Effects of Microbial Phytase Replacing Partial Inorganic Phosphorus Supplementation and Xylanase on the Growth Performance and Nutrient Digestibility in Broilers Fed Wheat-based Diets

Y. L. Peng, Y. M. Guo* and J. M. Yuan
College of Animal Science and Technology, China Agricultural University, Beijing 100094, P. R. China

ABSTRACT : Two experiments were conducted with broilers to investigate the feasibility of microbial phytase replacing partial inorganic phosphorus supplementation and the synergistic effects of xylanase (320 FTU/kg) supplementation alone or in combination with phytase (750 U/kg) replacing 0.08% dietary inorganic phosphorus, on the growth performance and utilization of nutrients in broilers fed wheat-based diets. In Experiment 1, 540 broilers were fed five diets for 6 weeks. Diets C0 and C1 were corn-based diets and 0.08% inorganic P supplementation was replaced with 750 U phytase/kg feed in Diet C1. Diets W0, W1 and W2 were wheat-based diets supplemented with microbial phytase 0, 750, 750 U/kg feed and 0, 0.08% and 0.16% dietary inorganic P were replaced, respectively. In Experiment 2, 432 broilers were divided into four treatments to determine the synergistic effects of supplemental xylanase and phytase replacing 0.08% inorganic P. Four experimental diets were arranged according to a 2×2 factorial design. The results indicated that addition of phytase increased the digestibility of phytic P by 31.0 to 55%, dramatically decreased the excretion of phytic P and total P by 31.6 to 55.0% and 13.8 to 32.9%, respectively ($p<0.01$). It is feasible to completely replace 0.08% inorganic phosphorus supplementation with microbial phytase 750 U/kg in corn- or wheat-based diets for broilers. Addition of xylanase alone or in combination with phytase replacing 0.08% dietary inorganic P, increased body weight gain and feed utilization efficiency of broilers fed wheat-based diets ($p<0.10$) and decreased overall mortality ($p<0.10$). In the groups of birds supplementing xylanase 320 FTU /kg feed, a marked elevation of the dietary AME was observed ($p<0.05$). Addition of phytase replacing 0.08% dietary inorganic phosphorus, concurrently with xylanase supplementation had additive effects on the apparent digestibility of dietary phytic P and overall feed conversion ratio ($p<0.05$). ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 2 : 239-247)***

Key Words : Broilers, Phytase, Xylanase, Wheat-based Diet, Inorganic Phosphorus

INTRODUCTION

The use of wheat in poultry diet as energy feed is very prevalent in some European and North American countries. Wheat contains various levels of arabinoxylan (approximately 5.27 to 8.18%; Annison, 1990), which depends on cereal variety and growing conditions (Scott et al., 1999). The digestibility of arabinoxylan for 2 week broilers was about 4%, whereas that for adult birds by 19% (Bolton, 1955). The anti-nutritive activities of these non-starch polysaccharides (NSP) were demonstrated by Marquardt and co-workers (Marquardt et al., 1996) who showed that growth performance of broilers were depressed when pentosans isolated from rye were added to experimental diets. Supplementation of the wheat-based diets with enzymes exhibiting xylanase activity improved the nutritional value and growth performance of broilers (Marquardt et al., 1996).

Phytate is another anti-nutritive factor in almost all plant feedstuffs. Approximate 60 to 80% of total phosphorus in plant feedstuffs was phytic Phosphorus (NRC, 1994). The bioavailability of phytic P for monogastrics such as pigs and birds were rather low, owing to the lack of endophytase in the gastrointestinal tract. Relatively high intrinsic phytase activity is present in some feedstuffs, such as wheat and its by-products (Nelson, 1976; Eeckhout and De Paepe, 1994), and this naturally occurring phytase also improves dietary phytic P utilization by broilers. Although the bioavailability of Phytic P in wheat may be close to 15 to 30% (NRC, 1994), most phytic P in feces remains indigestible. Supplemental microbial phytase in diets for broilers can improve the bioavailability of phytic P and other nutrients and reduce the concentration of these nutrients in poultry manure (Coelho and Kornegay, 1996).

Some studies have investigated the feasibility of replacing partial dietary inorganic P with phytase addition. The effects of supplemental microbial phytase replacing partial inorganic P in diets for laying hens were well comfirmed, but for broilers without consistent results (Nelson, 1976; Kornegay, 1999; Um et al., 2000). Limited published data are available on the interaction of phytase and xylanase (Ravindran et al., 1999; Zyla et al., 1999a,b). It is still unclear what and how much nutrients may be influenced by the combined application of two enzymes. The objectives of this study were: 1) to compare the efficacy of microbial phytase replacing for different levels of inorganic phosphorus supplementation in corn- or wheat-based diets fed to young broilers, and 2) to determine whether adding xylanase alone or in combination with

* Corresponding Author: Y. M. GUO. Tel: +86-10-6289-2712, Fax: +86-10-6289-3900, E-mail: guoyum@public.bta.net.cn
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phytase substituting 0.08% dietary inorganic P, additively improve the growth performance and nutrient digestion of broilers fed wheat-based diets.

MATERIALS AND METHODS

Enzymes and enzyme assays

A recombinant phytase (EC3.1.3.8) (Natuphos®) was obtained from BASF Corp and the specific activity was about 5,000 U/g of product. One unit is defined as the amount of enzyme that liberates 1 μmol of inorganic P from sodium phytate per minute at pH 5.5 and 37°C. Phytase activity in the microbial phytase product was assayed as described by Alko Biotechnology (1990).

Commercial preparations of xylanase (EC 3.2.1.8) (Ronozyme™) derived from *Humicola insolents* were provided by Roche Corp. Guaranteed analysis of the preparations contained endo-1,4-β-xylanase 800 FTU/g and endo-1,4-β-glucanase (EC 3.2.1.4) 75 FBU/g. The activity of endo-1,4-β-xylanase (EC 3.2.1.8) was assayed by the procedure of Yinbo et al. (1996) using a 1.6% suspension of oat spelt xylan as substrate. One unit of xylanase activity (FXU) was equivalent to 1 μM of reducing sugars released per minute under the assay conditions.

Birds and experimental design

Nine hundred and seventy two day-old Avian broiler chickens were obtained from a commercial hatchery and reared in an environment-controlled broiler-house. All broilers were randomly allotted into dietary treatment groups as hatched. All birds were raised in three-layer cages with wire bottom floors and followed the two-stage feeding system (wk 1 to 3 and wk 4 to 6). The birds were inoculated vaccines according to the normal immunization procedures of broilers.

In Experiment 1, 540 one-day-old broilers were used to determine the effectiveness of phytase, relative to supplemental inorganic P, in improving phytic P bioavailability. Five diets were arranged in a completely randomized design. Diet C0 and C1 were corn-based diet, whereas Diet W0, W1 and W2 were wheat-based diet. There are five dietary treatments in total, each treatment had six pen replicates of eighteen birds.

Experiment 2 was conducted with 432 day-old broilers to study the synergistic effects of xylanase and phytase added to wheat-based diets. In a completely randomized 2×2 factorial (xylanase×phytase) design, two levels of xylanase (0, 320 FXU/kg) and two levels of phytase (0, 750 U/kg replaced 0.08% dietary inorganic P supplementation) were added. Each treatment had six pen replicates of eighteen birds. In both experiments, diets were formulated to meet all nutrient requirements (NRC, 1994), except available phosphorus in treatments supplemented phytase. The composition of the experimental diets used in Experiment 1 and Experiment 2 were given in Table 1. Birds were given free access to mash feed and water.

Metabolism balance trial was conducted using a classical total collection method (Mollah et al., 1983) during 16 to 20 d age of broilers. An equal number of male and female birds were used in each cage for excreta collections. Total excreta output, per pen, was collected every 12 h and sampled, and then stored frozen (-20°C) prior to analysis. Then, the excreta samples were pooled by pen, dried in a 60°C oven and ground for analysis use. Food intake per pen was recorded during the collection period.

Total body weights and feed consumption were recorded per pen at 21 and 42 days of age. The mortality was recorded daily. At the end of week 6, six birds per treatments were killed, and Toe samples were obtained by severing the middle toe through the joint between the second and third tarsal bones from the distal end. Experimental procedures were evaluated and approved by the Institutional Animal Care and Use Committee at China Agricultural University.

Chemical analysis

Dried excreta and diet samples were ground to pass through a 1 mm sieve before analysis. Proximate analysis was performed according to standard procedures (AOAC, 1990). Gross energy of diet and excreta samples were determined using an adiabatic bomb calorimeter standardized with benzoic acid. Total phosphorus concentration was determined colorimetrically by the molybdo-vanadate method (AOAC, 1990). Phytic phosphorus in the feed was calculated based on amounts of phytic phosphorus determined in wheat and soybean meal by the method of Frühback et al. (1995). The toe samples were dried to a constant weight at 105°C and then ashed in a muffle furnace at 600°C for 12 h.

Statistical analysis

The data in Exp. 1 were subjected to analysis of variance using the one-way ANOVA procedure of Statistical Package for the Social Science (SPSS 10.0). Data from Exp. 2 were analyzed using a completely randomized design 2×2 factorial (phytase×xylanase) ANOVA of SPSS (10.0). Significance level was set as $p<0.05$ unless indicated otherwise.

RESULTS

Experiment 1

The growth performance of Experiment 1 is presented in Table 2. Addition of phytase replacing inorganic P supplementation had no significant effects on the feed intake during wk 1 to 3 or wk 4 to 6, but broilers fed wheat-

Table 1. Composition and nutrient levels of experimental diets[1] during week 1 to 3 in Experiment 1 and Experiment 2

		Experiment 1 (g/kg)					Experiment 2 (g/kg)			
	Diet	C0	C1	W0	W1	W2	W0	W1	W3	W4
	Available P,%	0.45	0.37	0.45	0.37	0.29	0.45	0.37	0.45	0.37
	Phytase, U/kg	0	750	0	750	750	0	750	0	750
Ingredient	Xylanase, FTU/kg	0	0	0	0	0	0	0	320	320
Corn		562.8	565.6							
Wheat				647.1	650.4	653.9	647.1	650.4	646.2	649.4
Soybean meal		341.3	340.8	239.2	238.2	237.1	239.2	238.2	239.5	238.5
Rapeseed meal		40	40	40	40	40	40	40	40	40
Vegetable oil		15.3	14.4	32.6	31.7	30.8	32.6	31.7	32.8	32.0
Dicalcium phosphate		18.8	14.0	18.9	14.0	9.1	18.9	14.0	18.9	14.0
Limestone		11.6	14.9	9.6	13.0	16.3	9.6	13.0	9.6	13.0
Lysine-HCl		0.76	0.77	3.25	3.27	3.29	3.25	3.27	3.25	3.27
DL-methionine		1.71	1.71	1.64	1.64	1.64	1.64	1.64	1.65	1.64
Sodium chloride		3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Choline chloride		1	1	1	1	1	1	1	1	1
Aureomycin		0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Antioxidant		0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix[2]		2	2	2	2	2	2	2	2	2
Vitamin premix[3]		0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total		1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Calculated nutritive values per kilogram (as fed)										
ME, MJ		12.39	12.39	12.39	12.39	12.39	12.39	12.39	12.39	12.39
CP, g		210	210	210	210	210	210	210	210	210
Ca, g		10	10	10	10	10	10	10	10	10
Available P, g		4.5	3.7	4.5	3.7	2.9	4.5	3.7	4.5	3.7
Lysine, g		11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0
Methionine, g		3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8

[1] The formula of experimental diets during week 4 to 6, omitted here, were used the same ingredients as that of week 1 to 3 and formulated according to the recommended nutrient levels for wk 4 to 6 broilers (NRC, 1994): ME 12.60 MJ/kg, CP 19%, Ca 0.9%, Available P 0.40%, lysine 1.00%, Methionine 0.38%. The available P level of dietary treatments with phytase supplementation was varied according to the experimental design.

[2] Trace elements premix (Supplied per kg feed): Cu 8 mg, Zn 75 mg, Fe 80 mg, Mn 100 mg, Se 0.15 mg, I 0.35 mg.

[3] Vitamin premix (Supplied per kg feed): Retinyl Acetate 12,500 IU, Cholecalciferol 2,500 IU, DL-α-tocopheryl Acetate 18.75 mg, Meneadione Sodium Bisulphite 2.65 mg, Thiamin Mononitrate 2 mg, Riboflavin 6 mg, Cyanocobalamin 0.025 mg, Biotin 0.0325 mg, Folic Acid 1.25 mg, Calcium Pantothenate 12 mg, Nicotinic Acid 50 mg.

based diets had higher feed intake ($p<0.05$) than broilers fed corn-based diets during wk 4 to 6. Addition of 750 U phytase/kg feed replacing 0.08% or 0.16% inorganic P supplementation, didn't reduce Average Daily Gain (ADG) throughout the experiment, though the birds fed corn-based diets (Diet C0 and C1) had higher ADG than birds fed wheat-based diets (Diet W0, W1 and W2). Broilers fed wheat-based diet replacing 0.08% inorganic P (Diet W1) had the highest Body weight at 21 d. Feed utilization efficiency of corn-based diets were higher than that of wheat-based diets ($p<0.01$). Feed: gain ratio of broilers fed the wheat-based diet reduced 0.16% inorganic P (Diet W2) were significantly higher than that of other treatments ($p<0.05$). The mortality was lowered by phytase addition ($p<0.05$).

The results of metabolism balance trial in Experiment 1 were showed in Table 3. There was a tendency for phytase supplementation to improve AME. This increase, however, was not statistically significant. Addition of phytase improved apparent digestibility of nitrogen by 1 to 3%. Supplemental phytase replacing 0.08% or 0.16% dietary inorganic P significantly improved the apparent digestibility of phytic P by 31.0 to 55% ($p<0.01$), but had no marked influence on the digestibility of total P. The excretion of total P and phytic P in feces were reduced significantly by 13.8 to 32.9% and 31.7 to 55.0% ($p<0.01$), respectively, owing to phytase addition and reduction of inorganic P supplementation. In experiment 1, identical toe ash at the end of wk 6 were observed among the broilers fed different experimental diets (Table 3).

Experiment 2

Addition of xylanase or phytase replacing 0.08% available P, improved ADG during wk 1 to 3 by 2.66% ($p<0.05$) and 2.91% ($p=0.051$), respectively (Table 4). There was a tendency for phytase or xylanase supplementation to improve ADG from wk 4 to 6 as well as overall ADG. This increase, however, was not statistically significant.

Xylanase or phytase added to wheat-based diets did not

Table 2. Effects of phytase replacing partial inorganic phosphorus supplementation on growth performance of broilers in Experiment 1

	Dietary treatments					P<	SEM
	C0	C1	W0	W1	W2		
Feed Intake (g/d)							
Wk 1 to 3	46.5	46.2	46.2	47.3	45.3	NS	0.34
Wk 4 to 6	130.2[a]	133.1[a]	139.6[ab]	140.0[ab]	146.4[b]	0.032	1.83
Overall	89.3[a]	89.6[a]	93.8[ab]	93.7[ab]	96.6[b]	NS	1.06
Average daily gain (g/d)							
Wk 1 to 3	30.86[a]	31.08[a]	31.20[a]	32.78[b]	30.88[a]	0.019	0.22
Wk 4 to 6	61.43[ab]	62.65[b]	57.97[a]	58.61[ab]	58.61[ab]	0.066	0.64
Overall	47.11	47.83	45.53	46.64	45.69	NS	0.32
Average body weight (g/d)							
21 d	688.3[a]	693.0[a]	695.0[a]	728.0[b]	688[a]	0.022	4.65
42 d	1,978.3	2,008.6	1,912.3	1,958.7	1,918.9	NS	5.60
Feed/gain							
Wk 1 to 3	1.42	1.40	1.40	1.37	1.39	NS	0.01
Wk 4 to 6	2.17[a]	2.13[a]	2.46[b]	2.39[b]	2.55[b]	0.000	0.04
Overall	1.90[a]	1.88[a]	2.06[bc]	2.01[b]	2.12[c]	0.000	0.02
Mortality (%)	4.56[a]	1.78[b]	5.48[a]	1.85[b]	3.70[ab]	0.05	0.05

[a,b] Means within a row lacking a common superscript letter differ (p<0.05).
NS: No significance.

Table 3. Effects of phytase replacing partial inorganic phosphorus supplementation on AME, nitrogen metabolism and the bioavailability of phosphorus in Experiment 1 (as fed)

	Dietary treatments					P<	SEM
	C0	C1	W0	W1	W2		
AME (MJ/ kg)	12.67	13.10	12.82	12.87	12.89	NS	0.08
Retained nitrogen (g/d)	1.43	1.53	1.53	1.47	1.40	NS	0.03
Apparent digestibility of nitrogen (%)	63.97	66.06	66.05	66.43	67.65	NS	0.54
Apparent digestibility of total P (%)	55.19	57.89	52.89	56.00	57.88	NS	0.75
Total P in feces (g/d)	0.225[c]	0.194[b]	0.241[c]	0.192[b]	0.162[a]	.000	0.005
Digestibility of phytic P (%)	18.18[a]	64.83[c]	25.51[b]	62.88[c]	80.78[d]	.000	1.85
Phytic P in feces (g/d)	0.112[d]	0.054[b]	0.138[e]	0.066[c]	0.034[a]	.000	0.007
Toe ash (%)	13.45	14.79	14.34	13.97	14.02	NS	0.04

[a,b] Means within a row lacking a common superscript letter differ (p<0.05).

significantly increase feed intake and feed : gain ratio during wk 1 to 3. Xylanase supplementation, however, reduced feed intake of broilers from wk 4 to 6 by 3.86% and improved feed utilization efficiency by 5.37% (p<0.05). No obvious reduction of feed intake during wk 4 to 6 was observed in dietary treatment with phytase supplementation, but feed: gain ratio was reduced by 3.33%. Addition of xylanase and phytase alone may reduce overall feed intake by 2.88% and 1.82%, and improve overall feed utilization efficiency by 3.94% (p<0.05) and 2.97% (p=0.085), respectively. The overall mortality of broilers receiving phytase, instead of 0.08% available P, and xylanase alone or in combination were inferior to the value observed in the control treatment (p<0.10) (Table 4).

Addition of xylanase and phytase simultaneously exhibited significantly additive effects on overall feed utilization efficiency (p<0.05), however no marked interaction in other measurements of growth performance. Xylanase and phytase supplementation alone improved overall feed utilization efficiency by 3.88% and 2.43%, respectively, while the combined application of the two enzymes increased by 6.80% (p<0.05).

The results of metabolism balance trial in Experiment 2 showed that supplemented xylanase improved dietary AME, retained nitrogen and apparent digestibility of nitrogen by 2.18% (p<0.05), 2.58% and 0.93%, respectively (Table 5). No significant improvements of dietary AME and N balance were observed with addition of phytase replaced 0.08% inorganic P. Phytase supplementation replacing 0.08% available P, reduced the excretion of total P and phytic P by16.6% and 55.0%, respectively, and improved the apparent digestibility of phytic P by 43.3% (p<0.01).

Addition of phytase replacing 0.08% inorganic P, alone or in combination with xylanase to wheat-based diet for broilers showed significantly additive effects on overall digestibility and excretion of phytic P (p<0.05). Overall digestibility of phytic P with phytase addition substituted for 0.08% available P, rather than xylanase supplementation,

Table 4. Effects of xylanase and phytase[1] supplementation on the growth performance of broilers fed wheat-based diets in Experiment 2

Items	Dietary treatments				Main effects				P<			SEM
	Phytase (-)		Phytase (+)		Phytase		Xylanase		Phytase	Xylanase	Phytase× Xylanase	
	Xylanase (-)	Xylanase (+)	Xylanase (-)	Xylanase (+)	-	+	-	+				
Feed intake (g/d)												
Wk 1 to 3	46.2	48.2	47.3	47.1	47.2	47.2	46.8	47.7				0.45
Wk 4 to 6	139.6	135.9	140.0	132.8	137.8	136.4	139.8	134.4	NS	NS	NS	1.60
Overall	93.8	92.6	93.7	89.4	93.2	91.5	93.7	91.0	NS	NS	NS	0.96
Average daily gain (g/d)												
Wk 1 to 3	31.20[a]	32.70[b]	32.78[b]	32.98[b]	31.95	32.88	31.99	32.84	0.051	0.013	NS	0.25
Wk 4 to 6	57.97	59.10	58.61	58.51	58.53	58.56	58.29	58.80	NS	NS	NS	0.66
Overall	45.53	46.86	46.64	46.70	46.20	46.66	46.08	46.78	NS	NS	NS	0.37
Average body weight (g)												
21 d	695.0[a]	727.0[b]	728[b]	732.5[b]	711.0	730.3	711.5	729.7	0.055	0.068	NS	5.40
42 d	1,912.9	1,968.5	1,959.1	1,961.1	1,940.6	1,960.1	1,936.0	1,964.8	NS	NS	NS	5.60
Feed/Gain												
Wk 1 to 3	1.40	1.39	1.37	1.35	1.40	1.36	1.38	1.37	NS	NS	NS	0.01
Wk 4 to 6	2.46[a]	2.33[ab]	2.39[ab]	2.14[b]	2.40	2.32	2.42	2.29	NS	0.016	NS	0.02
Overall	2.06[a]	1.98[ab]	2.01[ab]	1.92[b]	2.02	1.96	2.03	1.95	0.085	0.011	.047	0.02
Mortality (%)	5.48[a]	2.56[b]	1.85[b]	2.70[b]	4.08	2.28	3.67	2.61	0.058	0.065	NS	0.04

[1] 0.08%dietary inorganic phosphorus supplementation was replaced by addition of phytase (750 U/kg).
[a,b] Means within a row lacking a common superscript letter differ ($p<0.05$).
NS: No significance.

Table 5. Effects of xylanase and phytase[1] supplementation on AME, nitrogen metabolism and the availability of phosphorus (as fed) in Experiment 2

Items	Dietary treatments				Main effect				P<			SEM
	Phytase (-)		Phytase (+)		Phytase		Xylanase		Phytase	Xylanase	Phytase× Xylanase	
	Xylanase (-)	Xylanase (+)	Xylanase (-)	Xylanase (+)	-	+	-	+				
AME (MJ. kg^{-1})	12.84	13.19	12.89	13.09	13.01	12.98	12.86	13.14				0.09
Retained nitrogen (g/d)	1.53[ab]	1.62[b]	1.50[a]	1.55[ab]	1.58	1.52	1.55	1.59	NS	NS	NS	0.03
Apparent digestibility of nitrogen (%)	65.99	66.81	63.90	64.95	66.40	64.42	64.95	65.88	NS	NS	NS	0.74
Apparent digestibility of total P (%)	52.24	56.58	56.48	55.27	54.41	55.88	54.36	55.93	NS	NS	NS	0.79
Total P in feces (g/d)	0.243[a]	0.226[a]	0.188[b]	0.182[b]	0.235	0.196	0.216	0.212	0.001	NS	0.096	0.006
Digestibility of phytic P (%)	26.13[a]	29.91[a]	64.73[b]	77.79[c]	28.02	71.27	45.43	53.85	0.000	0.001	0.027	4.8
Phytic P in feces (g/d)	0.140[a]	0.136[a]	0.064[b]	0.042[b]	0.138	0.063	0.102	0.089	0.000	0.001	0.010	0.002

[1] 0.08%dietary inorganic phosphorus supplementation was replaced by addition of phytase (750 U/kg).
[a,b] Means within a row lacking a common superscript letter differ ($p<0.05$).
NS: No significance.

was enhanced by 38.6%, while the combination of the two enzymes may increase by 51.66% ($p<0.05$).

DISCUSSION

Microbial phytase substitutes for dietary partial supplementation of inorganic phosphorus

For more than 50 year, it has been known that phytase had the ability to hydrolyze phytate (McCance and Widdowson, 1944). Its effectiveness for improving phytase P digestibility in pigs and poultry has been shown (Bagheri and Gueguen, 1985). By using Natuphos® phytase, Simons et al. (1990) showed that the availability of P in low P diet for broilers increased to over 60%. Zhang et al. (2000) also reported that addition of phytase to the lower P basal diet increased the P retention 6.6 to 17.2% and improved P digestibility 9.1 to 20.5%. The bioavailablity of phytic P were linearly improved with dietary available P reduction (Zhang et al., 2000). Phytase supplementation, replaced partial dietary inorganic P of poultry, may improve the availability of phytic P and other nutrients and then improve the growth performance to various degrees (Simons et al., 1990; Kornegay, 1999).

In the study presented here, supplemental phytase may increase the availability of phytic P by 31.0 to 55.0%. Toe ash percentage was as sensitive as ash weight for assessing P bioavailability or effectiveness of microbial phytase (Qian et al., 1996). Phytase addition in the present trial, replacing 0.08% or 0.16% inorganic P supplementation, had no marked influence on the toe ash percentage or toe ash retention compared with the birds fed normal P level diets. It is feasible to completely replace 0.08% inorganic phosphorus supplementation with 750 U microbial phytase/kg in the corn- or wheat-based diets for broilers. Further evidences were found in Experiment 2. Those results were approximately consistent with Kornegay's result (Kornegay, 1999) that when an optimal level (500 to 750 U/kg) of phytase is included in the diet, the dietary P level needed might be reduced 0.1% below recommended levels (NRC, 1994). Though the additional microbial phytase replacing 0.16% inorganic P supplementation (Diet W2) significantly improved apparent digestibility of phytic P, broilers fed that diet still had lower ADG and feed efficacy than those fed the diets replacing 0.08% inorganic P or the control diet. Supplementing microbial phytase 750 U/kg, replacing 0.16% inorganic P supplementation, was insufficient to produce the same response as that of the replacing 0.08% inorganic P-supplemented diet.

Relatively high intrinsic phytase activity is present in feedstuffs, such as wheat and its by-product, that contributes to the availability of phytate phosphorus (Barrier-Guillot et al., 1996). Endogenous phytase activities in wheat were reported by 512 to 686 FTU/kg (Zyla et al., 1999a) or 1193 U/kg (Eeckbout and DePaepe, 1994). Although wheat phosphorus is more available to monogastrics than phosphorus of many other feedstuffs, most likely owing to high endogenous phytase activity, the activities of these phytases are often relatively low and a substantial fraction of phytate in wheat remains indigestible (Kiiskinen et al., 1994; Larsson et al., 1997). The effects of endogenous phytase activity were reduced with the increased level of exogenous phytase supplementation. Improvements in digested P per unit of phytase decreased as phytase levels increased (Zhang et al., 2000). Therefore, the exogenous phytase, rather than cereal phytase itself, was largely responsible for the observed improvements in broilers fed the wheat-based diet.

Phytate calcium was formed insoluble soap with starch and fatty acids in the gastrointestinal of broilers, which depress the digestibility of carbohydrate and lipid (Rama Rao et al., 2001). Supplemental phytase can have positive effects on Dry Matter digestibility by releasing bound organic nutrients such as protein and starch (Ravindran and Bryden, 1997). Simons et al. (1990) and Kornegay et al. (1997) have reported improvements in feed efficiency when phytase was supplemented to low P broiler diets, but others failed to show such effects of phytase (Schoner et al., 1993). The positive effect of phytase addition to wheat-based diet on AME and nitrogen digestibility did not occur in the experiment described here. Even though the digestibilities of dietary protein or amino acids improve a little, minimal improvements in growth performance were not always observed (Peter et al., 2001).

P excretions in feces of broilers with exogenous phytase addition were reduced by 20 to 50% (Jongbloed et al., 1992). Excretory total P and phytic P in the present research were reduced by 13.8 to 32.9% and 31.6 to 55.0%, respectively. This results agrees with the findings from the review of Kornegay (1999) that when an appropriate level (500 to 750 U/kg) of phytase is replaced 0.1% available P, P excretion is reduced 31.8 to 35.7% compared with P excretion when recommend P levels are fed.

Xylanase

The results of Experiment 1 (Table 2) showed that feed efficiency of wheat-based diets during wk 4 to 6 was significantly lower than that of corn-based diets ($p<0.01$). This discrepancy may be partially explained by the fact that wheat contains higher level of arabinoxylan (Annison, 1990).

As in most studies on xylanase application in poultry diets (Marquardt et al., 1996; Crouch et al., 1997), the enzyme dramatically increased the growth performance of birds in our present experiment. Wheat contains substantial amounts of arabinoxylans that impair nutrient bioavailability (Almirall et al., 1995) and decrease metabolisable energy and consequently lower the performance of birds fed on wheat-based diets. Adding of a suitable xylanase to wheat-based diets may depolymeryse arabinoxylan (Fengler and Marquardt, 1988) and reduces the detrimental impacts of intestinal viscosity, thereby increase metabolisable energy (Iji et al., 2001) (Table 5) and growth performance of broilers (Table 4).

The tendency of xylanase addition to improve dietary AME and the apparent digestibility of nitrogen observed in this study was consistent with findings reported by other researchers (Ravindran et al., 1999). The reasons of dietary AME improvement with xylanase supplementation in most reports (Fuente et al., 1998) were mainly due to the enhancement of dietary starch, fat and protein digestibility (Almirall et al., 1995; Marquardt et al., 1996; Ouhida et al., 2000). The starch digestibility in some wheat varieties was very low (Mollah et al., 1983; Rogel et al., 1987), and apparent digestibility of wheat starch was lower than that of corn starch (Maisonnier et al., 2001). The hypothesis supported by Annison (1993) was that wheat AME values were negatively correlated with soluble non-starch polysaccharide levels and the AME depression was a result of the inhibition of starch, lipid and protein digestion in the foregut. A close correlation between starch digestibility and fat digestibility of wheat-based diet for broiler were demonstrated, there were also significant correlation between digestibility of starch and that of protein (Maisonnier et al., 2001).

In the present work, xylanase supplementation may increase feed intake during wk 1 to 3. The underlying mechanism may be partially explained by that xylanase decomposed cell wall and arabinoxylan in wheat, reduced viscosity of digesta, accelerated digesta flux rate (Ouhida et al., 2000) and consequently increase feed intake. Feed utilization efficiency during wk 1 to 3, however, was not markedly increased with xylanase addition. Feed intake during wk 4 to 6, however, was reduced with xylanase inclusion and feed utilization efficiency was increased. Theoretically, birds adjusted feed intake according to energy intake. If dietary AME was improved by xylanase supplementation and superior to the requirement of broilers, feed intake of wk 4 to 6 broilers was reduced. At the same time, xylanase alleviates the anti-nutritive effects of wheat arabinoxylan and increase digestion and absorption of primary nutrients, and then improve feed : gain ratio. Therefore, the effects of xylanase on the early growing phase of broilers may be different from that of later growing phase. Xylanase application to wheat-based feeds resulted in improved performance primarily due to increased feed utilization efficiency. The effect, however, depended on the age of birds (Fuente et al., 1998) and wheat variety (Crouch et al., 1997).

A positive effect of adding phytase or xylanase to the wheat-based diets on overall survivability was clear. Two possible mechanisms for decreasing morality might be that (1) NSP in feedstuffs result in overproliferation of intestinal microorganism flora. Partial soluble NSP were hydrolyzed in jejunum with xylanase supplementation, while insoluble NSP was little hydrolyzation in jejunum. Those arabinoxylans fluxed into intestinal posterior meliorated the microorganism flora and reduced the incidence of the intestinal diseases of poultry, therefore increased the survivability of young birds. (2) It is unclear whether mediate products (such as olisaachirade) produced from arabinoxylan hydrolysis may influence the immunity or resistance of broilers.

Interaction between xylanase and phytase

Few literatures about the interaction of phytase and xylanase were found. First attempts to optimize simultaneous application of phospholytic and viscosity-reducing enzymes in poultry feeds have been made by Newkirk and Classen (1996) on laying hens. Further evidences confirmed that the synergistic effects between microbial phytase and xylanase on dietary P utilization or growth performance (Jongbloed et al., 1996; Piao et al., 1998). Recent report (Ravindran et al., 1999) was that addition of phytase and xylanase alone improved the AME of low-AME wheat by 9.7% and 5.3%, respectively; while the concurrent application of the two enzymes increased the AME by 19.0%. Zyla (1999b) also reported that the effectiveness of phytase added to wheat-based diet of broilers might be improved with the inclusion of xylanase levels.

Evidence in the present study confirmed those findings and also showed that the feed efficiency-promoting effect of xylanase supplied at the optimal level to diets can be significantly enhanced by concurrent phytase addition. The combination of the two enzymes has no beneficial effects on the growth performance of broilers except feed utilization efficiency. Under the experimental conditions employed in the present study, significant positive interactions of xylanase and phytase replacing 0.08% available P, on the apparent digestibility of phytic P and the overall feed efficiency were observed. Addition of xylanase or phytase alone may reduce overall feed: gain ratio by 3.88% and 2.43%, respectively, while supplementation simultaneously by 6.80% ($p<0.05$). It was unclear about the possible mechanism of the interaction between xylanase and phytase. The possible mechanism may be that cell walls of plant feedstuffs were decomposed by supplemental xylanase and arabinoxylans of wheat were hydrolyzed, which in favor of the effectiveness of phytase to phytic P and therefore improved phytate dephosphorylation. Concurrent application of phytase and xylanase to broiler feeds based on wheat may be also expected to reduce intestinal viscosity, enhance absorption of nutrients and possibly improve absorption of phosphorus released from phytate by phytase (Ravindran et al., 1999).

CONCLUSIONS

The current results indicate that adding 750 units of microbial phytase per kilogram feed may completely substitute for 0.08% inorganic phosphorus supplementation in corn- or wheat-based diets for growing broilers. The efficacy of phytase on the phytic P digestibility in wheat-based broiler diets can be enhanced by xylanase addition to feeds. Addition of phytase replacing 0.08% inorganic phosphorus, concurrently with xylanase supplementation have additive effects on the apparent digestibility of dietary phytate P and feed utilization improvement.

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