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Effects of exogenous phytase and xylanase, individually or in combination, and pelleting on nutrient digestibility, available energy content of wheat and performance of growing pigs fed wheat-based diets

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Objective: Two experiments were conducted to determine the effects of adding exogenous phytase and xylanase, individually or in combination, as well as pelleting on nutrient digestibility, available energy content of wheat and the performance of growing pigs fed wheat-based diets.
Methods: In Experiment 1, forty-eight barrows with an initial body weight of 35.9±0.6 kg were randomly assigned to a 2×4 factorial experiment with the main effects being feed form (pellet vs meal) and enzyme supplementation (none, 10,000 U/kg phytase, 4,000 U/kg xylanase or 10,000 U/kg phytase plus 4,000 U/kg xylanase). The basal diet contained 97.8% wheat. Pigs were placed in metabolic cages for a 7-d adaptation period followed by a 5-d total collection of feces and urine. Nutrient digestibility and available energy content were determined. Experiment 2 was conducted to evaluate the effects of pelleting and enzymes on performance of wheat for growing pigs. In this experiment, 180 growing pigs (35.2±9.0 kg BW) were allocated to 1 of 6 treatments according to a 2×3 factorial treatment arrangement with the main effects being feed form (meal vs pellet) and enzyme supplementation (0, 2,500 or 5,000 U/kg xylanase).
Results: In Experiment 1, there were no interactions between feed form and enzyme supplementation. Pelleting reduced the digestibility of acid detergent fiber (ADF) by 6.4 percentage units ($p<0.01$), increased the digestibility of energy by 0.6 percentage units ($p<0.05$), and tended to improve the digestibility of crude protein by 0.5 percentage units ($p = 0.07$) compared with diets in mash form. The addition of phytase improved the digestibility of phosphorus ($p<0.01$) and calcium ($p<0.01$) by 6.9 and 7.6 percentage units respectively compared with control group. Adding xylanase tended to increase the digestibility of crude protein by 1.0 percentage units ($p = 0.09$) and increased the digestibility of neutral detergent fiber (NDF) ($p<0.01$) compared with control group. Supplementation of the xylanase-phytase combination improved the digestibility of phosphorus ($p<0.01$) but impaired NDF digestibility ($p<0.05$) compared with adding xylanase alone. In Experiment 2, adding xylanase increased average daily gain ($p<0.01$) and linearly improved the feed:gain ratio ($p<0.01$) compared with control group.
Conclusion: Pelleting improved energy digestibility but decreased ADF digestibility. Adding xylanase increased crude protein digestibility and pig performance. Phytase increased the apparent total tract digestibility of phosphorus and calcium. The combination of phytase-xylanase supplementation impaired the effects of xylanase on NDF digestibility.

Keywords: Digestible and Metabolizable Energy, Feed Enzymes, Growing Pigs, Pelleting, Wheat, Performance

INTRODUCTION

The use of wheat in swine diets has been well documented [1]. It is well known that viscous grains such as wheat and barley contain non-starch polysaccharides (NSP) which cannot be

digested by mammalian digestive enzymes while their hindgut fermentation is considered to be inefficient [2,3]. Among all types of NSP, studies have shown that xylan is the most responsible factor for the anti-nutritional effects of NSP in wheat [4,5]. Because xylanase is capable of hydrolyzing xylan, adding xylanase to wheat-based diets is considered to be an effective means to enhance nutrient utilization and to improve pig performance. Kiarie et al [6] suggested that xylanase functions through two main mechanisms, including breaking down the cell wall and releasing the nutrients inside the cell as well as reducing digesta viscosity thereby promoting the mixing of digesta and enzymes.

In addition to xylan, the presence of phytate and phytate-P in plant-based feedstuffs (including wheat) has long been recognized [7,8]. Monogastric animals lack proper enzymes to hydrolyze phytate which results in large amounts of P being excreted in the manure of animals that are fed wheat-based diets [9]. The capacity of phytase to release phytate-bound P is well documented and has made phytase an effective, alternative and economical P source. Previous studies have also shown that dietary supplementation of phytase in pig diets can reduce P excretion and protect the environment from P pollution [10]. In addition to P, the digestibility of other nutrients can also be promoted when phytase is added. Former studies have proven that the best additional level of phytase and xylanase is 500 U/kg and 4,000 U/kg, respectively, while taking cost and advantages of enzymes into consideration [11-13]. In the present experiment, no inorganic P was added in the experimental diets in order to evaluate the influences of phytase on wheat itself. To make up for the low P level in feed, we added high dose phytase (10,000 U/kg) to the diet.

Feed processing is another factor which impacts nutrient digestibility and pig performance. The effects of pelleting on wheat-based diets in poultry has been well documented [14] while few studies have been conducted to estimate the effects of feed processing on nutrient digestibility and performance of pigs fed wheat-based diets. The objective of pelleting is to agglomerate small feed particles after grinding with heat, moisture and pressure, thus, to promote pig performance and enhance the economics of feed processing. Pelleting involves three processes including conditioning, pelleting and cooling [14]. Each change during the pelleting process can affect the nutritional value of ingredients and the activity of feed enzymes [15,16]. The objective of this experiment was to study the interactions between feed form and exogenous enzymes as well as to evaluate the effects of pelleting and the addition of exogenous enzymes on nutrient digestibility, energy metabolism and performance in growing pigs fed wheat-based diets.

MATERIALS AND METHODS

Animal care

The experimental protocol used in this study was approved by the Institutional Animal Care and Use Committee of China Agricultural University (Beijing, China). This study was conducted in the Metabolism Laboratory of the Fengning Pig Experimental Base (Hebei, China).

Animals and experiment design

In Experiment 1, 48 barrows (Duroc×Landrace× Yorkshire) with an initial average body weight (BW) of 35.9±0.6 kg were selected for study. The pigs were randomly allocated to a 2×4 factorial arrangement involving 2 feed forms (meal vs pellet) and 4 types of enzyme supplementation (none, 10,000 U/kg phytase, 4,000 U/kg xylanase or 10,000 U/kg phytase plus 4,000 U/kg xylanase). The pigs were fed at 4% of their initial BW determined 1 day before the beginning of the trial. The room temperature was maintained at 20°C±1°C. Pigs were housed individually in stainless steel metabolism crates (1.4×0.7×0.6 m^3). Pigs were allowed *ad libitum* access to water through a nipple waterer located at the front of the crate.

In Experiment 2, 180 crossbred pigs (Duroc×Landrace× Yorkshire) weighing 35.2±9.0 kg BW were used in a 28-d experiment. The 6 treatments were based on a similar wheat-based diet with Experiment 1, with 2 feed forms (meal vs pellet) and 3 xylanase levels (0, 2,500, or 5,000 U/kg). The xylanase was the same as used in Experiment 1. Pigs were allocated to 1 of 6 treatments on the basis of weight and gender in a randomized complete block design with 5 replicates (pens) per treatment and 6 pigs per replicate (pen). The pigs were housed in pens of 1.2×2 m^2 with half of the floor cement and the other half woven mesh. All pigs had free access to water and feed throughout the 28-d experiment period. The temperature of barn was set at 25°C. Pigs and feed were weighed at the beginning and the end of the trial. The average daily gain (ADG), average daily feed intake and feed:gain ratio (F:G) were calculated.

Diet composition and enzyme preparation

The enzymes used in this study included xylanase and phytase. The phytase was supplied by AB Vista Asia Pte Ltd (Beijing, China), named Enhanced high temperature resistant type-6 phytase. The xylanase used in this study was produced by the Ministry of Agriculture Feed Industry Center Lab located at China Agricultural University (Beijing, China), the patent of which is ZLCN201510033630.3. One phytase unit is defined as the amount of enzyme required to release 1 mmol of inorganic P/min from a 0.0015 M Na-phytate solution at pH 5.5 and 37°C. One xylanase unit was defined as the activity that releases 1 mol of xylose/min at pH 3.0 and 50°C.

The ingredient composition of the experimental diets and their nutrient content are shown in Table 1. The experimental diets were exact the same between different treatments in Experiment 1 and in Experiment 2 as well. Different measures were achieved by different feed processing process and additional level of enzymes. The pelleted diets were processed with

Table 1. Experimental diet composition and nutrient content[1] (as fed basis)

Item	Exp 1		Exp 2	
	Meal	Pellet	Meal	Pellet
Ingredients (%)				
Whole wheat	97.84		92.52	
Soybean meal	-		3	
Limestone	1		0.85	
Dicalcium phosphate	-		1.2	
L-Lysine-HCl (98%)	-		0.9	
DL-methionine (98%)	-		0.11	
Threonine (98%)	-		0.32	
NaCl	0.3		0.4	
Premix[2]	0.8		0.5	
Choline chloride	0.06		-	
Analyzed chemical content[2] (%)				
Dry matter	89.3	89.3	88.8	89.39
Organic matter	85.9	85.9	-	-
Crude protein	14.2	14.6	15.6	15.6
Neutral detergent fiber	17.7	17.1	14.87	12.82
Acid detergent fiber	4.9	4.4	4.13	4.24
Gross energy (MJ/kg DM)	15.6	16	15.9	16
Ash	3.4	3.4	-	-
Total phosphorus	0.3	0.3	0.31	0.31
Calcium	0.46	0.46	0.64	0.64

[1] Same experiment diet design was used during different measures. The data in this table are analytical values.

[2] Premix provided the following per kg of complete diet: vitamin A, 5,512 IU; vitamin D_3, 2,200 IU; vitamin E, 30 IU; vitamin K_3, 2.2 mg; vitamin B_{12}, 27.6 μg; riboflavin, 4 mg; pantothenic acid, 14 mg; niacin, 30 mg; choline chloride, 400 mg; folic acid, 0.7 mg; thiamin, 1.5 mg; pyridoxine, 3 mg; biotin, 44 μg; Mn, 40 mg (MnSO4); Fe, 75 mg ($FeSO_4 \cdot H_2O$); Zn, 75 mg ($ZnSO_4$); Cu, 100 mg ($CuSO_4 \cdot 5H_2O$); I, 0.3 mg (KI); Se, 0.3 mg (Na_2SeO_3).

a single layer conditioning pellet mill (MUZL350, FAMSUN, Yangzhou, Jiangsu, China). The wheat remained in the conditioning chamber for 15 seconds and left the conditioner at a temperature of 83°C. The pellet diameter was 3 mm. The ratio of the diameter to length was 1:11. In Experiment 1, the dose of exogenous enzymes were calculated according to the pigs' daily feed intake (xylanase and phytase were included at about 1.5 and 2 g/kg of finished feed and we believe the impact of enzymes on chemical composition of diets is negiligible). The enzymes were weighed and mixed with the diet immediately before feeding.

Sampling procedures

In Experiment 1, the daily feed allotment was divided into two equal portions which were fed at 08:00 and 15:00 h. After a 7-d-adaptation period, a 5-d-total collection of feces and urine was conducted. Feed refusals and feed spillage were collected, dried and weighed to calculate feed intake. Feces were collected immediately as they appeared in the metabolic crates, placed in plastic bags and stored at –20°C. Urine was collected in a bucket placed under the metabolic crates. The bucket contained 10 mL of 6 N HCl per 1,000 mL of urine in order to fix the nitrogen in the urine. The total volume of urine was measured daily and a 10% aliquot was filtered through gauze and 50 mL of the mixed urine sample was transferred into a screw-capped tube and immediately stored at –20°C to avoid bacterial alteration [17,18]. At the end of the collection period, the sampled feces and urine were pooled for each pig and sub-samples were collected for chemical analysis. The sub-samples of feces were dried for 72 h at 65°C and ground through a 1-mm screen.

Chemical analysis

All chemical analyses were conducted in duplicate according to AOAC [19]. Samples of wheat, diets and feces were analyzed for dry matter (DM), crude protein (CP), ash, calcium, and total phosphorus. Organic matter was analyzed as 100 minus the ash content. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using fiber filter bags and fiber analyzer equipment (Fiber Analyzer, Ankom Technology, Macedon, NY, USA) following an adaptation of the procedure described by Van Soest et al [20]. The concentration of NDF was analyzed using heat stable α-amylase and sodium sulphite without correction for insoluble ash. The ADF fraction was analyzed in a separate sample. Samples of wheat, diets, feces and urine, (after been quantitatively absorbed by a paper tower, and caulated according to the weight and energy of bag, added quantity of urine and total energy, same principle as Kim et al [21] were analyzed for gross energy (GE) with an Isoperibol Oxygen Bomb Calorimeter (Parr 6400 Calorimeter, Moline, IL, USA).

Calculations

Energy values determined from the excretion of GE in the feces and urine were subtracted from the intake of GE to calculate digestible energy (DE) and metabolizable energy (ME) for each diet. The apparent total tract digestibility (ATTD) of various chemical constituents was determined by subtracting the excretion of the chemical constituent in the feces from the dietary intake of the chemical constituent and then dividing by the intake of the chemical constituent [22]. The DE and ME in the wheat diets were divided by 0.9784 (wheat content in the diet) to calculate the DE and ME of the wheat itself.

Statistics

All data in Experiment 1 were analyzed as a 2×4 factorial design while data in Experiment 2 were analyzed as a 2×3 factorial treatment arrangement according to the analysis of variance procedure of SAS (SAS Institute; Cary, NC, USA) to evaluate the main effects of feed form, exogenous enzymes and their interaction. The experimental unit in Experiment 1 was the pig and the unit was pen in Experiment 2. The statistical model included the fixed effects of feed form, exogenous enzymes and their interactions as well as the random effect of pig. Mutiple comparisons among treatments were performed using the Student Newman Keuls (SNK) adjustment option of SAS in

Table 2. Effect of feed form and exogenous enzymes on total tract nutrient digestibility for growing pigs fed wheat-based diets[1)]

Items	Feed form		SEM	Enzyme				SEM	p values		
	Meal	Pellet		Control	Xylanase	Phytase	Combination		Feed form	Enzyme	Feed form×enzyme
Dry matter (%)	89.8	89.6	0.20	89.3	89.4	89.8	90.2	0.30	0.77	0.12	0.54
Organic matter (%)	92.0	92.0	0.16	91.8	91.9	92.1	92.4	0.24	1.00	0.31	0.58
Crude protein (%)	90.3	90.8	0.23	90.0	91.0	90.2	90.9	0.34	0.07	0.09	0.59
Neutral detergent fiber (%)	64.3	65.4	0.57	63.1[b]	67.9[a]	63.6[b]	64.8[b]	0.85	0.1	<0.01	0.46
Acid detergent fiber (%)	55.0	48.6	1.29	50.5	52.2	53.7	50.8	1.90	<0.01	0.54	0.18
Phosphorus (%)	72.7	71.4	1.47	68.0[b]	67.2[b]	74.9[a]	78.2[a]	2.18	0.60	<0.01	0.09
Calcium (%)	75.9	75.5	0.64	71.7[a]	73.8[a]	79.3[b]	78.0[b]	0.95	0.64	<0.01	0.65

SEM, stands for standard error mean.
[1)] Each treatment has 6 replicants.
[ab] Within main effect, means in the same row with different superscripts differ ($p < 0.05$).

Experiment 1. All results are reported as least squares means. For Experiment 2, control statement in general linear model model was applied to determine linear and quadratic effects of enzyme concentrations. The level of significance adopted was $p<0.05$ while $p<0.10$ was considered as indicative of a trend.

RESULTS

Effects of pelleting on nutrient digestibility and performance of pigs fed wheat-based diets

There were no interactions between feed form and exogenous enzymes in either Experiment 1 or Experiment 2. The CP digestibility tended to increase by 0.5 percentage units ($p = 0.07$) (Table 2) while digestibility of energy increased by 0.6 percentage units ($p<0.05$) (Table 3) for pigs fed pelleted diets compared with pigs fed mash diets. However, the digestibility of ADF was decreased by 6.4 percentage units ($p<0.01$) for pigs fed pelleted diets than mash diets. Pelleting had no effect on the performance of growing pigs fed wheat based diets (Table 4).

Effects of enzymes on nutrient digestibility and performance of pigs fed wheat-based diets

Adding xylanase to wheat tended to increase CP digestibility by 1.0 percentage units compared with the control group ($p = 0.09$). The digestibility of phosphorus and calcium was 6.9 ($p<0.01$) and 7.6 percentage units ($p<0.01$) higher respectively when the pigs were fed with phytase than those without enzymes. Adding phytase-xylanase together reduced NDF digestibility by 3.1 percentage units ($p<0.05$) compared with pigs fed with xylanase only. Xylanase and phytase didn't have any additive effects. Adding 2,500 and 5,000 U/kg xylanase linearly increased ADG by 3.9 and 8.3 ($p<0.01$) percentage units. Adding 2,500 and 5,000 U/kg xylanase also linearly improved F:G by 15.4% and 24.8% compared with control groups.

DISCUSSION

Pelleting

Pelleting can promote pig performance and nutrient digestibility by protein denaturation and starch gelatinization in feedstuffs and the elimination of enzyme inhibitors [23]. A trend of an increase in CP digestibility was observed in pigs fed pelleted diets in this experiment, which is consistent with Mauron [24] who revealed a small but significant increase in CP digestibility in finishing pigs when pelleted diets were fed. Abdollahi et al [25] observed a small but significant increase in ileal digestibility of nitrogen (N) when wheat-based broiler diets were steam-conditioned at 60°C or 75°C (0.85 and 0.85, respectively) com-pared with a basal diet without steam-conditioning (0.83). However, the improvement in N digestibility disappeared when the conditioning temperature was increased to 90°C (0.83). It was speculated that in their experiment, the improvement in N

Table 3. Concentration of digestible energy and metabolizable energy, and apparent total tract digestibility of energy in diets (as-fed basis)[1)]

Item	Feed form		SEM	Enzymes				SEM	p values		
	Meal	Pellet		Control	Xylanase	Phytase	Combination		Feed form	Enzyme	Feed form×enzyme
Feed intake (kg/d)	1.41	1.43	0.93	1.47	1.43	1.37	1.41	0.35	0.83	0.98	0.46
GE intake (MJ/d)	22.05	22.99	0.69	23.26	22.59	21.88	22.35	0.55	0.4	0.98	0.46
GE in feces (MJ/d)	2.46	2.39	0.80	2.54	2.33	2.34	2.47	0.89	0.69	0.58	0.81
GE in urine (MJ/d)	0.73	0.74	0.93	0.79	0.75	0.62	0.76	0.60	0.99	0.20	0.49
Digestible energy (MJ/kg)	13.88	14.36	0.07	14.10	14.19	14.11	14.08	0.06	<0.01	0.62	0.74
Metabolic energy (MJ/kg)	13.35	13.81	0.07	13.53	13.67	13.58	13.54	0.17	<0.01	0.22	0.98
ATTD of GE (%)	88.94	89.56	0.02	88.86	89.29	89.33	89.51	0.41	0.04	0.61	0.84

DE, Digestible energy; ME, Metabolizable energy; SEM, stands for standard error mean; ATTD, apparent total tract digestibility.
[1)] Each treatment has 6 replicants.

Table 4. Effects of pelleting and xylanase on performance of growing pigs fed wheat-based diets[1)]

Item	Feed form		SEM	Enzyme level (U/kg)			SEM	p values				
	Pellet	Meal		0	2,500	5,000		Feed form	Enzyme	Feed form×enzyme	Linear	Quadratic
Weight gain (g/d)	772	735	17.56	724	752	784	21.23	0.43	<0.01	0.79	0.06	0.95
Feed intake (g/d)	1,759	1,686	71.50	1,915	1,690	1,563	75.81	0.15	0.16	0.55	<0.01	0.60
Feed:gain	2.29	2.31	0.11	2.66	2.25	2.00	0.10	0.87	<0.01	0.97	<0.01	0.51

SEM, stands for standard error mean.
[1)] Each treatment has 6 replicants.

digestibility at 60°C could have been due to proper protein denaturation and enzyme-inhibitor inactivation, and the impaired N digestibility at 90°C was due to the formation of Maillard products which counteracted the positive effects of pelleting. Abdollahi et al [14] found the proportion of empty aleurone cells (utilized contents) was significantly greater in pellet diets compared with meal form. The level of non-utilized (residual) protein decreased as the proportion of empty cells increased.

Although pelleting can produce many advantages, it can also negatively affect the feeding value of wheat. Cowieson et al [26] reported a significantly higher diet viscosity for pelleted wheat-based broiler diets than meal form diets when no xylanase was added and the viscosity resulted in a higher conditioning temperature which may explain the decrease in ADF digestibility observed in the present experiment.

In our experiment, pelleting increased aparent digestible energy (ADE) and apparent metabolic energy (AME) in wheat which agrees with the results of Abdollahi et al [25]. Abdollahi et al [14] also reported that pelleting had a significant effect on AME. Increased protein and starch digestibility may be the two main factors leading to a higher GE digestibility in pigs fed pelleted diets [27,28]. Pelleting did not affect the performance of growing pigs fed wheat diets in this experiment which is contrary to Wondra et al [29], who reported pelleting significantly promoted the performance of pigs fed corn. Jensen and Becker [23] also reported no improvement in pig performance from pelleting wheat diets. In our experiment, because wheat contains more fiber than corn, this would have resulted in a larger friction force when the diet was extruded and thus a higher temperature during pelleting [14], and a higher pelleting temperature may cause Milliard reactions as well as denaturation of amino acids and vitamins, which may diminish the beneficial effects of pelleting compared with previous published studies conducted with corn-based diets.

Enzymes

Phytase: During the past decade, the inclusion of microbial phytase in pig diets has increased remarkably [30]. In the current experiment, consistent with most studies [11,31], phytase promoted phosphorus and calcium digestibility. However, the effects of phytase on the digestibility of other nutrients are still not clear [32]. Brady et al [33] reported an increase in dietary DE content, contrary to the reports of Shelton et al [34] and Nortey et al [35], who found no effect of phytase addition on dietary DE content. Some studies reported increases of 1% to 3% in CP and amino acids (AA) digestibility when phytase was fed [36,37] while other experiments showed no effect [9,12]. In this experiment, high dose of phytase was added, according to Zeng et al [38], the apparent ileal digestibility of IP_6 and other nutrients would be even greater with super dose phytase addition compared with a normal phytase level (500 U/kg) because more phosphorus resources could be released.

Xylanase: Xylanase supplementation has achieved inconsistent results [10,39]. In the present experiment, xylanase addition tended to improve CP digestibility and increased NDF digestibility. Most researchers believe that adding xylanase to wheat-based diets is beneficial for nutrient digestibility and gastro intestinal micro-ecology [30,35]. Woyengo et al [12] added graded levels of phytase and xylanase to the diet of pigs weighing 20 and 60 kg and found these enzymes only increased phosphorus, calcium and apparent AA digestibility which was similar to the results of Moehn et al [40] and Lindberg et al [9]. Atakora et al [13] added phytase-xylanase to a low protein and low phosphorus wheat-based diet and discovered an increased digestibility of NDF and phosphorus which was similar to our experiment (digestibility of CP and phosphorus were increased). Pedersen et al [41] reported xylanase increased the ileal digestibility of NSP and decreased ileal viscosity [42] in a wheat diet. However, the increased fiber digestibility did not result in higher energy digestibility as reported by Woyengo et al [12].

Adding xylanase to a wheat-based diet promoted the performance of growing pigs in this experiment which is in agreement with most studies [3,12]. Improvements in nutrient digestibility may be the main factor which contributes to this promotion.

Additive effects between xylanase and phytase: No additive effects were observed between xylanase and phytase in this experiment which is consistent with Nortey et al [43] and Woyengo et al [12], and in contrast to Kim et al [11], who believed that xylanase could break down cell walls and release more phytate acid for phytase. The low phosphorus level in these experiments and our experimental diet may have caused this difference.

Surprisingly, we observed a negative effect of phytase on xylanase's promotion effects on NDF digestibility. Atakora et

al [13] had similar results in their experiment, although it was not significant. It is speculated that this phenomenon was caused by a disturbance in the flora in the hindgut. Because the excessive phytase added in this experiment promoted ATTD of phosphorus, the phosphorus concentration was decreased in the hindgut. Metzler-Zebeli and Mosenthtin [44] and Metzler-Zebeli et al [45] found that the dietary level of calcium and phosphorus can affect the intestinal abundance of certain fermentation end products, bacterial numbers, bacterial activity and intestinal morphology [46] in young pigs.

Interactions between pelleting and enzymes: No significant interactions between feed form and enzymes were found in this study. Pelleting tended to decrease the digestibility of phosphorus in diets without enzymes while it was increased in diets with enzymes. Jongbloed and Kemme [47] believed this was caused by the elimination of phytase in wheat.

CONCLUSION

Under the conditions of this experiment, pelleting improved energy digestibility but decreased ADF digestibility. Adding xylanase to wheat increased CP digestibility and pig performance and had positive effects on NDF digestion. Phytase increased the ATTD of phosphorus and calcium. No interactions were observed between xylanase and phytase. The combination of phytase-xylanase supplementation impaired the effects of xylanase on NDF digestibility.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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