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Original Research Article

Diet xylo-oligosaccharide supplementation improves growth performance, immune function, and intestinal health of broilers

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ABSTRACT

The effects of xylo-oligosaccharides (XOS) on broiler growth performance, immune function, and intestinal health were investigated. A total of 540 one-d-old Arbor Acres Plus broilers were randomly divided into 5 groups with 6 replicates per group and 18 chickens per replicate. Broilers in the control (CON) group received a corn–soybean meal based basal diet, those in the antibiotics (ANT) group received the basal diet plus 500 mg/kg oxytetracycline, and those in XOS groups received the basal diet plus 150, 300, or 450 mg/kg XOS. Compared with CON, the body weight at 42 d and average daily gain from 1 to 42 d were significantly increased in the 150, 450 mg/kg XOS-added and ANT groups ($P = 0.018$), and the relative expression of claudin-1 and *ZO*-1 mRNA in the ileum was significantly higher in the 300 and 450 mg/kg XOS-added groups ($P < 0.001$). The feed conversion ratios ($P < 0.001$) and abdominal fat rates ($P = 0.012$) of broilers from 1 to 42 d of age were significantly lower in all XOS-added groups than in the control group. Splenic index ($P = 0.036$) and bursa of Fabricius index ($P = 0.009$) were significantly better in the ANT group and each XOS-added group than in the control group. Compared to CON and ANT, serum IgA ($P = 0.007$) and IgG ($P = 0.002$) levels were significantly higher in the 300 mg/kg XOS-added group, and the relative abundance of short-chain fatty acid-producing genera (*Alistipes*) was also significantly higher ($P < 0.001$). Meanwhile, ileal villus height ($P < 0.001$) and ratio of villus height to crypt depth (V:C) ($P = 0.001$) were significantly increased in XOS-added broilers. In analysis of relationships between cecal microbes and the physical barrier of the gut, *[Ruminococcus]_torques_group* was positively correlated with mRNA expression of ileal *ZO-1* and claudin-1 ($P < 0.05$), and *Bacteroides* was positively correlated with increased ileal villus height and V:C ($P < 0.05$). Overall, XOS addition to broiler diets improved growth performance, promoted intestinal health by enhancing intestinal barrier function and regulating cecal microbiota diversity, and had positive effects on immunity.

1. Introduction

Since their introduction, antibiotic growth promoters have rapidly increased in popularity in the livestock industry, because they can promote animal growth, reduce animal morbidity, and be used in small quantities (Li et al., 2018). However, with intensive use of antibiotic growth promoters, negative effects have gradually emerged, including increases in bacterial resistance, antibiotic residues in edible livestock products, and environmental pollution. Thus, antibiotic use is a serious threat to the development of a healthy farming industry and consumer health (Wang et al., 2021a).

China's Ministry of Agriculture and Rural Affairs has completely banned feed manufacturers from producing commercial feed containing growth-promoting drug feed additives (except for traditional Chinese medicines) (MARA, 2019). Therefore, it is vital that the feed industry explores and identifies effective alternatives to antibiotic growth promoters for healthy and sustainable development of livestock farming.

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Xylo-oligosaccharides (XOS) are composed of 2 to 10 xylose molecules connected by β-1,4 glycosidic bonds, and are a mixture of xylobiose, xylotriose and xylotetraose as the main components (Samanta et al., 2015). Xylo-oligosaccharides are derived from xylan-rich natural plants by biotechnology approaches (Carvalho et al., 2013). As a stimbiotic, XOS can have positive effects on the intestinal microbiota by increasing the number of beneficial bacteria and reducing the number of pathogenic bacteria (Macfarlane et al., 2006). It is an important supplement that improves animal growth performance, regulates intestinal flora balance, increases immunity, and regulates blood lipids (Patel and Goyal, 2011). Xylo-oligosaccharides can be obtained in corncob, bagasse, and straw, and the degradation products of xylan are extracted from those crop residues by chemical, physical, or enzymatic degradation (Jain et al., 2015). Physical and chemical properties of XOS include low viscosity, high water solubility, high temperature resistance, and acid and alkali resistance (Wei et al., 2018), and it also has potential biological functions that include antibacterial, anti-inflammatory, antioxidant, and anti-tumor activities (Chen et al., 2021). Therefore, XOS is widely used in food and beverage and health care products, as well as in other industries (Bali et al., 2015). Application of XOS in the livestock industry focuses on monogastric animals and aquatic animals. Xylanase is a nonstarch polysaccharide (NSP) degrading enzyme that cleaves the internal β-xylosidic glycosidic linkages of xylan into XOS. Therefore, it is a common practice to add xylanase to poultry diets to promote the conversion of dietary xylans to xylo-oligosaccharides (Cowieson and Bedford, 2009; Gonzalez-Ortiz et al., 2017). However, it has been found that XOS prepared by modern processes is usually better than the addition of xylanase, because the in-situ depolymerization of NSP is not instantaneous. So, producing XOS in the gut by using enzymes is not as effective as feeding arabinoxylo-oligosaccharides (AXOS) directly, hence XOS generation in the gut via the use of enzymes is not as efficient as feeding XOS directly (Morgan et al., 2019). Investigating the additional modes of action of dietary fiber supplementation in the form of fermentable AXOS in wheat-based broiler diets revealed that these AXOS boosted the development of microbial flora related to intestinal fiber fermentation in broilers, thus having a positive impact on the health and production performance of broilers (Bautil et al., 2020). Compared with other oligosaccharides, XOS has been studied as a potential prebiotic because it has been shown to promote the production of butyrate through cross-feeding of lactate to butyrate-producing bacteria, stimulating gut health and consequently performance through the beneficial effects of butyrate (De Maesschalck et al., 2015). In addition, supplementation with XOS can increase short-chain fatty acids (SCFA) in broilers, stimulate the immune system, and increase the population of beneficial bacteria (Ding et al., 2018; Morgan et al., 2019). Increasing the production of fermentation metabolites such as SCFA can promote the growth of intestinal villi and improve mucosal health. It was found that dietary supplementation of 75 and 100 mg/kg of XOS was beneficial to broilers fed a conventional corn–soybean meal diet, which could reduce the drip loss of thigh muscle, improve meat quality, and reduce the depth of duodenal crypts consequently contributing to intestinal health (Suo et al., 2015). XOS is also reported to enhance the immune response by reduced cecal colonization of the pathogen and production of inflammatory cytokines (Mohsen et al., 2017).

Poultry is one of the fastest-growing meat industries, of which broiler chickens account for a high population of the total poultry population in the world (Ali et al., 2021). There have been few studies on XOS supplementation in broiler production in recent years. Therefore, the effects of XOS on growth performance, slaughter traits, intestinal health, and immunity of broiler chickens were investigated in this study to provide a reference for the application of XOS to broiler chickens.

2. Materials and methods

2.1. Animal ethics

All procedures in the present study were performed in accordance with the guidelines of the Animal Care Committee of Henan Agricultural University (approval No. HENAU-2022-015).

2.2. Animals and experimental design

The trial was conducted with 540 Arbor Acres Plus broilers at 1 d old, randomly divided into 5 groups of 6 replicates each, with 18 birds per replicate. Male and female chicks were mixed at a ratio of 1:1. The control (CON) group was fed a basal diet, the antibiotic (ANT) group was fed the basal diet plus 500 mg/kg calcium oxytetracycline, and 3 XOS test groups were fed the basal diet plus 150, 300, or 450 mg/kg XOS for 42 d. Xylo-oligosaccharide was extracted from corncob with a purity of 20% (effective composition of XOS from heat-cracked corn kernel: 20% XOS; 37.93% cellulose; 14.96% lignin; 12.57% xylose; 2.89% dextran; 6.3% water; 5.35% others). The antibiotic was oxytetracycline calcium at 10%. The products used in the trial were supplied by Henan Kornbo Agricultural Technology Co., Ltd. (Zhumadian, Henan, 463000, China).

2.3. Diets and analyses

The ingredients and chemical analysis of the basal diets formulated to meet Arbor Acres Plus nutrient recommendations are shown in Table 1. The calcium content in feed was determined by EDTA disodium complexometric titration (GB/T 6436-2018). The total phosphorus content in feed was determined by spectrophotometry

Table 1
Ingredients and nutrient composition of the basal diet (%, air-dry basis).

Item	Starter (1 to 21 d of age)	Finisher (22 to 42 d of age)
Ingredients		
Corn	55.00	60.48
Soybean meal	32.70	25.80
Corn gluten meal	5.00	5.00
$CaHPO_4$	1.50	1.00
Limestone	1.10	1.00
NaCl	0.30	0.30
Soybean oil	3.00	5.30
L-Lys·HCl	0.46	0.31
DL-Met	0.21	0.16
Thr	0.13	0.05
Premix	0.60[1]	0.60[2]
Total	100.00	100.00
Nutrient levels[3]		
ME, MJ/kg	12.55	13.40
CP	23.42	19.78
Ca	0.76	0.67
TP	0.63	0.50
Lys	1.44	1.15
Met	0.56	0.47
Thr	0.97	0.78

[1] The premix provided the following per kilogram of the diet from 1 to 21 d of age: vitamin A 12,000 IU, vitamin D_3 3,500 IU, vitamin E 60 IU, vitamin K_3 4 mg, vitamin B_1 2.5 mg, vitamin B_2 10 mg, vitamin B_6 6 mg, vitamin B_{12} 8 μg, biotin 0.8 mg, folic acid 10 mg, D-pantothenic acid 40 mg, nicotinic acid 75 mg, choline 700 mg, Zn 90 mg, Fe 110 mg, Cu 20 mg, Mn 100 mg, Se 0.3 mg, I 0.5 mg, phytase 0.1 g.

[2] The premix provided the following per kilogram of the diet from 22 to 42 d of age: vitamin A 10,000 IU, vitamin D_3 3,000 IU, vitamin E 50 IU, vitamin K_3 3.5 mg, vitamin B_1 2 mg, vitamin B_6 5 mg, vitamin B_2 10 mg, vitamin B_{12} 6 μg, biotin 0.6 mg, folic acid 8 mg, D-pantothenic acid 20 mg, nicotinic acid 60 mg, choline 600 mg, Zn 80 mg, Fe 100 mg, Cu 15 mg, Mn 80 mg, Se 0.3 mg, I 0.5 mg, phytase (acid phytase of microbial origin) 0.1 g.

[3] ME values were calculated from data provided by Feed Database in China (2020), while the others were measured values.

(GB/T 6437-2018). The crude protein content in feed was determined by the Kjeldahl method (GB/T 6432-2018). The contents of lysine, methionine and threonine in feed were determined by the conventional acid hydrolysis method (GB/T 15399-2018).

At the beginning of the experiment, 540 one-d-old Arbor Acres broilers were weighed and randomly allocated to 180 cages, 3 broilers per cage, with an area of 0.44 square meters per cage. The experiment was conducted in a 3-tier cage, and each cage was randomly assigned to each treatment group. Each cage had a separate feeding trough and birds had access to nipple drinkers. The initial temperature was 33 °C, which was then reduced by 2 to 3 °C per week until reaching 26 °C. Relative humidity was approximately 60%, and daylight was natural light, and artificial light was added at night. Feed and water were provided ad libitum throughout the experiment, and immunization was according to normal immunization procedures. Broilers were fed with mash.

2.4. Performance measurement and sampling

During the experiment, the daily feed intake was recorded. At 1, 21 and 42 d of age, the broilers were weighed after fasting for 12 h (fasting for 12 h before weighing is to reduce the impact of feed and water in the digestive tract on weighing results), and the average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (feed intake to body weight gain ratio [F:G]) were calculated. Fasting weight was measured on a replicate basis.

2.5. Serum immunoglobulin

On d 43, one chicken with similar body weight (30 broilers in total) was selected from each replicate for jugular vein blood sampling; the sample was placed in a 5-mL coagulant tube. Blood was kept at room temperature for 3 h to allow clotting and then centrifuged (3,000 × *g* for 10 min) to separate serum. Serum samples were immediately frozen at −20 °C until ELISA assays were performed. The contents of IgA, IgG, and IgM in serum samples were determined by ELISA kits (Nanjing Jiancheng Bioengineering Institute, China) using a double antibody sandwich enzyme-linked immunosorbent assay with a Synergy LX multifunctional enzyme marker (Li et al., 2023).

2.6. Slaughtering performance

Thirty broilers in total were slaughtered and sampled after blood collection from the jugular vein. The chickens were euthanized by intravenous injection of 50 mg/kg body weight sodium pentobarbital solution under the wing.

Carcass weight was the weight of the carcass after bleeding and feathering. Semi-eviscerated weight was the weight of the carcass minus the trachea, esophagus, crop, intestines, spleen, and reproductive organs, leaving the heart, liver (bile removed), kidneys, lungs, glandular stomach, muscular stomach (contents and corpuscles removed), and abdominal fat (including abdominal plate oil and fat around the muscular stomach). Eviscerated weight was the semi-eviscerated weight minus heart, liver, glandular stomach, muscular stomach, and abdominal fat.

The following calculations were performed:

$$\text{Slaughter rate (\%)} = 100 \times \text{carcass weight/broiler live weight,}$$

$$\text{Semi-eviscerated percentage (\%)} = 100 \times \text{semi-eviscerated weight/broiler live weight,}$$

$$\text{Eviscerating percentage (\%)} = 100 \times \text{eviscerated weight/broiler live weight.}$$

2.7. Immune organ index

On d 43 of the experiment, euthanized broilers were slaughtered. The immune organs (bilateral thymus, spleen, and bursa of Fabricius) were dissected and separated, and the tissue attached to the thymus, spleen and bursa was removed. The organs were blotted dry with filter paper, and weighed accurately. The weights of thymus, spleen and bursa were recorded and the immune organ index was calculated as follows.

$$\text{Immune organ index (\%)} = 100 \times \text{immune organ weight/broiler live weight.}$$

2.8. Morphological observation of the intestinal tract

On the 43rd day of the experiment, after the blood samples, carcass and immune organs were taken, the intestinal samples were separated. Segments (2 cm) of the duodenum, jejunum, and ileum were removed and after gently rinsing off contents with saline, they were placed in 4% formaldehyde solution for fixation and then used to cut into serial 5 μm sections. Three orientated sections cutting vertically from the villus enterocytes to the muscularis mucosa were selected from each sample and the measurements were carried out as follows. The vertical distance from the villus tip to villus–crypt junction level was taken as the intestinal villus height (VH), and the vertical distance from the villus-crypt junction to the lower limit of the crypt as the crypt depth (CD). Ten loci per section were selected for the measurement of the VH and CD. The ratio of VH to CD was defined as V:C.

2.9. Gene expression assays

To determine the relative mRNA expression of intestinal tight junction proteins, ileal intestinal segments were cut open and contents rinsed out with saline. Segments were snap-frozen in liquid nitrogen and then stored at −80 °C. Total RNA was extracted from the tissues using the Trizol method (Qiu et al., 2021). The cDNA was prepared using Prime Script RT reagent Kit with gDNA Eraser (Perfect Real Time) (Takara Biomedical Technology (Beijing) Co., Ltd., China) according to the instructions. The reverse-transcription quantitative PCR (RT-qPCR) reaction was conducted using TB Green Premix Ex Taq II (Takara Biomedical Technology (Beijing) Co., Ltd., China). The reaction procedure was as follows: predenaturation at 95 °C for 30 s, and denaturation at 95 °C for 5 s and annealing at 60 °C for 34 s in 40 cycles. Primers were designed with Primer 5.0 (National Center for Biotechnology Information, Bethesda, MD, USA) and were synthesized by Sangon Biotech Co., Ltd (Shanghai, China). Primer sequences are shown in Table 2. Before the start of the formal test, a pre-test using glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as an internal reference found that the cycle threshold (CT) value (less than 1.5) and the multiplicity of difference (less than 1.2) did not vary much between different treatment groups and different samples, and were relatively stable, so *GAPDH* was used as an internal reference primer in this test (Hul et al., 2020; Sun et al., 2012). The data were presented as $2^{-\Delta\Delta CT}$.

2.10. Cecal sample processing and microbial diversity analysis

2.10.1. Sample collection

On d 43, after stripping the cecum of the above-mentioned slaughtered broiler, contents of the cecum were placed in 2-mL lyophilized tubes and quickly stored at −80 °C to measure microbial diversity.

Table 2
The primers for real-time quantitative PCR.

Gene	Login number	Primer sequence (5′ → 3′)	Product length, bp
Occludin	NM_205128.1	F: GATGGACAGCATCAACGACC R: CTTGCTTTGGTAGTCTGGGC	142
Claudin-1	NM_001013611.2	F: ACACCCGTTAACACCAGATTT R: GCATTTTTGGGGTAGCCTCG	152
ZO-1	XM_040680630.1	F: GCCTACTGCTGCTCCTTACAACTC R: GCTGGATCTATATGCGGCGGTAAG	129
GAPDH	NM_204305.2	F: AGCCATTCCTCCACCTTTGAT R: AGTCCACAACACGGTTGCTGTAT	112

ZO-1 = zonula occludens-1; *GAPDH* = glyceraldehyde-3-phosphate dehydrogenase.

2.10.2. Determination of cecal microbiota

Samples of cecum contents were sent to Beijing Biomarker Technologies Co., Ltd. (Beijing, China) for 16S rDNA sequencing. The paired-end sequencing method was used to perform high-throughput sequencing analysis of bacterial 16S rDNA based on the Illumina HiSeq 2500 platform.

2.10.3. Total DNA extraction and detection of cecal microbiota

Total microbial genomic DNA samples were extracted using the OMEGA DNA isolation kit (D5635-02; Omega, Norcross, GA, USA) following the manufacturer's instructions, and stored at −20 °C prior to further analysis. The quantity and quality of extracted DNA were measured using a NanoDrop NC-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis respectively (She et al., 2018).

2.10.4. 16S rRNA gene amplicon sequencing and product purification

PCR amplification of the bacterial 16S rRNA genes (V3 to V4 region) was performed using the forward primer 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and the reverse primer 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The extracted DNA was amplified with two-step PCR, with sample-specific 7-bp barcodes incorporated into the forward and reverse primers for multiplex sequencing in the second PCR step. After the individual quantification step, amplicons were pooled in equal amounts, and paired-end 2 × 250 bp sequencing was performed using the Illumina NovaSeq platform with NovaSeq 6000 SP Reagent Kit (500 cycles) at Shanghai Personal Biotechnology Co., Ltd (Shanghai, China).

2.10.5. Sequence analysis

By splicing and filtering the sequences, operational taxonomic unit (OTU) clustering, species annotation and abundance analysis, the species composition of the samples could be revealed. Sequence data analyses were mainly performed using QIIME2 and R packages (v3.2.0). OTU-level alpha diversity indices, such as Chao1 richness estimator, observed species, Shannon diversity index and ACE index were calculated using the OTU table in QIIME2 and visualized as a table. OTU-level ranked abundance curves were generated to compare the richness and evenness of OTU among samples. Alpha diversity analysis was used to study the species diversity within a single sample, to understand the species richness, sample coverage rate and evenness of the samples in each treatment group (the ACE, Shannon and Chao indices measure coverage rate, diversity and richness of bacteria, respectively).

Using QIIME2 software, the composition and abundance of each sample at the two taxonomic levels of phylum and genus were obtained, and the analysis results were presented by a bar graph. Based on the results of OTU classification and taxonomic status identification, the specific species composition of each sample at each taxonomic level was obtained. Then, at the phylum and genus level, the dominant microbial flora was selected to make a histogram for difference analysis.

2.11. Statistical analysis

The experimental data were analyzed by one-way ANOVA using SPSS 26.0 software (SPSS Inc., Chicago, IL), and then followed by Duncan's multiple range test. Results are expressed as treatment means with the pooled standard error of the mean (SEM). Significant differences were accepted at $P < 0.05$, with $0.05 < P < 0.10$ indicating a tendency.

To determine correlations between cecal bacteria and intestinal barrier functions, redundancy analysis (RDA) was performed at the genus level using the *R* language packet of Spearman correlation analysis. Benjamini–Hochberg's false discovery rate (FDR) correction was used to correct for multiple testing.

3. Results

3.1. Growth performance

The effects of XOS on growth performance are presented in Table 3. Compared with CON, body weight at 42 d of age and ADG from 1 to 42 d of age were significantly higher ($P < 0.05$) in ANT and 150 and 450 mg/kg XOS groups. Compared to ANT, there was no significant difference in body weight at 42 d of age and ADG at 1 to 42 d of age in XOS groups, and there was no significant change in F:G ratio at 22 to 42 d of age and 1 to 42 d of age in XOS groups. Compared with CON and ANT, ADFI was significantly lower in from 22 to 42 d of age and from 1 to 42 d of age in the 3 XOS groups ($P < 0.001$). In addition, the F:G ratio was significantly lower from 22 to 42 d of age and from 1 to 42 d of age in ANT and XOS groups than in the CON group ($P < 0.001$). In addition, no chicken mortality was observed during the test period.

3.2. Slaughter performance

The effects of XOS on the slaughter performance of broiler chickens are shown in Table 4. Compared with CON, abdominal fat percentage was significantly reduced in 150, 300 and 450 mg/kg XOS groups ($P < 0.05$). Compared to ANT, the abdominal fat percentage in the 300 mg/kg XOS group decreased significantly ($P < 0.05$), but there was no significant difference between the 150 mg/kg and 450 mg/kg XOS groups. Compared with CON and ANT, the breast muscle rate of broilers in the 3 XOS groups increased significantly ($P < 0.01$).

3.3. Immune organ index and serum immunoglobulin levels

As shown in Table 5, compared with CON, the addition of XOS significantly increased the spleen index ($P < 0.05$) and bursa of

Table 3
Effects of xylo-oligosaccharides (XOS) on growth performance of broilers.[1]

Item	Feeding stage	CON	ANT	XOS level			SEM	*P*-value
				LXOS	MXOS	HXOS		
BW, g	1 d	47.13	47.38	47.40	46.94	47.23	0.137	0.842
	21 d	764.60	745.54	775.71	757.30	751.63	6.295	0.625
	42 d	2144.69^{b}	2361.82^{a}	2346.63^{a}	2263.31^{ab}	2305.15^{a}	23.826	0.018
ADG, g	1 to 21 d	34.15	33.26	34.66	33.78	33.52	0.296	0.628
	22 to 42 d	66.63	76.06	74.80	71.72	73.98	1.118	0.051
	1 to 42 d	49.94^{b}	55.11^{a}	54.74^{a}	52.77^{ab}	53.76^{a}	0.567	0.018
ADFI, g	1 to 21 d	47.46	48.60	47.97	47.36	46.89	0.449	0.817
	22 to 42 d	136.74^{a}	133.76^{a}	120.40^{b}	113.96^{b}	115.39^{b}	2.365	<0.001
	1 to 42 d	92.67^{a}	90.61^{a}	84.19^{b}	80.66^{b}	81.14^{b}	1.234	<0.001
F:G	1 to 21 d	1.47	1.39	1.39	1.41	1.40	0.016	0.509
	22 to 42 d	2.07^{a}	1.76^{b}	1.61^{b}	1.60^{b}	1.58^{b}	0.048	0.001
	1 to 42 d	1.86^{a}	1.65^{b}	1.54^{b}	1.53^{b}	1.52^{b}	0.032	<0.001

SEM = standard error of the mean; BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; F:G = feed conversion ratio (feed intake to BW gain ratio). CON = basal diet; ANT = basal diet + 500 mg/kg calcium oxytetracycline; LXOS = basal diet + 150 mg/kg XOS; MXOS = basal diet + 300 mg/kg XOS; HXOS = basal diet + 450 mg/kg XOS.
^{a,b}In the same row, values with no letter or the same letter superscripts mean no significant difference ($P > 0.05$), while with different small letter superscripts mean significant difference ($P < 0.05$).
[1] Data is the mean of 6 replicates per treatment.

Table 4
Effects of xylo-oligosaccharides (XOS) on slaughter performance of 43-d-old broilers.

Item	CON	ANT	XOS level			SEM	*P*-value
			LXOS	MXOS	HXOS		
Dressing percentage, %	91.08	91.25	90.95	88.90	89.14	0.380	0.125
Semi-eviscerated percentage, %	83.20	82.61	82.04	79.28	81.71	0.616	0.325
Eviscerating percentage, %	69.01	70.33	67.84	64.57	66.97	0.676	0.068
Abdominal fat percentage, %	1.55^{a}	1.27^{ab}	1.03^{bc}	0.82^{c}	1.06^{bc}	0.077	0.012
Breast muscle percentage, %	27.07^{b}	27.08^{b}	30.73^{a}	29.77^{a}	30.63^{a}	0.477	0.008
Leg muscle percentage, %	23.26	22.74	22.97	21.77	22.66	0.417	0.857

SEM = standard error of the mean.
CON = basal diet; ANT = basal diet + 500 mg/kg calcium oxytetracycline; LXOS = basal diet + 150 mg/kg XOS; MXOS = basal diet + 300 mg/kg XOS; HXOS = basal diet + 450 mg/kg XOS.
$^{a-c}$In the same row, values with no letter or the same letter superscripts mean no significant difference ($P > 0.05$), while with different small letter superscripts mean significant difference ($P < 0.05$).

Fabricius index ($P < 0.01$) of broiler chickens. Moreover, the spleen index was also significantly higher in the ANT group than in the control group ($P < 0.05$).

As shown in Table 6, the serum IgA ($P < 0.01$) and IgG ($P < 0.01$) contents of broilers in the 300 mg/kg XOS group were significantly higher than those in other treatment groups, but the addition of XOS had no significant effect on serum IgM contents compared with the CON and ANT.

3.4. Intestinal morphology of duodenum, jejunum, and ileum

The depth of the duodenal crypts was significantly lower in the three XOS groups than in the CON group ($P < 0.01$), but the CD was not significantly different compared with that in the ANT group. The addition of XOS had no significant effect on broiler jejunum intestinal morphology. Compared with CON and ANT, the ileal VH of broilers in XOS groups increased significantly ($P < 0.001$), and the V:C ratio also increased significantly ($P < 0.01$). Also, ileal V:C ratio were significantly higher in the CON group than in the ANT group ($P < 0.01$) (Table 7).

3.5. Ileal tight junction protein gene expression

Compared with CON, the XOS supplementation groups significantly increased the relative expression of occludin mRNA in the ileum of broilers ($P < 0.001$). The relative expression levels of claudin-1 and zonula occludens-1 (*ZO-1*) mRNA in the ileum of 300 mg/kg XOS group and 450 mg/kg XOS group were also significantly higher than those in the control group ($P < 0.001$). Compared to ANT, the relative mRNA expression of occludin, claudin-1 and *ZO-1* in the ileum of each XOS addition group was significantly increased ($P < 0.001$). Furthermore, the mRNA relative expression levels of occludin, claudin-1 and *ZO-1* in the ileum of 300 mg/kg XOS group and 450 mg/kg XOS group were also significantly higher than those of CON and ANT ($P < 0.001$) (Fig. 1).

3.6. Microbial diversity in the cecum

As shown in Table 8, compared with CON, the ACE index was significantly higher in the 150 mg/kg and 300 mg/kg XOS groups ($P < 0.001$). Compared with ANT, the ACE index was significantly higher in the three XOS groups ($P < 0.001$), and the Shannon index was significantly higher in the three XOS groups ($P < 0.05$). Compared with CON and ANT, the Chao1 index was significantly higher in the 150 mg/kg and 300 mg/kg XOS groups ($P < 0.01$), and the Shannon index was significantly higher in the 150 mg/kg XOS group ($P < 0.05$).

3.7. Cecal microbiota

The distribution of the top 10 phyla of the broiler cecal bacterial community is shown in Fig. 2. The 10 most abundant phyla of bacteria in the test groups were Firmicutes, Bacteroidetes, Verrucomicrobia, Proteobacteria, Actinobacteria, Synergistetes,

Table 5
Effects of xylo-oligosaccharides (XOS) on immune organ index of 43-d-old broilers.

Item	CON	ANT	XOS level			SEM	*P*-value
			LXOS	MXOS	HXOS		
Thymus index, %	1.29	1.33	1.33	1.32	1.30	0.017	0.859
Spleen index, %	1.00[b]	1.27[a]	1.28[a]	1.38[a]	1.31[a]	0.055	0.036
Bursa of Fabricius index, %	0.47[b]	0.55[ab]	0.65[a]	0.67[a]	0.56[ab]	0.026	0.009

SEM = standard error of the mean.
CON = basal diet; ANT = basal diet + 500 mg/kg calcium oxytetracycline; LXOS = basal diet + 150 mg/kg XOS; MXOS = basal diet + 300 mg/kg XOS; HXOS = basal diet + 450 mg/kg XOS.
[a,b]In the same row, values with no letter or the same letter superscripts mean no significant difference ($P > 0.05$), while with different small letter superscripts mean significant difference ($P < 0.05$).

Table 6
Effects of xylo-oligosaccharides (XOS) on serum immunoglobulin content of 43-d-old broilers.

Item	CON	ANT	XOS level			SEM	*P*-value
			LXOS	MXOS	HXOS		
IgA, μg/mL	345.98[b]	310.87[b]	362.81[b]	509.58[a]	383.69[b]	22.178	0.007
IgG, μg/mL	3048.47[b]	2906.80[b]	3265.14[b]	4123.47[a]	2815.14[b]	164.092	0.002
IgM, μg/mL	793.25	881.98	833.34	847.30	825.22	16.841	0.767

SEM = standard error of the mean; IgA = immunoglobin A; IgG = immunoglobin G; IgM = immunoglobin M.
CON = basal diet; ANT = basal diet + 500 mg/kg calcium oxytetracycline; LXOS = basal diet + 150 mg/kg XOS; MXOS = basal diet + 300 mg/kg XOS; HXOS = basal diet + 450 mg/kg XOS.
[a,b]In the same row, values with no letter or the same letter superscripts mean no significant difference ($P > 0.05$), while with different small letter superscripts mean significant difference ($P < 0.05$).

Table 7
Effects of xylo-oligosaccharides (XOS) on intestinal morphology of 43-d-old broilers.

Item	CON	ANT	XOS level			SEM	*P*-value
			LXOS	MXOS	HXOS		
Duodenum							
VH, μm	1567.47	1282.47	1568.03	1598.73	1551.50	34.498	0.071
CD, μm	181.67[a]	143.80[b]	154.83[b]	156.11[b]	142.64[b]	5.104	0.009
V:C	8.66	8.93	10.14	10.22	10.93	0.347	0.056
Jejunum							
VH, μm	1268.13	1243.10	1266.26	1273.10	1299.63	32.507	0.990
CD, μm	141.30	149.94	133.69	138.53	140.17	1.456	0.065
V:C	8.97	8.29	9.46	9.20	9.28	0.226	0.553
Ileum							
VH, μm	947.04[b]	619.56[c]	1184.98[a]	1294.58[a]	1280.51[a]	44.777	<0.001
CD, μm	157.89	130.99	160.94	170.11	167.69	4.431	0.071
V:C	6.01[b]	4.73[c]	7.42[a]	7.67[a]	7.68[a]	0.278	0.001

SEM = standard error of the mean; VH = villus height; CD = crypt depth; V:C = ratio of VH to CD.
CON = basal diet; ANT = basal diet + 500 mg/kg calcium oxytetracycline; LXOS = basal diet + 150 mg/kg XOS; MXOS = basal diet + 300 mg/kg XOS; HXOS = basal diet + 450 mg/kg XOS.
[a–c]In the same row, values with no letter or the same letter superscripts mean no significant difference ($P > 0.05$), while with different small letter superscripts mean significant difference ($P < 0.05$).

Tenericutes, Cyanobacteria, Elusimicrobia, and Kiritimatiellaeota. The dominant phyla were Firmicutes and Bacteroidetes, which accounted for more than 80% of the total bacterial community. As shown in Fig. 3, compared with CON, the relative abundance of Firmicutes decreased significantly in the 300 mg/kg XOS and 450 mg/kg XOS groups, and the relative abundance of Bacteroidetes in the three XOS supplemental groups was significantly increased ($P < 0.001$). Compared to ANT, the relative abundance of Firmicutes decreased significantly in the three XOS addition groups, while the relative abundance of Bacteroidetes increased significantly in the three XOS addition groups ($P < 0.001$).

Distribution of the genera of bacteria with relative abundance greater than 1% in the cecum of broiler chickens is shown in Fig. 4. The total relative abundance of the top genera of bacteria with a relative abundance greater than 1% in each group of broilers accounted for 80%. As shown in Fig. 5, compared with CON, relative abundance of *Bacteroides* in the ANT group decreased significantly ($P < 0.001$), but it increased significantly in the XOS groups ($P < 0.001$), and the relative abundance of *Ruminococcaceae_UCG-014* ($P < 0.05$) was significantly higher in the 150 and 300 mg/kg XOS groups. Compared with CON and ANT, relative abundances of *Synergistes* ($P < 0.001$) and *Phascolarctobacterium* ($P < 0.001$) increased significantly in the XOS experimental groups. Compared with CON, relative abundance of *Alistipes* decreased significantly in the ANT group ($P < 0.001$) but increased significantly in the 300 and 450 mg/kg XOS groups ($P < 0.001$). And, the greater the amount of XOS added, the higher the relative abundance of *Alistipes* in the broiler intestine. The relative abundance of *Alistipes* in broilers was significantly higher in the high-dose XOS-added group (450 mg/kg) than in the medium-dose-added group (300 mg/kg) and the low-dose-added group (150 mg/kg) ($P < 0.001$).

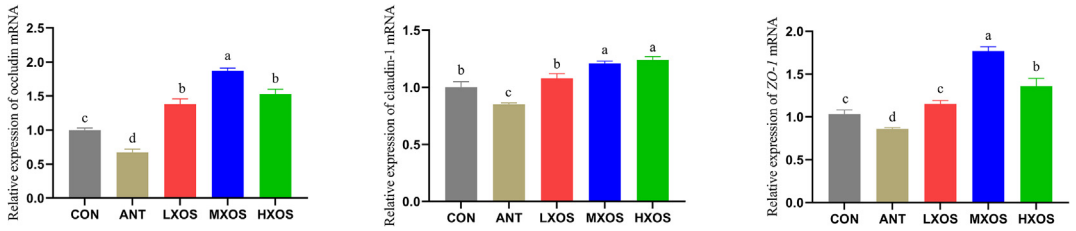

Fig. 1. Relative expression of ileal tight junction protein mRNA. *ZO-1* = zonula occludens-1. CON = basal diet; ANT = basal diet + 500 mg/kg calcium oxytetracycline; LXOS = basal diet + 150 mg/kg xylo-oligosaccharide; MXOS = basal diet + 300 mg/kg xylo-oligosaccharide; HXOS = basal diet + 450 mg/kg xylo-oligosaccharide. [a–c]Columns with different lowercase indicate a significant difference ($P < 0.05$).

Table 8
Effects of xylo-oligosaccharides (XOS) on the alpha diversity of the cecal microbiota of 43-d-old broilers.

Item	CON	ANT	XOS level			SEM	*P*-value
			LXOS	MXOS	HXOS		
ACE index, %	561.00^{bc}	552.60^{c}	584.17^{a}	578.99^{a}	568.23^{b}	2.880	<0.001
Chao1 index, %	565.05^{c}	558.73^{c}	588.37^{a}	580.95^{ab}	569.64^{bc}	3.181	0.003
Shannon index, %	6.78^{bc}	6.60^{c}	7.14^{a}	7.00^{ab}	7.06^{ab}	0.047	0.013

SEM = standard error of the mean.
CON = basal diet; ANT = basal diet + 500 mg/kg calcium oxytetracycline; LXOS = basal diet + 150 mg/kg XOS; MXOS = basal diet + 300 mg/kg XOS; HXOS = basal diet + 450 mg/kg XOS.
[a–c]In the same row, values with no letter or the same letter superscripts mean no significant difference ($P > 0.05$), while with different small letter superscripts mean significant difference ($P < 0.05$).

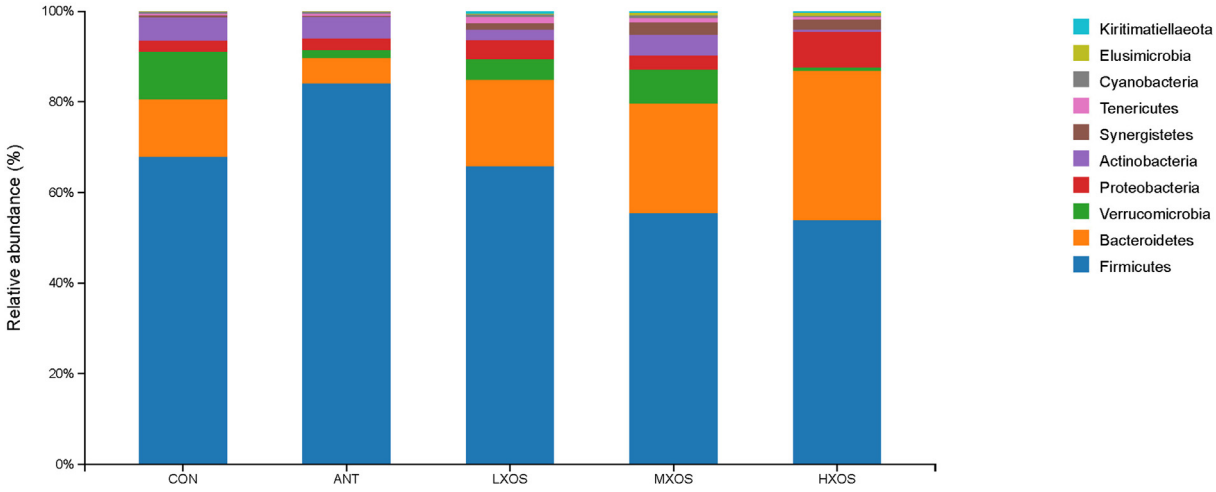

Fig. 2. Phylum level species distribution map. CON = basal diet; ANT = basal diet + 500 mg/kg calcium oxytetracycline; LXOS = basal diet + 150 mg/kg xylo-oligosaccharide; MXOS = basal diet + 300 mg/kg xylo-oligosaccharide; HXOS = basal diet + 450 mg/kg xylo-oligosaccharide.

3.8. Correlations between indicators of physical barrier function of the broiler intestine and cecal bacteria

To further investigate relations between cecal bacteria and intestinal barrier functions, the relative abundance of the top 20 differential flora at the level of cecal microorganism genus in broilers was selected for Spearman's correlation analysis with the indicators of ileal intestinal morphology and mRNA relative expression of ileal tight junction proteins, respectively (Fig. 6). *Bacteroides*, *Desulfovibrio*, and *Synergistes* were positively correlated with occludin ($P < 0.05$). *Ruminococcaceae_NK4A214_group* was negatively correlated with occludin ($P < 0.05$). *[Ruminococcus]_torques_group* and *Lachnoclostridium* were positively correlated with claudin-1 ($P < 0.05$). *[Ruminococcus]_torques_group* ($P < 0.01$) and *Ruminococcaceae_UCG–014* ($P < 0.05$) was positively correlated with *ZO-1*. *Olsenella* was negatively correlated with *ZO-1* ($P < 0.05$). The results indicated that those microbes had important interactions with the ileal physical barrier.

Correlations were also examined between gut morphology and cecal microbes. *Bacteroides* ($P < 0.01$) and *Phascolarctobacterium* ($P < 0.05$) were positively correlated with VH ($P < 0.05$). *Faecalibacterium* was negatively correlated with VH ($P < 0.05$). *Bacteroides* were positively correlated with V:C ($P < 0.05$). *Akkermansia* was negatively correlated with V:C ($P < 0.05$). These results suggested that those microbes were important in the gut morphology.

4. Discussion

Growth performance is the most direct and apparent measure of the positive effects of XOS. Studies have shown that because XOS are difficult to digest, they have a high residual rate in the intestinal tract (Ho et al., 2018). Therefore, XOS can be effective in the

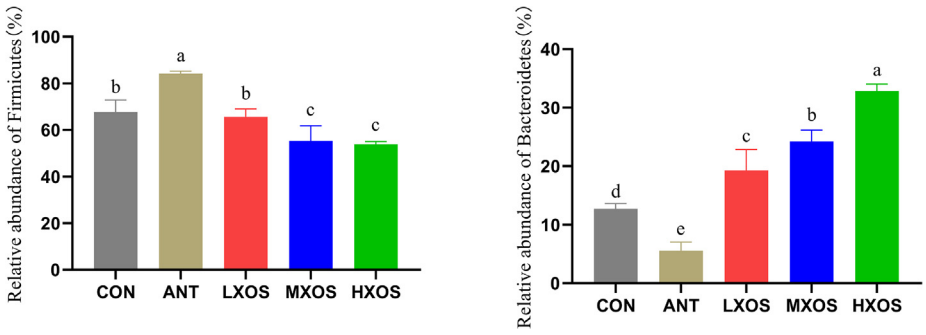

Fig. 3. Relative abundance of dominant bacteria at phylum level. CON = basal diet; ANT = basal diet + 500 mg/kg calcium oxytetracycline; LXOS = basal diet + 150 mg/kg xylo-oligosaccharide; MXOS = basal diet + 300 mg/kg xylo-oligosaccharide; HXOS = basal diet + 450 mg/kg xylo-oligosaccharide. $^{a-e}$Columns with different lowercase indicate a significant difference ($P < 0.05$).

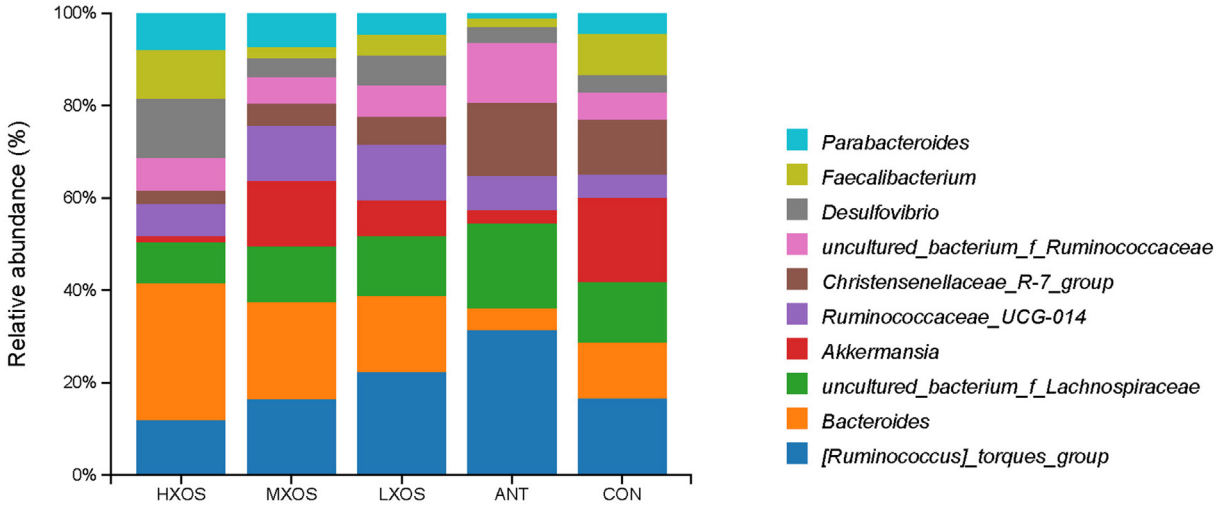

Fig. 4. Genus level species distribution map. CON = basal diet; ANT = basal diet + 500 mg/kg calcium oxytetracycline; LXOS = basal diet + 150 mg/kg xylo-oligosaccharide; MXOS = basal diet + 300 mg/kg xylo-oligosaccharide; HXOS = basal diet + 450 mg/kg xylo-oligosaccharide.

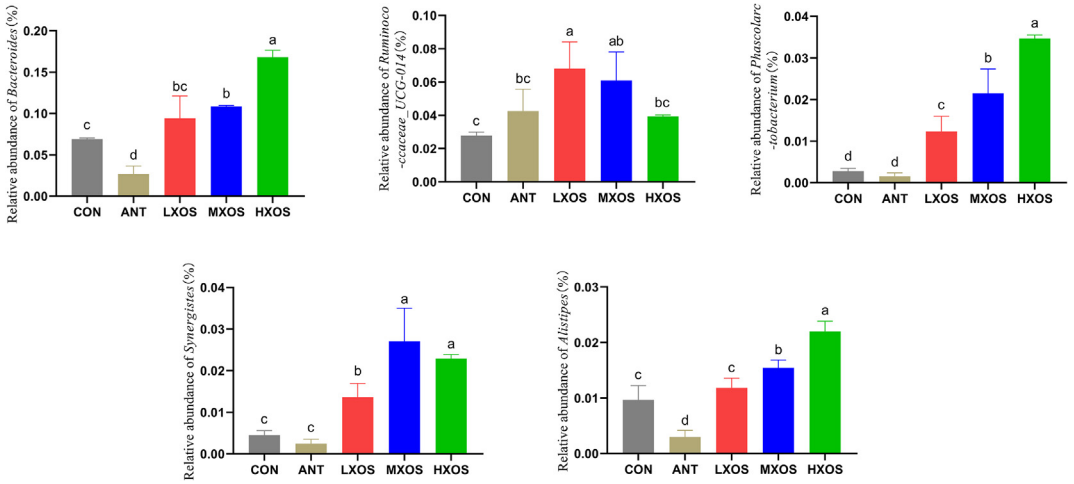

Fig. 5. Relative abundance of different bacteria at genus level. CON = basal diet; ANT = basal diet + 500 mg/kg calcium oxytetracycline; LXOS = basal diet + 150 mg/kg xylo-oligosaccharide. MXOS = basal diet + 300 mg/kg xylo-oligosaccharide. HXOS = basal diet + 450 mg/kg xylo-oligosaccharide. $^{a-d}$Columns with different lowercase indicate a significant difference ($P < 0.05$).

intestinal tracts of livestock and poultry. Xylo-oligosaccharides regulate the intestinal microbiota of livestock and poultry, promote intestinal health and improve intestinal function, thus improving the growth performance of livestock and poultry (de Figueiredo et al., 2020). In this experiment, the addition of 150 and 450 mg/kg XOS significantly increased the body weight at 42 d

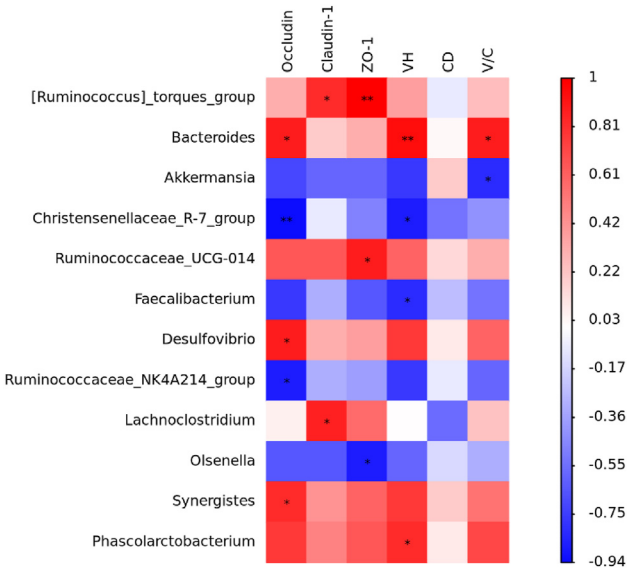

Fig. 6. Heat map of correlation between indexes related to intestinal physical barrier function and cecal microbiota (genus level). *UGG-014* and *NK4A214* belong to the Ruminococcaceae, both of which are part of Firmicutes, the main fiber-degrading bacteria in the intestine. And *[Ruminococcus]_torques_group* is another subgroup in the Ruminococcaceae family, which can produce short-chain fatty acids. *ZO-1* = zonula occludens-1; VH = villus height; CD = crypt depth; V:C = ratio of VH to CD. * indicating $P < 0.05$; ** indicating $P < 0.01$.

of age and the ADG from 1 to 42 d of age of broilers. It has been reported that adding 500 mg/kg XOS can alleviate the growth inhibition of broilers caused by Eimeria, improve weight gain and feed-to-gain ratio, and positively affect the growth performance of broilers without Eimeria infection (Lin et al., 2022). Dietary XOS supplementation significantly increased body weight of broilers (Zhenping et al., 2013). This is consistent with the results of this experiment. In the experiment, 150 mg/kg, and 450 mg/kg XOS were more effective than the medium-dose supplementation group (300 mg/kg XOS) in improving production performance, possibly because lower doses of XOS may be more easily digested and utilized by broilers, helping to promote production performance, whereas higher doses of oligo-xylulose may provide more nutrients within a certain concentration range to further improve production performance. However, supplementing with too many oligosaccharides is not easily digested and absorbed in the broiler's intestinal tract, and excessive intake may lead to increased fermentation in the intestinal tract and the production of excess acid (Liu et al., 2022), which can cause digestive problems.

In addition, the F:G of broilers in the XOS-added test group was significantly lower from 22 to 42 d of age and from 1 to 42 d of age. This supports the finding that XOS directly arrives at the mucosa of the small intestine to promote the absorption of nutrients (Orban et al., 1997) and that XOS could also improve the health status of animals (Gibson and Roberfroid, 1995). The addition of 0.2% and 0.5% oligosaccharides to wheat-based basal diets significantly increased feed conversion in the early and growing stages of broiler chicks, as well as throughout the trial (De Maesschalck et al., 2015). In addition, XOS supplementation significantly reduced the feed intake of Ross 308 broilers but significantly increased the feed conversion ratio, which are results consistent with those of this study. The results of this trial also showed that the addition of XOS had no significant effect on the growth performance of broilers in the early stages but significantly improved the growth performance in the later stages and overall. It was hypothesized that the absence of early XOS effects might be related to the incomplete development of the broiler gut in the early stages and the period needed to adapt to the addition of XOS. The ability of XOS to improve growth performance in broilers might be associated with its ability as a prebiotic to promote intestinal health. Xylo-oligosaccharides promote intestinal homeostasis by favoring the establishment of beneficial intestinal bacteria, which competitively suppress the number of harmful bacteria (Chen et al., 2021). It also acts as a fermentation substrate and promotes the production of SCFA in the intestine (Liu et al., 2022). Short-chain fatty acids reduce intestinal pH, inhibit pathogen growth, and promote animal health by regulating animal immunity through specific signaling molecules (Palani et al., 2021). Broiler cecum microbiota data shows that addition of XOS increased the abundance of SCFA-producing microbiota of *Bacteroides*, *Ruminococcaceae_UCG-014*, *Phascolarctobacterium*, and *Alistipes*, thus, indirectly improving broiler production performance.

In this study, the addition of XOS tended to increase the carcass rate of broilers, significantly increase the breast muscle rate, and significantly decrease the abdominal fat percentage rate. The reduction in abdominal fat percentage might be related to the ability of XOS to regulate lipid metabolism, reduce lipid synthesis, and promote lipolysis (Li et al., 2021). It has been shown that XOS was able to down-regulate the expression of CCAAT/enhancer-binding protein alpha (C/EBPa) and peroxisome proliferator-activated receptor-γ (PPAR-γ) in mouse epididymal tissues to inhibit lipogenesis, and increase the expression of adenosine monophosphate-activated protein kinase (AMPK) in mouse liver tissues to promote acetyl-coenzyme A carboxylase (ACC) phosphorylation, thus reducing fat accumulation, while XOS also up-regulated the expression of carnitine palmitoyltransferase-1 (CPT-1) to promote fatty acid β-oxidation to promote lipolysis (Lensu et al., 2020; Li et al., 2021). The enhancement of breast muscle rate by XOS in broiler chickens may be realized by the up-regulation of the expression of genes related to muscle growth and the increase of the apparent metabolic rate of crude protein by XOS (Zhou et al., 2021a; Zhu et al., 2022), and its mechanism needs to be further investigated.

Oligosaccharides can improve immune organ indices, increase inflammatory factor expression, significantly increase spleen weight and spleen index, and improve immunity in a cyclophosphamide-induced mouse model (Jo et al., 2023). The addition of XOS to diets significantly increases IgA and IgM in the serum of laying hens (Ding et al., 2018). The results of this test are consistent with those of previous studies. The addition of XOS increased the spleen index and the bursa of Fabricius index of broiler chickens, and the addition of 300 mg/kg XOS significantly increased the serum levels of IgA and IgG. Previous studies have shown that XOS is not readily digestible by the gastrointestinal tract or that it has low digestibility overall, it can be fermented by intestinal microbes to produce SCFA (Kabel et al., 2002). One study showed that in poultry, SCFA enhances intestinal, non-specific immune mechanisms by stimulating the expression of mucin glycoprotein in intestinal epithelial cells to combat pathogens, thus affecting growth performance (Willemsen et al., 2003). Butyrate can also interfere with the signal transduction of interferon-gamma (IFNγ) by inhibiting the activation of signal transducers and the activator of transcription 1 (STAT1) (Klampfer et al., 2003). The results of this experiment showed that the addition of XOS increased the relative abundance of SCFA-producing genera such as *Bacteroides*, and *Ruminococcaceae_UCG-014*. Therefore, XOS can be used as an immune adjuvant to promote the development and maturation of immune organs, increase humoral immunity, and improve the immune system of broiler chickens.

Some studies suggest that supplementation of 0.5% XOS with a purity of 35% to broiler chicken feed significantly increased the VH

in the ileum, suggesting an increase in gut health and improved nutrient absorption (De Maesschalck et al., 2015), and the addition of 100 mg/kg XOS to diets significantly increases VH and the V:C ratio in the small intestine (duodenum, jejunum, and ileum) of broiler chickens (Wang et al., 2021b). The results of this trial are similar to those of previous studies, and the addition of XOS significantly reduced the depth of duodenal crypts in broiler chickens and significantly increased the VH and V:C ratio in the ileum compared with CON. XOS as a prebiotic forms SCFA through the fermentation of broiler intestinal flora, and then SCFA were metabolized to produce butyrate, which can increase the activity of broiler intestinal protease, amylase, and lipase (Soumeh et al., 2019; Xu et al., 2003). Thus, it promotes the digestion and utilization of proteins in broiler intestines and provides nutrients for intestinal development. The reason for this may be that butyrate lowers intestinal pH, which activates proteinogen and increases protease activity (Rérat, 1981).

It has also been shown that XOS hinder the adhesion of pathogenic bacteria to the intestinal mucosa by competing for binding sites, improve intestinal morphology with increasing the height of the intestinal villus, promote the secretion of digestive enzymes, and increase the utilization of nutrients (Min et al., 2016). Ileal VH and V:C ratio were significantly higher in the control group than in the antibiotic group. The reason for this is that the ileum, which is at the end of the digestive tract, may have a high level of antibiotic residue. Antibiotics can affect the structure of the intestinal tract by inhibiting the growth and metabolic activity of intestinal bacteria and reducing the number of beneficial bacteria.

Therefore, the addition of XOS can improve the growth performance of broilers by increasing the absorption area of nutrients in the small intestine, improving the integrity of the small intestine epithelium, strengthening the intestinal mechanical barrier, reducing the risk of pathogen invasion, and increasing the ability to absorb nutrients (Swaggerty et al., 2019). Overall, the addition of XOS can promote the production of SCFA in the broiler intestine and influence the intestinal morphology through SCFA metabolism which in turn promotes protein digestion.

The addition of 400 mg/kg XOS significantly up-regulated the expression of claudin 1 mRNA in the ileal mucosa of laying hens fed oxidized fish oil in the diet and effectively alleviated the intestinal damage caused by oxidized fish oil (Zhou et al., 2021b). The addition of 150 mg/kg XOS to broiler feed also significantly upregulated ileal occludin and claudin 3 mRNA expression (Luo et al., 2021). In this study, the addition of XOS up-regulated the expression of occludin, claudin 1 and *ZO*-1 mRNA in broiler ileum. The increases in expression were most likely because XOS, as a prebiotic, could bind directly to pathogens, resulting in the loss of biological activity of pathogenic bacteria while XOS promotes the production of antimicrobial compounds in the organism, thus reducing the adhesion of pathogens in the mucus layer (Brink et al., 2006). It is also possible that XOS regulated the intestinal microbiota by increasing the number of beneficial bacteria and maintaining gut integrity.

The composition of intestinal flora significantly affects animal health and production performance. A stable intestinal flora has certain beneficial effects on the absorption and metabolism of nutrients, whereas an imbalance in intestinal flora can lead to negative effects such as diarrhea and malabsorption of nutrients. Therefore, the balance of intestinal flora is vital to organismal health, and maintaining that balance is crucial in livestock production (Sekirov et al., 2010; Yadav and Jha, 2019). In this study, compared with ANT, the addition of XOS significantly increased ACE, Chao1, and Shannon alpha diversity indices and increased homogeneity. Shannon and ACE indices represent the diversity of microbial community species, and the Chao1 index represents the species richness of the community (Kong et al., 2009). In most studies, the dominant phyla in the cecum of 42-d-old broilers are Firmicutes and Bacteroidetes, and the results of this experiment are consistent with those of previous reports. The phyla have important roles in energy production and metabolism. The addition of XOS reduces the proportion of Firmicutes and Bacteroidetes in the intestinal tract of mice fed a high-fat diet, reduces the body fat weight of mice, and improves lipid metabolism (Jla et al., 2019). In this experiment, the addition of XOS in the diet regulated cecal bacteria by significantly lowering the ratio of Firmicutes to Bacteroidetes and contributing to the balance of intestinal flora. At the genus level, *Bacteroides*, *Ruminococcaceae_UCG-014*, *Phascolarctobacterium*, and *Alistipes* are all involved in the formation of SCFA, and *Bacteroides* and *Alistipes* are involved in the formation of propionic acid (Huang et al., 2018; Polansky et al., 2015).

Short-chain fatty acids can absorb many nutrients from the intestinal mucosa and can also increase the intestinal barrier function. Simultaneously, SCFA also contribute to the absorption of minerals and regulate the composition of intestinal microorganisms, with anti-inflammatory and immune regulation functions (Brooks, 2018). *Alistipes* are also involved in the production of acetic acid and thus have good anti-inflammatory effects (Göker et al., 2011; Oliphant and Allen-Vercoe, 2019). *Phascolarctobacterium* produces butyric acid through the acetyl/propionyl-CoA carboxylase pathway (Polansky et al., 2015). *Synergistes* can inhibit toxins in ruminant diets (Halliday et al., 2019). In this experiment, the addition of XOS increased the relative abundances of SCFA-related genera, such as *Bacteroides* and *Ruminococcaceae_UCG-014*, and thus promoted the intestinal health of broilers.

Both *[Ruminococcus]_torques_group* and *Bacteroides* can use fermentation to produce SCFA, which act as special nutritional and energy components of the broiler intestine, increasing intestinal barrier function and promoting healthy growth in broilers (Morrison and Preston, 2016). The results of the correlation analysis showed that the addition of XOS increased relative abundance of beneficial genera, which positively correlated with increases in physical barrier function in the broiler ileum and modulated the structure of intestinal bacterial communities. In the ileum of broilers, *Ruminococcus_torque_group* was positively correlated with the relative expression of claudin 1 and *ZO-1*, and *Bacteroides* was positively correlated with the relative expression of occludin, as well as ileal VH and V:C ratio. *Phascolarctobacterium* can produce butyric acid via the acetyl/propionyl-coenzyme A carboxylase pathway (Polansky et al., 2015). Correlation analysis in this study showed that *Phascolarctobacterium* was positively correlated with VH. Also, the relative abundance of *Phascolarctobacterium*, Bacteroidetes *and Alistipes* was significantly and positively correlated with the amount of dietary XOS supplementation (150, 300 and 450 mg/kg). It was shown that high doses of XOS (450 mg/kg) had a positive effect on the broiler intestinal tract, promoting its intestinal development and increasing the abundance of beneficial bacterial flora.

5. Conclusions

In conclusion, the addition of 150 and 450 mg/kg of XOS to broiler diets significantly improved the production performance and slaughtering performance of broilers, and the effect was better than that of 300 mg/kg of XOS group. The addition of 150 and 300 mg/kg of XOS to the diet enhanced the immunity of broilers. Further, the addition of 150, 300 and 450 mg/kg of XOS to broiler diets could effectively improve the cecum microbiota and promote the intestinal health of broilers. According to the conditions of this experiment, the appropriate amount of XOS to add to broiler diets is 150 mg/kg, which achieved similar effects to those of the antibiotic.

Author contributions

Zhiyong Rao: Conceptualization, Investigation, Methodology, Formal analysis, Writing – original draft, Visualization. **Yue Li:** Formal analysis, Methodology, Software. **Xiaopeng Yang:** Formal analysis, Software, Validation. **Yongpeng Guo:** Writing – review & editing. **Wei Zhang:** Writing – review & editing. **Zhixiang Wang:** Conceptualization, Supervision, Funding acquisition, Project administration, Resources, Writing – review & editing.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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