105 (2026) 106506

Contents lists available at ScienceDirect

Poultry Science

journal homepage: www.elsevier.com/locate/psj

Antibiotic-free Wenchang chickens may promote blood levels of B vitamins by modulating the gut microbiota: An integrated analysis of cecal content metagenomics and serum metabolomics

Shifan Deng [a,1], Xinli Zheng [b,1], Han Chu [a], Liang Hong [a], Jianbin Zhang [a], Hua Yang [a], Lihong Gu [b], Lei Pu [a,*]

[a] Tianjin Key Laboratory of Agricultural Animal Breeding and Healthy Husbandry, College of Animal Science and Veterinary Medicine, Tianjin Agricultural University, Tianjin, China
[b] Institute of Animal Science and Veterinary Medicine, Hainan Academy of Agricultural Sciences, Haikou, China

ARTICLE INFO

Keywords:
Wenchang chicken
Gut microbiota
Metagenomics
Serum metabolites
Disease resistance

ABSTRACT

Through the selective breeding of superior strains, livestock and poultry can achieve enhanced disease resistance and production performance, thereby improving farming efficiency and increasing chicken meat yield. This study analyzed the expression of gut health-related genes, cecal microbiota, and untargeted serum metabolomics in Wenchang chickens from the **NS** strain (Normal strain) and the **AFS** strain (Antibiotic-free strain), and explored the relationships between their cecal microbiota and serum metabolites. Our results show that in the ileum, antioxidant-related indicators T-AOC ($P < 0.05$), T-SOD ($P < 0.05$), and GSH-PX ($P < 0.05$) were significantly higher in the AFS strain than in the NS strain, while MDA ($P < 0.05$) was significantly lower in the AFS strain than in the NS strain. The mRNA expression level of *RORγt/FoxP3*, which is related to immune regulation, was significantly lower in the AFS strain than in the NS strain ($P < 0.05$). The differential microorganisms in the cecum primarily included *Muribaculum, Cryptobacteroides, Blautia, Enterocloster, Lachnoclostridium, Hydrogenoanaerobacterium, Ruminococcus, Subdoligranulum, Clostridioides*, and *Evtepia*. The main differential metabolites in serum included folinic acid, biotin, lysophosphatidic acid (LPA), 3-hydroxy-3-methylbutanoic acid, 3-hydroxybutyric acid, and others. The differential metabolites are primarily enriched in the following metabolic pathways: gap junction, glycolipid metabolism, and fatty acid biosynthesis. In addition, the Pearson correlation analysis between the gut microbiota and serum metabolites showed that *Blautia* was positively correlated with folinic acid ($P < 0.05$) and biotin ($P < 0.05$); *Lachnoclostridium* was positively correlated with biotin ($P < 0.01$); and *Ruminococcus* was positively correlated with 3-hydroxybutyric acid ($P < 0.05$). This study mainly elucidates the metabolic characteristics of the antibiotic-free Wenchang chicken strain by analyzing gut microbiota and serum metabolites.

Introduction

Wenchang chicken, a unique indigenous breed from Hainan Province, not only reflects the region's cultural and culinary traditions but also boasts considerable market recognition and competitiveness. Wenchang chicken is renowned for its tender and flavorful meat, characterized by a distinctive taste (Zheng et al., 2024). These characteristics provide Wenchang chicken with a distinct advantage in the food market. Therefore, research on disease-resistant breeding of Wenchang chicken will help improve its disease resistance and production performance, ensuring the quality and safety of the meat.

In promoting the healthy growth of poultry, gut health is particularly important. In the research on gut health, gut microbiota, intestinal inflammation, antioxidant capacity, and gut barrier function are frequently investigated and considered important areas of study. There are multifarious microorganisms in the guts of chickens, forming a complex ecosystem (Yang et al., 2024). The gut microbiota of chickens plays a crucial role in growth and development, immune function, feed

* Corresponding author.
E-mail address: pulei87@126.com (L. Pu).
[1] Shifan Deng and Xinli Zheng are co-first authors.

https://doi.org/10.1016/j.psj.2026.106506
Received 2 July 2025; Accepted 22 January 2026
Available online 26 January 2026

efficiency, and fat deposition (Shen et al., 2024). Consequently, many studies have focused on enhancing the production performance of chickens through modulating gut microbiota composition. In-depth research on the types and functions of gut microbiota holds significant scientific value for promoting healthy growth and improving disease resistance in chickens (Abdel-Moneim et al., 2024; Zhang et al., 2023).

The metabolic activities of the gut microbiota can influence the host's metabolism (Luo et al., 2022). Metabolites produced by gut microbiota, such as short-chain fatty acids (SCFAs) and vitamins, can be absorbed by intestinal epithelial cells into the bloodstream, entering systemic circulation and influencing the physiological state of the host, thereby regulating the host's health (Xu et al., 2022; Mao et al., 2024). Untargeted serum metabolomics is an emerging field of biotechnology that analyzes the complete profile of small-molecule metabolites present in organisms. Serum metabolomics provides a more comprehensive perspective for research, helping to reveal the complex relationship between gut health and overall host health. Analyzing the differences in these metabolites contributes to our understanding of metabolic activities within the body. Therefore, the combined analysis of gut metagenomics and serum metabolomics facilitates a deeper understanding of how microorganisms impact the physiological processes of the organism through the metabolites.

To gain a more comprehensive understanding of the differences in gut health between the NS and AFS strains of Wenchang chickens, this study analyzed the cecal microbiota through metagenomics and employed liquid chromatography-mass spectrometry (LC-MS) for serum metabolite profiling. Additionally, Pearson correlation analysis was performed to identify potential associations between the gut microbiota and metabolites. This integrated analytical approach enables us to gain deeper insights into how microorganisms influence the physiological processes of the host through their metabolic products. It also reveals associations and interaction patterns that cannot be elucidated by single-omics analyses alone.

Despite numerous studies on gut microbiota and serum metabolites in poultry, research focusing on specific local breeds, such as Wenchang chickens, remains relatively limited. Research results indicate that there are significant differences in gut microbiota and serum metabolites between the two strains, and Pearson correlation analysis reveals correlations between these variables. This study focuses on the local breed of Wenchang chickens from Hainan Province, featuring regional characteristics and filling a knowledge gap regarding this breed. It provides a reference for future breeding efforts aimed at enhancing disease resistance in Wenchang chickens, with a focus on gut microbiota and serum metabolites.

Methods

Sample collection

The study focuses on the NS and AFS strains of Wenchang chickens. Sixty NS Wenchang chickens and sixty AFS Wenchang chickens were used, divided into six replicates with ten chickens in the same pen for each replicate. They were housed in the same environment with a temperature of 30°C and natural ventilation. Natural and artificial lighting was provided, and all chickens had free access to feed and water. At 120 days of age, six female chickens from each strain were randomly selected for blood sampling. The blood samples were allowed to stand at 4°C for 1 h, followed by centrifugation at 4,000 rpm for 15 min to obtain serum samples, which were then stored at −80°C. Cecal contents and intestinal samples were collected and immediately frozen in liquid nitrogen, followed by storage at −80°C. Ethical approval (reference number: HNXMSY-20240427) was granted by the Institutional Animal Care and Use Committee at the Experimental Animal Center of Hainan Academy of Agricultural Science, which is responsible for ensuring animal welfare.

Relative mRNA expression analysis by qRT-PCR

Total RNA was extracted from the ileum by using Trizol (Genes and Biotech Co., Ltd, Beijing, China). A cDNA reverse transcription kit was used to reverse transcribe the RNA into cDNA (Accurate Biology, Beijing, China). The concentration of total RNA and cDNA was determined using NanoDrop One Microvolume UV-Vis Spectrophotometer (Thermo Scientific, USA). qRT-PCR was performed using SYBR Green qPCR Mix (Baosharp, China) with the following program: 95°C for 5 min, 40 cycles of 95°C for 10 s, and 60°C for 30 s. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative mRNA expression levels. The primer sequences for the genes are provided in Table 1.

Antioxidant capacity

The ileum tissue homogenate was prepared using saline. After centrifugation, the supernatant was collected. According to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), the levels of malondialdehyde (MDA), total antioxidant capacity (T-AOC), total superoxide dismutase (T-SOD), and glutathione peroxidase (GSH-PX) activity were measured using the prepared supernatant.

Analysis of gut microbiota

Cecal contents samples were sent to Novogene (Beijing, China) for microbiota analysis. Following the extraction of genomic DNA, 1 μg of gDNA from each sample was subjected to sonication (Covaris, UK), which randomly fragmented the DNA into segments of approximately 350 bp in length. The fragmented DNA was then used for library construction. Sequencing was performed on the Illumina NovaSeq PE 150 platform to obtain raw sequence data reads. The raw data generated from the NovaSeq platform was filtered using fastp software to produce clean data for further analysis.

To analyze the gut metagenomic microbiota, the clean data were obtained and then assembled using MEGAHIT software, resulting in the generation of scaftigs. Based on the assembled scaftigs, fragments shorter than 500 bp were filtered out. ORF prediction was performed on the scaftigs of each sample using MetaGeneMark. Redundancy in the ORF prediction results was removed using CD-HIT software to construct a non-redundant initial gene catalogue. Subsequently, Bowtie2 was used to align the clean data from each sample to the initial gene catalogue. This alignment enabled the calculation of the number of reads mapped to each gene in each sample, allowing for the determination of gene

Table 1
Primer sequences of qRT-PCR.

primer name	primer sequence(5′−3′)	product size/bp
ZO-1 F	GTGACTCTTCACAGGGCTCC	139
ZO-1 R	GTAGCAGTCCTTCTGCTGGG	
CLAUDIN 1 F	ACCCACAGCCTAAGTGCTTC	200
CLAUDIN 1 R	AGGTCTCATAAGGCCCCACT	
OCCLUDIN F	ACGGCAGCACCTACCTCAA	123
OCCLUDIN R	GGGCGAAGAAGCAGATGAG	
NRF2 F	GTGTGTACTCATCGCGGTTC	103
NRF2-R	GGAGGGTCTTTCTTTGGTGTG	
HO-1 F	GAAACTTCGCAGCCACACAAC	233
HO-1 R	GGAGCATAGACAGGGTTGTCC	
CAT F	AGCAGGTGCCTTTGGCTATT	121
CAT R	TCCAGCAACAGTGGAGAACC	
SOD-1 F	GTGACCTCGGCAATGTGACT	86
SOD-1 R	GCAGTGTGGTCCGGTAAGAG	
FoxP3 F	CCGTGTCGGTGGCTATGATG	149
FoxP3 R	CTCTTGAAGCTGCTGCTGTTG	
RORyt F	CGCATTGGAGATTGAGCTGC	134
RORyt R	TTTTCTGGAAGGCGCAGACC	
β-actin F	GAGAAATTGTGCGTGACATCA	152
β-actin R	CCTGAACCTCTCATTGCCA	

abundance in each sample. Unigenes were aligned with commonly used databases that provide functional annotations (NCBI NR, KEGG) using DIAMOND software. For subsequent analysis, the highest score for each sequence was selected to obtain annotation information from each database.

Untargeted serum metabolomics

The serum samples were sent to Novogene (Beijing, China) for untargeted metabolomics analysis. A total of 100 μL of serum was placed in an EP tube, and 400 μL of 80% methanol was added to it. The mixture was vortexed and left on ice for 5 min, followed by centrifugation at 15,000 g for 20 min at 4°C. The supernatant was collected and diluted to a final methanol concentration of 53%. After centrifugation at 15,000 g for 20 min at 4°C, the supernatant was collected for analysis by LC-MS. Subsequently, the samples were separated using a Hypesil Gold column (100 × 2.1 mm, 1.9 μm) and analyzed using a Q Exactive™ HF/Q Exactive™ HF-X mass spectrometer. Chromatographic separation was conducted at a column temperature of 40°C, using a mobile phase consisting of 0.1% formic acid (A) and methanol (B) at a flow rate of 0.2 mL/min. The elution gradient was as follows: at 0 min, 98% A and 2% B; at 1.5 min, 98% A and 2% B; at 3 min, 15% A and 85% B; at 10 min, 0% A and 100% B; at 10.1 min, 98% A and 2% B; at 11 min, 98% A and 2% B; and at 12 min, 98% A and 2% B. The mass spectrometry conditions

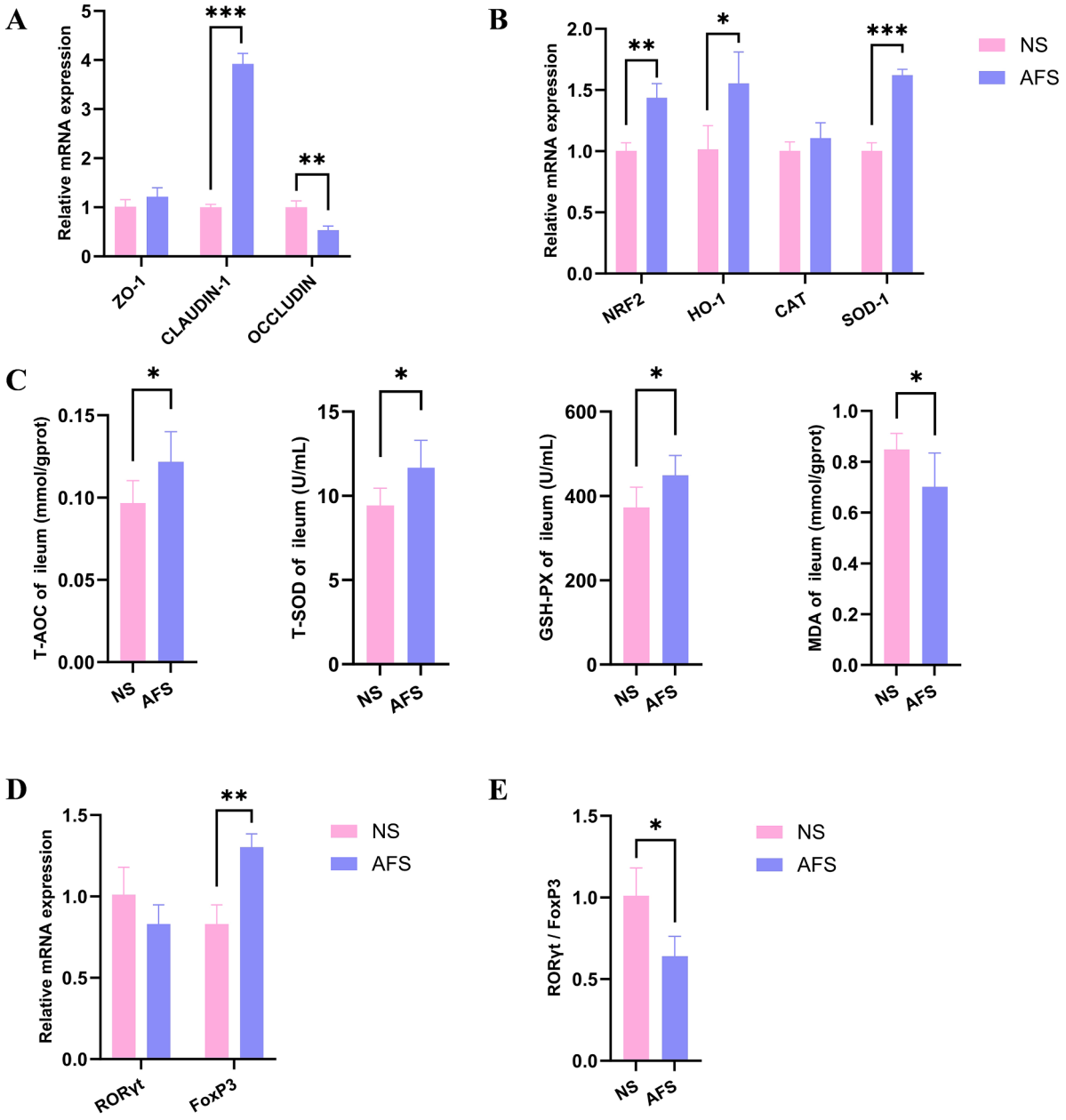

Fig. 1. The analysis of intestinal barrier, antioxidant capacity, and immunity in the ileum
(A)Gene expression levels related to gut barrier function.
(B)Gene expression levels related to antioxidant capacity.
(C)Measurement of antioxidant indicators MDA, T-AOC, T-SOD, and GSH-PX in the ileum.
(D)Expression levels of immune-related genes.
(E)The ratio of gene expression levels of *RORγt* to *FoxP3*.
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

were set to scan a range of m/z 100-1500. The settings for the ESI source were as follows: Spray Voltage: 3.5 kV; Sheath gas flow rate:35 psi; Aux Gas flow rate:10L/min; Capillary Temp:320°C; S-lens RF level:60; Aux gas heater temp:350°C; Polarity: positive, negative; MS/MS secondary scans: data-dependent scans.

Statistical analysis

Data from qRT-PCR and antioxidant capacity indicators were analyzed using GraphPad Prism 10 and SPSS 27. Intergroup differences were analyzed using a t-test. Differences were considered statistically significant when $P < 0.05$. A post-hoc power analysis was performed using SSPS AU to evaluate the adequacy of the selected sample size.

In the study of gut metagenomics, after annotating information at different levels, we conducted alpha diversity and beta diversity analyses. Next, we used Analysis of Similarity (ANOSIM) to assess the differences between groups. Subsequently, we employed the MetaGenomeSeq method to process and analyze the data, and used the LEfSe method to identify species with significant abundance differences between groups. After that, we applied MetaStat for statistical testing to further confirm the significance of these differential species.

For the analysis of metabolomics data, we employed the software metaX to process and transform the data, followed by PLS-DA analysis. Statistical differences among metabolites were calculated using the t-test. The default criteria for identifying differential metabolites were set as VIP > 1, P-value < 0.05, and $|\log_2 FC| \geq 0.58$.

Pearson correlation analysis was conducted to assess the top 10 differential microbiota identified at the genus level and the top 10 differential metabolites in serum under both positive and negative ionization modes. A heatmap was generated utilizing Origin software.

Result

Ileal analysis of intestinal barrier, antioxidant capacity, and immunity

As shown in Fig. 1, compared with the NS strain, the AFS strain exhibited significantly higher mRNA expression of *CLAUDIN-1* ($P < 0.001$) and significantly lower mRNA expression of *OCCLUDIN* ($P = 0.009$). The mRNA level of *ZO-1* in the AFS strain showed an upward trend, but this was not statistically significant (Fig. 1A). In the AFS strain, the mRNA expression levels of the antioxidant indicator *NRF2* ($P = 0.005$), *OH-1* ($P = 0.045$), and *SOD-1* ($P < 0.001$) were significantly higher than those in the NS strain, while there was no significant difference in *CAT* (Fig. 1B). Subsequently, we examined the antioxidant markers and found that the levels of T-AOC ($P = 0.018$), T-SOD ($P = 0.016$), and GSH-PX ($P = 0.020$) in the AFS strain were significantly higher than those in the NS strain, while MDA ($P = 0.036$) was significantly lower than that in the NS strain (Fig. 1C). The mRNA expression level of *FoxP3*, which is related to immune regulation, was significantly higher in the AFS strain ($P = 0.003$) compared to the NS strain, while the ratio of RORγt to *FoxP3* showed a significant decrease ($P = 0.038$) (Fig. 1D, E). These experimental results suggest that the AFS strain may have stronger intestinal antioxidant capacity in the ileum.

Gut microbiota composition and diversity

We collected cecal contents from two strains of Wenchang chickens, with six samples per strain. Metagenomic DNA was extracted, and a sequencing library was constructed. The obtained original data of 77.77 Gbps underwent quality control, resulting in an effective data volume of 76.55 Gbps after filtering. Next, gene fragments from a single sample were assembled, filtering out those shorter than 500 bp, resulting in fragments with an average length of 1320 bp. Subsequently, gene prediction was conducted to generate a non-redundant gene set. In the next step, the number of genes among the sample groups was quantified, revealing that the two groups share a total of 2,551,856 genes, with 195,298 genes unique to the NS strain and 322,717 genes unique to the AFS strain (Fig. 2A).

We conducted a selection of the top 10 microbial abundances from two strains of Wenchang chickens. The phyla with the highest relative abundance are *Bacteroidota, Bacillota, Spirochaetota, Actinomycetota, Thermodesulfobacteriota, Euryarchaeota, Uroviricota, Synergistota, Elusimicrobiota*, and *Deferribacterota* (Fig. 2B). The top ten genera with the highest relative abundance are *Bacteroides, Muribaculum, Alistipes, Brachyspira, Phocaeicola, Prevotella, Clostridium, Pseudoflavonifractor, Parabacteroides*, and *Flavonifractor* (Fig. 2C).

The results of the α-diversity analysis indicated that at the phylum level, the Simpson index of the AFS gut microbiota was significantly higher than that of the NS strain ($P = 0.0290$). However, there were no significant differences in the ACE, Chao1, Shannon, Observed species, and Good's coverage indices (Fig. 2D, E). At the genus level, the Simpson ($P = 0.0004$) and Shannon ($P < 0.0001$) indices of the gut microbiota in the AFS strain were extremely significantly higher than those in the NS strain, while there were no significant differences in ACE, Chao1, Observed species, and Good's coverage indices (Fig. 2F, G).

Beta diversity was primarily assessed using principal component analysis (PCA) to analyze the gut microbiota of the two strains of Wenchang chickens. The analysis indicated a slight overlap at the phylum level, while the overlap at the genus level was significantly lower (Fig. 2H, I). Additionally, through ANOSIM similarity analysis, we identified significant differences in microbial communities between the two strains, both at the phylum level ($R = 0.7630$, $P = 0.0060$) and the genus level ($R = 0.9352$, $P = 0.0020$). Overall, there is a significant separation of microbial communities at both the phylum and genus levels.

Differences in cecal microbiota

Employing LEfSe analysis to identify microorganisms that show significant differences between groups, the LDA value distribution histogram revealed differences in the composition of microbial communities between the AFS and NS strains. The results indicated that the hallmark microorganisms in the NS strain included *Muribaculum* and *Brachyspira*, while in the AFS strain, the hallmark microorganisms included *Massilistercora timonensis* and *Blautia* (Fig. 3A, B).

Additionally, MetaStat analysis was employed to assess differential microorganisms between the two groups at the genus level, as shown in the accompanying figure. The results demonstrated that *Muribaculum* and *Cryptobacteroides* were significantly more abundant in the NS strain compared to the AFS strain ($P < 0.05$) (Fig. 3C). Conversely, in the AFS strain, the relative abundances of *Blautia, Enterocloster, Lachnoclostridium, Hydrogenoanaerobacterium, Ruminococcus, Subdoligranulum, Clostridioides*, and *Evtepia* were significantly higher than those in the NS strain ($P < 0.05$) (Fig. 3D).

KEGG annotation and functional analysis of microbiota

Based on the analysis of KEGG annotation results, we observed that proteins associated with metabolic functions comprise the highest proportion of annotated genes, particularly those related to carbohydrate metabolism. The enriched KEGG pathways primarily include carbohydrate metabolism, amino acid metabolism, membrane transport, translation, energy metabolism, and metabolism of cofactors and vitamins (Fig. 4A).

We analyzed the top 10 abundances of annotated genes in the KEGG level 3 metabolic pathways of Wenchang chickens (Fig. 4B). To investigate the potential metabolic differences in functional composition, we utilized the MetaStat analysis method to identify the top 35 significantly different metabolic pathways between the groups (Fig. 4C). As shown in the figure, the relative abundance of ABC transporters (ko02010) in the AFS strain is significantly higher than that in the NS strain, while the relative abundances of pyrimidine metabolism (ko00240) and ribosome

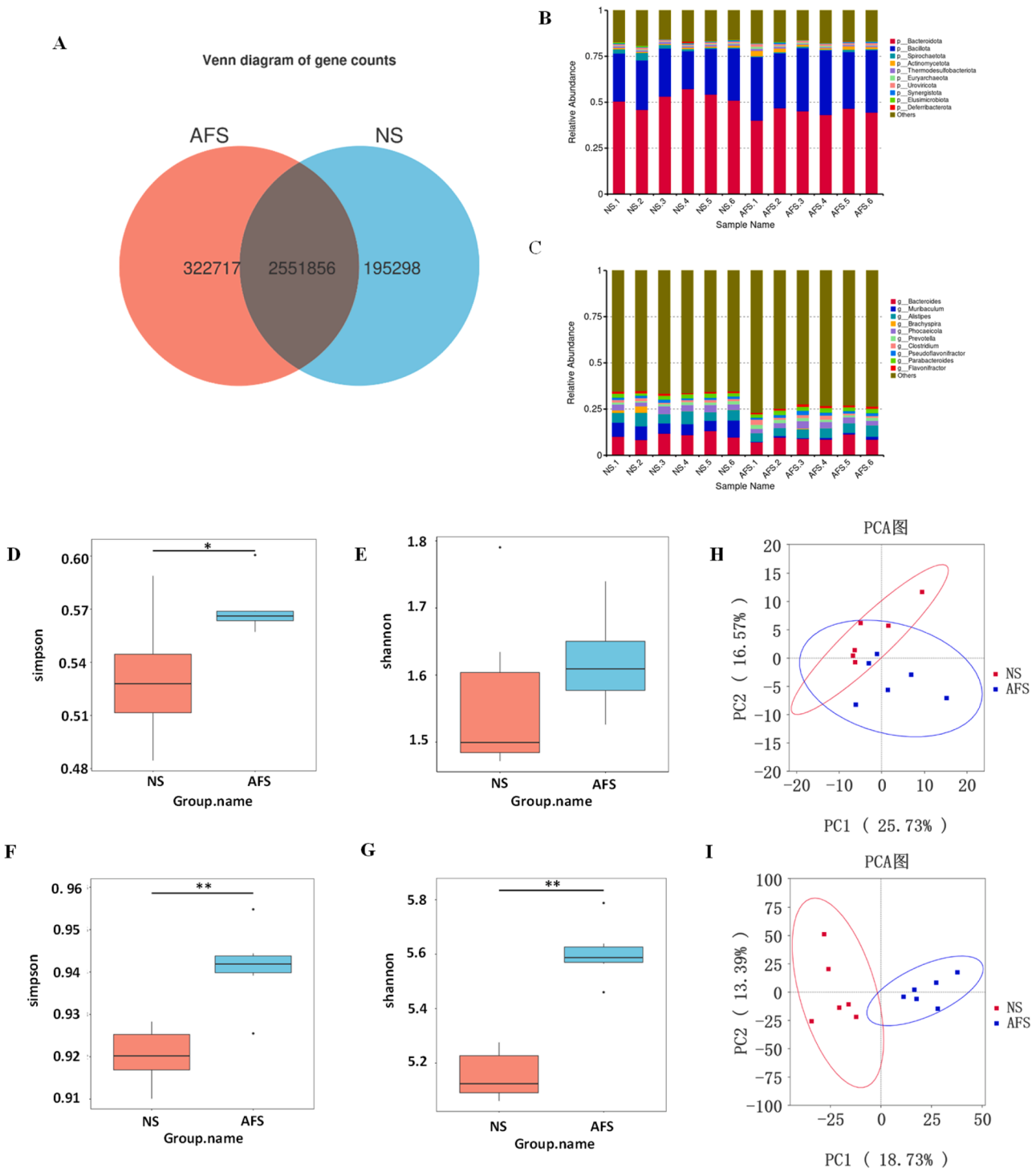

Fig. 2. Analysis of gut microbial community composition and diversity in NS and AFS strains of Wenchang chickens.
(A)Statistical analysis of gene numbers in the Venn diagram.
(B) The top 10 compositions of gut microbiota at the phylum level.
(C) The top 10 compositions of gut microbiota at the genus level.
(D)The Simpson index at the phylum level.
(E)The Shannon index at the phylum level.
(F)The Simpson index at the genus level.
(G)The Shannon index at the genus level.
(H) PCA of the β-diversity at the phylum level.
(I) PCA of the β-diversity at the genus level.
*$P < 0.05$, **$P < 0.01$. $N = 6$ in each group.

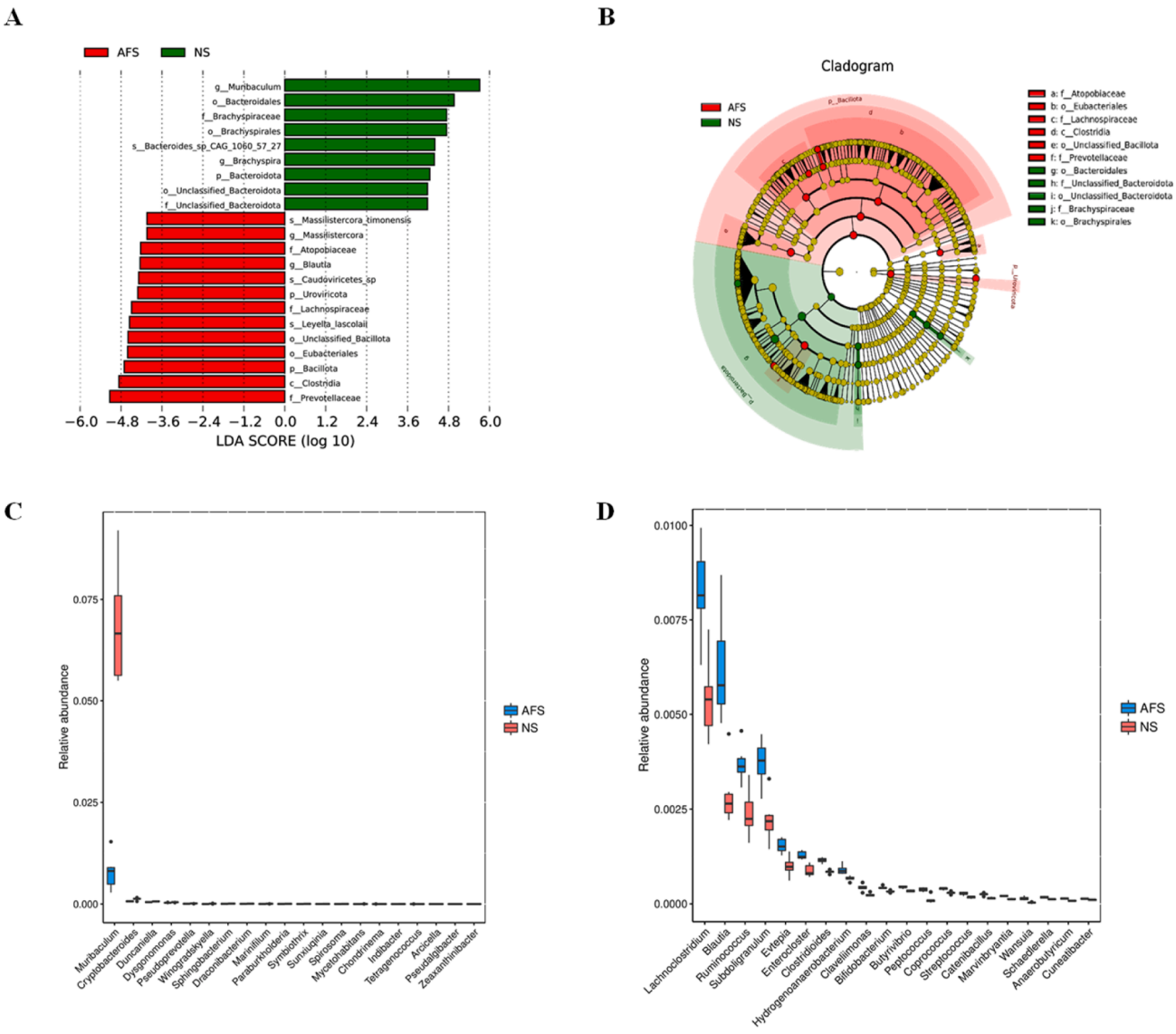

Fig. 3. Differential analysis of gut microbiota between the NS and AFS strains of Wenchang chickens.
(A) The LEfSe analysis of gut microbiota.
(B) Cladogram indicating the phylogenetic distribution of microbiota correlated with the NS or AFS strains.
(C, D) The MetaStat analysis of differential gut microbiota.

(ko03010) are lower than those in the NS strain.

Diversity and difference in serum metabolomics

This study utilized LC-MS to perform a metabolomic analysis of serum collected from different strains of Wenchang chickens, aiming to further assess the differences in metabolites. The PCA analysis indicates a trend in the differences of metabolites between the two strains of Wenchang chickens (Fig. 5A, B). In the PLS-DA model, the metabolomics of the NS strain and AFS strain exhibited clear separation in both positive and negative ion modes (Fig. 5C, D).

To visually represent the differential metabolites, a volcano plot was constructed with differentially expressed metabolites indicated by red and blue dots. A total of 95 differential metabolites were identified in the positive mode between the two groups, including 13 significantly up-regulated metabolites and 82 significantly down-regulated metabolites (Fig. 5E). In the negative mode, a total of 109 differential metabolites were identified, comprising 57 significantly up-regulated metabolites and 52 significantly down-regulated metabolites (Fig. 5F). Among these differential metabolites are lipids and lipid-like molecules, organoheterocyclic compounds, organic acids and derivatives, benzenoids, and others. Among these, compared to the NS strain, the significantly up-regulated metabolites in the AFS strain included 2,3-dinor prostaglandin E1, 3-hydroxy-3-methylbutanoic acid, 3-hydroxybutyric acid, 5 (S)-HpEPE, chaetocin, biotin, and folinic acid, while the significantly down-regulated metabolites included 5-sulfosalicylic acid and PE O-26:5_18:2.

The KEGG pathway enrichment analysis of differential metabolites revealed that there were three significantly enriched pathways: gap junction, glycolipid metabolism and fatty acid biosynthesis (Fig. 5G). Compared to the NS strain, six metabolites in the glycolipid metabolism pathway were significantly downregulated in the AFS strain, specifically LPA20:5, LPA20:4, LPA20:3, LPA18:3, LPA18:2, and LPA18:0. Moreover, the gap junction pathway, which has seven significantly down-regulated metabolites in total, includes the six metabolites that were downregulated in the glycolipid metabolism pathway.

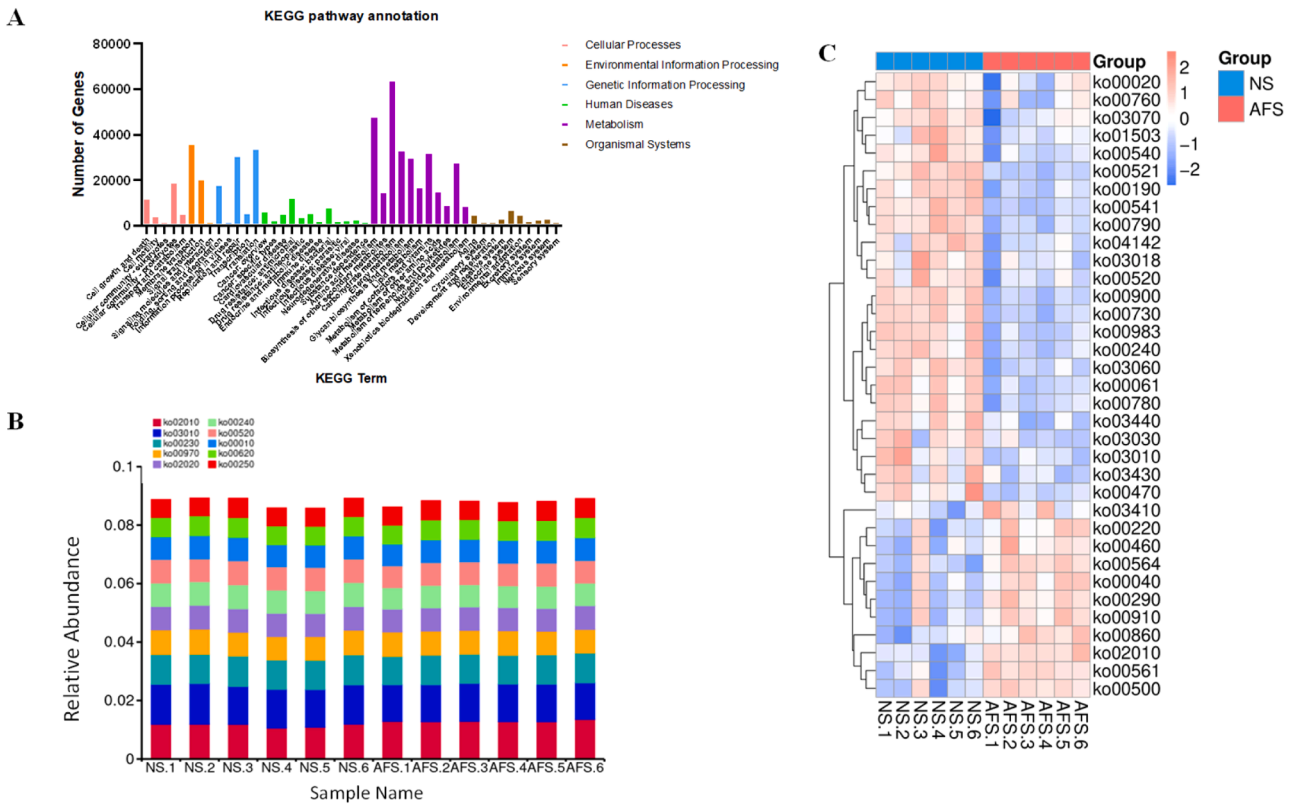

Fig. 4. Functional annotation of the cecal metagenome using the KEGG database.
(A) Statistics of the number of annotated genes at the KEGG metabolic pathway level 2.
(B) Analysis of the top 10 abundant KEGG pathways (level 3) in Wenchang chickens.
(C) The MetaStat analysis method was used to identify differential KEGG level 3 metabolic pathways.

Metabolomics-metagenomics association analysis

In addition, we conducted an association analysis between the metagenome and the metabolome, selecting the top 10 differential metabolites and microbiota at the genus level. Based on the Pearson correlation coefficient, we found a strong correlation between serum metabolites and the gut microbiota (Fig. 6A, B). This indicates a complex functional relationship between the gut microbiota and the metabolome.

Blautia is positively correlated with folinic acid, biotin, and 3-hydroxy-3-methylbutanoic acid ($P < 0.05$). *Lachnoclostridium* shows a positive correlation with biotin ($P < 0.01$) and folinic acid ($P < 0.05$). *Ruminococcus* is positively correlated with biotin ($P < 0.01$), folinic acid ($P < 0.05$), and 3-hydroxybutyric acid ($P < 0.05$). *Subdoligranulum* shows a positive correlation with biotin and 3-hydroxybutyric acid ($P < 0.05$). Conversely, *Muribaculum* exhibits negative correlations with folinic acid ($P < 0.01$), biotin ($P < 0.01$), 3-hydroxy-3-methylbutanoic acid ($P < 0.01$), and 3-hydroxybutyric acid ($P < 0.05$).

Discussion

In the development of livestock industry, healthy breeding has been receiving continuous attention. Enhancing animal immunity is the key to ensuring the health of livestock, and breeding varieties with stronger disease resistance is also an important part of animal breeding. The study screened for cecal microorganisms and serum metabolites that may enhance disease resistance in Wenchang chickens by conducting cecal metagenomic analysis and untargeted serum metabolomics analysis of the NS strain and AFS strain.

Intestinal tight junction proteins (TJPs) are primarily composed of molecules such as ZO-1, OCCLUDIN, and CLAUDIN (Sarker et al., 2025; Xie et al., 2024). ZO-1 is a scaffold for transmembrane tight junction (TJ) proteins and can recruit various signaling molecules (Cholewińska et al., 2023). CLAUDIN-1 can directly regulate paracellular permeability (Zhang et al., 2016). The main function of OCCLUDIN is to maintain the tight junction structure and protect the intestinal barrier (Zhang et al., 2024b). In our study, the mRNA expression level of *CLAUDIN-1* significantly increased, and the mRNA expression level of *OCCLUDIN* significantly decreased. Previous studies have also found an inconsistency phenomenon regarding OCCLUDIN and CLAUDIN-1, speculating that it may be related to compensatory effects (Zhou et al., 2022). The NRF2/HO-1 signaling pathway plays a crucial role in combating oxidative stress (Li et al., 2025b). NRF2 is a key transcription factor that regulates the expression of antioxidant enzymes (Chen et al., 2019). HO-1, SOD, and CAT work synergistically to maintain redox balance and alleviate oxidative stress damage (Chen et al., 2024). In this study, the mRNA expression levels of *NRF2, HO-1*, and *SOD* in the ileum of the AFS strain were significantly elevated. Therefore, the elevation of these indicators in the AFS strain may be related to its enhanced antioxidant capacity. In addition, we further measured the antioxidant-related indicators T-AOC, T-SOD, GSH-PX, and MDA. T-AOC is a major indicator for assessing the redox balance status in biological systems, while SOD and GPX are important antioxidant enzymes (Tian et al., 2025). MDA, as one of the final products of membrane lipid peroxidation, can indirectly reflect the extent of oxidative damage (Qi et al., 2024). SOD converts superoxide into hydrogen peroxide, while CAT and GSH-PX can further decompose hydrogen peroxide into water (Mavridi-Printezi et al., 2023). Although the mRNA level of CAT did not show a significant increase in our study, we speculate that this may be related to the compensatory effect of GSH-PX. The enhancement of antioxidant capacity is indicated by a decrease in MDA levels, an increase in T-AOC levels, and an increase in the activities of SOD and GSH-Px (Liu et al.,

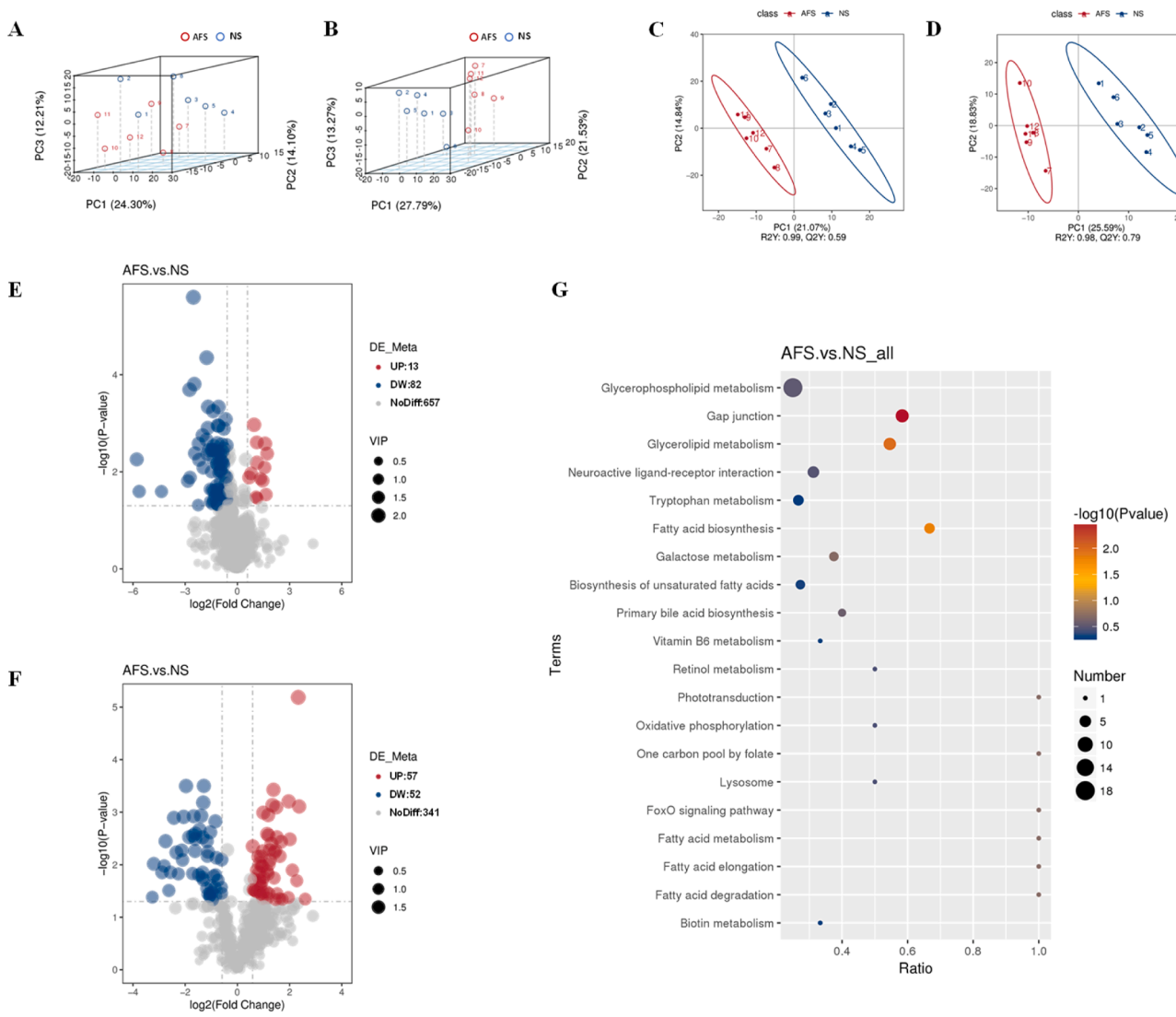

Fig. 5. Characteristics of metabolites in serum samples and KEGG enrichment analysis of differential metabolites.
(A, B) 3D maps of PCA analysis showing the differences between the two strains in positive (ESI+) and negative (ESI-) ionization modes.
(C, D) PLS-DA score plots comparing the two strains in positive and negative ionization modes.
(E, F) Significantly different metabolites between NS and AFS strains in positive and negative modes.
(G) Bubble diagram showing the results of the KEGG enrichment analysis.

2024). Our results further support the notion that the AFS strain may possess a stronger antioxidant capacity. Chronic intestinal inflammation is closely associated with the imbalance of Helper T cells 17 (Th17) and regulatory T cells (Treg) (Ma et al., 2023). RORγt, a key transcription factor for Th17 cells, and FoxP3, a specific transcription factor for regulatory T cells (Treg), respectively play crucial roles in promoting the immune response and suppressing immunity (Song et al., 2022). In this study, the mRNA expression level of *FoxP3* was significantly higher in the AFS strain compared to the NS strain, while the ratio of *RORγt* to *FoxP3* was significantly decreased. This suggests that the AFS strain may have some advantage in *FoxP3* expression in the ileum, and the change in the ratio of *RORγt* to *FoxP3* indicates a difference in immune regulatory status. These results may suggest a potential advantage for the AFS group in immune regulation; however, further experiments are needed to validate the specific impact of these observations on immune function.

When comparing the cecal microbiota, α diversity analysis showed that the microbial diversity of AFS strain was higher than that of the NS strain at the phylum level. At the genus level, the AFS strain had higher microbial diversity and more uniform species distribution. Furthermore, beta diversity analysis revealed significant differences in microbiota between the two strains.

Blautia is an anaerobic genus of bacteria that shows potential as a probiotic, capable of producing propionate and other short-chain fatty acids to enhance colonic mucus function (Murakami et al., 2024; Xie et al., 2025). *Blautia* can produce bacteriocins to inhibit pathogen adhesion and may regulate host health by upregulating T cells and the production of SCFAs (Gao et al., 2024). *Massilistercora timonensis* is a strict anaerobe negatively correlated with serum cholesterol and triglyceride levels (Li et al., 2025a). Studies indicate a significant interplay between lipid metabolism and the immune system (Schmitz et al., 2023). LEfSe analysis identifies *Blautia* and *Massilistercora timonensis* as marker microbes in the AFS strain that positively influencing host immunity and gut health. *Brachyspira*, a genus of anaerobic bacteria, has been shown to predominantly colonize individuals with compromised immune function. The *Brachyspira* genus includes some pathogenic spirochetes that affect poultry health (Regina et al., 2021). LEfSe results indicate a higher relative abundance of *Brachyspira* in the NS strain, which may suggest that the immune function of the NS strain is impaired.

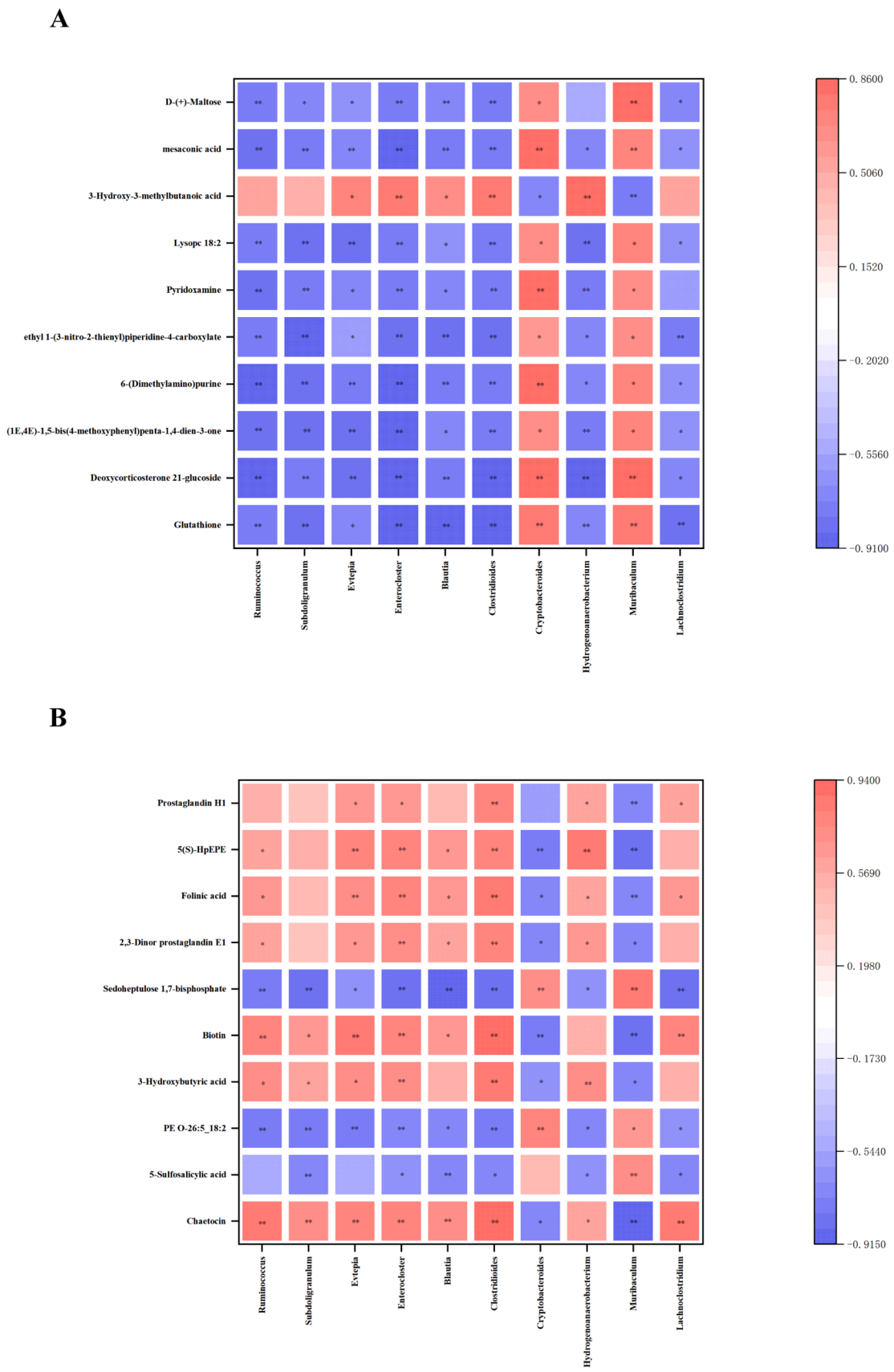

Fig. 6. The metabolomics-metagenomics association analysis based on Pearson correlation coefficients.
(A, B) Heatmaps of correlation analysis between differential microorganisms and differential metabolites under positive and negative ionization modes.

According to the differential microorganisms obtained from the MetaStat analysis, in addition to the aforementioned *Blautia* and *Muribaculum*, the three bacterial genera *Lachnoclostridium, Ruminococcus*, and *Subdoligranulum* also play important roles in immunity. *Lachnoclostridium* is associated with the production of SCFAs and can generate butyrate, which has anti-inflammatory properties and enhances gut barrier integrity by upregulating tight junction proteins (Özkan et al., 2024; Dai et al., 2023). *Ruminococcus*, a member of the Firmicutes phylum, is capable of hydrolyzing starch and other sugars to produce butyrate and secretes SCFAs such as acetate, propionate, butyrate, and lactate (Wang et al., 2024b). Studies have shown that *Subdoligranulum* is a butyrate-producing bacterium that is involved in the regulation of inflammatory responses and potentially contributes to the maintenance of gut barrier integrity (Wang et al., 2024a; Song et al., 2024, 2024d). The abundance of *Lachnoclostridium, Ruminococcus*, and *Subdoligranulum* were significantly higher in the AFS strain compared to the NS strain, which may indicate that the AFS strain has superior regulation of inflammatory responses, immune capacity, and gut barrier function.

In untargeted serum metabolomics, we found that significantly upregulated metabolites such as folinic acid, and biotin play crucial roles in anti-inflammatory and immune functions. Folinic acid, also known as leucovorin, is a naturally occurring compound and one of the active metabolites of vitamin B9 (Gristan et al., 2025). Insufficient dietary folate intake, hereditary malabsorption, or defects in folate transport can lead to combined immunodeficiency, and folinic acid replacement therapy may be beneficial for alleviating the symptoms of immunodeficiency in affected patients (Gök et al., 2023). Research has shown that folinic acid can enhance the accumulation of natural killer T cells in the tumor microenvironment, thereby improving the immune response in mouse models of pancreatic ductal adenocarcinoma (Li et al., 2024a). Although it is currently known that folinic acid plays an important role in enhancing immune cell activity, there is limited research on its specific effects in poultry. However, we have reason to speculate that folinic acid may also promote immunity and improve disease resistance in poultry.

Biotin, also known as vitamin B7 or vitamin H, is an important water-soluble vitamin that is essential for various metabolic processes. As a cofactor, biotin participates in various metabolic processes in the body, including gluconeogenesis, fatty acid synthesis, and the catabolism of odd-chain fatty acids and branched-chain amino acids (Yuan and Chen, 2025). Studies have demonstrated that the supplementation of vitamins in pig feed can enhance the immune system and gut health (Mainardi et al., 2024). Furthermore, adding different concentrations of biotin to broiler diets has been shown to enhance production and physiological performance (Hasan Kadhim et al., 2022). Moreover, the gut microbiota can synthesize various vitamins, including biotin (Petschow et al., 2015). Studies have indicated that the gut microbiota can influence the synthesis, metabolism, and transport of vitamins (Zhai et al., 2022). Therefore, we speculate that changes in biotin levels in serum may be associated with the composition of the gut microbiota.

Chaetocin is a natural product produced by fungi of the genus Chaetomium, which have been found to possess anticancer, anti-inflammatory, and anti-aging effects (Wang et al., 2025, 2024c; Vo et al., 2017). LPA is a bioactive lipids associated with T cell-regulated diseases (Mathew and Torres, 2020). Notably, in certain chronic viral infections, the levels of LPA are significantly elevated, and LPA may have a suppressive effect on $CD8^+$ T cell immunity (Kremer et al., 2022). 3-hydroxy-3-methylbutanoic acid and 3-hydroxybutyric acid are classified as butyric acid derivatives and fall under the category of SCFAs. SCFAs serve as an energy source and possess antibacterial properties, playing a crucial role in maintaining overall gut health (Li et al., 2024b). Research has indicated that 3-hydroxybutyric acid exerts beneficial effects on the gut mucosal barrier (Chen et al., 2025). The significant upregulation of metabolites such as folinic acid, biotin, 3-hydroxy-3-methylbutanoic acid, 3-hydroxybutyric acid and chaetocin, along with the significant downregulation of LPA, may have a positive impact on protecting gut health in chickens and are potential metabolites for enhancing the disease resistance of Wenchang chickens.

In the Pearson correlation analysis of cecal microbiota and serum metabolites, we observed complex association between microbiota and metabolites, suggesting that gut microbiota not only plays an important role in metabolic processes but may also regulate health status by influencing the physiological functions of the host. Previous studies have shown that *Blautia* is capable of producing various forms of folate (Kundra et al., 2024), and is positively correlated with folinic acid. Therefore, in our study, the positive correlation between *Blautia* in the cecum and folinic acid in the serum is particularly noteworthy. We reasonably speculate that the abundance of *Blautia* may enhance the host's immune function and increase the host's resistance to disease by increasing folate levels in the blood. Research has shown that the decreased ability to synthesize biotin in the intestines of mice is associated with a reduction in the abundance of beneficial bacteria such as *Blautia* and *Lachnoclostridium* (Leung et al., 2023). This also suggests that an increase in the abundance of *Blautia* and *Lachnoclostridium* may promote the production of biotin, which is then absorbed by the intestine into the host's bloodstream (Zhang et al., 2024a). This relationship further supports our speculation that *Blautia* and *Lachnoclostridium* enhance the host's resistance to disease by increasing the levels of biotin in the bloodstream. In addition, *Blautia* and *Ruminococcus* are widely recognized as bacterial genera that produce butyrate (Grosicki et al., 2020). Research has shown that *Ruminococcus torques* is positively correlated with 3-hydroxybutyric acid in patients with type 1 diabetes (Tan et al., 2023). In our study, we also found a positive correlation between *Ruminococcus* and 3-hydroxybutyric acid, which may be related to the disease resistance of Wenchang chickens. Although we observed a correlation between the microbiota and serum metabolites, further experimental studies are needed to explore whether these microorganisms may directly influence the synthesis of metabolites.

Conclusions

In summary, our study identified the differential cecal microorganisms and serum metabolites between the NS and AFS strains of Wenchang chickens. Notably, Pearson correlation analysis revealed that *Blautia* was positively correlated with folinic acid and biotin; *Lachnoclostridium* was positively correlated with biotin; and *Ruminococcus* was positively correlated with 3-hydroxybutyric acid. Therefore, we speculate that these differential microorganisms and metabolites may serve as potential markers for breeding Wenchang chickens with strong disease resistance. However, further functional studies are still needed to validate the specific roles of the identified microorganisms and metabolites in enhancing disease resistance in Wenchang chickens.

CRediT authorship contribution statement

Shifan Deng: Data curation, Formal analysis, Methodology, Visualization, Writing – original draft. **Xinli Zheng:** Data curation, Methodology, Project administration, Writing – review & editing. **Han Chu:** Data curation, Methodology, Writing – original draft. **Liang Hong:** Software, Visualization, Writing – review & editing. **Jianbin Zhang:** Data curation, Formal analysis. **Hua Yang:** Software, Writing – review & editing. **Lihong Gu:** Formal analysis, Visualization. **Lei Pu:** Funding acquisition, Methodology, Software, Writing – review & editing.

Disclosures

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Reports a relationship with that includes:. Has patent pending to. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was supported by Hainan Provincial Key R&D Program (ZDYF2024XDNY271), Tianjin Excellent Agricultural Science and Technology Special Commissioner (23ZYCGSN00230), Fuyang Normal University scientific research platform open topic (FYKFKT24042), Key Research, the Tianjin Pig Industry Technology System Innovation Team, China to JZ and National Key Research and Development Program Project (2021YFD1300100).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2026.106506.

References

Abdel-Moneim, A.E., Khidr, R.S., Badran, A.M.M., Amin, S.A., Badri, F.B., Gad, G.G., Thabet, H.A., Elbaz, A.M., 2024. Efficacy of supplementing Aspergillus awamori in enhancing growth performance, gut microbiota, digestibility, immunity, and antioxidant activity of heat-stressed broiler chickens fed diets containing olive pulp. BMC Vet. Res. 20, 205. https://doi.org/10.1186/s12917-024-04050-7.

Chen, C., Wang, J., Cheng, M., Xie, H., Li, W., Zhang, C., 2025. Muribaculum intestinale-derived 3-hydroxybutyric acid from Heterophyllin B attenuated pulmonary fibrosis through IDO1-mediated ferroptosis. Pharmacol. Res. 212, 107587. https://doi.org/10.1016/j.phrs.2025.107587.

Chen, X., Zheng, A., Li, S., Wang, Z., Chen, Z., Chen, J., Zou, Z., Liang, H., Liu, G., 2024. Bacillus amyloliquefaciens regulates the Keap1/Nrf2 signaling pathway to improve the intestinal (Caco-2 Cells and Chicken Jejunum) oxidative stress response induced by lipopolysaccharide (LPS). Antioxidants 13. https://doi.org/10.3390/antiox13121550.

Chen, Z., Zhong, H., Wei, J., Lin, S., Zong, Z., Gong, F., Huang, X., Sun, J., Li, P., Lin, H., Wei, B., Chu, J., 2019. Inhibition of Nrf2/HO-1 signaling leads to increased activation of the NLRP3 inflammasome in osteoarthritis. Arthritis Res. Ther. 21, 300. https://doi.org/10.1186/s13075-019-2085-6.

Cholewińska, E., Marzec, A., Sołek, P., Fotschki, B., Listos, P., Ognik, K., Juśkiewicz, J., 2023. The effect of copper nanoparticles and a different source of dietary fibre in the diet on the integrity of the small intestine in the rat. Nutrients 15. https://doi.org/10.3390/nu15071588.

Dai, W., Cai, D., Zhou, S., Li, A., Xie, J., Zhang, J., 2023. Uncovering a causal connection between the lachnoclostridium genus in fecal microbiota and non-alcoholic fatty liver disease: a two-sample mendelian randomization analysis. Front. Microbiol. 14, 1276790. https://doi.org/10.3389/fmicb.2023.1276790.

Gao, Y.Y., Liu, X.P., Zhou, Y.H., He, J.Y., Di, B., Zheng, X.Y., Guo, P.T., Zhang, J., Wang, C.K., Jin, L., 2024. The addition of hot water extract of juncao-substrate ganoderma lucidum residue to diets enhances growth performance, immune function, and intestinal health in broilers. Animals 14. https://doi.org/10.3390/ani14202926.

Gök, V., Erdem, Ş., Haliloğlu, Y., Bişgin, A., Belkaya, S., Başaran, K.E., Canatan, M.F., Özcan, A., Yılmaz, E., Acıpayam, C., Karakükcü, M., Canatan, H., Per, H., Patıroğlu, T., Eken, A., Ünal, E., 2023. Immunodeficiency associated with a novel functionally defective variant of SLC19A1 benefits from folinic acid treatment. Genes Immun. 24, 12–20. https://doi.org/10.1038/s41435-022-00191-7.

Gristan, Y.D., Patel, P., Moosavi, L., 2025. Folinic Acid. StatPearls Publishing LLC., Treasure Island (FL) in StatPearlsStatPearls Publishing. Copyright © 2025ineligible companies.

Grosicki, G.J., Riemann, B.L., Flatt, A.A., Valentino, T., Lustgarten, M.S., 2020. Self-reported sleep quality is associated with gut microbiome composition in young, healthy individuals: a pilot study. Sleep Med. 73, 76–81. https://doi.org/10.1016/j.sleep.2020.04.013.

Hasan Kadhim, A., Shamkhi Noor, A., Ali, M.A., 2022. The effectiveness of Biotin (Vitamin B (7)) added to the diet in improving the efficiency of productivity, and some physiological traits for broiler chickens (Ross-308) exposed to oxidative stress. Arch. Razi Inst. 77, 1805–1811. https://doi.org/10.22092/ari.2022.358365.2210.

Kremer, K.N., Buser, A., Thumkeo, D., Narumiya, S., Jacobelli, J., Pelanda, R., Torres, R. M., 2022. LPA suppresses T cell function by altering the cytoskeleton and disrupting immune synapse formation. Proc. Natl. Acad. Sci. USA 119, e2118816119. https://doi.org/10.1073/pnas.2118816119.

Kundra, P., Geirnaert, A., Pugin, B., Plüss, S., Kariluoto, S., Lacroix, C., Greppi, A., 2024. Microbially-produced folate forms support the growth of Roseburia intestinalis but not its competitive fitness in fecal batch fermentations. BMC Microbiol. 24, 366. https://doi.org/10.1186/s12866-024-03528-6.

Leung, H., Xiong, L., Ni, Y., Busch, A., Bauer, M., Press, A.T., Panagiotou, G., 2023. Impaired flux of bile acids from the liver to the gut reveals microbiome-immune interactions associated with liver damage. NPJ Biofilms Microbiomes 9, 35. https://doi.org/10.1038/s41522-023-00398-0.

Li, J., Moresco, P., Fearon, D.T., 2024a. Intratumoral NKT cell accumulation promotes antitumor immunity in pancreatic cancer. Proc. Natl. Acad. Sci. USA 121, e2403917121. https://doi.org/10.1073/pnas.2403917121.

Li, T., Wang, P., Zhi, Z., Guo, T., Zhou, J., Zhang, H., Cao, C., Cai, Y., Li, Y., Zhang, J., 2025a. Free-caged rearing modes regulate chicken intestinal metabolism by influencing gut microbial homeostasis. Poult. Sci. 104, 104381. https://doi.org/10.1016/j.psj.2024.104381.

Li, X., Ling, H., He, Z., Yang, Z., Jiang, T., Huang, P., Zeng, J., 2024b. Effects of edible grass (Rumex patientia L. × Rumex tianschanicus A. LOS) leaf powder on growth performance, antioxidant properties, Cecal short-chain fatty acids, and microbial community levels in broilers. Antioxidants 13. https://doi.org/10.3390/antiox13111291.

Li, Y., Wang, S., Feng, R., 2025b. Dietary selenium mitigates cadmium-induced apoptosis and inflammation in chicken testicles by inhibiting oxidative stress through the activation of the Nrf2/HO-1 signaling pathway. Poult. Sci. 104, 104990. https://doi.org/10.1016/j.psj.2025.104990.

Liu, T., Ma, W., Wang, J., Wei, Y., Wang, Y., Luo, Z., Zhang, Y., Zeng, X., Guan, W., Shao, D., Chen, F., 2024. Dietary protease supplementation improved growth performance and nutrients digestion via modulating intestine barrier, immunological response, and microbiota composition in weaned piglets. Antioxidants 13. https://doi.org/10.3390/antiox13070816.

Luo, Z., Yong, K., Luo, Q., Du, Z., Ma, L., Huang, Y., Zhou, T., Yao, X., Shen, L., Yu, S., Deng, J., Ren, Z., Zhang, Y., Yan, Z., Zuo, Z., Cao, S., 2022. Altered fecal microbiome and correlations of the metabolome with plasma metabolites in dairy cows with left displaced abomasum. Microbiol. Spectr. 10, e0197222. https://doi.org/10.1128/spectrum.01972-22.

Ma, Z., Akhtar, M., Pan, H., Liu, Q., Chen, Y., Zhou, X., You, Y., Shi, D., Liu, H., 2023. Fecal microbiota transplantation improves chicken growth performance by balancing jejunal Th17/Treg cells. Microbiome 11, 137. https://doi.org/10.1186/s40168-023-01569-z.

Mainardi, E., Corino, C., Rossi, R., 2024. The effect of vitamins on the immune systems of pigs. Animals 14. https://doi.org/10.3390/ani14142126.

Mao, L., Gao, B., Chang, H., Shen, H., 2024. Interaction and metabolic pathways: elucidating the role of gut microbiota in gestational diabetes mellitus pathogenesis. Metabolites 14. https://doi.org/10.3390/metabo14010043.

Mathew, D., Torres, R.M., 2020. Lysophosphatidic acid is an inflammatory lipid exploited by cancers for immune evasion via mechanisms similar and distinct from CTLA-4 and PD-1. Front. Immunol. 11, 531910. https://doi.org/10.3389/fimmu.2020.531910.

Mavridi-Printezi, A., Menichetti, A., Mordini, D., Amorati, R., Montalti, M., 2023. Recent applications of melanin-like nanoparticles as antioxidant agents. Antioxidants 12. https://doi.org/10.3390/antiox12040863.

Murakami, A., Watanabe-Yanai, A., Iwata, T., Namai, F., Sato, T., Fujii, T., Tochio, T., Khempaka, S., Shimosato, T., 2024. Oral administration of limosilactobacillus ingluviei C37 inhibits Campylobacter jejuni colonization in chicks. Front. Microbiol. 15, 1491039. https://doi.org/10.3389/fmicb.2024.1491039.

Özkan, S., Bay, V., Cömert Acar, M., Yalcın, S., 2024. Partial replacement of soybean with local alternative sources: effects on behavior, cecal microbiota, and intestinal histomorphometry of local chickens. Front. Vet. Sci. 11, 1463301. https://doi.org/10.3389/fvets.2024.1463301.

Petschow, B.W., Burnett, B.P., Shaw, A.L., Weaver, E.M., Klein, G.L., 2015. Dietary requirement for serum-derived bovine immunoglobulins in the clinical management of patients with enteropathy. Dig. Dis. Sci. 60, 13–23. https://doi.org/10.1007/s10620-014-3322-0.

Qi, J., Zhou, S., Wang, G., Hua, R., Wang, X., He, J., Wang, Z., Zhu, Y., Luo, J., Shi, W., Luo, Y., Chen, X., 2024. The antioxidant dendrobium officinale polysaccharide modulates host metabolism and gut microbiota to alleviate high-fat diet-induced atherosclerosis in ApoE (-/-) mice. Antioxidants 13. https://doi.org/10.3390/antiox13050599.

Regina, D.M.M., Patricia, G.A., Alessandra, d.C., Colombari, C.A., Amanda, G., Carla, F., Leonardo, G., Marco, V.A.D., 2021. Identification of brachyspira spp. in the cecum of broiler chickens using histology and *in situ* diagnostic assays. Semin. Cienc. Agrar. 42, 2813–2824. https://doi.org/10.5433/1679-0359.2021v42n5p2813.

Sarker, M.T., Wang, S., Wang, S., Xia, W., Zhang, Y., Jin, C., Huang, X., Li, K., Elokil, A., Lv, Y., Zheng, C., Chen, W., 2025. Sodium butyrate alleviates high ambient temperature-induced oxidative stress, intestinal structural disruption, and barrier integrity for growth and production in growing layer chickens. BMC Vet. Res. 21, 131. https://doi.org/10.1186/s12917-025-04583-5.

Schmitz, T., Freuer, D., Linseisen, J., Meisinger, C., 2023. Associations between serum cholesterol and immunophenotypical characteristics of circulatory B cells and tregs. J. Lipid Res. 64, 100399. https://doi.org/10.1016/j.jlr.2023.100399.

Shen, H., Wang, T., Dong, W., Sun, G., Liu, J., Peng, N., Zhao, S., 2024. Metagenome-assembled genome reveals species and functional composition of Jianghan chicken gut microbiota and isolation of pediococcus acidilactic with probiotic properties. Microbiome 12, 25. https://doi.org/10.1186/s40168-023-01745-1.

Song, B., Li, P., Yan, S., Liu, Y., Gao, M., Lv, H., Lv, Z., Guo, Y., 2022. Effects of dietary Astragalus polysaccharide supplementation on the Th17/treg balance and the gut microbiota of broiler chickens challenged with necrotic enteritis. Front. Immunol. 13, 781934. https://doi.org/10.3389/fimmu.2022.781934.

Song, R., Jiang, Y., Zhang, B., Jiao, Z., Yang, X., Zhang, N., 2024. Effects of Hypericum attenuatum Choisy extract on the immunologic function and intestinal microflora of broilers under oxidative stress. Poult. Sci. 103, 104189. https://doi.org/10.1016/j.psj.2024.104189.

Tan, H., Shi, Y., Yue, T., Zheng, D., Wang, C., Liu, Z., Yang, D., Ding, Y., Xu, W., Yan, J., Luo, S., Weng, J., Zheng, X., 2023. 185-OR: machine-learning approach reveals microbiome, metabolome, lipidome, and their interaction in type 1 diabetes mellitus. Diabetes 72. https://doi.org/10.2337/db23-185-or, 1-1.

Tian, Y., Liu, X., Chen, L., Zeng, T., Gu, T., Xu, W., Ren, J., Lu, L., 2025. Dietary resveratrol alleviates liver and intestinal injury in ducks under cage rearing system. Poult. Sci. 104, 105330. https://doi.org/10.1016/j.psj.2025.105330.

Vo, M.C., Nguyen-Pham, T.N., Lee, H.J., Jung, S.H., Choi, N.R., Hoang, M.D., Kim, H.J., Lee, J.J., 2017. Chaetocin enhances dendritic cell function via the induction of heat shock protein and cancer testis antigens in myeloma cells. Oncotarget 8, 46047–46056. https://doi.org/10.18632/oncotarget.17517.

Wang, C., Liu, X., Sun, X., Li, Y., Yang, X., Liu, Y., 2024a. Dietary betaine supplementation improved egg quality and gut microbes of laying hens under dexamethasone-induced oxidative stress. Poult. Sci. 103, 104178. https://doi.org/10.1016/j.psj.2024.104178.

Wang, K., Li, Y., Wang, L., 2025. Chaetocin inhibits the progression of neuroblastoma by targeting JAK2/STAT3 signaling pathway in SH-SY5Y cells. Naunyn Schmiedebergs Arch. Pharmacol. 398, 4237–4246. https://doi.org/10.1007/s00210-024-03426-8.

Wang, M., Zhong, J., Guo, Y., Zhao, S., Xia, H., Wang, G., Liu, C., Guo, A., 2024b. Effects of adding sphingomonas Z392 to drinking water on growth performance, intestinal histological structure, and microbial community of broiler chickens. Animals 14. https://doi.org/10.3390/ani14131920.

Wang, X., Ma, L., Lu, D., Zhao, G., Ren, H., Lin, Q., Jia, M., Huang, F., Wang, S., Xu, Z., Yang, Z., Chu, Y., Xu, Z., Li, W., Yu, L., Jiang, Q., Zhang, C., 2024c. Nuclear envelope budding inhibition slows down progerin-induced aging process. Proc. Natl. Acad. Sci. USA 121, e2321378121. https://doi.org/10.1073/pnas.2321378121.

Wang, Y., Zhang, J., Wang, X., Wang, R., Zhang, H., Zhang, R., Bao, J., 2024d. The inflammatory immunity and gut microbiota are associated with fear response differences in laying hens. Poult. Sci. 103, 103816. https://doi.org/10.1016/j.psj.2024.103816.

Xie, C., Liang, Q., Cheng, J., Yuan, Y., Xie, L., Ji, J., 2025. Transplantation of fecal microbiota from low to high residual feed intake chickens: impacts on RFI, microbial community and metabolites profiles. Poult. Sci. 104, 104567. https://doi.org/10.1016/j.psj.2024.104567.

Xie, Z., Lv, X., Zhong, C., Wang, F., Zhang, Y., Li, Y., Huang, Y., Yang, S., Shi, Y., 2024. Protective effect of phage pSal-4 on chicken intestinal epithelial cells injured by Salmonella enteritidis. BMC Microbiol. 24, 515. https://doi.org/10.1186/s12866-024-03641-6.

Xu, H., Pan, L.B., Yu, H., Han, P., Fu, J., Zhang, Z.W., Hu, J.C., Yang, X.Y., Keranmu, A., Zhang, H.J., Bu, M.M., Jiang, J.D., Wang, Y., 2022. Gut microbiota-derived metabolites in inflammatory diseases based on targeted metabolomics. Front. Pharmacol. 13, 919181. https://doi.org/10.3389/fphar.2022.919181.

Yang, J., Sun, Y., Wang, Q., Yu, S., Li, Y., Yao, B., Yang, X., 2024. Astragalus polysaccharides-induced gut microbiota play a predominant role in enhancing of intestinal barrier function of broiler chickens. J. Anim. Sci. Biotechnol. 15, 106. https://doi.org/10.1186/s40104-024-01060-1.

Yuan, Y., Chen, L., 2025. Transporters in vitamin uptake and cellular metabolism: impacts on health and disease. Life Metab. 4, loaf008. https://doi.org/10.1093/lifemeta/loaf008.

Zhai, Z., Dong, W., Sun, Y., Gu, Y., Ma, J., Wang, B., Cao, H., 2022. Vitamin-microbiota crosstalk in intestinal inflammation and carcinogenesis. Nutrients 14. https://doi.org/10.3390/nu14163383.

Zhang, L., Du, S.Y., Lu, Y., Liu, C., Tian, Z.H., Yang, C., Wu, H.C., Wang, Z., 2016. Puerarin transport across a Calu-3 cell monolayer - an *in vitro* model of nasal mucosa permeability and the influence of paeoniflorin and menthol. Drug Des. Dev. Ther. 10, 2227–2237. https://doi.org/10.2147/dddt.S110247.

Zhang, J., Yu, H., Zhang, H., Zhao, Q., Si, W., Qin, Y., Zhang, J., 2023. Dietary epimedium extract supplementation improves intestinal functions and alters gut microbiota in broilers. J. Anim. Sci. Biotechnol. 14, 14. https://doi.org/10.1186/s40104-022-00812-1.

Zhang, Y., Dong, L., Dai, X., Huang, Y., Gao, Y., Wang, F., 2024a. Modulation of intestinal metabolites by calorie restriction and its association with gut microbiota in a xenograft model of colorectal cancer. Discov. Oncol. 15, 46. https://doi.org/10.1007/s12672-024-00897-2.

Zhang, X., Sun, L., Wu, M., Yu, C., Zhao, D., Wang, L., Zhang, Z., Yi, D., Hou, Y., Wu, T., 2024b. Effect of supplementation with Lactobacillus rhamnosus GG powder on intestinal and liver damage in broiler chickens challenged by lipopolysaccharide. Front. Microbiol. 15, 1466274. https://doi.org/10.3389/fmicb.2024.1466274.

Zheng, L., Han, Z., Zhang, J., Kang, J., Li, C., Pang, Q., Liu, S., 2024. Lactiplantibacillus plantarum and saccharomyces cerevisiae-fermented coconut water alleviates dextran sodium sulfate-induced enteritis in Wenchang chicken: a gut microbiota and metabolomic approach. Animals 14. https://doi.org/10.3390/ani14040575.

Zhou, N., Tian, Y., Liu, W., Tu, B., Gu, T., Xu, W., Zou, K., Lu, L., 2022. Effects of quercetin and coated sodium butyrate dietary supplementation in diquat-challenged pullets. Anim. Biosci. 35, 1434–1443. https://doi.org/10.5713/ab.21.0493.