RESEARCH ARTICLE

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Effects of dietary xylanase supplementation on growth performance and gut health of weaned pigs fed a high non-starch polysaccharides diet

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Abstract

Non-starch polysaccharides (NSPs) in cereal grains can negatively affect the growth performance of early weaned pigs. Weaned pigs cannot digest NSPs due to a lack of endogenous enzymes. Feeds containing high levels of NSPs can decrease nutrient digestibility by increasing digesta viscosity and modulating the gut environment. Dietary xylanase (XYL) is used to increase nutrient utilization by degrading NSPs containing anti-nutritional factors. Therefore, this study was conducted to evaluate the effects of XYL on the high NSPs diet on growth performance, frequency of diarrhea, blood profiles, systemic immune responses, digesta characteristics, nutrient digestibility, and intestinal health parameters of weaned pigs. XYL improved ($p < 0.05$) the average daily gain (ADG) and gain to feed ratio from day 1 to 7 and day 1 to 14 compared with the control group (CON). Additionally, pigs fed XYL tended to have a higher ($p = 0.098$) ADG from day 8 to 14 than those fed CON. Pigs fed XYL tended to have a lower ($p = 0.093$) number of white blood cells on day 28 than those fed CON. The XYL group tended to increase ($p = 0.088$) digesta pH in the duodenum, but decreased digesta pH in the jejunum ($p = 0.069$) and cecum ($p < 0.05$) on day 28 compared with the CON. Pigs fed XYL had higher ($p < 0.05$) apparent total tract digestibility (ATTD) and apparent ileal digestibility of dry matter on day 28 than those fed CON. Additionally, the XYL group tended to improve ATTD of energy ($p = 0.083$) and crude protein ($p = 0.082$) compared with the CON. Dietary XYL decreased concentrations of tumor necrosis factor-α ($p < 0.05$), immunoglobulin G ($p = 0.066$), malondialdehyde ($p = 0.070$) in jejunal mucosa compared with CON. In conclusion, supplementation of high NSPs diet with XYL enhanced the growth performance of weaned pigs by enhancing nutrient digestibility through the modulation of the intestinal environment.

Keywords: Non-starch polysaccharides, Nutrients digestibility, Xylanase, Gut health, Weaned pigs

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Ethics approval and consent to participate
The experimental protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of Chungnam National University, Daejeon, Korea (approval #: 202112-CNU-182).

Declaration of generative AI
No AI tools were used in this article.

INTRODUCTION

Among the various factors affecting the swine industry, such as feed, disease, management, and environmental conditions, feed cost is regarded as the largest expense [1]. Cereal grain costs have also continued to increase over the past 50 years. Moreover, the quantity of cereal grain available in swine diets is limited due to increasing consumer demand for meat following population growth and rising industrial demand, such as bioethanol and biodiesel production [2,3]. Therefore, swine nutritionists have been seeking substitutes for conventional feed ingredients to reduce feed costs. Grain co-products such as corn distiller's dried grains with solubles (DDGS), wheat bran, and wheat middlings can be used as low-cost alternative ingredients [4,5]. However, these co-products have high concentrations of non-starch polysaccharides (NSPs) [6,7]. NSPs in feed encapsulate nutrients, limiting the accessibility of endogenous enzymes and reducing nutrient utilization [6,8].

Early weaned pigs are exposed to environmental, nutritional, physiological, and immunological changes during this period [9–11]. In particular, weaned pigs are commonly fed plant-based feedstuffs containing antinutritional factors such as NSPs [12]. However, pigs have an immature digestive tract, which results in poor nutrient utilization [13]. NSPs are primarily composed of cellulose, hemicellulose, and pectin. They constitute a notable portion of the plant cell wall and more than 90% of its structural strength [14]. NSPs are classified as soluble and insoluble NSPs based on their physicochemical properties. Excessive NSP inclusion in feed may negatively affect the gastrointestinal tract and cause morphological changes [15]. Soluble NSPs have a high water-holding capacity and can increase digesta bulk and viscosity, thereby affecting the passage rate. In contrast, insoluble NSPs may reduce nutrient digestibility and alter intestinal transit time and motility [10,12]. These functions reduce nutrient absorption and induce intestinal disorders associated with local inflammation, ultimately leading to diarrhea and pathogenic infections [16,17]. Xylanase (XYL) enzyme degrade NSPs, including xylan and arabinoxylan structures in cereal grains [12], thereby improving growth performance and nutrient digestibility. Additionally, XYL can release metabolites from indigestible nutrients through hydrolytic action, which may not only modulate the gut environment and immune status [10,18], but also but also provide additional energy sources for the host [19]. However, the effects of dietary XYL on growth and health of pigs vary depending on the dietary grain composition [10,12,20]. Moreover, supplementation of dietary XYL in diets containing high levels of NSPs may exert beneficial effects in pigs [21]. Therefore, the objective of this study was to evaluate the effect of XYL supplementation on growth performance, frequency of diarrhea, blood profiles, systemic immune responses, digesta characteristics, nutrients digestibility, and intestinal health parameters of weaned pigs fed high NSPs diets.

MATERIALS AND METHODS

Experimental design, animals, and diets

A total of 60 newly weaned pigs ([Landrace × Yorkshire] × Duroc; 8.04 ± 0.99 kg of average initial body weight [BW]; 4 weeks of age) were assigned to two dietary treatments (5 pigs per pen; 6 replicate pens per dietary treatment) using a randomized complete block design (block = initial BW). Control (CON) pigs were fed a high NSPs diet, and the other pigs were fed a CON diet with 0.03% XYL for 28 days. The experimental diet was formulated to meet the nutrient requirements of the weaned pigs, as estimated by the National Research Council [22] (Table 1). The experimental diet was designed with high NSPs content using corn DDGS and wheat. XYL product was obtained from a commercial company (CJ BIO). During the last week of the study, chromium oxide (Daejung Chemicals & Metals) was added to diet at a concentration of 3 g/kg

Table 1. Composition of experimental diets (as-fed basis)

Item	Control
Ingredient (%)	
Corn	12.50
Corn distillers dried grains with soluble	30.00
Soybean meal (44%)	14.00
Wheat	30.00
Whey permeate	5.00
Spray dried porcine plasma	0.60
Fish meal	1.00
Soybean oil	3.30
Limestone	1.30
Dicalcium phosphate	0.75
Iodized salt	0.20
Vitamin-mineral premix[1)]	0.35
Lysine-HCl	0.71
DL-Methionine	0.10
L-Threonine	0.17
L-Tryptophan	0.02
Total	100
Calculated energy and nutrient contents	
Dry matter (%)	88.44
Metabolizable energy (kcal/kg)	3,399
Crude protein (%)	21.93
SID lysine (%)	1.23
SID methionine (%)	0.40
SID cysteine + methionine (%)	0.69
SID threonine (%)	0.73
SID tryptophan (%)	0.20
Calcium (%)	0.78
Total phosphorus (%)	0.60
STTD phosphorous (%)	0.38
ATTD phosphorous (%)	0.33
Non-starch polysaccharides (%)	15.47

[1)] Provided per kilogram of diet: vitamin A, 12,000 IU; vitamin D_3, 2,500 IU; vitamin E, 30 IU; vitamin K3, 3 mg; D-pantothenic acid, 15 mg; nicotinic acid, 40 mg; choline, 400 mg; and vitamin B_{12}, 12 µg; Fe, 90 mg from iron sulfate; Cu, 8.8 mg from copper sulfate; Zn, 100 mg from zinc oxide; Mn, 54 mg from manganese oxide; I, 0.35 mg from potassium iodide; Se, 0.30 mg from sodium selenite.

SID, standardized ileal digestible; STTD, standardized total tract digestible; ATTD, apparent total tract digestible.

as an indigestible marker to determine nutrient digestibility [23]. All pigs had *ad libitum* access to feed and water and were housed in pens of equal size (2 × 2 m) with automatically controlled temperature, humidity, and lighting during the experimental period.

Data and sample collection

Feed intake and pigs' BW in each pen were recorded on day 1, 7, 14, and 28 to calculate growth performance parameters, including average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F). The frequency of diarrhea in pigs was recorded during the first 2 weeks after weaning by visual observation with a score ranging from 1 to 5 (1 = dry feces, 2 = normal feces, 3 = slightly mild feces, 4 = mild diarrhea, and 5 = watery severe diarrhea) and was calculated by counting the number of days with a pen average diarrhea score of 4 or higher [24]. Blood samples were collected from one randomly selected pig in each pen on days 1, 7, 14, and 28 using a 10 mL vacutainer tube with or without ethylenediaminetetraacetic acid (EDTA) to yield whole blood and serum, respectively [25]. Serum samples were obtained after centrifugation of non-EDTA tubes at 3,000×g for 15 min at 4℃. The supernatant after centrifugation was collected and stored at −80℃ until analysis of immune responses [26]. Fecal samples were collected from one randomly selected pig per pen by rectal stimulation for 3 days following a four days adaptation period and stored at −20℃ for subsequent analysis of apparent total tract digestibility (ATTD) [12]. On the last day of the study, two randomly selected pigs per pen were anesthetized with 2 mL of suxamethonium chloride (Succicholine, Ilsung Pharm). The pigs were euthanized by exposure to CO_2 gas. Ileal digesta samples were collected into 50 mL tubes from a site 30 cm proximal to the ileocecal junction and stored at −20℃ until apparent ileal digestibility (AID) analysis [27]. To measure the viscosity and pH of digesta, samples were collected from the stomach, duodenum, jejunum, ileum, and cecum. The samples for viscosity analysis were collected into 50 mL tubes, placed on ice, and immediately transported to the laboratory for viscosity measurement, while digesta pH was measured immediately after collection using a digital pH meter (Accumet, Thermo Fisher Scientific) [26]. Mid-jejunal segments were collected, rinsed with distilled water, and fixed in 50 mL conical tubes with 10% neutral buffered formalin solution for histomorphological measurements. Mucosal samples were scraped from the remaining mid-jejunum, placed into 2 mL microtubes, and stored at −80℃ freezer to determine the mucosal immune responses and oxidative stress indicators.

Blood profiles and systemic immune responses

Whole blood samples in EDTA tubes were analyzed by an automated hematology analyzer (scil Vet abc hematology analyzer, Scil Animal Care Company) for evaluating the number of white blood cells (WBC), platelet, hematocrit, red blood cells, mean corpuscular volume, hemoglobin, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. Serum samples were used to measure tumor necrosis factor-α (TNF-α; R&D Systems), transforming growth factor-β1 (R&D Systems), interleukin-10 (IL-10; R&D Systems), C-reactive protein (CRP; Aviva Systems Biology), immunoglobulin G (IgG; Bethyl Laboratories), and immunoglobulin A (IgA; Bethyl Laboratories) using porcine-specific enzyme-linked immunosorbent assay (ELISA) kits following the provided manufacturer protocols. Absorbance was measured at 450 nm using a microplate reader (Epoch microplate spectrophotometer, BioTek Instruments) and software (Gen5 Data Analysis Software, BioTek Instruments), and the concentrations were calculated based on the standard curve from each ELISA kit.

Viscosity of digesta

The procedure for determining the digesta viscosity was adapted from a previous study [15]. The viscosity of digesta from the stomach, jejunum, ileum, and cecum was measured using a viscometer (Model DV-II Version 2.0, Brookfield Engineering Laboratories). The sample tubes were centrifuged at 1,000×g for 10 min to obtain the liquid phase. The liquid phase was transferred to a 2 mL microtube to second centrifuge at 1,000×g for 10 min. The supernatant was collected for further analysis. Before viscosity measurement, the viscometer was equilibrated to 25℃. Viscosity values were determined using 0.5 mL of digesta supernatant and calculated as the average of shear rates at 45.0/s and 22.5/s, and recorded as millipascal-seconds.

Nutrient digestibility

Diets, ileal digesta, and fecal samples were dried using air-forced drying oven at 65℃ for 72

h. All samples were ground to powder using a grinder (80350, Hamilton Beach) for AID and ATTD analysis. The bomb calorimeter (Parr 1261EA Bomb Calorimeter, Parr Instrument) was used for measuring energy [28]. Dry matter (DM; method 930.15), crude protein method (CP; method 988.05), and crude fiber (method 962.09) were analyzed based on Association of Official Analytical Chemists [29]. NSPs were analyzed based on a previous report [30]. The chromium concentration was analyzed using an absorption spectrophotometer (Hitachi Z-5000 Absorption Spectrophotometer, Hitachi High-Technologies). The calculation methods for AID and ATTD have been described in a previous study [27,31].

Histomorphological analysis

Histomorphological analyses were performed as previously described [12,32]. The two sections from the mid-jejunum were dehydrated, embedded in paraffin wax, sectioned to 5 μm and stained using hematoxylin and eosin. The villus height to crypt depth ratio (VH:CD) was measured from stained slides using an Olympus CX31 microscope (Olympus Corporation) equipped with an Infinity 2-2 digital CCD camera (Lumenera Corporation). Fifteen well-oriented, intact villi and their associated crypt depths were measured on each slide. The length was measured from the top of the villi to the villus crypt junction, and the crypt depth was measured from the villus crypt junction to the bottom of the crypt. The images for counting Ki-67 positive cells in the crypt were cropped into 15 intact images from each slide, and the ImageJS software was used to calculate the percentage of Ki-67 positive cells to total cells in the crypt.

Intestinal immune responses and oxidative stress indicators

Mucosal samples were weighed, suspended in 1 mL of phosphate-buffered saline, homogenized on ice using a tissue homogenizer (Tissuemiser, Thermo Fisher Scientific), and centrifuged at 14,000×g at 4℃ for 3 min. After centrifugation, the supernatant was collected and stored at −80℃ until further analysis [10]. Jejunal mucosal immune responses (TNF-α [R&D Systems], interleukin-6 [IL-6; R&D Systems], interleukin-8 [IL-8; R&D Systems], IgG [Bethyl Laboratories], and IgA [Bethyl Laboratories]) and oxidative stress (malondialdehyde [MDA; Cell Biolabs], protein carbonyl [PC; Cell Biolabs], and endotoxin [Aviva Systems Biology]) were determined using ELISA kits following the manufacturer's instruction. The absorbance was measured using a microplate reader (Epoch microplate spectrophotometer, BioTek Instruments) and software (Gen5 Data Analysis Software, BioTek Instruments). All concentrations were calculated based on standard curve generated from the concentration and absorbance of each standard.

Statistical analyses

All data, except for the frequency of diarrhea, were analyzed using the GLM procedure in SAS, using a randomized complete block design (block = initial BW). The experimental unit used was a pen. Statistical models for growth performance, digesta characteristics, nutrient digestibility, jejunal health parameters, blood profiles, and systemic immune responses included effects of dietary treatments as the main effects and BW as a covariate. Chi-square test was used to determine the frequency of diarrhea. Statistical significance and tendency between dietary treatments were considered at $p < 0.05$ and $0.05 \leq p < 0.10$, respectively.

RESULTS

Growth performance and frequency of diarrhea

XYL increased ($p < 0.05$) ADG and G:F from day 1 to 7 and from day 1 to 14 compared with the CON (Table 2). Additionally, pigs fed XYL tended to have a higher (p = 0.098) ADG from day 8 to 14 than those fed CON. However, there was no difference on ADFI of weaned pigs during overall experimental period between the treatments. There was no difference in frequency of diarrhea between CON and XYL.

Blood profiles and systemic immune responses

Pigs fed XYL tended to have lower (p = 0.093) WBC counts on day 28 than those fed the CON (Table 3). However, no differences were observed in serum immune responses of weaned pigs between the CON and XYL (Table 4).

Table 2. Effects of dietary xylanase on growth performance of weaned pigs[1)]

Item[2)]	CON	XYL	SEM	*p*-value
Day 1 to 7				
Initial BW (kg)	8.04	8.05	0.44	0.985
Final BW (kg)	8.94	9.40	0.46	0.497
ADG (g/d)	128.57	192.86	17.12	0.026
ADFI (g/d)	225.52	245.90	12.84	0.288
G:F (g/g)	0.57	0.79	0.06	0.014
Day 8 to 14				
Initial BW (kg)	8.94	9.40	0.46	0.497
Final BW (kg)	10.86	11.76	0.53	0.255
ADG (g/d)	274.29	337.14	24.87	0.098
ADFI (g/d)	397.19	434.67	30.71	0.408
G:F (g/g)	0.69	0.78	0.06	0.306
Day 1 to 14				
Initial BW (kg)	8.04	8.05	0.44	0.985
Final BW (kg)	10.86	11.76	0.53	0.255
ADG (g/d)	201.43	265.00	15.25	0.014
ADFI (g/d)	311.36	340.29	19.96	0.330
G:F (g/g)	0.65	0.78	0.05	0.043
Day 15 to 28				
Initial BW (kg)	10.86	11.76	0.53	0.255
Final BW (kg)	16.82	18.22	0.93	0.311
ADG (g/d)	425.71	461.43	32.08	0.455
ADFI (g/d)	801.29	857.00	47.65	0.428
G:F (g/g)	0.53	0.53	0.02	0.644
Day 1 to 28				
Initial BW (kg)	8.04	8.05	0.44	0.985
Final BW (kg)	16.82	18.22	0.93	0.311
ADG (g/d)	313.57	363.21	19.33	0.100
ADFI (g/d)	556.32	598.64	31.80	0.369
G:F (g/g)	0.56	0.61	0.02	0.105
Frequency of diarrhea (%)	20.24	14.29	-	0.391

[1)] Each value is the mean value of 6 replicates (5 pigs/pen).

[2)] CON, high non-starch polysaccharides diet; XYL, CON + 0.03% dietary xylanase; BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain to feed ratio; Frequency of diarrhea for the first two weeks after weaning = (number of diarrhea with score of 4 or higher / number of pen days) × 100.

Table 3. Effects of dietary xylanase on blood profiles of weaned pigs[1)]

Item[2)]	CON	XYL	SEM	*p*-value
White blood cell (× 10^3/μL)				
Day 1	13.95	14.65	2.67	0.859
Day 7	19.70	18.50	1.53	0.592
Day 14	24.95	22.95	2.55	0.591
Day 28	25.45	21.42	1.54	0.093
Platelet (× 10^3/ μL)				
Day 1	426.25	480.00	48.54	0.463
Day 7	430.00	514.50	122.13	0.652
Day 14	719.00	637.33	108.32	0.591
Day 28	485.00	515.17	515.17	0.612
Hematocrit (%)				
Day 1	32.15	31.18	1.81	0.716
Day 7	34.98	28.65	3.06	0.175
Day 14	29.33	28.60	2.08	0.808
Day 28	30.68	30.32	1.05	0.810
Red blood cell (× 10^6/μL)				
Day 1	5.65	5.53	0.30	0.783
Day 7	5.53	7.34	0.99	0.226
Day 14	4.68	5.63	0.46	0.178
Day 28	6.11	5.95	0.23	0.627
MCV (fL)				
Day 1	56.75	56.50	0.91	0.852
Day 7	49.67	50.50	2.64	0.828
Day 14	48.50	50.83	2.33	0.494
Day 28	50.33	51.00	1.29	0.722
Hemoglobin (g/dL)				
Day 1	10.95	10.70	0.59	0.775
Day 7	9.65	12.78	1.98	0.289
Day 14	7.88	9.85	0.88	0.143
Day 28	10.50	10.35	0.35	0.770
MCH (pg)				
Day 1	19.33	19.45	0.51	0.868
Day 7	16.95	17.17	0.82	0.855
Day 14	16.85	17.47	0.80	0.597
Day 28	17.27	17.38	0.47	0.864
MCHC (g/dL)				
Day 1	34.03	34.50	0.52	0.544
Day 7	34.28	34.12	0.45	0.796
Day 14	34.88	34.32	0.50	0.443
Day 28	34.20	34.12	0.20	0.772

[1)] Each value is the mean value of 6 replicates (1 pig/pen).

[2)] CON, high non-starch polysaccharides diet; XYL, CON + 0.03% dietary xylanase; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

Digesta characteristics and nutrient digestibility

Pigs fed XYL tended to have a higher (p = 0.088) digesta pH in the duodenum than those fed the

Table 4. Effects of dietary xylanase on systemic immune responses of weaned pigs[1)]

Item[2)]	CON	XYL	SEM	*p*-value
Day 1				
TNF-α (pg/mL)	150.24	148.24	1.33	0.398
TGF-β1 (pg/mL)	2,124.29	1,888.61	100.22	0.172
IL-10 (pg/mL)	48.49	37.26	4.43	0.215
CRP (ng/mL)	18.44	18.88	0.82	0.740
IgG (mg/mL)	20.02	20.12	13.79	0.996
IgA (mg/mL)	0.15	0.17	0.06	0.831
Day 28				
TNF-α (pg/mL)	130.61	96.29	19.81	0.249
TGF-β1 (pg/mL)	2,014.28	1,840.31	167.33	0.479
IL-10 (pg/mL)	74.00	145.17	55.92	0.389
CRP (ng/mL)	114.28	108.16	5.75	0.469
IgG (mg/mL)	22.29	13.17	4.09	0.146
IgA (mg/mL)	0.20	0.26	0.06	0.493

[1)] Each value is the mean value of 6 replicates (1 pig/pen).

[2)] CON, high non-starch polysaccharides diet; XYL, CON + 0.03% dietary xylanase; TNF-α, tumor necrosis factor-α; TGF-β1, transforming growth factor-β1; IL-10, interleukin-10; CRP, C-reactive protein; IgG, immunoglobulin G; IgA, immunoglobulin A.

CON (Table 5). In contrast, the XYL group digesta pH decreased in the jejunum ($p = 0.069$) and cecum ($p < 0.05$). However, no differences were observed in the digesta viscosity of the stomach, duodenum, jejunum, ileum, and cecum between the dietary treatments. Dietary XYL increased the AID and ATTD of DM ($p < 0.05$) and ATTD of energy ($p = 0.083$) and CP ($p = 0.082$) on day 28 compared with the CON (Table 6).

Intestinal histomorphology, immune responses, and oxidative stress indicators

No differences were found in VH:CD and percentage of Ki-67 positive cells in the jejunum between the dietary treatments (Table 7). However, pigs fed XYL had lower ($p < 0.05$) concentrations of TNF-α and IgG in the jejunal mucosa than those fed CON. In addition, dietary

Table 5. Effects of dietary xylanase on pH and viscosity of gut digesta of weaned pigs[1)]

Item[2)]	CON	XYL	SEM	*p*-value
Digesta pH				
Stomach	3.42	3.66	0.32	0.600
Duodenum	6.20	6.43	0.10	0.088
Jejunum	6.25	6.07	0.07	0.069
Ileum	7.00	7.15	0.13	0.419
Caecum	6.07	5.91	0.05	0.048
Digesta viscosity				
Stomach	1.15	1.05	0.05	0.183
Duodenum	1.16	1.23	0.05	0.278
Jejunum	1.17	1.12	0.04	0.304
Ileum	1.50	1.55	0.08	0.663
Caecum	1.88	1.88	0.15	0.985

[1)] Each value is the mean value of 6 replicates (2 pigs/pen).

[2)] CON, high non-starch polysaccharides diet; XYL, CON + 0.03% dietary xylanase.

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Table 6. Effects of dietary xylanase on nutrient digestibility of weaned pigs[1)]

Item[2)]	CON	XYL	SEM	*p*-value
Apparent ileal digestibility (%)				
Dry matter	73.16	75.41	0.52	0.013
Energy	70.36	71.84	1.62	0.531
Crude protein	67.56	69.54	2.07	0.514
Crude fiber	40.79	41.59	2.95	0.852
Non-starch polysaccharides	38.38	43.09	2.17	0.156
Apparent total tract digestibility (%)				
Dry matter	81.95	84.43	0.54	0.008
Energy	82.96	86.33	1.24	0.083
Crude protein	79.79	81.32	0.56	0.082
Crude fiber	51.32	52.52	5.30	0.876
Non-starch polysaccharides	51.69	52.05	3.12	0.936

1)Each value is the mean value of 6 replicates (1 pig/pen).

2)CON, high non-starch polysaccharides diet, XYL, CON + 0.03% dietary xylanase.

Table 7. Effects of dietary xylanase on intestinal health parameters of weaned pigs[1)]

Item[2)]	CON	XYL	SEM	*p*-value
Histomorphology				
VH:CD (μm/μm)	3.45	3.47	0.22	0.952
Ki-67 positive (%)	35.28	34.32	1.21	0.575
Mucosal immune responses				
TNF-α (pg/mg)	0.56	0.14	0.05	< 0.001
IL-6 (pg/mg)	0.49	0.62	0.11	0.404
IL-8 (pg/mg)	0.58	0.57	0.09	0.927
IgG (μg/mg)	7.02	4.41	0.97	0.066
IgA (μg/mg)	1.51	2.54	0.45	0.110
Mucosal oxidative stress				
MDA (μmol/mg)	0.34	0.21	0.05	0.070
PC (nmol/mg)	0.57	0.50	0.05	0.341
Endotoxin (EU/mL)	94.90	60.28	17.50	0.167

1)Each value is the mean value of 6 replicates (2 pigs/pen).

2)CON, high non-starch polysaccharides diet; XYL, CON + 0.03% dietary xylanase; VH:CD, villus height to crypt depth ratio; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; IL-8, interleukin-8; IgG, immunoglobulin G; IgA, immunoglobulin A; MDA, malondialdehyde; PC, protein carbonyl.

XYL tended to decrease (p = 0.070) MDA level in the jejunal mucosa compared with CON.

DISCUSSION

The present study demonstrated that supplemental dietary XYL in weaned pigs fed a high NSPs diet improved growth performance and nutrient digestibility, modulated digesta pH in the gut, and reduced local immune responses and oxidative stress indicator. These positive effects of dietary XYL may be attributed to the increased nutrient utilization efficiency and the regulation of the gut environment through the enzymatic breakdown of NSPs.

The high NSPs diets used in this study included corn DDGS and wheat, which have been reported to contain approximately 3.1% and 2.4% soluble NSPs and 25.2% and 9.0% insoluble NSPs, respectively [33–35]. The negative effects of DDGS inclusion in swine diets on growth performance and nutrient digestibility reported in previous studies could be explained by the increased NSPs levels in the feed, which reduce nutrients availability and gut functions [17,35,36]. Additionally, wheat is a relatively viscous grain compared to corn, and wheat-based diets have a higher total NSP content than corn-based diets [13,37]. Because NSPs are not degraded by endogenous digestive enzymes in pigs, they may reduce utilization [38]. Dietary XYL, an exogenous enzyme, has been used to break down the structural bonds of NSP, thereby modulating digesta characteristics and enhancing nutrient utilization in the feed [7]. However, the effects of dietary XYL on the growth performance of pigs have been inconsistent [15,20]. In the present study, the addition of dietary XYL improved ADG and G:F in pigs for the first 2 weeks after weaning. The differences in the effects of XYL on growth performance may be attributed to the differences in NSPs content in the diet, and the effects of XYL were relatively pronounced when supplemented with high NSP diet [18,20]. The high NSP diet used in this study is considered sufficient as a substrate for enzymatic XYL activity, thereby improving nutrient utilization and resulting in beneficial performance in weaned pigs. Thus, our results showed that although supplemental XYL did not influence the frequency of diarrhea for the first 2 weeks after weaning, the growth performance indicated a positive effect of dietary XYL during the critical period after weaning.

The viscosity of digesta in the small intestine is related to the structure and molecular weight of the polysaccharides, which can have a greater influence on viscosity than the type of linkage or sugar composition of the polysaccharide [13]. The high digesta viscosity in the digestive tract can disturb nutrient digestibility and absorption and can also increase the level of oxidative stress and inflammatory responses, which results in damage to the intestinal histomorphology [12]. Previous studies have demonstrated the functional effects of supplemental XYL on the digestive tract of weaned pigs fed a high NSPs diet [10,12]. However, the effect of dietary XYL on digesta viscosity can vary depending on the presence of primary cereal grains. Several studies have evaluated the effects of supplemental XYL in corn-soybean meal diets with or without corn DDGS on intestinal digesta viscosity [10,20,39] and have also assessed the absence of interactions between dietary XYL and corn DDGS [18]. In this study, we observed that the addition of dietary XYL to corn-soybean meal diets containing corn DDGS and wheat with high NSP contents did not affect the viscosity of gastrointestinal digesta in weaned pigs. Cereal co-products generally do not elevate digesta viscosity to a similar extent as conventional cereal grains because of their higher levels of insoluble NSPs [13,33]. Nevertheless, corn DDGS and wheat in the basal diets of this study not only had higher levels of soluble NSPs than corn and soybean meal but also contained more insoluble NSPs than soluble NSPs [34,40]. The lack of a substantial effect on digesta viscosity despite XYL supplementation may be attributed to the high content of insoluble NSPs in the experimental diets, which may not have been readily degraded by exogenous XYL. Previous studies have shown that insoluble NSPs are less susceptible to degradation by exogenous XYL than soluble NSPs [41,42]. In addition, unlike soluble NSPs, which mainly increase digesta viscosity, insoluble NSPs primarily act as physical barriers to digestive enzymes in the gastrointestinal tract [43], thereby impeding nutrient utilization. Therefore, XYL supplementation may have improved nutrient utilization through partial degradation of NSP structures, despite limited effects on digesta viscosity.

The results that dietary XYL modulated intestinal digesta pH in weaned pigs. Soluble NSPs are primarily degraded in the proximal intestine [44], and the increased duodenal digesta pH observed following XYL supplementation may reflect altered digestion dynamic associated with NSP hydrolysis. This process may increase the accessibility of nutrients to endogenous digestive enzymes. In contrast, the lower digesta pH in the jejunum and cecum of XYL supplemented pigs

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may indicate the fermentation of NSPs and/or their degradation products. In general, the water-holding capacity of fibers affects fermentability, soluble NSPs are more fermentable than insoluble NSPs [39] supplemental XYL indirectly provides fermentable xylooligosaccharides (XOS) via hydrolysis of the xylan backbone [45]. Fermentation of NSPs end-products, such as XOS, by commensal gut microbiota leads to the production of short-chain fatty acids (SCFAs), which lower the pH of the gut [46,47]. In addition, SCFAs produced by gut microbiota play crucial roles in gut health by modulating barrier function and immune responses [47]. Therefore, it is suggested that the pH of the gut digesta modified by XYL supplementation may contribute not only to improved accessibility to nutrients but also to changes in the gut environment of weaned pigs.

Our findings on nutrient digestibility further support the beneficial effects of XYL supplementation. Although supplemental XYL did not improve NSP digestibility, it increased the AID of DM and ATTD of DM, energy, and CP. These improvements may be explained by partial hydrolysis of NSP structures, which can reduce the physical barrier effect of NSPs and increase accessibility of nutrients to endogenous digestive enzymes [48]. As a result, nutrients may become more available for digestion and absorption in the gastrointestinal tract. However, the improvement in nutrient digestibility did not translate into improved overall growth performance, suggesting that additional factors such as nutrient absorption efficiency may need to be considered. Furthermore, further studies are needed to evaluate optimal dietary XYL dosages and feeding durations across different growth stages to maximize long-term growth performance. Nevertheless, XYL supplementation in high-NSP diets may improve nutrient availability by mitigating the NSP-induced physical barrier to digestion rather than directly enhancing NSP digestibility. Additionally, the marked improvement in ATTD, relative to AID, suggests that the large intestinal microbiota may contribute to nutrient digestibility through fermentation, as supported by the digesta pH in the hindgut. Further studies are needed to investigate the effect of dietary XYL on different diet compositions and its effects on the proximal to distal gut may provide its potential to enhance growth by improving nutrient utilization in weaned pigs.

The inclusion of DDGS in the feed can cause oxidative stress in pigs, which may modulate their immune responses [49,50]. In addition, highly viscous soluble NSP may promote the proliferation of intestinal *Escherichia coli*, thereby increasing oxidative stress and inflammatory responses [51–53], and a positive correlation with post-weaning colibacillosis has been reported [54,55]. MDA and PC are considered end products of lipid and protein peroxidation and are commonly used as indicators of oxidative stress [10], which can demage the intestinal environment. Excessive levels of reactive oxygen species can damage cellular components such as the cell membrane, DNA, and proteins, leading to chronic inflammation [56]. Oxidative stress can induce the production of pro-inflammatory cytokines by intestinal immune and epithelial cells, thereby modulating intestinal immune responses [10,57]. In the present study, mucosal TNF-α, IgG, and MDA were reduced following dietary XYL addition, indicating that XYL appears to have anti-inflammatory and anti-oxidant effects in weaned pigs. In addition, the decrease in jejunal mucosal IgG levels following XYL supplementation suggests that the local immune response was modulated. As high levels of NSP in feed can act as anti-nutritional factors [58], effective degradation and fermentation of NSPs by dietary XYL may reduce the amount of antigenic components reaching the intestinal immune system. Furthermore, the reduced WBC count in XYL-supplemented pigs may suggest a potential systemic immunomodulatory effect, because the number of WBC can be used as an indicator of systemic inflammatory responses. However, as no effects were found on the levels of systemic inflammatory cytokines, the impact of dietary XYL on systemic immune responses requires further investigation.

CONCLUSION

Our findings suggest that supplementation of high NSP diet with dietary XYL including corn DDGS and wheat, can improve the early growth performance and nutrient digestibility of weaned pigs. Dietary xylanase may play an important role in enhancing gut health by degrading and fermenting complex NSPs, thereby regulating nutrient utilization and the biochemical environment in the gut. In addition, the reduction in inflammatory markers and oxidative stress indicators suggests that dietary xylanase helps maintain intestinal integrity and functions, demonstrating its potential as a feed additive to improve the growth and health of weaned pigs. Further studies are needed to evaluate the effects of dietary xylanase on the growth performance, intestinal health, and local and systemic immune responses in weaned pigs fed in various cereal grain-based diets.

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