarriage and stillbirth due to dehydration and toxin absorption, even though the diarrhea may be self-healing. Previous studies have revealed that gut microbiota can regulate immune system and nutrient absorption to maintain porcine health and development [23, 24], but most of studies for the function of gut microbiota mainly focused on piglets. In this study, we characterized the gut microbiota and metabolites profiles of non-diarrheal and diarrheal pregnant sows naturally infected with PEDV. The results indicated that the composition and diversity of gut microbiota significantly differed between the two groups. There is a lower abundance of *Prevotella, Treponema* and *Bacteroides,* but the relative abundance of *Lactobacillus* and *Ruminococcus* was considerably higher in diarrheal pregnant sows. Microbial community structure analysis had released a reduction percentage in *Prevotella* and *Treponema*, and a significant increase in the abundances of *Lactobacillus* in diarrhea piglets compared to healthy piglets [25]. A lower percentage of *Prevotella* and the higher abundance of *Lactobacillus* was found in Enterotoxigenic Escherichia coli (ETEC)-induced diarrheal piglets [26]. Moreover, the recovered piglet from ETEC-induced diarrheal showed more higher percentage of *Lactobacillus* than the diarrheal piglets [17]. In addition, PEDV infection reduced the abundance of *Bacteroides* but increase the *Lactobacillus* abundance in Min pigs and Landrace piglets [27], which is consistent with our findings, suggesting that the alteration of *Prevotella* and *Lactobacillus* might play a conservative key role in regulating diarrhea.

A growing investigation has showed that bacterial species participated in intestinal immune responses and host adaptation against disease processes. *Lactobacillus_johnsonii enriched in diarrhea sows* has been proved

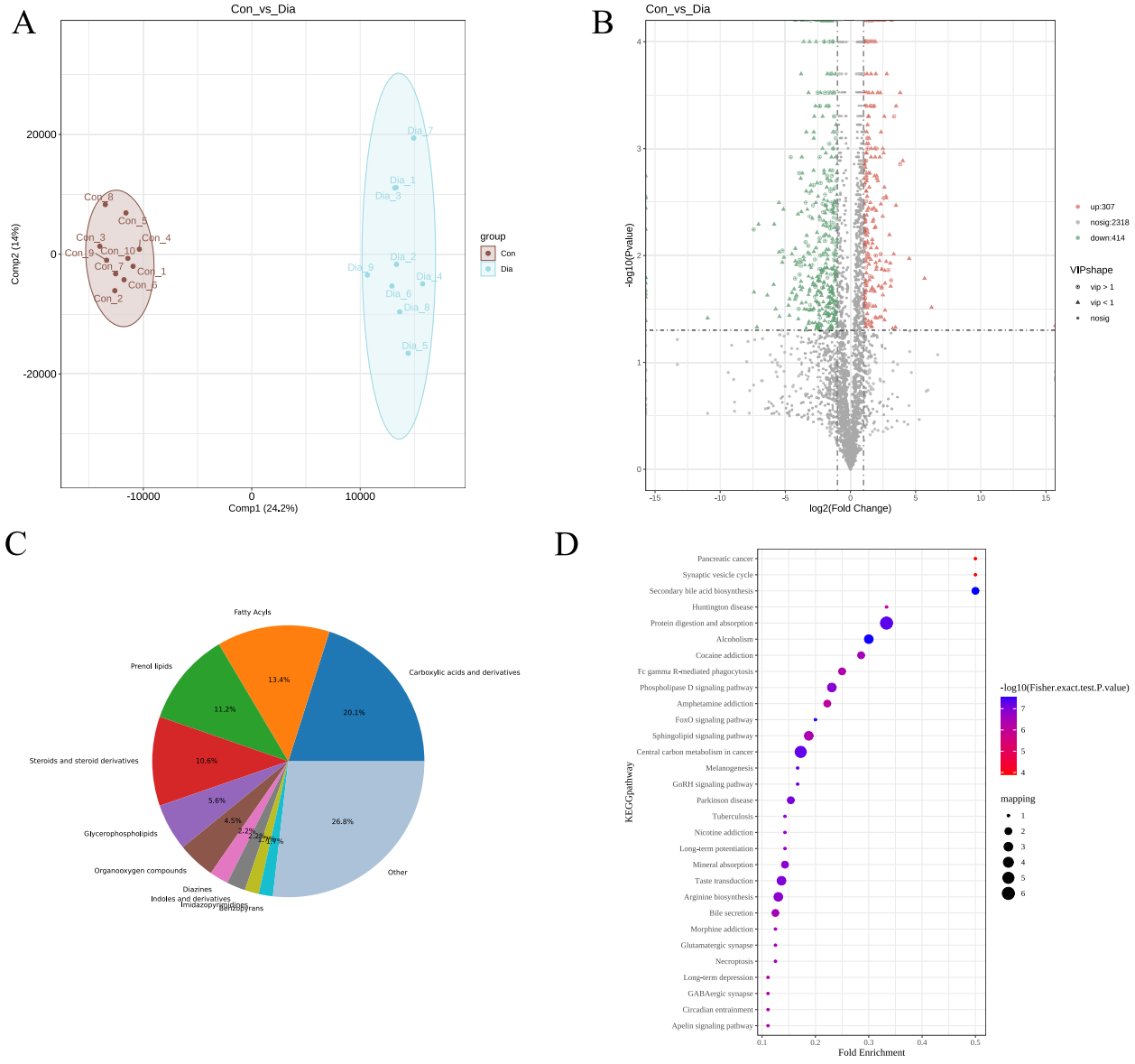

Fig. 5 Analysis of intestinal microbial-derived metabolites profiling between non-diarrheic and diarrheic pregnant sows in PEDV infection. **A** Orthogonal partial least squares discriminant analysis (OPLS-DA) score plots showing clear separation between non-diarrheic and diarrheic groups in PEDV infection. **B** Volcano plot showing the differences of gut microbial metabolites based on VIP > 1.0 and FDR-adjusted $P < 0.05$. **C** The classification of differential metabolites. **D** Functional enrichment analysis of differential metabolites based on KEGG database

as anti-inflammatory bacterium to alleviate colitis and kidney lesion [28, 29]. Extracellular vesicles derived from *Lactobacillus_johnsonii inhibited the gut inflammation induced by ETEC K88* and activate M2 macrophages by inhibiting NLRP3 activation [30]. Another enriched bacterium *Limosilactobacillus mucosae* and its derived Extracellular vesicles can significantly alleviate growth retardation and villus to crypt length ratio caused by ETEC K88 infection by inhibiting inflammatory cytokines expression (IL-1β, IL-6, TNF-α, p-AKT and p-NF-κB) [31]. *Treponema_bryantii* has recently been found to be associated with feed efficiency in sows and was the most contributors of antibiotic resistance genes in the cecum high feed efficiency group [32]. In addition, it has been showed that PEDV infection can reduce the absorptive capacity of nutrients and energy causing lower growth performance [33]. Collecting these data, we hypothesized that the reduced abundance of *Treponema_bryantii* in diarrheic sows might contribute to impaired dietary fiber digestion, potentially exacerbating diarrhea.

Our fecal metabolomics revealed metabolic dysregulation in diarrheal pregnant sows after PEDV infection, and identified several difference metabolites, such as pantothenate, xanthine and 5-aminovaleric acid,

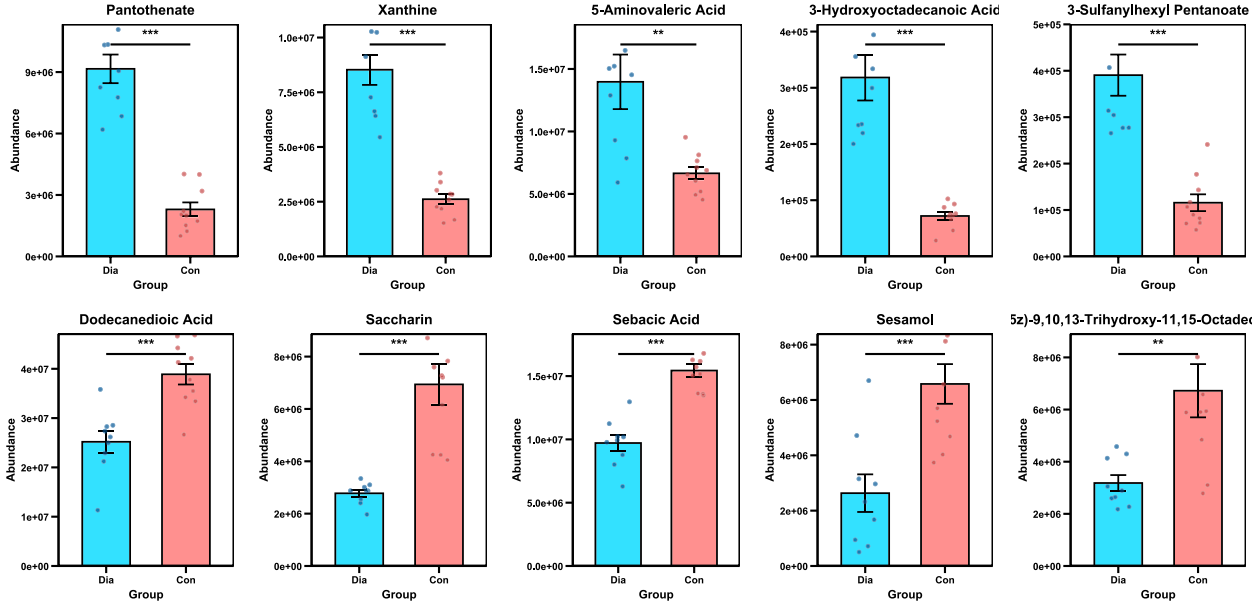

Fig. 6 The expression levels of differential metabolites between non-diarrheic and diarrheic pregnant sows in PEDV infection

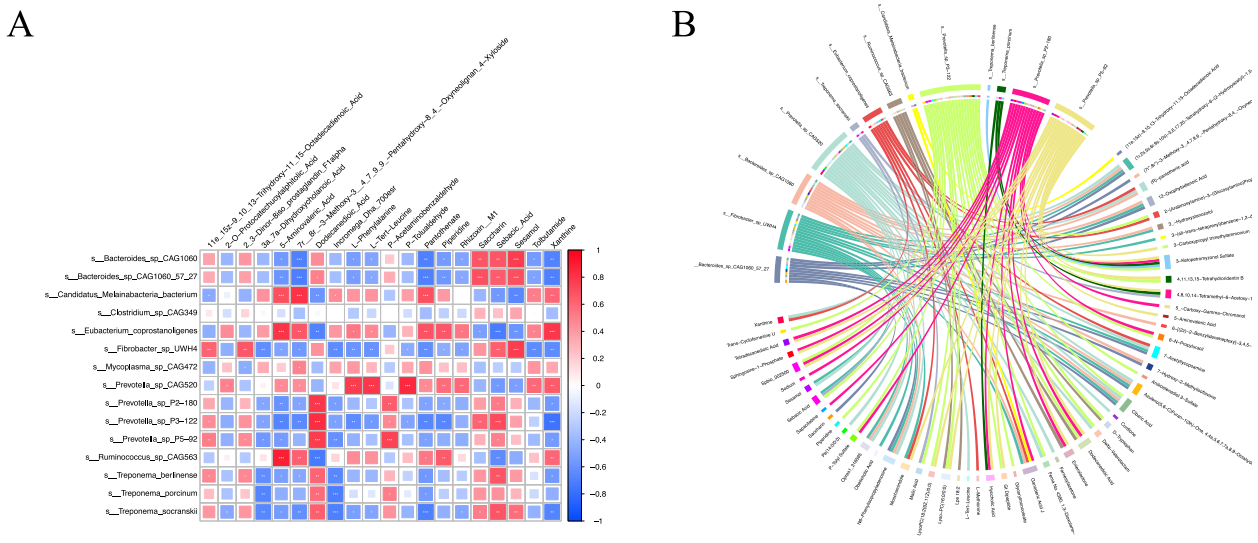

Fig. 7 Correlation analysis between metabolic patterns and species. **A** The correlation coefficients between 15 differentially enriched species and 20 differentially expressed metabolic were calculated. **B** Chord diagram showed the interrelationships between differentially expressed metabolic and species according to the correlation |r|> = 0.7 and *p*-value < 0.05 was showed

dodecanedioic acid, sebacic acid, sesamol, involved in secondary bile acid biosynthesis, biosynthesis of amino acids, protein digestion and absorption, ABC transporters, 2-Oxocarboxylic acid metabolism and phenylalanine metabolism. Moreover, our integrative analysis revealed the significant relationship between these metabolites and the identified key microbial genera. Xanthine, pantothenate, xyloside, 5-aminovaleric acid was strongly negatively correlated with *Bacteroides, Fibrobacter, Prevotella* and *Treponema,* but they were strongly positively correlated with *Eubacterium_coprostanoligenes, Candidatus_Melainabacteria_bacterium, Mycoplasma_sp_CAG472, Ruminococcus_sp_CAG563.* Xanthine is known as a purines-derived metabolite and contribute to several symptoms in chronic kidney disease and inflammatory bowel disease [34], and the accumulation of xanthine promoted the differentiation of Th17 cells aggravating gut inflammatory responses in inflammatory bowel disease [35]. It has been shown that the reduced levels of *Prevotella sp.* and *Bacteroides sp.* can increase the abundances of xanthine [36]. Pantothenate (vitamin B5) is an essential vitamin for CoA metabolic and gut microbiota-derived pantothenate can regulate purine metabolism for blood–brain barrier and intestinal

barrier [37]. Pantothenate supplementation enhanced the morphological structure of intestinal and reduced the level of isobutyric acid and isovaleric acid by inhibiting harmful bacteria abundance [38]. In addition, *Prevotella_sp_CAG520* was strongly positively correlated with L-phenylalanine and L-Tert-Leucine, whereas *Bacteroides_sp_CAG1060* and *Bacteroides_sp_CAG1060_57_27* were demonstrated as the important microbial species involving a decrease in L-phenylalanine and L-Tert-Leucine production, which is consistent with other result in HIV patients [39]. Sesamol is the key natural phenols, and its supplementation can inhibit DSS-induced colitis by inhibiting inflammatory response, gut barrier damages and increasing gut microbiome-derived short-chain fatty acid (SCFAs) contents [40]. In our study, we found sesamol is significantly reduced in diarrheal pregnant sows after PEDV infection and associated with gut microbiome, suggesting that sesamol may influence PEDV-induced diarrhea. Sebacic acid is a medium-chain fatty acid (MCFA), which shows anti-inflammatory effect by reducing the level of TNF-α and can also decrease LPS-induced inflammation by inhibiting IRF3/IFN-β/STAT axis [41, 42]. Together, these findings suggest microbiota–metabolite regulatory axis affects the occurrence of diarrhea in sows during PEDV infection.

Although our results showed intestinal microbiota structure and metabolite contents were associated with PEDV-induced diarrhea in pregnant sows, there were several limitations in this study. The sample size was relatively small, and the independent validation cohort has not yet been performed. In addition, metagenomic and metabolomic analyses cannot establish causal relationships between the gut microbiota, metabolites, and PEDV-induced diarrhea. Future studies should investigate the functional roles of these key differential microbes and metabolites in regulating diarrhea.

Conclusion

In this study, we combined shotgun metagenomics and metabolomics data to demonstrate that PEDV-induced diarrhea alters intestinal microbiota structure and metabolite contents in pregnant sows. Additionally, we found that the differentially enriched microbiota and metabolite-enriched pathway functions were closely associated with secondary bile acid biosynthesis, amino acids biosynthesis and metabolism, protein digestion and absorption. Our findings provide insights into the host-gut microbiota interaction during PEDV infection and offer potential therapeutic strategies for PEDV-induced diarrhea by targeting the gut microbiota and its derived metabolites.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-026-05043-2.

Supplementary Material 1: Supplementary data 1: the differentially expressed metabolites.

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Authors' contributions

XD, JY, AZ designed the experiments, Ao and CW revised the manuscript, XD and JY performed the experiments, JY, YW and CW collected the samples and analyzed the data, JZ and LS provided guidance on experimental procedures and data analysis, AZ supervised this research. All authors reviewed and approved the manuscript.

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Data availability

All the data presented by this study have been submitted with this research paper. The data was available and reproducible in the OMIX repository at National Genomics Data Center (CNCB-NGDC) (OMIX ID: OMIX015321) via https://ngdc.cncb.ac.cn/omix/preview/oQBP79fZ.

Declarations

Ethics approval and consent to participate

The animal study was reviewed and approved by the Animal Care and Use Committee of Wuhan polytechnic university (WHPU). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Competing interests

The authors declare no competing interests.

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