The effects of xylo-oligosaccharides on regulating growth performance, nutrient utilization, gene expression of tight junctions, nutrient transporters, and cecal short chain fatty acids profile in *Eimeria*-challenged broiler chickens

Yang Lin, Po-Yun Teng, and Oluyinka A. Olukosi[1]

Department of Poultry Science, University of Georgia, Athens, GA 30602, USA

ABSTRACT A 21-d experiment was conducted to investigate the effects of xylo-oligosaccharides (**XOS**) on growth performance, nutrient utilization, gene expression of tight junctions, nutrient transporters, and cecal short chain fatty acids (**SCFA**) profile of broiler chickens challenged with mixed *Eimeria* spp. Two hundred fifty-two zero-day-old chicks were allocated to 6 treatments in a 3 × 2 factorial arrangement (corn-soybean meal diets supplemented with 0, 0.5, or 1.0 g/kg XOS; with or without *Eimeria* challenge). Challenged groups were inoculated with a solution containing *E. maxima, E. acervulina*, and *E. tenella* oocysts on d 15. During the infection period (d 15 to d 21), there was a significant ($P < 0.05$) *Eimeria* × XOS interaction for weight gain (**WG**). XOS significantly ($P < 0.05$) increased WG in the unchallenged birds but not in the challenged treatments. There was no significant *Eimeria* × XOS interaction for N and minerals utilization responses. XOS supplementation at 0.5 g/kg tended to alleviate *Eimeria*-induced depression in apparent ileal digestibility of DM ($P = 0.052$). Challenged birds had lower ($P < 0.01$) AME, AMEn, and total retention of N, Ca, and P. *Eimeria* upregulated ($P < 0.01$) gene expression of tight junction proteins claudin-1, junctional adhesion molecule-2, and glucose transporter GLUT1; but downregulated ($P < 0.01$) the peptide transporter PepT1, amino acid transporters rBAT, CAT2, y+LAT2, and zinc transporter ZnT1. XOS alleviated ($P < 0.05$) *Eimeria*-induced claudin-1 upregulation. *Eimeria* decreased ($P < 0.05$) cecal saccharolytic SCFA acetate, butyrate, and total SCFA, but increased ($P < 0.05$) branched chain fatty acids isobutyrate and isovalerate. The supplementation of XOS tended to decrease the concentration of isobutyrate ($P = 0.08$) and isovalerate ($P = 0.062$). In conclusion, 0.5 g/kg XOS supplementation alleviated depression in growth performance and nutrient utilization from the *Eimeria* challenge. In addition, supplemental XOS reversed the gene expression changes of claudin-1, also showed the potentials of alleviating the negative cecal fermentation pattern induced by *Eimeria* infection.

Key words: *Eimeria*, xylo-oligosaccharides, growth performance, short chain fatty acid, broiler chicken

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INTRODUCTION

Prebiotic was first defined by Gibson and Roberfroid in 1995 as "a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and, or, activity of one or a limited number of bacteria in the colon". The concept has been developed and expanded as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" in 2016 ISAPP (Gibson and Roberfroid, 1995; Gibson et al., 2017). Prebiotic application viability is based on the observation that beneficial and pathogenic bacteria prefer different nutrient sources (Gibson et al., 2004). Unlike probiotics, which directly alter the intestinal microbial population, prebiotics are substrates selectively utilized by beneficial intestinal bacteria thereby enhancing their competitive strength against pathogenic bacteria. In poultry, the most commonly used prebiotics are oligosaccharides such as fructo-oligosaccharides (**FOS**), mannan-oligosaccharides (**MOS**), galacto-oligosaccharides (**GOS**), and xylo-oligosaccharides (**XOS**) (Adhikari and Kim, 2017). Oligosaccharides are carbohydrates containing 3 to 10 sugar monomers (Mussatto and Mancilha, 2007).

Avian coccidiosis is a global disease that is caused by *Eimeria* spp., including 3 most prevalent species *E. acervulina*, *E. maxima,* and *E. tenella* in broiler chickens (Allen and Fetterer, 2002). As the most economically significant disease in the poultry industry, coccidiosis globally leads to

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[1]Corresponding author: oaolukosi@uga.edu

14.5 billion US dollar losses annually, 95.1% of which was from depressed broiler production (Dalloul and Lillehoj, 2006; Blake et al., 2021). The economic losses in morbidity which causes reduced growth and feed efficiency took the majority, varying from 51% to 90%, of the total cost. After decades of use of anticoccidial drugs, the resistance to all anticoccidial drugs is developing rapidly. Furthermore, driven by public and legislative pressure, the use of several anticoccidial drugs such as ionophores, is not permitted in the "No antibiotics, ever" production systems. Probably as a consequence thereof, there has been no new anticoccidial drug introduced to the market after Diclazuril in 1990 (Blake et al., 2021).

Several nutritional approaches as complementary methods for coccidiosis control are being studied. The main point of prebiotic effects on gut health is modifying the composition and activities of gut microbiota, in particular stimulating the growth of *bifidobacteria* and *lactobacilli*, thus creating a healthy microbial flora situation (Baurhoo et al., 2007; Roberfroid et al., 2010; Ding et al., 2018). Based on the prebiotic effects on modulating composition or activities of intestinal microflora, it is generally accepted that prebiotics have the capacity to alleviate pathogenic coccidia-induced negative effects, especially in ameliorating dysbacteriosis which is the typical symptom of coccidiosis (Baurhoo et al., 2007; Mookiah et al., 2014; Abd El-Hack et al., 2021). In addition, carbohydrate fermentation stimulated by prebiotics acidify the cecal environment which mitigates adverse effects of *Eimeria* infection on cecal fermentation (Adhikari and Kim, 2017; Lin and Olukosi, 2021a).

Xylo-oligosaccharides (XOS) are hydrolytic degradation oligomers from arabinoxylans which are hemicellulosic polymers universally present in plants and consist of xylose units (Moure et al., 2006). As promising prebiotics, XOS have been shown to stimulate the activity of the intestinal microbiota and alter microbial composition, improving the gut health and animal growth performance (De Maesschalck et al., 2015a; Craig et al., 2020a). XOS are capable of protecting intestinal integrity and stimulating immunomodulation (Yuan et al., 2018; Yin et al., 2019). Specifically, XOS increased plasma concentrations of IgA, IgM, IL-2, and TNF-α, and decreased the expression of proinflammatory cytokines, such as IFN-γ and IL-1β (Hansen et al., 2013). XOS-increased beneficial microbiota or microbial metabolites such as SCFA is likely the main driver in their immunomodulation effects in chickens (Lecerf et al., 2012; Shokryazdan et al., 2017).

The objective of this experiment was to investigate the potential and mechanisms of prebiotic xylo-oligosaccharides on mitigating the harmful effects of *Eimeria* challenge on growth performance, nutrient utilization, gene expression of tight junctions and nutrient transporters, and cecal SCFA profile in broiler chickens.

MATERIALS AND METHODS

The experiment was conducted at the University of Georgia Poultry Research Center. All the experimental animal procedures used in this study were approved by the Institutional Animal Care and Use Committee of the University of Georgia, Athens, GA.

Birds, Diets, Experimental Design, and Eimeria Challenge

Two hundred fifty-two zero-day-old Cobb 500 (off-sex) broiler chicks were used in the 21-d experiment to study the possibility of xylo-oligosaccharides (XOS) alleviating *Eimeria*-induced adverse effects in broiler chickens. Corn-soybean meal diets were formulated with phytase at 500 FTU/kg (Quantum Blue, AB Vista, Marlborough, UK; 5,000 FTU/g). Titanium dioxide was added as inert marker for nutrient digestibility calculations by the index method. Birds were allocated to 6 treatments in a 3 × 2 factorial arrangement. The factors were 3 levels of xylo-oligosaccharides (0, 0.5, or 1.0 g/kg) (AIDP Inc., City of Industry, CA) supplementation on top of the basal diet and the *Eimeria* challenge (with or without). The food-grade prebiotic XOS used in the current study comprises xylo-oligosaccharides molecules containing from 2 to 6 linked xyloses (xylobiose, xylotriose, xylotetraose, xylopentaose, and xylohexaose) obtained from non-genetically modified corn and was previously characterized (Yang et al., 2015; Silva et al., 2020). The birds in all the treatments had similar initial body weight (41.3 ± 0.25 g) on d 0. Each of the 6 treatments had 6 replicate cages and 7 birds per replicate cage.

On d 15, birds were gavaged with 1 mL water as placebo or 1 mL mixed-species *Eimeria* oocysts solution based on the treatments. The concentration of mixed-species *Eimeria* spp. in the water-based solution was 12,500 oocysts of *E. maxima*, 12,500 oocysts of *E. tenella*, and 62,500 oocysts of *E. acervuline* per 1 mL, in order to produce a mild infection (Teng et al., 2020). The ingredient composition and the analyzed chemical composition of the experimental diets are presented in Table 1.

Growth Performance, Intestinal Permeability, and Lesion Scoring

The weight of birds and feed intake (**FI**) were measured on d 0, 15, and 21. The body weight gain (**WG**) and gain: feed were calculated from d 0 to d 15 and d 15 to d 21. An intestinal permeability test referenced from a previous study (Teng et al., 2020) was conducted on 5-d postinfection (**DPI**). One bird was randomly selected from each challenged cages and gavaged with 1 mL of previously prepared 2.2 mg/mL fluorescein isothiocyanate dextran (FITC-d, MW 4,000; Sigma-Aldrich, Oakville, Canada) solution. Extra birds (provided with the same basal diet without XOS) were used to collect blank

Table 1. Ingredients and analyzed compositions (g/kg) of the experimental diets.

Diets[1]	Basal
Corn	580
Soybean meal	339
Soybean oil	40
Titanium dioxide	5.0
Di-calcium phosphate	8.5
Limestone	15.0
Lysine	0.8
Methionine	1.5
Threonine	0.4
$NaHCO_3$	2.0
Salt	3.0
Vitamin premix[2]	2.5
Trace minerals premix[3]	2.5
Phytase	0.15
Xylo-oligosaccharides	0
Total	1,000
Crude protein	212
ME, kcal/kg	2,966
Ca	9.1
Total P	5.2
Available P[4]	2.7
Met	4.8
Cys	3.5
Met + Cys	8.3
Lys	12.2
His	5.7
Trp	2.5
Thr	8.5
Arg	14.2
Dry matter	898
Crude protein	203
Ca	9.25
Total P	4.89
$(Hex)_3$	77.3
$(Hex)_4$	75.5
$(Hex)_5$	3.6
$(Pent)_3$	13.1
$(Pent)_5$	1.1

[1]C— diet without xylo-oligosaccharides; XOSL— diet with 0.5 g/kg xylo-oligosaccharides; XOSH— diet with 1 g/kg xylo-oligosaccharides.

[2]Vitamin A, 5,484 IU; vitamin D3, 2,643 ICU; vitamin E, 11 IU; menadione sodium bisulfite, 4.38 mg; riboflavin, 5.49 mg, d-panthothenic acid, 11 mg; niancin, 44.1 mg, choline chloride, 771 mg; vitamin B12, 13.2 μg; biotin, 55.2 μg; thiamine mononitrate, 2.2 mg; folic acid, 990 μg; pyridoxine hydrochloride, 3.3 mg.

[3]Iodine, 1.11 mg; manganese, 66.06 mg; copper, 4.44 mg; iron, 44.1 mg; zinc, 44.1 mg; selenium, 300 μg.

[4]Available P level included the matrix for the phytase (1.5 g/kg for non-phytate P). Hexose (Hex); Pentose (Pent). (Hex)6, (Pent)4 and (Pent)6 were not detected in diet.

blood sample to dilute FITC-d for the preparation of the standards curve. After 2 h of administration, blood samples were collected from the heart of euthanized birds. Clotted blood was centrifuged at 1,000 × *g* for 12 min and the serum was measured by spectrophotometer (Spectramax M5, Molecular Devices, San Jose, CA) at 485 nm excitation wavelength and 528 nm emission wavelength. All the procedures of blood sample preparation and measurements were conducted in a dark room.

At the end of the study, 3 birds per cage were randomly chosen to score intestinal lesion based on a 0 to 4 (no lesion to severe lesion) scale grading. Scoring was done for birds in *Eimeria*-challenged treatments only. The duodenum, jejunum and ileum, and ceca were scored separately (Johnson and Reid, 1970).

Sample Collection

Excreta samples were collected on d 20 (5 DPI). The excreta were subsequently oven-dried, ground, and later used for nutrient utilization measurements including total tract retention of N and minerals. On d 21 (6 DPI), the ileal digesta were collected from five birds per cage and oven-dried for ileal digestibility measurements. Cecal contents were collected from 2 birds per cage and stored at −20°C for later short chain fatty acids (**SCFA**) analysis. Jejunal tissues were collected from 2 birds per cage, snap-frozen in liquid N immediately, and later stored at −80°C before further gene expression analysis.

Oocyst Shedding

Excreta at d 21 (6 DPI) were quantitatively collected from cage for oocyst shedding measurement. After thorough mixing, approximately 5 g of sample from each cage were weighed and diluted with water in 1: 9 ratio. The dilution was repeated again to make 1:99 diluted sample. After vortexing, 5 mL of diluted samples were mixed with 45 mL of saturated salt solution in a centrifuge tube. The vortexed samples were loaded in a McMaster chamber and observed under a microscope. The total oocyst shed was counted and standardized as oocysts per gram excreta.

Quantitative Real-Time PCR Analysis

Quantitative real-time PCR was used to analyze gene expression of jejunal tight junction proteins and nutrient transporters. After being homogenized in QiAzol lysis reagent (QIAGEN, Hilden, Germany), total RNA of jejunal tissue was extracted according to the manufacturer's instructions. Extracted RNA was converted to cDNA in a 20 μL reaction volume by high-capacity cDNA reverse transcription kit (Thermo Fisher Scientific, Waltham, MA) after quantity measurement in Bio-Tek Synergy HTX spectrophotometer (Agilent, Santa Clara, CA) and diluted to equal concentration. The quantitative reverse-transcriptase polymerase chain reaction was performed in Step One Plus real-time PCR system (Thermo Fisher Scientific, Waltham, MA) with reaction master mix iTaq Universal SYBR Green Supermix (Bio-Rad, Hercules, CA). Samples were run in duplicate, and the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) was applied to analyze the results. All the primers used and function of genes in the experiments are listed in Table 2.

Chemical Analysis

Oven-dried diets, excreta, and ileal digesta were ground (0.5 mm) to measure dry matter (**DM**) (AOAC Method 934.01), nitrogen (**N**) content, gross energy (**GE**), and titanium dioxide. Nitrogen analyzer (LECO, St. Joseph, MO) was used to measure N content in diets,

Table 2. List of primers used for qPCR.

Gene symbol	Accession number	Full name	Function	Forward primer	Reverse primer
Beta-actin	NM_205518.1	Beta-actin	Housekeeping gene	5-CAACACAGTGCTG TCTGGTGGTA-3	5-ATCGTACTCCTGCTT GCTGATCC-3
CLDN1	NM_001013611.2	Claudin 1	Tight junction	5-TGGAGGATGACCAGGTGAAGA-3	5-CGAGCCACTCTGTTGC-CATA-3
JAM2	XM_025149444.1	Junctional adhesion molecule 2	Tight junction	5-AGCCTCAAATGGGATTGGATT-3	5-CATCAACTTG-CATTCGCTTCA-3
PepT1 (SLC15A1)	KF366603.1	Peptide transporter-1	Peptide transporter	5-CCCCTGAGGAGGATCACTGTT-3	5-CAAAAGAGCAGCAG-CAACGA-3
GLUT1 (SLC2A1)	NM_205209.1	Glucose transporter-1	Glucose transporter	5-CTTTGTCAACCGC TTTGG-3	5-CAGAATACAGGCCG ATGAT-3
GLUT2 (SLC2A2)	XM_010716927.3	Glucose transporter-2	Glucose transporter	5-TCATTGTAGCTGAGCTGTT-3	5-CGAAGACAACGAACA-CATAC-3
GLUT5 (SLC2A5)	XR_005855627.1	Glucose transporter-5	Glucose transporter	5-TTGCTGGCTTTGG GTTGTG-3	5-GGAGGTTGAGGGC-CAAAGTC-3
SGLT1 (SLC5A1)	NM_001293240.1	Sodium glucose transporter-1	Glucose transporter	5-GCCGTGGCCA GGGCTTA-3	5-CAATAACCTGATCTGTG-CACCAGT-3
rBAT (SLC3A1)	XM_040667709.1	Solute carrier family 3-member 1	Dimerize with $b^{0,+}$AT	5-CCCGCCGTTCAACAAGAG-3	5-AATTAAATCCATC-GACTCCTTTGC-3
$b^{0,+}$AT (SLC7A9)	NM_001199133.1	Solute carrier family 7-member 9	Na^+-independent neutral/cysteine, cationic amino acid exchanger	5-CAGTAGTGAATTCTCTGAGTGT-GAAGCT-3	5-GCAATGATTGCCACAAC-TACCA-3
CAT2 (SLC7A2)	XM_040699005.1	Cationic amino acid transporter-2	Amino acid transporter	5-TGCTCGCGTT CCCAAGA-3	5-GGCCCACAGTTCACCAA-CAG-3
y+ LAT1 (SLC7A7)	XM_040665181.1	y+ L amino acid transporter-1	Na^+-dependent neutral/cationic amino acid exchanger	5-CAGAAAACCTCA-GAGCTCCCTTT-3	5-TGAGTACAGAGC-CAGCGCAAT-3
y+ LAT2 (SLC7A6)	XM_040681086.1	y+ L amino acid transporter-2	Na^+-dependent neutral/cationic amino acid exchanger	5-GCCCTGTCAGTAAATCAGA-CAAGA-3	5-TTCAGTTG-CATTGTGTTTTGGTT-3
ZnT1 (SLC22A18)	XM_040673965.1	Zinc transporter-1	Zinc transporter	5-TCCGGGAGTAATGGAAATC TTC-3	5-AATCAGGAACAAACC-TATGGGAAA-3

ileal digesta, and excreta (AOAC Method 968.06). Gross energy and mineral profile of diets and excreta were analyzed using an isoperibol bomb calorimeter and inductive coupled plasma-optical emission spectrometry, respectively. Titanium dioxide concentration in the samples was determined according to the method of Short et al. (1996). Cecal SCFA composition was analyzed by gas chromatography. Briefly, around 1 g cecal content sample was diluted in deionized water in 1: 3 ratio in 15 mL tubes. The solution was votexed and 1.5 mL of the mix was centrifuged at 10,000 × *g* for 10 min. The supernatant was collected and mixed well with 25% meta-phosphoric acid. After being frozen overnight, the mixture was thawed, centrifuged and the supernatant was mixed with ethyl acetate in a ratio of 1: 2. After being vortexed and and allowed to settle for 5 min, the mixture's top layer was transferred to a glass vial and analyzed on gas chromatography. Dietary oligosaccharides profile was analyzed using matrix-assisted laser desorption ionization mass spectrometry detection as described previously (Lin and Olukosi, 2021a, 2021b).

Calculations and Statistical Analysis

Index method was used to calculate total tract retention and apparent ileal digestibility of energy, DM, and crude protein using the following equation:

$$Digestibility = 100 \times \left\{1 - \left[\left(\frac{C_i}{C_o}\right) \times \left(\frac{N_o}{N_i}\right)\right]\right\}$$

Marker-corrected minerals concentration (mg /100 g dry matter intake) in excreta was calculated using the following equation:

$$Mineral\ components = N_o \times \frac{C_i}{C_o}$$

where C_i is the concentration of titanium in the diet, N_i is the nutrient content in the diet, C_o is the concentration of titanium in excreta or digesta, and N_o is the nutrient content in excreta or ileal digesta.

Apparent metabolizable energy and AMEn were calculated by the following 2 equations:

$$AME = GE_i - \left[\left(\frac{C_i}{C_o}\right) \times GE_o\right];\ \text{and}\ AMEn = AME - \left(8.22 \times \frac{NR}{DMI}\right);$$

where GE_i is gross energy of the diet, GE_o is gross energy of the excreta. NR is the retained nitrogen (g), and DMI is the dry matter intake (kg).

Distribution and normal quantile plot in JMP were used to test the data normality. The data were analyzed by the mixed model procedure of JMP Pro 14.1.0 (SAS Institute Inc., Cary, NC) as appropriate for a randomized complete block design and a factorial treatment arrangement. The comparison of treatments was subjected to two-way ANOVA. The two factors were *Eimeria* challenge (2 levels) and XOS supplementation (3 levels). Tukey's honest significant difference test was

Table 3. Growth performance of broiler chickens in response to feeding with graded levels of prebiotic xylose oligosaccharides when challenged or unchallenged with mixed *Eimeria* spp.

		Pre-challenge phase (d 0 to 15)			Challenge phase (d 15 to 21)		
Eimeria	XOS, g/kg	WG, g	FI, g	Gain: Feed, g/kg	WG, g	FI, g	Gain: Feed, g/kg
-	0				373[b]	432	864
-	0.50				425[a]	453	943
-	1.00				399[ab]	439	911
+	0				200[c]	329	612
+	0.50				203[c]	336	607
+	1.00				210[c]	340	618
Pooled SEM					10.2	9.98	24.9
Means for main effect of *Eimeria* challenge							
-					399	441	906
+					205	335	612
Pooled SEM					5.89	5.76	14.35
P-values					< 0.001	< 0.001	< 0.001
Means for main effect of XOS supplementation							
	0	404[a]	506	802[a]	287	380	738
	0.50	383[ab]	498	769[ab]	314	394	775
	1.00	370[b]	506	732[b]	305	390	764
Pooled SEM		9.49	10.6	13.0	7.22	7.06	17.6
P-values for main effect of XOS		0.046	0.829	0.005	0.052	0.363	0.313
P-values for interactions					0.047	0.625	0.245

n = 6, 18, or 12 replicates for the simple effects, main effects of *Eimeria* challenge or XOS, respectively.
[a-c] Means within a group in a column but with different superscripts are significantly different ($P \leq 0.05$).
Abbreviations: FI, feed intake; WG, weight gain; XOS, xylose oligosaccharide.

used to separate means if there is a significant interaction; otherwise, main effects means are discussed. Intestinal lesion scores were analyzed by Kruskal-Wallis nonparametric statistical method. Statistical significance was set at $P \leq 0.05$, and trends were set at $P < 0.10$.

RESULTS

Growth Performance and Nutrient Utilization

During pre-infection period (d 0 to d 15), XOS supplemented at 1 g/kg significantly decreased WG ($P < 0.05$) and gain:feed ratio ($P < 0.05$) in broiler chickens (Table 3). During the infection period (d 15 to d 21), there was significant ($P < 0.05$) *Eimeria* × XOS interaction for WG. Supplementation with XOS at 0.5 g/kg significantly ($P < 0.05$) increased WG in unchallenged but not in challenged treatment.

There was no significant *Eimeria* × XOS interaction for nutrient utilization response (Table 4). *Eimeria* challenge significantly ($P < 0.01$) lowered ileal DM and N digestibility by 28.8 and 37.5% units, respectively. XOS supplementation at 0.5 g/kg level tended to increase ($P = 0.052$) ileal DM digestibility by 7% units compared with treatment without XOS. In addition, chickens in challenged treatments showed depressed ($P < 0.01$) AME, AMEn, and total tract retention of N, Ca, and P. On the contrary, supplemental XOS at 0.5 g/kg increased ($P < 0.05$) total tract retention of N by 8.7% units and P by 11.6% units; and tended to increase ($P < 0.10$) AME, AMEn, and Ca total tract retenton.

Eimeria challenge significantly ($P < 0.01$) increased the post-cecal marker-corrected concentrations of trace minerals including Fe, K, Mg, Na, and S, whereas supplemental XOS in 0.5 g/kg significantly reduced the marker corrected concentrations of Fe, K, and Mg (Table 5). On the contrary, 1 g/kg XOS supplementation significantly increased the concentrations of Mn and Zn, relative to the control diet.

Intestinal Permeability, Lesion Scores, and Oocyst Shedding

Figure 1 shows the gastrointestinal permeability response on d 20 (5 DPI). Birds challenged with mixed *Eimeria* species showed numerically higher serum FITC-d levels, indicating intestinal leakage due to gut damage caused by *Eimeria* spp. invasion. XOS supplementation had no significant effect on intestinal permeability. The results of intestinal lesion scores are presented in Figure 2. *Eimeria* challenge resulted in severer ($P < 0.01$) intestinal lesions in the upper intestine, middle intestine, and ceca. XOS alleviated ($P < 0.05$) intestinal lesion in upper-intestinal section. Figure 3 shows the effect of XOS on excreta oocyst shedding in challenged birds. Oocyst shedding was observed in all *Eimeria*-challenged birds, whereas supplemental XOS had no effect on oocyst numbers.

Gene Expression of Tight Junction Proteins and Nutrients Transporters

The significant *Eimeria* × XOS interaction ($P < 0.05$) for claudin 1 showed that both 0.5 and 1 g/kg XOS supplementation were able to alleviate ($P < 0.05$) claudin 1 upregulation induced by *Eimeria* infection (Table 6). *Eimeria* challenge upregulated ($P < 0.01$) the expression

Table 4. Ileal digestibility and total tract nutrient retention for the broiler chickens in response to feeding with graded levels of prebiotic xylose oligosaccharides when challenged or unchallenged with mixed *Eimeria* spp.

			Ileal digestibility, %		Total tract retention, %				
Treatment	*Eimeria*	XOS, g/kg	DM	CP	Nitrogen	AME, kcal/kg	AMEn, kcal/kg	Ca	P
1	-	0	71.9	75.1	60.8	2,975	2,876	51.6	56.6
2	-	0.50	71.0	79.3	66.5	3,062	2,953	52.0	62.1
3	-	1.00	69.0	77.3	59.0	2,906	2,792	47.6	54.3
4	+	0	36.3	30.0	29.4	1,783	1,603	38.2	15.9
5	+	0.50	51.1	50.9	41.0	1,974	1,814	45.6	33.8
6	+	1.00	36.9	38.0	32.4	1,790	1,699	33.7	21.6
Pooled SEM			7.20	11.8	8.60	192	192	7.50	7.00
Means for main effect of Eimeria challenge									
	-		71.0	78.0	62.1	2,981	2,874	50.4	57.7
	+		42.2	40.5	35.8	1,876	1,705	39.2	23.8
Pooled SEM			1.70	2.78	2.03	45.3	45.3	1.77	1.64
P-values for main effect of challenge			< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Means for main effect of XOS supplementation									
		0	54.1	52.5	45.1[b]	2,379	2,239	44.9	36.3[b]
		0.50	61.1	65.1	53.8 [a]	2,518	2,384	48.8	47.9[a]
		1.00	53.0	57.7	48.0 [ab]	2,389	2,246	40.7	38.0[b]
Pooled SEM			2.08	3.41	2.48	55.4	55.4	2.17	2.02
P-values for main effect of XOS			0.052	0.104	0.019	0.051	0.093	0.062	< 0.001
P-values for interactions			0.690	0.798	0.838	0.942	0.915	0.498	0.225

n = 6, 18, or 12 replicates for the simple effects, main effects of *Eimeria* challenge or XOS, respectively;
[ab] Means within a group in a column but with different superscripts are significantly different (P ≤ 0.05).
Abbreviations: CP, crude protein; XOS, xylose oligosaccharide.

of JAM2, whereas XOS supplementation had no effect on JAM2 expression. With respect to nutrients transporters, the significant *Eimeria* × XOS interaction for GLUT2 ($P < 0.05$) and GLUT5 ($P < 0.01$) is explained by 0.5 g/kg XOS supplementation downregulating the expression of GLUT2 and GLUT5 in unchallenged treatments but not in challenged treatments. In addition, *Eimeria* upregulated ($P < 0.01$) the expression of GLUT1 and SGLT1; but downregulated ($P < 0.01$) the expression of peptide transporter PepT1, amino acid transporters rBAT, CAT2, y+LAT2, and zinc transporter ZnT1. However, XOS supplementation had no effect on these transporters.

Cecal Volatile Fatty Acids Profile

No significant interactions were observed on the profile of SCFA in the cecal content of birds (Table 7). The profile of SCFA indicates that birds challenged with *Eimeria* spp. had lower ($P < 0.01$) concentrations of saccharolytic SCFA acetate, butyrate, and total SCFA, but higher ($P < 0.01$) concentrations of branched chain fatty acids (**BCFA**) isobutyrate and isovalerate. On the contrary, the supplementation of XOS tended to decrease the concentration of isobutyrate ($P < 0.10$) and isovalerate.

Table 5. Post-cecal marker-corrected concentration of trace minerals (mg/100 g dry matter intake) of broiler chickens in response to feeding with graded levels of prebiotic xylose oligosaccharides when challenged or unchallenged with mixed *Eimeria* spp.

Treatment	*Eimeria*	XOS, g/kg	Fe	K	Mg	Mn	Na	S	Zn
1	-	0	122	7,133	1,347	311	552	920	259
2	-	0.50	110	6,197	1,231	302	500	862	249
3	-	1.00	176	10,974	2,069	488	772	1,480	403
4	+	0	154	8,574	1,639	296	852	1,498	260
5	+	0.50	133	7,417	1,497	291	801	1,414	249
6	+	1.00	160	8,830	1,677	321	903	1,578	273
Pooled SEM			6.78	429	60.3	8.09	44.9	51.9	5.7
Means for main effect of Eimeria challenge									
	-		136	8,101	1,549	1,549	608	1,088	303
	+		149	8,274	1,604	1,604	852	1,496	261
Pooled SEM			2.40	167	23.6	3.21	17.9	23.5	2.36
P-values for main effect of challenge			< 0.001	< 0.001	< 0.001	0.071	< 0.001	< 0.001	0.6725
Means for main effect of XOS supplementation									
		0	138[a]	7,854[a]	1,493[a]	1,493[b]	702	1,209[ab]	259[b]
		0.50	121[b]	6,807[b]	1,364[b]	1,364[b]	651	1,138[b]	249[b]
		1.00	168[a]	9,902[a]	1,873[a]	1,873[a]	838	1,529[a]	338[a]
Pooled SEM			3.35	233	32.9	4.44	24.8	32.8	3.29
P-values for main effect of XOS			< 0.001	0.001	0.003	< 0.001	0.123	0.009	< 0.001
P-values for interactions			0.535	0.908	0.550	0.708	0.887	0.815	0.322

n = 6, 18, or 12 replicates for the simple effects, main effects of *Eimeria* challenge or XOS, respectively.
[ab] Means within a group in a column but with different superscripts are significantly different (P ≤ 0.05).
Abbreviation: XOS, xylose oligosaccharide.

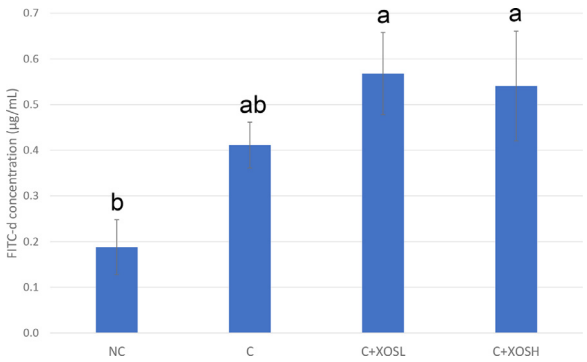

Figure 1. Fluorescein isothiocyanate dextran concentration (FITC-d, μg/mL) in serum of broiler chickens in response to *Eimeria* challenge and feeding with or without supplementation. a,b Treatments with different letters are significantly different ($P < 0.05$). N = 6. NC, unchallenged-no supplementation treatment; C, challenged-no supplementation treatment; C+XOSL, challenged and supplemented with 0.5g/kg xylo-oligosaccharides, C+XOSH, challenged and supplemented with 1g/kg xylo-oligosaccharides. The error bars represent the SEM values.

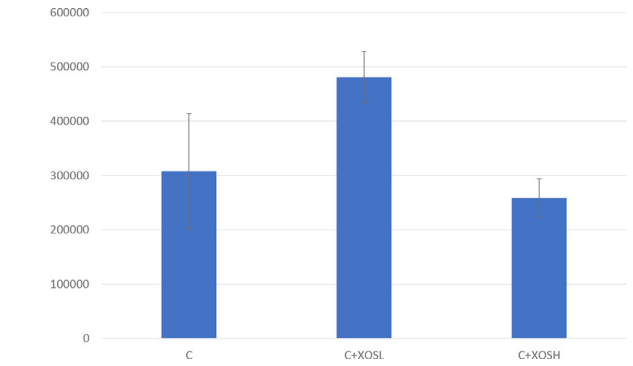

Figure 3. Oocyst shedding (oocysts/g) of broiler chickens in response to *Eimeria* challenge and feeding with or without supplementation (6 DPI). N = 6. C, challenged-no supplementation treatment; C+XOSL, challenged and supplemented with 0.5g/kg xylo-oligosaccharides, C+XOSH, challenged and supplemented with 1g/kg xylo-oligosaccharides. The error bars represent the SEM values.

DISCUSSION

The objective of this experiment was to investigate the mechanisms of a prebiotic, xylo-oligosaccharides, on mitigating the negative effects of *Eimeria* challenge on growth performance, nutrient utilization, gene expression of tight junctions and nutrient transporters, and cecal SCFA profile. In the current study, XOS was more effective at the lower inclusion (0.5 g/kg) in eliciting positive responses (nutrient and energy utilization, alleviating *Eimeria*-induced lesion in the upper intestine, altering gene expressions of jejunal tight junctions and transporters, and cecal SCFA profile).

The positive effects of xylo-oligosaccharides on growth performance and nutrient utilization have been widely demonstrated in studies with broilers and layers with or without disease challenges. For example, Craig et al. (2020a) reported that XOS supplementation increased broiler chickens WG and decreased FCR in a 21-d experiment in an *Eimeria* vaccine challenge model. A 9.4% improvement in WG was reported by d 59 in the study of Sun et al. (2013). XOS also improved FCR, AME and N digestibility in laying hens (Zhou et al., 2021). However, significant improvement of nutrient utilization is not consistently observed (Wang et al., 2019; Craig et al., 2020b; Singh et al., 2021). The inconsistency may be due to application of different levels and, or, the quality of xylo-oligosaccharides products.

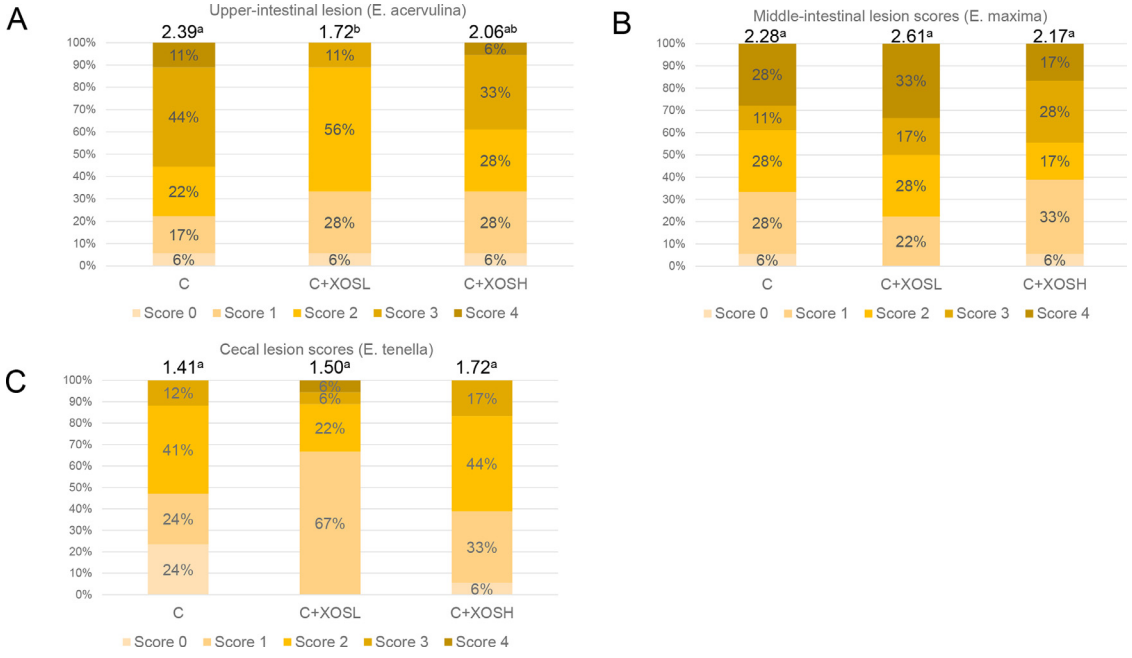

Figure 2. Percentage of lesion scores in the upper intestine, middle intestine, and ceca of broiler chicken in response to feeding diet with or without supplementation (6 DPI). Average scores of each treatment are present at the top of the bar. a,b Treatments with different letters are significantly different ($P < 0.05$). (A) Upper-intestine; (B) middle-intestine; (C) ceca. N = 6. C, challenged-no supplementation treatment; C+XOSL, challenged and supplemented with 0.5g/kg xylo-oligosaccharides, C+XOSH, challenged and supplemented with 1g/kg xylo-oligosaccharides.

Table 6. Gene expression of tight junctions and nutrient transporters in the jejunum of broiler chickens 6-d postchallenge in response to feeding with graded levels of prebiotic xylose oligosaccharides when challenged or unchallenged with mixed *Eimeria* spp.

Treatment	*Eimeria*	XOS g/kg	CLDN1	JAM2	GLUT1	GLUT2	GLUT5	SGLT1	PepT1	rBAT	$b^{0+}AT$	CAT2	y+LAT1	y+LAT2	ZnT1
1	-	0	1.000[b]	1.000	1.000	1.000[ab]	1.000[a]	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
2	-	0.50	0.777[b]	0.750	0.924	0.741[b]	0.584[b]	0.889	0.980	1.189	0.757	0.788	0.884	0.753	1.204
3	-	1.00	0.981[b]	0.847	1.013	1.190[a]	0.831[ab]	0.886	0.859	0.875	0.790	0.795	1.006	0.771	1.179
4	+	0	7.766[a]	1.477	4.211	0.112[c]	0.084[c]	0.519	0.455	0.424	0.270	0.638	1.061	0.533	0.691
5	+	0.50	4.055[b]	1.329	3.952	0.075[c]	0.152[c]	0.667	0.569	0.468	0.347	0.462	1.032	0.554	0.599
6	+	1.00	3.227[b]	1.455	3.085	0.043[c]	0.095[c]	0.597	0.633	0.445	0.270	0.520	1.042	0.594	0.850
Pooled SEM			0.768	0.139	0.653	0.099	0.065	0.087	0.164	0.146	0.098	0.109	0.195	0.083	0.132
Means for main effect of Eimeria challenge															
	-		0.919	0.865	0.979	0.977	0.805	0.925	0.947	1.021	0.849	0.861	0.963	0.841	1.128
	+		5.016	1.420	3.749	0.077	0.110	0.594	0.552	0.446	0.295	0.540	1.045	0.560	0.714
Pooled SEM			0.443	0.080	0.377	0.057	0.038	0.050	0.095	0.084	0.057	0.063	0.112	0.048	0.076
P values for main effect of challenge			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.015	<0.001	<0.001	0.002	0.625	0.001	<0.001
Means for main effect of xylo-oligo supplementation															
		0	4.383	1.238	2.605	0.556	0.542	0.760	0.727	0.712	0.635	0.819	1.031	0.767	0.846
		0.50	2.416	1.039	2.438	0.408	0.368	0.778	0.774	0.829	0.552	0.625	0.958	0.653	0.902
		1.00	2.104	1.151	2.049	0.616	0.463	0.741	0.746	0.660	0.530	0.657	1.024	0.682	1.015
Pooled SEM			0.543	0.098	0.462	0.070	0.046	0.061	0.116	0.103	0.070	0.077	0.138	0.059	0.093
P values for main effect of XOS			0.018	0.404	0.760	0.070	0.054	0.919	0.968	0.540	0.566	0.194	0.925	0.380	0.300
P-values for interactions			0.024	0.897	0.611	0.040	0.006	0.334	0.618	0.645	0.304	0.927	0.960	0.183	0.329

n = 6, 18 or 12 replicates for the simple effects, main effects of *Eimeria* challenge or XOS, respectively.
[a–c] Means within a group in a column but with different superscripts are significantly different ($P \leq 0.05$).
Abbreviations: XOS, xylose oligosaccharide.

Secondly, in the current study, it is worth noting that xylo-oligosaccharides showed contrasting effects at different ages of the chickens, as shown by the supplemental XOS decreasing WG and gain: feed ratio at young age but increasing WG in the older birds. Similar observations were found in previous studies (Suo et al., 2015; Bautil et al., 2020), where XOS tended to improve growth performance in older ages (>23 d) but inhibited or had no effect on growth performance in younger birds. Thus, the significant effects of xylo-oligosaccharides in older ages could be masked by the negative effects of the product in an earlier age period, if the performance is measured only at the beginning and end of the entire growth phase.

In view of the fact that performance was only measured at d 15 and d 21 in the current study, the exact time point at which xylo-oligosaccharides started playing the beneficial roles in growth performance could not be determined. Therefore, multipoint measurement is recommended in the future xylo-oligosaccharides functional studies. The early phase negative effect of XOS on the growth performance is likely due to the immature microbiome, lacking efficient fermentation, and specific microbial communities to utilize the supplemented materials. The composition and diversity of intestinal bacterial communities are age-dependent. Older birds have more biodiverse and complex microbial communities (Awad et al., 2016; Ocejo et al., 2019). Also, XOS induces greater viscosity in young birds' intestine due to their smaller gut size (Bautil et al., 2020), which could interfere with intestinal nutrient utilization. Even though the XOS supplementation in early life may negatively influence the growth of young birds, the product can possibly condition gut microbial communities to acclimatize to arabinoxylan utilization and benefit the growth in later life (Bautil et al., 2020).

On the other hand, *Eimeria* infective dose is negatively correlated to bird growth performance and nutrient utilization (Teng et al., 2020, 2021a). A medium-low level challenge of mixed *Eimeria* spp. was used in the current study to develop a mild infection situation. In this study, the *Eimeria* infection resulted in losses of 23% WG, 40% AME, and 40% ileal digestibility of N at 6 DPI, compared to the nonchallenged chickens. The depressed nutrient utilization also included reducing total tract retention of Ca (22% reduction) and P (58% reduction). Reduced retentions of other minerals such as Fe, K, Mg, Na, and S were also observed. However, supplementation of XOS in the current study partly reversed the negative effect of *Eimeria* on nutrient utilization, as shown by improvement in total tract retention of N, P and AME during the infection; and the observation is consistent with previous studies (Morgan et al., 2019).

Increased blood concentration of $1,25(OH)_2$ D_3, which promotes Ca absorption, has been observed in response to XOS supplementation (Ding et al., 2018). There are several proposed mechanisms for this observation. First, supplementation of prebiotics can increase SCFA levels in the ceca which lower the cecal pH and increases the

Table 7. Caecal short chain fatty acid profile (mM) of broiler chickens 6-d postchallenge in response to feeding with graded levels of prebiotic xylose oligosaccharides when challenged or unchallenged with mixed *Eimeria* spp.

Treatment	*Eimeria*	Xylo-oligosaccharide, g/kg	Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate	Total VFA
1	-	0	73.9	6.85	0.742	20.88	0.855	1.511	105
2	-	0.50	80.1	5.13	0.272	23.66	0.312	1.048	111
3	-	1.00	88.1	5.31	0.338	21.23	0.459	1.061	117
4	+	0	41.7	9.33	1.164	11.45	1.653	1.399	67
5	+	0.50	49.6	10.53	0.941	15.54	1.448	1.236	79
6	+	1.00	55.6	11.18	0.894	14.60	1.225	1.269	85
Pooled SEM			7.72	1.21	0.155	3.23	0.245	0.196	11.4
Means for main effect of Eimeria challenge									
	-		78.6	5.66	0.567	21.92	0.568	1.207	109
	+		44.3	9.54	1.140	13.86	1.552	1.477	74
Pooled SEM			4.45	0.70	0.090	1.86	0.141	0.113	6.60
P values			< 0.001	< 0.001	< 0.001	0.008	< 0.001	0.112	0.002
Means for main effect of xylo-oligo supplementation									
		0	57.8	8.09	0.953	16.16	1.254	1.455	86
		0.50	64.9	7.83	0.606	19.60	0.880	1.142	95
		1.00	71.8	8.24	0.616	17.92	0.842	1.165	101
Pooled SEM			5.46	0.857	0.110	2.281	0.173	0.139	8.08
P values for main effect of XOS			0.377	0.895	0.080	0.588	0.062	0.101	0.460
P-values for interactions			0.827	0.305	0.664	0.916	0.749	0.774	0.966

n = 6, 18 or 12 replicates for the simple effects, main effects of *Eimeria* challenge or XOS, respectively.
Abbreviation: XOS, xylose oligosaccharide.

solubility of minerals such as Ca and Mg, hence improving passive diffusion and absorption of macro minerals (Roberfroid et al., 2010). Moreover, Li et al. (2017) studies showed that prebiotic can produce improvement in mineral absorption, especially Ca, which resulted in enhanced eggshell quality (Han et al., 2017; Whisner and Castillo, 2018).

The current experiment studied intestinal permeability by application of fluorescein isothiocyanate-dextran (**FITC-d**). In the current study, XOS supplementation had no effect on *Eimeria*-induced gut leakage or oocyst shedding. However, lower lesion score was observed in the XOS-supplemented treatments, which may be explained by the prebiotic effects of XOS to inhibit intestinal inflammation. It was found that XOS counteracted high fat diet-induced inflammation with decreased plasma inflammatory cytokines TNF-α, MCP-1, and LPS, as well as decreased colonic inflammatory cytokines TNF-α and IL-10 (Fei et al., 2020).

The current experiment showed that the parasite challenge significantly upregulated gene expression of claudin-1 and JAM2. Similar changes in tight junctions have been reported in previous *Eimeria* challenge studies (Lin and Olukosi, 2021a; Teng et al., 2021b). It has been demonstrated that upregulation of claudin-1 is elevated in inflammatory bowel disease conditions (Poritz et al., 2011). Moreover, the expressions of claudin-1 and JAM2 correlated positively with *Eimeria*-induced inflammatory activity (Teng et al., 2020). Alteration in tight junction proteins may induce intestinal permeability defects and barrier dysfunction, which agrees with the observation on *Eimeria*-induced increase in gut permeability. The current study demonstrated that XOS could partly reverse the *Eimeria*-induced elevated claudin-1 expression. It can be speculated therefore that the XOS partly alleviated *Eimeria*-induced intestinal barrier impairment.

In agreement with the present study, it has been previously reported that at the peak of the infection (6 DPI), the *Eimeria* challenge influenced the expression of nutrient transporters by downregulating an extensive range of brush border-located transporters such as PepT1, sugar transporters GLUT5 and SGLT1, and amino acids transporters rBAT and $b^{0,+}$AT. The downregulated expression partly explains the depression on growth performance and nutrient utilization during *Eimeria* infection (Su et al., 2014, 2015a; Miska and Fetterer, 2017). It is proposed that the downregulation of most of the transporters located in the brush border membrane is an action employed by host cells to "starve" the parasite of nutrients (Su et al., 2015b). In addition, decrease of basolateral zinc transporter, ZnT1 results in toxic Zn accumulation in epithelial cells, stimulating cell death (Su et al., 2014). The upregulation of GLUT1 may also be related to the host's strategy to transport more nutrients out of cells and lead to cell nutrient depletion and apoptosis. Additionally, a similar finding of downregulation of CAT2 and y +LAT2 with *Eimeria* infection has been previously reported (Lin and Olukosi, 2021b; Teng et al., 2021a). However, the mechanism behind the observation is unclear. In the current study, the effects of XOS is mainly reflected in sugar transporters GLUT2 and GLUT5, which is in agreement with the previous findings that prebiotic upregulates the expression of SGLT1, GLUT5 and PepT1 in broiler chickens (Biswas et al., 2022). Supplemental XOS partly reversed the *Eimeria*-related depression of GLUT2 and GLUT5 expressions, indicating the ability of the additive to partly alleviate negative effect of *Eimeria* challenge on sugar absorption.

The SCFA profile in chicken hindgut is associated with the intestinal fermentation condition which is managed by microbial activities. For example, some bacteria such as *Bacteroidetes* are saccharolytic and

preferentially degrade carbohydrates as substrate, producing SCFA which can be utilized by enterocytes as an energy source (Adhikari and Kim, 2017) and contribute to animal growth. On the other hand, proteolytic bacteria such as *Firmicutes* and *Proteobacteria* preferentially utilize protein and produce BCFA Neis et al., 2015; Coffey, 2018). Therefore, the production of SCFA is considered a marker for cecal carbohydrate fermentation whereas BCFA is for cecal protein fermentation (Macfarlane et al., 1992).

In the current study, the concentration of cecal BCFA isobutyrate and isovalerate increased when the birds were challenged with coccidia, indicating increased protein fermentation under this condition. On the contrary, the *Eimeria* challenge decreased the concentration of saccharolytic SCFA including acetate, butyrate, and total SCFA, indicating a depression in carbohydrate fermentation. A similar SCFA pattern shift was observed in previous *Eimeria* challenge studies (Lin and Olukosi, 2021a). The increased BCFA can be explained by *Eimeria*-induced depression in N utilization (37.5% units' reduction in the ileum). The undigested N reaching the hindgut would have been utilized by proteolytic bacteria, elevating the cecal content of BCFA. In addition, *Eimeria* stimulates mucin production and induces gastrointestinal hemorrhage, which could also contribute to the increased N quantity in the ceca and excreta (Teng et al., 2021a).

In the current study, XOS tended to decrease the concentration of BCFA. XOS have also been shown to increase the production of acetate (Singh et al., 2021), butyrate (Ding et al., 2018), valerate, and total SCFA (Craig et al., 2020b). These observations suggest that XOS could shift cecal fermentation to predominantly carbohydrate fermentation. Because of their resistance to the hosts' endogenous digestive enzymes, prebiotics can "survive" small intestinal digestion and ultimately flow into hindgut to act as additional carbohydrate substrates for cecal bacteria (Manning and Gibson, 2004). The above possibility cannot fully explain the increased SCFA production, since a low level of inclusion such as 0.5 g/kg of XOS is not enough to produce a meaningful amount of SCFA (Bedford, 2018). A new possible prebiotic mode of action was proposed that XOS act as signal molecules to train microbial communities to acclimatize to arabinoxylan digestion (Bedford, 2018; Bautil et al., 2020), which is also supported by the finding that XOS stimulate butyrate-producing bacteria through cross-feeding action (De Maesschalck et al., 2015b). Therefore, the increased quantity of cecal SCFA is probably a result of both supplemental XOS as well as dietary fibers which can serve as feedstuff-related oligosaccharides source in the digestive tract.

In conclusion, XOS at 1 g/kg produced adverse effects on growth performance in the early phase of chicken's growth but XOS at 0.5 g/kg produced positive effects on growth performance in the later growth phase. In contrast to negative effects of *Eimeria* challenge, XOS supplementation improved ileal nutrient digestibility, AME, and total tract mineral retention. In addition, XOS supplementation alleviated *Eimeria*-induced lesion in the upper intestine, beneficially altered gene expression of tight junctions and sugar transporters, as well as cecal SCFA profile. These responses are speculated as modes of action by which the additive supports gut health in broiler chickens.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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