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***Lactobacillus* CNCM I-4622 modulates gut microbiota and restores barrier  
function in *Salmonella*-infected Haidong chickens**

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**Abstract:** The gastrointestinal tract regulates nutrient absorption and immune homeostasis in poultry. *Salmonella* infection severely impairs intestinal barrier function, induces systemic inflammation, and disturbs microbial balance, representing a major challenge for the poultry industry. Excessive antibiotic use further contributes to antimicrobial resistance, increasing the demand for safe alternative strategies. Although *Lactobacillus* confers gut-protective

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properties, its effects against *Salmonella*-induced caecal damage in Haidong chickens remain poorly defined. This study aimed to evaluate the protective effects of dietary supplementation with *Lactobacillus* CNCM I-4622 on caecal function, inflammation, digestive metabolism, and gut microbiota in *Salmonella*-challenged Haidong chickens. A *Salmonella* infection model was established, and birds were allocated to a *Salmonella*-infected group (SIG), a *Lactobacillus* CNCM I-4622-treated infected group (LTG), and a normal control group (NDG). Samples were analysed using biochemical assays, histomorphometry, qRT-PCR, and 16S rRNA sequencing. Compared to the SIG group, *Lactobacillus* CNCM I-4622 significantly increased serum total protein, immunoglobulins A, G and M, and reduced IL-6 levels ( $P < 0.05$ ). It also enhanced caecal amylase, carboxymethyl cellulase, and trypsin activities, restored villus height, mucosal thickness, villus/crypt ratio ( $P < 0.05$ ), and increased the expression of tight-junction and metabolic genes while downregulating proinflammatory-related genes ( $P < 0.05$ ). Moreover, *Lactobacillus* CNCM I-4622 significantly increased caecal microbial  $\alpha$ -diversity and improved microbial community structure and functional pathways ( $P < 0.05$ ). Dietary supplementation with *Lactobacillus* CNCM I-4622 effectively alleviated *Salmonella*-induced caecal damage, suppressed inflammation, improved digestive function, and stabilised microbial homeostasis in Haidong chickens. These results support *Lactobacillus* CNCM I-4622 as a promising alternative to antibiotics for maintaining poultry health under pathogenic stress.

**Keywords:** barrier function, caecum microbiota, Haidong chicken, *Salmonella* colonization, resistance, *Lactobacillus* CNCM I-4622

## Introduction

The poultry gastrointestinal tract plays a central role in nutrient absorption and immune

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regulation and its proper function is essential for maintaining host homeostasis, growth and development. It is continuously exposed to environmental stressors and pathogens, which may significantly increase disease susceptibility (Wickramasuriya et al., 2022). Viral, bacterial, and parasitic pathogens are among the main causes of intestinal diseases in poultry. Diverse microbial communities maintain a dynamic balance within the gastrointestinal ecosystem (Qamar et al., 2021), and their symbiotic interactions with the host are important for immune and metabolic regulation. Gut microbiota facilitate the enzymatic degradation of complex polysaccharides and participate in the regulation of lipid metabolism, bile acid circulation, and amino acid utilisation through metabolite exchange and signalling pathway modulation (Jia et al., 2024). In contrast, microbial dysbiosis, characterised by reduced abundance of barrier-associated genera such as *Bifidobacterium* and *Lactobacillus*, together with overgrowth of pathogenic endotoxin-producing bacteria, may impair intestinal barrier function (Capuano, 2016; Hays et al., 2024). Gut microbial communities also change in response to host maturation and dietary modifications (Onafowokan, 2017). In addition, inflammatory responses can significantly impair nutrient absorption efficiency (Harrell et al., 2021). Collectively, these observations highlight the importance of targeted strategies supporting gut health in poultry production.

*Salmonella* represents a major pathogenic threat in the poultry industry, with infections occurring through the faecal-oral route and primarily colonising the jejunum, ileum, and caecum. *Salmonella* adheres to the intestinal mucosa, invades epithelial cells, and releases virulence factors that disrupt tight junctions and impair gut barrier function (Onafowokan, 2017). Additionally, *Salmonella* may damage internal organs and suppress the development of immune structures such as the thymus and bursa of Fabricius, weakening immune responses

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(Harrell et al., 2021). In infected tissues, activation of the I $\kappa$ B (IkB) kinase complex, extracellular signal-regulated protein kinases 1 and 2 (ERK1/2), and c-Jun N-terminal kinases (JNKs) has been reported to interfere with phosphorylation of tight-junction proteins (Li et al., 2023). Although antibiotics have traditionally been used to prevent *Salmonella* infection in poultry, their appropriate use remains effective for disease prevention (Gut et al., 2018). However, excessive or inappropriate antibiotic application leads to antibiotic resistance development and compromises sustainable poultry production (Yang et al., 2023).

As one of the most important beneficial bacterial groups in the gastrointestinal tract of animals, *Lactobacillus* species provide multiple health benefits, including maintenance of microbial balance, improvement of gut motility, reduction of inflammatory responses, and support of overall intestinal function (Huang et al., 2022). Research has shown that *Lactobacillus* can inhibit the growth of harmful intestinal bacteria and strengthen mucosal immunity, contributing to better intestinal health, feed utilisation, and livestock performance (Iorizzo et al., 2022). Notably, the caecum in poultry contains a more diverse, abundant, and stable microbial community than other intestinal segments due to slower peristalsis and a neutral to mildly acidic pH, conditions that favour microbial diversity (Fan, 2023). The caecal barrier is regarded as the primary defence mechanism against enteric pathogens in poultry. *Salmonella* infection disrupts colonisation resistance mediated by the indigenous microbiota, whereas *Lactobacillus*-associated changes in microbial composition may alter short-chain fatty acid production and affect metabolic activity of the gut epithelium, increasing oxygen and nitrate availability within the mucus layer.

Haidong chicken is a relatively primitive dual-purpose chicken breed native to the Qinghai-Tibet Plateau in China. It has not undergone systematic artificial selection and is well-

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adapted to the ecological conditions of high-altitude, cold agro-pastoral regions (Ding et al., 2020). In the present study, a *Salmonella* infection model was established in Haidong chickens, and caecal tissue morphology, digestive metabolism, inflammatory factor expression, and caecal microbiota composition were systematically evaluated. The study investigated the effects of dietary supplementation with *Lactobacillus* CNCM I-4622 on gut microbial composition and intestinal barrier function during *Salmonella* infection. The findings may contribute to optimisation of feeding strategies and management practices in poultry exposed to pathogenic stress.

## **Material and methods**

### **Ethics statement**

All animal procedures were approved by the Animal Care Committee of Qilu Normal University. Approval for animal slaughter was additionally obtained in accordance with the National Administration of Poultry Slaughtering and Quarantine Regulations (Jinan, China; No. 202308026-11).

### **Animal models and experimental design**

Sixty two-week-old Haidong male chickens were obtained from Chenggui Farming Cooperatives (Xining, China) and acclimatised to the experimental environment under a standard diet for 3 days. *Lactobacillus* CNCM I-4622 was purchased as the BACTOCELL strain (*Pediococcus acidilactici* CNCM I-4622 – MA 18/5M,  $1.0 \times 10^9$  CFU/g) from Canada Lallemand Inc. (Beijing, China). Following acclimatisation, chickens were randomly assigned to three treatment groups as follows: a Normal Diet Group (NDG, negative control), a *Salmonella*-infected group (SIG, positive control; standard diet supplemented with  $1 \times 10^9$  CFU/g *Salmonella* administered by oral gavage) (Shaodi et al., 2024), and

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a *Lactobacillus* CNCM I-4622 treatment group (LTG; SIG diet supplemented with 0.02% *Lactobacillus* CNCM I-4622 at  $1 \times 10^9$  CFU/g). All treatments were administered for 5 weeks. To account for potential solvent effects, the NDG received an equivalent volume of physiological saline via oral gavage. Chickens were maintained according to standard immunisation protocols and had *ad libitum* access to feed and water throughout the experiment. Each group was housed in standard plastic cages (4 cages per group, with 5 birds per cage as the experimental unit) under a 12-h light-dark cycle at a controlled temperature of 20–24 °C and appropriate relative humidity. The NDG was fed commercial layer compound feed (No. 511HM), purchased from Shandong Hemei Group Co., Ltd. (Shandong, China).

### **Sample collection**

Blood samples (5 ml) were collected from the left wing vein of each chicken into heparinised vacutainer tubes and centrifuged at 3 000 rpm for 20 min. The resulting plasma was subsequently separated and stored at –20 °C for further biochemical analysis.

Six chickens were selected from each group, with at least one bird randomly chosen from each cage to ensure balanced sampling across replicates. Birds were humanely euthanized by cervical dislocation followed by arterial exsanguination and transferred on dissecting trays. The abdominal cavity was immediately opened, and the caecum was carefully excised. Caecal digesta samples were collected from individual birds and stored at –80 °C for microbiome sequencing and digestive enzyme activity assays. Following rinsing with phosphate-buffered saline (PBS), portions of the caecal tissue were fixed in 4% neutral buffered formalin for histological analysis, while the remaining tissues samples were rapidly frozen in liquid nitrogen and stored at –80 °C for PCR analysis.

### **Sample measurement**

Total protein (TP), albumin (ALB), globulin (GLB), and total cholesterol (TC) were measured using an SK9600 automatic veterinary haematology analyser (Kanghua Biological

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Medical Co.,Ltd. Shandong, China). Immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), malondialdehyde (MDA), interleukin-2 (IL-2), interleukin-6 (IL-6), and tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) were determined using colorimetric assays with commercially available kits (Solarbio Science & Technology Co., Ltd. Beijing, China).

Caecal tissues were fixed in 4% buffered formaldehyde for 72 h. Following fixation, samples were dehydrated through a graded ethanol series, embedded in frozen medium, and sectioned into serial 5–7  $\mu$ m-thick transverse slices. Tissue sections were stained with haematoxylin and eosin (HE) to assess morphological parameters, including villus height, crypt depth, and mucosal thickness.

The enzymatic activities of amylase (AMS), trypsin (TRS), lipase (LPS), and carboxymethyl cellulase (CLS) were analysed using commercially available assay kits (Takara Biomedical Technology Co., Ltd., Dalian, China) according to the manufacturer's instructions.

RNA extraction from caecal digesta samples, construction of total bacterial RNA library, and microbiome sequencing were performed by Shanghai Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Microbial community profiling was conducted using 16S rRNA gene sequencing on the Illumina NovaSeq/Hiseq Xten platform (Illumina Inc., San Diego, CA, USA). The V3-V4 hypervariable regions of the 16S rRNA gene were amplified using the following universal primers: forward (5'-CCTACGGGNGGCWGCAG-3') and reverse (5'-GGACTACHVGGGTATCTAAT-3'). PCR amplification program consisted of an initial denaturation at 95 °C for 2 min; followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s; with a final extension at 72 °C for 5 min.

Total RNA was extracted from caecal tissues using the One-Step RT-PCR Kit (TaKaRa, China) in accordance with the manufacturer's instructions. Gene expression levels were

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analysed by quantitative real-time PCR (qRT-PCR) using SYBR Green PCR Master Mix (Tiangen, Beijing, China). Expression of target genes was normalised to glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). Specific primers for the target genes were designed using Primer 5.0 software and synthesised by Shanghai Biological Engineering Ltd. (Table 1). PCR reactions were carried out using a LightCycler 480 Real-Time PCR System (Fritz Hoffmann-La Roche Ltd., Basel, Switzerland). Amplification efficiencies of target genes and *GAPDH* were confirmed to be comparable, and relative gene expression levels were calculated using the  $2^{-\Delta\Delta C_t}$  method.

### Statistical analysis

16S rRNA gene sequences were analysed to determine microbial community composition and diversity. Multiplexed sequencing reads were clustered into operational taxonomic units (OTUs) at a 97% sequence similarity threshold according to the UPARSE pipeline. Taxonomic classification of 16S rRNA gene sequences was performed using the RDP Classifier (version 2.2). Sequence processing and analyses were conducted using R software (version 3.1.2), QIIME (version 1.9.1), and UPARSE software. For alpha diversity analysis OTUs were rarified and evaluated using OTU rank abundance curves, rarefaction curves, and diversity indices, including Shannon, Chao1, Simpson, and ACE. Beta diversity was evaluated by principal coordinates analysis (PCoA) and hierarchical clustering using the unweighted pair group method with arithmetic mean (UPGMA) based on weighted UniFrac distances in QIIME. Microbial functional profiles were predicted using PICRUSt. Comparisons of bacterial taxa at the domain, phylum, and genus levels were conducted using the Wilcoxon rank-sum test, with statistical significance defined as a false discovery rate (FDR)-adjusted P value < 0.05.

All experimental data were screened for outliers prior to statistical analyses. Data distributions were visualised using box-and-whisker plots, and normality was assessed using

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the Shapiro-Wilk test. Results are presented as mean  $\pm$  SEM. Statistical analyses were carried out using a randomised complete block design in SAS software (version 19.0; SAS Institute Inc., Cary, NC, USA). Duncan's post hoc test was employed to determine significant differences between groups. Differences were considered significant at  $P < 0.05$  and indicative of a trend at  $P < 0.1$ .

## Results

### Blood physiological and biochemical parameter analysis

Results of blood physiological and biochemical parameters (Tables 2a and 2b) demonstrated that the levels of TP, ALB, GLB, SOD, MDA, TNF- $\alpha$ , IL-2, IL-6, IgA, IgG, and IgM gradually decreased in the SIG, LTG, and NDG groups, while the A/G ratio and TC levels showed an increasing trend. Dietary supplementation with *Lactobacillus* CNCM I-4622 significantly affected TP, IgA, IgG, IgM, and IL-6 levels compared to the SIG group ( $P < 0.05$ ). These findings suggest that incorporating *Lactobacillus* CNCM I-4622 into chicken diets can effectively alleviate disturbances in systemic inflammatory markers induced by *Salmonella* infection and may also exert beneficial effects on antioxidant status.

### Caecal digestive enzyme activity analysis

AMS, LPS, TRS, and CLS activities in the SIG, LTG, and NDG groups are summarised in Table 3. AMS and CLS activities in the NDG group were significantly higher than those in the SIG and LTG groups ( $P < 0.05$ ). In contrast, LPS activity in the SIG group was significantly higher compared to the LTG and NDG groups ( $P < 0.05$ ). TRS activity in both the LTG and NDG groups was significantly higher than in the SIG group ( $P < 0.05$ ); however, no significant differences in LPS or TRS activities were observed between the LTG and NDG groups ( $P > 0.05$ ). Dietary supplementation with *Lactobacillus* CNCM I-4622 increased

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caecal digestive enzyme activity, which could contribute to improved nutrient digestion and absorption. Importantly, TRS and LPS activities in the LTG group reached levels comparable to those observed in the NDG group, suggesting partial restoration of digestive activity following *Lactobacillus* CNCM I-4622 supplementation.

### **Histomorphological analysis of the caecum**

Villous height, crypt depth, mucosal thickness, and villous height-to-crypt depth ratio in the three experimental groups are presented in Table 4. The NDG group showed significantly greater caecal villous height, mucosal thickness, and villous height-to-crypt depth ratio compared to the SIG and LTG groups ( $P < 0.05$ ). In contrast, crypt depth was significantly reduced in both the LTG and NDG groups relative to the SIG group, although no significant differences were detected between the LTG and NDG groups ( $P > 0.05$ ). These findings indicate that dietary supplementation with *Lactobacillus* CNCM I-4622 may alleviate Salmonella-induced alterations in caecal villus and crypt morphology, improve the villous height-to-crypt depth ratio, promote mucosal maturation, and support maintenance of the intestinal mucosal structure.

To evaluate the effects of lactic acid bacteria supplementation on gut barrier function, expression levels of genes associated with tight junctions, inflammation, and digestive metabolism were analysed in the NDG, LTG, and SIG groups (Figures 1a–c). Gene expression analysis by qRT-PCR showed that mRNA levels of inflammatory factor-related genes (*IL-2*, *IL-6*, *TNF- $\alpha$* , *I $\kappa$ B $\alpha$* , *CD86*, and *CASP1*) were significantly lower in the LTG compared to the SIG group. In contrast, expression of tight junction-related genes (*OCN*, *CLDN1*, and *TJPI*) and digestive metabolism-associated genes (*NQO1*, *HMOX1*, and *CAT*) was significantly higher in the LTG. Although *CXCL12* mRNA expression in the LTG did not

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differ significantly from that in the SIG group, a decreasing trend was observed. The abundance of *NF-κB*, *FNI*, *TLR4*, *MyD88*, and *NLRP3* transcripts in caecal tissues from the *Lactobacillus* CNCM I-4622-treated groups were significantly lower than those in the control groups (SIG and NDG). These findings demonstrate that *Lactobacillus* CNCM I-4622 affects expression of genes associated with tight junctions, inflammatory responses, and digestive metabolism, suggesting a protective effect against *Salmonella*-induced disruption of caecal barrier function.

### Caecal microbial community and function

After removal of low-quality reads and chimeric sequences from the Illumina sequencing data, a total of 615 345 high-quality clean tags were obtained. The number of sequence reads per sample among the 18 samples ranged from 48 071 to 83 591. Good's coverage values ranged from 0.9729 to 0.9913, indicating sufficient sequencing depth. High-quality sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity threshold using UPARSE software. A total of 1 348 OTUs were identified and classified into 5 phyla, 41 classes, 99 orders, 169 families, 367 genera, and 632 species (Figure 2). The 10 most abundant microbial families in caecal digesta are presented in Figure 3. *Lactobacillaceae*, *Enterococcaceae*, *Leuconostocaceae*, and *Mycoplasmataceae* were the dominant bacterial families, followed by *Streptococcaceae*, *Pasteurellaceae*, *Bacteroidaceae*, *Clostridiaceae*, *Peptostreptococcaceae*, and *Lachnospiraceae*. In the NDG group, relative abundance of beneficial microorganisms was significantly higher than in other groups ( $P < 0.05$ ), particularly for *Lactobacillaceae*. In contrast, relative abundance of *Enterococcaceae*, *Leuconostocaceae*, and *Streptococcaceae* was significantly lower in the LTG group compared to the SIG group ( $P < 0.05$ ), whereas the population size of *Mycoplasmataceae* and *Pasteurellaceae* was significantly higher ( $P < 0.05$ ). These findings suggest that dietary supplementation with *Lactobacillus* CNCM I-4622 alters the caecal microbial community in

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*Salmonella*-infected Haidong chickens by increasing the abundance of beneficial bacteria and reducing potentially harmful taxa (Figures 4a and b).

Alpha diversity was assessed using the Shannon and Simpson indices together with the Chao1 and ACE richness estimators. Both richness estimators (ACE and Chao1) were higher in the LTG compared to the SIG and NDG groups (Table 5). The Simpson index was significantly lower in the LTG than in the NDG group ( $P < 0.05$ ), while the Shannon index was significantly higher. These outcomes indicate that *Lactobacillus* CNCM I-4622 supplementation increased both richness and diversity of the caecal microbiota. Differences in beta diversity between groups were evaluated using binary Jaccard distances (Figure 5). The SIG and LTG groups showed greater beta-diversity compared to the NDG group, suggesting higher microbial community dispersion and reduced stability. Beta diversity was also higher in the LTG than in the SIG group, indicating that *Lactobacillus* CNCM I-4622 supplementation affected microbial composition in Haidong chicken infected with *Salmonella*. Principal coordinates analysis (PCoA) based on weighted UniFrac distances revealed that PC1 and PC2 explained 67.48 and 16.11% of variation, respectively (Figure 6). Caecal digesta samples from different treatment groups formed distinct and clearly separated clusters in the ordination space.

LefSe analysis was performed to identify taxa showing significance differences in relative abundance (>1%) among groups. Results of linear discriminant analysis (LDA) from LefSe are presented in Figure 7. In the SIG group, *Clostridia*\_UCG-014, *Erysipelotrichaceae*, *Anaerofustaceae*, *Planococcaceae*, and *Pseudomonadaceae* were significantly enriched. In the LTG group, *Oscillospiraceae*, *Veillonellaceae*, and *Atopobiaceae* showed significant enrichment. In the NDG group, *Clostridiaceae* and *Sporomusaceae* were identified as the main taxa showing differential abundance. To explore the functional potential of the caecal digesta microbiota, PICRUSt was employed to predict COG pathway composition (Figure 8).

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The 'Replication, recombination and repair' category was significantly enriched ( $P < 0.05$ ). Additionally, 'Inorganic ion transport and metabolism,' 'Nucleotide transport and metabolism,' 'Defence mechanisms,' and 'Cell cycle control, cell division, and chromosome partitioning' showed trends towards significance ( $P < 0.1$ , Figure 9). These results indicate differences in microbial composition between treatment groups. Functional predictions further suggest that supplementation with *Lactobacillus* CNCM I-4622 may positively affect caecal microbiota function and support intestinal barrier.

## Discussion

The gut microbiota plays an important role in immune regulation, and maintenance of microbial balance is essential for overall health. Healthy poultry production is critical for providing high-quality protein for human nutrition, and proper intestinal structure and function are fundamental for nutrient absorption, immune regulation, and disease resistance. Disruption of intestinal function may negatively affect feed efficiency, production performance, and animal health (Xu et al., 2021). Pathogenic bacteria such as *Salmonella* and *Campylobacter* can infect poultry and may also be transmitted to humans. These pathogens are important reservoirs and vectors of antimicrobial resistance, posing substantial risks to both poultry production and public health. In this context, probiotics, especially *Lactobacillus*, are increasingly investigated as alternatives to antibiotics in poultry production. The present study examined mechanisms through which *Lactobacillus* CNCM I-4622 may alleviate the effects of *Salmonella* infection. Previous research has shown that *Lactobacillus* strains can support growth and development, improve intestinal morphology, and enhance immune maturation (Salam et al., 2021). The current findings additionally demonstrate the anti-inflammatory, antioxidant, and digestive effects associated with *Lactobacillus* CNCM I-4622 supplementation. Treatment significantly reduced expression of pro-inflammatory cytokines, improved antioxidant status, and increased caecal digestive enzyme activity, which may

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contribute to alleviation of *Salmonella*-induced inflammatory damage and improved nutrient absorption. These findings suggest that *Lactobacillus* CNCM I-4622 may help reduce adverse effects associated with *Salmonella* infection, consistent with previous reports showing protective effects of *Lactobacillus* strains against pathogenic infections (Lantz, 2022). The results also provide further information on mechanisms underlying anti-inflammatory, antioxidant and digestion-related effects of lactobacilli and may support their application in poultry disease prevention and health management.

In terms of intestinal barrier repair mechanisms, the findings of this study further support the role of probiotics in regulation of gut health. Probiotics have long been recognised for their role in forming protective barriers by adhering to the intestinal epithelium and stimulating immune system development. These mechanisms are important for nutrient absorption, gut physiology, and maintenance of intestinal function (Hao et al., 2023). Symbiotic interactions between host microbiota and probiotics may limit pathogen colonisation by promoting formation of a protective layer on the intestinal epithelium (Khaneghah et al., 2020). Moreover, probiotics can support development of the host immune system, including the mucus layer and mucosal immune cells such as antibody-producing cells and phagocytic cells. Together, these components form a functional barrier between the host and the external environment, helping to defend against pathogenic microorganisms, including bacteria and viruses (Debnath et al., 2022). Proper structural and functional maturation of intestinal villi is essential for optimal nutrient absorption, and preservation of villus architecture is an important prerequisite for normal intestinal function (Shini & Bryden, 2021). This represents one of the mechanisms through which probiotics improve animal physiological status and gut health. The present study specifically investigated *Salmonella* infection and confirmed that *Lactobacillus* CNCM I-4622 contributed to the restoration of caecal villus length and crypt depth caused by *Salmonella* infection. Supplementation also improved the villus height-to-

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crypt depth ratio and supported maintenance of normal villus morphology. Tight junction proteins and adherens junction proteins are closely associated with epithelial barrier dysfunction. PCR results showed that *Lactobacillus* CNCM I-4622 treatment significantly upregulated the expression of tight junction-related genes such as *OCN*, *CLDN1*, and *TJPI*, while simultaneously reducing serum DAO activity. This suggests that *Lactobacillus* CNCM I-4622 may support mechanical barrier function in the intestine. The present results are consistent with previous studies reporting improved intestinal health in chickens following *Lactobacillus* CNCM I-4622 supplementation through promotion of villus development (Ding et al., 2021).

The gut microbiota is closely associated with host nutrition, metabolism, and immune function. Owing to its major role in regulation of host health and development of the ‘superorganism’ phenotype, it is often referred to as the ‘second genome’ (Chevrette et al., 2022). Maintaining a balanced and stable gut microbiota is essential for normal intestinal function and protection against infections (Kunst et al., 2023). Disruption of microbial balance (dysbiosis) may impair intestinal barrier function, including alterations in villus structure, and reduce nutrient absorption, thereby increasing the risk of bacterial translocation and inflammation (Wang, 2021). The poultry gastrointestinal microbiota is highly complex and includes bacteria, fungi, archaea, protozoa, and viruses, with bacteria representing the dominant microbial group (Rychlik, 2020). The caecum, as the most microbiologically diverse segment, plays an important role in acquiring energy and nutrients, such as vitamins, amino acids, and short-chain fatty acids (SCFAs), which subsequently become available to the host. Disruption of caecal homeostasis may therefore directly contribute to metabolic dysfunction (Huang et al., 2021). The present study found that *Lactobacillus* CNCM I-4622 affects gut

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microbiota composition through selective regulation of microbial populations. *Lactobacillus* CNCM I-4622 supplementation helps maintain microbiota balance by increasing the abundance of beneficial bacteria, including *Bifidobacterium* and *Lactobacillaceae*, while inhibiting the proliferation of potentially pathogenic microorganisms (Hallagan et al., 2020). Supplementation with *Lactobacillus* CNCM I-4622 altered gut microbial composition and reduced the abundance of potential pathogens such as *Salmonella*, *E. coli*, *Pasteurella*, *Clostridiaceae*, *Peptostreptococcaceae*, and *Lachnospiraceae*. These findings suggest that dietary supplementation with *Lactobacillus* CNCM I-4622 may reduce susceptibility to *Salmonella* infection, consistent with previous reports describing its role in limiting pathogen colonisation (Li et al., 2020). Additionally, reduced microbial diversity and abundance are frequently associated with pathological conditions such as diarrhoea and intestinal inflammation. In the current work, *Lactobacillus* CNCM I-4622 significantly increased microbial diversity and abundance in *Salmonella*-infected chickens, consistent with previous findings showing that *Lactobacillus* CNCM I-4622 can restore microbial balance and diversity (Li et al., 2020). Although the dominant bacterial families remained similar between experimental groups, their relative abundance differed substantially. Compared to the SIG group, the *Lactobacillus* CNCM I-4622-supplemented group showed a significant increase in the relative abundance of *Oscillospiraceae*, *Veillonellaceae*, and *Atopobiaceae* ( $P = 0.0096$ ,  $0.0242$ , and  $0.0281$ , respectively). Bacterial groups enriched in the LTG and NDG groups, including *Oscillospiraceae*, *Veillonellaceae*, *Atopobiaceae*, *Lactobacillaceae*, and *Bacteroidaceae*, are generally associated with anti-inflammatory activity and regulation of digestive processes. Consistent with the present findings, Shang et al. (2018) also reported inhibitory effects of *Lactobacillus* CNCM I-4622 against pathogenic bacteria. In summary,

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*Lactobacillus* CNCM I-4622 supplementation significantly promoted gut microbiota balance and was associated with antibacterial, anti-inflammatory, and digestion-related effects.

## **Conclusions**

This study demonstrated that supplementation with *Lactobacillus* CNCM I-4622 in Haidong chickens was associated with multiple beneficial effects. Supplementation alleviated alterations induced by *Salmonella* infection, contributing to restoration of blood physiological and biochemical parameters, caecal function, and intestinal morphology. In addition, *Lactobacillus* CNCM I-4622 supplementation increased the abundance of beneficial bacteria, enhanced cellular defence mechanisms, reduced inflammatory activity and promoted maturation of the intestinal mucus layer, thereby contributing to maintenance of gut health. *Lactobacillus* CNCM I-4622 also altered gut microbiota composition, improved caecal and intestinal barrier functions, increased expression of barrier-associated genes, and supported intestinal immune responses. From a practical farming perspective, these results suggest that dietary supplementation with *Lactobacillus* CNCM I-4622 may represent a useful strategy for poultry production by partially reducing dependence on antibiotics for prevention of *Salmonella* infection. Such an approach may help limit development of antimicrobial resistance while maintaining production performance in Haidong chickens and other local breeds, particularly under high-altitude farming conditions.

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## Conflict of interest

The Authors declare that there is no conflict of interest.

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**Table 1.** The primer sequences of target genes

Gene type	Gene name	Primer sequences (5'→3')
Tight junction	<i>OCN</i>	F:TACCAGGATGTGGCAGAGGA; R:CCATGCGCTTGATGTGGAAG
	<i>CLDN1</i>	F:AGAAGATGCGGATGGCTGTC; R:AAGGGTCATAGAAGGCCCGA
	<i>TJP1</i>	F:AGCCCCTTGTAATGTGTGG; R:TTGGCGTGACGTATAGCTG
Immune factor	<i>IL-2</i>	F: ACCTCACTGCAAGAATGTGGAA; R: GAAGCTGAATGGCGTTGTCT
	<i>IL-6</i>	F:CTGTCACCGCTTCTTCACCT; R:GAACTCCCCATGGCTTTGT
	<i>TNF-α</i>	F:CAGGACAGCCTATGCCAACA; R:AACTCATCTGAACTGGGCGG
	<i>FN1</i>	F: CAATGGGGCAGTTGACGTTG; R: GCCTCGGGAATGGAAAAGGA
	<i>TLR4</i>	F:TGACCTACCCATCGGACACT; R:TGCCTGAGAGAGGTCAGGTT
	<i>MyD88</i>	F:TACTTACGAAGGAAGCAGCAGG; R:GTTCCATGCCCATACGGATCA
	<i>NLRP3</i>	F:CGTGTGGGCGAGTTTCACAG; R:GCCCCTGCTTGATGGAGAA
	<i>CASP1</i>	F:CTCTGACAGCACCTTCCTGG; R:GCATCTCAGGGTCGATGGAG
	<i>NF-κB</i>	F:AAGATCTGGTGGTGTGCCTG; R:AGTGGAACTTTTCGCGGATT
	<i>IκBα</i>	F:CAGCACTACACTTGGCCGTA; R:GGAGTAGCCCTGGTAGGTCA
	<i>CXCL12</i>	F: AACCACGTCATCGCACTCAT; R: TTAGTGGTGTTCACGGGAA
	<i>CD86</i>	F: GCCGCTAGTCATAGAGATACCG; R: AAGCCAGAAGAAGCGTGACC
Digestive metabolism	<i>NQO1</i>	F:GCACCCCGAGAAAACCTCTT; R:GAGATGGTGACTTCCCAGCC
	<i>HMOX1</i>	F:AGTTCAAGCTGGTCACGGC; R:GGTGCAGCTCCATCGGAAAA
	<i>iNOS</i>	F:ATTGTGGAAGGACCGAGCTG; R:CCTCGCACACGGTACTCATT
Reference gene	<i>CAT</i>	F:TCAGGAGATGTGCAGCGTTT; R:TCTTACACAGCCTTTGGCGT
	<i>GAPDH</i>	F:TCGGAGTCAACGGATTTGGC; R: TTCCCCTTCTCAGCCTTGAC

*OCN* – occluding, *CLDN1* – claudin 1, *TJP1* – tight junction protein 1, *IL-2* – interleukin 2, *IL-6* – interleukin 6, *TNF-α* – tumour necrosis factor alpha, *FN1* – fibronectin 1, *TLR4* – toll like receptor 4, *MyD88* – myeloid differentiation primary response gene 88, *NLRP3* – NLR family pyrin domain containing 3, *CASP1* – caspase 1, *NF-κB* – nuclear factor kappa B subunit, *IkBa* – inhibitor of kappaB kinase alpha, *CXCL12* – C-X-C motif chemokine ligand 12, *CD86* – CD86 molecule, *NQO1* – NAD(P)H quinone dehydrogenase 1, *HMOX1* – heme oxygenase 1, *iNOS* – inos orthologs from arthropods, *CAT* – catalase, *GAPDH* – glyceraldehyde-3-phosphate dehydrogenase

**Table 2a.** Blood biochemical parameters analysis

Group	TP, g/l	ALB, g/l	GLB, g/l	A/G	TC, mmol/l
SIG	35.89±1.62 <sup>A</sup>	15.21±1.85	23.76±1.23 <sup>A</sup>	0.64±0.09	3.06±0.72
LTG	31.88±1.17 <sup>B</sup>	14.88±1.93	21.88±1.44 <sup>A</sup>	0.68±0.11	3.22±1.01
NDG	29.56±1.03 <sup>B</sup>	12.53±2.11	17.65±1.56 <sup>B</sup>	0.71±0.13	3.51±0.89

SIG – *Salmonella*-infected group, LTG – *Lactobacillus* CNCM I-4622-treated infected group, NDG – normal control group, TP – total protein, ALB – albumin, GLB – globulin, A/G – albumin-globulin ratio, TC – total cholesterol

**Table 2b.** Blood physiological parameters analysis

Group	GSH-Px, U/ml	SOD, U/ml	MDA, nmol/ml	TNF-α, ng/l	IL-2, ng/l	IL-6, ng/l	IgA, g/l	IgG, g/l	IgM, g/l
SIG	410.52±23.68 <sup>B</sup>	79.11±9.97 <sup>A</sup>	4.85±1.01	56.15±4.63	32.38±8.76	12.86±1.12 <sup>A</sup>	0.545±0.022 <sup>C</sup>	3.166±0.12 <sup>2B</sup>	0.514±0.056 <sup>B</sup>
LTG	378.56±43.96 <sup>B</sup>	65.39±8.51 <sup>A</sup>	3.95±0.83	54.23±7.24	28.55±8.11	10.78±1.06 <sup>B</sup>	0.439±0.034 <sup>B</sup>	2.970±0.21 <sup>3A</sup>	0.498±0.061 <sup>B</sup>
NDG	315.84±35.74 <sup>A</sup>	56.45±5.93 <sup>B</sup>	3.05±0.93	54.19±5.26	27.86±5.32	10.03±0.93 <sup>B</sup>	0.215±0.029 <sup>A</sup>	2.433±0.18 <sup>4A</sup>	0.407±0.045 <sup>A</sup>

SIG – *Salmonella*-infected group, LTG – *Lactobacillus* CNCM I-4622-treated infected group, NDG – normal control group, GSH-Px – glutathione peroxidase, SOD – superoxide dismutase, MDA – malondialdehyde, TNF-α – tumor necrosis factor-α, IL-2 – interleukin-2, IL-6 – interleukin-6, IgA – immunoglobulin A, IgG – immunoglobulin G, IgM – immunoglobulin M;

**Table 3.** Caecal digestive enzyme activity analysis

Group	AMS, U/mg	TRS, U/mg	LPS, U/mg	CLS, U/mg
SIG	6.38±0.11 <sup>A</sup>	506.11±11.56 <sup>A</sup>	0.73±0.02 <sup>A</sup>	0.95±0.03 <sup>A</sup>
LTG	6.56±0.11 <sup>A</sup>	553.89±13.24 <sup>B</sup>	0.69±0.01 <sup>B</sup>	1.12±0.03 <sup>B</sup>
NDG	6.95±0.13 <sup>B</sup>	570.64±12.39 <sup>B</sup>	0.68±0.02 <sup>B</sup>	1.23±0.01 <sup>C</sup>

SIG – *Salmonella*-infected group, LTG – *Lactobacillus* CNCM I-4622-treated infected group, NDG – normal control group, AMS – amylase, TRS – trypsin, LPS – lipase, CLS – carboxymethyl cellulose;

**Table 4.** Histological analysis of caecal morphology

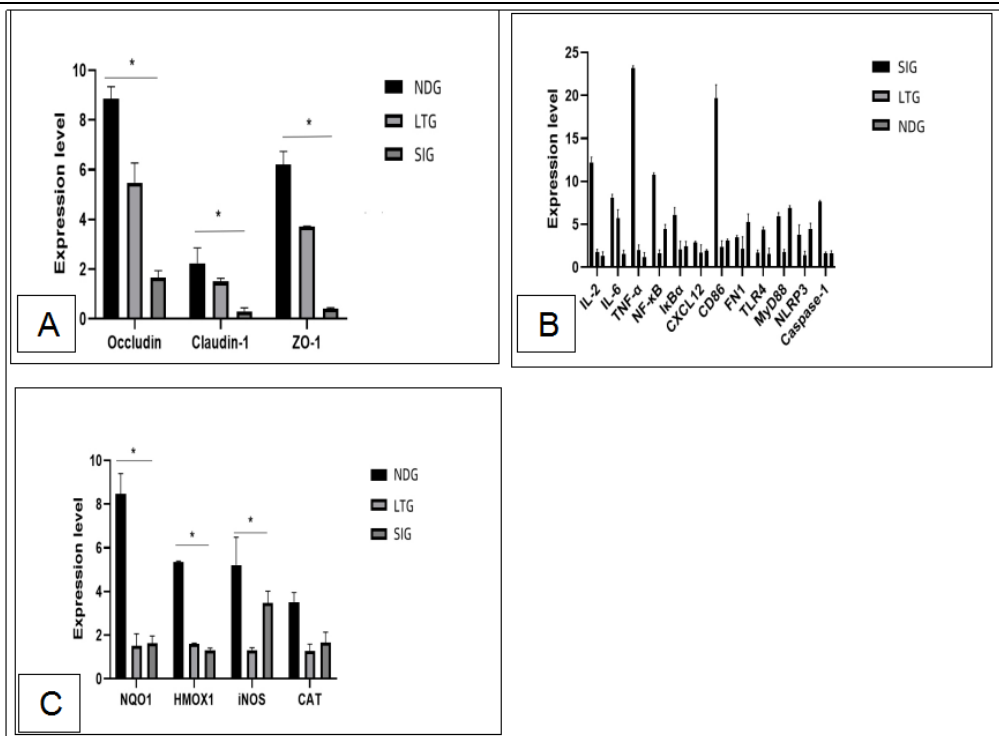
Group	Villus height (VH)	Crypt depth (CD)	Mucosal thickness	VH/CD
SIG	118.73±19.83 <sup>A</sup>	133.43±19.94 <sup>A</sup>	213.54±29.73 <sup>A</sup>	0.89±0.08 <sup>A</sup>
LTG	149.43±21.76 <sup>AB</sup>	92.15±14.08 <sup>B</sup>	259.65±31.24 <sup>A</sup>	1.62±0.11 <sup>B</sup>
NDG	164.92±22.41 <sup>B</sup>	81.36±10.52 <sup>B</sup>	331.26±38.96 <sup>B</sup>	2.03±0.14 <sup>C</sup>

SIG – *Salmonella*-infected group, LTG – *Lactobacillus* CNCM I-4622-treated infected group, NDG – normal control group;

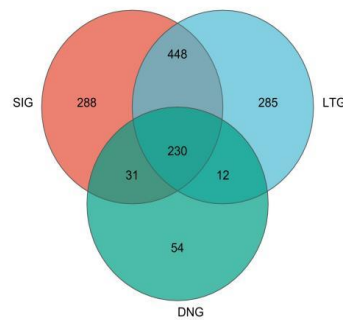
**Table 5.** The alpha diversity of different treatment group

Items	SIG	LTG	NDG
Shannon	2.21±0.39	1.98±0.51	1.34±0.26
Simpson	0.27±0.05 <sup>A</sup>	0.28±0.06 <sup>A</sup>	0.59±0.18 <sup>B</sup>
ACE	84.72±5.69	72.58±4.75	74.87±11.81
Chao1	86.63±7.25	71.14±4.57	76.79±9.76
Coverage, %	99.98	99.99	99.98

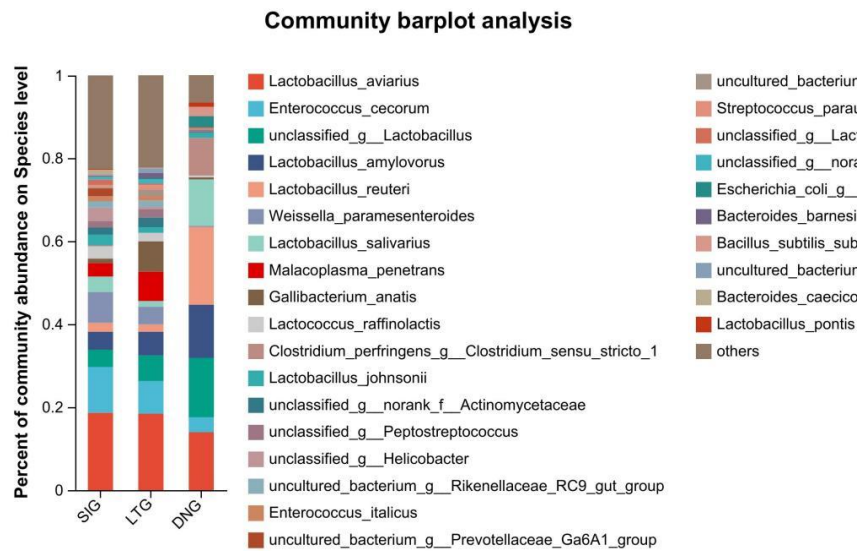
SIG – *Salmonella*-infected group, LTG – *Lactobacillus* CNCM I-4622-treated infected group, NDG – normal control group, ACE – abundance-based coverage estimator, Chao1 – Chao1 richness estimator;



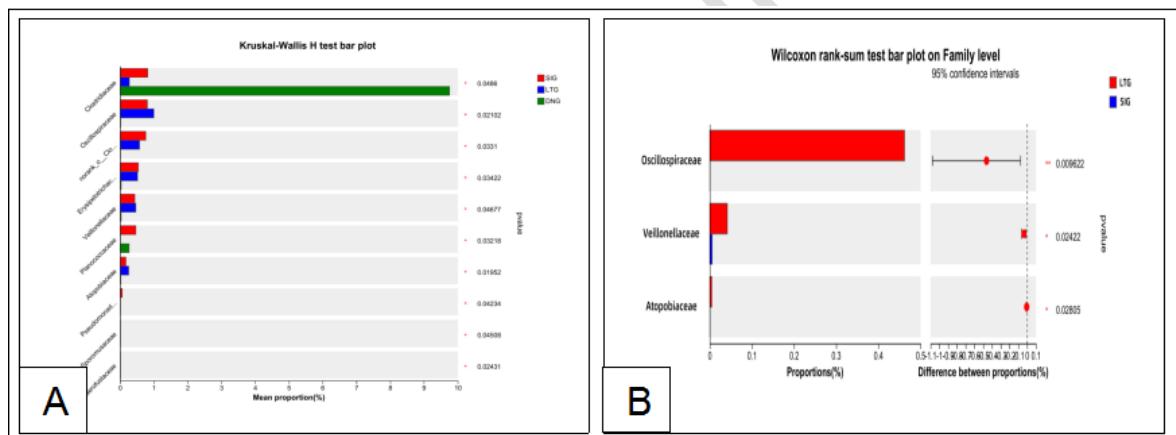
**Figure 1.** Effects of *Lactobacillus* CNCM I-4622 supplemented diets on the target genes in salmonella-infected Haidong chicken caecum. A – tight junction proteins coding genes, B –inflammatory factor proteins coding genes, C – digestive metabolism proteins coding genes  
 SIG – *Salmonella*-infected group, LTG – *Lactobacillus* CNCM I-4622-treated infected group, NDG – normal control group,



**Figure 2.** Caecal digesta samples microbiota venn diagram  
 SIG – *Salmonella*-infected group, LTG – *Lactobacillus* CNCM I-4622-treated infected group, NDG – normal control group

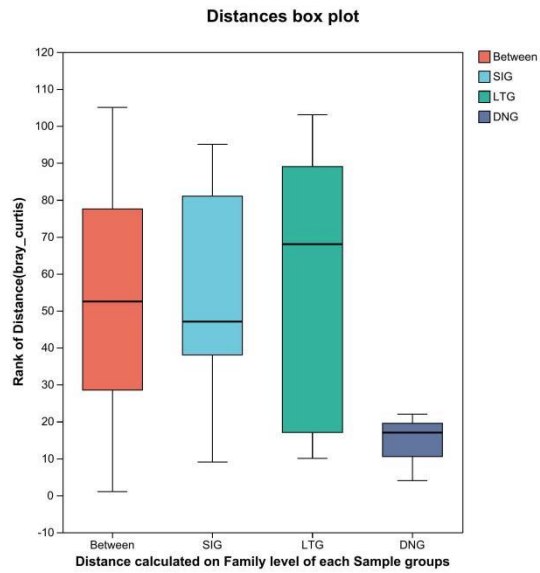


**Figure 3.** Percent of community abundance of microorganism



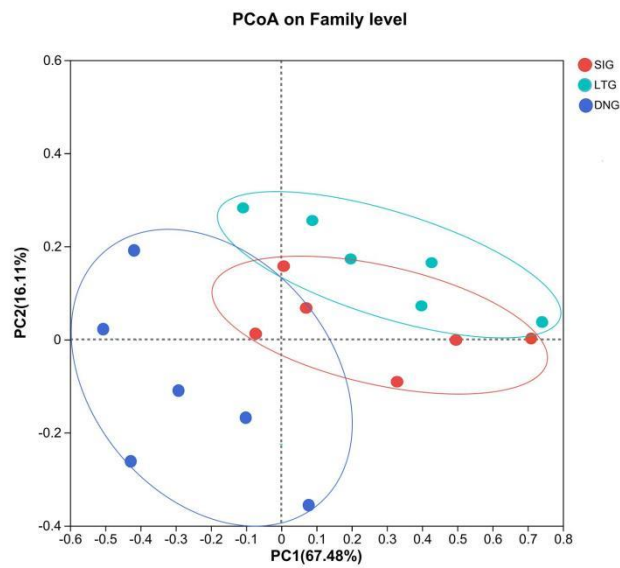
**Figure 4.** The significance analysis of microorganism community abundance. A – significance analysis among SIG, LTG and NDG, B – significance analysis among SIG and LTG.

SIG – *Salmonella*-infected group, LTG – *Lactobacillus* CNCM I-4622-treated infected group, NDG – normal control group



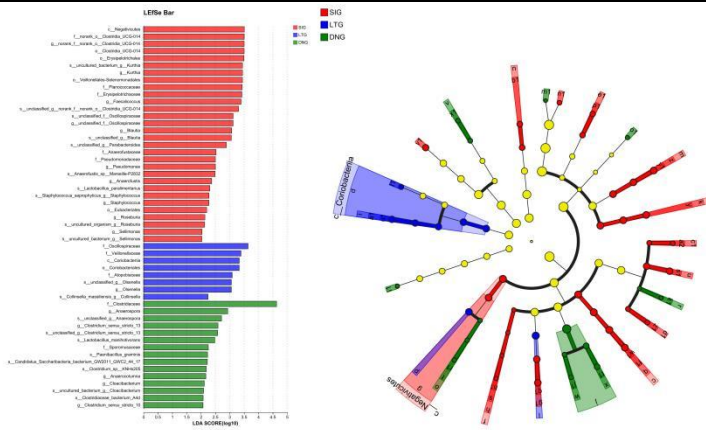
**Figure 5.** Caecal digesta samples microbiota beta-diversity analysis

SIG – *Salmonella*-infected group, LTG – *Lactobacillus* CNCM I-4622-treated infected group, NDG – normal control group



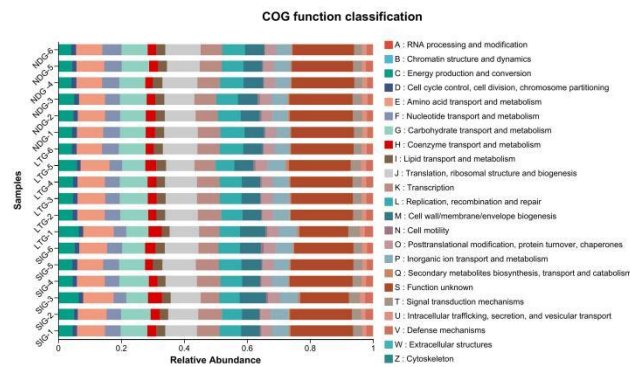
**Figure 6.** Caecal digesta samples microbiota beta-diversity analysis

SIG – *Salmonella*-infected group, LTG – *Lactobacillus* CNCM I-4622-treated infected group, NDG – normal control group

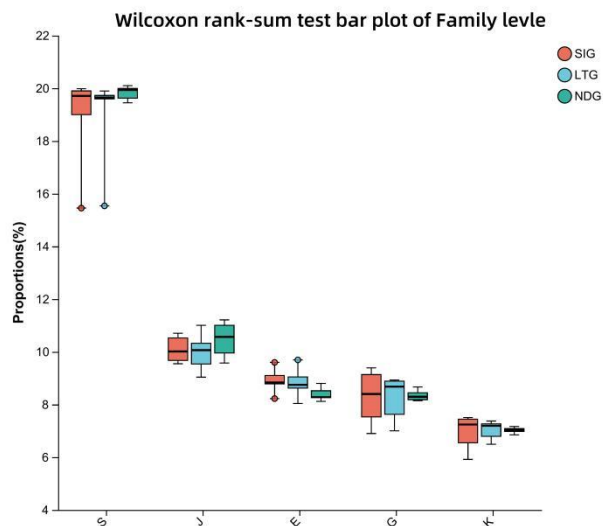


**Figure 7.** The linear discriminant analysis effect size (LEfSe) method identifies the significantly different abundant taxa of bacteria.

SIG – *Salmonella*-infected group, LTG – *Lactobacillus* CNCM I-4622-treated infected group, NDG – normal control group



**Figure 8.** Caecal digesta samples microbiota (COG) analysis



**Figure 9.** The significance analysis of (COG) analysis pathways

SIG – *Salmonella*-infected group, LTG – *Lactobacillus* CNCM I-4622-treated infected group, NDG – normal control group