Effects of dietary xylooligosaccharides supplementation on the intestinal morphology, nitrogen metabolism, faecal ammonia release, antioxidant capacity, and immune organ indices of broilers

Xixi Li, Xiaohong Wu, Wenfeng Ma, Wei Chen and Furong Zhao

ABSTRACT
This aim of the present study was to evaluate the effects of dietary xylooligosaccharides (XOS) on the intestinal morphology, nitrogen metabolism, faecal ammonia release, antioxidant capacity, and immune organ indices of broilers fed corn-soybean meal diet. A total of 240 healthy 1-day-old Arbor Acres (AA) broilers were randomly divided into four treatment groups receiving XOS supplementation of 0 (Control), 150, 300, and 450 mg/kg to basal diet for 6 weeks. Six replicates with 10 birds each were prepared for each treatment. Results showed that the villus height of duodenum, jejunum, and ileum and the villus height to crypt depth ratio of jejunum were increased significantly in the broilers receiving 150 mg/kg XOS compared with those in the Control ($p < 0.05$). Nitrogen utilisation from day 19 to 21 was higher in the 150 mg/kg XOS group than in the Control ($p < 0.05$). Moreover, the addition of 150 mg/kg XOS reduced the nitrogen excretion from day 40 to 42 ($p < 0.05$), and all XOS treatments increased the nitrogen utilisation from day 40 to 42 ($p < 0.05$). All dietary XOS supplementations decreased the faecal ammonia concentration on day 21 ($p < 0.05$), and the addition of 150 and 450 mg/kg XOS induced a lower faecal ammonia concentration compared with that of the Control on day 42 ($p < 0.05$). Dietary supplementation of 300 and 450 mg/kg XOS enhanced the activity of serum total antioxidant capacity compared with that in the Control and 150 mg/kg XOS groups at the age of 42 days ($p < 0.05$). Supplementation with 450 mg/kg XOS significantly increased the index of thymus ($p < 0.05$) and the content of total protein compared with those of the Control at the age of 21 days ($p < 0.05$). Therefore, dietary supplementation with 150 mg/kg XOS have a beneficial effect on broilers.

HIGHLIGHTS
- Dietary supplementation with XOS increased the villus height of duodenum, jejunum and ileum, and the VH/CD ratio of jejunum.
- Dietary supplementation with XOS enhanced nitrogen metabolism and reduced faecal ammonia release.

Introduction
Intestinal disease, oxidant damage, and poor immune defense systems have become major problems in poultry production (Borsoi et al. 2015; Zhang et al. 2018; Tarradas et al. 2020). Once the animals suffer from diseases, they will be harmed to varying degrees whether acute or chronic and infectious or non-infectious and bring certain losses to the farmers and the breeding industry. Antibiotics are widely used as animal feed additives because of their role in promoting growth and resisting diseases (Dibner and Richards 2005; Afsharmanesh et al. 2013) for the rapid development of animal husbandry. Unfortunately, the exposure of livestock and poultry to antibiotics poses risks to human health due to the emergency of antibiotic-resistant bacteria and the passage of antibiotics through faeces and residues in food of animal origin from organisms to humans (Hu and Cowling 2020). The long-term use of antibiotics has caused a series of negative problems, such as intestinal flora imbalance, decreased immunity, drug residues, drug-resistant strains, and environmental pollution. Therefore, exploring and promoting non-polluting and non-residue antibiotic alternatives are necessary for the further development of green ecological animal husbandry.
Gibson and Roberfroid (1995) defined prebiotics as a non-digestible food component that produce beneficial effects on the health of the host by selectively stimulating the growth and/or activity of one or a limited number of gastrointestinal microflora already existing in the colon. In general, non-digestible oligosaccharides are prebiotics. At present, some functional oligosaccharides, such as mannoooligosaccharides (MOS), fructooligosaccharides (FOS), chitoooligosaccharides (COS), pectic oligosaccharides (POS), and soybean oligosaccharides (SBO), have been widely used in poultry diets as growth promoters instead of antibiotics (Corrigan et al. 2015; Shang et al. 2018; Xu et al. 2020). Oligosaccharides are prebiotics. At present, some functional oligosaccharides, such as mannoooligosaccharides (MOS), fructooligosaccharides (FOS), chitoooligosaccharides (COS), pectic oligosaccharides (POS), and soybean oligosaccharides (SBO), have been widely used in poultry diets as growth promoters instead of antibiotics (Corrigan et al. 2015; Shang et al. 2018; Xu et al. 2020).

Xylooligosaccharides (XOS) are composed of 2–8 xylose molecules linked by β-1,4 glycosidic bonds, and its main components include xylobiose, xylotriose and xylotetraose (Pu et al. 2016; Zidan et al. 2021). XOS are mainly produced by enzymatic hydrolysis and thermal cracking from lignin-rich fibre raw materials, such as corn cob, peach palm waste, cauliflower stalk, wheat bran, and sugarcane bagasse (Seesuriyachan et al. 2017; Majumdar et al. 2021; Sonkar et al. 2021; Vieira et al. 2021; Nascimento et al. 2022). They have good stability and heat resistance under acidic conditions, a low calorie content, non-toxicity, and biological effects even at low daily doses (Carvalho et al. 2013). Given that XOS cannot be digested and absorbed via the gastrointestinal tract of animals, the short-chain fatty acids (SCFAs) produced through XOS fermentation by beneficial bacteria regulate lipid metabolism, antioxidant defense systems, and immune factors and relieve intestinal inflammation (Wang et al. 2011; Hansen et al. 2013; De Maesschalck et al. 2015; Fei et al. 2019). Using a human colon simulator, Christophersen et al. (2013) added XOS to ferment soybean protein by mixing the faecal microbiota and it was found that XOS could modulate the protein-induced genotoxicity of the colonic environment through specific microbiota and SCFAs. XOS administration could beneficially improve the production performance, increase the content of SCFAs in the hindgut, enhance the egg quality, and modulate the nutrient digestibility and ileum morphology of laying hens (Xiao et al. 2020; Zhou et al. 2021). However, previous studies obtained inconsistent results regarding the effectiveness of XOS as prebiotics in broilers. Suo et al. (2015) reported that XOS supplemented at 75 mg/kg decreases duodenal crypt depth. Yuan et al. (2018) found that the dietary addition of 2 mg/kg XOS affects immune function by stimulating SCFAs to reduce jejunal cytokine gene expression. Sun et al. (2013) observed that supplementation with 10 g/kg XOS could increase growth performance and endocrine metabolism and upregulate the titre of avian influenza H5N1 antibody to enhance humoral immunity. Yang et al. (2022) demonstrated that supplementation with 200 mg/kg XOS could strengthen the activity of total superoxide dismutase (T-SOD) in serum and glutathione peroxidase (GSH-Px) in breast muscle. The above disparities might be related to the supplementation dose and source of XOS and diet type and breed of broilers.

The objective of the current study was to investigate the effects of dietary XOS supplementation of 0, 150, 300 and 450 mg/kg, on the intestinal morphology, nitrogen metabolism, faecal ammonia release, antioxidant capacity, and immune organ indices of broilers.

**Materials and methods**

**Experiment design and dietary treatments**

All the animal experiments were approved by the Institutional Animal Care and Use Committee of the Henan University of Science and Technology (HAUST-EAW-2021-C00227). A total of 240 healthy 1-day-old Arbour Acres (AA) broilers purchased from a local breeding farm (Luoyang, China) were randomly divided into four treatment groups with six replicates per treatment and 10 broilers per replicate. The birds were fed a basal diet supplementation with 0 (Control), 150, 300, and 450 mg/kg XOS. The basal diet (Table 1) was formulated to meet the nutrient requirement of broilers in accordance with the Management Guide of National Research Council (National Research Council (NRC) 1994). XOS was purchased from Zhengzhou Yicong Biotechnology Co., Ltd (Zhengzhou, China). The main components of XOS are xylobiose, xylotriose and xylotetraose, and the content of XOS is 20%. The trial lasted for 42 days and divided into two stages: early (day 1–21) and later (day 22–42). Over the entire period (day 1–42) mortality rate was evaluated.

**Experimental conditions**

The birds in each replicate were housed in a wire cage (95 cm length × 90 cm width × 40 cm height) with ad libitum access to food and water. Natural light and artificial supplementary light (16L: 8D) were adopted. The room temperature was set at 33°C–35°C for the 1st week and gradually decreased by 2°C–3°C every week until the room temperature reached 25°C. Good ventilation was kept throughout the trial period.
Sample collection
Blood was collected from the wing vein of broilers on 21 and 42 days of age. Two chickens were selected from each replicate, and 5 mL of blood was collected to a vacuum blood collection tube and centrifuged at 3000 x g/min for 15 min. The serum was stored in a refrigerator. On day 42, two broilers from each replicate were randomly selected and stabbed in the jugular vein to bleed to death. Approximately 2 cm portions in the middle part of duodenum, jejunum, and ileum were excised, and their contents were gently flushed with physiological saline. The intestinal segments were preserved in 4% paraformaldehyde tissue fixing solution. The thymus, spleen, liver, and bursa of Fabricius were weighed and recorded separately.

Intestinal morphology
The fixed intestinal segments were dehydrated, transparent, immersed in wax, embedded, sliced, stained with hematoxylin-eosin, then mounted and observed under a microscope (Olympus, Olympus, Inc., Tokyo, Japan). The villus height (VH), crypt depth (CD) and the VH/CD ratio of duodenum, jejunum and ileum were measured (Motic image plus 2.0, Motic, Inc., Wetzlar, Germany).

Nitrogen metabolism and faecal ammonia release
Total protein (TP), uric acid (UA), and urea nitrogen (UN) levels in serum were determined with an automatic biochemical analyser (Toshiba TBA-2000FR, Japan). The total faecal method with manure trays set up for each replicate was performed on day 19–21 and 40–42. The daily feed intake was recorded, and the faeces were collected in repeat units. Hairy debris was removed from the collected fresh faecal samples. The samples were fix in nitrogen with 10% HCl (20 mL HCl per 100 g of faecal sample) and mixed thoroughly. Afterward, the faecal sample were taken out, dried in an oven at 65 °C for 72 h to a constant weight, crushed and passed through a 40 mesh sieve, and stored for measurement. The nitrogen content of the rations and manure was determined using a semi-automatic Kjeldahl nitrogen tester (Shengsheng K1301, Shanghai, China). In brief, 100 g of fresh chicken faeces were collected per replicate on day 21 and 42, placed in a 500 ml conical flask, and allowed to stand for 24 h at 25 °C. A portable ammonia gas detector (Xima AR8500, Shenzhen, China) was used to measure ammonia release from the samples.

Nitrogen intake (g/d) = daily feed intake × nitrogen content in the diet
Nitrogen excretion (g/d) = daily excretion × nitrogen content in the fecal
Nitrogen utilization % = (nitrogen intake – nitrogen excretion) / nitrogen intake

Antioxidant capacity
T-AOC, SOD and GSH-Px activities and malondialdehyde (MDA) content in serum were assayed following the instructions of the commercial kits from Nanjing Jiancheng Bioengineering Research Institute (Nanjing, China).

Immune organ indices
Immune organ index = immune organ weight (g)/live body weight (kg)
**Statistical analysis**

Data were analysed with SPSS 20.0 software (IBM Inc., NY) using one-way ANOVA and Duncan’s multiple range test to identify the differences among treatments. Significant difference was determined as $p < 0.05$.

**Results**

**Intestinal morphology**

The effects of dietary XOS on the morphology of small intestinal are summarised in Table 2. The dietary supplementation of 150 mg/kg XOS significantly increased the VH of duodenum, jejunum and ileum compared with that of the Control ($p < 0.05$). All XOS treatments did not have a significant effect on CD regardless of intestine part ($p > 0.05$). Nonetheless, the VH/CD ratio of the jejunum in the 150 mg/kg XOS treatment group was significantly improved compared with that in the Control ($p < 0.05$).

**Table 2.** Effects of dietary supplementation Xylooligosaccharides (XOS) on the intestinal morphology of broilers at 42 days of age.

| Items          | XOS mg/kg$^c$ | 0   | 150 | 300 | 450 | SEM$^d$ | p-Value |
|----------------|---------------|-----|-----|-----|-----|---------|---------|
| Duodenum       |               |     |     |     |     |         |         |
| Villus height (μm) | 1253.2$^{ab}$ | 1687.9$^a$ | 1507.9$^{ab}$ | 1433.2$^{ab}$ | 162.0 | 0.028   |         |
| Crypt depth (μm)      | 173.9         | 175.9 | 170.9 | 183.5 | 19.22 | 0.531   |         |
| V/C                | 7.61          | 9.84  | 8.84  | 8.12  | 1.74  | 0.255   |         |
| Jejunum          |               |     |     |     |     |         |         |
| Villus height (μm) | 1239.0$^{ab}$ | 1726.7$^a$ | 1429.7$^{ab}$ | 1411.1$^{ab}$ | 151.5 | 0.012   |         |
| Crypt depth (μm)      | 184.1         | 169.9 | 157.2 | 176.1 | 27.34 | 0.353   |         |
| V/C                | 6.76$^{ab}$  | 10.23 | 9.3$^{ab}$ | 8.29$^{ab}$ | 1.39  | 0.037   |         |
| Ileum             |               |     |     |     |     |         |         |
| Villus height (μm) | 971.1$^{ab}$ | 1252.3$^a$ | 1136.4$^{ab}$ | 1094.0$^{ab}$ | 97.01 | 0.020   |         |
| Crypt depth (μm)      | 175.7         | 175.5 | 159.8 | 157.3 | 23.05 | 0.447   |         |
| V/C                | 5.76          | 7.16  | 7.14  | 6.99  | 0.77  | 0.105   |         |

$^a$In the same row, values with the same or no letter superscripts means no significantly difference ($p > 0.05$), while values with the different letter superscripts means significantly difference ($p < 0.05$).

$^b$Basal diet supplementation with 0, 150, 300, and 450 mg/kg XOS.

$^c$Basal diet supplementation with 0, 150, 300, and 450 mg/kg XOS.

$^d$SEM: standard error of mean.

**Nitrogen metabolism and faecal ammonia release**

Table 3 shows the serum biochemical parameters on day 21 and 42. The concentration serum TP in 450 mg/kg XOS treatment group was the highest ($p < 0.05$) at the age of 21 days. XOS had no effect on the concentration of serum UA and UN ($p > 0.05$). The nitrogen utilisation in day 19–21 was higher ($p < 0.05$) in the 150 mg/kg treatment group than that in the Control (Table 4). The 150 mg/kg XOS supplementation significantly reduced ($p < 0.05$) the nitrogen excretion from day 40 to 42 and notably increased the nitrogen utilisation from day 40 to 42 ($p < 0.05$) compared with those of the Control. XOS supplementation decreased the faecal ammonia release at day 21; in particular, the faecal ammonia release in the 150 and 450 mg/kg XOS treatment groups were still lower ($p < 0.05$) than that in the Control at day 42 (Figure 1).

**Table 3.** Effects of dietary Xylooligosaccharides (XOS) supplementation on the serum biochemical parameters of broilers at 21 and 42 days of age.

| Items          | XOS mg/kg$^c$ | 0   | 150 | 300 | 450 | SEM$^d$ | p-Value |
|----------------|---------------|-----|-----|-----|-----|---------|---------|
| 21 days        |               |     |     |     |     |         |         |
| TP (g/L)       | 22.37$^{ab}$  | 25.97$^a$ | 20.17$^b$ | 27.33$^b$ | 1.83  | 0.013   |         |
| UA (μmol/L)    | 376.67        | 269.33 | 417.33 | 219.0 | 105.7 | 0.098   |         |
| UN (mmol/L)    | 0.38          | 0.41  | 0.50  | 0.33  | 0.072 | 0.060   |         |
| 42 days        |               |     |     |     |     |         |         |
| TP (g/L)       | 27.28         | 26.58 | 31.05 | 31.70 | 3.73  | 0.194   |         |
| UA (μmol/L)    | 418.50        | 330.50 | 472.25 | 430.25 | 85.34 | 0.123   |         |
| UN (mmol/L)    | 0.39          | 0.38  | 0.34  | 0.43  | 0.05  | 0.123   |         |

$^a$In the same row, values with the same or no letter superscripts means no significantly difference ($p > 0.05$), while values with the different letter superscripts means significantly difference ($p < 0.05$).

$^b$Basal diet supplementation with 0, 150, 300, and 450 mg/kg XOS.

$^c$SEM: standard error of mean.

$^d$SEM: standard error of mean.

**Antioxidant capacity**

The effects of dietary XOS supplementation on T-AOC, SOD and GSH-Px activities and MDA content in the serum at the age of 21 and 42 days are presented in Figure 2. No significant difference ($p > 0.05$) was observed on serum T-AOC, SOD, and GSH-Px activities and MDA concentration among the treatments on day 21 and 42 days.

**Table 4.** Effects of dietary Xylooligosaccharides (XOS) supplementation on the nitrogen metabolism of broilers from day 19 to 21 and from day 40 to 42.

| Items          | XOS mg/kg$^c$ | 0   | 150 | 300 | 450 | SEM$^d$ | p-Value |
|----------------|---------------|-----|-----|-----|-----|---------|---------|
| 19–21 days     |               |     |     |     |     |         |         |
| Nitrogen intake (g/day) | 1.98         | 2.10  | 2.16  | 2.02  | 0.193 | 0.391   |         |
| Nitrogen excretion (g/day) | 0.70         | 0.62  | 0.74  | 0.63  | 0.053 | 0.052   |         |
| Nitrogen utilisation (%) | 64.49$^{ab}$ | 70.48$^{b}$ | 65.38$^{b}$ | 68.63$^{ab}$ | 1.79  | 0.010   |         |
| 40–42 days     |               |     |     |     |     |         |         |
| Nitrogen intake (g/day) | 5.29         | 4.73  | 4.93  | 5.07  | 0.439 | 0.236   |         |
| Nitrogen excretion (g/day) | 2.10$^{a}$ | 1.54$^{b}$ | 1.71$^{ab}$ | 1.74$^{ab}$ | 0.202 | 0.025   |         |
| Nitrogen utilisation (%) | 60.36$^{b}$ | 67.34$^{ab}$ | 65.27$^{a}$ | 65.70$^{b}$ | 1.99  | 0.008   |         |

$^a$In the same row, values with the same or no letter superscripts means no significantly difference ($p > 0.05$), while values with the different letter superscripts means significantly difference ($p < 0.05$).

$^b$Basal diet supplementation with 0, 150, 300, and 450 mg/kg XOS.

$^c$SEM: standard error of mean.
21. At the age of 42 days, serum T-AOC activity was significantly enhanced ($p < 0.05$) in the 300 and 450 mg/kg XOS treatment groups compared with that in the Control and 150 mg/kg XOS treatment group.

**Immune organ indices**

Table 5 shows that the effects of dietary XOS on the thymus, spleen, liver and bursa of Fabricius of broilers. Compared with that in the Control, the thymus index was significantly improved in the broilers receiving 450 mg/kg XOS ($p < 0.05$). No significant difference in spleen, liver, and bursa of Fabricius was observed among the treatment groups ($p > 0.05$).

**Discussion**

The intestinal is an important place for the digestion and absorption of animal nutrients. Nutrients enter the animal body and are finally absorbed by small
intestinal epithelial cells through physical reaction. Intestinal villus height, crypt depth, the villus height to crypt depth ratio are important indicators reflecting the integrity of intestinal structure (Paiva et al. 2014). Yang et al. (2022) reported that 200 mg/kg XOS supplementation in broiler diet significantly increased the jejunal and ileal villus height. Other studies also found that the addition of 150 mg/kg XOS in broiler diets increased the ileum villus height (Luo et al. 2021). However, Wu et al. (2006) indicated that XOS supplementation had no effect on jejunum morphology of broilers. Long intestinal villus and shallow crypt depth suggest that cells have a high ability to proliferate and absorb nutrients (Ghalwash et al. 2022). Current results showed that the villus height of duodenum, jejunum, and ileum and the VH/CD ratio of jejunum were significantly increased in the broilers fed with 150 mg/kg XOS. Similar to our findings, Wang et al. (2021) found that corn-soybean meal diet supplemented with 100 mg/kg XOS has beneficial effects on the intestine of broilers; the villus height and the VH/CD ratio in all intestinal segments displayed notable increases. The improvement in intestinal morphology was due to XOS stimulating the populations of *Bifidobacterium* and *Lactobacilli* and the production of acetate and butyrate (Li et al. 2015; Ribeiro et al. 2018; Yin et al. 2019). In vitro experiment showed that probiotics can utilise XOS for growth and reproduction by competing with pathogenic bacteria for substrates and attachment sites, thus leading to the large accumulation of SCFAs and other metabolites of probiotics (de Figueiredo et al. 2020). SCFAs provide energy for the proliferation of intestinal epithelial cells (Venegas et al. 2019), the VH/CD ratio is generally considered to be directly connected with epithelial cell turnover (Fan et al. 1997). Cell proliferation occurs primarily in the lower half of the crypt, in which the mitotic pressure forces cells to ascend along the crypt axis (Inan et al. 2000); the cells that promote crypt division move towards the exfoliated intestinal villus epithelium, thus increasing villus height. Given that all intestinal segment villus height were stimulated, we believed that XOS may accelerate the renewal rate of intestinal epithelial cells. The large absorption area leads to the enhancement of intestinal nutrient absorption ability, thus keeping the broiler intestine in a healthy state.

The digestibility of plant-based protein feeds is lower than that of animal-based protein feeds mainly due to their fibre levels and anti-nutritional factors. During active carbohydrate breakdown, amino acid fermentation end products, such as ammonia, are used by bacteria for protein synthesis during microbial growth; however, amines, ammonia, phenols, and indoles accumulate during carbon-limited fermentation (Cummings and Bingham 1987). The combination of xylanase and XOS stimulates the microbial community in the gut to efficiently degrade fibre, thereby increasing the intake of digestible nutrients and energy (González-Ortiz et al. 2021).

Prebiotic oligosaccharides may have anti-adhesive activity that allow them to compete with enteric pathogens for binding sites, coat the intestinal epithelial surface, and inhibit pathogen infection (Shoaf et al. 2006). XOS are selectively utilised by beneficial gut flora without being fermented by potential pathogens, such as toxigenic *Escherichia coli*, proteolytic *Bacteroides*, and toxigenic *Clostridium* (Manning and Gibson 2004). In this study, 150 mg/kg XOS supplementation significantly improved the nitrogen utilisation and reduced the faecal ammonia release of broilers. XOS can exert its effect by inhibiting intestinal ammonia-producing anaerobic bacteria, such as *Bacteroides*, and improve nutrient utilisation by increasing the activity of intestinal digestive enzymes (Kajihara et al. 2000; Li et al. 2021). The improved nitrogen utilisation and reduced faecal ammonia could be attributed in part to the increased height of the intestinal villi that widens the absorption area of nutrients, thereby improving protein utilisation. The supplementation of 150 mg/kg XOS can increase the height of small intestinal villi and improve nitrogen utilisation, and a further increase in XOS had no significant difference compared with that in the Control, which may be related to the fact that XOS promotes the proliferation of beneficial bacteria (*Bifidobacterium* and *Lactobacillus*). The increase in the number of beneficial bacteria and the secretion of SCFAs reduce the intestinal pH, provide a suitable working environment for digestive enzymes, and promote the absorption and utilisation of nutrients. However, an over acid

### Table 5. Effects of dietary Xylooligosaccharides (XOS) supplementation on the immune organ indices of broilers at 42 days of age (g/kg).

| Items                  | 0   | 150 | 300 | 450 | SEM | p-Value |
|------------------------|-----|-----|-----|-----|-----|---------|
| Bursa of Fabricius index | 2.00 | 2.05 | 1.90 | 2.35 | 0.258 | 0.098   |
| Liver index            | 21.12 | 21.20 | 22.26 | 22.83 | 1.18 | 0.163   |
| Spleen index           | 2.00 | 2.05 | 1.90 | 2.35 | 0.258 | 0.098   |
| Thymus index           | 2.63<sup>a</sup>  | 3.31<sup>b</sup>  | 3.24<sup>ab</sup>  | 4.24<sup>a</sup>  | 0.465 | 0.003   |
| p-Value                |      |      |      |      |      |         |

<sup>a</sup>Indicates significantly different (p < 0.05).
<sup>b</sup>Indicates no significantly difference (p > 0.05).
<sup>c</sup>Dietary Xylooligosaccharides (XOS) supplementation with 0, 150, 300, and 450 mg/kg XOS.
<sup>d</sup>SEM: standard error of mean.
gut environment can affect digestive enzyme activity and inhibit nutrient absorption.

The oxidation of polyunsaturated phospholipids present in cell membranes causes lipid peroxidation which increases the permeability of cell membranes; protein oxidation changes the structure and impairs the functional properties of the cell membrane, and DNA oxidation produces mutations through aberrations and damage repair mechanisms (Shrivastava et al. 2021). T-AOC is a comprehensive index used to measure the antioxidant function and reflects the compensatory ability of the antioxidant enzyme system and non-enzymatic system to external stimuli and the body’s free radical metabolism. SOD and GSH-Px constitute the enzymatic antioxidant defense system that prevents cells and tissues from being damaged by oxidative stress, resists the destruction of peroxides, and balances oxidative and antioxidant status (Xu et al. 2021; Wu et al. 2022). XOS derived from corn cob has good 2,2'-diphenyl-1-pyridinium hydrazine (DPPH) and 2,2'-azido-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity and iron-reducing antioxidant capacity (FRAP) (Boonchuay et al. 2021). The phenolic substituents of XOS show antioxi-

dant activity is primarily due to the antioxidant properties of XOS. With the increase in the amount of XOS added, the active ingredients exhibiting antioxidant activity also increase. Our results indicated that supplementation of 300 and 450 mg/kg XOS can be effective to attenuate the oxidative stresses. These inconsistent results may be associated with the type and amount of XOS supplementation and the breed of broilers. Hence, the antioxidant mechanism of XOS needs further study.

The immune organ index is an important indicator of the immune status of poultry, the great absolute weight and relative weight of immune organs indicates the strength of the body’s cellular and humoral immune function. The liver contains the largest collection of phagocytic cells in the body and can generate a rapid and powerful immune response under the right conditions. Spleen is important in regulating the immune system and maintaining peripheral tolerance by eliminating circulating apoptotic cells, differentiating and activating T and B cells, and producing antibodies in the white pulp (Tarantino et al. 2013). The bursa of Fabricius is the major lymphoid organ in birds and is responsible for the expansion and differentiation of B lymphoid progenitors in its follicular microenvironment (Fellah et al. 2014). Broilers infected with Salmonella typhimurium and fed XOS diets showed a reduced abundance of Salmonella in their immune organs such as liver, spleen, and bursa of Fabricius, indicating their enhanced immune response (Jazi et al. 2019). Zhao (2013) indicated that 75 mg/kg superfine XOS had no effect on the thymus and spleen index of broiler chickens at 42 days but significantly increased the bursa of the Fabricius index. Our present results indicated that dietary supplementation with 450 mg/kg XOS could significantly increase the thymus index of broilers. Thymus is closely related to the selection, development, proliferation, and differentiation of T cells (Xue et al. 2019). XOS can be used as antigens to effectively and lastingly stimulate the immune system, thus promoting the division and development of immune organ cells (Licht et al. 2012). O-acetylated XOS and its deacetylated derivatives formed by almond hulls hydrolysis are linked to (4-O-
methyl-D-glucuronide)-D-xylan, exert mitogenic activity, and improve T-mitogen-induced thymocyte proliferation (Bhatia et al. 2019). Immune organs play an important role in the defense of poultry against bacterial invasion, so the immune organ index can reflect the immune response ability of birds. In the present work, supplementation of 450 mg/kg XOS significantly increased the thymus index, thereby enhancing the immunity of broilers to a certain extent, and without adversely affecting the development of other
immune organs. A possible explanation is that the body obtained the immune ability from thymus and had reached the required level of immunity; therefore, the index of other immune organs can no longer be up-regulated. In summary, XOS might promote the development of immune organs.

Conclusion

This study demonstrated that dietary supplementation with 150 mg/kg XOS in broilers could increase the villus height of duodenum, jejunum, and ileum and the VH/CD ratio of jejunum, enhance the nitrogen utilisation, and reduce the faecal ammonia release. The dietary addition of 450 mg/kg XOS can improve the serum T-AOC activity and thymus index on day 42. Owing to its effects and breeding benefits, the dietary addition level of 150 mg/kg XOS is beneficial to broiler production.

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Ethical approval

The experimental protocol in this study was approved by the Animal Care and Use Committee of Henan University of Science and Technology.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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