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# Effects of Qiamagu (Brassica rapa L.) polysaccharide on growth performance, immunity and gut health of yellow-feathered broilers

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## Abstract

This study aimed to investigate the effects of adding Qiamagu polysaccharides to feed on the growth performance, immunity and gut health of yellow-feathered broilers. A total of 240 healthy, day-old, male yellow-feathered broiler chickens with similar body weights were randomly divided into four groups, each with six replicates and 10 chickens per replicate. The groups consisted of a control group (CON) and three treatment groups: TP1 (250 mg/kg), TP2 (500 mg/kg), and TP3 (1000 mg/kg) of Qiamagu polysaccharide in the diet, with a feeding period of 64 days. The average daily weight gain of all experimental groups were significantly higher than those of the CON group ($P < 0.05$) in 1–21 days. The average daily gain of TP1 and TP2 groups was increased ($P < 0.05$), and the ratio of feed to gain was decreased ($P < 0.05$), at 43–63 days. The levels of IgM, IgA, IgG, IL-1β, IL-2 and TNF-α in the serum of broilers in the TP1 and TP2 groups exhibited a consistent increase at 21,42 and 63 day ($P < 0.05$). At 22–42 days, the villus length of duodenum and ileum and the ratio of Villus height to crypt depth ratio were increased in TP2 group ($P < 0.05$). The ileal mucosa IL-1β and IL-2 mRNA levels of broilers in TP3 group were down-regulated in 1–42 days ($P < 0.05$). The mRNA levels of MUC2, ZO-1 and Occluding in ileum mucosa of broilers in TP3 group were up-regulated in 1-63days ($P < 0.05$). The total number of cecal flora species in TP1, TP2 and TP3 groups was higher than that in control group ($P < 0.05$). The relative abundance of Lactobacillus in TP2 group was increased ($P < 0.05$). In conclusion, this study showed that the addition of Qiamagu polysaccharide improved the growth performance, enhanced the immune response and had better impact on intestinal health of yellow-feathered broilers.

**Keywords** Growth performance, Immune function, Qiamagu polysaccharide, Yellow-feathered broiler

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## Introduction

In modern poultry farming systems, broiler chickens are often affected by external factors such as diseases, nutrition, and environmental challenges. Due to their underdeveloped gut function and immature immune systems, broilers experience stunted growth, poor health, and high mortality rates [1, 2]. Additionally, pathogens present in the farming environment can cause diseases, further affecting the health of broilers [3]. In the past, antibiotics were widely used as feed additives to enhance poultry production performance and prevent diseases. However, their improper and unregulated use has led to antibiotic resistance and drug residues [4–5]. To promote sustainable and healthy farming, animal feed and its additives have become a focus of research [6]. Plant polysaccharides are considered safe, with low resistance and relatively few side effects. As such, they are used as feed additives to improve overall performance and health in broilers [7, 8]. Polysaccharides extracted from plants have a positive impact on regulating gut health and the intestinal microbiota of broilers [9, 10].

Qiamagu (Brassica rapa L.), also known as turnip, is a traditional medicinal and edible plant with a long history. It is a folk medicine used to treat cough and asthma. Qiamagu (Brassica rapa L.), provide health-promoting phytochemicals, including glucosinolates, anthocyanins, flavonoids, terpenes, coumarins, and some antioxidants (vitamins C, E, and antioxidant enzymes such as catalase and peroxidase [11]. Current research on Qiamagu polysaccharides mainly focuses on extraction, purification, structural characterization, and biological activity, with limited studies on their effects on poultry growth, immunity, and gut health. Therefore, on the basis of previous studies [12], different dosages of Qiamagu polysaccharide were added to the diets of yellow-feathered broilers to determine the growth performance, serum immune indexes, immune organ indexes, intestinal flora composition and intestinal morphology of yellow-feathered broilers, providing theoretical support for its application as a green feed additive in poultry breeding.

**Table 1** Composition and nutrient levels of the diet (DM basis)

| Item | 1–21 days old | 22–42 days old | 43–63 days old |
|---|---|---|---|
| Ingredients | | | |
| Com | 55.00 | 66.50 | 70.00 |
| Soybean meal | 32.20 | 21.00 | 17.50 |
| Bran | 2.50 | 2.00 | 1.50 |
| Fish meal | 2.00 | 2.00 | 2.00 |
| Soya-bean Oil | 3.30 | 3.50 | 4.00 |
| Premix[1)] | 5.00 | 5.00 | 5.00 |
| Total | 100.00 | 100.00 | 100.00 |
| Nutrient levels[2] | | | |
| ME(MJ/kg) | 12.24 | 12.66 | 12.88 |
| CP(Crude Protein) | 21.54 | 17.68 | 16.40 |
| Ca | 0.88 | 0.84 | 0.83 |
| TP(Total Phosphorus) | 0.80 | 0.75 | 0.73 |
| AP(Available Phosphorus) | 0.40 | 0.39 | 0.39 |
| Lys( Lysine) | 1.12 | 0.85 | 0.76 |
| Met(Methionine) | 0.49 | 0.45 | 0.43 |
| Thr( Threonine) | 0.80 | 0.64 | 0.59 |

## Materials and methods

### Ethical considerations

All broilers are raised and euthanized in strict accordance with the guidelines of the Animal Experiment Ethics Committee of the College of Animal Science and Technology of Shihezi University, approval number: 2023-031.

### Animal and experimental design

Day-old yellow-feathered broilers were purchased from Xinjiang Taikun Group, and Qiamagu polysaccharide (50% purity) was obtained from Shaanxi Hanna Biotechnology Co., Ltd. The experiment was conducted at the experimental station and microbiology laboratory of Shihezi University in Xinjiang. Two hundred and forty (240) healthy one-day-old male yellow-feathered broilers were randomly divided into four treatment groups, with six replicates per group and 10 chickens per replicate. The CON group served as the control group, fed only a basal diet, while the TP1, TP2, and TP3 groups were the experimental groups, with 250, 500, and 1000 mg/kg of Qiamagu polysaccharide added to the basal diet, respectively. The feeding period lasted 63 days, during which the broilers were housed in three-layered cages (1.4 m×0.7 m×1.6 m), with free access to feed and water. House temperature was maintained around 32 °C during the first 4 days and was gradually reduced to 18 °C until the end of the experiment [13]. Incandescent light was continuously (24 h) provided during the first 10 days of age, after which 9 h lighting and 15 h darkness was followed. Immunization and disinfection followed standard farm procedures. The formulated diets and related nutritional indicators refer to the United States NRC (1994) Nutritional requirements for poultry are shown in Table 1.

Per kg of feed, the premix provides: VitA 180,000 IU; VitD 70,000 IU; VitE 450 IU; VitK 30 mg; VitB 70 mg; niacin 600 mg; calcium pantothenate 260 mg; biotin 1.7 mg; folic acid 17 mg; Fe 10,000 mg; Cu 350 mg; Mn 1500 mg; Zn 2000 mg; Ca 14 mg; P 6 mg; sodium chloride 7 mg; and methionine 3 mg.

### Fluency indices

#### *Growth performance*

At the start of the experiment, the initial weight of one-day-old chicks in each replicate group was measured. The

body weight of each bird in the replicate group was precisely recorded at 10:00 AM on days 21, 42, and 63.

Daily feed intake for each replicate group was recorded, and the average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio (F/G) of yellow-feathered broilers were calculated.

#### *Serum biochemical indices*

On days 21, 42, and 63 of the experiment, three chicken was randomly selected for each replicate for wing vein blood collection. The blood was allowed to clot at room temperature for 40 min, then centrifuged at 3000 g for 10 min at 4 °C [14]. The levels of total protein (TP), albumin (ALB), globulin (GLB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and glucose (GLU) in the serum of yellow-feathered broilers were measured using an AU480 automatic biochemical analyzer.

#### *Serum immune index*

On days 21, 42, and 63 of the experiment, three chicken was randomly selected for each replicate for wing vein blood collection. The blood was allowed to clot at room temperature for 40 min, then centrifuged at 3000 g for 10 min at 4 °C. The serum supernatant was stored at -20 °C. ELISA (Nanjing Jiancheng Biotechnology Co., LTD) kits were used to measure immunoglobulins (IgA, IgG, IgM) and cytokines (IL-1β, IL-2, TNF-α) in the serum. All procedures were strictly performed according to the kit instructions.

#### *Determination of immune organ index*

After blood collection, each sample was weighed, and the animals were euthanized by cervical dislocation. The thymus, spleen, and bursa of Fabricius were then weighed.

#### *Analysis of intestinal tissue morphology*

On days 21, 42, and 63,three chicken were randomly selected for each replicate for wing vein blood collection. Blood was collected via jugular venipuncture, followed by slaughter. Duodenum, jejunum, and ileum tissue samples, each 5 cm long, were collected, washed with saline, and fixed in 4% paraformaldehyde. Tissue samples were processed into paraffin sections and stained with hematoxylin and eosin (H & E) [15]. For each sample, ten villus lengths and ten crypt depths were measured, and the villus height-to-crypt depth ratio (V/C) was calculated.

**Table 2** qPCR amplification parameters

| Step | Temperature | Time |
|---|---|---|
| Step 1. Predegeneration | 95℃ | 30 S |
| Step 2. Circular reaction | 95℃ | 10Sx40cycles |
| | 60℃ | 30Sx40cycles |
| Step 3. Solubility curve | 95℃ | 15 S |
| | 60℃ | 60 S |
| | 60℃ | 60 S |

#### *Determination of intestinal flora structure*

At the 63rd day, three yellow-feathered broilers were randomly selected from each replicate,. The selected yellow-feathered broilers were euthanized, dissected, and their abdominal organs were isolated. The cecum was removed, opened, and its contents were extracted, flash-frozen in liquid nitrogen, and stored at -80 °C. 16 S rDNA sequencing was outsourced to Shanghai Majorbio Bio-pharm Technology Co. Ltd. for analysis. The cecal content samples were sent to Shanghai Majorbio Biopharm Technology Co., Ltd. for analysis. Total DNA was extracted from the samples, and the extracted genomic DNA was analyzed using 1% agarose gel electrophoresis. PCR amplification was performed using universal primers 338 F and 806R.

#### *Relative mRNA expression of ileum mucosa gene*

During the slaughter tests conducted on days 21, 42, and 63, the ileum mucosa was scraped using clean slides and placed in cryotubes. Total RNA from various tissues was extracted using the Trizol reagent kit (Nanjing Jiancheng Biotechnology Co., LTD). The purity and concentration of the total RNA were measured using a spectrophotometer (Beijing Puyang General Instrument Co., LTD). The total RNA was reverse-transcribed into cDNA using a reverse transcription kit (Nanjing Jiancheng Biotechnology Co., LTD China) and stored at -80 °C for future real-time quantitative PCRanalysis. BioGold SYBR qPCR Master Mix (REF: VQ881-02) kit was used for real-time fluorescence quantitative PCR (RT-qPCR) detection on a PCR machine (Thermo Field). The steps for real-time quantitative PCR are listed in Table 2. In this experiment, β-actin was used as the reference gene, and relative gene quantification was performed using the $2-\Delta\Delta CT$ method. The sequences of the relevant gene primers are shown in Table 3.

### Statistical analysis

A one-way ANOVA was performed on the experimental data using SPSS 20.0 software [16]. The data were repeated three times, and the average value was taken. Multiple comparisons between groups were conducted using the LSD method. Results are expressed as mean ± standard error of the mean (SEM), and a *P*-value of less than 0.05 was considered statistically significant.

## Results

### Effects of Qiamagu polysaccharide on growth performance of yellow-feathered broilers

As shown in Table 4, the average daily weight gain of all experimental groups were significantly higher than

**Table 3** Primers sequence of target gene and reference gene

| Gene name | Primer sequence(5, ~ 3, ) |
|---|---|
| TNF-α | F: GGCAATGAACCCTCCCCAGTA<br>R:GGTTACAGGAAGGGCAACTCATC |
| IL-1β | F: CAGCCTCAGCGAAGAGACCTT<br>R: ACTGTGGTGTGCTCAGAATCC |
| IL-2 | F: TGCTTTTAACCGTCTTTG<br>R: GATGCTCCATAAGCTGTAGT |
| Occludin | F: ACGGCAGCACCTACCTCAA<br>R: GGGCGAAGAAGGAGATGAG |
| MUC2 | F: TTCATGATGCCTGCTCTTGTG<br>R: CCTGAGCCTTGGTACATTCTTGT |
| ZO-1 | F: GGGATGTTTATTTGGGCGGC<br>R: TCACCGTGTGTTGTTCCCAT |
| β-Actin | F: GAGAAATTGTGCGTGACATCA<br>R: CCTGAACCTCTCATTGCCA |

those of the CON group ($P<0.05$) in 1–21 days. The average daily gain of TP1 and TP2 groups was increased ($P<0.05$), and the ratio of feed to gain was decreased ($P<0.05$), at 43–63 days.

### Effects of Qiamagu polysaccharide on serum biochemical indices of yellow-feathered broilers

The results regarding serum biochemical indices has been presented in Table 5. The AST and ALT in TP2 group were significantly reduced ($P<0.05$) at day 63.

### Effects of Qiamagu polysaccharide on serum immune indices of yellow-feathered broilers

As shown in Table 6, Compared with CON group, serum IL-1β and IgG concentrations in TP1, TP2 and TP3 groups were significantly increased at 21 and 42 days of age; Serum levels of IL-2, IgA, IgM and TNF-α in TP1 and TP2 broilers were significantly increased. In 63d, serum IL-1β, IL-2, IgA and IgGTNF-α of broilers in TP1, TP2 and TP3 groups were significantly higher than those in control group; Serum IgM of broilers in TP1 and TP2 groups was significantly increased.

### Effects of Qiamagu polysaccharide on immune organ index of yellow-feathered broilers

The impact of feeding varying doses of Qiamagu polysaccharides on the immune organ index of yellow-feathered broilers is presented in Table 7. 1–21 days, the spleen relative weight of broilers in the TP1and TP2 group was higher ($P<0.05$) than CON group. 22–42 days, the bursa of Fabricius indices of broilers in the TP1 group were higher than those in the CON group ($P<0.05$). 43 -63days, the thymus index of broilers in the TP2 group and the bursa of Fabricius index in the TP1and TP2 groups was higher than that of the CON group ($P<0.05$).

### Effects of Qiamagu polysaccharide on intestinal villus length and crypt depth of yellow-feathered broilers

Figure 1 shows that the addition of Qiamagu polysaccharides in the feed increased the villus length (V) and crypt depth (C) of the small intestine. As can be seen from Table 8, compared with the control group, the addition of different levels of Qiamagu polysaccharide can improve the intestinal villus length and crypt depth of duodenum, jejunum and ileum of yellow feathered broilers at different growth stages.

### Effects of Qiamagu polysaccharide on cecal intestinal flora of yellow-feathered broilers

#### *OTUs( operational taxonomic units) analysis of cecum microbial community*

As shown in Fig. 2, a total of 669 microbial OTUs were identified in the cecal contents of Yellow-feathered broilers across the CON, TP1, TP2, and TP3 groups. Specifically, 326 OTUs were unique to the CON group, 427 to

**Table 4** Effects of Qiamagu polysaccharides on growth performance of yellow-feathered broilers (Mean ± SEM)

| Item | Control group | TP1 group | TP2 group | TP3 group | *P*-value |
|---|---|---|---|---|---|
| 1–21 days old | | | | | |
| ADFI/g | 33.90 ± 2.23 | 35.76 ± 2.50 | 34.86 ± 1.83 | 34.43 ± 1.98 | 0.162 |
| ADG/g | 23.59 ± 1.49$^{b}$ | 28.47 ± 2.03$^{a}$ | 29.26 ± 1.71$^{a}$ | 26.91 ± 1.71$^{a}$ | 0.013 |
| FCR | 1.44 ± 0.19 | 1.25 ± 0.14 | 1.19 ± 0.02 | 1.28 ± 0.11 | 0.274 |
| 22–42 days old | | | | | |
| ADFI/g | 90.87 ± 1.22 | 92.72 ± 1.52 | 91.62 ± 1.87 | 92.50 ± 2.66 | 0.489 |
| ADG/g | 36.19 ± 4.59 | 40.90 ± 7.07 | 37.06 ± 4.86 | 41.58 ± 7.34 | 0.637 |
| F/G | 2.51 ± 0.27 | 2.26 ± 0.31 | 2.47 ± 0.40 | 2.22 ± 0.18 | 0.279 |
| 43–63 days old | | | | | |
| ADFI/g | 143.54 ± 1.22 | 143.45 ± 3.39 | 141.32 ± 3.52 | 140.32 ± 1.51 | 0.361 |
| ADG/g | 49.04 ± 6.92$^{b}$ | 61.50 ± 3.70$^{a}$ | 62.14 ± 8.14$^{a}$ | 49.52 ± 5.70$^{b}$ | 0.029 |
| F/G | 2.92 ± 0.58$^{a}$ | 2.33 ± 0.23$^{b}$ | 2.57 ± 0.62$^{b}$ | 2.83 ± 0.53$^{ab}$ | 0.025 |

Note: CON: Control group; TP1: basal feed + 250 mg/kg Qiamagu polysaccharides; TP2: basal feed + 250 mg/kg Qiamagu polysaccharides; TP3:basal feed + 1000 mg/kg Qiamagu polysaccharides; ADFI: Average daily feed intake; ADG: Average daily gain; F/G: Feed conversion ratio;

Different lowercase superscripts in the same row indicate significant differences ($P<0.05$), while identical superscripts or no superscripts indicate no significant difference ($P>0.05$)

**Table 5** Effects of Qiamagu polysaccharide on serum biochemical indices of yellow-feathered broilers

| Item | Control group | TP1 group | TP2 group | TP3 group | *P*-value |
|---|---|---|---|---|---|
| 21 days old | | | | | |
| TP(g/L) | 22.20 ± 2.50 | 26.35 ± 0.45 | 24.90 ± 2.20 | 24.55 ± 1.35 | 0.841 |
| ALB(g/L) | 8.60 ± 1.60 | 11.00 ± 0.40 | 10.00 ± 1.20 | 10.05 ± 0.55 | 0.256 |
| GLB(g/L) | 13.60 ± 0.90 | 15.35 ± 0.05 | 14.90 ± 1.00 | 14.50 ± 0.80 | 0.379 |
| AST(U/L) | 267.50 ± 9.00 | 242.00 ± 15.00 | 217.50 ± 8.65 | 236.00 ± 7.5 | 0.865 |
| ALT(U/L) | 7.00 ± 2.00 | 5.00 ± 1.00 | 6.00 ± 2.50 | 6.50 ± 2.23 | 0.914 |
| GLU(mmol/L) | 10.15 ± 1.15 | 11.35 ± 0.65 | 10.70 ± 0.40 | 11.85 ± 0.65 | 0.718 |
| 42 days old | | | | | |
| TP(g/L) | 24.33 ± 0.55 | 28.40 ± 1.21 | 25.37 ± 3.55 | 22.43 ± 4.08 | 0.492 |
| ALB(g/L) | 8.77 ± 0.68 | 10.23 ± 0.20 | 7.90 ± 1.38 | 8.63 ± 1.88 | 0.887 |
| GLB(g/L) | 15.57 ± 0.20 | 18.17 ± 1.01 | 17.47 ± 2.18 | 13.80 ± 2.20 | 0.928 |
| AST(U/L) | 182.00 ± 3.51 | 171.00 ± 4.58 | 176.00 ± 25.94 | 179.67 ± 26.43 | 0.647 |
| ALT(U/L) | 5.00 ± 0.57 | 4.33 ± 0.33 | 3.67 ± 0.33 | 4.00 ± 0.57 | 0.976 |
| GLU(mmol/L) | 8.13 ± 1.27 | 8.63 ± 0.43 | 8.33 ± 1.33 | 8.13 ± 1.41 | 0.394 |
| 63days old | | | | | |
| TP(g/L) | 30.43 ± 3.15 | 34.00 ± 2.65 | 32.03 ± 3.27 | 31.40 ± 2.93 | 0.408 |
| ALB(g/L) | 10.70 ± 1.01 | 11.80 ± 1.37 | 11.73 ± 1.37 | 11.18 ± 0.83 | 0.217 |
| GLB(g/L) | 19.7 ± 2.13 | 22.43 ± 1.59 | 20.30 ± 1.95 | 20.23 ± 0.41 | 0.710 |
| AST(U/L) | $220.33 \pm 16.91^{a}$ | $208.50 \pm 8.08^{ab}$ | $175.67 \pm 4.91^{b}$ | $190.50 \pm 2.84^{ab}$ | 0.013 |
| ALT(U/L) | $4.00 \pm 0.33^{a}$ | $2.67 \pm 0.332^{ab}$ | $2.33 \pm 0.66^{b}$ | $3.50 \pm 0.33^{ab}$ | 0.025 |
| GLU(mmol/L) | 10.96 ± 0.13 | 11.93 ± 0.80 | 11.80 ± 0.15 | 11.63 ± 0.53 | 0.091 |

Note: CON: Control group; TP1: basal feed + 250 mg/kg Qiamagu polysaccharides; TP2: basal feed + 250 mg/kg Qiamagu polysaccharides; TP3:basal feed + 1000 mg/kg Qiamagu polysaccharides; TP: Total Phosphorus. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALB: Albumin; GLB: Globulin; GLU: Glucose

**Table 6** Effects of Qiamagu polysaccharide on serum immune indices of yellow-feathered broilers

| Item | CON group | TP1 group | TP2 group | TP3 group | *P*-value |
|---|---|---|---|---|---|
| 21 days old | | | | | |
| IL-1β(pg/mL) | $84.38 \pm 2.00^{c}$ | $164.78 \pm 4.73^{a}$ | $132.28 \pm 4.32^{b}$ | $114.96 \pm 5.78^{b}$ | 0.025 |
| IL-2(pg/mL) | $269.55 \pm 13.32^{c}$ | $442.54 \pm 11.57^{a}$ | $364.93 \pm 16.15^{b}$ | $319.11 \pm 10.68^{bc}$ | 0.038 |
| IgA(μg/mL) | $222.01 \pm 16.81^{b}$ | $369.13 \pm 10.84^{a}$ | $326.38 \pm 13.37^{a}$ | $259.36 \pm 6.50^{b}$ | 0.021 |
| IgG(μg/mL) | $1653.41 \pm 127.33^{c}$ | $3069.64 \pm 50.89^{a}$ | $2725.20 \pm 70.09^{a}$ | $2221.85 \pm 105.84^{b}$ | 0.032 |
| IgM(μg/mL) | $513.75 \pm 43.58^{c}$ | $1002.64 \pm 40.45^{a}$ | $842.96 \pm 43.21^{ab}$ | $681.03 \pm 46.61^{bc}$ | 0.040 |
| TNF-α(pg/mL) | $59.61 \pm 3.21^{c}$ | $97.92 \pm 2.68^{a}$ | $84.78 \pm 2.24^{b}$ | $69.40 \pm 1.98^{c}$ | 0.027 |
| 42 days old | | | | | |
| IL-1β(pg/mL) | $86.13 \pm 7.02^{c}$ | $157.65 \pm 5.29^{a}$ | $126.45 \pm 3.61^{b}$ | $115.25 \pm 4.56^{b}$ | 0.033 |
| IL-2(pg/mL) | $280.32 \pm 17.32^{b}$ | $456.35 \pm 18.60^{a}$ | $400.36 \pm 16.87^{a}$ | $313.34 \pm 7.21^{b}$ | 0.020 |
| IgA(μg/mL) | $261.85 \pm 4.56^{c}$ | $391.25 \pm 11.60^{a}$ | $327.09 \pm 6.01^{b}$ | $294.14 \pm 8.69^{bc}$ | 0.028 |
| IgG(μg/mL) | $1633.26 \pm 113.05^{d}$ | $3068.53 \pm 103.24^{a}$ | $2542.20 \pm 99.99^{b}$ | $2081.29 \pm 90.58^{c}$ | 0.019 |
| IgM(μg/mL) | $561.73 \pm 29.62^{b}$ | $958.16 \pm 18.05^{a}$ | $876.95 \pm 33.28^{a}$ | $670.90 \pm 15.53^{b}$ | 0.039 |
| TNF-α(pg/mL) | $57.30 \pm 3.99^{b}$ | $96.56 \pm 2.41^{a}$ | $87.35 \pm 3.76^{a}$ | $64.32 \pm 1.15^{b}$ | 0.041 |
| 63 days old | | | | | |
| IL-1β(pg/mL) | $83.68 \pm 8.04^{c}$ | $158.29 \pm 4.76^{a}$ | $136.36 \pm 2.64^{ab}$ | $114.45 \pm 6.35^{b}$ | 0.034 |
| IL-2(pg/mL) | $276.54 \pm 19.21^{c}$ | $460.55 \pm 15.66^{a}$ | $395.11 \pm 10.02^{b}$ | $295.67 \pm 7.19^{ab}$ | 0.042 |
| IgA(μg/mL) | $226.35 \pm 14.81^{c}$ | $391.61 \pm 7.92^{a}$ | $335.31 \pm 14.10^{b}$ | $298.65 \pm 8.12^{b}$ | 0.037 |
| IgG(μg/mL) | $1679.93 \pm 146.18^{c}$ | $3101.71 \pm 90.11^{a}$ | $2733.33 \pm 61.63^{a}$ | $2262.21 \pm 56.78^{b}$ | 0.042 |
| IgM(μg/mL) | $560.61 \pm 26.79^{b}$ | $953.67 \pm 19.24^{a}$ | $861.73 \pm 15.98^{a}$ | $667.11 \pm 39.27^{b}$ | 0.033 |
| TNF-α(pg/mL) | $53.60 \pm 2.19^{c}$ | $98.67 \pm 3.64^{a}$ | $84.52 \pm 3.87^{b}$ | $70.97 \pm 3.31^{b}$ | 0.029 |

Note: CON: Control group; TP1: basal feed + 250 mg/kg Qiamagu polysaccharides; TP2: basal feed + 250 mg/kg Qiamagu polysaccharides; TP3: basal feed + 1000 mg/kg Qiamagu polysaccharides; IgA: Immunoglobulin A; IgG: Immunoglobulin G; IgM: Immunoglobulin M; IL-1β:Interleukin-1β; IL-2: Interleukin-2; TP: Total protein; TNF-α: Tumor Necrosis Factor-α. Results are presented as mean ± standard error of the mean (SEM). Different lowercase superscripts in the same row indicate significant differences ($P < 0.05$), while identical superscripts or no superscripts indicate no significant difference ($P > 0.05$)

**Table 7** Effects of Qiamagu polysaccharides on the immune organ index of yellow feathered broilers

| Item | Control group | TP1 group | TP2 group | TP3 group | *P*-value |
|---|---|---|---|---|---|
| 21 days old | | | | | |
| Thymus index(%) | 2.46 ± 1.66 | 3.68 ± 1.24 | 2.27 ± 1.06 | 3.46 ± 0.57 | 0.412 |
| Spleen index(%) | 0.96 ± 0.24$^{b}$ | 2.07 ± 0.46$^{a}$ | 1.26 ± 0.46$^{a}$ | 1.48 ± 0.49$^{ab}$ | 0.031 |
| Bursa index(%) | 2.68 ± 0.94 | 3.46 ± 0.95 | 3.50 ± 0.75 | 2.78 ± 0.33 | 0.292 |
| 42 days old | | | | | |
| Thymus index(%) | 4.21 ± 1.87 | 5.03 ± 1.48 | 5.59 ± 1.87 | 5.18 ± 2.00 | 0.703 |
| Spleen index(%) | 1.47 ± 0.31 | 1.72 ± 0.67 | 1.67 ± 1.08 | 1.49 ± 0.37 | 0.525 |
| Bursa index(%) | 1.52 ± 0.58$^{b}$ | 2.98 ± 1.58$^{a}$ | 1.92 ± 0.79$^{ab}$ | 2.24 ± 0.59$^{ab}$ | 0.028 |
| 63 days old | | | | | |
| Thymus index(%) | 2.82 ± 0.88$^{b}$ | 3.34 ± 0.48$^{ab}$ | 4.05 ± 1.09$^{a}$ | 3.68 ± 0.68$^{ab}$ | 0.013 |
| Spleen index(%) | 1.94 ± 0.34 | 1.92 ± 0.44 | 2.02 ± 0.66 | 2.42 ± 1.74 | 0.331 |
| Bursa index(%) | 1.07 ± 0.21$^{b}$ | 1.58 ± 0.32$^{a}$ | 1.63 ± 0.46$^{a}$ | 1.46 ± 0.30$^{ab}$ | 0.017 |

Note: CON: Control group; TP1: basal feed + 250 mg/kg Qiamagu polysaccharides; TP2: basal feed + 250 mg/kg Qiamagu polysaccharides; TP3: basal feed + 1000 mg/kg Qiamagu polysaccharides; Different lowercase superscripts in the same row indicate significant differences ($P < 0.05$) among groups

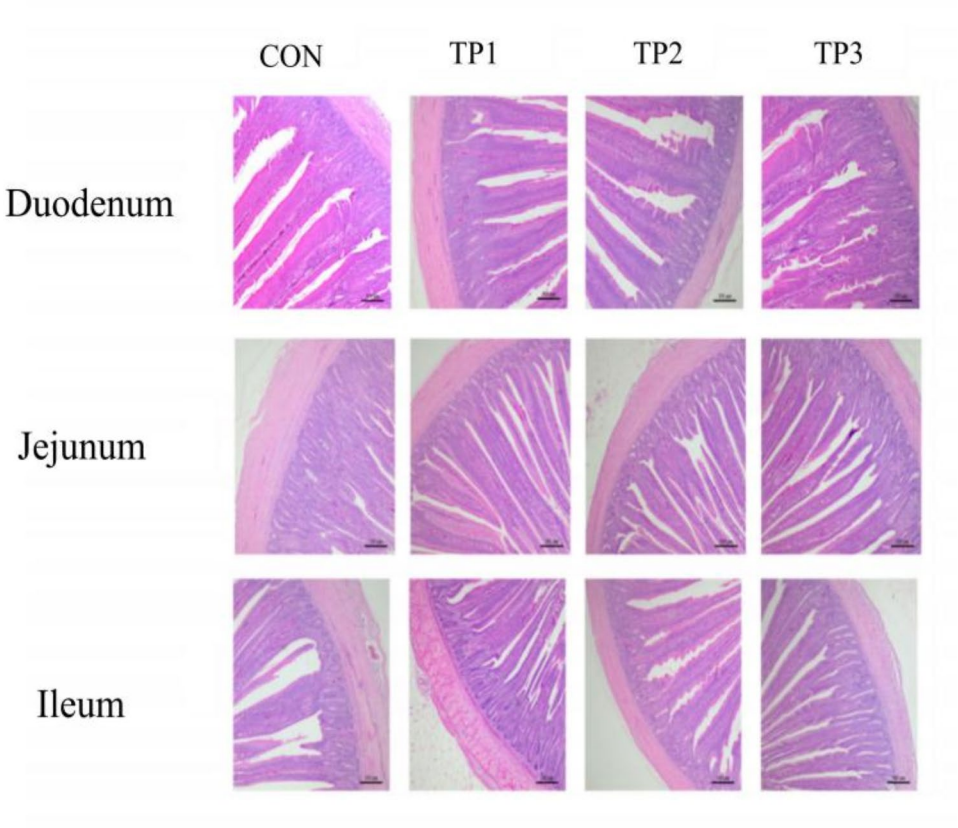


**Fig. 1** The effect of Qiamagu polysaccharides on the morphology of small intestine tissue in yellow feathered broiler chickens (100x)

the TP1 group, 191 to the TP2 group, and 341 to the TP3 group.

#### *Analysis of microbial α-diversity in the appendix*

According to Table 9, the ACE and Chao1 indices in both the TP1 and TP3 groups were higher than those in the CON group, but the differences were not statistically significant ($P > 0.05$).

#### *Analysis of microbial β diversity in cecum*

The PCoA (Principal Coordinates Analysis) results, as shown in Fig. 3, there was differences in cecal microbiota among groups ($P < 0.05$), as determined by ANOSIM (Analysis of Similarities) and PCoA of β-diversity.

#### *Analysis of intestinal flora composition in cecum*

Figure 4 shows the composition of the top ten microbial communities in terms of relative abundance at the

**Table 8** Effects of Qiamagu polysaccharide on intestinal villus length and crypt depth of yellow-feathered broilers (Means ± SE)

| Item | Control group | TP1 group | TP2 group | TP3 group | *P*-value |
|---|---|---|---|---|---|
| **21 days old** | | | | | |
| Duodenum | | | | | |
| V(μm) | 1226.43 ± 40.32 | 1257.76 ± 220.14 | 1266.65 ± 70.99 | 1227.35 ± 50.43 | 0.071 |
| C(μm) | 243.91 ± 13.12$^{a}$ | 237.02 ± 18.15$^{a}$ | 227.31 ± 16.10$^{b}$ | 238.14 ± 16.34$^{a}$ | 0.027 |
| V/C | 5.03 ± 0.35$^{c}$ | 5.30 ± 0.50$^{b}$ | 5.57 ± 0.86$^{a}$ | 5.15 ± 0.17$^{bc}$ | 0.036 |
| Jejunum | | | | | |
| V(μm) | 832.12 ± 7.72 | 845.40 ± 4.57 | 853.97 ± 4.49 | 838.92 ± 2.71 | 1.136 |
| C(μm) | 167.86 ± 12.58 | 173.01 ± 6.50 | 177.26 ± 3.04 | 165.13 ± 4.64 | 0.173 |
| V/C | 4.97 ± 0.33 | 4.89 ± 0.16 | 4.82 ± 0.06 | 5.08 ± 0.12 | 0.271 |
| Ileum | | | | | |
| V(μm) | 816.23 ± 3.89 | 823.34 ± 13.15 | 831.32 ± 8.70 | 815.22 ± 5.08 | 0.063 |
| C(μm) | 162.76 ± 6.92 | 169.50 ± 9.58 | 174.43 ± 6.12 | 165.55 ± 4.40 | 0.096 |
| V/C | 5.01 ± 0.19 | 4.86 ± 0.20 | 4.77 ± 0.12 | 4.92 ± 0.10 | 0.065 |
| **42 days old** | | | | | |
| Duodenum | | | | | |
| V(μm) | 1262.62 ± 25.87$^{b}$ | 1310.34 ± 42.53$^{b}$ | 1612.46 ± 43.51$^{a}$ | 1395.14 ± 27.04$^{ab}$ | 0.037 |
| C(μm) | 280.64 ± 3.65$^{a}$ | 239.78 ± 2.90$^{b}$ | 220.54 ± 2.93$^{b}$ | 246.98 ± 0.68$^{b}$ | 0.022 |
| V/C | 4.51 ± 0.03$^{c}$ | 5.43 ± 0.11$^{bc}$ | 7.35 ± 0.12$^{a}$ | 5.65 ± 0.12$^{b}$ | 0.031 |
| Jejunum | | | | | |
| V(μm) | 866.67 ± 6.15 | 942.68 ± 11.12 | 1116.14 ± 14.77 | 975.16 ± 10.49 | 0.233 |
| C(μm) | 178.44 ± 21.06 | 179.54 ± 12.10 | 194.74 ± 15.35 | 184.24 ± 15.23 | 0.345 |
| V/C | 4.86 ± 0.54 | 5.25 ± 0.29 | 5.73 ± 0.38 | 5.29 ± 0.38 | 0.512 |
| Ileum | | | | | |
| V(μm) | 923.16 ± 74.20$^{b}$ | 913.45 ± 31.87$^{b}$ | 1096.60 ± 32.42$^{a}$ | 918.30 ± 37.81$^{b}$ | 0.032 |
| C(μm) | 176.60 ± 8.48 | 172.36 ± 16.60 | 185.34 ± 16.82 | 174.48 ± 15.43 | 0.444 |
| V/C | 5.23 ± 0.17$^{b}$ | 5.30 ± 0.33$^{b}$ | 5.92 ± 0.36$^{a}$ | 5.26 ± 0.25$^{b}$ | 0.035 |
| **43–63 days old** | | | | | |
| Duodenum | | | | | |
| V(μm) | 1368.60 ± 22.84 | 1412.23 ± 25.30 | 1403.50 ± 26.31 | 1395.77 ± 31.17 | 0.115 |
| C(μm) | 221.12 ± 18.27 | 232.64 ± 15.53 | 224.36 ± 17.28 | 225.02 ± 22.18 | 0.077 |
| V/C | 6.19 ± 0.41 | 6.07 ± 0.30 | 6.26 ± 0.36 | 6.23 ± 0.48 | 0.068 |
| Jejunum | | | | | |
| V(μm) | 1111.21 ± 24.15 | 1123.34 ± 13.78 | 1134.34 ± 33.16 | 1153.80 ± 23.17 | 0.086 |
| C(μm) | 211.71 ± 10.66 | 187.24 ± 13.06 | 215.41 ± 18.29 | 220.36 ± 16.02 | 0.063 |
| V/C | 5.25 ± 0.15 | 6.00 ± 0.34 | 5.27 ± 0.29 | 5.24 ± 0.27 | 0.057 |
| Ileum | | | | | |
| V(μm) | 1163.23 ± 25.57 | 1177.04 ± 35.60 | 1150.13 ± 13.66 | 1153.80 ± 31.44 | 0.062 |
| C(μm) | 198.01 ± 20.10 | 193.47 ± 11.99 | 186.35 ± 12.23 | 189.13 ± 12.85 | 1.33 |
| V/C | 5.87 ± 0.47 | 6.08 ± 0.19 | 6.17 ± 0.35 | 6.10 ± 0.25 | 0.074 |

Note: CON: Control group; TP1: basal feed + 250 mg/kg Qiamagu polysaccharides; TP2: basal feed + 250 mg/kg Qiamagu polysaccharides; TP3: basal feed + 1000 mg/kg Qiamagu polysaccharides; Different lowercase superscripts in the same row indicate significant differences ($P < 0.05$)

different levels. At Phylum level (Fig. 4.A), the main gut microbiota in the four groups primarily belonged to the phyla Bacteroidota and Firmicutes. The relative abundance of Bacteroidota in the CON, TP1, TP2, and TP3 groups was 42.86%, 44.90%, 45.54%, and 46.32%, respectively, while the relative abundance of Firmicutes was 54.27%, 44.99%, 48.39%, and 48.15%. Notation analysis at the family level (Fig. 4.B) revealed that the four most abundant microbial families in the cecal content samples were Lachnospiraceae, Bacteroidaceae, Ruminococcaceae, and Rikenellaceae. The relative abundance in the CON group was 12.00%, 20.59%, 17.76%, and 6.18%; in the TP1 group: 11.80%, 20.79%, 15.28%, and 11.09%; in the TP2 group: 10.42%, 26.96%, 14.08%, and 8.91%; and in the TP3 group: 10.40%, 24.16%, 6.84%, and 11.68%. It can be observed that Qiamagu polysaccharides reduced the proportion of Ruminococcaceae and increased the proportions of Bacteroidaceae and Rikenellaceae. At Genus level (Fig. 4.C), the four most abundant bacterial genera in the samples are Bacteroides, Faecalibacterium, Lactobacillus, and Phascolarctobacterium. In the CON group, the relative abundances were 22.90%,

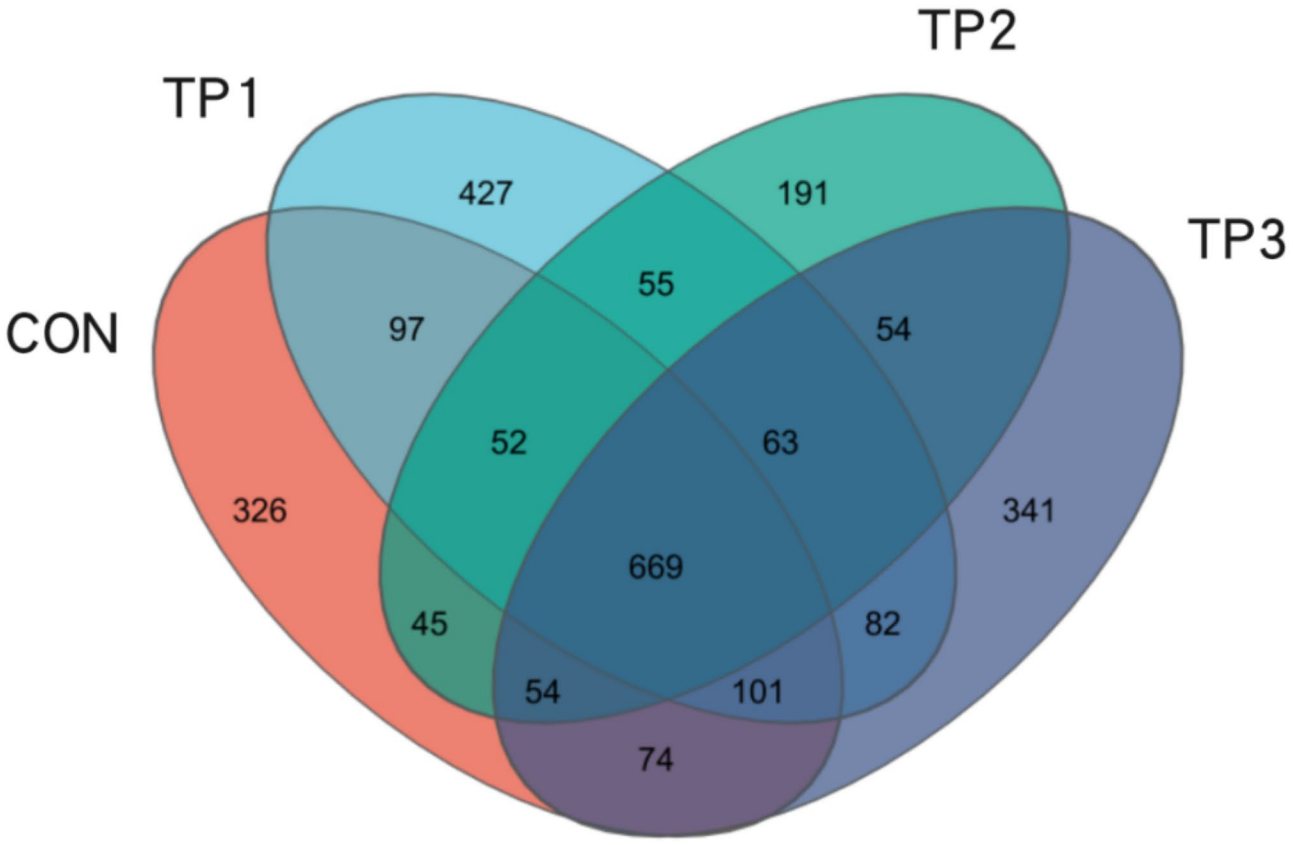


**Fig. 2** Venn diagram of cecal intestinal flora in yellow-feathered broilers

**Table 9** Effects of Qiamagu polysaccharide on α diversity of cecal microorganisms in yellow-feathered broilers (Means ± SE)

| Item | Control group | TP1 group | TP2 group | TP3 group | *P*-value |
|---|---|---|---|---|---|
| Shannon | 4.22 ± 0.43 | 4.36 ± 0.35 | 3.94 ± 0.31 | 4.62 ± 0.21 | 0.140 |
| Simpson | 0.05 ± 0.04 | 0.03 ± 0.01 | 0.05 ± 0.02 | 0.02 ± 0.01 | 0.824 |
| Ace | 858.10 ± 151.82 | 875.16 ± 242.01 | 701.57 ± 181.58 | 928.04 ± 63.55 | 0.609 |
| Chao1 | 830.94 ± 136.04 | 838.46 ± 220.15 | 681.91 ± 162.91 | 897.07 ± 52.95 | 0.917 |
| Good coverage | 0.996 | 0.996 | 0.996 | 0.996 | - |

Note: CON: Control group; TP1: basal feed + 250 mg/kg Qiamagu polysaccharides; TP2:basal feed + 250 mg/kg Qiamagu polysaccharides; TP3:basal feed + 1000 mg/kg Qiamagu polysaccharides; Different lowercase superscripts in the same row indicate significant differences ($P < 0.05$) among groups

14.10%, 3.61%, and 2.88%, respectively. In the TP1, TP2, and TP3 groups, the relative abundances of Bacteroides were 24.85%, 20.65%, and 26.79%, respectively; the relative abundances of Faecalibacterium were 12.68%, 9.05%, and 4.80%; the relative abundances of Lactobacillus were 2.89%, 11.06%, and 2.74%; and the relative abundances of Phascolarctobacterium were 2.10%, 3.41%, and 2.71%. It can be observed that in the TP1 and TP3 groups, the relative abundance of Bacteroides in the cecal microbiota of broilers increased, while the relative abundance of Faecalibacterium decreased. The TP2 group significantly increased the relative abundance of Lactobacillus in the cecal microbiota of broilers ($P < 0.05$).

### *Prediction of cecum microbial function in yellow-feathered broilers*

The LEfSe(Linear discriminant analysis Effect Size) analysis, also referred to as LDA Effect Size analysis, is a powerful tool designed for comparing multiple groups in order to identify microbial species exhibiting significant differences in abundance between the groups. It can be seen from Fig. 5 that Oribacterium, Fournierella, Christensenella and Paludicola in the control group have a high relative abundance. Butyricimonas in group TP1 has a high abundance. Christensenellaceae, Christensenellales, Christensenellaceae R-7group, unclassified-f-Rikenellaceae, UCG-005, Patescibacteria, Saccharimonadales, Saccharimonadia, Veillonella, Eubacteriales, Anaerofustis, Anaerofustaceac, Unclassified-o-Rhodospirillales,

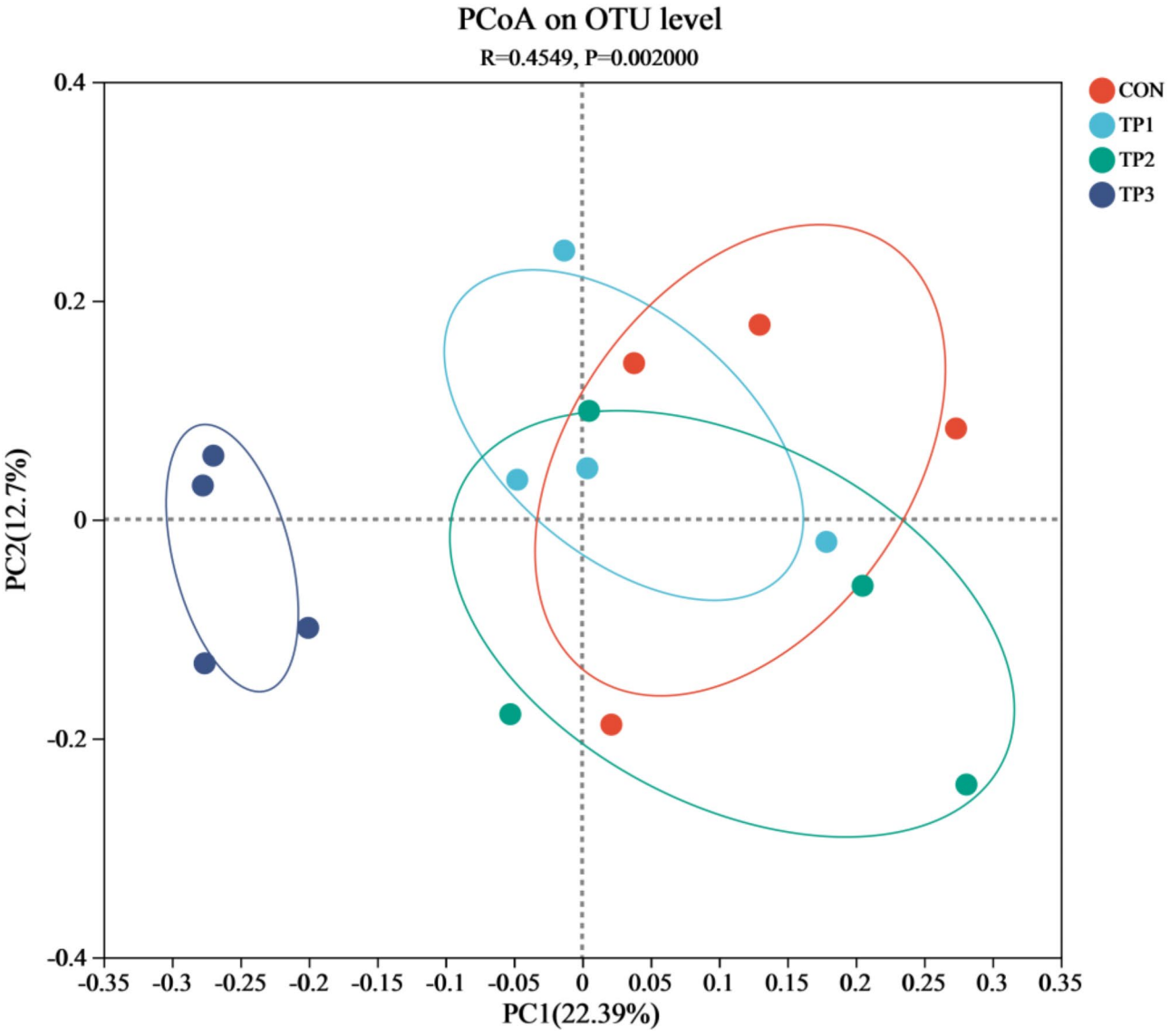


**Fig. 3** Results of PCoA analysis. Note: CON: Control group; TP1: basal feed + 250 mg/kg Qiamagu polysaccharides; TP2: basal feed + 250 mg/kg Qiamagu polysaccharides; TP3:basal feed + 1000 mg/kg Qiamagu polysaccharides

Norank-f-Peptococcaceae have higher relative abundance in group TP3.

#### *mRNA expression in ileum of yellow-feathered broilers in treated group*

As shown in Table 10,The mRNA expression of IL-1β in the TP2 group from 1 to 21 days of age and in the TP3 group from 22 to 42 days of age exhibited a significant reduction when compared to the control group ($P < 0.05$).

As shown in Table 11, Compared to the control group, mRNA expression levels of Occludin, MUC2, and ZO-1 in the ileum mucosa of yellow-feathered broilers in the TP3 group exhibited differential increases at 21and 63 d.

## Discussion

### Effects of Qiamagu polysaccharide on growth performance of yellow-feathered broilers

The daily gain and feed-to-weight ratio of broilers serve as important indicators of growth performance [17], reflecting the efficiency of feed digestion, absorption, and utilization. The results of this experiment show that supplementation of 500 mg/kg Qiamagu polysaccharide in the diet of yellow-feathered broilers can significantly increase the average daily gain of broilers aged 1–21 days and 42–64 days, which is consistent with the results of Eghbaldost-Jadid et al. [18] and Zhang et al. [19]. This may be related to the fact that Qiamagu polysaccharide can improve the growth performance of broilers by increasing the digestibility of dry matter and nitrogen

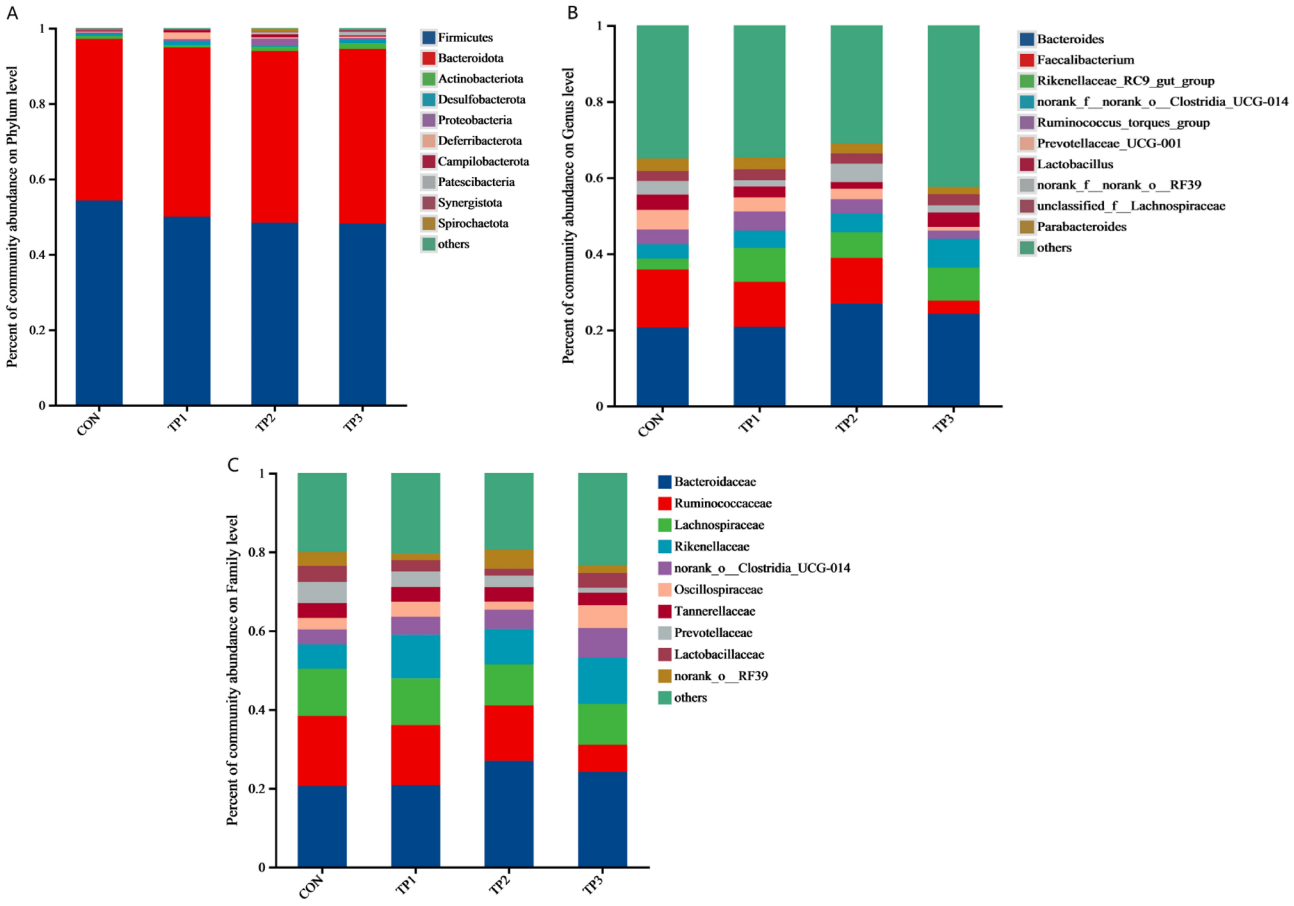


**Fig. 4** Effects of Qiamagu polysaccharides on microflora abundance at Phylum(A), Genus (B) and family(C) level in yellow-feathered broilers. Note: CON: Control group; TP1: basal feed + 250 mg/kg Qiamagu polysaccharides; TP2: basal feed + 250 mg/kg Qiamagu polysaccharides; TP3: basal feed + 1000 mg/kg Qiamagu polysaccharides

and increasing digestive enzyme activity [20]. The addition of Qiamagu polysaccharide increased the average daily gain of broilers, thereby increasing the feed conversion rate and improving the growth performance of broilers. In addition, at 42–64 days of age, the average daily feed intake of broilers supplemented with Qiamagu polysaccharide was significantly lower than that of the control group, thereby improving feed conversion rate. This may be due to the fact that the addition of Qiamagu polysaccharide improved the abundance of intestinal microorganisms in broilers, increased the abundance of beneficial bacteria and decreased the abundance of harmful bacteria [21].

### Effects of Qiamagu polysaccharide on immune performance of yellow-feathered broilers

Serum biochemical indicators reflect the metabolic status of broilers and the function of vital organs like the liver and kidneys [22]. Serum contains various proteins that play an important role in physiological and pathological activities in animals [23]. Studies have shown that plant-derived polysaccharides can improve serum biochemical indicators in animals [24]. For example, Achyranthes bidentata polysaccharide can reduce serum TG and TC levels in broilers, promote fat metabolism by oxidizing excess fat, and provide energy for enhancing protein synthesis and growth [25]. Glycyrrhiza polysaccharide can increase serum albumin concentration in broilers, enhancing protein synthesis, metabolic energy capacity, and immune function [26]. This study shows that at 63 days of age, the serum AST levels in the TP2 group of broilers increased significantly, while AST levels in the TP1 group also rose significantly. The levels of TP, ALB, GLB, and GLU in the serum of broilers in the experimental group increased, although not significantly. This suggests that Cistanche polysaccharides have a positive effect on serum protein and lipid metabolism in yellow-feathered broilers, providing a better metabolic state for their growth.

Serum immune indicators reflect the immunity and health status of broilers [27]. Studies show that adding Moringa leaf polysaccharides to the diet of Sanhuang chickens significantly increases serum IL-1β and IL-4 levels, regulates the inflammatory response, boosts

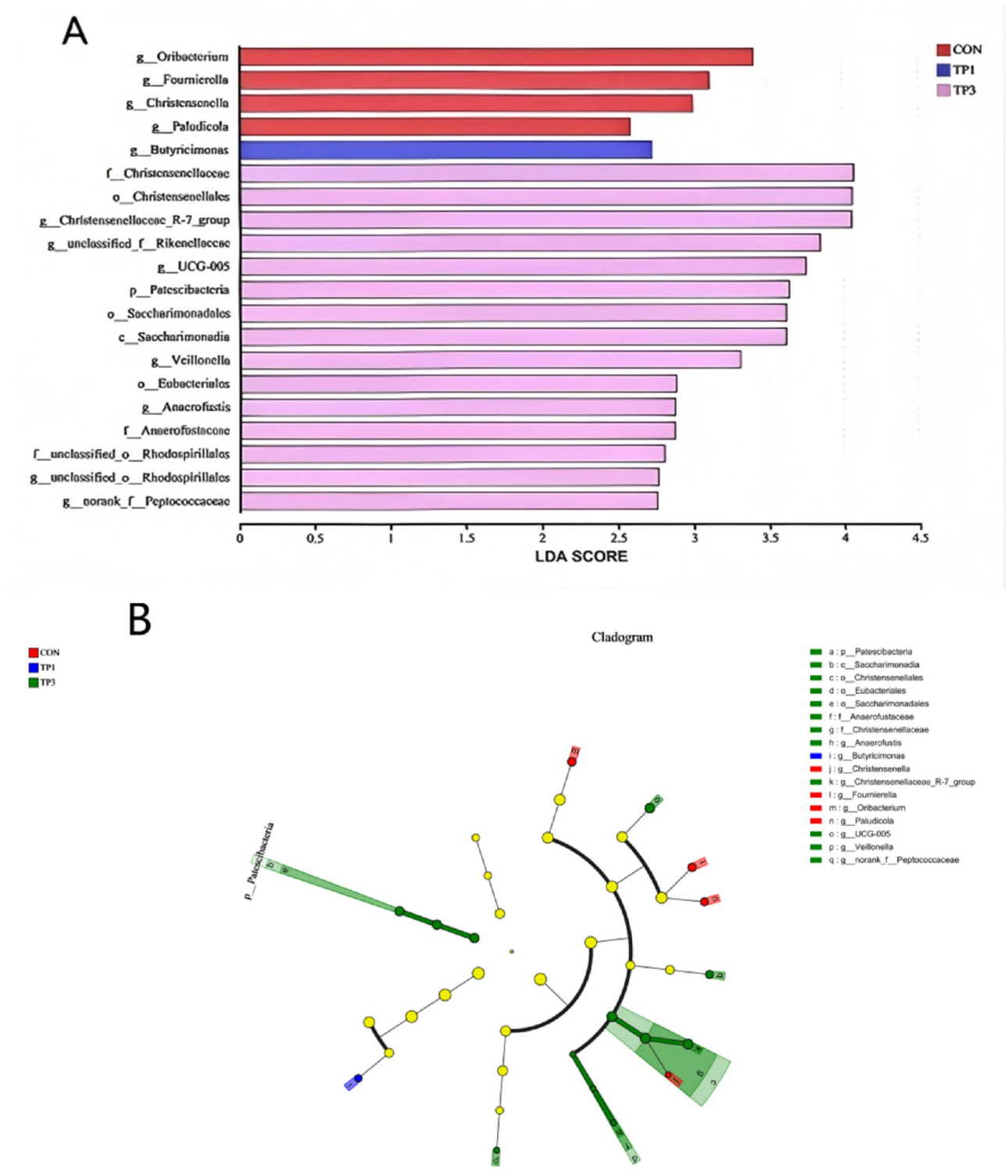


**Fig. 5** Effects of Qiamagu polysaccharide on different microbe in cecum of yellow-feathered broilers. Note: CON: Control group; TP1: basal feed + 250 mg/kg Qiamagu polysaccharides; TP2:: basal feed + 250 mg/kg Qiamagu polysaccharides; TP3: basal feed + 1000 mg/kg Qiamagu polysaccharides; Red indicated that the abundance of these bacteria was higher in CON group; Blue indicates that these bacteria are more abundant in group TP1; Pink indicates bacteria that are more abundant in the TP3 group

**Table 10** The mRNA expression levels in ileum of yellow feathered broilers (Mean ± SE)

| Item | CON group | TP1 group | TP2 group | TP3 group | *P*-value |
|---|---|---|---|---|---|
| 21 days old | | | | | |
| IL-1β | 1.00 ± 0.23$^{a}$ | 0.75 ± 0.83$^{ab}$ | 0.48 ± 0.93$^{b}$ | 0.66 ± 0.49$^{b}$ | 0.022 |
| IL-2 | 1.00 ± 0.32 | 0.69 ± 0.43 | 0.66 ± 0.79 | 1.16 ± 0.79 | 0.497 |
| TNF-α | 1.00 ± 0.04 | 0.72 ± 0.48 | 0.91 ± 0.30 | 1.14 ± 0.31 | 0.305 |
| 42 days old | | | | | |
| IL-1β | 1.00 ± 0.65 | 1.04 ± 0.51 | 0.70 ± 0.53 | 0.50 ± 0.57 | 0.576 |
| IL-2 | 1.00 ± 0.64$^{a}$ | 0.72 ± 1.01$^{ab}$ | 1.68 ± 0.33$^{a}$ | 0.25 ± 0.46$^{b}$ | 0.039 |
| TNF-α | 1.00 ± 0.13 | 0.96 ± 0.36 | 1.03 ± 0.18 | 0.95 ± 0.46 | 0.290 |
| 63 days old | | | | | |
| IL-1β | 1.00 ± 0.51 | 1.10 ± 0.50 | 1.31 ± 0.81 | 1.03 ± 0.79 | 0.701 |
| IL-2 | 1.00 ± 0.45 | 1.33 ± 0.78 | 1.40 ± 0.22 | 1.44 ± 0.65 | 0.853 |
| TNF-α | 1.00 ± 0.77 | 1.03 ± 0.57 | 0.22 ± 2.28 | 0.90 ± 1.05 | 0.669 |

Note: CON: Control group; TP1: basal feed + 250 mg/kg Qiamagu polysaccharides; TP2:: basal feed + 250 mg/kg Qiamagu polysaccharides; TP3:: basal feed + 1000 mg/kg Qiamagu polysaccharides; Different lowercase superscripts in the same row indicate significant differences ($P < 0.05$). IL-1β:Interleukin-1β; IL-2: Interleukin-2; TNF-α: (Tumor Necrosis Factor-α)

**Table 11** The mRNA expression levels in ileum of yellow feathered broilers (Mean ± SE)

| Item | CON group | TP1 group | TP2 group | TP3 group | *P*-value |
|---|---|---|---|---|---|
| 21 days old | | | | | |
| Occludin | 1.00 ± 0.64 | 1.05 ± 0.34 | 0.96 ± 0.52 | 1.89 ± 0.10 | 0.418 |
| MUC2 | 1.00 ± 1.11$^{b}$ | 2.04 ± 1.42$^{a}$ | 1.79 ± 1.05$^{a}$ | 4.22 ± 1.31$^{a}$ | 0.027 |
| ZO-1 | 1.00 ± 0.69$^{b}$ | 0.71 ± 0.13$^{b}$ | 1.08 ± 0.22$^{b}$ | 2.17 ± 0.01$^{a}$ | 0.032 |
| 42 days old | | | | | |
| Occludin | 1.00 ± 0.77 | 1.50 ± 0.93 | 0.91 ± 0.75 | 0.71 ± 0.29 | 0.617 |
| MUC2 | 1.00 ± 0.11 | 1.00 ± 0.59 | 1.50 ± 0.87 | 0.78 ± 0.32 | 0.395 |
| ZO-1 | 1.00 ± 0.91 | 1.21 ± 0.96 | 0.99 ± 0.42 | 0.54 ± 0.28 | 0.610 |
| 63 days old | | | | | |
| Occludin | 1.00 ± 0.88$^{b}$ | 2.59 ± 0.84$^{a}$ | 1.35 ± 0.76$^{ab}$ | 2.27 ± 0.61$^{a}$ | 0.029 |
| MUC2 | 1.00 ± 0.95 | 1.77 ± 0.16 | 0.86 ± 0.66 | 1.20 ± 0.77 | 0.493 |
| ZO-1 | 1.00 ± 0.13 | 1.65 ± 0.27 | 0.57 ± 1.60 | 1.35 ± 0.44 | 0.862 |

Note: CON: Control group; TP1: basal feed + 250 mg/kg Qiamagu polysaccharides; TP2:: basal feed + 250 mg/kg Qiamagu polysaccharides; TP3:: basal feed + 1000 mg/kg Qiamagu polysaccharides; Different lowercase superscripts in the same row indicate significant differences ($P < 0.05$) among groups

immunity, and reduces disease occurrence in broilers [28]. Consistent with the above findings, this experiment showed that feeding yellow-feathered broilers different doses of Qiamagu polysaccharides significantly increased serum IL-1β, IL-2, and TNF-α levels. Immunoglobulins (IgM, IgA, IgG) are key immune-active substances and the main components of humoral immunity, enhancing the body's immune response. Zhao et al. [29] found that adding 0.6 g/kg of mulberry polysaccharides to the diet significantly increased serum IgG levels in weaned piglets, effectively enhancing their immunity. Liu et al. [30] confirmed that adding 0.2 g/L of Hericium erinaceus polysaccharides significantly increased serum IgM, IgA, and IgG levels in Muscovy ducks, improving their immune function. This study showed that compared with the control group, the levels of pro-inflammatory factors IL-1β, IL-2 and TNF-α in serum of broilers in each Qiamagu polysaccharide group were reduced, which consistent with the results of above studies.

The immune organ index is a key indicator for assessing the strength of an organism's immune capacity. The results of this study indicate that the immune organ index of yellow-feathered broilers in the TP1 and TP2 groups significantly improved. Nan et al. and Xing et al. [31–32] found that adding plant polysaccharides (such as Lycium barbarum polysaccharides and camellia cake polysaccharides) can enhance the immune organ index in mice and yellow-feathered broilers, thus improve the immune performance of the body which is consistent with our findings.

### Effects of Qiamagu polysaccharide on intestinal villus length and crypt depth of yellow-feathered broilers

The length of the intestinal villi and the depth of the crypts play a crucial role in the gut health and function of yellow-feathered broilers. The longer the villi, the greater the surface area, and the stronger the nutrient absorption capacity [33]. This study shows that, in the early stages of the experiment, the crypt depth in the duodenum of broilers in the TP2 group was significantly reduced compared to the CON group, while the villus-to-crypt ratio in both the TP1 and TP2 groups significantly increased. This study shows that, in the early stages of the experiment, the crypt depth in the duodenum of broilers in the TP2 group was significantly reduced compared to the CON group, while the villus-to-crypt ratio in both the TP1 and TP2 groups significantly increased. In the mid-experiment period, the villus length and villus-to-crypt ratio in the duodenum and ileum of the TP2 group were significantly higher than those in the CON group, indicating that Qiamagu polysaccharides can stimulate the development of the small intestine in broilers and optimize intestinal structure.

### Effects of Qiamagu polysaccharide on cecal intestinal flora of yellow-feathered broilers

Gut microbiota play a crucial role in nutrient absorption in broiler chickens [34]. Microorganisms can stimulate the intestinal mucosa to trigger immune responses, enhancing the immune system's function [35]. Beneficial microbes like Bifidobacterium and Lactobacillus can inhibit the growth and invasion of pathogens by competing for nutrients and attachment sites [36]. β-diversity analysis revealed significant differences in microbial composition between the CON and TP3 groups, indicating that Qiamagu polysaccharides had a noticeable impact on the gut microbiota structure of broilers. β-diversity

analysis revealed significant differences in microbial composition between the CON and TP3 groups, indicating that Qiamagu polysaccharides had a noticeable impact on the gut microbiota structure of broilers. α-diversity is a metric used to measure species richness and evenness within a single sample of microorganisms. This metric includes methods like the Shannon index, Chao1 index, and ACE index. In this experiment, the ACE and Chao1 indices of the TP1 and TP3 groups were both higher than those of the CON group. The results differ from Liu et al.'s study [37], which found that feeding rats Qiamagu polysaccharides significantly increased the Shannon and Chao1 indices in the ileum, suggesting that Qiamagu polysaccharides enhance gut flora diversity in mice. These differences may be related to variations in the extraction process of Qiamagu polysaccharides and the region where Qiamagu was sourced.

In the cecum of broilers, the proportion of Bacteroidetes increased while Firmicutes decreased, potentially improving intestinal immune status, enhancing mucosal health, and possibly reducing excessive energy absorption, which may have a positive impact on the health and growth performance of broilers [38]. A study indicated that adding inulin to broiler diets increased the relative abundance of Bacteroidetes while reducing the abundance of Firmicutes and Proteobacteria, consistent with our experiment's findings [39].

### Effect of Qiamagu polysaccharide on mRNA relative expression in ileum mucosa of yellow-feathered broilers

Pro-inflammatory factors can promote protein degradation and inhibit protein synthesis, leading to a decline in broiler growth performance. Interleukin-1β (IL-1β), Interleukin-2 (IL-2), and Tumor Necrosis Factor-α (TNF-α) are key pro-inflammatory factors that play significant immunoregulatory roles in the intestinal tract of broilers. In the early stages of this experiment, compared to the CON group, the TP2 and TP3 Qiamagu polysaccharide groups significantly reduced IL-1β levels in the ileal mucosa of broilers. In the middle stage of the experiment, the TP3 Qiamagu polysaccharide group showed lower IL-2 levels in the ileal mucosa compared to the CON group. This finding is consistent with Xing et al. [35].,Who demonstrated that Artemisia polysaccharides could activate the Nrf2/Keap1 signaling pathway and inhibit the TLR4/NF-κB pathway, enhancing antioxidant enzyme activity and reducing pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) [32]. Qiamagu polysaccharides may enhance the function of anti-inflammatory cells in the immune system while inhibiting pro-inflammatory cells, thereby reducing the expression of pro-inflammatory factors and improving both the immune function and gut health of broilers.

Occludin, MUC2, and ZO-1 play crucial protective roles in the intestines of broiler chickens, helping to maintain the integrity of the intestinal barrier and prevent the invasion of pathogens and toxins [40]. The results of this experiment showed that, in the early stages, compared to the CON group, MUC2 mRNA levels in the ileal mucosa of broiler chickens were significantly upregulated in the TP1, TP2, and TP3 groups, while ZO-1 mRNA levels were significantly upregulated in the TP3 group. In the mid-experiment stage, compared to the CON group, the TP3 group showed a significant increase in Occludin mRNA expression in broiler chickens. The study by Li et al. [41] demonstrated that feeding broiler chickens alfalfa polysaccharides increased the expression of tight junction proteins (claudin-1, occludin, and MUC2) in the duodenal mucosa. The results of this study are largely consistent with Li et al.'s findings.

## Conclusions

In summary, under the conditions of this study, adding Qiamagu polysaccharide to the basic diet of yellow-feathered broilers improved the growth performance, immune performance, intestinal morphology and the relative abundance of intestinal beneficial bacteria of yellow-feathered broilers. Among them, 500 mg/kg of Qiamagu polysaccharide had the best effect.

#### Author contributions

T.l. and S.W. completed the main experimental content and wrote the manuscript; J.Y assisted in the experiment; YM to revise the manuscript; Z.L and J.Z discussed the experimental method; S.C and M.G analyzed and statistically analyzed the experimental results. H.S and J.W provide financial support and technical guidance. All authors reviewed the manuscript。.

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#### Data availability

Data is provided within the manuscript or supplementary information files.

## Declarations

#### Institutional Review Board Statement

All procedures in this study were approved by the Animal Experiment Ethics Committee of Animal Science and Technology of Shihezi University China, approval number: 2023-031.

#### Informed consent

Informed consent was obtained from all subjects involved in the study.

#### Competing interests

The authors declare no competing interests.

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**References**


1. Wang Q, Wang XF, Xing. T. The combined impact of xylo-oligosaccharides and gamma-irradiated Astragalus polysaccharides on growth performance andintestinal mucosal barrier function of broilers[J]. Poult Sci. 2021;100(3):100909.
2. Zhang W, Tang Y, Fan R. Effects of the pesticide carbendazim on broiler chicken health and carbendazim residue levels in broiler tissues. Vet Res Commun (2024).
3. He CL, Fu BD, Shen HQ. Xiang-qi-Tang increases avian pathogenic *Escherichia coli*-induced survival rate and regulates serum levels of tumor necrosis factor alpha, interleukin-1 and soluble endothelial protein C receptor in chicken[J]. Biol Pharm Bull. 2011;34(3):379–82.
4. Ha-Anh N, Thao TP. Hang Thuy Dam. Whole genome sequence analysis of *Bacillus amyloliquefaciens* strain S2.5 as a potential probiotic for feed supplement in livestock production. J Genetic Eng Biotechnol 2024, 22(3).
5. Lin J, Hunkapiller AA, Layton AC. Response of intestinal microbiota to antibiotic growth promoters in chickens[J]. Foodborne Pathog Dis. 2013;10(4):331–7.
6. Suresh G, Das RK, Kaur. Brar. S. Alternatives to antibiotics in poultry feed: molecular perspectives[J]. Crit Rev Microbiol. 2018;44(3):318–35.
7. Yasodha T, Jeevitha S, Yogesh S. Growth performance parameters of broiler chickens as influenced by herbal poultry feed[J]. Int Res J Anim Vet Sci. 2019;1:26–30.
8. Wang X, Wang S, Li Y. Sulfated Astragalus polysaccharide can regulate the inflammatory reaction induced by LPS in Caco2 cells[J]. Int J Biol Macromol. 2013;60:248–52.
9. Kallon S, Li X, Ji J. Astragalus polysaccharide enhances immunity and inhibits H9N2 avian influenza virus in vitro and in vivo[J]. J Anim Sci Biotechnol. 2013;4:1–11.
10. Yang J, Sun Y, Wang Q. Astragalus polysaccharides-induced gut microbiota play a predominant role in enhancing of intestinal barrier function of broiler chickens. J Anim Sci Biotechnol. 2024;15:106. https://doi.org/10.1186/s40104-024-01060-1.
11. Eghbaldost-Jadid R, Nosrati M, Rasouli B. The effects of Turnip (Brassica rapa) extract on the growth performance and Health of Broilers. Animals. 2021;11:867. https://doi.org/10.3390/ani11030867.
12. Abdugafurova DG, Oripova MZ, Amanlikova DA, et al. Study of the Immunomodulatory Effect of Polysaccharides isolated from seeds of Turnip BRASSICA RAPA. Pharm Chem J. 2024;57:1552–6.
13. Zhang XW, Li X, Yin Y, et al. Effects of ursolic acid on growth performance, serum biochemistry, antioxidant capacity, and intestinal health of broilers. Animal. 2024;101385. https://doi.org/10.1016/j.Animal.2024.101385. ISSN 1751–7311.
14. Kim D-H, Yang HM, Song J-Y et al. Effects of dietary mangosteen peel powder and extract on the growth performance, meat quality and indicators for immunity, gut health and antioxidant activity in broiler chicks,Poultry Science, 2024;103(12):104477,ISSN 0032-5791,https://doi.org/10.1016/j.psj.2024.104477
15. Fisayo Oretomiloye L, Lahaye. Deborah Adewole,Effect of a microencapsulated complex of biofactors and antioxidants on the growth performance, plasma biochemistry, intestinal health, and immune and antioxidant status of broiler chickens challenged with cold stress,Canadian. J Anim Sci 2024 ISSN 0008–3984,https://doi.org/10.1139/cjas-2024-0091
16. Caiwei Luo J, Wang W, Jiang et al. Different starch sources and amino acid levels on growth performance, starch and amino acids digestion, absorption and metabolism of 0- to 3-week-old broilers fed low protein diet,Animal Nutrition,2024,ISSN 2405–6545,https://doi.org/10.1016/j.aninu.2024.11.004
17. Zukiwsky NM, Afrouziyeh M, Robinson FE, Zuidhof MJ. Broiler growth and efficiency in response to relaxed maternal feed restriction. Poult Sci. 2021;100:100993.
18. Eghbaldost-Jadid R, Nosrati M, Rasouli B. The effects of Qiamagu (Brassica rapa) extract on the growth performance and health of broilers[J]. Animals. 2021;11(3):867.
19. Zhang S, Zhu C, Xie H. Effect of Gan Cao (Glycyrrhiza Uralensis Fisch) polysaccharide on growth performance, immune function, and gut microflora of broiler chickens[J]. Poult Sci. 2022;101(10):102068.
20. Wang XY, Xue JJ, Zhang R. Prebiotic characteristics of degraded polysaccharides from Acanthopanax senticosus polysaccharide on broilers gut microbiota based on in vitro digestion and fecal fermentation. Poult Sci. 2024;103(7):103807. ISSN 0032–5791.
21. Yang B, Li XF, Noura M. The impact of dietary supplementation of polysaccharide derived from Polygonatum sibiricum on growth, antioxidant capacity, meat quality, digestive physiology, and gut microbiota in broiler chickens. Poult Sci. 2024;103(6):103675. ISSN 0032–5791.
22. Zhang X, Huang K, Zhong H, Ma Y, Guo Z, Tang Z. Effects of Lycium barbarum polysaccharides on immunological parameters, apoptosis, and growth performance of Nile tilapia (Oreochromis niloticus). Fish Shellfish Immunol. 2020;97:509–14.
23. Lou AG, Cai JS, Zhang XM, Cui CD, Piao YS. The aflatoxin-detoxifizyme specific expression in the parotid gland of transgenic pigs. Transgenic Res. 2017;26:677–87.
24. Bing Yang X, Li, Noura M et al. The impact of dietary supplementation of polysaccharide derived from *Polygonatum sibiricum* on growth, antioxidant capacity, meat quality, digestive physiology, and gut microbiota in broiler chickens. Poult Sci 2024;103(6):103675, ISSN 0032–5791,https://doi.org/10.1016/j.psj.2024.103675
25. Kim HYSYM. In Ho Kim,evaluation of Achyranthes japonica Nakai extract on growth performance, nutrient utilization, cecal microbiota, excreta noxious gas emission, and meat quality in broilers fed corn–wheat.–soybean meal diet Poult Sci 2020;99(11):5728–5735,ISSN 0032-5791,https://doi.org/10.1016/j.psj.2020.07.023
26. Xiaoyu Ji H, Hou X, Wang et al. Effect of dietary Glycyrrhiza polysaccharides on growth performance, hepatic antioxidant capacity and anti-inflammatory capacity of broiler chickens,Research in Veterinary Science, 2024;167:105114, ISSN https://doi.org/10.1016/j.rvsc.2023.105114
27. Xin Zhu L, Tao. Haiying Liu,effects of fermented feed on growth performance, immune organ indices, serum biochemical parameters, cecal odorous compound production, and the microbiota community in broilers,Poultry Science, 2023;102(6):102629, ISSN 0032–5791,https://doi.org/10.1016/j.psj.2023.102629
28. Xingjiao Jiang J, Yang Z, Zhou et al. Moringa oleifera leaf improves meat quality by modulating intestinal microbes in white feather broilers,Food Chemistry: X, 2023;20:100938, ISSN 2590– 1575,https://doi.org/10.1016/j.fochx.2023.100938
29. Zhao X, Yang R, Bi Y. Effects of dietary supplementation with mulberry (Morus alba L.) leaf polysaccharides on immune parameters of weanling pigs[J]. Animals. 2019;10(1):35.
30. Li T, Qin W, Wu. Baiyila. Effects of glycyrrhiza polysaccharides on growth performance, meat quality, serum parameters and growth/meat quality-related gene expression in broilers. Front Veterinary Sci. 2024;11:2297– 1769.
31. Nan Y, Wang R, Yuan L. Effects of Lycium barbarum polysaccharides (LBP) on immune function of mice[J]. Afr J Microbiol Res. 2012;6(22):60–4757.
32. Xing YY, Zheng YK, Yang S. Artemisia Ordosica polysaccharide alleviated lipopolysaccharide-induced oxidative stress of broilers via Nrf2/Keap1 and TLR4/NF-κB pathway[J]. Ecotoxicol Environ Saf. 2021;223:112566.
33. Gopinger E, Xavier EG, Elias MC. The effect of different dietary levels of canola meal on growth performance, nutrient digestibility, and gut morphology of broiler chickens[J]. Poult Sci. 2014;93(5):1130–6.
34. Waters JL, Ley RE. The human gut bacteria Christensenellaceae are widespread, heritable, and associated with health[J]. BMC Biol. 2019;17:1–11.
35. Wu YT, Yang WY, Wu YHS. Modulations of growth performance, gut microbiota, and inflammatory cytokines by trehalose on *Salmonella Typhimurium*-challenged broilers[J]. Poult Sci. 2020;99(8):4034–43.
36. Zhu Q, Sun P, Zhang B. Progress on gut health maintenance and antibiotic alternatives in broiler chicken production[J]. Front Nutr. 2021;8:692839.
37. Liu L, Liu C, Hua H. Effect of polysaccharides from tibetan Qiamagu (Brassica rapa L.) on the gut microbiome after in vitro fermentation and in vivo metabolism[J]. Volume 13. Food & Function; 2022;76(5):3063–.
38. Wang Q, Wang XF, Xing. T.The combined impact of xylo-oligosaccharides and gamma-irradiated Astragalus polysaccharides on growth performance andintestinal mucosal barrier function of broilers[J]. Poulty Sci. 2021;100(3):100909.
39. Wang Q, Guo LY, Zhou GQ. Effects of chicory polysaccharides on the growth, antioxidant activity, and disease resistance in the Chinese mitten crab Eriocheir sinensis H. Milne Edwards, 1853 (Decapoda: Brachyura: Varunidae). J Crustacean Biology. 2021;41(2):ruab023.
40. Yang J, Sun Y, Wang Q. Astragalus polysaccharides-induced gut microbiota play a predominant role in enhancing of intestinal barrier function of broiler chickens. J Anim Sci Biotechnol. 2024;15:106.

41. Li Z, Zhang C, Li B. The modulatory effects of alfalfa polysaccharide on intestinal microbiota and systemic health of *Salmonella serotype* (ser.) Enteritidis-challenged broilers[J]. Scientific reports, 2021;11(1):109.

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