R. Bras. Zootec., 49:e20190170, 2020
https://doi.org/10.37496/rbz4920190170

Non-ruminants
Full-length research article

Revista Brasileira de Zootecnia

Brazilian Journal of Animal Science
e-ISSN 1806-9290
www.rbz.org.br

Effects of dietary xylo-oligosaccharide on growth performance, serum biochemical parameters, antioxidant function, and immunological function of nursery piglets

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Received: September 17, 2019
Accepted: April 9, 2020

How to cite: Hou, Z.; Wu, D. and Dai, Q. 2020. Effects of dietary xylo-oligosaccharide on growth performance, serum biochemical parameters, antioxidant function, and immunological function of nursery piglets. Revista Brasileira de Zootecnia 49:e20190170. https://doi.org/10.37496/rbz4920190170

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ABSTRACT - This study investigated the effects of dietary xylo-oligosaccharide (XOS) on growth performance, serum biochemical parameters, antioxidant function, and immunological function of nursery piglets. In total, three groups including 72 nursery piglets were designed and fed one of three diets: a control basal diet, basal diet supplemented with 0.2% ZnO, or basal diet supplemented with 0.04% XOS, for 28 days. Compared with the control group, the XOS group significantly increased the final body weight and average daily weight gain. No significant differences were found about these parameters between the control and ZnO groups. Compared with the control group, the ZnO group showed no changes in the serum content of total protein (TP), albumin (ALB), albumin:globulin (ALB:GLB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), glucose, triglyceride, total cholesterol (TC), or in the serum activity of amylase and alkaline phosphatase. However, in the XOS group, serum glucose content increased and blood urea nitrogen and triglyceride content decreased significantly. Compared with the control group, dietary supplementation with XOS significantly increased the serum activity of total antioxygenic capacity, superoxide dismutase, and catalase and decreased the serum activity of malondialdehyde. At the same time, serum IgG content in XOS group was significantly higher than that in control group. From the current study, supplementation of 0.04% XOS in the diet could improve the antioxidant and immune function of piglets, promotes nitrogen deposition, and accelerates lipid and glucose metabolism, thereby improving piglet growth performance.

Keywords: antioxidant, feed additive, immune function, metabolism, Ningxiang pig

1. Introduction

Weaning is critical to pigs due to physiological and immunological immaturity that has been associated with increased susceptibility to infection and disease, especially diarrhea, and reduced growth performance (Campbell et al., 2013).Thus, the use of feed additives has become a common practice as modulator of immune system and intestinal microbiota, thereby promoting health and performance of pigs during the immediately post-weaning period (Lallès et al., 2007). Pharmacological levels of Zn oxide (ZnO), above 2000 mg ZnO/kg, have been widely used to control diarrhea (Owusu-Asiedu et al., 2003) as well as to improve feed efficiency and growth performance in weanling pigs (Walk et al., 2015; Payne et al. 2006). These beneficial effects of ZnO supplemented in the diet are effective until up to three weeks after weaning; however, some authors (Janczyk et al., 2013; Starke et al., 2014) observed the adverse effects due to toxicity. Because of its low digestibility, about 80% of ZnO is expelled through the feces (Buff et al., 2005). High dietary ZnO leads to the development of Zn resistance by animal intestinal bacteria and may play a role (Cavaco et al., 2011; EFSA, 2014) in the co-occurrence of antibiotic resistance and excessive fecal Zn content (Case and Carlson, 2002; Buff et al., 2005); therefore, Zn accumulation in soil after intensive pig farming (Romeo et al., 2014) will be a serious environmental concern.

The European Union has voted for the phase-out pharmacological ZnO by 2022 (Wang et al., 2019). The Chinese government has also explicitly proposed that all drug feed additives should be withdrawn from livestock and poultry breeding by 2020 (Ministry of Agriculture and Rural Areas of the People's Republic of China, 2018). Therefore, it has become a major research topic to investigate preferable alternative growth promoters.

Xylo-oligosaccharide (XOS) is a functional oligosaccharide that consists of two to seven xylose molecules linked by a beta-1,4 glycoside bond. It can pass through the stomach and small intestine, travels directly into the hindgut, then is utilized by beneficial bacteria in the gut (Jacobsen and Wyman, 2002). It is also fermented into short-chain fatty acids (SCFA) such as acetic, propionic, butyric, and valeric acids. These SCFA can reduce intestinal pH, inhibit the growth of harmful bacteria, regulate the intestinal flora balance, and protect intestinal health (Zhao et al., 2018). Xylo-oligosaccharide is widely used in healthy animal husbandry breeding and has become a new type of green feed additive due to its many advantages, such as safety, effectiveness, and low toxicity. In July 2003, XOS was first certified as a new feed product and feed additive by the Ministry of Agriculture of China (Announcement of the Ministry of Agriculture of the People's Republic of China, No. 288). Many studies (Wang and Lu, 2013; Yang et al., 2015; Jain et al., 2015) have showwn that it preferentially stimulates the growth or activity of beneficial bacteria (such as *Bifidobacterium* and other lactic acid bacteria) in the gut, enhances immune function, improves the growth of the intestinal mucosa, meanwhile increasing the microbiota diversity of the intestine (Tan et al., 2016) of weanling pigs.

However, little research has been performed regarding the application of XOS for nursery piglets. The purpose of the present study was to determine the effects of XOS on growth performance, serum biochemical parameters, antioxidant function, and immunological function of nursery piglets. This study aims to provide new insights for the wide application of XOS as a new type of green feed additive.

2. Material and Methods

The experiment was conducted in Yuanjiang (28°50'40.84" N, 112°20'55.60" E), Hunan province, China. All experimental procedures used in the present study were approved by the Animal Care Committee (case no. 2018009).

Xylo-oligosaccharide was obtained in powdered form from Beijing Strowin Biotechnology Co., Ltd. (Beijing, China). Its main components are xyldisaccharides, xyltrisaccharides, and xyltetrasaccharides, and the content of XOS is > 35%.

Seventy-two nursery pigs (Ningxiang pig, a local breed of Hunan, China; Castrate pig;average body weight [BW] = 18.94±0.24 kg) were randomly divided into three treatment groups. Each treatment consisted of four replicate pens and six pigs per replicate. Pigs were fed either a control basal diet, basal diet supplemented with 0.2% ZnO, or basal diet supplemented with 0.04% XOS. Each compound was uniformly mixed into the basal diet. These diets without antibiotics were formulated to provide the nutrients needed to meet or exceed the NRC requirements (Table 1) (NRC, 2012).

All diets were pelleted. Piglets were fed two times (8.00 and 18.00 h) every day. Access to feed and water were provided *ad libitum* throughout the experiment. Pretest period was seven days and experimental period was 28 days.

Piglets were weighed on day 1 and 28 of experimental period. Feed intake was recorded daily per pen to determine the average daily gain (ADG), average daily feed intake (ADFI), and feed intake to body gain ratio (F:G). A 10 mL of blood sample (three pigs per pen) was taken from the anterior vena cava of each animal on day 28 and centrifuged at 3 000 rpm for 10 min to collect serum (stored at −20 °C).

Table 1 - Composition and nutrient levels of the basal and experimental diets

Item	Diet[1]		
	Control	ZnO	XOS
Ingredient (g kg^{-1} as fed)			
Corn	435.00	435.00	435.00
Extruded corn	100.00	100.00	100.00
Soybean meal	140.00	140.00	140.00
Extruded rice	120.00	120.00	120.00
Extruded soybean	70.00	70.00	70.00
Fermented soybean meal	56.00	56.00	56.00
Fish meal	5.00	5.00	5.00
Rice protein peptide	10.00	10.00	10.00
Whey powder	20.00	20.00	20.00
ZnO	0.00	2.00	0.00
XOS	0.00	0.00	0.40
Premix compound[2]	44.00	42.00	43.60
Nutrient composition[3]			
Digestive energy (MJ/kg)	13.67		
Crude protein (%)	18.29		
Ca (%)	0.73		
Total P (%)	0.58		
Digestible P (%)	0.38		
Lys (%)	1.41		
Met (%)	0.32		
Thr (%)	0.80		
Trp (%)	0.21		

[1] Control - basal diet; ZnO - basal diet supplemented with 0.2% ZnO; XOS - basal diet supplemented with 0.04% xylo-oligosaccharide.
[2] The premix contained the following nutritional components (values are given per kg feed): Mn, 25 mg; Fe, 100 mg; Cu, 15 mg; Zn, 179 mg; I, 0.29 mg; Se, 0.3 mg; choline chloride, 500 mg; vitamin A, 4,500 IU; vitamin D3, 3,300 IU; vitamin E, 22.5 IU; vitamin K3, 3 mg; vitamin B1, 3 mg; vitamin B2, 7.5 mg; vitamin B6, 4.5 mg; vitamin B12, 0.03 mg; niacin 30, mg; pantothenate, 15 mg; folic acid, 1.5 mg; biotin, 0.12 mg.
[3] Calculated values.

Routine blood tests were performed using a Mindry automatic biochemical analyzer (BS-408, Mindry, Shenzhen, China). The indicators were as follows: total serum protein (TP), albumin:globulin ratio (ALB:GLB), albumin (ALB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose (GLU), blood urea nitrogen (BUN), total cholesterol (TCH), triglyceride (TG), amylase (AMY), and alkaline phosphatase (AKP). ELISA was performed to determine total antioxidant capacity (T-AOC), as well as the concentrations of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), immunoglobulin G (IgG), catalase (CAT), and malondialdehyde (MDA); ELISA kits were purchased from Nanjing Jincheng Bioengineering Research Institute Co., Ltd. (Nanjing, China).

The experimental data were initially collated by Excel 2016 and then analyzed by one-way ANOVA with IBM SPSS Statistics 21.0 (IBM, Armonk, New York, US). The results were expressed as means. Significant means were separated by Duncan's Multiple Range test method. The significance level was $P \leq 0.05$, whereas $0.05 < P < 0.1$ was considered as a tendency of significant difference.

3. Results

The group fed XOS showed a significant increase in final BW and ADG ($P<0.05$) comparing to the control group (Table 2). No significant difference was observed in growth performance between the control and ZnO groups.

To evaluate the effects of XOS on blood composition, biochemical parameters (Table 3) were analyzed. Xylo-oligosaccharide significantly decreased ($P<0.05$) BUN and TG concentrations compared with the control group, but significantly increased ($P<0.05$) the GLU concentration compared with with control and ZnO groups. There were no significant effects on any other biochemical parameters.

Compared with the control group, serum T-AOC, SOD, and CAT activities were significantly higher ($P \leq 0.05$), but serum MDA activity was significantly lower ($P<0.05$) in the groups fed ZnO and XOS (Table 4). Dietary addition of XOS had a tendency to increase GSH-Px ($P = 0.061$) and IgG content in serum ($P = 0.097$).

Table 2 - Effects of xylo-oligosaccharide (XOS) on growth performance of nursery piglets

Item	Diet[1]			SEM	P-value
	Control	ZnO	XOS		
Initial BW (kg)	18.96	18.94	18.93	0.25	1.000
Final BW (kg)	28.05b	29.76ab	31.03a	1.40	0.034
ADG (g)	324.70b	386.38ab	432.14a	46.43	0.032
ADFI (g)	1184.16	1303.42	1302.90	36.29	0.095
F:G	3.69	3.38	3.08	0.37	0.173

BW - body weight; ADG - average daily gain; ADFI - average daily feed intake; F:G - feed to gain ratio; SEM - standard error of the means.
[1] Control - basal diet; ZnO - basal diet supplemented with 0.2% ZnO; XOS - basal diet supplemented with 0.04% XOS.
a,b - Values in the same row with different letters were significantly different ($P<0.05$).

Table 3 - Effects of xylo-oligosaccharide (XOS) on serum biochemical parameters of nursery piglets

Item	Diet[1]			SEM	P-value
	Control	ZnO	XOS		
TP (g/L)	67.65	71.42	77.14	3.57	0.117
ALB (g/L)	39.80	37.15	35.74	1.90	0.132
ALB/GLB	1.27	1.08	1.07	0.04	0.876
ALT (U/L)	63.71	63.56	43.96	12.02	0.095
AST (U/L)	65.88	50.31	49.82	11.44	0.164
BUN (mmol/L)	3.25a	2.54ab	1.630b	0.33	0.002
GLU (mmol/L)	4.01b	4.34b	6.06a	0.70	0.002
TG (mmol/L)	1.55a	0.83b	0.79b	0.08	0.000
TCH (mmol/L)	8.12	6.74	5.77	0.70	0.67
AMY (U/dL)	5.73	6.09	8.75	0.96	0.059
AKP (U/L)	13.67	14.60	16.43	4.18	0.728

TP - total protein; ALB - albumin; ALB:GLB - albumin to globulin ratio; ALT - alanine aminotransferase; AST - aspartate aminotransferase; BUN - blood urea nitrogen; GLU - glucose; TG - triglyceride; TCH - total cholesterol; AMY - amylase; AKP - alkaline phosphatase; SEM - standard error of the means.
[1] Control - basal diet; ZnO - basal diet supplemented with 0.2% ZnO; XOS - basal diet supplemented with 0.04% XOS.
a,b - Values in the same row with different letters were significantly different ($P<0.05$).

Table 4 - Effects of xylo-oligosaccharide (XOS) on antioxidant and immunological function of nursery piglets

Item	Diet[1]			SEM	P-value
	Control	ZnO	XOS		
T-AOC (mmol/L)	0.13b	0.21a	0.23a	0.03	0.043
SOD (U/mL)	174.92b	180.60ab	218.51a	22.47	0.050
GSH-Px (U/mL)	1874.04	1983.55	2108.56	26.60	0.060
CAT (U/mL)	6.73b	13.73a	11.71a	2.33	0.003
MDA (nmol/mL)	11.48a	5.220b	5.23b	1.33	0.001
IgG (mg/mL)	10.75	12.32	12.49	1.43	0.097

T-AOC - total antioxidant capacity; SOD - superoxide dismutase; GSH-Px - glutathione peroxidase; CAT - catalase; MDA - malondialdehyde; SEM - standard error of the means.
[1] Control - basal diet; ZnO - basal diet supplemented with 0.2% ZnO; XOS - basal diet supplemented with 0.04% XOS.
a,b - Values in the same row with different letters were significantly different ($P<0.05$).

4. Discussion

Xylo-oligosaccharide plays a beneficial role in intestinal function by targeting tight junction proteins (Nawaz et al., 2018) and exhibits a probiotic effect by promoting the proliferation of beneficial microbes (Ho et al., 2018). Therfore, XOS is widely used as a probiotic to promote animal growth in animal production. Dietary supplementation of 0.01% XOS (Tan et al., 2016) could significantly increase ADG and ADFI in weanling pigs. Similarly, dietary supplementation with 20 mg/kg XOS in weanling piglets gained in a greater ADG, ADFI, and FCR (Zhao et al., 2018). Some researchers reported that inclusion of 200 mg/kg XOS in diet significantly improved the growth performance of nursery pigs (Fang et al., 2015; Fan et al., 2016; Guo et al., 2017; Liu et al., 2018). The current study obtained similar results that dietary supplementation with 0.04% XOS for nursery pigs could significantly improve the final BW and ADG ($P<0.05$). However, the present results were contradictory to the literature reported, in which supplementary 0.01% XOS affected growth performance weakly (Yin et al., 2019). This may be due to the growth stage of the pigs, as well as different sources and dosage of XOS.

High serum TP content is beneficial for enhancing the metabolic function and immunity of animals. A decreased ALB:GLB indicates (Sun et al., 2009) that the synthesis of immunoglobulin is accelerated, accompanied by enhanced disease resistance. Researchers (Zhao et al., 2018; Yin et al., 2019) reported that XOS failed to affect serum TP, ALB, or ALB:GLB in pigs. The results of this study showed that diet supplementation with XOS for nursery piglets did not significantly affect serum TP, ALB, and ALB:GLB. However, serum TP content was higher and ALB:GLB was lower in XOS-fed pigs.

The present results may have been due to the breed of the piglets used, which could easily adapt to feeding management conditions and have strong anti-stress mechanisms.

Alanine aminotransferase and AST are two important amino acid transferases found in the mitochondria and cytoplasm of hepatocytes. When animals are damaged by liver injury or acute stress, the serum activities of these two enzymes will increase (Chen et al., 1995). Therefore, the activity of ALT and AST in the serum can reflect damage to hepatocytes (Lv et al., 2015; Liu et al., 2015). Tan et al. (2016) reported that the activities of AST in plasma of 500 g/t XOS group were significantly higher than those of positive control group on day 7 and 56 of experiment, and the activity of ALT in plasma of 100 and 500 g/t XOS groups was significantly higher than that of blank control group on day 21 of experiment. As the number of XOS addition increases, the activities of ALT and AST in serum had a tendency to improve, but there were no significant differences among all groups (Zhao et al., 2018). Yin et al. (2019) found that supplementation with XOS in diet did not affect the serum activities of ALT and AST. The results of this experiment were consistent with Yin et al. (2019), in which no significant differences were observed in the serum activities of ALT and AST in the nursery piglets among groups, indicating that the piglets had strong stress resistance and XOS added in the diet had no harmful effects on their livers. This is contrary to other findings (Tan et al., 2016; Zhao et al., 2018) because it may be due to pig breed or XOS types and doses, etc.

A previous study reported that the appropriate dietary supplementation with XOS could improve serum GLU content (Singh and Cresswell, 2010). Some researchers found that the addition of XOS to the pig diet could significantly decrease serum BUN and TG contents (Pan, 2011; Tan et al., 2016; Zhao et al., 2018). In the present study, it was found that the addition of XOS in diet could significantly increase GLU contents and reduce the contents of serum BUN and TG. Under normal conditions, the serum GLU content of high-yield animals has been found to be higher than that of low-yield animals (Hou et al., 2015), which reflects the glucose metabolism status of the body. The serum content of BUN is an important indicator of protein synthesis efficiency and amino acid balance (Coma et al., 1995), which is negatively correlated with muscle growth and ADG in piglets. Serum TG levels are known to increase when the body is under stress, which could result in decreasing fat utilization (Chen et al., 2016). Our results showed that XOS added to the diet could enhance the digestion and absorption of nutrients, increase the efficiency of protein biosynthesis and fat utilization, and simultaneously reduce adverse stress reactions in piglets and improve their health status.

Activity of AMY reflects the digestive and absorptive capacity of starch and affects the speed of chemical reactions, growth, developmental health, and the adaptability of the body. Furthermore, AKP promotes the deposition of calcium and phosphorus in bone and also regulates the metabolic functions of the body. Pan (2011) reported that adding 0.02% XOS in diet significantly increased the activities of AKP and AMY. The activity of AMY in plasma of group fed 500 g/t XOS was significantly higher than that of positive control group on day 56 of experiment (Tan et al., 2016). In another study, it was found that the activities of AMY and AKP in serum of dietary XOS (200 mg/kg) group for 28 days in weaned pigs were significantly higher than those of control group (Zhao et al., 2018). The results of this study showed that the 0.04% XOS addition to diet had a tendency to increase the activity of AMY and AKP in serum. However, no significant differences were observed among groups in the current study, which were contrary to previous results (Pan, 2011; Zhao et al., 2018), possibly because of the duration of this experiment and growth stages of the piglets, as well as the composition and source of the diet.

Serum T-AOC is an indicator of the overall level of enzymatic and non-enzymatic antioxidants *in vivo*. Enzymatic oxygen free radical scavengers SOD, CAT, and GSH-Px are the major components of the enzymatic antioxidant system. Malondialdehyde is a metabolic product of lipid peroxidation and indirectly reflects the degree of damage by oxygen free radicals to cells. When the concentration of free radicals increases in the body, tissues will enhance the endogenous activity of the antioxidant system to prevent free radical damage. Fan et al. (2016) found that the diets supplemented with 200 mg/kg XOS markedly enhanced the serum T-AOC, liver SOD, and CAT activity and decreased MDA contents of serum and liver. From the present results, dietary supplementation with 0.04% XOS significantly improved serum T-AOC, SOD, and CAT activities and significantly decreased serum MDA activity. Therefore, under this experimental condition, the results indicated that 0.04% XOS added to diet could enhance the antioxidant capability *in vivo*.

Serum Ig is a non-specific antibody found in animal serum. It mainly plays a protective role in the immune systems, as they are stimulated by the invasion of foreign pathogens and viruses to protect the body against infection. Supplementation of XOS in pig diets could significantly increase serum IgG content (Fang et al., 2015; Pan, 2011; Wang et al., 2006). The current study showed similar results, as dietary supplementation with XOS could improve the serum IgG content to a certain extent. This result showed that the suitable addition of 0.04% XOS in diet might have anti-infective effect which needs to be confirmed with further experiments.

Conclusions

Under the current experiment conditions, dietary 0.04% xylo-oligosaccharide supplementation could improve the antioxidant and immune function of piglets, promote nitrogen deposition, and accelerate lipid and glucose metabolism, thereby improving their growth performance.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Data curation: Z. Hou. Formal analysis: Z. Hou. Methodology: Z. Hou and D. Wu. Project administration: Q. Dai. Software: Z. Hou. Supervision: D. Wu. Writing-original draft: Z. Hou. Writing-review & editing: Z. Hou and D. Wu.

Acknowledgments

This study was supported by Chinese Academy of Agricultural Sciences and Central Public-interest Scientific Institution Basal Research Fund (No. 1610242019002), Huxiang High-level Talents Gathering Project 2018-Innovative Talents (2018RS3130), and the Agricultural Science and Technology Innovation Program (ASTIP-IBFC02).

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