Effects of dietary Trollius chinensis Bunge residue on performance, meat quality, antioxidant capacity, intestinal health, and cecal microbiota in weaned rabbits

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Abstract

The present study aimed to investigate the effects of *Trollius chinensis Bunge* residues (TCBR) on growth, meat quality, antioxidant capacity, intestinal healthy and cecal microbiota in weaned rabbits. In total, 48 30-day-old rabbits were randomly allocated into 4 groups, with 12 replicates per group. Four diets were formulated with graded levels of TCBR: 2.0%, 4.0%, and 6.0% represented as TCBR2, TCBR4, and TCBR6 groups alongside a control group without TCBR. The results showed that TCBR2 significantly alleviated adverse clinical manifestations in weaned rabbits and improved survival rate, growth performance, and meat quality. while reducing the feed conversion ratio compared with the Mock group. TCBR2 also enhanced carcass yield, semi-eviscerated carcass yield, liver index, and liver antioxidant capacity, and increased jejunal villus height and villus/crypt ratio compared with that in the Mock group, whereas no differences were observed between the TCBR4 and TCBR6 groups. Notably, 16S RNA analysis revealed that Bacteroidota levels were significantly elevated in the TCBR2 groups, with Akkermansia, Clostridium, and Succiniclasticum also upregulated in the TCBR2 group. Furthermore, TCBR2 significantly increased the expression levels of occludin and ZO-1 in jejunal tissue. In conclusion, TCBR2 supplementation improved growth performance and attenuated adverse clinical symptoms in rabbits.

1. Introduction

Rabbit meat is highly nutritious and healthy, containing high levels of polyunsaturated fatty acids, proteins, and essential amino acids, which benefit the human diet[1]. Rabbit meat production primarily occurs in Asia, Europe, the Americas, and Africa, with Asia accounting for 70% of global production. China leads the Asian rabbit meat market [2]. The first 10–15 days after weaning are the most critical period of postnatal development for rabbits. During this period, weaned rabbits are susceptible to gastrointestinal infections, leading not only to increased mortality, but also to growth retardation and consequently severe economic losses [3]. Especially, the implementation of the “Prohibition of Antibiotic Use,” policy has worsened health issues in Chinese rabbit farms [4, 5]. Therefore, nutritional regulation through exogenous supplements, aiming to promote the healthy development of the livestock and poultry industry, has become a research focus [6–9].

Traditional Chinese medicine, natural substances known for their safety, simple preparation, affordability, and minimal side effects [10, 11], has been widely used to prevent and treat various diseases in humans and animals, including as herbal antimicrobial, anti-inflammatory, antiparasitic, and antidiarrheal agents [12–15]. Several studies have suggested that herbal ingredients in animal feed can promote growth, intestinal integrity, antioxidant effects, nutrient absorption, and immunity [16–20]. *Trollius chinensis Bunge*, a perennial herb of the buttercup family, has high medicinal value owing to its anti-inflammatory, antioxidant, antibacterial, and antiviral properties. These benefits are attributed to its rich bioactive compounds, such as flavonoids, organic acids, and alkaloids, evaluated the toxicity of T. chinensis, confirming its low toxicity and safety in animal feed [21–24]. *Trollius chinensis Bunge* residues (TCBR), byproducts of the plant’s extraction process, can cause environmental pollution if not properly managed.

A previously study shown that Xiasangju residues supplementation on diets significantly reduced the average daily feed intake and the diarrhea score in weaned piglets, moreover, Xiasangju residues improves intestinal health [25]. In addition, Chen et al. also found that dietary Isatidis Root residue alleviated diarrhea in weaned piglets and improved gut function and microbial compositions. Traditional Chinese medicine residue fermentation broth inhibited porcine origin K. pneumoniae Capsular polysaccharide (CPS) production and downregulated transcription of CPS-related biosynthesis genes [26]. However, there are no reports on the use of TCBR in meat-rabbit breeding.

This study aimed to investigate the effects of TCBR supplementation on the growth performance, slaughter performance, meat quality, serum biochemical indexes, liver function, and cecal microbiota of weaned rabbits, providing a theoretical basis for using TCBR as a feed additive to promote healthy rabbit breeding.

2. Results

2.1 Growth performance

TCBR2 significantly increased the BW of rabbits (13.4%) compared with those in the Mock group ($P < 0.05$). However, the TCBR4 and TCBR6 groups did not show significant differences (Fig. 1A). TCBR2 also improved the rabbit survival rate and reduced their adverse clinical manifestations (diarrhea and death; Fig. 1B and C). The Mock group had the highest diarrhea index (Fig. 1B), whereas the TCBR6 group had the highest mortality rate (33.3%) (Fig. 1C).

Compared with that in the Mock group, TCBR2 increased the BW, average daily BW gain, and feed conversion ratio of rabbits between 37 and 72 days of age ($P < 0.05$; Table 1). However, TCBR4 and TCBR6 showed no notable benefits.

Table 1

Effects of *Trollius chinensis Bunge* residue supplementation on the growth performance of rabbits[1]

Item	Mock[2]	TCBR2	TCBR4	TCBR6	SEM	*P*-value
Initial BW, g	850	837.5	779.2	875	0.02	0.19
BW, g	2371.4[a]	2688.9[b]	2514.3	2250.0[a]	0.06	0.01
Average daily gain, g	42.0[a]	52.9[b]	49.6	39.6 [a]	1.89	0.01
Average daily feed intake, g	151.8	147.9	145.3	156.7	2.58	0.43
Feed conversion ratio	3.7[a]	2.7[b]	3.0	3,8	0.18	0.02

[1]Values are means of eight replicate samples.

[2]The mock group was fed basal diet; TCBR2, TCBR4, and TCBR6 indicate that 2, 4, and 6% of the *Trollius chinensis Bunge* residue supplementation on the control diet, respectively.

[ab] Different superscript letters between treatments indicate significant differences at $P < 0.05$.

2.2 Slaughter performance

TCBR2 significantly increased carcass yield and half-eviscerated carcass yield by 3.1% and 4.1%, respectively, compared with the Mock group ($P < 0.05$; Table 2). No significant differences were observed in the carcass yield and half-eviscerated carcass yield between the TCBR4 and TCBR6 groups ($P > 0.05$). Furthermore, the gastric, small intestine, and appendiceal indexes were notably lower in the TCBR2 group compared with the Mock group ($P < 0.05$). Interestingly, TCBR2 significantly improved the spleen and liver indexes ($P < 0.05$).

Table 2

Effects of *Trollius chinensis Bunge* residues on the carcass traits and organ indexes of rabbits[1].

Item	Mock[2]	TCBR2	TCBR4	TCBR6	SEM	*P*-value
Carcass yield, %	81.8[a]	84.9[b]	83.0	81.7[a]	0.44	0.01
Semi-eviscerated carcass yield, %	56.4[a]	60.5[b]	59.6	58.3	0.70	0.03
Eviscerated carcass yield, %	47.0	49.3	48.8	47.8	0.67	0.67
Gastric index, %	12.4[a]	11.4[b]	11.2[b]	10.8[b]	0.19	0.003
Small intestine index, %	49.5[a]	40.2[b]	40.4[b]	38.0[b]	2.55	0.001
Appendiceal index, %	32.6[a]	29.3[b]	25.5[b]	26.1[b]	0.86	0.001
Splenic index,	0.7[a]	0.8[b]	0.6	0.6	0.03	<0.001
Liver index, %	38.5[a]	46.3[b]	44.4	36.4[a]	1.42	0.021

[1]Values are means of eight replicate samples.

[2]The mock group was fed basal diet; TCBR2, TCBR4, and TCBR6 indicate that 2, 4, and 6% of the *Trollius chinensis Bunge* residue supplementation on the control diet, respectively.

[ab] Different superscript letters between treatments indicate significant differences at $P < 0.05$.

2.3 Meat quality index

TCBR2 decreased the drip loss rate in muscle by 5.1%, compared to the Mock group (Table 3). In the dorsal muscle, TCBR2 reduced the pH value, although there was no significant change in muscle tenderness. However, TCBR6 significantly reduced the tenderness index ($P < 0.05$). In the leg muscle, TCBR2 and TCBR4 supplementation decreased the pH index, but no significant differences in tenderness were observed among treatments.

Table 3

Effects of *Trollius chinensis Bunge* residues on the muscle quality traits of rabbits[1].

Item	Mock[2]	TCBR2	TCBR4	TCBR6	SEM	*P*-value
Drip loss/%	59.1[b]	54.0[a]	63.2[b]	58.2[b]	0.93	<0.001
Dorsal muscle						
pH index	6.25[a]	6.07[b]	6.17	6.32[a]	0.03	0.007
Tenderness	28.03	30.08[a]	22.84	21.93[b]	1.19	0.018
Thigh muscle						
pH index	6.66[a]	6.34[b]	6.42[b]	6.44	0.04	0.006
Tenderness	33.51	30.94	29.02	29.40	1.21	0.039

[1] Values are means of eight replicate samples.

[2]The mock group was fed basal diet; TCBR2, TCBR4, and TCBR6 indicate that 2, 4, and 6% of the *Trollius chinensis Bunge* residue supplementation on the control diet, respectively.

[ab] Different superscript letters between treatments indicate significant differences at $P < 0.05$.

2.4 Serum biochemistry

Different levels of TCBR supplementation reduced the serum glucose levels in 72-day-old rabbits (Table 4). TCBR2 and TCBR4 lowered the activities of ALP and ALT, as well as the blood urea nitrogen concentration in serum, compared with the Mock treatment. TCBR2 also increased the levels of total protein compared with the Mock treatment. Additionally, TCBR6 significantly increased serum total cholesterol concentration ($P < 0.05$).

Table 4

Effects of *Trollius chinensis Bunge* residues on the serum biochemical indexes of rabbits[1].

Item	Mock2	TCBR2	TCBR4	TCBR6	SEM	*P*-value
GLU, mmol/L	10.2[a]	6.2[b]	7.1[b]	7.4[b]	0.44	<0.001
TG, mmol/L	1.1	0.4[a]	0.8	1.8[b]	0.18	0.015
T-CHO, mmol/L	0.7[a]	1.0[a]	1.1[a]	2.1[b]	0.16	0.001
ALB, g/L	15.5	19.2	18.0	18.3	0.63	0.197
ALT, U/L	24.0[a]	11.3[b]	13.8[b]	26.0	1.95	<0.001
BUN, mmol/L	9.2[a]	5.6[b]	5.7[b]	10.5	0.63	<0.001
ALP, U/ml	16.2[a]	9.9[b]	11.1[b]	20.8	1.42	0.007
TP, g/L	21.2[a]	22.1[b]	20.0	20.4	0.24	<0.001

[1] Values are means of eight replicate samples.

[2]The mock group was fed basal diet; TCBR2, TCBR4, and TCBR6 indicate that 2, 4, and 6% of the *Trollius chinensis Bunge* residue supplementation on the control diet, respectively.

[ab] Different superscript letters between treatments indicate significant differences at $P < 0.05$.

2.5 Antioxidant capacity

TCBR4 and TCBR6 significantly increased CAT activity in serum ($P < 0.05$; Table 5). However, no significant differences were observed in the serum activities of SOD, GSH-Px, and MDA among treatments. In the liver, Different levels of TCBR supplementation increased SOD activity, with TCBR6 also enhancing GSH-Px activity. But TCBR2 and TCBR4 decreased the concentration of MDA in the liver. However, TCBR supplementation had no effect on CAT activity in the liver.

Table 5

Effects of *Trollius chinensis Bunge* residues on the antioxidant capacity of rabbits[1].

Item	Mock[2]	TCBR2	TCBR4	TCBR6	SEM	*P*-value
Serum						
SOD, U/ml	13.8	14.2	13.6	13.5	0.22	0.681
GSH-Px, U/ml	596.5	714.8	853.8	519.7	56.68	0.171
CAT, U/ml	2.3[a]	1.9	4.4[b]	4.9[b]	0.40	0.002
MDA, nmol/ml	0.09	0.10	0.12	0.11	0.01	0.026
Liver						
SOD, U/mg protein	69.8[a]	389.3[b]	411.9[b]	458.3[b]	56.45	0.043
GSH-Px, U/mg protein	26.4[a]	28.0	27.2	31.4[b]	0.74	0.049
CAT, U/mg protein	2.7	3.2	3.3	2.6	0.14	0.182
MDA, nmol/mg protein	0.60[a]	0.29[b]	0.27[b]	0.64	0.46	<0.001

[1] Values are means of eight replicate samples.

[2]The mock group was fed basal diet; TCBR2, TCBR4, and TCBR6 indicate that 2, 4, and 6% of the *Trollius chinensis Bunge* residue supplementation on the control diet, respectively.

[ab] Different superscript letters between treatments indicate significant differences at $P < 0.05$.

2.6 Jejunal morphology

The jejunal morphology of tested rabbits is shown in Fig. 2. Compared with Mock rabbits, those supplemented with TCBR2 showed significantly increased villus height and V/C ratio ($P < 0.001$; Fig. 2A). However, no significant differences were found in villus height or the V/C ratio with TCBR4 and TCBR6 supplementation ($P > 0.05$) compared with the Mock treatment. Jejunal sections, stained with hematoxylin and eosin, are shown in Fig. 2B.

2.7 Cecal microbiota

In total, 8081 ASV were obtained through sequencing, with the number of operational taxonomic units decreasing as TCBR concentration increased (Fig. 3). ASV Venn analysis identified 2 174, 2 169, 1 948, and 451 unique ASV in the Mock, TCBR2, TCBR4, and TCBR6 groups, respectively (Fig. 3A). Compared with the Mock group, both the ACE and Chao1 indexes were reduced in the TCBR6 groups ($P < 0.05$). The Shannon and Simpson indexes were lower in all TCBR-treated groups relative to the Mock group ($P <$

0.05). Principal coordinate analysis indicated a progressive shift in the microbial community with increasing dietary TCBR levels (Fig. 3C).

TCBR2 and TCBR4 supplementation reduced the relative abundance of the cecal Firmicutes phylum while increasing the relative abundance of the cecal Bacteroidota phylum ($P < 0.0001$; Fig. 4). TCBR2 also increased the relative abundance of the cecal Verrucomicrobiota phylum ($P < 0.0001$). All TCBR levels increased the relative abundance of the cecal Akkermansia genus ($P < 0.001$). TCBR2 further increased the relative abundance of the cecal Clostridium, Alistipes, and Succiniclasticum genera ($P < 0.05$), whereas TCBR4 reduced the relative abundance of the cecal Clostridium genus ($P < 0.05$).

2.8 Gene expression

The effects TCBR supplementation on gene transcript levels in the jejunal tissue of rabbits are shown in Fig. 5. Compared with the Mock treatment, TCBR2 supplementation significantly upregulated the expression levels of occludin and ZO-1 but had no significant effect on claudin-1 expression. In contrast, TCBR4 and TCBR6 supplementation downregulated the expression levels of occludin, ZO-1, and claudin-1 in the jejunal tissue. Additionally, TCBR2 and TCBR4 supplementation significantly downregulated the relative expression levels of TNF-α and IL-8 ($P < 0.05$).

3. Discussion

Rabbits are considered ideal meat-producing animals owing to their short life cycle, short gestation period, high reproductive rates, and efficient feed conversion. As an important source of meat protein, the rabbit farming industry is receiving increasing attention [1]. However, gut health of weaned rabbits is important for rabbit growth, with the growing emphasis on reducing antibiotic use in the livestock industry, there are higher demands for the sustainable and healthy development of rabbit farming. Traditional Chinese medicine plays a role in promoting animal health and improving production efficiency, and its byproducts, i.e., residues left after the extraction of active herbal ingredients, may still contain active compounds beneficial to livestock [9, 27, 28]. A previous study showed that adding turmeric residue improved survival rates in Chinese soft-shelled turtles exposed to high ambient temperatures [29]. He et al. found that supplementing Shengxuebao herbal residues in the diets of heat-stressed New Zealand rabbits increased BW [30]. Similarly, our study found that adding 2% TCBR significantly increased final BW, reduced adverse clinical symptoms, and improved survival rates in Japanese white rabbits under standard rearing conditions. TCBR2 also significantly increased the average daily BW gain of the rabbits. These effects may be linked to the presence of bioactive substances, such as brassinoids, organic acids, and alkaloids, in TCBR [21]. However, high dose of *Trollius chinensis Bunge* residues, with 6% into rabbits diet, showed adverse clinical syndrome, implying a toxic side effect. The study had found that flavonoids produced cellular damage at 450 μM cellular viability, isolation of guinea pig enterocytes, decreased 12–60% and LDH leakage 28–41% greater [31]. In addition, The total flavonoids (TFs) from Rosa laevigata Michx fruit could cause side effects at the dose of 2000 mg/kg/day in males and females, such as increased intercellular space of myocardial cells and

the relative cardiac weight [32]. Therefore, our study suggested that supplementation of 2% TCBR may be a relatively safe dose in rabbit basal diets.

Slaughter rate is a key indicator of growth and performance. A previous study found that supplementation with traditional Chinese medicine significantly increased the slaughter and evisceration rates of broiler chickens [33]. Similarly, research on Eucommia ulmoides polysaccharides found that adding them to diets improved growth and carcass performance in Songliao black pigs [34]. In our study, TCBR2 supplementation significantly increased the carcass and semi-eviscerated carcass yield in rabbits, consistent with findings from previous studies.

Blood biochemical markers, such as sugars, fats, and proteins, provide insight into an animal's growth, nutrient digestion, absorption, and metabolism. For instance, albumin, total protein, and urea nitrogen levels reflect protein and amino acid utilization and metabolism, whereas blood glucose levels indicate an animal's glucose regulation and overall health, and triglyceride and total cholesterol levels suggest the efficiency of fat metabolism [35, 36]. Our study revealed that TCBR2 supplementation significantly increased serum total protein levels while decreasing urea nitrogen and triglyceride levels. Moreover, TCBR supplementation lowered serum glucose levels. Chen et al. reported that the Chinese medicine Tongxinluo significantly reduced lipid levels in rabbits [37]. However, Li et al. found no effect of Ligustrum lucidum supplementation on glucose, total protein, or albumin levels in Hy-Line Brown hens during the late laying period [38], which may be attributed to differences in the animal species and herbs used.

ALT and ALP are key markers of liver function [39]. TCBR2 supplementation in rabbits reduced serum ALT and ALP levels but significantly increased the liver index and liver SOD activity; however, it had no significant effect on GSH-Px and catalase levels. A previous study showed that supplementing with Ilicis Chinensis folium extract significantly reduced ALT levels and increased SOD activity in broiler chickens [40]. Similarly, acidic polysaccharides from Schisandra chinensis significantly reduced ALT levels and increased SOD activity in cases of liver injury [41], aligning with the results of our study. Furthermore, supplementation with 4% and 6% TCBR significantly increased serum catalase levels. These results imply that TCBR possess antioxidant properties.

As a crucial organ for nutrient absorption, the intestines play a key role in maintaining overall health through their structure, mucosal barrier, and microbial composition. Our study demonstrated that supplementing with TCBR significantly reduced the digestive organ indices of rabbits, including the gastric, small intestinal, and appendiceal indexes. We hypothesize that components within TCBR can regulate intestinal flora and modify the intestinal index. Previous research has shown that various compounds, such as patchouli alcohol, luteolin, and other therapeutic agents, can regulate gut flora and reduce intestinal inflammation and damage [42–44]. Our findings suggested that TCBR2 supplementation significantly increased jejunal villus height and decreased crypt depth compared with the Mock treatment. The intestinal barrier, consisting of microbial, mechanical, and chemical components, plays a key role in preventing harmful substances from entering the bloodstream and

maintaining the body's internal stability [45]. TCBR supplementation also influenced the intestinal flora of rabbits. Notably, TCBR2 and TCBR4 supplementation significantly increased the relative abundance of Bacteroidetes at the phylum level, whereas TCBR6 supplementation had the opposite effect. Additionally, TCBR2 supplementation significantly increased the relative abundance of the Verrucomicrobiota phylum, particularly the Akkermansia genus. Niu et al. found that Pulsatilla decoction alleviated clinical symptoms and increased the relative abundance of Mycobacterium and Clostridium in DSS-induced colitis models [46]. Similarly, Yu et al. demonstrated the significant regulatory impact of Ginkgo biloba extract on intestinal microflora and microbial metabolism in Alzheimer's disease model mice, reducing the Firmicutes/Bacteroides ratio and increasing Bacteroidetes abundance [47]. Importantly, TCBR2 supplementation in our study significantly upregulated the relative expression of occludin-1 and ZO-1 while downregulating the relative expression of TNF-αand IL-8. The tight junction proteins occludin and ZO-1 are vital for maintaining the integrity of the intestinal mucosa [45]. Modified Gegen Qinlian decoction has been shown to improve symptoms and pathological damage in ulcerative colitis model mice by upregulating occludin and ZO-1 expression [48]. Mulberry extract was found to attenuate colonic damage and inflammation, suppress colonic oxidative stress, and restore intestinal tight junction protein expression in mice with DSS-induced colitis [19]. Our results are consistent with these findings, suggesting that TCBR2 enhances intestinal barrier function.

In conclusion, the supplementation TCBR2 in the diets of weaned rabbits alleviated adverse clinical symptoms and improved survival rates, growth performance, and meat quality. These benefits may be attributed to enhanced antioxidant activity in the liver, improved jejunal morphology, and a higher relative abundance of beneficial intestinal flora. These findings highlight the potential of TCBR as an effective feed additive in rabbit production.

4. Material and methods

4.1 Experimental design, diets, growth performance, and clinical records

In total, 48 weaned (30-days-old) weaned rabbits were used in a 42-day experiment. Each rabbit was housed individually, with each cage representing a replicate experimental unit. After assessing their initial body weight (BW), the weaned rabbits were randomly assigned to one of four dietary groups (n = 12/group), with TCBR added to their diets as follows: (1) Mock without TCBR, (2) TCBR2 containing 2% TCBR, (3) TCBR4 containing 4% TCBR, and (4) TCBR6 containing 6% TCBR. The basal diet, containing corn and soybean meal, was formulated to meet or exceed the nutrient requirements for growing rabbits. The ingredients and proximate composition of the basic diets are provided in Table 6. Rabbits had free access to feed and water without any vaccinations during the experiment. Individual feed intake was recorded daily, and BW was recorded once every five days daily feed intake, body weight gain, and feed conversion ratio (FCR = feed intake/ body weigh gain) were calculated. Clinical scores were based on the Vesikari rating system with slight modifications [49]. A five-point scale from 1 to 5 was used, reflecting

normality (0 score), non-feeding (1 score), mild diarrhea (2 score), severe diarrhea (3 score), and death (5 score).

Table 6
Composition and nutrient content of the diet for rabbits aged 30–72 days (as-fed basis).

Item	Value
Ingredients	
Corn, %	28.0
Soybean meal, %	12.0
NaCl, %	0.3
Trace element, %	0.5
$CaHPO_4.2H_2O$, %	1.8
Multi-vatimins, %	0.05
choline chloride, %	0.2
lucerne, %	36.0
Semipowder, %	17.0
Rice brane, %	3.2
vegetable oil, %	0.5
talcum powder, %	0.5
Total, %	100.5
Nutrition contents[1]	
Moisture, g/kg	72.0
Crude protein, g/kg	170.5
Crude fat, g/kg	30.0
Crude fiber, g/kg	142.0
Crude ash, g/kg	81.0
Calcium, g/kg	10.4
Total phosphorus, g/kg	5.6

[1] Values were derived from the Chinese Nutrient Requirements of Rabbits [50].

4.2 Sample collection and calculation of slaughter performance

After 42 days of feeding, the rabbits were fasted overnight before sampling and slaughter. The rabbits were sacrificed via intravenous air injection at the ear margin. Blood samples were collected via heart puncture, centrifuged to collect serum, and stored at − 20°C for further analysis. Eight rabbits from each group were selected to assess slaughter performance. The rabbits were sequentially skinned, limbed, decapitated, and eviscerated. The following measurements were recorded: carcass weight, semi-eviscerated carcass weight, eviscerated carcass weight, gastric weight, small intestine weight, appendiceal weight, spleen weight, and liver weight. Carcass traits and organ indexes were calculated relative to live BW. Index = weight of Carcass traits or organ / live body weight.

4.3 Evaluation of meat quality

A portable pH meter (Crison MicropH 2001) was used to measure the pH of the dorsal and thigh muscles. Muscle tenderness was measured following the Warner-Bratzler shear force protocol with slightly revisions [51]. Briefly, the muscle was boiled to an internal temperature of 80°C (measured using a thermometer) before being cooled to room temperature. Once cooled, three 1-cm cores were extracted from each muscle, oriented along the muscle fiber direction according to the guidelines in Section VIII. Appendix B.a. (AMSA, 2015). Each core was sheared perpendicular to the muscle fiber direction, and the peak shear forces were recorded. The average peak shear force was calculated for each muscle sample.

Drip loss was measured using the bag method and a standardized sample from the dorsal muscle after slaughter [52]. Briefly, samples were cut into 2.5-cm-thick slices, weighed, suspended in a plastic bag, and stored at 4°C for 48 h. They were then reweighed, and drip loss was calculated as a percentage of weight loss.

4.4 Measurement of serum biochemistry

Serum levels of glucose (GLU), triglyceride (TG), total cholesterol (T-CHO), albumin (ALB), alanine aminotransferase (ALT), blood urea nitrogen (BUN), alkaline phosphatase (ALP), and total protein (TP) were assayed using ELISA kits (F006-1-1, A110-1-1, A111-1-1, A028-2-1, C009-2-1, C013-2-1, A059-2-2, and A045-2-2; Nanjing Jiancheng Institute of Bioengineering, Nanjing, PRC) and a microplate reader (Tecan Austria GmbH, Vienna, Austrian).

4.5 Determination of antioxidant parameters

Serum and liver antioxidant parameters were measured using a previously described method [53]. Briefly, serum samples were directly analyzed, whereas liver samples were homogenized with precooled phosphate-buffered saline (1:10, v/v) and centrifuged (8000 rpm and 4°C for 10 min) to obtain clarified homogenates. The activities of total superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), along with the concentration of malondialdehyde (MDA), were assayed using

ELISA kits (A001-3-2, A007-1-1, A005-1-2, and A003-1-1; Nanjing Jiancheng Institute of Bioengineering) and a spectrophotometer, as described above.

4.6 Morphological observation of the ileum wall

Fixed ileum samples were dehydrated, embedded in overheated paraffin, and cut into 4-μm-thick sections (RM2235, Leica Microsystems, Germany). After drying, dewaxing, and staining with hematoxylin and eosin, the sections were scanned using a Pannoramic Scanner (C13210-01, HAMAMATSU, Japan). Villus height and crypt depth were measured using ImageJ software (version 1.54), and the villus-to-crypt (V/C) ratio was calculated.

4.7 RNA extraction, cDNA synthesis, and quantitative real-time PCR

Tissue RNA was extracted according to methods described in a previous study [54]. Ileum tissues were homogenized with precooled PBS, and the homogenates were lysed using RNA isolator total RNA extraction reagent (Vazyme Biotech, Nanjing, China). RNA concentration was measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, MA, USA). Subsequently, a cDNA template was synthesized from 1000 ng of RNA using the HiScript®II Q RT SuperMix for quantitative PCR (qPCR; + gDNA wiper; Vazyme Biotech). Real-time qPCR was performed on an ABI system (Thermo Fisher Scientific) using the ChamQTM SYBR® qPCR Master Mix (Vazyme Biotech). PCR conditions comprised 40 cycles of 95°C for 5 s, 60°C for 30 s, and 72°C for 60 s. The sequences of the primers used are listed in Table 7. Gene relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Table 7
Primers used for gene expression analysis via qPCR.

Gene	Primer sequence (5'-3')	Accession No.	Product size (bp)
TNF-α	F: AAGGTCAACCTCCTCTCT	NM_001082263	93
	R: CAGGTAGATGGGCTCGTACC		
IL-8	F: TGATGGAAGAGAACTCTGC	NM_001082293	92
	R: ATGACTCTTGCTGCTCAG		
IL-1β	F: GAATTTGAGTCTGCCCAGTT	NM_001082201	104
	R: CCATGCTGAAGTCAATTAGGT		
ZO-1	F: GAGAACAAGAAGGAGGTGAA	NM_100346390	317
	R: CACTGAACTGGCTCTGAG		
Occludin	F: TTGAGCAGCAGCAGTAAC	NM_100338492	427
	R: TGTAGTCCGTCTCGTAGTG		
Claudin-1	F: AATTCGGTCAGGCTCTTT	NM_001089316	96
	R: GAGGACAAGAACAGCAAAG		
GAPDH	F:TTCCCGTTCTCAGCCTTGACC	NM_001082253.1	118
	R:TGCTGATGAGTACAACCGACT		

4.8 Analysis of the cecal microbial community

Total DNA from cecal samples was extracted using the CTAB method according to the manufacturer's instructions. Sequences of the 16S rRNA genes from the V3–V4 hypervariable regions were amplified using the universal primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3'). PCR products were confirmed via 2% agarose gel electrophoresis, purified using AMPure XT beads (Beckman Coulter Genomics, Danvers, MA, USA), and quantified using Qubit (Invitrogen, CA, USA). Amplicon pools were prepared for sequencing, and the size and quantity of the amplicon library were assessed using an Agilent 2100 Bioanalyzer (Agilent, USA) and the Library Quantification Kit for Illumina (Kapa Biosciences, MA, USA), respectively. Libraries were sequenced on the NovaSeq PE250 platform. Paired-end reads were merged using FLASH, filtered, and replicated to obtain the feature table and feature sequence. Alpha and beta diversity were calculated by normalizing to the same sequences randomly. Feature abundance was normalized using the SILVA database (release 138) based on relative sample abundance. Sequence alignment was performed using BLAST, and feature sequences were annotated using the SILVA database for each representative sequence.

4.9 Statistical analysis

The statistical analysis was conducted using SPSS software (version 16.0; SPSS Inc., Chicago, IL, USA). One-way ANOVA was used to determine the differences among the treatments, and Tukey's tests were used to compare the treatments' means. All data were presented as mean ± standard error (S.E.). $P < 0.05$ indicated that the differences were statistically significant.

Abbreviations

ALB	albumin
ALP	alkaline phosphatase
ALT	alanine aminotransferase
BUN	blood urea nitrogen
BW	body weight
CAT	catalase
CTAB	Cetyltrimethylammonium Bromide
FCR	feed conversion ratio
GLU	glucose
GSH-Px	glutathione peroxidase
MDA	malondialdehyde
SOD	superoxide dismutase
TCBR	*Trollius chinensis Bunge* residues
T-CHO	total cholesterol
TG	triglyceride
TP	total protein
V/C	villus-to-crypt
ZO-1	Zona Occludens 1

Declarations

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Author Contributions: Conceptualization, L.C.D., and J.Y.Y.; methodology, Y.D.; software, K.L.H.; validation, X.Z.; formal analysis and investigation, J.N.L.; resources and data curation, X.Y.; writing—original draft

preparation, L.C.D.; writing—review and editing, J.Y.Y.; visualization, D.L.F.; supervision, X.B.H.; project administration, M.X.M.; funding acquisition, J.Y.B. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest: No conflict to declare.

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Institutional Review Board Statement: The animal study protocol was approved by the Institutional Review Board (or Ethics Committee) of the Jilin Genet-Med Biotechnological Co., Ltd. (Number: IACUC-2024-006).

Informed Consent Statement: Not application.

References

1. Cullere, M. & Dalle Zotte, A. Rabbit meat production and consumption: State of knowledge and future perspectives. *Meat Sci.* **143**, 137–146. https://doi.org/10.1016/j.meatsci.2018.04.029 (2018).
2. Li, S. et al. Rabbit meat production and processing in China. *Meat Sci.* **145**, 320–328. https://doi.org/10.1016/j.meatsci.2018.06.037 (2018).
3. Bivolarski, B. L. et al. Morphological and functional events associated to weaning in rabbits. *J. Anim. Physiol. Anim. Nutr.* **98** (1), 9–18. https://doi.org/10.1111/jpn.12058 (2014).
4. Sun, C. et al. Antibiotic resistance spectrums of Escherichia coli and Enterococcus spp. strains against commonly used antimicrobials from commercial meat-rabbit farms in Chengdu City, Southwest China. *Front. veterinary Sci.* **11**, 1369–1655. https://doi.org/10.3389/fvets.2024.1369655 (2024).
5. Elghandour, M. M. Y. et al. Saccharomyces cerevisiae as a probiotic feed additive to non and pseudo-ruminant feeding: a review. *J. Appl. Microbiol.* **128** (3), 658–674. https://doi.org/10.1111/jam.14416 (2020).
6. Jiang, S. Q. et al. Protective effects of protocatechuic acid on growth performance, intestinal barrier and antioxidant capacity in broilers challenged with lipopolysaccharide. *Animal: Int. J. Anim. bioscience*. **17** (1), 100–693. https://doi.org/10.1016/j.animal.2022.100693 (2023).
7. Song, Z. et al. Effects of Dietary Ginsenoside Rg1 Supplementation on Growth Performance, Gut Health, and Serum Immunity in Broiler Chickens. *Front. Nutr.* **8**, 705–279. https://doi.org/10.3389/fnut.2021.705279 (2021).
8. Abdelatty, A. M. et al. Sun-dried Azolla leaf meal at 10% dietary inclusion improved growth, meat quality, and increased skeletal muscle Ribosomal protein S6 kinase β1 abundance in growing rabbit.

Animal: Int. J. Anim. bioscience. **15** (10), 100–348. https://doi.org/10.1016/j.animal.2021.100348 (2021).

9. Abdallah, A. et al. Application of Traditional Chinese Herbal Medicine By-products as Dietary Feed Supplements and Antibiotic Replacements in Animal Production. *Curr. Drug Metab.* **20** (1), 54–64. https://doi.org/10.2174/1389200219666180523102920 (2019).
10. Yu, W. et al. Traditional Chinese Medicine and Constitutional Medicine in China, Japan and Korea: A Comparative Study. *Am. J. Chin. Med.* **45** (1), 1–12. https://doi.org/10.1142/S0192415X1750001X (2017).
11. Su, Y. et al. Prospects for the application of traditional Chinese medicine network pharmacology in food science research. *J. Sci. Food. Agric.* **103** (11), 5183–5200. https://doi.org/10.1002/jsfa.12541 (2023).
12. Huang, K. et al. Traditional Chinese Medicine (TCM) in the treatment of COVID-19 and other viral infections: Efficacies and mechanisms. *Pharmacol. Ther.* **225**, 107–843. https://doi.org/10.1016/j.pharmthera.2021.107843 (2021).
13. Dai, Y. J. et al. Recent advances of traditional Chinese medicine on the prevention and treatment of COVID-19. *Chin. J. Nat. Med.* **18** (12), 881–889. https://doi.org/10.1016/S1875-5364(20)60031-0 (2020).
14. Du, H. Z. et al. Classic mechanisms and experimental models for the anti-inflammatory effect of traditional Chinese medicine. *Anim. models experimental Med.* **5** (2), 108–119. https://doi.org/10.1002/ame2.12224 (2022).
15. Li, P. et al. Discovery of ligustrazine and chalcone derivatives as novel viral nucleoprotein nuclear export inhibitors against influenza viruses. *J. Med. Virol.* **95** (7), e28968. https://doi.org/10.1002/jmv.28968 (2023).
16. Song, X. et al. Traditional chinese medicine prescriptions enhance growth performance of heat stressed beef cattle by relieving heat stress responses and increasing apparent nutrient digestibility. *Asian-Australasian J. Anim. Sci.* **27** (10), 1513–1520. https://doi.org/10.5713/ajas.2014.14058 (2014).
17. Li, D. et al. Modified Sijunzi granule decreases post-weaning diarrhea in Rex rabbits via promoting intestinal development. *Front. veterinary Sci.* **9** https://doi.org/10.3389/fvets.2022.972326 (2022). 972 – 326.
18. Mo, J. et al. Mulberry Anthocyanins Ameliorate DSS-Induced Ulcerative Colitis by Improving Intestinal Barrier Function and Modulating Gut Microbiota. *Antioxid. (Basel Switzerland)*, **11**(9), 16–74. https://doi.org/10.3390/antiox11091674 (2022).
19. Zhu, M. et al. Manipulating Microbiota in Inflammatory Bowel Disease Treatment: Clinical and Natural Product Interventions Explored. *Int. J. Mol. Sci.* **24** (13), 110–104. https://doi.org/10.3390/ijms241311004 (2023).
20. Wang, Y. et al. Antitumor effects of immunity-enhancing traditional Chinese medicine. *pharmacotherapy = Biomedecine pharmacotherapie*, **121**, 109–570.

https://doi.org/10.1016/j.biopha.2019.109570 (2020).

21. He, L. et al. Trollius chinensis Bunge: A Comprehensive Review of Research on Botany, Materia Medica, Ethnopharmacological Use, Phytochemistry, Pharmacology, and Molecules (Basel, Switzerland), 29(2), 421. (2024). https://doi.org/10.3390/molecules29020421
22. Guo, L. et al. Investigation of the effective components of the flowers of Trollius chinensis from the perspectives of intestinal bacterial transformation and intestinal absorption. *Pharm. Biol.* **55** (1), 1747–1758. https://doi.org/10.1080/13880209.2017.1321023 (2017).
23. Yang, K. et al. A Comprehensive Research Review of Herbal Textual Research, Phytochemistry, Pharmacology, Traditional Uses, Clinical Application, Safety Evaluation, and Quality Control of Trollius chinensis Bunge. *Pharmaceuticals (Basel Switzerland)*. **17** (6), 800. https://doi.org/10.3390/ph17060800 (2024).
24. Sun, W. et al. Effects of dietary traditional Chinese medicine residues on growth performance, intestinal health and gut microbiota compositions in weaned piglets. *Front. Cell. Infect. Microbiol.* **13**, 1283–1789. https://doi.org/10.3389/fcimb.2023.1283789 (2023).
25. Chen, Z. et al. Dietary Isatidis Root Residue Improves Diarrhea and Intestinal Function in Weaned Piglets. *Animals: open. access. J. MDPI*. **14** (19), 27–76. https://doi.org/10.3390/ani14192776 (2024).
26. Niu, K. et al. Stepwise co-fermented traditional Chinese medicine byproducts improve antioxidant and anti-inflammatory effects in a piglet model. *J. Sci. Food. Agric.* **104** (2), 1166–1177. https://doi.org/10.1002/jsfa.13002 (2024).
27. Yu, F. et al. Dregs of Cardamine hupingshanensis as a feed additive to improve the egg quality. *Front. Nutr.* **9**, 915865. https://doi.org/10.3389/fnut.2022.915865 (2022).
28. Chen, Y. et al. Supplementation with turmeric residue increased survival of the Chinese soft-shelled turtle (Pelodiscus sinensis) under high ambient temperatures. *J. Zhejiang Univ. Sci. B*. **19** (3), 245–252. https://doi.org/10.1631/jzus.B1600451 (2018).
29. He, Y. et al. Anti-oxidant effects of herbal residue from Shengxuebao mixture on heat-stressed New Zealand rabbits. *J. Therm. Biol*. **119**, 103–752. https://doi.org/10.1016/j.jtherbio.2023.103752 (2024).
30. Canada, A. T. et al. The toxicity of flavonoids to guinea pig enterocytes. *Toxicol. Appl. Pharmcol.* **99** (2), 357–361. https://doi.org/10.1016/0041-008x(89)90018-5 (1989).
31. Zhang, S. et al. Subchronic toxicity study of the total flavonoids from Rosa laevigata Michx fruit in rats. *Regul. Toxicol. pharmacology: RTP*. **62** (2), 221–230. https://doi.org/10.1016/j.yrtph.2011.12.009 (2012).
32. Xie, Z. et al. Effect of Chinese herbal medicine treatment on plasma lipid profile and hepatic lipid metabolism in Hetian broiler. *Poult. Sci.* **96** (6), 1918–1924. https://doi.org/10.3382/ps/pew456 (2017).
33. Liang, Y. et al. Effect of dietary Eucommia ulmoides oliver polysaccharide on immune function and meat quality of Songliao Black Pigs. *Sci. Rep.* **14** (1), 139–101. https://doi.org/10.1038/s41598-024-

64257-4 (2024).

34. Tang, H. et al. Genome-wide association study reveals the genetic determinism of serum biochemical indicators in ducks. *BMC Genom.* **23** (1), 856. https://doi.org/10.1186/s12864-022-09080-9 (2022).
35. Zhang, Q. Q. et al. Dietary calcium and non-phytate phosphorus levels affect the performance, serum biochemical indices, and lipid metabolism in growing pullets. *Poult. Sci.* **102** (2), 102–354. https://doi.org/10.1016/j.psj.2022.102354 (2023).
36. Chen, W. Q. et al. Chinese medicine tongxinluo significantly lowers serum lipid levels and stabilizes vulnerable plaques in a rabbit model. *J. Ethnopharmacol.* **124** (1), 103–110. https://doi.org/10.1016/j.jep.2009.04.009 (2009).
37. Li, X. L. et al. Effect of dietary supplementation of Ligustrum lucidum on performance, egg quality and blood biochemical parameters of Hy-Line Brown hens during the late laying period. *Animal: Int. J. Anim. bioscience*. **11** (11), 1899–1904. https://doi.org/10.1017/S1751731117000532 (2017).
38. Laker, M. F. Liver function tests. BMJ (Clinical research ed.), **301**(6746), 250–251. (1990). https://doi.org/10.1136/bmj.301.6746.250
39. Zhong, Y. et al. Effects of Ilicis Chinensis folium extract supplementation on growth performance, serum parameters, intestinal morphology, and antioxidant capacity of broiler chickens. *BMC Vet. Res.* **19** (1), 94. https://doi.org/10.1186/s12917-023-03667-4 (2023).
40. Yuan, R. et al. Protective effect of acidic polysaccharide from Schisandra chinensis on acute ethanol-induced liver injury through reducing CYP2E1-dependent oxidative stress. *Biomed. pharmacotherapy = Biomedecine pharmacotherapie*. **99**, 537–542. https://doi.org/10.1016/j.biopha.2018.01.079 (2018).
41. Wu, J. et al. Patchouli alcohol attenuates 5-fluorouracil-induced intestinal mucositis via TLR2/MyD88/NF-kB pathway and regulation of microbiota. *Biomed. pharmacotherapy = Biomedecine pharmacotherapie*. **124**, 109–883. https://doi.org/10.1016/j.biopha.2020.109883 (2020).
42. Li, B. et al. Luteolin alleviates inflammation and modulates gut microbiota in ulcerative colitis rats. *Life Sci.* **269**, 119–008. https://doi.org/10.1016/j.lfs.2020.119008 (2021).
43. Wang, L. et al. Therapeutic effects of paeoniflorin on irritable bowel syndrome in rats. *J. Vet. Sci.* **24** (3), e23. https://doi.org/10.4142/jvs.22083 (2023).
44. Che, Q. et al. Mechanisms by Which Traditional Chinese Medicines Influence the Intestinal Flora and Intestinal Barrier. Frontiers in cellular and infection microbiology, 12, 863 – 779. (2022). https://doi.org/10.3389/fcimb.2022.863779
45. Niu, C. et al. Pulsatilla decoction improves DSS-induced colitis via modulation of fecal-bacteria-related short-chain fatty acids and intestinal barrier integrity. *J. Ethnopharmacol.* **300**, 115–741. https://doi.org/10.1016/j.jep.2022.115741 (2023).
46. Yu, T. et al. Ginkgo biloba Extract Drives Gut Flora and Microbial Metabolism Variation in a Mouse Model of Alzheimer's Disease. *Pharmaceutics* **15** (12), 27–46.

https://doi.org/10.3390/pharmaceutics15122746 (2023).

47. Wang, Y. et al. Modified Gegen Qinlian decoction ameliorated ulcerative colitis by attenuating inflammation and oxidative stress and enhancing intestinal barrier function in vivo and in vitro. *J. Ethnopharmacol.* **313**, 116–538. https://doi.org/10.1016/j.jep.2023.116538 (2023).
48. Chen, L. et al. Inhibition of porcine origin Klebsiella pneumoniae capsular polysaccharide and immune escape by BY3 compounded traditional Chinese medicine residue fermentation broth. *Microb. Pathog.* **195**, 106–853. https://doi.org/10.1016/j.micpath.2024.106853 (2024).
49. Kabue, J. P. et al. Norovirus-Associated Gastroenteritis Vesikari Score and Pre-Existing Salivary IgA in Young Children from Rural South Africa. *Viruses* **15** (11), 21–85. https://doi.org/10.3390/v15112185 (2023).
50. Ministry of Agriculture of the People's Republic of China (PRC). *Nutrition requirement of meat rabbit* (China Agriculture, 2021).
51. Heimbuch, M. L. et al. Evaluation of growth, meat quality, and sensory characteristics of wool, hair, and composite lambs. *J. Anim. Sci.* **101**, skad076. https://doi.org/10.1093/jas/skad076 (2023).
52. Kayan, A. & Koomkrong, N. Expression levels of filaggrin-2 in relation to drip loss in pigs. *Anim. bioscience*. **35** (4), 624–630. https://doi.org/10.5713/ab.21.0220 (2022).
53. Jiang, S. Q. et al. Protective effects of protocatechuic acid on growth performance, intestinal barrier and antioxidant capacity in broilers challenged with lipopolysaccharide. *Animal: Int. J. Anim. bioscience*. **17** (1), 100–693. https://doi.org/10.1016/j.animal.2022.100693 (2023).
54. Deng, L. et al. Interference of pseudorabies virus infection on functions of porcine granulosa cells via apoptosis modulated by MAPK signaling pathways. *Virol. J.* **21** (1), 25. https://doi.org/10.1186/s12985-024-02289-y (2024).

Figures

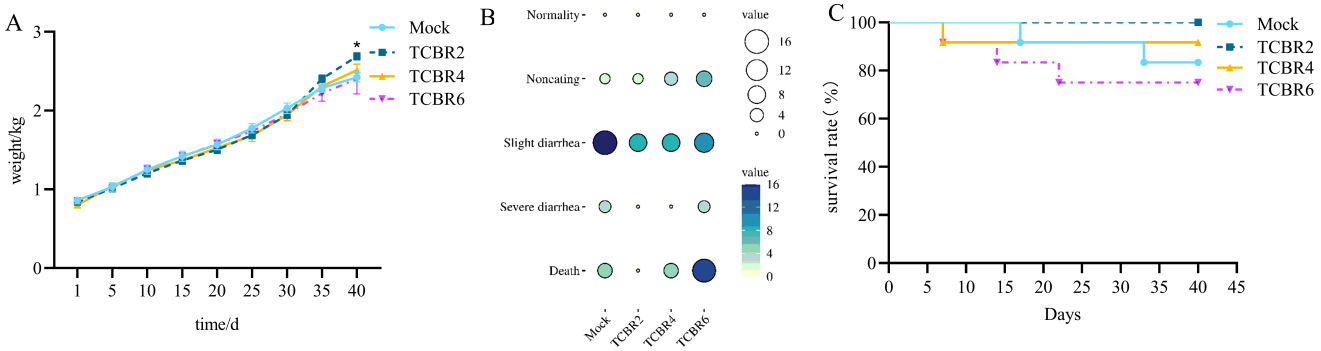

Figure 1

Clinical condition documentation for rabbits. (A) Recorded body weights of rabbits. Values represent means of eight replicate samples. *P < 0.05 compared to the Mock group. (B) Clinical performance score statistics: scores range from 0 to 5, with higher scores indicating more severe disease; darker colors represent more disease cases. (C) Survival rates of rabbits.

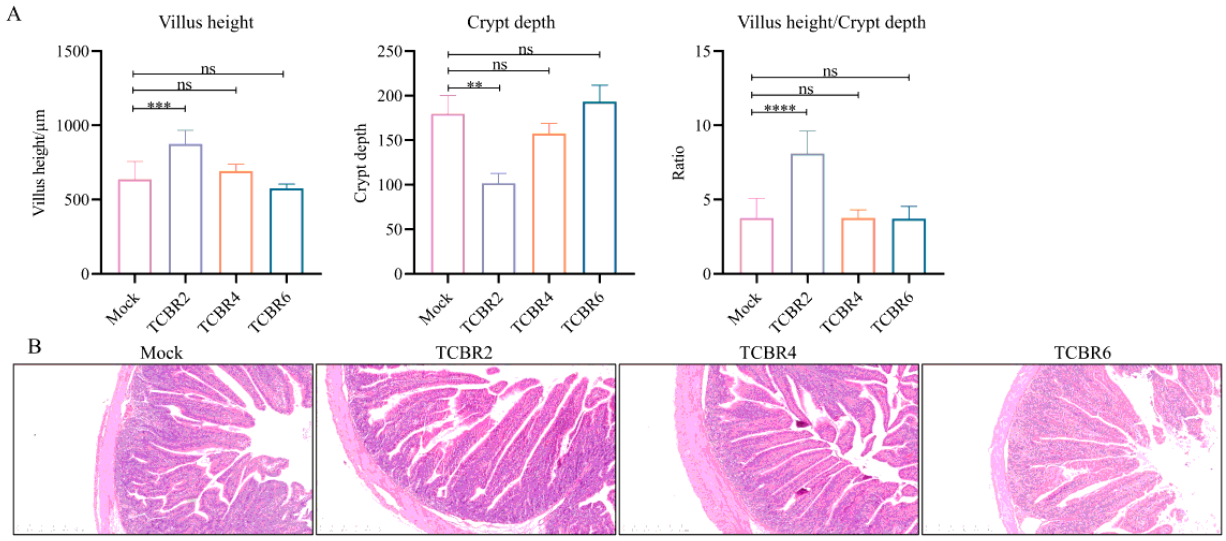

Figure 2

Effects of *Trollius chinensis Bunge* residue supplementation on the jejunal morphology of rabbits. (A) Villus height, crypt depth, and the villus-to-crypt ratio among treatments. Values represent means of eight replicate samples. *P < 0.05, **P < 0.01, ***P< 0.001, and ****P < 0.0001 compared to the Mock group. (B) Representative images of hematoxylin and eosin–stained jejunal tissue. Scale bar: 500 μm.

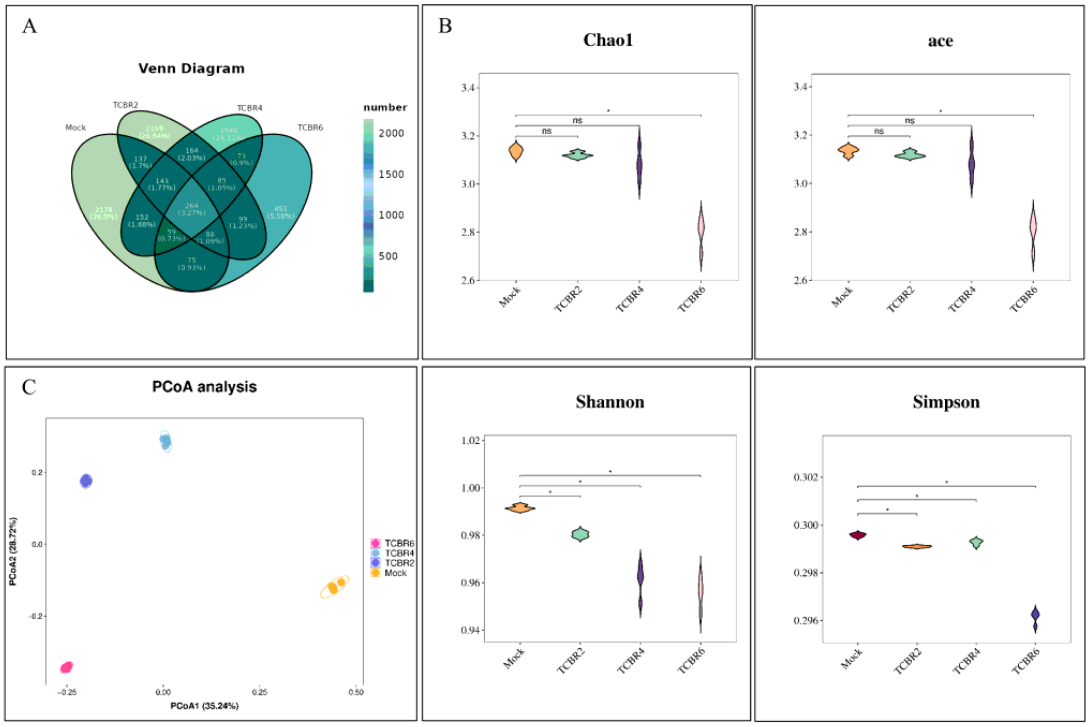

Figure 3

Cecal microbiota richness and diversity. (A) ASV Venn diagram. (B) Comparison of alpha diversity indexes presented as box plots (median, first, and third quartiles). *Significant difference at $P < 0.05$. The Mock group was fed a basal diet; TCBR2, TCBR4, and TCBR6 indicate diets supplemented with 2, 4, and 6% *Trollius chinensis Bunge* residues, respectively.

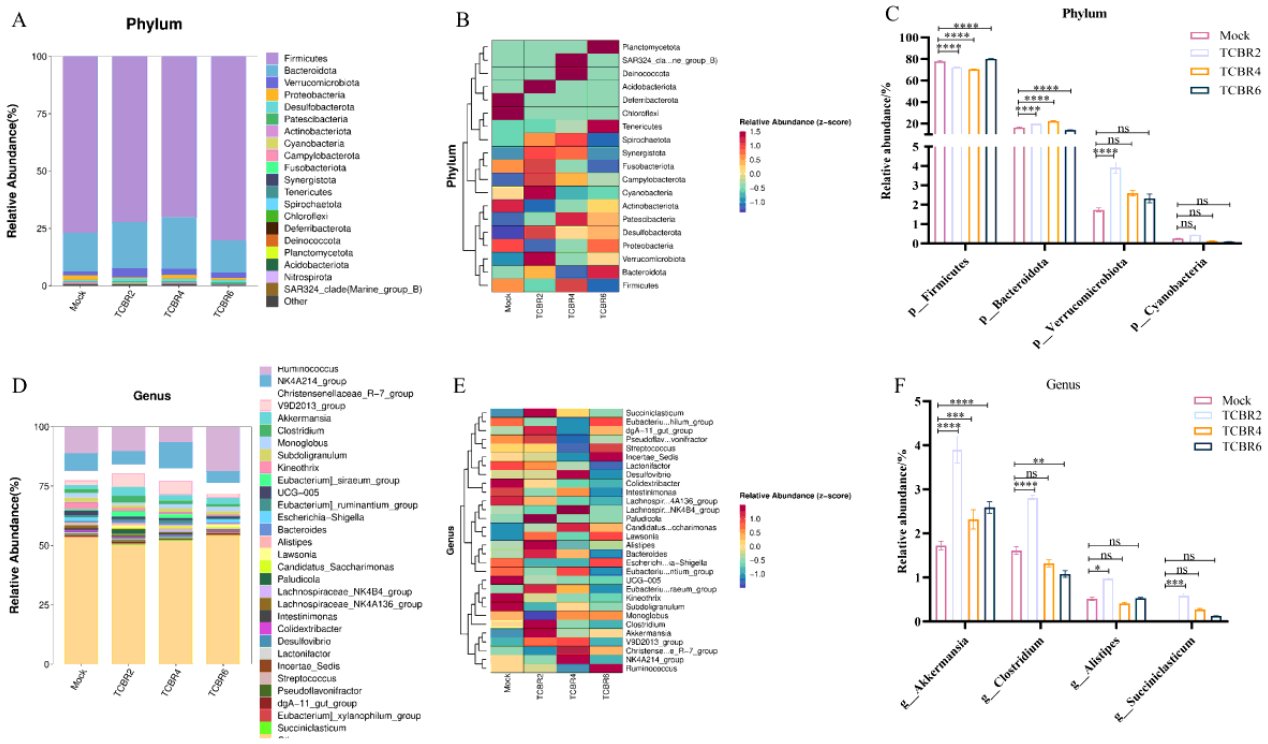

Figure 4

Relative abundance and differences in cecal microbiota. (A) Relative abundance of the top 20 phyla. (B) Heatmap of the top 20 phyla. (C) Differences in microbiota composition at the phylum levels. (D) Relative abundance of the top 30 genera. (E) Heatmap of the top 30 genera. (F) Differences in microbiota composition at the genus levels. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 compared to the Mock group. The Mock group was fed a basal diet; TCBR2, TCBR4, and TCBR6 indicate diets supplemented with 2, 4, and 6% *Trollius chinensis Bunge* residues, respectively.

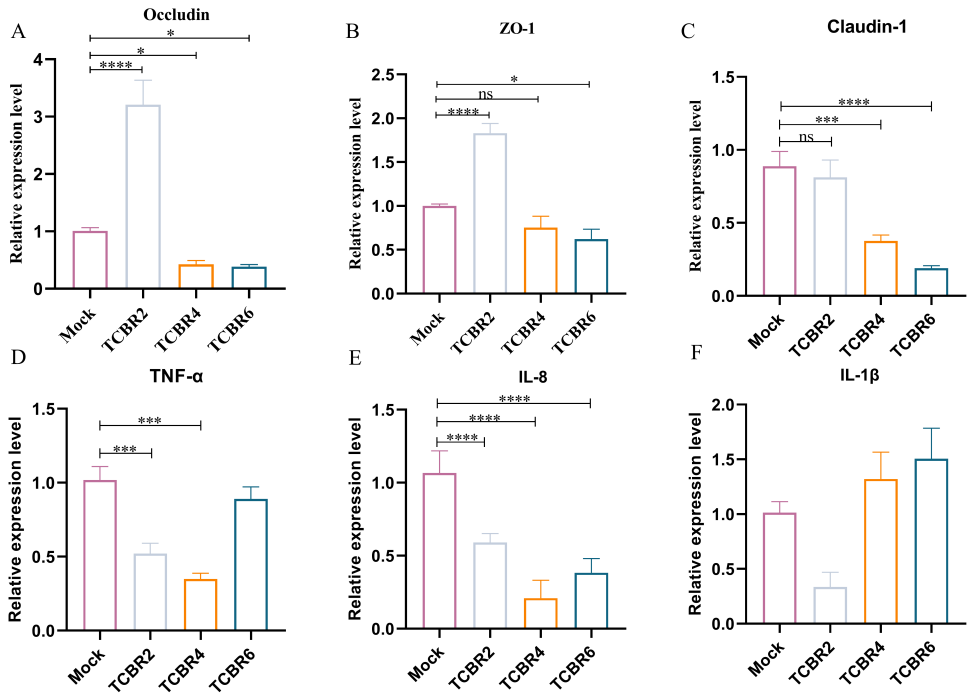

Figure 5

Relative mRNA expression levels of genes in the jejunal mucosa of rabbits. (A), (B), and (C) show jejunal mechanical barrier gene expression levels. (D), (E), and (F) indicate jejunal inflammatory gene expression levels. *P < 0.05, **P < 0.01, ***P< 0.001, and ****P < 0.0001 compared with the Mock group. The Mock group was fed a basal diet; TCBR2, TCBR4, and TCBR6 indicate diets supplemented with 2, 4, and 6% *Trollius chinensis Bunge* residues, respectively.