Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2025.810

RESEARCH ARTICLE

Matrine Enhances the Intestinal Barrier and Regulates Gut Microbiota in Chickens

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ARTICLE HISTORY (25-855)

Received: September 07, 2025
Revised: October 30, 2025
Accepted: November 08, 2025
Published online: December 08, 2025

Key words:
Antioxidant function
Intestinal flora
Intestinal Immune
Matrine
Transcriptome

ABSTRACT

Matrine, an alkaline polysaccharide, is the main bioactive component of *Sophora flavescens* and has been shown to possess pharmacological activities. In our previous research, we found that matrine exerted significant antibacterial activity *in vitro* and enhanced the efficacy of antibiotics and mitigated bacterial resistance. Nevertheless, the mechanisms by which matrine influences intestinal health are not well understood. Thus, we explored how matrine influences the intestinal well-being of yellow-feathered chickens and the mechanisms involved. Our current findings reveal that matrine supplementation can boost goblet cell quantity in the intestine, enhance intestinal barrier function, and strengthen immune response. Furthermore, we found that matrine treatment modulated the abundance of specific intestinal bacteria. Transcriptomic profiles further confirmed that dietary matrine altered the gene expression profile. Examining the DEGs revealed unique signaling pathways involved in regulating immune responses, lipid metabolism, and neural conduction. Adding matrine to the diet can improve the intestinal mucosal barrier integrity and strengthen immune function.

To Cite This Article: Wu H, Liu J and Hao H, 2025. Matrine enhances the intestinal barrier and regulates gut microbiota in chickens. Pak Vet J. http://dx.doi.org/10.29261/pakvetj/2025.310

INTRODUCTION

Intestinal health is a vital factor in growth performance and overall animal health status of livestock (Beumer and Clevers, 2021). The intestine is responsible for breaking down and absorbing nutrients, being a barrier against pathogenic microorganisms, and harmful metabolites (Diaz Carrasco *et al*., 2019). In contemporary intensive breeding practices, several factors contribute to intestinal oxidative stress, disrupt intestinal integrity, and compromise poultry health. These factors include pathological issues, nutritional imbalances, and management practices that can negatively impact feed efficiency and reduce production performance (González-González *et al*., 2019). Antibiotics are often incorporated in feed additives, thereby increasing poultry production, as they can eliminate harmful intestinal bacteria. However, the indiscriminate antibiotics use can cause increased bacterial resistance to antibiotics, potentially posing significant risks to food safety (Zhang *et al*., 2018). Given these concerns, it is essential to develop safe, effective, and environmentally sustainable alternatives to antibiotics. By focusing on these innovative strategies, the poultry industry can improve animal welfare and ensure safer food production practices.

Matrine is a polypeptide alkaloid, which is an alkaline polysaccharide extracted from the traditional Chinese herbal medicine *Sophora flavescens* (Fabaceae). It has shown potential to improve animal production performance and modulate immune responses, while also exerting anti-inflammatory, antioxidant, and anti-pathogenic effects. Its application in livestock and poultry breeding has been reported to improve overall production efficiency and health. It can help prevent and treat several diseases, including diarrhea, ulcerative colitis, dairy mastitis, endometritis, as well as parasitic infections. Additionally, matrine is effective in mitigating heat and immune stress, making it a valuable alternative to conventional treatments (Sun *et al*., 2022). Its multifaceted benefits highlight its potential as a sustainable and natural solution in animal husbandry. Incorporating 1% *Sophora flavescens* extract into livestock feed has been shown to significantly enhance the immune function and antibacterial capacity of mutton sheep (Na *et al*, 2021). Additionally, feed containing matrine has been effective in preventing diarrhea in weaned piglets (Yao *et al*.2011). In weaned piglets and fattening pigs, matrine increased daily weight gain from 9.14 to 16.98% and improved feed utilization from 7.56

to 9% (Wu, 2001; Wang *et al*., 2002). Elsewhere, administration of 1500 mg/kg of a compound herbal additive composed of (Maqimai (*Pipturus albidus*), *S. flavescens* and Fulonggan (*Terra Flava Usta*) enhanced broiler chicken growth performance, boosted antioxidant capacity and immune function, enhanced intestinal villi stability and development, and strengthened intestinal barrier function (Liu *et al*., 2023). Furthermore, matrine displayed a variety of antibacterial properties and hindered the formation of biofilms. *In vitro* tests assessing drug susceptibility have shown that matrine possesses the ability to combat various bacteria. Furthermore, matrine can synergize with antibiotics to enhance their efficacy against these pathogens (Wu *et al*., 2024). For instance, a combination of amoxicillin and matrine decreased the required dosage of amoxicillin (Sun *et al*., 2023). These findings highlight the potential benefits of matrine in livestock production.

Previous research into matrine has concentrated primarily on its immunomodulatory, alongwith anti-inflammatory properties. However, few studies have explored its impact on poultry production performance and the associated underlying mechanisms. In response to this gap, we conducted research to investigate the impact of a matrine-containing basal diet on yellow-feather broilers. Our aim was to assess its effects on production performance and to elucidate the potential mechanisms of action involved. This research provides important perspectives on how matrine can be effectively utilized in poultry production.

MATERIALS AND METHODS

Experimental Animals: For this research,healthy one-day-old fast large yellow-feathered broilers (purchased from Sangao Agriculture and Animal Husbandry Co., Ltd, Henan, China) with similar body weights were chosen as the experimental subjects. Matrine (MAR, Purity 98%) employed in the experiment was sourced from a supplier located in Shaanxi Province, China.

Experimental design and feeding management: A complete randomized design was adopted in the study in which 360 broilers were allocated into three treatment groups: a blank control group (CON), test group I (ML group), and test group II (MH group). Each category consisted of six replicates, with 20 chickens assigned to each replicate, ensuring that the initial average body weight was comparable across groups. The CON group received only a standard diet, while the experimental groups, ML and MH groups, were supplemented with 200 and 400 mg/kg of matrine, respectively, incorporated into the basal diet through a stepwise premixing method. The dosage of matrine was established based on preliminary trials that comprised a 7-day pretest phase followed by a 35-day trial period, which included a brooding phase from days 7 to 21 and a growing phase from days 22 to 42. The Experimental Animal Ethics Committee at Xinyang Agriculture and Forestry University conducted a thorough review and granted approval for all facets of animal feeding management and the procedures used in experiments. Additionally, all protocols concerning the use of experimental animals adhered to the ethical standards set for animal research.

Detection of Serum Antioxidant: At the ages of 21 and 42 days, a random selection of six test chickens was made from each replicate group to obtain cardiac blood. These samples were then tested using their corresponding commercial ELISA kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) to determine superoxide dismutase (SOD) and malondialdehyde (MDA) levels.

Histology: At 21 and 42 days of age, six test chickens were randomly selected based on average weight from each replicate and euthanized via jugular vein exsanguination and dissected. The duodenum, jejunum, and ileum were excised from consistent anatomical locations with each segment measuring 2 cm in length. The intestinal tissues were preserved in a 10% formaldehyde solution and subsequently embedded in paraffin for sectioning. After staining with hematoxylin and eosin (HE), the pathological alterations in the intestinal tissues were evaluated microscopically. The Image-Pro Plus 6.0 software ware was utilized to quantify the villus height and crypt depth. Statistical analyses were conducted on both the villus height and crypt depth, and the V/C ratio (villus height to crypt depth) was calculated for each intestinal segment.

Detection of intestinal goblet cells: Samples of tissue obtained from the duodenum, jejunum, and ileum were preserved for 24 hours and subsequently dehydrated. Next, they were embedded and sectioned as described above and stained with periodic acid-Schiff (PAS) reagent. The distribution and abundance of goblet cells within these intestinal segments were examined using light microscopy.

Detection of Intestinal Immune Index and Mechanical Barrier Gene Expression: Total RNA was extracted from these tissues using a Total RNA Extraction Kit (Wuhan Sevier Biotechnology) and cDNA was synthesized by reverse transcription. For each sample, three replicate wells were established, and the cycle threshold (CT) values were documented. Relative levels of IL-10, IL-1β, TNF-α, Occludin, and ZO-1 were calculated utilizing the $2^{-\Delta\Delta CT}$ method. Primer sequences provided by Sangon Bioengineering (Zhengzhou, China) are shown in Table1.

Table 1: Primer pairs for qPCR

Target	Primer sequence (5'-3')	Annealing temperature, ℃	Accession number
IL-10	F: GCTGAGGGTGAAGTTTGAGGA	60	XM_025143715.1
	R: GCTCTGCTGATGACTGGTGCT		
IL-1β	F: TACATGTCGTGTGTGATGAGCG	60	NM_204524.2
	R: TGGTCGGGTTGGTTGGTGAT		
TNF-α	F: TTGACTTGGCTGTCGTGTGG	60	NM_204267.1
	R: TATAAGAACCAACGTGGGCATT		
Occludin	F:GAGTTGGATGAGTCCCAGTATGAG	60	NM_205128.1
	R: ATTGAGGCGGTCGTTGATG		
ZO-1	F: GGAGGATCCAGCCATGAAAC	60	XM_015278981.2
	R: CTTGAGGTCTCTGTGGTTCTGG		
β-actin	F: CTGACTGACCGCGTTACTCC	60	NM_205518.1
	R: TTGCACATACCGGAGCCATT		

Bioinformatics Analysis of Intestinal Flora: 16S rRNA gene amplicon sequencing and analysis were performed by OE Biotech (Shanghai, China). Utilize the NEBNext®

Ultra™ DNA Library Prep Kit for library construction, in accordance with the selected sequencing regions. Conduct quality control on the constructed libraries using the Agilent Bioanalyzer 2100 and Qubit systems. Amplify and sequence the V3-V4 variable regions employing the Illumina MiSeq sequencing platform. Perform expression analysis using Linear Discriminant Analysis Effect Size (LEfSe) and R software.

Transcriptome analysis of jejunal mucosal tissue: The sequencing of the transcriptome and subsequent analysis were carried out by OE Biotech Co., Ltd. (Shanghai, China). the analysis of differential expression was conducted utilizing DESeq2. Q value<0.05 and fold-change>2 or<0.5 was established to identify significantly differentially expressed genes (DEGs). To further investigate the expression profiles of the identified DEGs across different groups, hierarchical cluster analysis was performed using R version 3.2.0. This visualization was accomplished through the use of the ggradar package in R, providing a clear and informative representation of the expression dynamics of these key genes. Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis were carried out using the GO and KEGG databases, respectively.

Statistical Analysis: Data were sorted using Excel 2010 and analyzed using SPSS 24.0 software (IBM, Chicago, IL, USA), and R (v3.0.3) and GraphPad Prism (v 8.0c). The data are presented as mean±standard deviation (SD), and a p-value<0.05 was considered statistically significant.

RESULTS

Effect of matrine on intestinal mucosa: Examinations of intestinal tissue samples indicated that the intestinal mucosal architecture in chickens across experimental groups remained unchanged. The intestinal villi were orderly and dense with occasional shedding. The demarcation between villi and crypts was clearly defined, with no signs of edema or swelling in the intestinal wall. At 21 and 42 days old, the villus density observed in the duodenum and jejunum of the ML group was greater than that seen in both the blank control and MH groups, displaying clearly defined borders (Fig. 1A and 1B). By the 21st day of the study, a notable reduction in the crypt depths of the duodenum, jejunum, and ileum was observed in the ML group relative to the control group, while the V/C ratio demonstrated an increase. In the ileum, there was a reduction of 45.83% in crypt depth, whereas the V/C ratio saw an increase of 134.67% when compared to the CON. In the MH group, a reduction of 30.14% in duodenal villus length was observed when compared to the CON, while ileal and jejunal villi lengths showed an increase of 76.70%. Additionally, crypt depths in the duodenum, jejunum, and ileum were markedly reduced in the MH group. Notably, the control group exhibited a significant decrease of 32.93% in jejunal crypt depth. Moreover, ileum depth and ileal crypt depth rose by 18.95% and 144.89%, respectively, in the MH group, compared to the CON (Fig. 1C-K).

The ML group showed longer villus lengths compared to the CON group on the 42nd day of the experiment. Specifically, the ileal villus length increased by 49.57% relative to the control group (P<0.05). In the MH group, the duodenal villus length decreased by 24.39% compared to the CON group, while ileal villi length showed an increase of 69.62% (Fig. 1C-K).

Effect of matrine on goblet cells of Small Intestine: On day 21, the ML group had more goblet cells per unit length in the jejunum and ileum relative to controls, though not significantly. Similarly, the MH group showed an increase in these cells in the duodenum, jejunum, and ileum relative to controls (P>0.05). On day 42, the ML group had more goblet cells per unit length in the duodenum and ileum, but fewer in the jejunum, relative to controls (P>0.05). By contrast, the MH group illustrated a notably higher duodenal goblet cell count than controls (P<0.05) (Fig. 2C-E).

Effect of matrine on antioxidant function: In the assessment of oxidative stress markers, we found that on day 21, SOD levels increased in ML and MH groups relative to controls while the concentration of MDA was decreased, but not significantly (P>0.05). By the 42nd day, there was a notable rise in SOD levels within both the ML and MH groups (P<0.05) (Fig. 3).

Effect of matrine on Immune Function of Small Intestine: On the 21st day, matrine-treated animals showed decreased expression of IL-10, IL-1β, and TNF-α within the duodenum, jejunum, and ileum. Importantly, the ML group had notably lower IL-1β levels within the jejunum, as well as decreased three cytokines in the ileum. In the MH group, IL-1β was significantly decreased in the duodenum, with all three cytokines reduced in the ileum (Fig. 4).

On the 42nd day of the experiment, the IL-1β mRNA level in the ML group was down-regulated compared to the levels in the control group, while the levels of IL-10 and TNF in the duodenum were raised. However, the IL-α mRNA levels were not significantly altered (P>0.05). Furthermore, IL-1β mRNA levels were down-regulated in the MH group, but the IL-10 mRNA levels were increased in all three intestinal segments, but not statistically significant (Fig.4).

Effect of matrine on Mechanical Barrier Gene of Small Intestine: On day 21, occludin and ZO-1 mRNA levels in the duodenum showed a reduction in the ML group, though not significantly. Similarly, the ZO-1 mRNA level in the jejunum was significantly reduced. By contrast, the occludin mRNA level was up-regulated in the jejunum. Meanwhile, occludin and ZO-1 mRNA levels were significantly reduced in the MH group (P>0.05). By day 42, matrine supplementation had increased occludin and ZO-1 mRNA expression in the duodenum, jejunum, and ileum compared to controls. However, further analysis revealed a non-significant down-regulation of occludin and ZO-1 mRNA levels within the jejunum and ileum (Fig. 4).

Effect of matrine on microbiota diversity: Moreover, the Goods coverage index was examined to analyze the intestinal microbiomes in all groups. The indices of Chaol, Ace, Simpson, and Shannon for the ML and MH groups illustrated a non-significant rise compared with the control (Fig. 5A1-4 and 5B1-4). Therefore, we examined the

changes in intestinal microbiota structure through β-diversity analysis via PCoA. This revealed notable differences in the intestinal microbiota characteristics between the matrine test groups and the controls at days 21 and 42 (Fig. 5A5 and 5B5). The Non-metric multidimensional scaling (NMDS) is an extensively utilized clustering analysis technique that evaluates similarities in species composition across samples and serves as a representation of β-diversity. Significant differences in the structure and distribution of microbial communities between the matrine and control groups were identified (P<0.05) (Fig. 5A6 and 5B6).

The intestinal microbiota's composition at the phylum level remained stable across all three groups at both 21 and 42 days, primarily dominated by *Firmicutes*, *Bacteroidota*, *Proteobacteria*, *Actinobacteriota*, and *Desulfobacterota*, with a cumulative relative abundance exceeding 99%. At 21 days, the relative abundance of *Actinobacteriota* was found to be reduced in both ML and MH groups compared to controls, with a notable reduction in the MH group (P<0.05). Additionally, the ML group had a significantly lower abundance of Desulfobacterota, while the MH group showed a marked rise (P<0.05) (Fig. 5A7). At 42 days, the ML group showed an increase in the relative abundance of

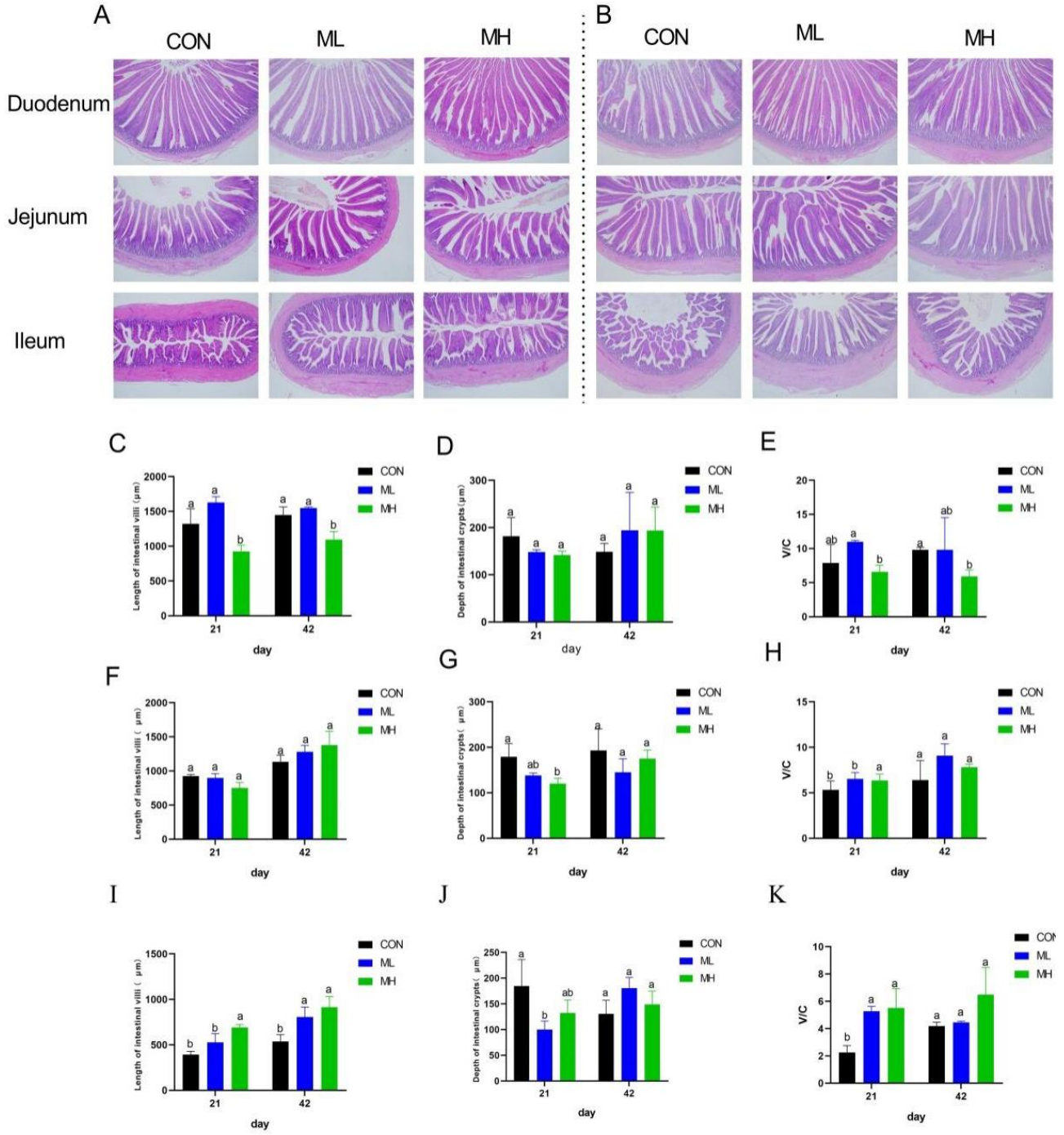


Fig. 1: Effects of matrine dietary supplementation on intestinal function in yellow broiler chickens. The effects of matrine on intestinal histomorphology of duodenum, jejunum and ileum in (A) 21 and (B) 42-day-old yellow broiler chicken (×40). Analysis of the duodenal (C) villi lengths (D) crypt depths and (E) villi length/ crypt depth ratios. Jejunum measurements of (F) villi lengths. (G)crypt depths and (H) villi length/ crypt depth ratios. Ileum measurements of (I) villi lengths (J) crypt depths and (K) villi length/ crypts ratios. n=6 (C–K). Values with different small letter superscripts represent P<0.05, while superscripts with the same letter indicate P>0.05. the same as below.

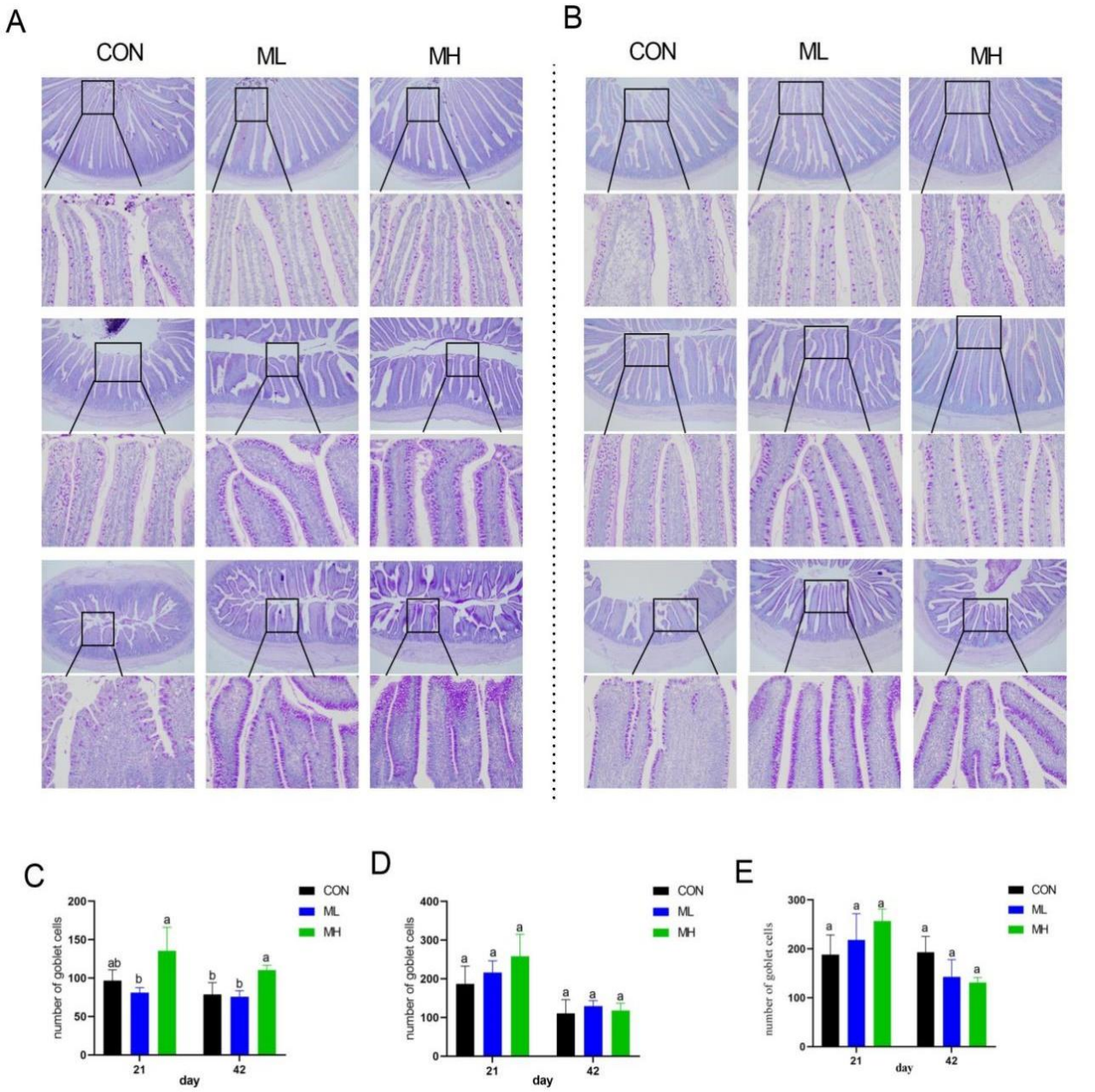


Fig. 2: Effects of matrine on goblet cells in yellow broiler chickens. Goblet cell AB-PAS staining (bluish violet) in (A) 21-day-old chickens (×40; ×200) and (B) 42-day-old chickens (×40; ×200). Goblet cell number in (C) duodenum (D) jejunum and (E) in ileum of blank and matrine group.

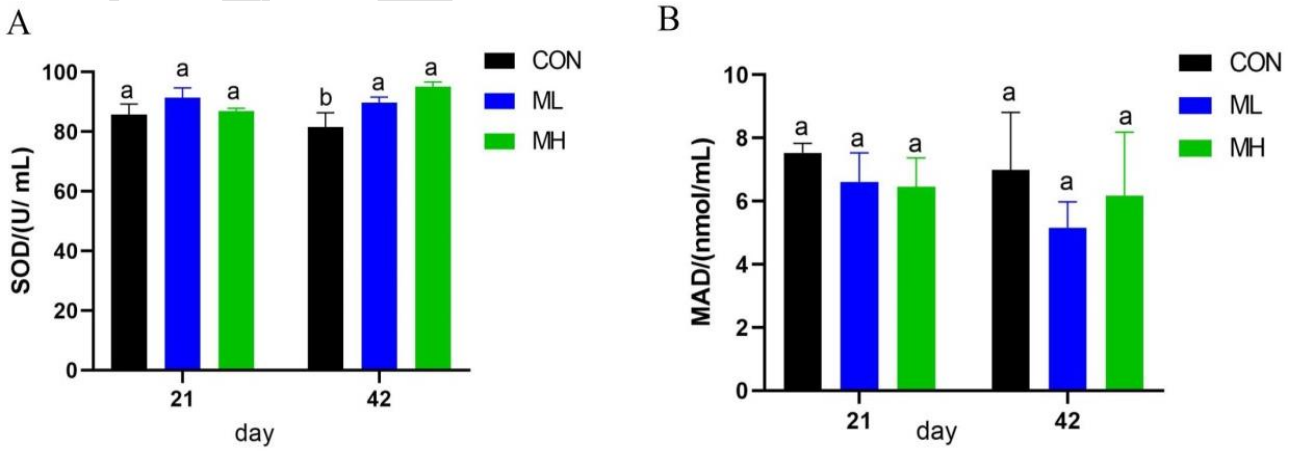


Fig. 3: Changes in the antioxidant enzymes (SOD activity and MDA content) of plasma with different concentrations of matrine in yellow broiler chickens. (A) SOD and (B) MDA levels. n=6.

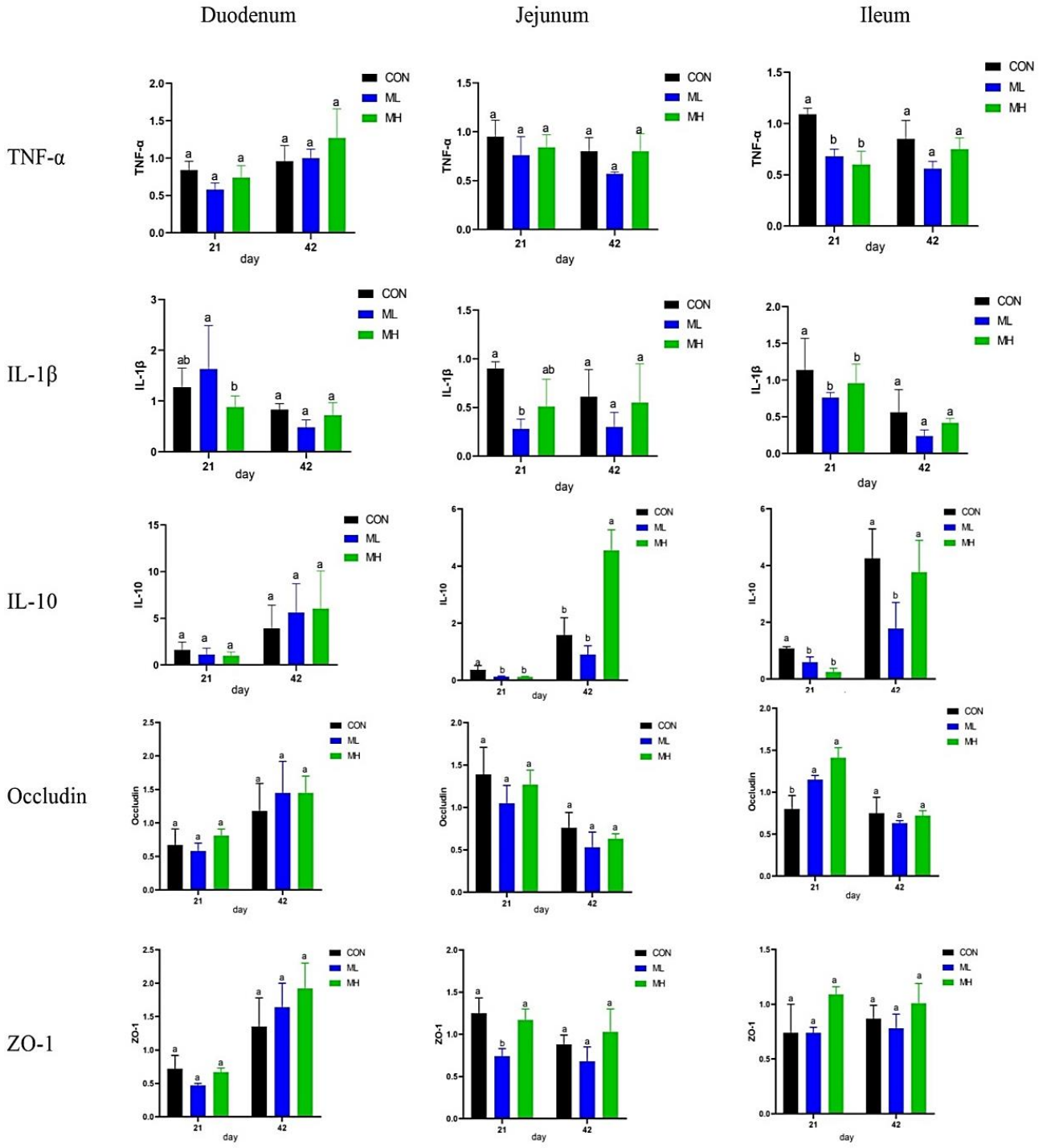
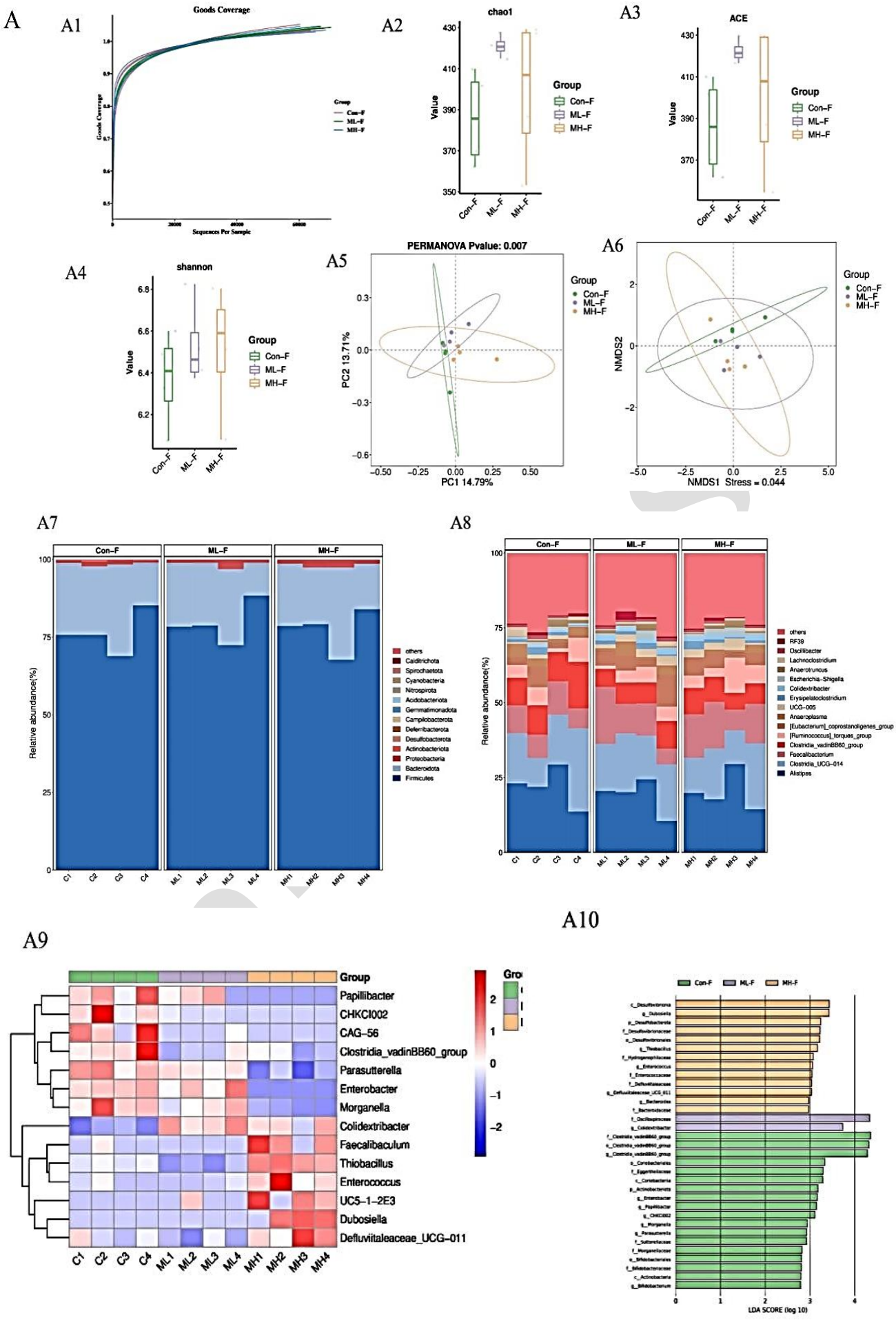


Fig. 4: Effects of matrine on immunoglobulins and cytokine levels. The relative mRNA expression of IL-10, IL-1β, TNF-α, as well as occludin and ZO-1, was analyzed for both the control and matrine-treated groups. n=6.

Actinobacteriota and Desulfobacterota compared to controls (P<0.05). Conversely, Campilobacterota abundance was significantly lower in both ML and MH groups (P<0.05) (Fig. 5B7).

After 21 days, the microbiomes observed in the control and matrine test groups exhibited the highest relative abundance of the following genera: *Alistipes*, *Clostridia*_UCG-014, *Faecalibacterium*, and *Clostridia*_vadinBB60_group. Moreover, when compared to the control groups, those treated with matrine demonstrated significantly decreased abundance of *Clostridia*_vadinBB60_group, CHKCI002, and *Parasutterella*, while *Colidextribacter* increased notably (P<0.05). Additionally, the MH group exhibited a significant rise in *Defluviitaleaceae*_UCG-011 (P<0.05) (Fig.5A8-9). At 42 days, the abundance of *Bacteroides*, *Clostridia*_UCG-014, *Faecalibacterium*, and *Alistipes* was found to be the most abundant in the intestines of both groups. Interestingly, the presence of *Shuttleworthia*, *Thiobacillus*, *Virgibacillus*, *Kroppenstedtia*, and *Morganella* was considerably greater in the ML group relative to the controls (P<0.05). Moreover, *Virgibacillus*, *Kroppenstedtia*, and *Morganella* abundance were found to be greater in the MH group than the controls (P<0.05). Conversely, Family_XIII_UCG-001 exhibited a significantly reduced presence (P<0.05) than controls (Fig. 5B8-9).


A
A1
Goods Coverage
Goods Coverage
Sequences Per Sample
Group
Con–F
ML–F
MH–F
A2
chao1
Value
Group
Con–F
ML–F
MH–F
A3
ACE
Value
Group
Con–F
ML–F
MH–F
A4
shannon
Value
Group
Con–F
ML–F
MH–F
A5
PERMANOVA Pvalue: 0.007
PC2 13.71%
PC1 14.79%
Group
Con–F
ML–F
MH–F
A6
NMDS2
NMDS1 Stress = 0.044
Group
Con–F
ML–F
MH–F
A7
Con–F
ML–F
MH–F
Relative abundance(%)
others
Calditrichota
Spirochaetota
Cyanobacteria
Nitrospirota
Acidobacteriota
Gemmatimonadota
Campilobacterota
Deferribacterota
Desulfobacterota
Actinobacteriota
Proteobacteria
Bacteroidota
Firmicutes
C1 C2 C3 C4 ML1 ML2 ML3 ML4 MH1 MH2 MH3 MH4
A8
Con–F
ML–F
MH–F
Relative abundance(%)
others
RF39
Oscillibacter
Lachnoclostridium
Anaerotruncus
Escherichia–Shigella
Colidextribacter
Erysipelatoclostridium
UCG–005
Anaeroplasma
[Eubacterium]_coprostanoligenes_group
[Ruminococcus]_torques_group
Clostridia_vadinBB60_group
Faecalibacterium
Clostridia_UCG–014
Alistipes
C1 C2 C3 C4 ML1 ML2 ML3 ML4 MH1 MH2 MH3 MH4
A9
Group
Papillibacter
CHKCI002
CAG–56
Clostridia_vadinBB60_group
Parasutterella
Enterobacter
Morganella
Colidextribacter
Faecalibaculum
Thiobacillus
Enterococcus
UC5–1–2E3
Dubosiella
Defluviitaleaceae_UCG–011
C1 C2 C3 C4 ML1 ML2 ML3 ML4 MH1 MH2 MH3 MH4
A10
Con–F
ML–F
MH–F
LDA SCORE (log 10)

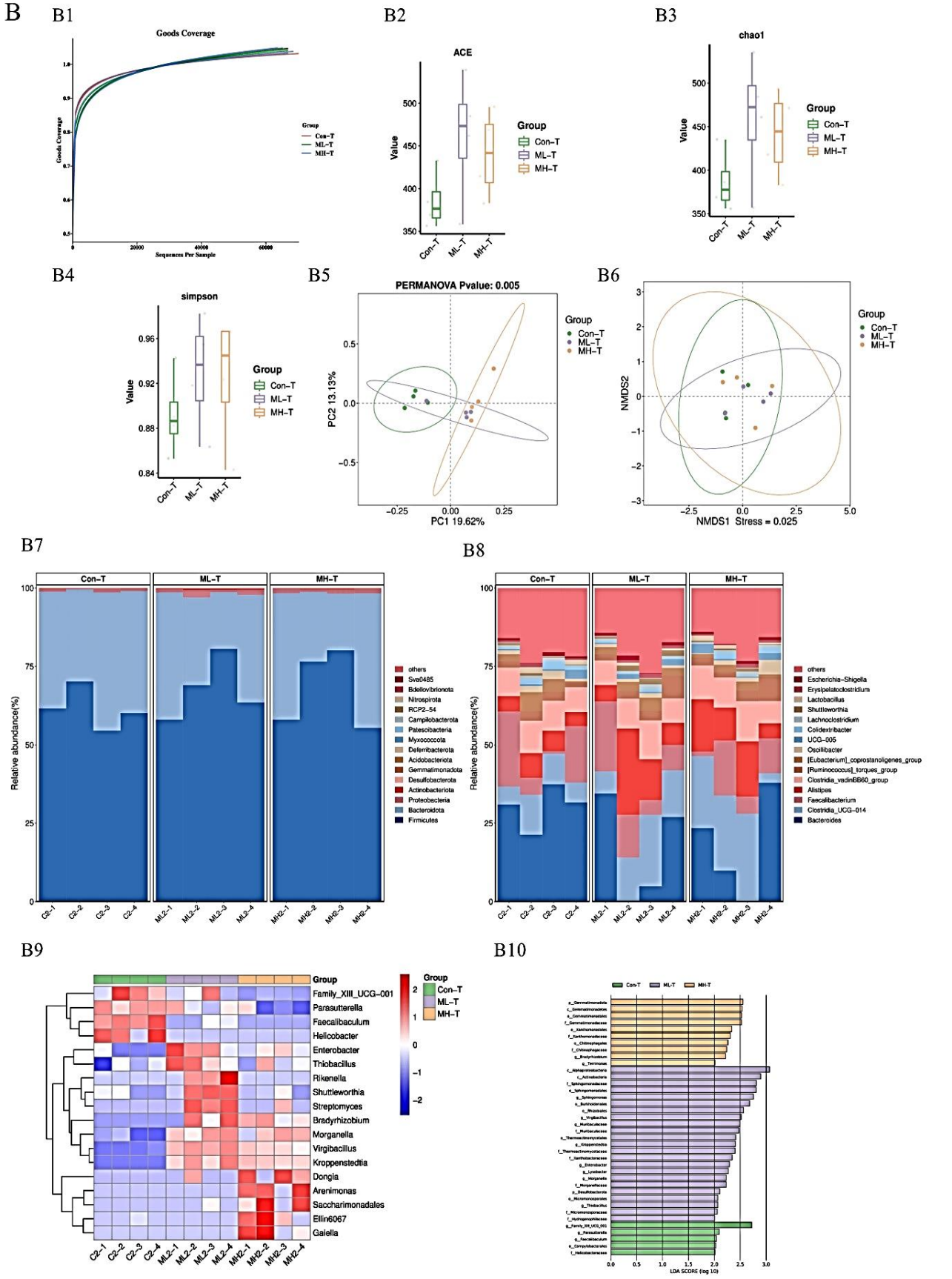


Fig. 5: The effects of matrine on gut bacterial taxonomic composition of both the control and the matrine-treated groups. Analysis of intestinal bacteria in (A) 21-day-old and (B) 42-day-old yellow-feathered broilers. Numbers associated with group designations are as follows: (1) Coverage; (2) Chao1 richness (Chao1) index;(3) Abundance-based coverage estimator (ACE) index; (4) Shannon index; (5) PCoA analysis based on unweighted unifrac algorithm; (6) NMDS analysis based on weighted_unifrac; Gut bacterial taxonomic compositions at the (7) phylum and (8) genus; (9) Heatmap showing the relative richness of bacterial communities at the genus level; (10) LEFSe analysis. n=4.

During the LEfSe analysis conducted at the genus level, we discovered bacterial groups that exhibited significant differences among the experimental groups. Furthermore, we applied a linear discriminant analysis (LDA) with a threshold set at 2. At the age of 21 days, 20 bacterial genera displayed notable differences between the ML and control groups, while 31 bacterial genera were significantly different between the MH and control groups. The predominant bacterial taxa in the ML group were *f__Oscillospiraceae* and g__*Colidextribacter*, while *c__Desulfovibrionia* and g__*Dubosiella* were dominant in the MH-F group (Fig. 5A10). At 42 days of age, notable differences were observed in the abundance of 28 and 15 bacterial genera in ML and MH groups compared to controls, respectively. The dominant bacterial groups in the ML group were *c__Alphaproteobacteria* and *c__Actinobacteria*, whereas the predominant taxa in the MH group were *f__Gemmatimonadaceae* and *p__Gemmatimonadota* (Fig.5B10). The findings suggested that matrine modulated the abundance of specific intestinal bacteria.

Effect of matrine on transcriptome analysis of jejunal mucosal tissue: In further analyses, we explored the transcriptome profiles of the 3 groups to compare DEGs. At 21 days, we found 728 DEGs, among which 303 were up-regulated and 425 were down-regulated. By 42 days, 613 DEGs were discovered, comprising 268 upregulated and 345 downregulated genes (Fig. 6A1,A4,B1,B4).

We further performed a Gene Ontology (GO) analysis to categorize and describe all DEGs across a range of biological processes, molecular functions and cellular components. After 21 days, the genes that were up-regulated in the ML group showed significant enrichment in pathways linked to negative regulation of endopeptidase activity, fascia adherens (cardiac cell-cell junctions) and serine-type endopeptidase inhibitor activity. Conversely, the genes that were down-regulated were mainly linked to pathways involved in the processing of antigens and the presentation of peptide antigens through MHC class I, the MHC class I protein complex, and the activity of aspartic-type endopeptidases. Within the MH group, genes that were up-regulated predominantly linked to pathways associated with cytolysis, specific granule binding, and lipopolysaccharide interactions. In contrast, the genes that showed down-regulation were largely concentrated in pathways pertaining to immune responses, MHC class I protein complexes, and chemokine activity. At 42 days, up-regulated ML genes were predominantly associated with pathways that regulate protein hetero-tetramerization, MHC class I protein complex and pyridoxal phosphate binding. In contrast, pathways that govern collagen-activated signaling, collagen receptor activity, and the extracellular region were found to be enriched in the downregulated genes. In the MH group, the upregulation of genes showed significant enrichment in pathways related to the regulation of antigen processing and the presentation of the peptide/MHC class I protein complex, along with activities related to bicarbonate transmembrane transport. Conversely, the genes that were down-regulated were mainly linked to pathways associated with the defense response against Gram-negative bacteria, as well as specific granule functions and the binding of the CCR6 chemokine receptor (Fig. 6 A2, A5, B2, B5).

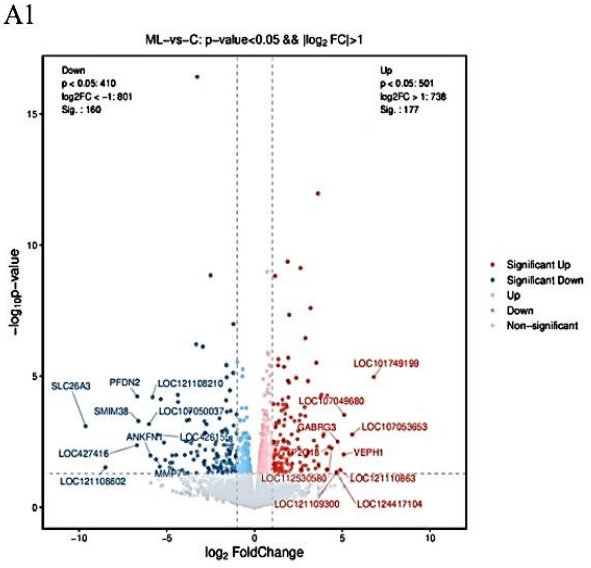
The KEGG pathway enrichment analysis for the DEGs at day 21 identified four KEGG first-level categories for the ML and control groups: Environmental Information Processing, Metabolism, Organismal Systems and Cellular Processes. The pathways with the most significant enrichment included PPAR signaling and neuroactive ligand-receptor interaction, with differential expression observed in a total of five and nine genes, respectively. Conversely, six KEGG first-level categories: Human Diseases, Metabolism, Environmental Information Processing, Cellular Processes, Organismal Systems and Genetic Information Processing were identified for the MH group and control groups. The most significantly enriched pathways for this group were cytokine-cytokine receptor interaction, the Toll-like receptor (TLR) pathway, the NOD-like receptor (NLR) pathway. Within the TLR and NLR pathways, there were six and seven differentially expressed genes, respectively. At 42 days, four KEGG first-level categories, Environmental Information Processing, Metabolism, Organismal Systems and Cellular Processes, were uncovered for the ML group and control groups. The most significantly enriched pathways at this stage included neuroactive ligand-receptor interaction, cytokine-cytokine receptor interaction, drug metabolism by other enzymes and the calcium signaling pathway. Specifically, within the neuroactive ligand-receptor interaction and cytokine-cytokine receptor interaction pathways, there were ten and seven differentially expressed genes, respectively. Five KEGG first-level categories: Human Diseases, Metabolism, Environmental Information Processing, Cellular Processes and Organismal Systems, were identified for MH and control groups. The most significantly enriched pathways in these groups at 42 days of age included Influenza A, nitrogen metabolism, pentose and glucuronate interconversions, linoleic acid metabolism, cell adhesion molecules, the NLR pathway and mucin type O-glycan biosynthesis (Fig. 6 A3,A6,B3,B6).

DISCUSSION

The intestinal villi constitute a unique anatomical feature in broilers that enhances the processes of nutrient digestion and absorption. Nutrients pass into the bloodstream and lymphatic system via the epithelial cells located in these villi. Therefore, the structure of intestinal tissue is a vital marker for assessing intestinal health, injury, and healing(Lang etal,2019). A healthy and intact small intestinal mucosa is vital for normal nutrient absorption. It guarantees efficient processing and uptake of nutrients critical for animal growth and overall health. Metrics such as villus height, crypt depth and the V/C ratio serve as measures for assessing the intestinal mucosa integrity (Liu *et al*., 2020). Research indicates that Sophora seeds have the potential to increase the length of villi and V/C ratio in both the duodenum and jejunum to different extents, while also reducing crypt depth. This implies that Sophora may affect the development of villus structure and the muscular layer of the small intestine, consequently enhancing digestive and absorptive capabilities (Zhao, 2024). Consumption of matrine-containing feed can exacerbate jejunal damage associated with coccidiosis, increase jejunal villus heights and decrease crypt depth

A

A1



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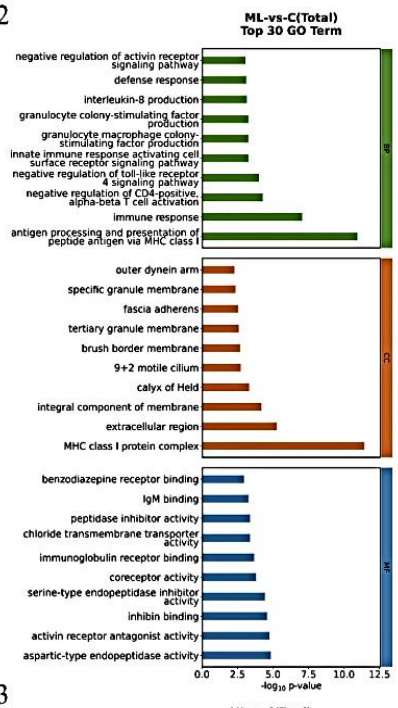

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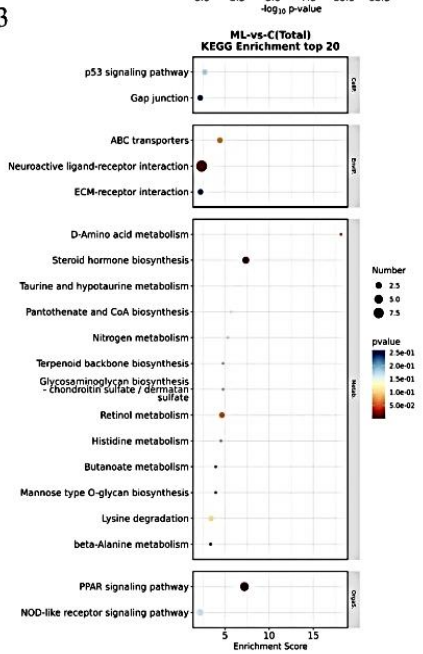

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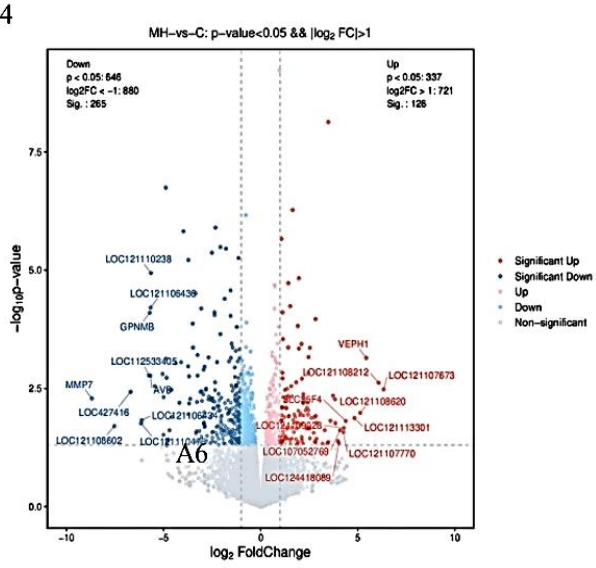

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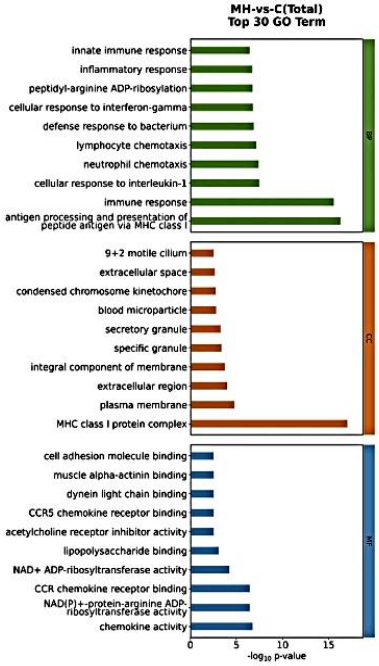

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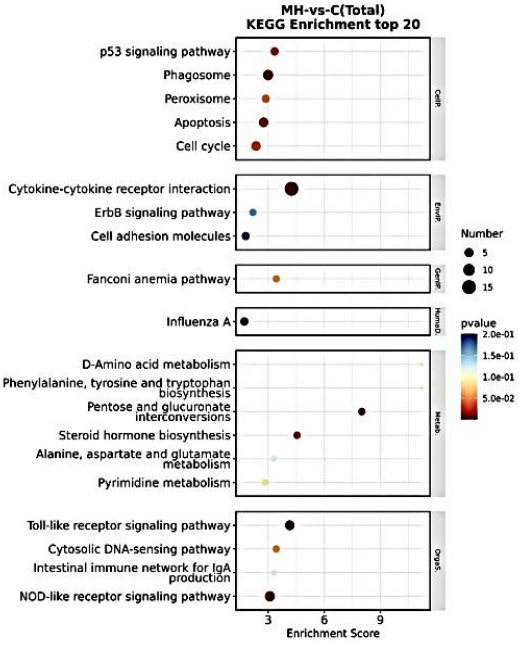

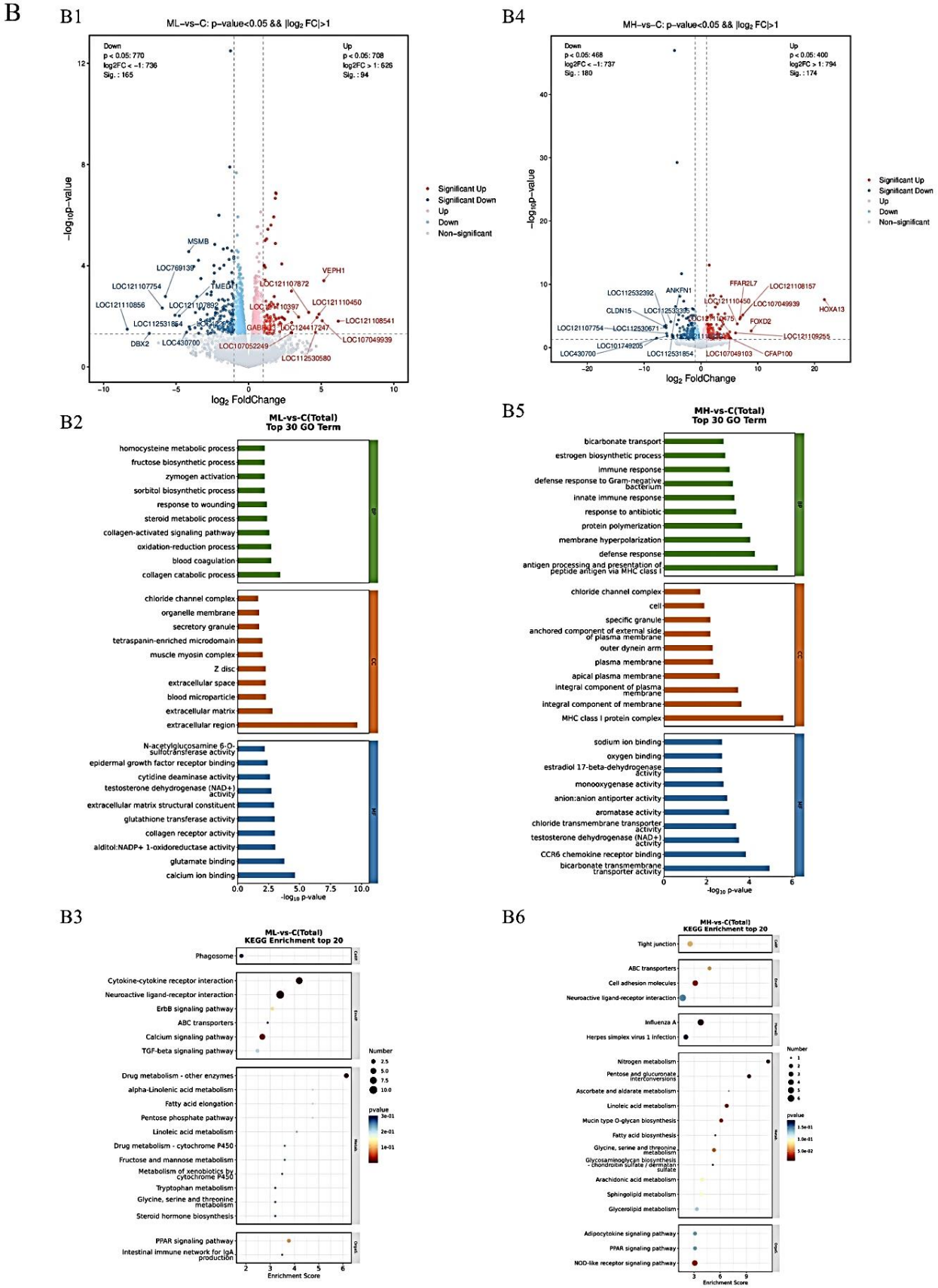

Fig. 6: The effects of matrine on the transcriptome profile of jejunal mucosal tissue. Transcriptomic analysis of jejunal mucosal tissue in (A) 21-day-old and (B) 42-day-old yellow-feathered broilers.ML and control 21-day-old chicken group gene expression analysis (A1)Volcano diagram (A2) GO functional enrichment analysis and (A3) KEGG functional classification. MH and control 21-day-old chicken group gene expression analysis. (A4) Volcano diagram (A5) GO functional enrichment and (A6) KEGG functional classification. ML and control 42-day-old chicken group gene expression analysis (B1) Volcano diagram (B2) GO functional enrichment and (B3) KEGG functional classification. MH and control 42-day-old chicken group gene expression analysis. (B4) Volcano diagram (B5) GO functional enrichment and (B6) KEGG functional classification. n=3.

(Zan *et al*., 2024). In our research, we discovered that adding matrine (200 mg/kg) to the feed of chickens notably enhanced the heights of villi in the duodenum, jejunum, and ileum. However, this supplementation reduced crypt depth and elevated the V/C ratio. Conversely, using 400 mg/kg of matrine caused a marked reduction in duodenal villi height and increased crypt depth within the jejunum and ileum. The findings indicated that a suitable amount of matrine can strengthen the intestinal mucosa's integrity, thereby improving feed digestion and absorption in yellow-feathered broiler chickens.

Goblet cells within the intestinal epithelium are crucial for preserving intestinal health, primarily through the secretion of mucus and the prevention of intestinal damage. Several factors, including age (Birchenough *et al*., 2016) nutritional status(Liu *et al*., 2018), intestinal microbiota (Gāliņa *et al*., 2020) and pharmacological interventions (Wei *et al*., 2019), significantly affect goblet cell quantity and functionality. Goblet cell development and the repair of intestinal tissue in inflammatory conditions, including Clostridium perfringens type A infection (Li *et al*., 2024), coccidiosis, and ulcerative colitis, have been shown to be enhanced by S. flavescens (Yuan *et al*., 2023; Mao *et al*., 2024). Our finding indicated that matrine inclusion in feed raised goblet cell quantity in both the jejunum and ileum at 21 days of age relative to the controls. Furthermore, goblet cell quantity in the jejunum was higher at 42 days of age than the CON group, whereas in the ileum, it was found to be lower. This finding showed that matrine exerted antibacterial effects, which inhibited intestinal inflammation. Except for enhancing goblet cell quantity, matrine treatment elevated tight junction (TJ) protein gene expression, specifically ZO-1 and Occludin. The intestine serves as a natural barrier, ensuring a stable intestinal environment and preventing harmful bacteria and toxins.

Regulating the release of cytokines and chemokines by epithelial cells in response to antigenic triggers is crucial for intestinal barrier integrity and protecting the host from pathogenic threats. This regulatory mechanism is a critical marker of intestinal mucosal immune function. Matrine has been demonstrated to downregulate interleukins (IL), tumor necrosis factor (TNF-α), chemokine macrophage inflammatory protein-3α, antibodies and IL receptors in phagocytes, lymphocytes and damaged tissue cells, thereby exerting anti-inflammatory effects (Hu *et al*., 2012). In vitro investigations revealed that matrine at a concentration of 50 μM (24.8 mg/L) significantly inhibited the expression of IL-6 and TNF-α in colon epithelial cells stimulated by lipopolysaccharide, while a concentration of 100 μM effectively ameliorated the inflammatory damage to these cells (Chen *et al*., 2020). Our findings demonstrated that by day 21, matrine supplementation significantly decreased the relative expression levels of IL-10, IL-1β and TNF-α mRNA in the duodenum, jejunum and ileum compared to the control group. By day 42, matrine continued to downregulate IL-1β mRNA expression in these intestinal regions while upregulating IL-10 mRNA expression. Dietary supplementation of matrine downregulates inflammatory mediator expression within the intestinal tissues of yellow-feather broilers, which in turn alleviates inflammation and enhances overall intestinal function.

The gut microbiota is a critical factor in breaking down and absorbing nutrients. It contributes to the formation of the intestinal epithelial barrier, helps develop and maintain the host immune system, and prevents pathogenic microorganisms from proliferating. This suggests that matrine may enhance the abundance of beneficial flora that regulate cellular differentiation and growth, potentially improving overall gut health and immune function. Additionally, Firmicutes/Bacteroidetes value in the gut affects both growth performance and the digestion of nutrients (Singh *et al*., 2013). In our study, supplementation of feed with matrine increased the Firmicutes/ Bacteroidetes ratio in the cecum, promoting nutrient digestion capacity and the production performance of chickens. Incorporating matrine into the diet reduced the relative abundance of the genera *Alistipes*, *Clostridia*_UCG-014, and *Clostridia*_vadinBB60_group in the cecum of the control group at 21 days old, whereas the relative abundance of *Faecalibacterium* saw an increase. Previously, it was observed that an increase in *Clostridia* species such as *Clostridia*_UCG-014 and *Clostridia*_vadinBB60_group disrupted intestinal microecology in animals, inducing diarrhea (van Kuijk *et al*., 2021). In contrast, *Faecalibacterium*, which produces butyric acid, can exert anti-inflammatory effects and enhance bacterial enzyme activity, conferring protection on the digestive system against intestinal pathogens (Martín *et al*., 2023). Notably, *Colidextribacter* abundance was markedly elevated in matrine-received groups. This bacterium demonstrates anti-inflammatory properties in the intestines, and its prevalence has been linked to improved feed efficiency (Zhu *et al*., 2023). At 42 days, the abundance of *Alistipes* and *Ruminococcus torques* groups was raised in the treated groups. *Alistipes* is a beneficial bacterium belonging to the *Bacteroidetes* phylum involved in breaking down dietary fiber and producing acetic acid. *Ruminococcus* belongs to the *Firmicutes* phylum and produces cellulase and hemicellulase enzymes that break down lignocellulose, resulting in the production of considerable quantities of acetic and butyric acids. In the ML group, there was a notable rise in the population of *Shuttleworthia* found in the cecum of yellow-feathered chickens. This is an intestinal symbiotic microbe that helps maintain intestinal morphology and produces anti-inflammatory volatile fatty acids (Song *et al*., 2023). In our study, the abundance of *Bacteroides* was lower in all groups administered with matrine compared with controls. Berry and colleagues discovered that *Bacteroides* breaks down mucus oligosaccharides. This weakens the intestinal mucosal barrier and results in inflammation (Berry *et al*., 2015). Nevertheless, the addition of matrine resulted in a notable rise in the populations of *Virgibacillus*, *Kroppenstedtia*, and *Morganella* within the cecum. These genera play a role in wine fermentation, suggesting that matrine may promote protein fermentation in the cecum and facilitate nutrient absorption. Consequently, matrine inclusion could promote the intestinal health of chickens through boosting beneficial bacteria presence while decreasing harmful bacteria levels, ultimately leading to better growth performance.

In this study, we utilized jejunal tissue samples for transcriptomic analysis, discovering 728 and 613 DEGs at 21 and 42 days of age, respectively. Additionally, we identified dietary matrine as a factor contributing to these changes. KEGG pathway enrichment analysis of DEGs

revealed distinct signaling pathways, primarily associated with immune response, lipid metabolism and neural conduction. The peroxisome proliferator-activated receptor (PPAR) pathway functions as a nuclear hormone receptor activated by fatty acids and their derivatives. It regulates lipid metabolism, adipogenesis, metabolic homeostasis, and modulates the expression of inflammatory genes (Wagner *et al*., 2020). Research has shown that steroid hormone biosynthesis can influence carbohydrate metabolism and salt balance, thereby altering the metabolism of carbohydrates, lipids, glucose and amino acids (Zhao *et al*., 2024). Moreover, it has been found that matrine treatment can suppress the expression of TLR 2 and inhibit its downstream nuclear factor NF-κB, thereby reducing inflammatory damage (Zhao *et al*., 2022). In inflammatory bowel disease (IBD), matrine can modulate the inflammatory response associated with IBD, via mechanisms involving neuroactive ligand-receptor interactions (Chen *et al*., 2023). Notably, there was an upregulation of MAPK10 gene expression, which may potentiate the antibacterial response via the downstream NF-κB pathway activation. Furthermore, the differential expression of CATH1-3 (antimicrobial peptides) and DEFB4A (β-defensin) suggests that matrine modulates innate immune effector molecules. At 42 days of age, a downregulation of IL-18 and CATH2/3 expression within the NOD-like receptor pathway was observed in the MH group. This reduction, coupled with decreased IL-1β mRNA levels, indicates that matrine may mitigate intestinal inflammation by inhibiting NLRP3 inflammasome activity. Microbiome analysis demonstrated that matrine increased the abundance of Faecalibacterium and Shuttleworthia while decreasing Bacteroides levels. Research indicates that butyrate can improve the expression of proteins responsible for intestinal tight junctions, including Occludin, by activating the GPR109A receptor. This finding aligns with the increased levels of Occludin mRNA noted in the ML group during the later phases of the study. Moreover, the proliferation of Colidextribacter might attenuate the secretion of pro-inflammatory mediators through blocking the TLR4/MyD88 pathway, thereby enhancing the immunomodulatory effects of matrine. Collectively, these results indicate that matrine can enhance intestinal mucosal structure, immune function and intestinal microbiota in yellow-feathered broilers. More research is needed to analyze the regulatory mechanisms.

Conclusions: This research demonstrated that dietary supplementation of matrine significantly enhanced both intestinal conditions and immune functions in yellow-feathered broiler chickens. Incorporating 200 mg/kg of matrine into their feed markedly raised intestinal villus height, reduced crypt depth, and elevated V/C ratios in the duodenum, jejunum, and ileum, thereby enhancing mucosal integrity and the ability to absorb nutrients. Moreover, it enhanced goblet cell number, antioxidant activity (evidenced by elevated SOD levels and reduced MDA content), and modulated immune responses by downregulating IL-1β while upregulating IL-10 expression. Additionally, matrine altered the intestinal microbiota composition through increasing beneficial genera abundance while decreasing the presence of harmful taxa, thereby promoting a balanced microbial environment. Transcriptomic analysis revealed enrichment of pathways involved in immune regulation, lipid metabolism, and neural conduction, further revealing the molecular mechanisms associated with matrine's effects.

Funding: National key research and development program (2022YFA1304104), National natural science foundation of China (U23A20241/32172914), the earmarked fund for CARS-41, Key Research and Development Program Project of Xinjiang Production and Construction Corps (2024AB034).

CRediT Author Contribution Statement: Haigang Wu: Methodology, Data curation, Writing-original draft, Writing-review and editing, Supervision; Haihong Hao: Conceptualization, Supervision, Funding acquisition; Jinni Liu: Data curation, Formal analysis, Writing-original draft, Funding acquisition;

Declaration of competing interest: The authors declare no competing interests.

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