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Effects of dietary *Solanum nigrum* L. supplementation on growth performance, antioxidant capacity, ileal transcriptomic profile, and cecal microbiome in Wuhua yellow-feathered chickens

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Although studies have investigated *Solanum nigrum* L. (SNL) in mice, its effects on broilers remain unclear. This study examined how dietary SNL influences growth performance, antioxidant capacity, ileal transcriptome, and gut microbiota in broilers. A total of 200 one-day-old healthy Wuhua yellow-feathered chickens were randomly divided into four groups of five replicates (10 birds each). The groups received: a basal diet (CON), a basal diet with 500 mg/kg amoxicillin (AMO), a basal diet with 1000 mg/kg SNL grass meal (0.1% SNL), and a basal diet with 2000 mg/kg SNL grass meal (0.2% SNL). The experiment lasted 35 days. SNL supplementation modestly improved feed efficiency and jejunal villus height ($p = 0.019$). It also altered cecal microbiota by increasing *Bacteroidetes*, *Bacteroides*, and *Faecalibacterium*, while decreasing *Firmicutes* and *Oscillibacter*. Ileal transcriptomics identified multiple differentially expressed genes (DEGs) across comparisons, which were enriched in intestinal immune network pathways for IgA production. Correlation analysis linked cecal microbiota changes to ileal gene expression. In conclusion, SNL exhibits the potential as an alternative to antibiotics in chickens, and this study provides empirical support for its broader adoption in poultry industry.

KEYWORDS

Solanum nigrum L., growth performance, antioxidant capacity, transcriptome, microbiome, Yellow-feathered chicken

1 Introduction

Antibiotics play a pivotal role in safeguarding animal health and improving feed conversion efficiency owing to their antimicrobial activity, which inhibits or eliminates pathogenic microorganisms (Fernández Miyakawa et al., 2024). However, the overuse and misuse of antibiotics have contributed to a marked rise in antibiotic-resistant bacterial strains, posing a substantial public health threat (Abd El-Ghany, 2020; Obianwuna et al., 2024; Oladeji et al., 2025). In light of growing concerns over environmental sustainability and food safety, the development of antibiotic alternatives has become a major focus of scientific research. Among these alternatives, Chinese herbal medicines have garnered significant interest as cost-effective feed additives, particularly due to their demonstrated benefits in enhancing intestinal health in broiler chickens (Xu et al., 2022; Wang et al., 2024a), along with their antioxidative, immunomodulatory, and growth-promoting properties (Wang et al., 2021; Xu et al., 2022; Li et al., 2023).

Solanum nigrum L. (SNL), a dicotyledonous weed belonging to the Solanaceae family, is a spontaneous plant commonly found in wastelands, along fences and roadsides, and as an agricultural weed throughout China. SNL exhibits a range of pharmacological effects, including antimicrobial, anticancer, antioxidant, anti-inflammatory, antimetastatic, antiproliferative, and antitumor activities (Sivaraj et al., 2020; Bhuvaneshwari et al., 2022), attributable to its rich and diverse phytochemical composition. Its primary bioactive constituents include alkaloids, flavonoids, steroids, glycoproteins, tannins, polysaccharides, and polyphenols, with notable compounds such as caffeic acid, catechin, esculin, protocatechuic acid, rutin, and epicatechin (Zeeshan et al., 2023). To date, numerous studies have explored the effects of SNL in murine models. For instance, one study indicated that extracts from SNL berries can modulate the gut microbiota by reducing the Firmicutes-to-Bacteroidetes ratio and restoring beneficial microbial populations, while concurrently mitigating oxidative stress in mice (Wang et al., 2024b). Another study demonstrated that SNL exerts anti-inflammatory effects by inhibiting *NF-κB* nuclear translocation and downregulating the expression of *iNOS*, *COX-2*, *IL-1β*, and *IL-6* in a concentration-dependent manner (Deng et al., 2023). Given these pharmacological characteristics and the presence of medicinally active secondary metabolites, SNL holds promising potential as an antibiotic alternative in poultry production.

However, to the best of our knowledge, no studies have specifically examined the effects of SNL supplementation in

broiler chickens. To bridge this gap, the current study employed integrated microbiome and transcriptome analyses to evaluate how SNL influences intestinal health and production performance in broilers. By uncovering the mechanistic basis of its probiotic activity, this research seeks to establish a scientific foundation for the resource-efficient use of SNL and its application as an eco-friendly feed additive that supports animal health in antibiotic-free poultry production systems.

2 Materials and methods

2.1 Ethics statement

The animal study protocol was approved by the Ethics Committee of Guangdong Meizhou Vocational and Technical College (protocol code: GDMZVTC-2023-003 and date of approval: 2023.02.17).

2.2 Experimental design and diets

A total of 200 one-day-old healthy Wuhua yellow-feathered chickens with similar initial body weight (BW; 31.5±0.09g) were obtained from a commercial hatchery and randomly divided into 4 groups, with 5 replicate floor pens per group and 10 birds per pen. The four groups were: group A (CON group, fed the basal diet as control), group B (AMO group, fed the basal diet+500mg/kg amoxicillin), group C (SNL group, fed the basal diet+1000mg/kg *Solanum nigrum* L. grass meal), group D (SNL group, fed the basal diet+2000mg/kg *Solanum nigrum* L. grass meal), respectively. The basal diets were formulated referring to Chinese Nutrient Requirements of Yellow broilers (Ministry of Agriculture and Rural Affairs of the People's Republic of China, 2020).

The experiment lasted for 35 days, consisted of a 7 days pre-trial period and a 28 days experimental period. Birds were housed and fed in disease-free pens (200 cm × 200 cm × 60 cm) and had free access to feed and water. During the first week, the indoor temperature was maintained at approximately 33°C under continuous artificial lighting. From day 8 to day 35, the temperature was gradually reduced by 2°C per week until it stabilized within the range of 26–27°C. Body weight was measured on a weekly basis, and feed intake was recorded daily.

2.3 Growth performance and sample collection

On the 36th day, after an eight-hour fasting period, measurements were made of the body weight (BW) and the amount of feed remaining in each replicate. Afterwards, the growth performance parameters for each group were computed, which included the mean average daily feed intake (ADFI), mean average daily gain (ADG), and the feed conversion ratio (F/G). F/G = feed intake (F)/weight gain (G).

Abbreviations: BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; F/G, feed-to-gain ratio; DEG, differentially expressed gene; SNL, *Solanum nigrum* L.; T-AOC, total antioxidant capacity; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; H&E, hematoxylin and eosin; VH, villus height; CD, crypt depth; ASV, amplicon sequence variant; PCA, principal component analysis; NMDS, non-metric multidimensional scaling.

Then, five birds per group (1 bird per pen, 20 birds in total) were randomly selected for sample collection, including blood samples, jejunum tissue, ileum tissue and cecal digesta. The samples were cryopreserved at -80°C after undergoing the necessary processing for subsequent analysis.

2.4 Determination of serum oxidative stress related indices

The serum total antioxidant capacity (T-AOC), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) activities and the level of malondialdehyde (MDA) were performed by appropriate assay kits (Nanjing Jiancheng Biotechnology Institute, Nanjing, China) using the builder's standard method (Bai et al., 2021).

2.5 Intestinal morphology

The jejunum tissue samples (1-2cm) were fixed with 4% paraformaldehyde and prepared on tissue slides (Tang et al., 2022). The tissues were embedded in paraffin blocks, cut into 4-μm slices, and stained with hematoxylin and eosin (H&E). Then, the slices were dried and sealed for follow-up observation and analysis (the villus height (VH) and crypt depth (CD)) through optical microscopy.

2.6 16s rRNA gene sequencing analysis

2.6.1 DNA extraction and amplification

Total genomic DNA of cecal contents was extracted using MagPure Soil DNA KF Kit (Magan) following the manufacture's instructions. DNA concentration and integrity were measured with NanoDrop 2000 (Thermo Fisher Scientific, USA) and agarose gel electrophoresis. Extracted DNA was stored at -20°C until further processing. The extracted DNA was used as template for PCR amplification of bacterial 16S rRNA genes with the barcoded primers and Takara Ex Taq (Takara). For bacterial diversity analysis, V3-V4 variable regions of 16S rRNA genes was amplified with universal primers 343F (5' - TACGGRAGGCAGCAG-3') and 798R (5'-AGGGTATCTAATCCT-3') (Nossa et al., 2010).

2.6.2 Library construction and sequencing

The Amplicon quality was visualized using agarose gel electrophoresis. The PCR products were purified with AMPure XP beads (Agencourt) and amplified for another round of PCR. After purified with the AMPure XP beads again, the final amplicon was quantified using Qubit dsDNA Assay Kit (Thermo Fisher Scientific, USA). The concentrations were then adjusted for sequencing. Sequencing was performed on an Illumina NovaSeq 6000 with 250 bp paired-end reads. (Illumina Inc., San Diego, CA; OE Biotech Company; Shanghai, China).

2.6.3 Bioinformatic analysis

Raw sequencing data, in FASTQ format, were preprocessed using Cutadapt software to detect and cut off the adapter. After trimming, quality control of the adapter-removed paired-end reads, including low quality sequences removal, denoised, merged and chimera reads removal, was conducted by using DADA2 plugin (Callahan et al., 2016) with the default parameters of QIIME2 (Bolyen et al., 2019) (2020.11). At last, the software output the representative reads and the amplicon sequence variant (ASV) abundance table. The representative read of each ASV was selected using QIIME2 package. All representative reads were annotated and blasted against Silva database (Version 138) using q2-feature-classifier with the default parameters.

QIIME2 software was used for alpha and beta diversity analysis. Then the R package was used to analyze the significant differences between different groups using ANOVA/Kruskal Wallis statistical test. The linear discriminant analysis effect size (LEfSe) method was used to compare the taxonomy abundance spectrum.

2.7 Transcriptome sequencing analysis

2.7.1 RNA isolation and library preparation

Total RNA was extracted from ileum tissue using the TRIzol reagent (Invitrogen, CA, USA) according to the manufacture's protocol. RNA purity and quantification were evaluated using the NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). RNA integrity was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Then the libraries were constructed using VAHTS Universal V6 RNA-seq Library Prep Kit according to manufacture's instructions.

2.7.2 RNA sequencing and differentially expressed genes analysis

RNA Sequencing was facilitated in collaboration with OE Biotech Co., Ltd. (Shanghai, China). The libraries were sequenced on an Illumina Novaseq 6000 platform and 150 bp paired-end reads were generated (the sequencing depth/volume is 6 G). Raw reads of fastq format were firstly processed using fastp (Chen et al., 2018) and the low quality reads were removed to obtain the clean reads. Then the clean reads were mapped to the chicken reference genome using HISAT2 (Kim et al., 2015). FPKM (Roberts et al., 2011) of each gene was calculated and the read counts of each gene were obtained by HTSeq-count4. PCA analysis were performed using R (v 3.2.0) to evaluate the biological duplication of samples.

Differential expression analysis was performed using the DESeq2 (Love et al., 2014). Q value < 0.05 and foldchange > 2 was set as the threshold for significantly DEGs. Based on the hypergeometric distribution, Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of DEGs were performed to screen the significant enriched term using R (v 3.2.0), respectively. R (v 3.2.0) was used to draw the column diagram and bubble diagram of the significant enrichment term.

2.8 Comprehensive analysis of the cecal microbiome and ileal transcriptome

To explore the potential relationship between the cecal microbiome and its hosts, the Spearman correlation coefficient was employed to assess the correlation between differentially abundant microbiota and DEGs. *P-value* < 0.05 is considered indicative of significant relationships. The correlation heatmap of cecal microbiota and DEGs was constructed using the R package. To decrease the risk of false positives, correction for multiple testing is also applied.

2.9 Statistical analysis

Quantitative data were analyzed using IBM SPSS 22.0 software (SPSS Inc., USA) and shown as the mean±SEM. Shapiro - Wilk test was used to test the normality of data. For the analysis of growth performance data, in addition to considering the grouping factors, initial body weight was incorporated into the analysis model as a covariate. For intestinal morphology data and the serum induces, only the grouping information was taken into account. Among different treatments, comparisons of means were performed using ANOVA followed by the least significant difference (LSD) *post hoc* test. Significance was established at *p* ≤ 0.05, and 0.05 < *P* < 0.10 was considered a trend.

3 Results

3.1 Growth performance

Group C was excluded from further analysis due to its growth performance showing no substantial deviation from that of the control group (Group A, CON). Consequently, only Group A (CON), Group B (AMO), and Group D (SNL) were included in the subsequent statistical analyses.

Table 1 presents the effects of dietary AMO and SNL supplementation on the growth performance of Wuhua yellow-feathered broilers. No statistically significant differences in growth performance were observed among the three groups during the overall experimental period (*P* > 0.05). Although the differences did not reach statistical significance, certain numerical trends were noted in BW, ADFI, ADG and F/G across the groups. Both the AMO and SNL groups exhibited numerically higher BW and ADG, as well as lower ADFI and F/G, compared to the CON group. Notably, the SNL group demonstrated a lower F/G (1.86±0.04) than the CON group (1.97±0.01), while its F/G was comparable to that of the AMO group (1.81±0.12).

3.2 MDA and antioxidant enzyme activity changes

The effects of AMO and SNL supplementation on serum antioxidant parameters in Wuhua yellow-feathered broilers are

TABLE 1 Effects of different treatments on broiler growth performance.

Items	Groups				P-value
	A	B	C	D	
BW/g	823.90 ±40.39	884.10 ±29.28	818.82 ±100.08	847.77 ±18.81	0.515
ADFI/g	49.97±1.64	48.29±2.98	48.39±4.34	48.41±1.42	0.867
ADG/g	25.37±0.80	26.64±0.92	25.22±2.08	26.00±0.19	0.499
F/G	1.97±0.01 ^a	1.81±0.12 ^b	1.92±0.02 ^{ab}	1.86 ±0.04 ^{ab}	0.065

^{ab} Means within same row carrying different superscript letters indicate significant differences (*p*<0.05).

shown in Table 2. No statistically significant differences (*p* > 0.05) were observed in the levels of SOD, GPX, T-AOC, GSH, or MDA among the treatment groups. However, compared with other dietary treatments, the SNL-supplemented group exhibited a notable increasing trend in both GPX activity and T-AOC.

3.3 Jejunum morphological change

The jejunal tissues were subjected to H&E staining to evaluate the effects of different experimental treatments on intestinal morphology (Figure 1 and Table 3). Significantly longer VH (about 9.5%, *p* = 0.019) was observed in the SNL-supplemented group compared to the AMO-treated group, with a notable increasing trend relative to the control group. Consequently, the V/C ratio was significantly improved in the SNL treatment group compared to the AMO-supplemented group, while no significant differences were detected between the CON and SNL treatment groups.

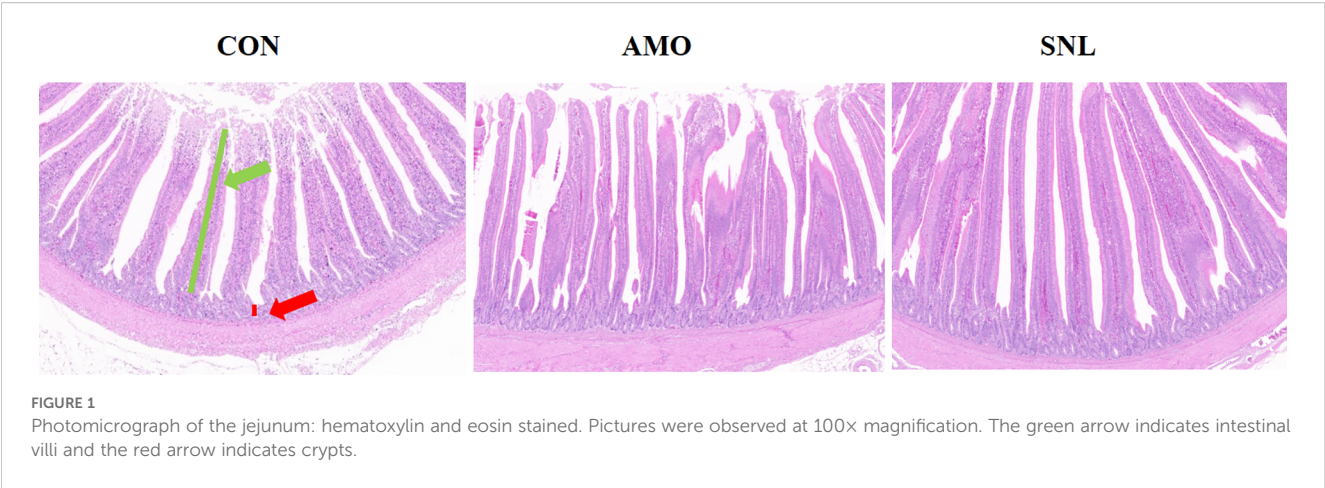
3.4 Caecal microbiota analysis

3.4.1 Analysis of the caecal microbiota diversity

The Illumina MiSeq high-throughput sequencing platform was employed to characterize the cecal microbial communities across

TABLE 2 Effects of different treatments on antioxidant enzyme activity and MDA.

Items	Groups			P-values
	CON	AMO	SNL	
SOD/(U/ml)	328.22±33.48	331.52 ±22.47	318.89±35.72	0.515
MDA/(nmol/ml)	2.29±0.39	1.96±0.24	2.35±0.26	0.086
GPX/(U/ml)	16.01±2.25	12.97±2.45	17.33±7.58	0.154
GSH/(umol/L)	905.14 ±188.29	864.00 ±82.61	838.29 ±131.04	0.448
T-AOC/(mmol/L)	0.70±0.05	0.70±0.13	0.71±0.13	0.829



different experimental groups. The raw data ranges from 78,484 to 81,729 reads across the samples, as detailed in [Supplementary Table S1](#). Following stringent quality filtering and the removal of chimeric sequences, the number of processed reads per sample averaged at 52,990 ([Supplementary Table S1](#)). A total of 1,084 ASVs were yielded with confirmed domain-level taxonomic assignments, ranging from 281 to 338, from 98 to 381, from 214 to 284 in CON, AMO, SNL group, respectively. Based on a 99% species similarity threshold, the α -diversity indices at the ASV level for each group are presented in [Figure 2A](#). No statistically significant differences were observed in either the Chao1 (richness) or Simpson (evenness) indices among the three treatments, suggesting comparable community diversities. However, the AMO group exhibited a significantly higher Shannon index compared to the SNL-supplemented group, indicating greater microbial richness in the AMO-treated caecal microbiota.

For β -diversity analysis, multivariate statistical approaches—including PCA and non-metric multidimensional scaling (NMDS)—were applied to assess compositional similarities among the treatment groups. The PCA and NMDS plots revealed distinct clustering patterns (at the ASV level) of caecal microbial communities across treatments. While the CON and AMO groups displayed partial overlap, the SNL group formed a separate cluster, distinctly differentiated from the other groups ([Figure 2B](#)).

TABLE 3 Effects of different treatments on jejunum villus height and crypt depth.

Items	Groups			P-values
	CON	AMO	SNL	
Villus height/ μm	1257.25 $\pm 174.09^{\text{ab}}$	1055.57 $\pm 65.31^{\text{b}}$	1376.09 $\pm 237.79^{\text{a}}$	0.019
Crypt depth/ μm	141.51 $\pm 14.94^{\text{b}}$	141.76 $\pm 20.87^{\text{b}}$	169.78 $\pm 16.66^{\text{a}}$	0.021
V/C	8.90 ± 0.93	7.58 ± 1.16	8.12 ± 1.41	0.183

^{ab}Means within same row carrying different superscript letters indicate significant differences ($p < 0.05$).

A Venn diagram illustrated the distribution of unique and shared intestinal ASVs among the cecal microbiota of different groups. Among the identified ASVs, 337 were common to all groups (core microbiota), with 201 ASVs unique to the control group, 196 ASVs exclusive to the AMO group, and 140 ASVs specific to the SNL group ([Figure 2C](#)).

3.4.2 Composition and structure analysis of the cecal microbiota

[Figures 3A, B](#) illustrate the relative abundance of gut microbiota in each group at the phylum and genus levels, respectively. At the phylum level, the cecal microbiota of all groups were predominantly composed of 10 major bacterial phyla, with *Bacteroidetes* and *Firmicutes* being the two most abundant ([Supplementary Table S2](#)). Compared with the CON and AMO groups, the SNL group exhibited a significant increase in the relative abundance of *Bacteroidetes*, whereas the relative abundances of *Proteobacteria* and *Firmicutes* decreased. Notably, the SNL group displayed the highest relative abundance of *Bacteroidetes* and the lowest abundances of *Firmicutes* and *Deferribacterota*. At the genus level, *Bacteroides*, *Clostridia_UCG-014*, *Faecalibacterium*, *[Eubacterium]_coprostanoligenes_group*, and *[Ruminococcus]_torques_group* were the predominant taxa in the ceca of broilers across all groups ([Supplementary Table S3](#)). The SNL treatment significantly promoted the growth of *Bacteroides*, *Faecalibacterium*, and *[Ruminococcus]_torques_group*, while markedly suppressing the abundance of *Oscillibacter* relative to other groups.

3.4.3 Analysis of cecal microbiota composition and metabolic function changes

The LEfSe revealed the dominant microbial taxa among different experimental groups. As illustrated in [Figures 3C, D](#), the SNL, AMO, and CON groups exhibited 3, 4, and 7 significantly different taxonomic units at various classification levels (LDA score > 3), respectively. Specifically, the predominant bacterial phylotypes in the SNL group comprised *Erysipelotrichaceae*, *Bacillales*, and *Bacillaceae*. In the AMO group, the dominant taxa included *CHKCI001*, *Lachnoclostridium*, *Shuttleworthia*, and *Eubacterium_hallii_group*. Meanwhile, the CON group was characterized by the predominance of *Lactobacillales*,

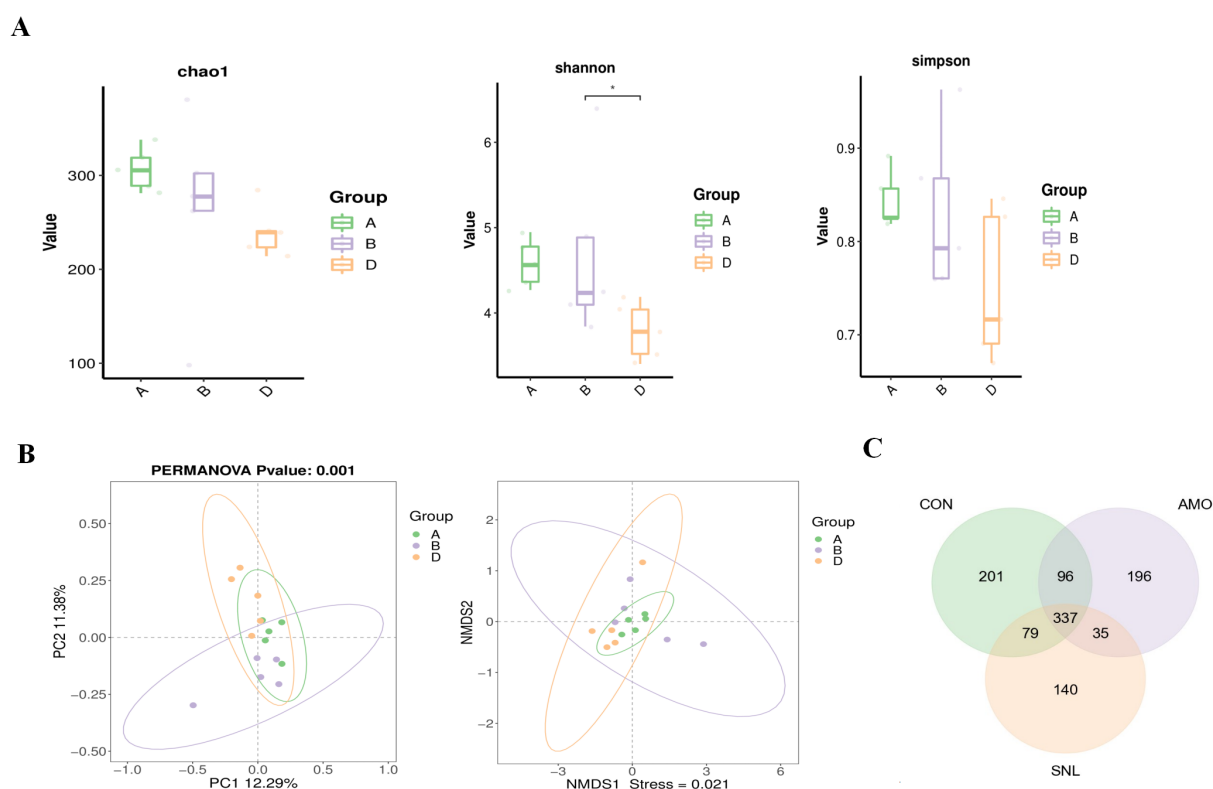


FIGURE 2

Caecal microbiota diversity analysis. CON (Group A), AMO (Group B), SNL (Group D). (A) The α -diversity of the microbial communities (Chao1, Shannon and Simpson) a bar with the asterisk (*) level suggests the degree of significant difference, ($*p < 0.05$); (B) β -diversity metrics: Principal component analysis (PCA) and non-metric multidimensional scaling (NMDS); (C) Venn diagram.

Lactobacillaceae, *Lactobacillus*, *Butyrivibrio*, *Actinobacteriota*, *Lachnospiraceae_NK4A136_group*, and *Peptostreptococcaceae*.

Significant intergroup differences in KEGG functional pathways, as predicted by PICRUST-based metagenomic analysis, are presented in Figure 3E. Comparative analysis demonstrated that the SNL dietary intervention elicited elevated heatmap scores for functional gene clusters associated with the MAPK signaling pathway – yeast, steroid biosynthesis, and sesquiterpenoid/triterpenoid biosynthesis, relative to both the AMO and CON groups. Furthermore, microbial gene functions linked to metabolic pathways—including pancreatic secretion and salivary secretion—were significantly upregulated in the SNL group compared to the AMO group, whereas pathways related to *Staphylococcus aureus* infection, shigellosis, and nitrotoluene degradation exhibited downregulation.

3.5 Transcriptome analysis of intestinal tissue

To elucidate the probiotic mechanisms of SNL, we conducted ileal transcriptomic profiling using RNA sequencing (RNA-seq). As depicted in Figure 4A, differential expression analysis (FDR < 0.05 and $|\log_2FC| > 1$) identified 174 DEGs (100 upregulated and 74 downregulated) in the CON vs. AMO comparison, 135 DEGs (76

upregulated and 59 downregulated) in the CON vs. SNL comparison, and 219 DEGs (88 upregulated and 131 downregulated) in the AMO vs. SNL comparison.

To further investigate the functional implications of these DEGs, we performed GO and KEGG pathway enrichment analyses. The GO analysis of upregulated DEGs in CON vs. SNL chickens suggested potential involvement in glucose homeostasis, insulin receptor signaling, oxaloacetate decarboxylase activity, and various enzymatic processes. KEGG enrichment highlighted pathways including the peroxisome proliferator-activated receptor (PPAR) signaling pathway, FoxO signaling pathway, butanoate metabolism, sphingolipid metabolism, and other amino acid metabolic processes. Conversely, downregulated DEGs in CON vs. SNL chickens were predominantly enriched in hormone-mediated signaling, lipid metabolic regulation, complement activation (lectin pathway), and carboxypeptidase activity, with KEGG analysis emphasizing the PPAR signaling pathway, Toll-like receptor signaling, RIG-I-like receptor signaling, peroxisome-related functions, and additional amino acid metabolism.

In the AMO vs. SNL comparison, GO analysis indicated that upregulated DEGs may participate in TGF- β sequestration within the extracellular matrix and cellular calcium ion homeostasis. KEGG enrichment identified pathways related to *Salmonella* infection, neuroactive ligand-receptor interactions, adrenergic

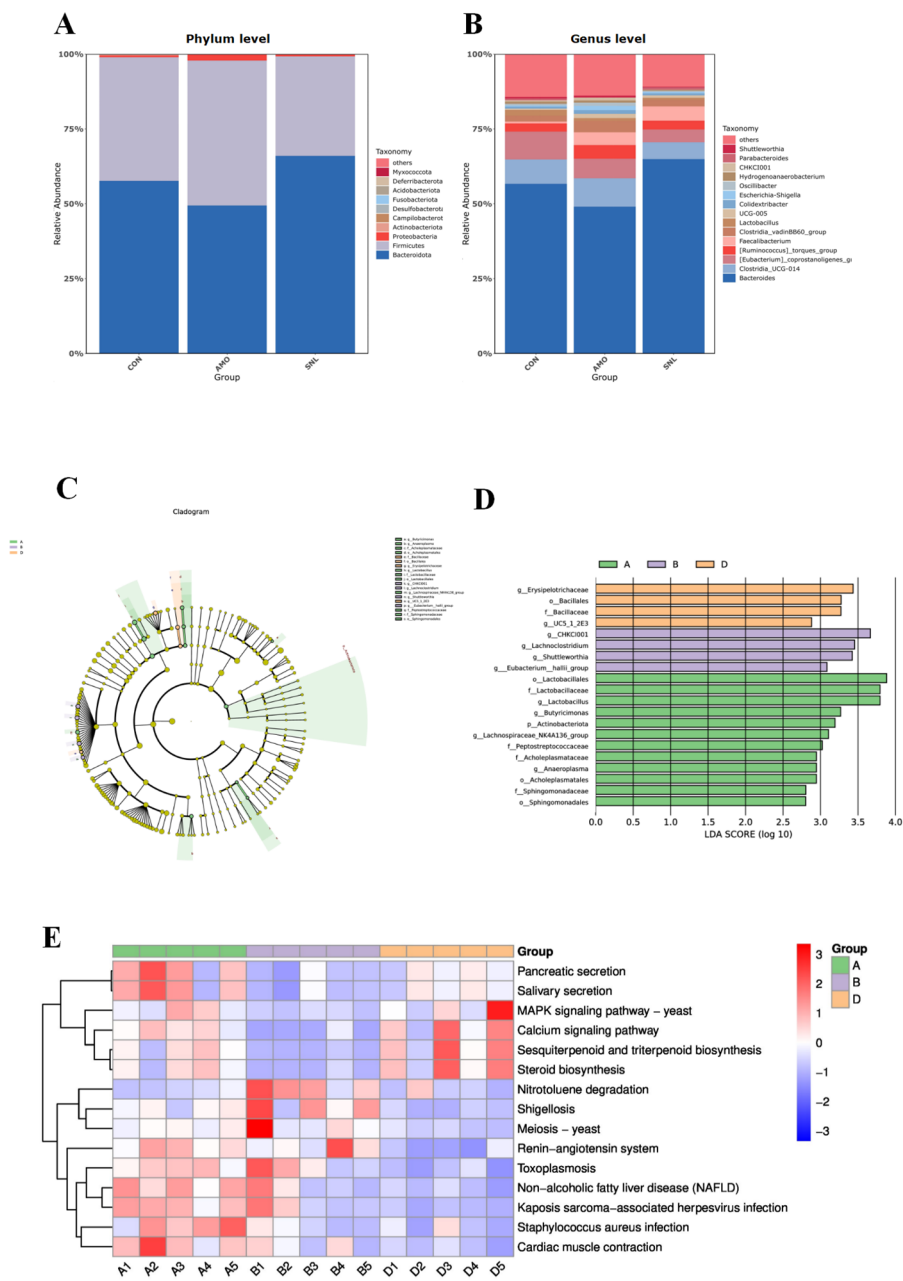


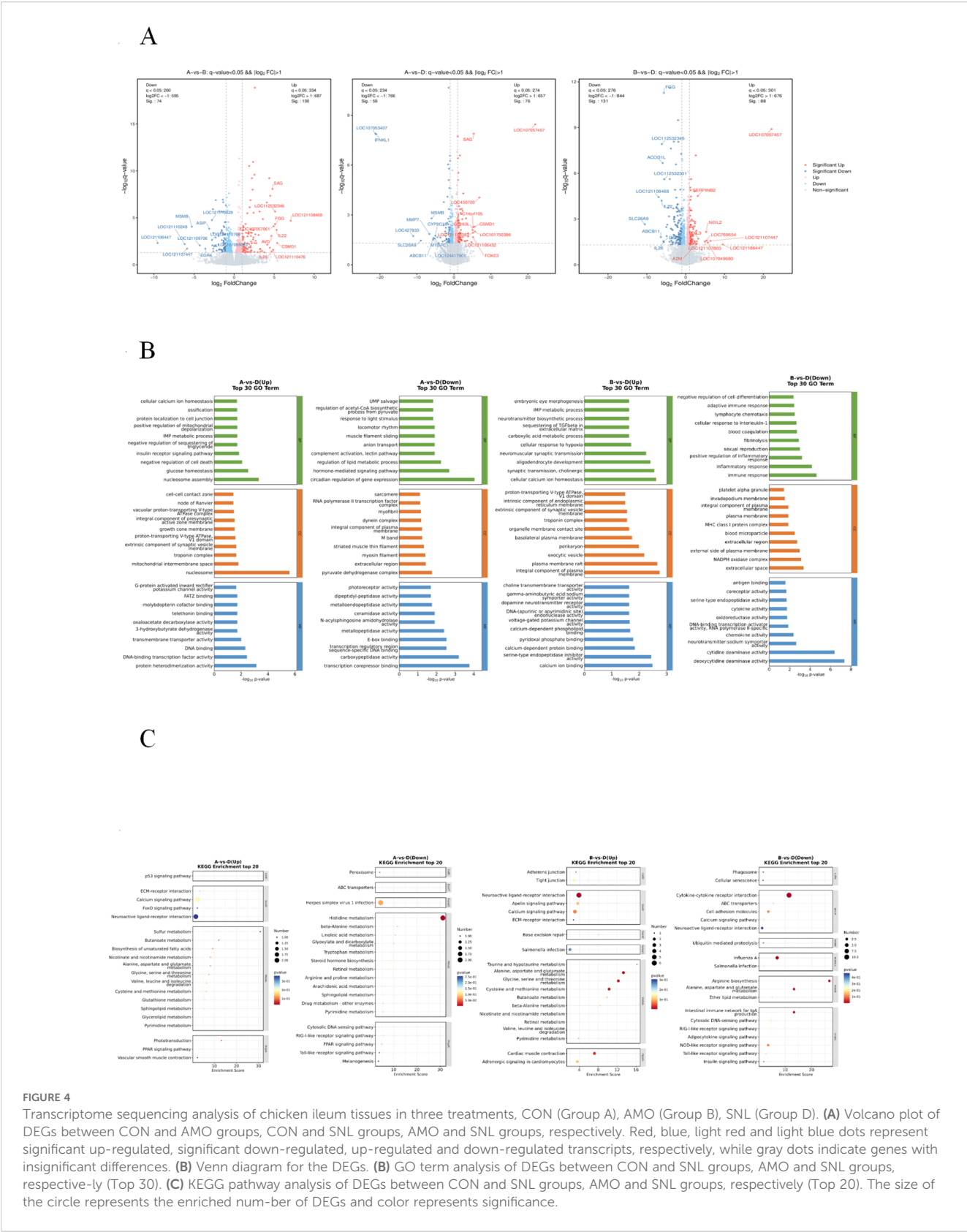
FIGURE 3 Microbiota analysis in cecum chyme. CON (Group A), AMO (Group B), SNL (Group D). **(A)** Community column diagram at the phylum level. **(B)** Community column diagram at the genus level. **(C, D)** Multilevel species differences from the phylum to genus level in different groups determined using the linear discriminant analysis (LDA) effect size (LEfSe) algorithm. **(E)** Difference in the metabolic functions of the cecal microbiota.

signaling in cardiomyocytes, and cardiac muscle contraction. Meanwhile, downregulated DEGs were primarily associated with immune and inflammatory responses (including positive regulation of inflammation, cellular responses to IL-1, and adaptive immunity), oxidoreductase activity, and various enzymatic processes. KEGG analysis further revealed enrichment in *Salmonella* infection, neuroactive ligand-receptor interactions, the intestinal immune network for IgA production, RIG-I-like receptor signaling, adipocytokine signaling, NOD-like receptor signaling,

Toll-like receptor signaling, insulin signaling, and additional amino acid metabolism (Figures 4B, C).

3.6 Correlation analysis of host transcriptome and cecal microbiome

Subsequently, we investigated the potential correlations between the microbiome and transcriptome by calculating



Spearman correlation coefficients in CON, AMO, and SNL chickens, respectively. Based on the results of 16S rRNA amplicon sequencing, the top 30 most abundant bacterial genera were selected for analysis. The relationships between differentially abundant

bacterial genera and key DEGs were visualized as heatmaps (Figures 5A-C, Supplementary Table S4-S6). Based on *P*-value, our analysis revealed that *Oscillibacter* exhibited a significant positive correlation with *MAP3K7CL* and *PON1*. In contrast,

MAP3K7CL displayed a significant negative correlation with *Bacteroides*, which in turn showed a significant negative correlation with *PRLHRL*.

4 Discussion

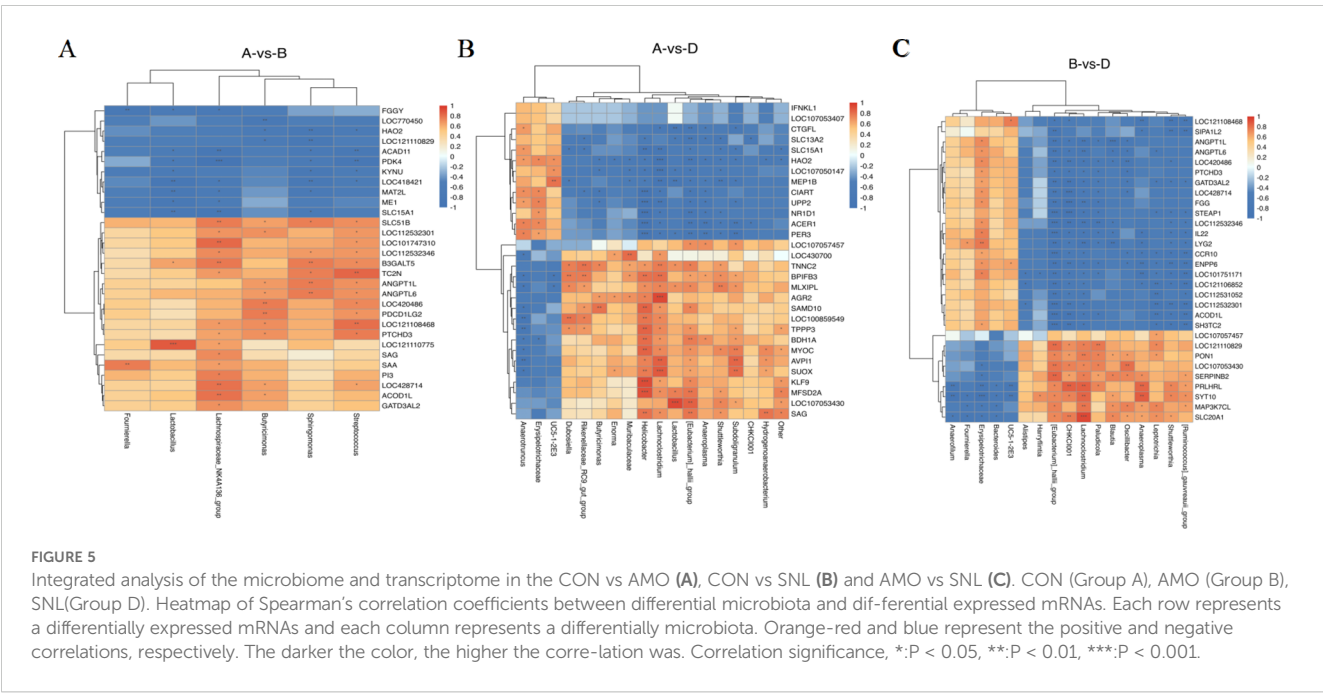
Historically, the use of antibiotics in the management of microbial infections and intestinal regulation has been a standard practice. However, due to the adverse environmental and food chain impacts of antibiotics, there is an urgent need for antibiotic alternatives in poultry production. Accumulating evidence suggests that phytogenic feed additives can modulate gut microbiota composition and activity, thereby conferring beneficial physiological effects on the host (Abdelli et al., 2021). While numerous studies have investigated the probiotic properties of Chinese herbal medicines in broilers, knowledge regarding the effects of SNL in avian species remains limited.

The present study aimed to evaluate the effects of SNL supplementation on broiler growth performance, intestinal barrier function, antioxidant status, cecal microbiota, and ileal gene expression. Compared to the CON group, SNL supplementation at 0.2% improved, although not significantly, body weight gain (BW, ADG) while reducing the F/G and ADFI, indicating enhanced nutrient utilization. No significant differences in growth performance or feed efficiency were observed between the AMO and SNL groups, suggesting that SNL is comparably effective to AMO in supporting broiler development. We hypothesize that SNL contains bioactive compounds, including polysaccharides and saponins, which may enhance digestive and absorptive efficiency, improve nutrient utilization, reduce feed wastage, and optimize dietary efficiency (Yao et al., 2020; Anzoom et al., 2023).

Although the growth performance is not significantly improved, the numeral trends demonstrate potential economic benefits from a production standpoint. In poultry industry, the cost of feed accounts for about 70% of the total costs, which means even a small improvement in feeding efficiency can significantly reduce production costs. Given that SNL is typically a byproduct, its utilization as a feed additive presents a cost-effective strategy for poultry farming without compromising growth performance, while also yielding high-quality broilers.

The antioxidant capacity of the organism plays a crucial role in suppressing free radical generation, preventing free radical chain reactions, and mitigating oxidative damage to the host. Enhanced antioxidant capacity is positively correlated with optimal health status and improved poultry production performance (Yang et al., 2016). Key antioxidant enzymes, including SOD for scavenging superoxide radicals ($O_2^{\cdot-}$), GPX and CAT for decomposing hydrogen peroxide (H_2O_2), are recognized as essential defense mechanisms against free radical-induced damage (Shang et al., 2023). The overall antioxidant status is comprehensively assessed through T-AOC, while MDA, a terminal product of lipid peroxidation induced by free radicals, serves as a reliable biomarker for oxidative stress intensity (Liu et al., 2021).

Our experimental results demonstrated no statistically significant differences in the levels of SOD, GPX, T-AOC, GSH, or MDA among the treatment groups. This is likely associated with the added concentration. Here, we added 0.2% SNL grass meal to the basal diet, the concentration of the bioactive constituents is relatively lower compared to other studies. A recent research indicated that dietary supplementation with SNL berries extract (200 mg/kg BW/d) can potentiate the body's antioxidant defense system via modulation of NRF2-related protein expression (Wang et al., 2024b). Such effects are likely attributable to the bioactive



constituents in SNL—namely polysaccharides, saponins, and flavonoids—which have been demonstrated to exert potent antioxidant properties (Guediri, 2021; Anzoom et al., 2023).

The intestine serves as the primary site for animals to absorb exogenous nutrients, with its fundamental structural components comprising crypts and villi. Intestinal villi extend the mucosal surface area, thereby enhancing nutrient absorption (Kai, 2021). Consequently, VH, CD, and their V/C have long been recognized as critical indicators for assessing intestinal physiological function and tissue integrity (Zhao et al., 2023). Generally, a longer villus height, higher V/C ratio, and shallower crypt depth reflect a superior structural and functional state of the intestinal epithelium, correlating with enhanced digestive and absorptive capacity as well as improved disease resistance (Banday et al., 2024). In the present study, dietary supplementation with 0.2% SNL elicited a marked trend toward increased jejunal VH in chickens compared to the CON group, although no statistically significant difference was observed in the V/C ratio. Notably, when compared to the AMO group, the 0.2% SNL treatment significantly elevated jejunal VH and improved the V/C ratio. These results indicate that incorporating an optimal dosage of SNL into the diet can enhance intestinal morphology and preserve structural integrity in chickens, thereby promoting intestinal health in yellow-feathered broilers. The mechanism underlying this difference is needed further investigation, and one possible reason for this may be the consequence of the alternation of the cecal microbiota. The observed improvements in VH and the consequent expansion of the small intestinal absorptive surface area likely underlie the enhanced growth performance observed following SNL supplementation.

The intestinal microbiota plays a pivotal role in enhancing nutrient assimilation, conferring disease resistance, and maintaining intestinal homeostasis (Ślizewska et al., 2020; Martin-Gallausiaux et al., 2021). In the present investigation, cecal microbiota analysis in broilers demonstrated that multiple α -diversity metrics, as well as the observed number of ASVs, were significantly reduced in the SNL group relative to both the CON and AMO groups. These findings indicate that dietary supplementation with SNL reduces both microbial species richness and diversity, implying a disruption in microbial evenness and potential dysbiosis (Zhang et al., 2025). The decrease in microbial species richness and diversity may be attributed to the antimicrobial characteristics mentioned before. This outcome contrasts with previous studies on other Chinese herbal preparations containing similar bioactive compounds (Che et al., 2024; Huang et al., 2024). Although the addition of SNL reduced intestinal microbial diversity, when combined with other results of this study, no significant impairment of intestinal health was observed. This observation was further supported by the metabolic function analysis of the cecal microbiota, which revealed that, compared to both the CON and AMO groups, the SNL group exhibited enrichment in metabolic pathways associated with reduced *Staphylococcus aureus* infection and *shigellosis* (Figure 3E). Besides, we observed SNL supplementation may potentiate the chicken's innate resistance to *Salmonella* infections from the transcriptome data in the present study. However, further investigation is still needed to clarify the reasons behind the decrease in α -diversity observed in the SNL group and to evaluate the impact on gut health.

Furthermore, PCA and NMDS revealed distinct compositional variations among the microbial communities of the three experimental groups. At the phylum level, consistent with extensive research on the gut microbiota of healthy broilers, *Bacteroidetes* and *Firmicutes* emerged as the predominant microbial phyla in our study (Bai et al., 2023). Notably, we observed a significant elevation in the relative abundance of both the phylum *Bacteroidetes* and its constituent genus *Bacteroides* in the SNL group, concomitant with significant reductions in the phyla *Firmicutes*, *Proteobacteria*, and *Deferribacterota*. Additionally, a marked decrease in the *Firmicutes*-to-*Bacteroidetes* (F/B) ratio was evident.

Bacteroidetes plays an indispensable role in complex carbohydrate degradation and propionate production, while the genus *Bacteroides* not only furnishes essential nutrients to other microbial populations but also contributes to host protection against intestinal pathogens through polysaccharide degradation and butyrate salt formation (Chen et al., 2019; Zafar and Saier, 2021). As the dominant gut microbiota in healthy individuals, an elevated F/B ratio is widely recognized as an indicator of intestinal microbiome dysbiosis (Xia et al., 2022). *Proteobacteria* has been identified as a potential diagnostic biomarker for malnutrition and disease susceptibility (Chen et al., 2021), while *Deferribacterota* exhibits an association with obesity (Walker et al., 2014). At the genus level, the cecal microbiota of broilers across all three experimental groups was predominantly composed of *Bacteroides*, *Clostridia_UCG-014*, *Faecalibacterium*, [*Eubacterium*] *_coprostanoligenes_group*, and [*Ruminococcus*] *_torques_group*. Among these, *Bacteroides*—a genus well-established for its role in complex carbohydrate degradation and short-chain fatty acid (SCFA) production (Yan et al., 2023a)—was the most abundant taxon in the SNL group. This genus has been positively correlated with weight gain and improved growth performance in poultry (Chang et al., 2016), suggesting that its elevated abundance in the SNL group may contribute to the observed higher ADG and lower F/G.

As a key butyrate-producing bacterium, *Faecalibacterium* plays a pivotal role in gut health evaluation (Li et al., 2019). Notably, *Faecalibacterium prausnitzii*, a prominent butyrate-producing species, exhibits a positive association with feed conversion efficiency (Kameyama and Itoh, 2014; Ye et al., 2019). The SNL-supplemented diet not only significantly enhanced the abundance of *Faecalibacterium* but also increased its proportional representation within the microbial community, which may explain the improved F/G ratio in the SNL group.

Furthermore, the cecal abundance of [*Ruminococcus*] *_torques_group* was higher in the SNL group compared to the COM group. Given that reduced abundance of *Ruminococcaceae* has been linked to inflammatory bowel disease (IBD) in humans (Joossens et al., 2011), this elevation may confer a protective effect. Conversely, the decreased abundance of *Oscillibacter* in the SNL group is likely beneficial, as this genus has been associated with impaired gut barrier function and intestinal inflammation (Li et al., 2021). Collectively, the dietary inclusion of SNL exerted favorable modulatory effects on the cecal microbiota of broilers, characterized

by an enrichment of beneficial bacterial taxa and a reduction in potentially detrimental ones. These microbial shifts likely underpin the observed enhancements in growth performance and feed efficiency. The SNL-supplemented diet was demonstrated to suppress the proliferation of Gram-negative and potentially pathogenic bacterial populations while concurrently enhancing the growth of Gram-positive bacteria. These observations are congruent with the documented structural remodeling of the cecal microbial community induced by dietary SNL intervention in the present investigation. Furthermore, metabolic pathway analysis revealed that SNL supplementation significantly attenuated the pathogenic potential associated with *Staphylococcus aureus* infections, shigellosis, and nitrotoluene biodegradation processes. This protective effect is partially attributable to the inherent anti-inflammatory bioactivity of SNL (Xiang et al., 2018; Deng et al., 2023).

The substantial number of DEGs suggests that dietary supplementation with SNL exerted a measurable influence on the ileal transcriptome of chickens. Comparative analysis of the AMO and SNL groups revealed that the top 30 significantly enriched GO terms and top 20 KEGG pathways in both groups prominently featured the *Salmonella* infection and neuroactive ligand-receptor interaction pathways. Notably, the *Salmonella* infection pathway warrants particular attention, given that *Salmonella* constitutes a primary poultry pathogen, contributing to considerable economic losses and posing significant public health risks. The observed upregulation of genes within this pathway implies that SNL supplementation may potentiate the chicken's innate resistance to *Salmonella* infections, thereby potentially diminishing the reliance on antibiotics in poultry production (Zhao et al., 2022). Similarly, the neuroactive ligand-receptor interaction pathway—which plays an indispensable role in cellular communication and signal transduction—was also significantly enriched. This enrichment suggests that SNL may modulate diverse physiological processes, including immune regulation and stress responses, ultimately contributing to enhanced poultry health (Han et al., 2022; Qiu et al., 2025). Transcriptomic analyses further indicated that SNL may confer health benefits by regulating multiple immune-associated pathways, notably the RIG-I-like receptor signaling pathway, NOD-like receptor signaling pathway, Toll-like receptor signaling pathway, and adipocytokine signaling pathway, with particular emphasis on the intestinal immune network for IgA production. As a pivotal immunoglobulin in mucosal immunity, IgA constitutes the first line of defense against gastrointestinal pathogens (Mantis et al., 2011). The enrichment of DEGs within this pathway implies that SNL supplementation may strengthen mucosal immune barriers, consequently reducing the incidence of gastrointestinal infections in chickens (Pabst, 2012; Jiao et al., 2023). Additionally, the RIG-I-like receptor signaling pathway, which mediates the detection of viral RNA and orchestrates antiviral responses, was also enriched. This finding suggests that the SNL-enriched diet may augment the chicken's antiviral defenses, providing protection against viral infections (Zhao et al., 2022). Furthermore, the identification of DEGs linked to the insulin signaling pathway highlights the potential role of SNL in modulating metabolic processes, including

glucose homeostasis and energy utilization (He et al., 2024). This is particularly pertinent in poultry production, where metabolic efficiency critically influences growth performance and feed conversion rates (Zhang et al., 2023; Guo et al., 2024). Comparative analysis of the CON and SNL groups demonstrated that DEGs were predominantly enriched in the PPAR signaling pathway, lipid metabolic process regulation, and other immune-related pathways, including the RIG-I-like receptor signaling pathway and Toll-like receptor signaling pathway. The PPAR signaling pathway governs multiple biological functions, encompassing energy metabolism, inflammation, cellular differentiation, and lipid homeostasis (Contreras et al., 2013; Fuior et al., 2023; Kim et al., 2023). The observed improvement in production performance within the SNL group is, at least in part, attributable to the functional modulation of the PPAR signaling pathway.

To investigate the interactions between the gut microbiota and host transcriptome following dietary supplementation with AMO and SNL in chickens, this study employed Spearman correlation analysis to examine the associations between transcriptomic and metagenomic profiles. Notably, chickens fed with SNL exhibited a significant increase in cecal *Bacteroides* abundance, which demonstrated a significant negative correlation with the gene *MAP3K7CL* (based on *P*-value).

MAP3K7CL is implicated in the activation of NF- κ B and MAPK signaling pathways, both of which are central to inflammatory and immune responses (Ling et al., 2022). The downregulation of *MAP3K7CL* in response to elevated *Bacteroides* levels suggests that these bacteria may modulate host inflammatory pathways, potentially through the production of short-chain fatty acids (SCFAs) or other microbial metabolites (Yan et al., 2023b, 2023a). This observation aligns with previous studies demonstrating that certain *Bacteroides* species, such as *B. vulgatus* and *B. fragilis*, can attenuate inflammation by regulating cytokine production and immune cell differentiation (Liu et al., 2022). Additionally, *MAP3K7CL* exhibited a positive correlation with the abundance of *Oscillibacter* (based on *P*-value). In hybrid yellow catfish subjected to transport stress, *MAP3K7CL* was significantly downregulated, indicating its involvement in immune signaling pathways, particularly those mediated by Toll-like and Nod-like receptors (Zheng et al., 2021). Furthermore, reduced *MAP3K7CL* expression has been observed in Chinese patients with non-small cell lung cancer, suggesting that leukocyte-derived *MAP3K7CL* may contribute to disease pathogenesis by modulating inflammatory processes, mitochondrial reactive oxygen species signaling, and the Wnt pathway (Niu et al., 2021).

The decreased abundance of *Oscillibacter* in SNL-fed chickens may indicate a diet-induced shift in gut microbiota composition that could mitigate inflammation and improve metabolic outcomes. This hypothesis is further supported by the positive correlation between *Oscillibacter* and *PON1*, a gene encoding paraoxonase 1—an enzyme with anti-inflammatory and antioxidant properties that protects against oxidative stress and lipid peroxidation (Xue et al., 2022).

PRLHRL, a gene associated with prolactin signaling, may also influence metabolic and immune responses, although its specific role in poultry remains incompletely understood. The enrichment

of *Bacteroides* in the ceca of SNL-fed chickens suggests that microbial metabolites may modulate host gene expression, thereby influencing immune and metabolic pathways (Fan et al., 2023). For example, SCFAs such as butyrate have been shown to regulate gene expression by inhibiting histone deacetylases and activating G protein-coupled receptors, which may indirectly affect *PRLHRL* expression (Cheng et al., 2022).

5 Conclusions

The results of the current study indicate that supplementation of broiler diets with 0.2% SNL might modestly improve growth performance. In addition, SNL increases jejunal villus height, alters microbial diversity or richness and consequently modulates host gene expression in broilers. Due to the relatively small sample size and the other limitations in the present study, further investigations are warranted to clarify the potential of SNL as an alternative to antibiotics in chickens.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://www.ncbi.nlm.nih.gov/sra/PRJNA1311735>.

Ethics statement

The animal study was approved by the Ethics Committee of Guangdong Meizhou Vocational and Technical College. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

MZ: Writing – original draft, Methodology, Data curation, Conceptualization. YL: Writing – original draft, Software, Visualization, Conceptualization. MC: Writing – original draft, Validation. ML: Validation, Writing – original draft. RH: Validation, Writing – original draft. WX: Writing – original draft, Formal Analysis. XL: Writing – original draft, Investigation. YX: Resources, Writing – original draft. CJ: Project administration, Supervision, Funding acquisition, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fanim.2025.1698465/full#supplementary-material>

SUPPLEMENTARY TABLE 1
The statistics of the sequencing data.

SUPPLEMENTARY TABLE 2
Mean of microbial community relative abundance at the phylum level in the cecum.

SUPPLEMENTARY TABLE 3
Mean of microbial community relative abundance at the genus level in the cecum.

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