Investigation of xylanase, diet formulation method for energy, and choice of digestibility index marker on nutrient and energy utilization for broiler chickens and pigs

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ABSTRACT: The aim of this study was to investigate the growth performance and nutrient utilization responses of broiler chickens and the nutrient utilization of pigs to xylanase, experimental diet formulation method for energy (FME), and digestibility index marker (DIM). In Exp. 1, a total of 448 male broiler chickens were used in a randomized complete block design with body weight (BW) as a blocking factor. Seven dietary treatments were prepared in a $3 \times 2 + 1$ factorial arrangement with inclusion of sand, diatomaceous earth (DAE), or wheat bran (WB) as FME and without or with xylanase (26,400 units/kg of diet) plus positive control. Each of chromic oxide and titanium dioxide was incorporated at 5 g/kg in diets. There were eight replicate cages of eight birds per cage. Birds were weighed on d 7, 14, and 21, and feed intake was recorded. Excreta samples were collected from d 19 to 21. On d 21, birds were euthanized, and ileal digesta samples were collected. In Exp. 2, twenty-one barrows (initial BW = 33.0 ± 0.3 kg), fitted with simple T-cannulas at the distal ileum, were used in a triplicate 7×2 incomplete Latin Square design with seven dietary treatments, which were prepared by the same arrangement as in broiler chickens, and

two periods giving six replicates per diet. Fecal samples were collected on d 4 and 5, and ileal digesta samples were collected on d 6 and 7 of each period. In Exp. 1, the growth performance of birds was not affected by xylanase. There were interactions ($P < 0.05$) between xylanase and FME for apparent ileal digestibility (AID) of crude protein, His, Met, Thr, and Trp. Inclusion of xylanase decreased ($P < 0.05$) the AID of crude protein and Trp in sand diet and AID of His and Thr in DAE diet, but increased ($P < 0.05$) AID of Met in WB diet. The AID of energy and nutrients except Arg and Met were greater ($P < 0.05$) observed with Cr than Ti as DIM. In Exp. 2, there were interactions ($P < 0.05$) between xylanase and FME for AID of dry matter, energy, Arg, and Lys. Inclusion of xylanase decreased ($P < 0.05$) the AID of Lys in DAE or WB diet, but increased ($P < 0.05$) AID of Arg in sand diet and AID of energy in WB diet. The DIM type had no effect on responses in pigs. In conclusion, the efficacy of xylanase on ileal energy and amino acids digestibility depends on the choice of FME in broiler chickens and pigs, and DIM affects the determination of ileal digestibility in broiler chickens.

Key words: chromic oxide, digestibility, poultry, swine, titanium dioxide, xylanase

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INTRODUCTION

Xylanase is one of the exogenous enzymes that have been widely used for commercial feeds in poultry and swine industry (Adeola and Cowieson, 2011). The evaluation of xylanase has been conducted in

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wheat-based diets (Yin et al., 2000b; Cowieson and Masey O'Neill, 2013) and corn–soybean meal-based (CSBM) diets (Stefanello et al., 2016; Cho et al., 2017) in both chickens and pigs. Negative control diets formulated to contain less energy than positive control (PC) diets are commonly used to evaluate the efficacy of xylanase supplement (Liu and Fowler, 2017). To replace part of the energy-contributing components, wheat bran (WB), sand, or diatomaceous earth (DAE) has been used in previous studies (Karimi et al., 2013; Liu and Fowler, 2017). However, whether the experimental diet formulation method for energy (FME) reduction of the test diet achieved by including WB, sand, or DAE affects the evaluation of xylanase on growth performance or efficiency of nutrient utilization in chickens and pigs is still unknown.

The interaction between dietary fiber and digestibility index marker (DIM) type has been reported in previous studies (Yin et al., 2000b; Wang et al., 2017). The main function of xylanase is to hydrolyze dietary fiber (Adeola and Cowieson, 2011), which might interfere with the interaction between fiber and DIM, and it is reasonable to speculate that the effect of FME and xylanase may depend on the choice of DIM. Therefore, the objective of the present study was to evaluate the effect of inclusion of xylanase, FME, and DIM on growth performance in broiler chickens and nutrient and energy utilization in both broiler chickens and pigs.

MATERIALS AND METHODS

All animal procedures used in this study were approved by the Purdue University Animal Care and Use Committee.

Dietary Treatments

Seven diets were prepared in a $3 \times 2 + 1$ factorial arrangement with 50 g/kg sand, 50 g/kg DAE, or 75 g/kg WB as FME and without or with xylanase plus PC for chickens and pigs, respectively (Table 1). The xylanase activity was 26,400 units/kg of complete diet. All diets were formulated to meet or exceed the vitamin and mineral requirement estimates for both broiler chickens (NRC, 1994) and pigs (NRC, 2012). Both chromic oxide and titanium dioxide were added into experimental diets as the DIM at 5 g/kg of diet, respectively.

Exp. 1: Growth Performance and Nutrient and Energy Utilization in Broiler Chickens

A total of 448 male broiler chicks (Cobb 500; Cobb-Vantress Inc., Siloam Springs, AR)

were obtained from a commercial hatchery. On d 0, tagged birds were individually weighed and assigned to seven experimental diets in a randomized complete block design with body weight (BW) as a blocking factor. There were eight replicate cages for each treatment and eight birds were housed in each cage. Birds had free access to feed and water during the experimental period. Birds were raised in electrically heated battery brooders (model SB 4 T; Alternative Design Manufacturing, Siloam Springs, AR), which maintained the temperature at 35, 31, and 27°C from d 0 to 7, d 7 to 14, and d 14 to 21, respectively. On d 7, 14, and 21, all birds were weighed individually, and feed consumption was recorded for each cage. On d 19, 20, and 21, uncontaminated excreta samples were collected daily. On d 21, birds were euthanized by CO₂ asphyxiation and dissected for the collection of ileal digesta samples from the distal two-thirds of ileum. Both excreta samples and ileal digesta samples were pooled within cages and stored at –20°C until further analyses.

Exp. 2: Apparent Nutrient and Energy Utilization in Pigs

Twenty-one barrows (initial BW = 33.0 ± 0.3 kg) surgically fitted with simple T-cannulas at the distal ileum according to the description of Dilger et al. (2004) were used. Pigs were allotted to three blocks based on BW and assigned to a triplicate 7×2 incomplete Latin square design with seven experimental diets and two periods, which provided six observations for each treatment. All pigs were individually housed in stainless steel crates (1.22×1.22 m) equipped with a feeder and a nipple drinker. Daily feed allowance was calculated as 4% of the initial smallest BW in each block and period, and pigs were fed at 0800 and 1700 h in two equal meals, and water was available all the time. Each experimental period consisted of a 3-d adaptation, a 2-d feces collection, and a 2-d ileal digesta collection. On d 4 and 5, representative fecal samples were collected after the meal in the morning. On d 6 and 7, Whirl-Pak bag (Nasco, Fort Atkinson, WI) containing 10 mL of 10% formic acid was attached to the T-cannula to collect ileal digesta from 0800 to 1700 h. Attached bags were inspected every 30 min, and the filled bags were changed and stored at –20°C immediately. After each experimental period, both ileal digesta samples and fecal samples were pooled within periods and pigs, and subsamples were stored at –20°C until further processing.

Table 1. Ingredient and analyzed nutrient composition of experimental diets for broiler chickens and pigs (as-fed basis)¹

Diet	Broiler chickens								Pigs							
	PC	Sand		DAE		Wheat bran		PC	Sand		DAE		Wheat bran		PC	Wheat bran
		–	+	–	+	–	+		–	+	–	+	–	+		
Xylanase																
Ingredients, g/kg																
Corn	569.2	554.1	524.1	554.1	524.1	538.4	508.4	560.2	545.8	515.8	545.8	515.8	539.6	509.6		
Soybean meal	336.0	327.1	327.1	327.1	327.1	317.8	317.8	336.0	327.4	327.4	327.4	327.4	323.6	323.6		
Soybean oil	36.0	10.0	10.0	10.0	10.0	10.0	10.0	45.0	18.0	18.0	18.0	18.0	3.0	3.0		
Sand	—	50.0	50.0	—	—	—	—	—	50.0	50.0	—	—	—	—		
DAE	—	—	—	50.0	50.0	—	—	—	—	—	50.0	50.0	—	—		
Wheat bran	—	—	—	—	—	75.0	75.0	—	—	—	—	—	75.0	75.0		
Salt	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0		
DL-Methionine	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8		
L-Lysine•HCl	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.9		
L-Threonine	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1		
Limestone	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0		
Monocalcium phosphate	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0	17.0		
Mineral-vitamin premix ²	3.0	3.0	3.0	3.0	3.0	3.0	3.0	—	—	—	—	—	—	—		
Vitamin premix ³	—	—	—	—	—	—	—	1.5	1.5	1.5	1.5	1.5	1.5	1.5		
Mineral premix ⁴	—	—	—	—	—	—	—	1.0	1.0	1.0	1.0	1.0	1.0	1.0		
Selenium premix ⁵	—	—	—	—	—	—	—	0.5	0.5	0.5	0.5	0.5	0.5	0.5		
Chromic oxide ⁶	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0		
Titanium dioxide ⁶	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0		
Xylanase premix ⁷	—	—	30.0	—	30.0	—	30.0	—	—	30.0	—	30.0	—	30.0		
Analyzed nutrient and energy, g/kg																
Dry matter	890.7	894.8	892.5	891.4	891.0	890.2	889.2	891.1	892.7	890.1	890.2	892.0	885.3	887.4		
Crude protein ⁸	214.9	210.1	210.3	199.2	205.6	208.0	203.4	216.3	217.0	212.9	210.6	220.3	227.1	220.5		
Gross energy, MJ/kg	16.3	14.8	15.0	14.8	15.0	15.8	15.9	16.7	15.1	15.1	14.9	14.8	15.6	15.6		
Acid detergent fiber	22.6	77.2	68.2	42.5	45.1	33.4	34.9	24.9	76.9	77.8	43.6	44.7	38.8	35.4		
Neutral detergent fiber	72.8	123.9	116.4	95.5	97.4	114.2	106.4	85.2	122.7	120.5	85.1	89.2	129.6	133.8		

¹PC = positive control; DAE = diatomaceous earth.

²Provided the following quantities per kilogram of complete diet: vitamin A, 8,575 IU; vitamin D₃, 4,300 IU; vitamin E, 28.6 IU; menadione, 7.30 mg; riboflavin, 9.15 mg; pantothenic acid, 18.3 mg; niacin, 73.5 mg; choline chloride, 1,285 mg; vitamin B₁₂, 0.02 mg; biotin, 0.09 mg; thiamine mononitrate, 3.67 mg; folic acid, 1.65 mg; pyridoxine hydrochloride, 5.50 mg; I, 1.85 mg; Mn, 180 mg; Cu, 7.40 mg; Fe, 73.5 mg; Zn, 180 mg; Se, 0.43 mg.

³Provided the following quantities per kilogram of complete diet: vitamin A, 3,960 IU; vitamin D₃, 396 IU; vitamin E, 26.4 IU; menadione, 1.30 mg; riboflavin, 5.30 mg; pantothenic acid, 13.2 mg; niacin, 19.8 mg; vitamin B₁₂, 0.02 mg.

⁴Provided the following quantities per kilogram of complete diet: I, 0.33 mg; Mn, 15.4 mg; Cu, 8.13 mg; Fe, 175 mg; Zn, 134 mg.

⁵Provided 0.3 mg Se/kg of complete diet.

⁶Provided 5 g chromic oxide/kg and 5 g titanium dioxide/kg of complete diet.

⁷A 0.165 g xylanase (160,000 units/g) was added to 29.835 g ground corn to form xylanase premix.

⁸Crude protein = nitrogen × 6.25.

Chemical Analysis and Calculation

Ileal digesta samples from Exp. 1 and 2 were lyophilized, and excreta samples from Exp. 1 and fecal samples from Exp. 2 were forced-air dried at 55°C to constant weight. Diet, excreta, and fecal samples were ground through a 0.5-mm screen in a centrifugal grinder (Retsch ZM 200; Retsch GmbH, Haan, Germany) and ileal digesta samples were ground in a coffee grinder. The concentration of dry matter (DM) in diets, freeze-dried ileal

digesta, and oven-dried excreta and feces samples were determined by drying in a forced-air oven at 105°C for 24 h (Precision Scientific Co., Chicago, IL; method 934.01; AOAC, 2006). Nitrogen (N) content in diets and ileal digesta were determined with the combustion method (TruMac N; LECO Corp., St. Joseph, MI; method 990.03; AOAC, 2000), and the crude protein (CP) content was calculated as N × 6.25. Gross energy (GE) content was analyzed by isoperibol bomb calorimetry using a Parr 6200 calorimeter (PARR Instrument

Co., Moline, IL). The acid detergent fiber [method 973.18 (AD); AOAC, 2006] and neutral detergent fiber (Van Soest et al., 1991) contents in diets were analyzed with a fiber analyzer (ANKOM A2000 Fiber Analyzer; ANKOM Technology, Macedon, NY). The contents of amino acids (AA) in diets and ileal digesta samples were determined by high performance liquid chromatography [method 982.30 E (a, b, c); AOAC, 2006], which was performed by the University of Missouri Experiment Station Chemical Laboratories (Columbia, MO). The concentrations of Cr and Ti were determined as described by Saha and Gilbreath (1991) and Myers et al. (2004), respectively, with few modifications as indicated by Wang and Adeola (2018).

The apparent ileal digestibility (AID), apparent total tract digestibility (ATTD) for pigs, and apparent total tract utilization (ATTU) for chickens of GE were calculated using the following equations (Adeola, 2001):

$$\text{AID, \%} = [1 - (\text{DIM}_{\text{diet}} / \text{DIM}_{\text{ileal}}) \times (\text{GE}_{\text{ileal}} / \text{GE}_{\text{diet}})] \times 100;$$

$$\text{ATTD, \%} = [1 - (\text{DIM}_{\text{diet}} / \text{DIM}_{\text{feces}}) \times (\text{GE}_{\text{feces}} / \text{GE}_{\text{diet}})] \times 100;$$

$$\text{ATTU, \%} = [1 - (\text{DIM}_{\text{diet}} / \text{DIM}_{\text{excreta}}) \times (\text{GE}_{\text{excreta}} / \text{GE}_{\text{diet}})] \times 100;$$

where DIM_{diet} , $\text{DIM}_{\text{ileal}}$, $\text{DIM}_{\text{feces}}$, and $\text{DIM}_{\text{excreta}}$ are the DIM concentration in diet, ileal digesta, feces, and excreta, respectively (mg/kg DM); GE_{diet} , GE_{ileal} , GE_{feces} , and $\text{GE}_{\text{excreta}}$ are the GE concentrations (MJ/kg DM) of diet, ileal digesta, feces, and excreta, respectively. The AID of DM, CP, or AA was calculated with GE replaced by DM, CP, or AA (mg/kg DM), and the ATTD or ATTU of DM was calculated with GE replaced by DM (mg/kg).

Statistical Analysis

The data on growth performance in broiler chickens were analyzed using GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC). In the model, fixed effects were control and factors nested in control, which included xylanase, FME, and the interaction between xylanase and FME; and random effect was block.

The data on energy and nutrients utilization in both broiler chickens and pigs were analyzed using GLIMMIX procedure of SAS as a split-plot arrangement, with dietary treatment as

whole-plot factor and the DIM type as split-plot factor. For broiler chickens, fixed effects were control and factors nested in control, which included xylanase, FME, interaction between them, and DIM; and random effects were block and factors nested in control, which included two-way interaction of block and DIM, three-way interaction of block, DIM, and xylanase, three-way interaction of block, DIM, and FME, and four-way interaction of block, DIM, xylanase, and FME. In the model used in pigs, the fixed effects were same with those in broiler chicken study, and the random effects were period, replication, pig, and factors nested in control, which included two-way interaction of period and DIM, three-way interaction of replication, FME, and DIM, and three-way interaction of pig, FME, and DIM.

Least squares means were calculated and separated by the SLICEDIFF option with Bonferroni's correction when there was interaction between FME and xylanase, or separated by the PDIFF option with Bonferroni's correction when there was no interaction between them. The experimental unit was cage in Exp. 1 and pig in Exp. 2 and statistically significant difference was set at $P \leq 0.05$.

RESULTS

In Exp. 1, the PC treatment had greater ($P < 0.001$) BW gain from d 0 to 7, and greater gain-to-feed ratio (G:F) ($P < 0.001$) during the whole experimental period compared with other treatments as shown in Table 2. There was no interaction between xylanase and FME and the inclusion of xylanase did not affect the growth performance of broiler chicken during the whole experimental period. During d 0 to 7, however, the chickens fed the diet containing DAE had lower ($P < 0.05$) BW gain and G:F compared with the chickens fed the diet containing WB. During d 14 to 21 and d 0 to 21, the chickens fed the diet containing DAE had lower ($P < 0.05$) BW gain compared with the chickens fed the diet containing sand and lower ($P < 0.05$) feed intake compared with the chickens fed the diet containing WB.

Compared with other treatments, the PC treatment had greater ($P < 0.05$) AID of DM and GE, but similar AID of CP and ATTU of DM and GE (Table 3). There was an interaction ($P < 0.05$) between xylanase and FME for AID of CP and the inclusion of xylanase in the diet containing sand decreased ($P < 0.001$) the AID of CP but had no effect when alternate FME were used. There was no effect of inclusion of xylanase on AID and ATTU of DM

Table 2. The effect of xylanase (0 or 26,400 units/kg of diet) and diet formulation method for energy (FME) on growth performance of broiler chickens¹

FME	Xylanase	n	d 0 to 7			d 7 to 14			d 14 to 21			d 0 to 21		
			BW gain, g/bird	Feed intake, g/bird	G:F, g/kg	BW gain, g/bird	Feed intake, g/bird	G:F, g/kg	BW gain, g/bird	Feed intake, g/bird	G:F, g/kg	BW gain, g/bird	Feed intake, g/bird	G:F, g/kg
PC		8	114.7	133.9	856.1	239.9	305.8	785.0	386.1	501.7	770.5	740.7	941.4	787.5
Sand	-	8	111.0	140.8	788.6	234.2	319.4	721.0	390.3	547.9	712.6	735.5	1008.0	729.3
Sand	+	8	103.3	133.3	776.7	226.9	311.3	727.4	398.1	550.4	723.3	728.4	994.9	731.9
DAE	-	8	99.9	132.0	756.5	214.0	295.8	720.6	380.9	530.0	721.0	694.8	957.8	725.8
DAE	+	8	98.3	130.8	751.8	217.4	294.7	735.9	338.7	487.5	690.1	654.4	913.0	715.9
Wheat bran	-	8	105.1	134.1	783.0	234.1	316.6	738.5	383.2	548.1	699.1	722.3	998.7	723.5
Wheat bran	+	8	108.5	135.1	803.2	241.4	322.8	747.2	400.0	570.8	700.3	749.8	1028.8	729.0
	-	24	105.3	135.6	776.0	227.4	310.6	726.7	384.8	542	710.9	717.5	988.2	726.2
	+	24	103.4	133.1	777.2	228.6	309.6	736.8	378.9	536.2	704.6	710.9	978.9	725.6
Sand		16	107.2 ^A	137.0	782.7 ^{AB}	230.6	315.3	724.2	394.2 ^A	549.1 ^{AB}	718.0	731.9 ^A	1001.5 ^{AB}	730.6
DAE		16	99.1 ^B	131.4	754.2 ^B	215.7	295.2	728.3	359.8 ^B	508.7 ^B	705.5	674.6 ^B	935.4 ^B	720.9
Wheat bran		16	106.8 ^A	134.6	793.1 ^A	237.7	319.7	742.8	391.6 ^{AB}	559.4 ^A	699.7	736.1 ^A	1013.7 ^A	726.2
SEM														
Xylanase			1.76	1.79	7.63	7.19	7.77	7.87	8.95	12.09	5.90	13.19	17.42	3.41
FME			2.01	2.04	9.32	8.81	9.52	9.63	10.84	14.57	7.23	16.15	21.34	4.17
P-value														
PC vs. others			< 0.001	0.865	< 0.001	0.380	0.769	< 0.001	0.795	0.090	< 0.001	0.289	0.203	< 0.001
Xylanase			0.335	0.195	0.909	0.914	0.929	0.369	0.634	0.727	0.453	0.722	0.709	0.900
FME			0.002	0.071	0.014	0.209	0.166	0.364	0.047	0.036	0.202	0.017	0.028	0.264
Xylanase × FME			0.081	0.188	0.445	0.834	0.868	0.945	0.117	0.257	0.116	0.341	0.467	0.388

^{A,B}Main effect means for FME within a column without a common superscript letter differ ($P < 0.05$).¹n = number of observations; PC = positive control; DAE = diatomaceous earth; BW = body weight; G:F = gain-to-feed ratio.

Table 3. The effect of xylanase (0 or 26,400 units/kg of diet), diet formulation method for energy (FME), and digestibility index marker (DIM) on apparent ileal digestibility (AID) and apparent total tract utilization (ATTU) of nutrients and gross energy (GE) in broiler chickens¹

FME	Xylanase	DIM	n	AID			ATTU	
				DM	CP	GE	DM	GE
Positive control			16	73.4	86.8	76.2	70.3	77.5
Sand	–		16	69.7	88.5 ^x	76.6	67.1	76.4
Sand	+		16	69.3	87.2 ^y	76.6	70.8	75.6
Diatomaceous earth	–		16	69.5	86.2	75.4	69.3	75.9
Diatomaceous earth	+		16	68.9	86.8	75.1	69.7	74.0
Wheat bran	–		16	69.8	85.3	72.0	71.4	77.7
Wheat bran	+		16	70.5	85.2	72.9	71.4	78.0
	–		48	69.7	86.7	74.6	69.3	76.7
	+		48	69.6	86.4	74.9	70.7	75.9
Sand			32	69.5 ^{AB}	87.9	76.6 ^A	68.9 ^B	76.0 ^B
Diatomaceous earth			32	69.2 ^B	86.5	75.2 ^B	69.5 ^{AB}	74.9 ^B
Wheat bran			32	70.2 ^A	85.3	72.4 ^C	71.4 ^A	77.9 ^A
		Cr	48	70.6	87.0	75.5	70.4	76.6
		Ti	48	68.7	86.1	74.0	69.5	75.9
SEM								
Xylanase				0.26	—	0.26	0.58	0.39
FME				0.30	—	0.31	0.71	0.47
Xylanase × FME				—	0.49	—	—	—
DIM				0.26	0.41	0.26	0.58	0.39
P-value ²								
Positive control vs. others				< 0.001	0.673	0.012	0.818	0.226
Xylanase				0.787	0.362	0.487	0.097	0.150
FME				0.048	< 0.001	< 0.001	0.047	< 0.001
Xylanase × FME				0.175	0.031	0.297	0.129	0.308
DIM				< 0.001	0.032	< 0.001	0.262	0.207

^{A,B,C}Main effect means for FME within a column without a common superscript letter differ ($P < 0.05$).

^{x,y}Simple effect for xylanase within FME within a column without a common superscript letter differ ($P < 0.05$).

¹n = number of observations; DM = dry matter; CP = crude protein.

²P-values for interactions among FME, xylanase, and DIM (except xylanase × FME) were all greater than 0.10 and are not presented.

and GE. The chickens fed the diet containing WB had greater ($P < 0.05$) AID of DM compared with the chickens fed the diet containing DAE and greater ($P < 0.05$) ATTU of DM compared with the chickens fed the diet containing sand. As for the digestibility of GE, the chickens fed the diet containing WB had the lowest ($P < 0.05$) AID of GE, but greatest ($P < 0.05$) ATTU of GE compared with the chickens fed the diet containing DAE or sand. The AID of DM, CP, and GE observed with Cr as DIM was greater ($P < 0.05$) compared with Ti, but similar ATTU of DM and GE were observed with either Cr or Ti as DIM.

Except for the AID of Trp ($P < 0.05$), there were no differences between the PC and other treatments for AID of AA in broiler chickens (Table 4). There were interactions ($P < 0.05$) between xylanase and FME for AID of His, Met, Thr, and Trp. Inclusion of xylanase decreased ($P < 0.05$) AID of Trp in the diet containing sand and decreased ($P < 0.05$) AID of His and Thr in the diet containing DAE, but

increased ($P < 0.05$) AID of Met in the diet containing WB. The AID of Arg, Ile, Leu, Lys, Phe, and Val were not affected by the inclusion of xylanase in diet, but were affected by FME, where the diet containing WB had lower ($P < 0.05$) AID values compared with the diet containing either sand or DAE. The AID of all AA except Arg and Met was greater ($P < 0.05$) observed with Cr compared with Ti as DIM.

In Exp. 2, the PC treatment had greater ($P < 0.05$) ATTD of DM compared with other treatments as shown in Table 5. There were interactions ($P < 0.05$) between xylanase and FME for AID of DM and GE. Inclusion of xylanase decreased ($P < 0.05$) AID of DM in the diet containing sand, however, increased ($P < 0.05$) AID of GE in the diet containing WB. The ATTD of DM in the diet containing WB was greater ($P < 0.05$) than that in the diet containing sand or DAE, oppositely, the ATTD of GE in the diet containing WB was lower ($P < 0.05$) than that in the diet containing either sand or DAE. The choice

Table 4. The effect of xylanase (0 or 26,400 units/kg of diet), diet formulation method for energy (FME), and digestibility index marker (DIM) on apparent ileal digestibility (AID) of amino acids (AA) in broiler chickens¹

FME	Xyl	DIM	n	AID of indispensable AA										AID of dispensable AA							
				Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Ala	Asp	Cys	Glu	Gly	Pro	Ser	Tyr
PC			16	91.8	89.4	86.6	87.7	90.1	95.7	87.7	83.5	90.1	84.4	86.9	84.8	79.7	90.5	83.4	87.6	85.6	88.2
Sand	–		16	92.1	90.4	87.8	88.2	91.0	96.3	88.3	84.3	90.6 ^x	86.0	87.7	85.4	81.6	91.0	84.4	88.3	85.5	88.4
Sand	+		16	92.1	90.1	87.7	88.0	90.9	96.2	88.2	83.5	88.8 ^y	85.8	87.5	85.2	81.0	91.0	84.0	87.8	85.5	88.2
DAE	–		16	90.9	89.1 ^x	87.9	88.0	90.3	96.1	88.6	85.3 ^x	88.2	85.4	87.9 ^x	85.5	80.1 ^x	90.3	86.0 ^x	86.3	89.6 ^x	89.1 ^x
DAE	+		16	91.4	87.5 ^y	87.0	87.6	89.9	95.8	88.0	82.4 ^y	87.7	84.4	86.9 ^y	84.8	78.7 ^y	90.6	82.6 ^y	86.5	84.9 ^y	87.9 ^y
Wheat bran	–		16	90.8	87.8	85.8	86.4	88.8	94.9 ^y	86.4	81.8	87.4	83.2	85.0	83.5	79.7	89.5	81.3	86.8 ^y	84.1	86.7
Wheat bran	+		16	90.5	88.7	85.9	86.7	89.5	95.5 ^x	86.5	82.2	87.6	83.3	85.9	83.7	80.8	89.9	82.1	88.1 ^x	84.3	87.0
	–		48	91.2	89.1	87.2	87.5	90.0	95.8	87.8	83.8	88.7	84.9	86.9	84.8	80.5	90.3	83.9	87.2	86.4	88.1
	+		48	91.3	88.7	86.9	87.4	90.1	95.8	87.5	82.7	88.0	84.5	86.8	84.6	80.2	90.5	82.9	87.4	84.9	87.7
Sand			32	92.1 ^A	90.3	87.8 ^A	88.1 ^A	91.0 ^A	96.2	88.3 ^A	83.9	89.7	85.9 ^A	87.6	85.3 ^A	81.3	91.0 ^A	84.2	88.0	85.5	88.3
DAE			32	91.2 ^{AB}	88.3	87.4 ^A	87.8 ^A	90.1 ^B	96.0	88.3 ^A	83.9	87.9	84.9 ^A	87.4	85.2 ^A	79.4	90.5 ^A	84.3	86.4	87.3	88.5
Wheat bran			32	90.6 ^B	88.2	85.9 ^B	86.5 ^B	89.1 ^C	95.2	86.5 ^B	82.0	87.5	83.3 ^B	85.5	83.6 ^B	80.3	89.7 ^B	81.7	87.5	84.2	86.8
	Cr		48	91.6	89.3	87.4	87.9	90.4	95.9	88.0	83.8	88.7	85.2	87.2	85.2	80.9	90.7	83.9	87.7	86.1	88.3
	Ti		48	91.0	88.6	86.6	87.1	89.8	95.7	87.3	82.7	88.0	84.2	86.4	84.2	79.7	90.1	82.9	86.9	85.2	87.5
SEM																					
Xyl				0.28	—	0.25	0.24	0.22	—	0.23	—	—	0.53	—	0.23	—	0.19	—	—	—	—
FME				0.32	—	0.28	0.26	0.24	—	0.26	—	—	0.56	—	0.26	—	0.21	—	—	—	—
Xyl × FME				—	0.33	—	—	—	0.20	—	0.42	0.33	—	0.39	—	0.49	—	0.40	0.37	0.39	0.29
DIM				0.28	0.23	0.25	0.24	0.22	0.14	0.23	0.29	0.22	0.53	0.27	0.23	0.34	0.19	0.29	0.27	0.28	0.21
P-value ²																					
PC vs. others				0.354	0.258	0.312	0.630	0.823	0.735	0.949	0.580	0.001	0.569	0.826	0.844	0.278	0.783	0.968	0.554	0.919	0.315
Xyl				0.758	0.142	0.308	0.632	0.708	0.775	0.360	0.002	0.007	0.282	0.750	0.388	0.363	0.378	0.003	0.259	<0.001	0.076
FME				0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Xyl × FME				0.600	0.001	0.273	0.493	0.094	0.048	0.516	<0.001	0.005	0.298	0.029	0.268	0.020	0.646	<0.001	0.020	<0.001	0.020
DIM				0.113	0.011	0.008	0.006	0.008	0.092	0.008	0.004	0.009	0.011	0.010	0.002	0.004	0.006	0.003	0.009	0.006	0.002

^{A,B,C}Main effect means for FME within a column without a common superscript letter differ ($P < 0.05$).^{x,y}Simple effect for xylanase within FME within a column without a common superscript letter differ ($P < 0.05$).¹n = number of observations; PC = positive control; DAE = diatomaceous earth; Xyl = xylanase.²P-values for interactions among FME, xylanase, and DIM (except xylanase × FME) were all greater than 0.10 and are not presented.

Table 5. The effect of xylanase (0 or 26,400 units/kg of diet), diet formulation method for energy (FME), and digestibility index marker (DIM) on apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of nutrients and gross energy (GE) in pigs¹

FME	Xylanase	DIM	n	AID			ATTD	
				DM	CP	GE	DM	GE
Positive control			12	68.9	76.7	71.6	86.8	88.4
Sand	–		12	68.5 ^x	78.1	71.6	82.0	88.0
Sand	+		12	66.0 ^y	77.4	70.0	81.4	88.0
Diatomaceous earth	–		12	65.9	78.4	70.7	81.2	87.6
Diatomaceous earth	+		12	65.3	79.1	71.3	82.0	87.7
Wheat bran	–		12	65.1	76.3	66.3 ^y	83.0	84.6
Wheat bran	+		12	66.1	76.4	68.5 ^x	83.2	84.4
	–		36	66.5	77.6	69.5	82.1	86.7
	+		36	65.8	77.6	69.9	82.2	86.7
Sand			24	67.3	77.8	70.8	81.7 ^B	88.0 ^A
Diatomaceous earth			24	65.6	78.8	71.0	81.6 ^B	87.7 ^A
Wheat bran			24	65.6	76.3	67.4	83.1 ^A	84.5 ^B
		Cr	36	66.3	77.7	69.9	82.2	86.8
		Ti	36	66.0	77.5	69.6	82.0	86.6
SEM								
Xylanase				—	0.57	—	0.48	0.67
FME				—	0.78	—	0.51	0.70
Xylanase × FME				0.73	—	0.81	—	—
DIM				0.56	0.65	0.65	0.48	0.67
P-value ²								
Positive control vs. others				0.110	0.554	0.229	0.009	0.090
Xylanase				0.046	0.916	0.199	0.573	0.925
FME				0.110	0.061	0.001	0.001	< 0.001
Xylanase × FME				0.005	0.142	0.004	0.112	0.845
DIM				0.693	0.786	0.706	0.492	0.590

^{A,B}Main effect means for FME within a column without a common superscript letter differ ($P < 0.05$).

^{x,y}Simple effect for xylanase within FME within a column without a common superscript letter differ ($P < 0.05$).

¹n = number of observations; DM = dry matter; CP = crude protein.

²P-values for interactions among FME, xylanase, and DIM (except xylanase × FME) were all greater than 0.10 and are not presented.

of DIM had no effect on the determination of AID of DM, CP, and GE and ATTD of DM and GE.

There were interactions ($P < 0.05$) between xylanase and FME for AID of Arg and Lys (Table 6). Inclusion of xylanase decreased ($P < 0.05$) AID of Arg and Lys in the diet containing DAE and decreased ($P < 0.05$) AID of Lys in the diet containing WB, but increased ($P < 0.05$) AID of Arg in the diet containing sand. Regardless of FME, xylanase decreased ($P < 0.05$) AID of Ile and Met but increased ($P < 0.05$) AID of Val. There was no difference between the values of AID of all AA observed with either Cr or Ti.

DISCUSSION

The appropriate choice of feed ingredient plays important role in the strategic use of xylanase in nonruminant animals (Adeola and Cowieson, 2011). Beneficial effects of inclusion of exogenous xylanase

have been reported in wheat-based diets in both poultry (Adeola and Bedford, 2004; Olukosi et al., 2007) and pigs (Barrera et al., 2004; Vahjen et al., 2007) due to the high concentration of nonstarch polysaccharide (NSP) in wheat. Xylanase has also been evaluated in CSBM diets in both chickens and pigs (Stefanello et al., 2016; Cho et al., 2017). Similar to the results of several previous studies conducted in broiler chickens (Nian et al., 2011), no effect of exogenous xylanase on growth performance in broiler chickens was observed in the current study. However, Kiarie et al. (2014) reported that the addition of xylanase (1,250 xylanase units/kg of diet) to CSBM diet improved growth performance of broiler chickens, where more NSP substrate was provided by including corn distillers' dried grains with solubles. Therefore, the lower concentration of NSP might be the reason for the lower magnitude of response to supplemental enzyme in CSBM diets in the current study as discussed by Bedford and Schulze (1998).

Table 6. The effect of xylanase (0 or 26,400 units/kg of diet), diet formulation method for energy (FME), and digestibility index marker (DIM) on apparent ileal digestibility (AID) of amino acids (AA) in pigs¹

FME	Xyl	DIM	n	AID of indispensable AA										AID of dispensable AA									
				Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Ala	Asp	Cys	Glu	Gly	Pro	Ser	Tyr		
PC			12	90.4	85.2	82.4	82.8	86.6	92.1	83.3	76.8	80.2	76.1	77.0	79.9	72.1	85.4	70.6	80.9	81.6	83.4		
Sand	−		12	89.1 ^Y	86.2	83.5	83.6	86.4	94.0	83.6	78.5	80.5	77.4	78.7	81.3	72.5 ^Y	86.0	74.0	82.6	82.8	82.6 ^Y		
Sand	+		12	90.5 ^X	86.7	82.6	84.1	87.0	92.9	84.2	78.7	81.4	78.0	78.5	80.8	74.7 ^X	85.3	72.8	82.5	83.7	84.9 ^X		
DAE	−		12	91.4 ^M	86.7	83.3	84.1	88.8 ^X	93.3	84.1	78.9	81.7	77.2	79.4	81.4	73.0	87.3 ^X	74.1	81.4	83.9 ^X	85.0 ^M		
DAE	+		12	89.9 ^N	86.3	83.3	83.9	87.2 ^Y	92.2	84.2	78.6	81.3	78.7	78.3	81.0	72.5	85.3 ^Y	73.6	82.3	82.4 ^Y	83.8 ^N		
WB	−		12	89.2	86.0	83.0	82.9	86.5 ^M	92.9	82.5	76.9	80.2	76.6	78.2	79.6	73.0	85.2	72.6	82.0	81.5 ^M	82.7		
WB	+		12	88.6	85.5	82.3	82.5	85.4 ^N	92.3	82.8	76.3	79.7	77.1	76.4	78.6	72.4	84.2	72.4	81.6	79.5 ^N	82.7		
	−		36	89.9	86.3	83.3	83.5	87.2	93.4	83.4	78.1	80.8	77.1	78.8	80.8	72.8	86.1	73.6	82.0	82.7	83.4		
	+		36	89.7	86.2	82.7	83.5	86.5	92.5	83.7	77.9	80.8	77.9	77.7	80.1	73.2	85.0	72.9	82.1	81.9	83.8		
Sand			24	89.8	86.5	83.1	83.9	86.7	93.4	83.9	78.6	81.0	77.7	78.6	81.0 ^{AB}	73.6	85.7	73.4	82.5	83.3	83.8		
DAE			24	90.6	86.5	83.3	84.0	88.0	92.8	84.2	78.8	81.5	78.0	78.8	81.2 ^A	72.7	86.3	73.9	81.8	83.2	84.4		
WB			24	88.9	85.8	82.7	82.7	85.9	92.6	82.7	76.6	79.9	76.8	77.3	79.1 ^B	72.7	84.7	72.5	81.8	80.5	82.7		
		Cr	36	89.8	86.3	83.1	83.6	86.9	93.0	83.7	78.1	80.9	77.6	78.3	80.5	73.1	85.6	73.4	82.1	82.4	83.7		
		Ti	36	89.7	86.2	82.9	83.4	86.8	92.9	83.5	77.9	80.7	77.4	78.2	80.4	72.9	85.5	73.1	82.0	82.2	83.6		
SEM																							
Xyl				—	0.55	0.51	0.48	—	0.30	0.44	0.55	0.69	0.54	0.70	0.51	—	—	1.25	0.94	—	—		
FME				—	0.70	0.69	0.66	—	0.39	0.61	0.79	0.88	0.80	0.99	0.66	—	—	1.42	1.08	—	—		
Xyl × FME				0.47	—	—	—	0.91	—	—	—	—	—	—	—	1.32	0.89	—	—	0.75	0.66		
DIM				0.39	0.61	0.58	0.55	0.81	0.34	0.51	0.65	0.76	0.65	0.82	0.56	1.09	0.82	1.31	1.27	0.60	0.53		
P-value ²																							
PC vs. others				0.413	0.411	0.622	0.558	0.822	0.299	0.799	0.483	0.704	0.433	0.535	0.617	0.685	0.848	0.232	0.568	0.590	0.877		
Xyl				0.063	0.572	0.022	0.744	0.003	<0.001	0.110	0.427	0.984	0.014	0.004	0.030	0.340	<0.001	0.093	0.609	0.007	0.077		
FME				0.017	0.556	0.720	0.237	0.090	0.163	0.124	0.087	0.263	0.539	0.381	0.025	0.760	0.044	0.503	0.691	0.009	0.118		
Xyl × FME				<0.001	0.084	0.281	0.239	0.001	0.141	0.496	0.607	0.099	0.276	0.092	0.678	0.016	0.017	0.595	0.172	0.004	<0.001		
DIM				0.815	0.850	0.839	0.830	0.864	0.879	0.820	0.821	0.834	0.845	0.858	0.768	0.842	0.732	0.800	0.942	0.822	0.826		

^{AB}Main effect means for FME within a column without a common superscript letter differ ($P < 0.05$).^{XY} Simple effect for xylanase within FME within a column without a common superscript letter differ ($P < 0.05$).^{M,N} Simple effect for xylanase within FME within a column without a common superscript letter differ ($P < 0.05$).¹_n = number of observations; PC = positive control; DAE = diatomaceous earth; WB = wheat bran; Xyl = xylanase.² P -values for interactions among FME, xylanase, and DIM (except xylanase × FME) were all greater than 0.10 and are not presented.

Efficacy of supplemental xylanase on energy and nutrient digestion was influenced by the choice of FME in this study. In the broiler chicken study, supplemental xylanase in the diet containing sand did not show any beneficial effect, and the reason is unclear. Presumably, the grit effect of sand with large particle size was beneficial in the gizzard, which independently increased the ileal energy digestibility of diets (Liu and Fowler, 2017) by increasing the contact area between endogenous digestion enzyme and ileal digesta. In the current study, the chickens fed the diet containing sand had the greatest AID of GE and Lys regardless of the inclusion of xylanase, which also supports this speculation. Therefore, the inclusion of sand in diet might restrain the potential improvement space for exogenous xylanase.

In the experimental diets containing DAE, inclusion of exogenous xylanase negatively affected the AID of His and Thr in broiler chickens and AID of Arg and Lys in pigs, and the reason is unclear. Exogenous xylanase could specifically cleave and hydrolyze the xylose backbone of arabinoxylans and release oligosaccharides consisting of xylose or xylose-arabinose residues (Svihus, 2010). On the other hand, due to the specific physical properties of DAE including high oil absorption capacity, large active surface, and high amorphous silicon dioxide content (Korunić, 1997), DAE has been included in poultry diets to treat parasites (Bennett et al., 2011). Furthermore, most of the indispensable AA which responded negatively to xylanase inclusion in the current study are basic AA (Nagai and Taniguchi, 2014) and share the y⁺L transporter system (Bröer, 2008). Therefore, it is reasonable to speculate that the xylo-oligosaccharides released during the degradation of NSP by exogenous xylanase might function together with DAE as some unidentified factor associated with disturbing the process of either AA digestion or absorption.

The main substrate for exogenous xylanase is arabinoxylans (Svihus, 2010), which is the major component of NSP in wheat (Steenfeldt et al., 1998). The amount of soluble and insoluble NSP in WB has been reported to be 14 and 350 g/kg (Yin et al., 2000a), and therefore the inclusion of WB increased the concentration of NSP in the diet, which might account for the beneficial effect of exogenous xylanase observed in both broiler chicken and pig experiments. Yin et al. (2000b) have reported that if a large amount of substrate is available to exogenous enzyme, there might be more scope for this enzyme to increase the nutrient digestibility. One of the possible modes of action

of xylanase in wheat-based diet fed to poultry is decreasing the soluble NSP-induced viscosity of ileal digesta (Bedford and Schulze, 1998; Adeola and Bedford, 2004), which might also be the mode of action in the diet containing WB due to the soluble NSP in WB. Furthermore, another possible mode of action of xylanase in the diet containing WB might be the increasing release of xylo-oligosaccharides from the degradation of NSP in WB contained in diet, could be utilized by beneficial bacteria, and as a result improved the utilization of nutrients (Liao and Nyachoti, 2017).

High dietary fiber level could increase the endogenous N losses by increasing the endogenous losses of epithelial cell or mucus secretion, which might be relieved by including exogenous enzymes (Sauer, 1976; Adeola, 2001; Gabert et al., 2001). Therefore, the modest improvement in the AID of Met and Pro in chickens fed the diet containing WB might be due to a reduction in the endogenous losses of these AA with the addition of xylanase as discussed by Adeola and Cowieson (2011). However, the beneficial effect of hydrolyzing fiber by exogenous xylanase was not evident for Lys, Arg, Ile, Leu, Val, and Phe in broiler chickens.

In the current pig experiment, a beneficial effect of exogenous xylanase in the diet containing WB was observed in AID of GE. In broiler chickens, it has been reported that the fermentable xylo-oligosaccharides from the hydrolysis of NSP by exogenous xylanase could be used by beneficial bacteria as prebiotics to increase the count of beneficial bacteria and the amount of volatile fatty acids, which could contribute energy to host animals (Choct et al., 1996; Nian et al., 2011). Yin et al. (2000b) indicated that the effect from microbial xylanase on NSP degradation may be biologically significant, and the relatively long small intestine in pigs compared with chickens might increase the chance for microbial colonization in the small intestine. Therefore, it is reasonable to speculate that the prebiotics effect from hydrolysis by exogenous xylanase could be utilized by beneficial bacteria in the small intestine of pigs, as a result, the ileal digestibility of energy was increased in pigs. Furthermore, the main contributor to ileal endogenous loss of Lys in pig originates from bacteria (Lien et al., 1997), and the increased population of beneficial bacteria might be the reason for the decrease in AID of Lys in pigs.

In broiler chickens, Olukosi et al. (2012) reported that the AID of AA in CSBM diets calculated with Ti was greater than with Cr as DIM, whereas the AID of GE and AA observed with Cr was greater compared with Ti, but the ATTD of

GE and DM observed with Cr and Ti were similar. The varying results comparing Cr and Ti might be related to the inclusion of different exogenous enzymes, which was phytase in the study reported by Olukosi et al. (2012) and xylanase in the current study. In pigs, greater AID and ATTD of GE in pigs fed CSBM diets was observed with Ti compared with Cr as DIM, and the possible reason might be the greater recovery of Ti compared with Cr (83.4% vs. 78.9%; Wang and Adeola, 2018). However, the choice of DIM had no effect on the determination of AID and ATTD of GE in the current pig study. This is explained by a reduced interference from dietary fiber due to the hydrolysis of arabinoxylans backbone and reduced degree of polymerization with the addition of xylanase in this study (Adeola and Cowieson, 2011). Overall, the choice of DIM affected AID but not ATTU in broiler chickens and had no effect on digestibility in pigs. This different effect of DIM type might be explained by dissimilar procedure of sampling, where ileal digesta samples of broiler chickens were collected one time following euthanasia, whereas ileal digesta samples in pigs were collected over a 2-d period, which may be more representative of attainment of equilibrium.

In conclusion, the choice of of formulation method for energy in experimental diets affects the evaluation of xylanase on efficiency of energy and nutrients in broiler chickens and growing pigs, and choice of digestibility index marker affects observed ileal digestibility values in broiler chickens.

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