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Research Article

Effect of Xylo-Oligosaccharides (XOS) on Growth Performance, Blood Biochemistry and Total Viable Count in Ileum and Caecum of Broiler Chickens from Day 13-26

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ABSTRACT

Objective: This study was undertaken to investigate the effect of xylo-oligosaccharides (XOS) on the growth performance, blood biochemistry and total bacterial count in the intestine of broiler chicken from 13 to 26 days of age. **Materials and Methods:** A total of 96 day-old Cobb 500 broiler chicks were distributed randomly into four treatment groups with four replicates (6 birds replicate⁻¹) and fed basal diet (control) from day 1-12. Test diets were formulated with four different levels of XOS (0, 2.5, 5.0 and 7.5 g kg⁻¹) and offered to the birds from day 13-26. On day 26, the data on growth performance, serum biochemical profile, total viable count in the ileum and cecum, visceral organ weight and carcass yields of the broiler chickens were assessed. **Results:** There was no effect (p>0.05) of XOS on weight gain and feed intake. However, supplementation of 2.5 g XOS kg⁻¹ of diet significantly improved the feed conversion ratio (FCR) of the birds. This group of diet also increased (p<0.05) the serum concentration of T_3 and T_4 but reduced (p<0.05) the glucose level. Diets containing 2.5 and 5 g XOS kg⁻¹ increased the total viable count in both ileum and cecum. The dressing percentage and relative weight of the pancreas were significantly improved in birds consumed a diet containing 2.5 g XOS kg⁻¹. The abdominal fat content was low (p<0.05) in birds fed diets containing 2.5 and 5 g XOS kg⁻¹. **Conclusion:** Dietary XOS supplementation (2.5 and 5 g kg⁻¹ of diet) can improve the thyroid hormone activity and total viable count in ileum and cecum, thus improved the FCR of broiler chickens.

Key words: Xylo-oligosaccharide, glucose, poultry diet, gut microbiome, intestinal bacterial count

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The use of antibiotic growth promoters (AGPs) in poultry feed has been a growing concern among the consumers due to the emergence of antibiotic-resistant strains of microorganisms¹. The European Union has already restricted the use of antibiotic as growth promoters in poultry feed². However, birds become more prone to get sick in the absence of in-feed antibiotic. Hence, alternatives to antibiotics are needed in the poultry industry to promote the performance of birds. In this guest, several alternatives, including probiotics, prebiotics and phytobiotic etc., have been suggested to be the potential alternative of AGPs in poultry diet³. Among the prebiotics, xylo-oligosaccharide (XOS) is one of the promising AGP replacers due to their beneficial effects on gut health maintenance⁴ and productive performance of broiler chicken⁵. XOS is a hydrolytic degradation product of arabinoxylans (plant or microorganism origin) that can be fermented by the gut microbiota. A previous study observed that XOS could promote the growth of beneficial bacteria, such as Bifidobacterium spp. and Lactobacillus spp. and subsequently improve the gut microbial ecology and intestinal health of poultry⁶ and pig⁷.

Supplementation of straw-derived XOS (5-20 g kg $^{-1}$) improved not only the serum triiodothyronine (T_3), thyroxine (T_4), insulin concentration but also the immune function and FCR of broilers 8 . Another study 9 also reported that supplementation of XOS (2 g kg $^{-1}$) in the male broiler chicken diet increased the concentrations of acetate, propionate and the proportion of *Lactobacillus* spp. in the cecum. In contrast, several studies reported no effect of XOS on broiler performance 10,11 . Diet supplemented with 50 mg kg $^{-1}$

XOS showed no effect on broiler performance¹⁰. Similarly, Suo *et al.*¹¹ reported that there was no significant effect of XOS supplementation (50-100 mg kg⁻¹) on average daily gain (ADG), average daily feed intake (ADFI) on days 1-21, 22-42 and 1-42 and feed conversion rate on days 1-21.

Limited studies have been done so far on the beneficial effects of XOS on the overall gut microbiome and blood chemistry of broiler chickens. Besides, the effective dose of XOS in the broiler diet is yet to be explored. Therefore, the present study was carried out to investigate the effect of different levels of XOS on growth performance, blood biochemistry and intestinal bacterial count of broiler chickens.

MATERIALS AND METHODS

All experimental procedures were approved by the CVASU (Chattogram Veterinary and Animal Sciences University) Ethics Committee (EC) and the EC Approval NO is CVASU/Dir(R&E) EC/2019/94(6).

Dietary management: Four experimental diets were formulated with four levels; 0, 2.5, 5.0 and 7.5 g of XOS kg⁻¹ of diet, respectively. At first, a single batch of corn-soybean meal-based mashed diet (Table 1) was formulated according to the recommendation of the Cobb 500 Broiler performance and nutrition supplement guide to meet or exceed the nutrient requirements¹². After that, the basal diet was divided into four aliquots according to the experimental diet arrangement. Each supplemental XOS level was mixed on top with each aliquot of the basal diet. The diets were made as mash and offered to the birds from day 13-26. The corn-derived XOS was purchased from Henan Heagreen

Table 1. Composition and	d nutriant laval of the h	asal diets for broiler chicken ¹
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Ingredient compositions (%)	Basal diet	Nutrient composition (calculated)			
Maize	61.030	ME(kcal kg ⁻¹)	3025		
Palm oil	3.800	Crude protein (%)	21		
Protein concentrate	3.760	Digestible lysine (%)	1.12		
Soybean meal	27.840	Digestible methionine (%)	0.45		
Limestone	1.130	Ca%	0.84		
Dicalcium phosphate	0.320	AvP%	0.42		
Vitamin and trace mineral mix ²	0.250	Nutrient composition (analyzed)			
NaCl	0.250	Dry matter (%)	85.91		
Lysine	0.160	Crude protein (%)	21.78		
Methionine	0.500	Crude fiber (%)	3.6		
Toxin binder	0.400	Ash (%)	6.11		
Coccidiostat	0.500	Ether extract (%)	8.25		
Choline chloride	0.040				
Rena-phytase	0.025				

The basal diet was divided equally in four parts and XOS was added at the dose of 0, 25, 5.0 and 7.5 g kg $^{-1}$ of diet in D0, D1, D2 and D3 experimental diets, respectively. Supplied per kg of diet (mg), 11,998.8 IU vitamin A (as all-trans retinol), 3,600 IU cholecalciferol, 65.56 IU vitamin E (as day- α -tocopherol), 2 mg vitamin K3, 2 mg thiamine, 6 mg riboflavin, 5 mg pyridoxine hydrochloride, 0.2 mg vitamin B12, 0.1 mg biotin, 50 mg niacin, 12 mg D-calcium pantothenate, 2 mg folic acid, 80 mg Mn, 60 mg Fe, 8 mg Cu, 1 mg I, 0.3 mg Co, 1 mg Mo

Bio-technology Co., Ltd. (China) that contains <5% moisture, >95% XOS (as dry basis), <5% xylose, glucose and arabinose.

Birds' managements and diets: A total of 96 Cobb 500 day-old broiler chicks (40±0.10 g) were purchased from a local hatchery and randomly distributed into four groups with four replicates per dietary group (6 birds/replicates). From day 1-12, the birds received a basal (control) diet (Table 1). The experimental diets were offered to the birds during 13-26 days of age. The birds were reared in cages with well-equipped feeders and drinkers. Birds had free access to feed and water. Standard vaccination schedules and management procedures were maintained throughout the trial period. Feed intake (FI) and live weight were recorded weekly. Mortality was recorded as it happened. Feed conversion ratio (FCR; feed intake/body weight gain) was corrected for mortality.

Sample collection and processing: On day 26, three birds from each replicate were sacrificed by cutting the jugular vein after 12 h of fasting. The blood sample was collected in a falcon tube for separation of serum by centrifugation at 5000 revolutions per minute. Harvested serum samples were taken into the 2 mL eppendorf tubes and stored at -20°C in the laboratory for further analysis. The weight of the visceral organs (liver, spleen, bursa, breast, gizzard, intestine and abdominal fat content) was recorded after opening the abdominal cavity of the same birds. The ileal (from the duodenum to Meckel's diverticulum) and cecal content were collected by gently pressing and stored in a separate labeled container at -20°C for further analysis. The weight of the different body parts of the dressed birds was recorded accordingly.

Culture and total viable count: The collected intestinal samples of three individual birds/replicates were mixed and pooled. Around 1 g of ileal and caecal content was taken into two separate labeled sterile test tubes containing 2 mL of 0.9% saline solution with a stick. A 10-fold serial dilution was done for each pooled sample (0.1 mL) from 10-1 to 10-10. MacConkey agar, Violet red bile agar and KF streptococcus agar were used to enumerate the Enterobacteriaceae, Streptococci and Enterococci, respectively. Baird Parker agar and Mannitol salt agar were used for the enumeration of Staphylococci. All the plates were incubated at 37°C aerobically for 24-48 h and the number of colonies was counted accordingly¹³.

Chemical analysis: The protein, CF, ash and moisture percentage of the diet were analyzed by using the Association

of Official Analytical Chemists method 14 . The nitrogen content of the samples was determined by the Kjeldahl method. The obtained nitrogen value was multiplied by 6.25 to convert it to crude protein. The weight difference methods were used to determine moisture and ash content levels. The crude fat of the diet was determined using the AOAC procedure with petroleum ether as a solvent. The serum glucose, triglyceride, total protein (TP), GPT (glutamic pyruvic transaminase), GOT (glutamic oxaloacetic transaminase), cholesterol, creatinine, T_3 (triiodothyronine) and T_4 (thyroxine) level was analyzed by using their respective standard assay kit (Randox Laboratories Ltd, UK) and semi-automated Humalyzer (Humalyzer 4000 Merck $^{\circ}$, Germany).

Statistical analysis: Data was analyzed using one-way ANOVA. Differences between means were tested by the least significant difference (LSD) using SPSS v.16 statistical software (SPSS, Chicago, Illinois, USA) for windows. The linear and quadratic responses of dependent variables to dietary supplemental XOS levels were assessed by using orthogonal polynomials. The difference between means was considered significant at $p \le 0.05$.

RESULTS

Growth performance: The effects of different levels of XOS on the growth performance of broiler chickens are presented in Table 2. Supplementation of XOS had no (p>0.05) effect on BWG and FI of broiler chickens during 13-26 days of age. However, the FCR was significantly different among the treatment groups. Chicken received a diet with 2.5 g XOS kg⁻¹ showed better FCR than those of the birds fed diet with 7.5 g XOS kg⁻¹. A significant linear response was observed between supplementation of XOS and FCR. With the increased level of XOS supplementation, the FCR also linearly increased (p<0.021).

Table 2: Effect of XOS on performance of broiler chickens (day 13-26)

Added XOS (g kg ⁻¹)	BWG (g bird ⁻¹)	FI (g bird ⁻¹)	FCR
0	916.21	1640.83	1.79ab
2.5	936.21	1595.63	1.71 ^b
5.0	927.71	1705.92	1.84 ^{ab}
7.5	887.83	1742.96	1.96ª
SEM	8.26	23.73	0.03
p-value	0.167	0.109	0.037
¹ Linear value			0.021
² Quadratic value			0.075

Data represents the means of 4 replicate cages (6 birds/replicate). Data with different letters within the same column differ significantly (p<0.05), SEM: Standard error mean. ¹Linear effects of added XOS levels. ²Quadratic effects of added XOS levels

Table 3: Effect of XOS on serum biochemical profile of broiler chickens (day 13-26)

Parameters	Added XOS (g k	g ⁻¹)				
	0	2.5	5.0	7.5	SEM	p-value
Glucose (mg dL ⁻¹)	273.97 ^b	263.65 ^b	313.15ª	262.08ab	6.21	0.011
Triglyceride (mg dL ⁻¹)	104.60	102.32	126.95	90.18	7.02	0.335
Total protein (g dL^{-1})	3.06	3.53	3.55	3.29	0.12	0.499
GPT (U L ⁻¹)	10.12	9.77	9.32	9.90	0.40	0.930
$GOT (U L^{-1})$	197.03	189.27	168.02	215.92	9.70	0.404
Cholesterol (mg dL^{-1})	124.60	140.32	122.10	132.67	3.55	0.266
Creatinine (mg dL ⁻¹)	0.38	0.40	0.35	0.32	0.02	0.634
T_3 (µg mL $^{-1}$)	6.82	7.66	6.71	6.36	0.24	0.298
$T_4 (\mu g dL^{-1})^{1,2}$	1.68 ^b	2.61ª	1.38 ^b	1.61 ^b	0.13	0.037

Data represents the means of 4 replicate cages (3 birds/replicate). Data with different letters within the same row differ significantly (p<0.05). SEM = Standard error mean. 1 Linear effects of added XOS levels (p = 0.001). 2 Quadratic effects of added XOS levels (p = 0.027). GPT: Glutamic pyruvic transaminase, GOT: Glutamic oxaloacetic transaminase, T_3 : Triiodothyronine, T_4 : Thyroxine

Serum biochemical profile: Supplementation of XOS had no significant effect on blood parameters except for blood glucose and T_4 level of birds (Table 3). The serum glucose level of the birds fed diets containing 5 g XOS kg $^{-1}$ was higher than those of the birds fed diet with 0 or 2.5 g XOS kg $^{-1}$. Supplementation of 2.5g XOS kg $^{-1}$ of the diets increased (p<0.001) the serum T_4 level compared to the other diet groups. Dietary XOS supplementation resulted in linear (p>0.37) and quadratic (p = 0.027) response on serum T_4 concentration.

Total bacterial count in ileum and caecum: Table 4 shows the effect of different levels of XOS on the total bacterial count in the ileum and caecum. Birds that received diet containing 7.5 g XOS kg^{-1} showed lower TVC count in both ileum (p<0.023) and caecum (p<0.012) than those of the birds fed on other diet groups.

Relative weight of the visceral organ: Table 5 shows the effect of different levels of XOS on visceral organ development in broiler chicken. Birds fed diet with 5.0g XOS kg $^{-1}$ showed greater (p<0.05) proventriculus than those of the birds fed diet containing 7.5g XOS kg $^{-1}$. The diet containing 7.5 g XOS kg $^{-1}$ reduced (p<0.05) the gizzard weight compared to diets without XOS. A linear response (p = 0.044) was observed between gizzard weight and XOS supplementation. The liver and pancreas weight was higher in the birds fed diets with 2.5 g XOS kg $^{-1}$ than those of the birds fed diet containing 5 or 7.5 g XOS kg $^{-1}$. Supplementation of XOS had no (p>0.05) effect on the relative weight of the small intestine, heart, spleen and bursa. Significant (p<0.05) quadratic response was observed between supplementation of XOS and development of visceral organs (proventriculus, liver, pancreas and heart).

Carcass traits: Birds fed diet with 2.5 g XOS kg⁻¹ showed the highest dressing percentage compared to birds fed on other

Table 4: Effect of XOS on total viable count in ileum and caecum of broiler chickens form 13-26 days of age

emekens form 15 20 days of age					
Added XOS (g kg ⁻¹)	lleum TVC log₁₀	Caecum TVC log ₁₀			
0	7.40±0.40 ^{ab}	6.02 ± 0.48^{ab}			
2.5	7.46 ± 0.40^{a}	6.98 ± 0.09^{a}			
5.0	7.43 ± 0.46^{a}	6.79±0.85°			
7.5	6.45±0.53 ^b	5.57±0.52 ^b			
SEM	0.14	0.19			
p-value	0.023	0.012			

Data represents the means of 4 replicate cages (3 birds/replicate). Data with different letters within the same column differ significantly (p<0.05). SEM: Standard error mean

experimental diets (Table 6). Diet supplemented with 2.5 or 5 g XOS kg $^{-1}$ increased (p<0.05) the thigh percentage. Diet with 2.5 g XOS kg $^{-1}$ improved (p<0.05) the shank growth compared to the other experimental diets. The abdominal fat content was lower in birds fed diets with 2.5 or 5 g XOS kg $^{-1}$ than those of the birds fed diets with 0 or 7.5 g XOS kg $^{-1}$. A quadratic response was observed between thigh (p = 0.001), abdominal fat content (p = 0.011) and XOS supplementation.

DISCUSSION

Growth response: The current study observed that different levels of XOS in diet had no significant effect on BWG and FI of broiler chicken, which is consistent with the previous studies^{6,10,11}. In contrast, Zhenping *et al.*⁸ stated that broiler chickens fed diet contained 2.1 g XOS kg⁻¹ gained more BW than those of the birds fed on the other diets. In the present study, birds offered diets containing 2.5 g XOS kg⁻¹ showed better FCR. These findings are consistent with a previous study which reported that the dietary inclusion of 5.0 g XOS kg⁻¹ improved the FCR⁶. Although not significant, the diet with 2.5 g XOS kg⁻¹ showed better weight gain despite less feed consumption compared to the diet with 5 or 7.5 g XOS kg⁻¹. The mechanism of how XOS improved the FCR is unclear but may be due to the improved intestinal health, increased digestion and absorption of nutrients¹⁵.

Table 5: Effect of XOS on relative weights (g/100 g BW) of visceral organ to body weight of broiler chickens (day 13-26)

Parameters		Added XOS (g kg ⁻¹)								
	0	2.5	5.0	7.5	SEM	p-value	¹ Linear value	² Quadratic value		
Small intestine	2.30	2.26	2.86	2.44	0.17	0.657				
Proventriculus	0.63ab	0.61ab	0.73ª	0.46 ^b	0.03	0.020	0.120	0.035		
Gizzard	3.97ª	3.07 ^{ab}	3.80 ^{ab}	2.97 ^b	0.15	0.017	0.044	0.879		
Liver	2.79ab	3.11 ^a	2.52 ^b	2.43 ^b	0.16	0.048	0.501	0.024		
Heart	0.58	0.62	0.65	0.58	0.02	0.250	0.743	0.069		
Spleen	0.11	0.12	0.12	0.08	0.01	0.581				
Pancreas	0.24 ^b	0.34ª	0.26 ^b	0.22 ^b	0.02	0.039	0.957	0.014		
Bursa	0.05	0.05	0.06	0.05	0.01	0.812				

Data represents the means of 4 replicate cages (3 birds/replicate). Data with different letters within the same row differ significantly (p<0.05). SEM: Standard error mean 1 Linear effects of added XOS levels. 2 Quadratic effects of added XOS levels

Table 6: Effect of XOS on carcass trait (% BW) of broiler chickens (day 13-26)

Added XOS (g kg ⁻¹)	Dressing (%)	Breast (%)	Drumstick (%)	Thigh (%)	Shank (%)	Neck (%)	Wing (%)	Abdominal fat (%)
0	63.440 ^b	22.650	8.260	9.080 ^b	4.110 ^b	2.660	4.830	2.440a
2.5	75.290°	25.020	9.230	11.050°	4.890°	2.880	4.980	1.890 ^b
5.0	64.960 ^b	22.210	8.410	11.390°	4.160 ^b	2.910	5.080	1.820 ^b
7.5	64.530 ^b	22.980	8.710	9.240 ^b	4.330ab	2.830	4.850	2.430 ^a
SEM	1.600	0.680	0.200	0.310	0.100	0.050	0.110	0.010
p-value	0.011	0.511	0.365	0.001	0.013	0.153	0.889	0.024
¹ Linear value	0.208			0.590	0.062			0.766
² Quadratic value	0.020			0.001	0.069			0.011

Data represent the means of 4 replicate cages (3 birds/replicate). Data with different letters within the same column differ significantly (p<0.05). SEM: Standard error mean. ¹Linear effects of added XOS levels. ²Quadratic effects of added XOS levels

Serum biochemical profile: In the present study, supplementation of XOS (2.5 g kg⁻¹ of diet) reduced the serum glucose level in the broiler chickens, which is consistent with previous studies in the broiler^{8,10} and rats¹⁶. This finding could be due to the production of short-chain fatty acids (SCFA). XOS enters the distal intestinal tract without digestion because the broiler lacks digestive enzymes for hydrolyzing XOS¹⁷. The microbiota in the distal gastro intestinal tract (GIT) utilizes this intact XOS and produces different SCFA, like acetic, propionic and butyric acids¹⁸⁻²¹. SCFA can prevent the gluconeogenesis process by increasing the gut hormone peptide (PYY) and GLP-1 (glucagon-like peptide-1) through activation of receptor Ffar2 and Ffar3, resulting in a reduction in blood glucose level^{18,22,23}, which partially supports the present study findings.

In the current study, birds that received the diet with $2.5 \, \mathrm{g} \, \mathrm{XOS} \, \mathrm{kg}^{-1}$ showed higher concentration of serum T4 and T3 than those of the birds fed on other diet groups. Previous studies reported a similar trend^{8,10}. It has been claimed that the T_3 and T_4 hormones are closely related to the bird's metabolism and growth rates. Supplementation of XOS can improve the serum thyroid hormone activity, therefore, leads to better metabolism and subsequent growth of poultry²⁴, which supports the current study findings. Improved growth performance with a decreased serum glucose level in the birds fed diet with $2.5 \, \mathrm{g} \, \mathrm{XOS} \, \mathrm{kg}^{-1}$ could partly be attributed to the increased T_3 and T_4 activity.

Total viable count in ileum and cecum: Birds consumed diets containing 2.5 and 5 g XOS kg⁻¹ showed the highest TVC count in ileum and caecum. The beneficial effect of XOS on altering the composition and activity of ileal and caecal microbiota has been reported in previous studies^{25,26}. XOS supplementation significantly increased the number of Lactobacillus in broilers⁶ and Bifidobacteria in layer chickens²⁷. Pourabedin *et al.*²⁸ reported that dietary supplementation of 2 g XOS kg⁻¹ not only increased the SCFA concentrations but also the ileocaecal lactobacillus populations. The SCFA, especially butyrate maintains the intestinal integrity and microbial ecosystem balance of broiler chickens⁶. The gut microbial profiling and SCFA concentration in GIT was not analyzed in the current research and warrant further study.

Visceral organ development: In the current study, dietary supplementation of XOS (5 g XOS kg⁻¹) increased the relative weight of the proventriculus of broiler chickens. The liver and pancreas were the largest in birds that received diets with 2.5 g XOS kg⁻¹. The diet containing 7.5 g XOS kg⁻¹ increased the gizzard weight of the birds. Prebiotic supplementation increased the intestinal metabolic activity and increased visceral organ weight²⁹. In contrast, a previous study reported no effect of XOS supplementation on visceral organ development¹⁰. The discrepancy can be due to the differences in the source and dosage of XOS used over the studies.

Carcass yield: Dietary supplementation of 2.5 g XOS kg⁻¹ increased the thigh, shank and dressing percentage of the broiler chickens. This result is in line with the growth performance and serum metabolic profile data of this study. The mechanism of how prebiotics reduces the abdominal fat content is still unknown but the abundance of Lactobacillus in the intestine that decreases the activity of acetyl-CoA carboxylase can be one of the possible explanations²⁹.

CONCLUSION

The present study indicated that dietary supplementation of 2.5 g XOS kg $^{-1}$ significantly improved the FCR and TVC in ileum and caecum. The diet containing 2.5 g XOS kg $^{-1}$ decreased the serum glucose but increased the T3 and T4 levels. Moreover, the inclusion of XOS (2.5 g kg $^{-1}$) into the diet increased the dressing percentage but decreased the abdominal fat content of broiler chickens. In a nutshell, XOS supplementation can improve the overall performance of broiler chickens when supplemented at a dose of 2.5 g XOS kg $^{-1}$.

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