Effects of Compound Chinese Herbal Medicine on Pig Gut Microbiota and Metabolic Ecology

Min YAO[1,2], Jinrong SHEN[2], Ren OU[2], She WANG[2], Mingzong TANG[2], Fei GONG[2], Qingmeng LONG[2*], Xiao ZOU[1*]

1. College of Life Sciences, Guizhou University, Guiyang 500025, China; 2. Guizhou Breeding Livestock and Poultry Germplasm Determination Center, Guiyang 550018, China

Abstract [**Objectives**] This study was conducted to investigate the effects of a compound Chinese herbal medicine pig gut microbiota and metabolic ecology. [**Methods**] Through 16S rRNA high-throughput sequencing and metabolomics, the effects of administing a 5% compound mixture of *Pseudostellaria heterophylla*, *Uncaria rhynchophylla* and Lonicera japonica for 60 d on the intestinal microbiota and metabolites of three-way crossbred pigs were evaluated. [**Results**] The Chinese herbal medicine (CHM) group exhibited improved α-diversity indices (Ace = 541.22, Shannon = 3.36, Simpson = 0.10), though not significant ($P > 0.05$), compared with the CK group (Ace = 511.46, Shannon = 2.75, Simpson = 0.12). β-diversity analysis revealed a clear separation between the groups. At the phylum level, the overall differences in gut microbiota composition were minor. However, the CHM group showed a substantial increase in Bacteroidota in the cecum and a significant rise in *Myxobacteria*, which have anti-inflammatory and anti-obesity potential, suggesting a positive effect on intestinal health. Meanwhile, the CHM group exhibited 67 unique OTUs, higher than 27 in the CK group, primarily concentrated in the ileum and cecum. Additionally, metabolomic analysis revealed 210 unique metabolites in the CHM group, surpassing 77 in the CK group. A total of 653 metabolites showed significant changes, involving 11 metabolic pathways. Correlation analysis revealed that metabolites such as PC, PE, LysoPC, LysoPE and dehydrocarpaine were closely associated with microbiota. In the CHM group, key metabolites exhibited complex interactions with gut microbiota. Dehydrocarpaine showed positive correlations with probiotics such as *Lactobacillus* and *Bifidobacterium*. [**Conclusions**] These results demonstrated that CHM potentially enhanced pig gut health by improving microbial diversity and regulating metabolic networks, providing a scientific basis for further optimization of swine farming strategies.

Key words Compound Chinese herbal medicine; Intestine; Flora; Metabolite; Pig

DOI:10.19759/j.cnki.2164-4993.2025.04.001

It is reported that polyphenols, flavonoids, polysaccharides, lectins, proteins and peptides in Chinese herbal medicine can stimulate the immune system and possess antioxidant properties[1]. In recent years, Chinese herbal medicine has often been used as a phytogenic feed additive, serving as a nutritional supplement and antibiotic alternative to improve the health and growth performance of livestock and poultry[2-5]. *Pseudostellaria heterophylla*, *Uncaria rhynchophylla* and *Lonicera japonica* are common Chinese herbal medicines. Modern research has shown that they can effectively alleviate intestinal inflammation, regulate the structure and composition of gut microbiota, promote short-chain fatty acid production, and possess antibacterial, antiviral, immunomodulatory, antioxidant, and digestive function-improving properties[6-9]. Generally, compound Chinese herbal formulas composed of multiple herbs exhibit synergistic effects among their bioactive components, demonstrating greater biological efficacy than single-herb extract[2]. In this study, the effects of a compound Chinese herbal formula on the gut microbiota and metabolic ecology of pigs were investigated, as well as its potential positive and negative impacts, aiming to provide scientific evidence for optimizing swine production strategies.

Received: March 25, 2025 Accepted: May 28, 2025

Supported by Guizhou Provincial Department of Agriculture and Rural Affairs Project (QNYZZZ[2017]No.12, GZSZCYJSTX-04); 2024 Quality Supervision and Sampling Project of Normal Temperature Semen for Breeding Pigs.

Min YAO (1986-), female, P. R. China, senior engineer, PhD, devoted to research about food and inspection and food ecology.

*Corresponding author. Qingmeng LONG (1973-), female, P. R. China, senior veterinarian, devoted to research about animal husbandry and veterinary, genetic breeding and inspection and testing; Xiao ZOU (1977-), male, P. R. China, professor, doctoral supervisor, research interests include microbial ecology.

Materials and Methods

Experimental materials

The experiment was carried out at the experimental base of Guizhou University in Huaxi District, Guiyang City, Guizhou Province, China. Twenty healthy castrated three-way crossbred pigs of the same age were selected and randomly divided into two groups for separated raising and standardized management. All experimental operations strictly adhered to the ARRIVE guidelines and relevant animal ethics regulations.

Experimental methods

Experimental design The control group (CK) was fed a basal diet, while the experimental group (CHM) received the basal diet supplemented with 5% Chinese herbal mixture (*Pseudostellaria heterophylla*, *Uncaria rhynchophylla*, and *Lonicera japonica*). The trial lasted for 60 d. Pigs were randomly slaughtered ($n = 3$/group), and the contents of the duodenum, jejunum, ileum, cecum, colon and rectum were collected and pooled according to the same intestinal segment within each group. The samples were subjected to high-throughput sequencing and UHPLC-MS metabolomics analysis, labeled as CK1 to CK6 and CHM1 to CHM6.

Table 1 Sample name

Sample	Duodenum	Jejunum	Ileum	Cecum	Colon	Rectum
CK	CK1	CK2	CK3	CK4	CK5	CK6
CHM	CHM1	CHM2	CHM3	CHM4	CHM5	CHM6

PCR and Illumina MiSeq sequencing Microbial community genomic DNA was extracted from the samples using the E. Z. N. A. Soil DNA Kit (Omega Bio-tek, USA). The concentration and purity of the DNA were measured using a NanoDrop 2000 machine (Thermo Scientific, USA). The V3-V4 region of the bacterial 16S rRNA gene was amplified using universal primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). Sequencing was performed on the Illumina MiSeq PE300 platform (Illumina, USA). Primer sequences were removed, and OTU clustering analysis was conducted at 97% similarity. Species classification was analyzed by Bayesian algorithm of ribosome database project (RDP) classifier. The Majorbio Cloud Platform was employed to assess α-diversity, β-diversity, and bacterial abundance differences. Additionally, biochemical indices were screened using variance inflation factor (VIF) analysis (VIF < 10), followed by Spearman correlation analysis between significantly altered bacterial genera and biochemical parameters.

Metabolomics analysis After sample pretreatment, metabolites were detected and comparatively analyzed using a Vanquish Horizon UHPLC system coupled with a Q Exactive HF-X mass spectrometer (Thermo Scientific, USA) in both positive and negative ion modes. Separation was performed on an ACQUITY UPLC HSS T3 column (100 mm × 2.1 mm, 1.8 μm) with the column temperature set at 40 °C and an injection volume of 2 μl. Metabolomics data processing was completed on the Majorbio Cloud Platform. Metabolites were identified and annotated based on accurate relative molecular mass and MS/MS fragment ions using the HMDB and KEGG databases. Metabolic pathway enrichment analysis was performed on MetaboAnalyst with the KEGG database (Fisher's exact test, $P_{adjust} < 0.05$). Multivariate statistical analyses including PCA, PLS-DA and OPLS-DA were conducted using the R package "ropls" to screen differential metabolites (VIP ≥ 1, $P < 0.05$), and KEGG and HMDB pathway enrichment analyses were conducted.

Results and Analysis

Effects of Chinese herbal medicine on pig gut microbiota

Chinese herbal medicine affects the abundance and diversity of pig gut microbiota To investigate the effects of CHM on pig gut microbiota, 16S rRNA high-throughput sequencing was performed for the analysis of intestinal microorganisms. The sequencing results showed that a total of 310 426 optimized sequences were obtained from 8 experimental samples, comprising 129 536 329 bases with an average sequence length of 417 bp. Through OTU clustering analysis, a total of 911 OTUs were obtained, from 411 species, 231 genera, 100 families, 54 orders, 22 classes, 14 phyla, 1 domain and kingdom. The α diversity was used to represent the richness and diversity of microbial communities, and the results are shown in Table 2.

Table 2 Effects of CHM on the α-diversity of pig gut microbiota

Estimators	CK	CHM	*P*-value	*Q*-value	Change rate//%
Ace index	511.46 ± 166.66	541.22 ± 61.13	0.748 8	0.862 7	5.82
Chao richness	510.04 ± 173.62	541.95 ± 64.81	0.742 4	0.862 7	6.26
Shannon index	3.03 ± 0.55	3.36 ± 0.75	0.511 5	0.862 7	10.69
Simpson index	0.12 ± 0.04	0.10 ± 0.05	0.585 1	0.862 7	−16.23
Sobs index	421.25 ± 141.25	466.5 ± 60.1	0.577 0	0.862 7	10.74
Coverage	0.996 77 ± 0.000 89	0.996 86 ± 0.000 48	0.862 7	0.862 7	0.01

Indices including Ace and Shannon were used for evaluation. Compared with the control group (CK) (Ace = 511.46, Shannon = 2.75, Simpson = 0.12), the CHM group (Ace = 541.22, Shannon = 3.36, Simpson = 0.10) showed non-significant differences in diversity indices. However, the Ace index increased by 5.82%, suggesting enhanced community richness. The Shannon index increased by 10.69%, while the Simpson index decreased by 16.23%, indicating improved community diversity (Table 1). Additionally, as shown in Fig. 1A and Fig. 1B, the gut microbial diversity in the control group (CK) remained relatively stable from the proximal to distal segments, with a slight decrease observed in the colon. In contrast, the addition of Chinese herbal medicine significantly enhanced both the abundance and diversity levels in the ileum and cecum. The impact of Chinese herbal medicine on microbial community structure was further investigated through β-diversity analysis (PCA and PCoA). The PCA (Fig. 1C) and PCoA (Fig. 1D) score plots revealed clear separation between the Chinese herbal medicine group (CHM) and the control group (CK), particularly between samples CK3 and CHM3, as well as CK4 and CHM4, demonstrating the impact on microbial community structure. In conclusion, CHM might positively influence pig intestinal microorganisms by enhancing the diversity of flora and changing the structure of flora.

Effects of Chinese herbal medicine on the microbial composition in pig intestines To further elucidate the changes induced by Chinese herbal medicine in gut microbiota, we analyzed the relative abundance of dominant microbial taxa in both groups, with particular focus on significantly altered strains. As shown in Fig. 2A, the intestinal microbiota was primarily composed of Firmicutes, Bacteroidota, Actinobacteriota, Spirochaetota, and

Campylobacterota (TOP5), among which Firmicutes and Bacteroidota collectively accounted for over 80% of the total composition. Notably, compared with the control group (CK), the CHM group showed a significant increase in Bacteroidota abundance in the cecum, consistent with previous findings suggesting beneficial effects on porcine gut health[2,6]. Overall, no significant differences were observed between groups at the phylum level. As shown in Fig. 2B, at the genus level, dominant taxa with relative abundance exceeding 1% included *Clostridium*_sensu_stricto, *Streptococcus*, *Lactobacillus*, *Romboutsia*, and *Bifidobacterium*. Fig. 2D shows that at the genus level, bacterial genera showing significant differences ($P < 0.05$) included *Staphylococcus* (decreased by 6% in CHM group), *Veillonella* (decreased by 3% in CHM group), *Aerococcus* (decreased by 2% in CHM group), *Blautia* (increased in CHM group, a potential anti-inflammatory and anti-obesity genus), as well as unclassified *Micrococcales* and *Streptomyces*. As shown in Fig. 2D, at the OTU level, the CHM group exhibited 67 unique OTUs (accounting for 7.25%), significantly more than the 27 unique OTUs (accounting for 2.97%) in the control group (CK), representing an increase of 40 OTUs primarily concentrated in the ileum and cecum regions.

Effects of Chinese herbal medicine on pig intestinal metabolism

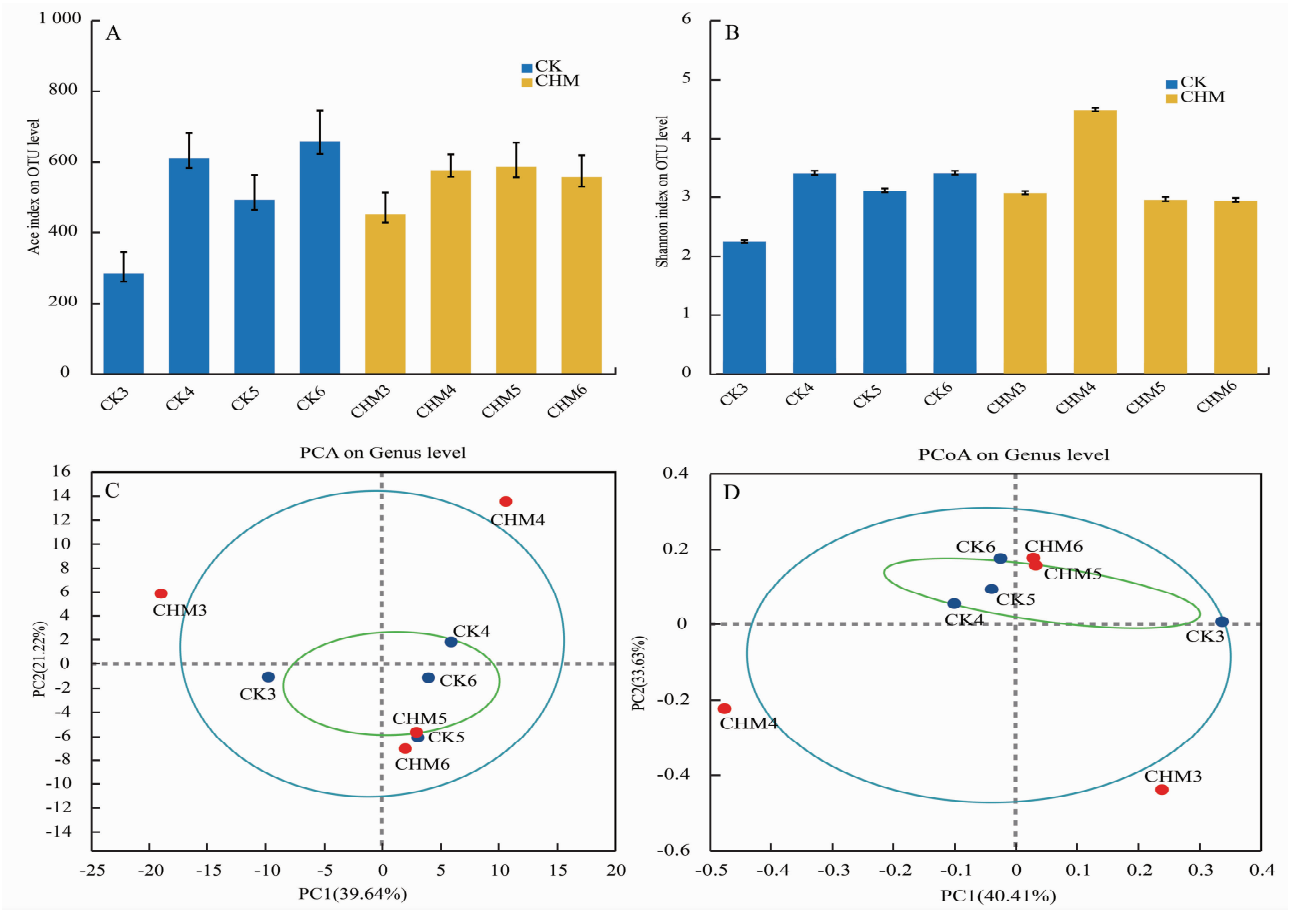
Effects of Chinese herbal medicine on pig intestinal metabolic profiles To investigate the effects of Chinese herbal medicine on pig intestinal metabolism, differential metabolomic analysis was conducted using UHPLC-MS/MS. After low-mass peak filtering, missing value processing and data normalization, a total of 3 631 peaks were detected, including 1 549 in positive ion mode and 2 082 in negative ion mode. Fig. 3A shows that compared with the control group (CK), the CHM group contained 210 unique metabolites while the CK group had 77. The two groups shared 1 537 common metabolites. A total of 653 metabolites showed significant changes, indicating that CHM induced distinct metabolic responses. Fig. 3B represents the correlation (similarity) of metabolite composition and abundance among samples. The analysis revealed that metabolite differences generally exist between intestinal segments, with adjacent segments showing higher similarity. In the distal colon, the CHM group exhibited high similarity with the CK group, particularly demonstrating stronger intra-group correlation within the CHM group. It likely reflected that CHM increased the diversity and complexity of intestinal digestion and absorption. Fig. 3C and Fig. 3D demonstrate the distinction in intestinal metabolite classification between the CHM group and the control group (CK) using PCA and PLS-DA. Both positive and negative ion modes exhibited clear separation, indicating that CHM significantly influenced the intestinal metabolites of pigs.

Effects of Chinese herbal medicine on intestinal metabolites and metabolic pathways Fig. 4A shows that through cluster heatmap and VIP bar chrat analysis (VIP > 2.0, $P < 0.05$), 13 significantly-different metabolites were identified between the CHM group and the control group (CK). They could serve as potential biomarkers for CHM's intestinal effects. Compared with the CK group, 7 metabolites were significantly upregulated in the CHM group, including austalide K, 15-KETE (15-oxo-ETE), GPEtn(2∶0/20∶0), 12(R)-HETE, monotropein, ricinoleic acid, and glaudine. Among these, 15-KETE and 12(R)-HETE are arachidonic acid metabolites formed through the lipoxygenase pathway, known to stimulate leukocyte aggregation and enhance chemotaxis, and may also play roles in regulating ocular transparency and secretion. GPEtn(2∶0/20∶0) is believed to enhance brain vitality, stabilize the nervous system, balance endocrine function, while also boosting immunity and regenerative capacity. Monotropein, as a component of Chinese herbal medicine, exhibits multiple pharmacological effects including kidney-yang tonification, bone strengthening, and rheumatism relief. Additionally, 6 metabolites were significantly downregulated in the CHM group, including [4-(3-hydroxybutyl)-2-methoxyphenyl] oxidanesulfonic acid, mono-isopropyl-disopyr amide, codeine N-oxide, cephalotaxine, dipropyl hexanedioate, and 6-hydroxy-5-methoxy indole glucuronide. The differentially-expressed metabolites were primarily categorized as follows: lipids and lipid-like molecules (41.56%, 128 compounds), organic acids and derivatives (18.18%, 56 compounds), organoheterocyclic compounds (12.66%, 39 compounds), organic oxygen compounds (10.39%, 32 compounds), benzenoids (5.19%, 16 compounds), phenylpropanoids and polyketides (5.19%, 16 compounds), nucleosides/nucleotides/analogs (2.92%, 9 compounds), alkaloids and derivatives (2.60%, 8 compounds), and organic nitrogen compounds (1.30%, 4 compounds).

Fig. 4B metabolic pathway enrichment analysis revealed that, compared with the CK group, the CHM group significantly altered 11 metabolic pathways, including N-glycan biosynthesis, taurine and hypotaurine metabolism, glutathione metabolism, vitamin B6 metabolism, starch and sucrose metabolism, pyrimidine metabolism, nicotinate and nicotinamide metabolism, glycerophospholipid metabolism, arginine and proline metabolism, pantothenate and CoA biosynthesis, and citrate cycle. These changes demonstrated the profound impact of Chinese herbal medicine on the intestinal and overall metabolism of pigs.

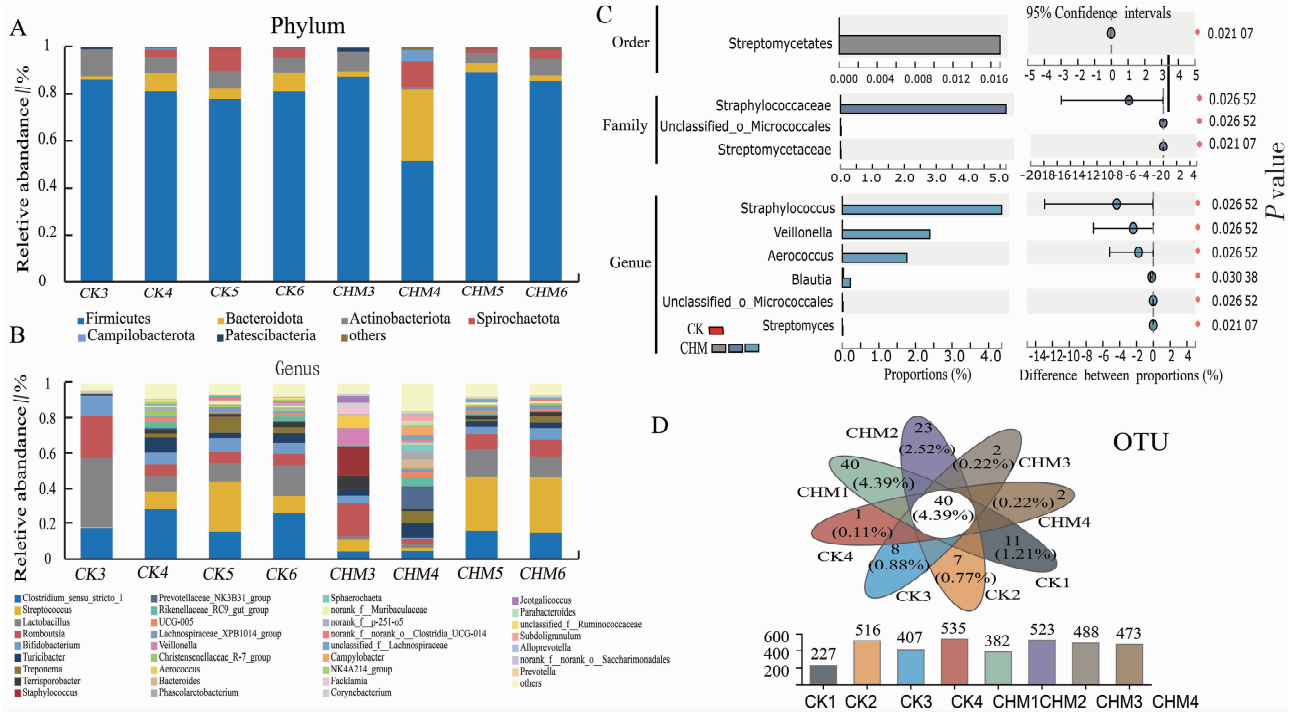
Correlation analysis between metabolites and gut microbiota

To explore the correlation between intestinal metabolites and bacterial communities in pigs from the CHM group and the normal diet group (CK), Spearman correlation analysis was performed. Fig. 5 shows that metabolites closely associated with bacterial communities include PC, PE, LysoPC, LysoPE, dehydrocarpaine, and 1-(8Z,11Z,14Z-eicosatrienoyl)-glycero-3-phosphate. The main bacterial groups involved were *Treponema*, *Romboutsia*, *Staphylococcus*, *Streptococcus*, *Terrisporobacter*, *Turicibacter*, *Veillonella*, *Clostridium*_sensu_stricto_1, *Lactobacillus*, and *Bifidobacterium*. Analysis revealed that *Treponema* was generally negatively correlated with 10 metabolites and showed a significant negative correlations with PC (22∶4/0∶0) and 1-(8Z,11Z,14Z-eicosatrienoyl)-glycero-3-phosphate. LysoPC(16∶0) exhibited negative correlations with *Veillonella*, *Staphylococcus*, and *Terrisporobacter*, but only showed significant negative correlations with the first two. Additionally, its positive correlations with *Clostridium*_sensu_stricto_1 and *Streptococcus* were not significant. Dehydrocarpaine showed positive correlations with *Romboutsia*, *Lactobacillus*, and *Bifidobacterium*, but negative correlations with



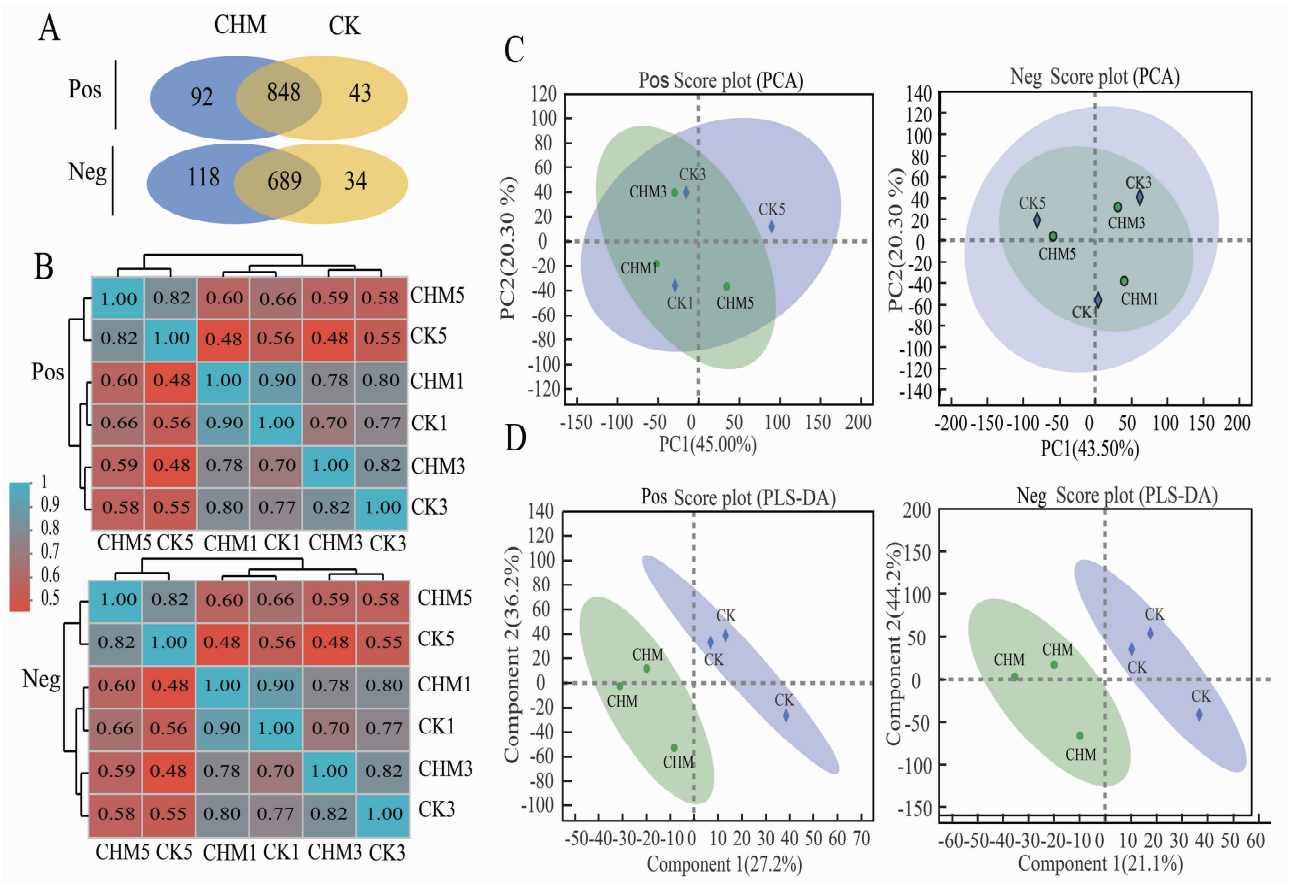
α-diversity assessed via Ace (A) and Shannon (B) indices at the OTU level. PCA (C) and PCoA (D) analyses were based on unweighted UniFrac.

Fig. 1 Effects of CHM on pig gut microbiota



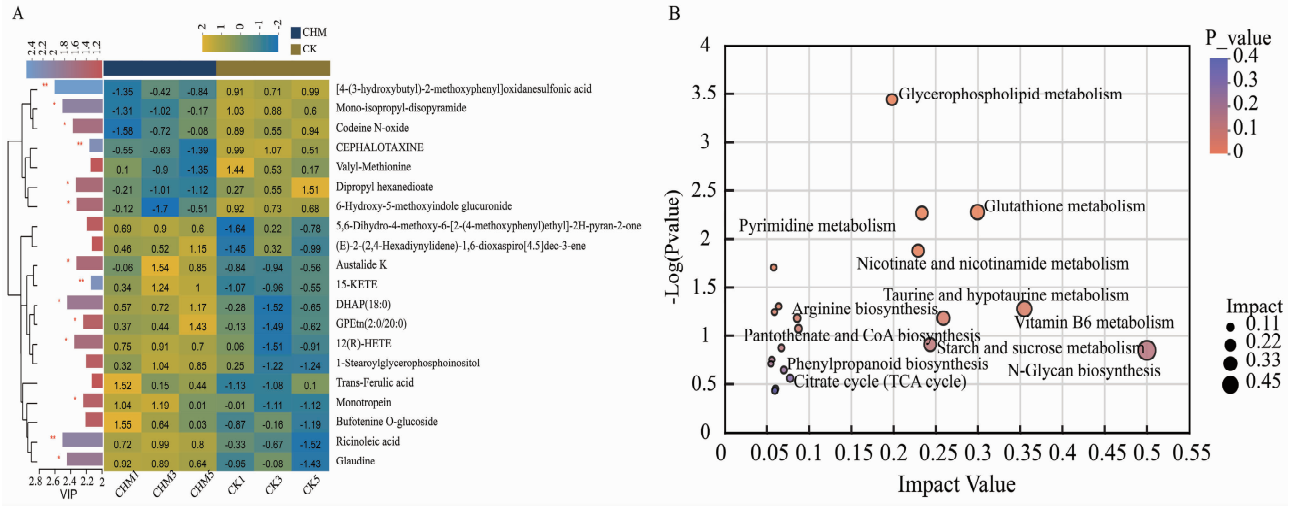
(A) and (B) show microbiota composition at phylum and genus levels; (C) is a species Venn diagram; (D) highlights the top 15 microbial taxa.

Fig. 2 Effects of CHM on the composition of pig gut microbiota



(A) Metabolite composition in positive (above) and negative (below) ion modes; (B) Sample correlation plots for positive (above) and negative (below) ion modes; (C) PCA plots for positive (left) and negative (right) ions; (D) PLS-DA plots for positive ions (left; $R^2Y = 0.949$; $Q^2 = -0.584$) and negative ions (right; $R^2Y = 0.94$; $Q^2 = -0.49$).

Fig. 3 Effects of CHM on intestinal metabolic profile in pigs



(A) Heatmap of the relative abundance of differential metabolites with VIP values (VIP > 2.0, $P < 0.05$, 95% confidence interval); (B) KEGG-based predictive analysis of intergroup metabolic pathways.

Fig. 4 Effects of CHM on pig intestinal metabolites and metabolic pathways

Turicibacter, *Streptococcus*, and *Treponema*. Except for LysoPC (16 : 0) and dehydrocarpaine, other eight metabolites exhibited similar correlations with the same bacterial genera, though the strength of correlation varied. For example, they generally showed positive correlations with *Romboutsia*, *Staphylococcus*, *Terrisporobacter*, and *Veillonella*, while displaying negative correlations with *Treponema*, *Bifidobacterium*, and *Clostridium*_sensu_stricto_1.

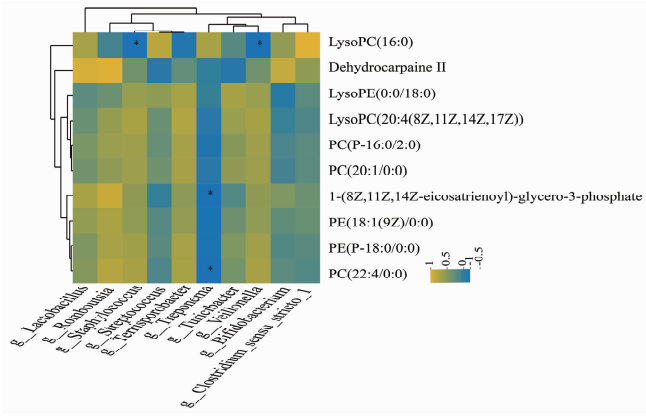


Fig. 5 Spearman correlation analysis linking the abundance of specific genera with intestinal metabolites at the genus level

Conclusions and Discussion

This study comprehensively evaluated the effects of Chinese herbal medicine (CHM) on pig gut microbiota and metabolism. The possible changes of CHM on pig intestinal health were analyzed from many aspects. The CHM group exhibited increased microbial richness and diversity, with the Ace and Shannon indices rising by 5.82% and 10.69%, respectively, while the Simpson index decreasing by 16.23%. Additionally, the microbial community structure significantly differed from that of the CK group, indicating a microbial community structure in pigs. The relative abundance of Bacteroidota in the cecum significantly increased, along with a rise in *Akkermansia*, which has potential anti-inflammatory and anti-obesity effects. Meanwhile, *Staphylococcus* and *Veillonella* decreased, and the number of unique OTUs in the CHM group was significantly higher than in the CK group, further demonstrating the specific regulatory effects of Chinese herbal medicine on gut microbiota[10-11]. Additionally, metabolomic analysis revealed significant metabolic changes induced by CHM, identifying 653 significantly differential metabolites spanning various categories such as lipids and lipid-like molecules. These alterations affected 11 metabolic pathways, including N-glycan biosynthesis and glutathione metabolism. These findings suggest that CHM may enhance intestinal digestive and absorptive functions as well as metabolic complexity through diversified metabolic regulation. Furthermore, Spearman correlation analysis further revealed associations between metabolites in the CHM group and bacterial groups. Key metabolites such as PC, LysoPC and dehydrocarpaine showed distinct correlations with bacterial groups such as *Treponema* and *Romboutsia*. Notably, *Treponema* showed negative correlations with most metabolites, while dehydrocarpaine exhibited positive correlations with probiotics (*e.g.*, *Lactobacillus* and *Bifidobacterium*). It suggests CHM may improve intestinal function and health status by regulating the relationship between microbiota and metabolites.

References

[1] YEH HS, WENG BC, LIEN TF. Effects of Chinese traditional herbal medicine complex supplementation on the growth performance, immunity and serum traits of pigs[J]. Animal Science Journal, 2011, 82: 747 – 752.

[2] XU Q, CHENG M, JIANG R, *et al.* Effects of dietary supplement with a Chinese herbal mixture on growth performance, antioxidant capacity, and gut microbiota in weaned pigs[J]. Front. Vet. Sci., 2022, 9: 971647.

[3] HUANG Y, YANG K, CAO JJ, *et al.* Integration of metabolomics and transcriptomicsto comprehensively evaluate the metabolic effects of Gelsemium elegans on pigs[J]. Animals, 2021, 11: 1192.

[4] KONG XF, WU G, YIN YL. Roles of phytochemicals in amino acid nutrition[J]. Frontiers in bioscience: Scholar edition, 2011, 3: 372 – 384.

[5] LI Y, LI H, CHU Q, *et al. Macleaya cordata* extracts suppressed the increase of a part of antibiotic resistance genes in fecal microorganism of weaned pigs[J]. Canadian Journal of Animal Science, 2018, 98: 884 – 886.

[6] XIAO Q. Polysaccharides from *Pseudostellaria heterophylla* modulate gut microbiota and alleviate syndrome of spleen deficiency in rats[J]. Scientific Reports, 2022, 12: 20217.

[7] PANG W. Antitussive activity of *Pseudostellaria heterophylla* (Miq.) Pax extracts and improvement in lung function via adjustment of multi-cytokine levels[J]. Molecules, 2011, 16: 3360 – 3370.

[8] LI W. Alleviation of colitis by honeysuckle MIR2911 via direct regulation of gut microbiota[J]. Journal of Controlled Release, 2024, 376: 123 – 137.

[9] LI HJ, WEI WL, LI ZW, *et al.* Systematic comparison of metabolic differences of *Uncaria rhynchophylla* in rat, mouse, dog, pig, monkey and human liver microsomes[J]. Anal Bioanal Chem, 2020, 412: 7891 – 7897.

[10] LIU S, XIAO GS, WANG Q, *et al.* Effects of dietary *Astragalus membranaceus* and *Codonopsis pilosula* extracts on growth performance, antioxidant capacity, immune status, and intestinal health in broilers[J]. Frontiers in Veterinary Science, 2023, 10: 1302801.

[11] YANG XF, LU M, YOU LJ, *et al.* Herbal therapy for ameliorating nonalcoholic fatty liver disease via rebuilding the intestinal microecology [J]. Chinese Medicine, 2021, 16(1): 62.

Editor: Yingzhi GUANG Proofreader: Xinxiu ZHU