Influence of Phytase and Xylanase Supplementation on Growth Performance and Nutrient Utilisation of Broilers Offered Wheat-based Diets

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ABSTRACT : Individual and combined supplementation of phosphorus-adequate, wheat-based broiler diets with exogenous phytase and xylanase was evaluated in three experiments. The effects of the enzyme combination in lysine–deficient diets containing wheat and sorghum were more pronounced than those of the individual feed enzymes. The inclusion of phytase plus xylanase improved ($p<0.05$) weight gains (7.3%) and feed efficiency (7.0%) of broilers (7-28 days post-hatch) and apparent metabolisable energy (AME) by 0.76 MJ/kg DM. Phytase plus xylanase increased ($p<0.05$) the overall, apparent ileal digestibility of amino acids by 4.5% (0.781 to 0.816); this was greater than the responses to either phytase (3.6%; 0.781 to 0.809) or xylanase (0.7%; 0.781 to 0.784). Absolute increases in amino acid digestibility with the combination exceeded the sum of the individual increases generated by phytase and xylanase for alanine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, phenylalanine, threonine, tyrosine and valine. These synergistic responses may have resulted from phytase and xylanase having complementary modes of action for enhancing amino acid digestibilities and/or facilitating substrate access. The two remaining experiments were almost identical except wheat used in Experiment 2 had a higher phytate concentration and a lower estimated AME content than wheat used in Experiment 3. Individually, phytase and xylanase were generally more effective in Experiment 2, which probably reflects the higher dietary substrate levels present. Phytase plus xylanase increased ($p<0.05$) gains (15.4%) and feed efficiency (7.0%) of broiler chicks from 4-24 days post-hatch in Experiment 2; whereas, in Experiment 3, the combination increased ($p<0.05$) growth to a lesser extent (5.6%) and had no effect on feed efficiency. This difference in performance responses appeared to be 'protein driven' as the combination increased ($p<0.05$) nitrogen retention in Experiment 2 but not in Experiment 3; whereas phytase plus xylanase significantly increased AME in both experiments. In Experiments 2 and 3 the combined inclusion levels of phytase and xylanase were lower that the individual additions, which demonstrates the benefits of simultaneously including phytase and xylanase in wheat-based poultry diets. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 3 : 394-402)***

Key Words : Phytase, Xylanase, Broilers, Growth Performance, Nutrient Utilisation

INTRODUCTION

During the last decade, the inclusion of non-starch polysaccharide (NSP) degrading enzymes, with predominantly xylanase activity, in wheat-based poultry diets has become routine (Silversides and Bedford, 1999). More recently, the acceptance of microbial phytase feed enzymes has increased in response to increasing concerns over phosphorus (P) pollution in the environment. The hydrolysis of phytate by phytase increases the utilisation of phytate-bound P (phytate-P) and reduces P excretion (Simons et al., 1990) but increasingly more evidence suggests that phytase also improves protein and energy utilisation (Selle et al., 2000).

Possible interactions between phytase and xylanase following their simultaneous inclusion in wheat-based broiler diets have attracted interest in recent years. Xylanase, by reducing digesta viscosity and releasing nutrients entrapped within the cell wall matrix, could increase the access of phytase to its substrate and facilitate the absorption of liberated nutrients. Ravindran et al. (1999b) reported that the simultaneous inclusion of phytase and xylanase was beneficial in wheat-based broiler diets on the basis of enhanced apparent metabolisable energy (AME) of wheat and digestibility of dietary protein. Zyla et al. (1999) found the simultaneous addition of phytase and xylanase to P-deficient broiler diets significantly increased growth rates and toe ash, but had no effect on either parameter when added individually. Despite the increasing likelihood of phytase and xylanase being used simultaneously in practice, published reports on their combined application are limited.

Consequently, three experiments were conducted to evaluate the simultaneous inclusion of microbial phytase and a NSP degrading enzyme, with predominantly xylanase activity, in P-adequate, wheat-based broiler diets. The individual and combined effects of phytase and xylanase on growth performance of broilers were determined. Additional parameters evaluated included apparent ileal digestibility of amino acids, AME, nitrogen retention, intestinal digesta viscosity, toe ash and pancreatic weights.

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Received August 2, 2002; Accepted November 4, 2002

MATERIALS AND METHODS

Experiment 1

The effects of adding microbial phytase and xylanase, individually and in combination, on the performance, apparent ileal amino acid digestibility and AME in broilers fed a P-adequate, lysine-deficient diet was investigated. The basal diet, based on wheat and sorghum (Table 1), was formulated to 80% of the lysine level recommended for broiler starters. The diet met the recommended requirements for all other amino acids and contained adequate levels (4.50 g/kg) of nonphytate-P. Celite, a source of acid-insoluble ash, was included as an inert digesta marker.

Table 1. Composition and analysis (g/kg as fed basis, unless specified otherwise) of the basal diets used in experiments 1 to 3

	Experiment 1	Experiment 2	Experiment 3
Wheat	330.50	702.05	702.05
Sorghum	222.60	-	-
Soybean meal	225.00	193.00	193.00
Meat and bone meal	-	57.50	57.50
Canola meal	50.00	-	-
Rice bran	26.00	-	-
Maize gluten meal	25.00	-	-
Vegetable oil	47.00	6.00	6.00
Tallow	-	20.00	20.00
Dextrose	7.50	-	-
Celite	20.00	-	-
Monocalcium phosphate	16.40	-	-
Limestone	16.60	3.50	3.50
Lysine·HCl	-	3.50	3.50
DL-methionine	2.90	2.35	2.35
L-threonine	0.70	-	-
Sodium bicarbonate	-	4.00	4.00
Potassium carbonate	-	1.75	1.75
Salt	3.00	1.00	1.00
Vitamin-mineral premix[1]	5.00	5.00	5.00
Choline chloride	2.00	0.35	0.35
Chemical composition			
AME, MJ/kg[2]	13.1	12.0	12.0
Crude protein[3]	197.5	211.9	223.0
Lysine[2]	10.0	12.9	12.9
Methionine+cysteine[2]	9.3	8.8	8.8
Threonine[2]	8.3	7.1	7.1
Tryptophan[2]	2.5	2.5	2.5
Calcium[3]	10.3	10.8	8.2
Total phosphorus[3]	7.5	7.7	6.0
Phytate-phosphorus[4]	3.0	2.5	2.1

[1] Supplied per kilogram diet: *trans*-retinol, 3.3 mg; cholecalciferol, 87.5 µg; dl-α-tocopheryl acetate, 20 mg; menadione, 2 mg; thiamine, 1.5 mg; riboflavin, 8 mg; calcium pantothenate, 15 mg; niacin, 30 mg; pyridoxine, 5 mg; folic acid, 2 mg; cyanocobalamin, 15 g; biotin, 100 µg; Mn, 75 mg; Zn, 50 mg; Cu, 5 mg; Mo, 1.6 mg; Co, 300 µg; I, 1 mg; Fe, 20 mg; Se, 100 µg; choline chloride, 300 mg; ethoxyquin, 125 mg.

[2] Calculated based on published tabular values for individual ingredients.

[3] Determined values.

[4] Calculated based on analysed values for individual ingredients.

Four experimental diets (nil, 500 FTU/kg phytase, 4,400 EXU/kg xylanase, 500 FTU/kg phytase plus 4,400 EXU/kg xylanase) were fed in mash form to male broiler chicks (Cobb) from 7 to 28 days post-hatch. Granular formulations of the enzymes were used. Each of the four treatments was randomly assigned to six pens of ten chicks each. Body weights and feed intake were recorded at weekly intervals. From days 24 to 27, feed intake and total excreta output were measured quantitatively per pen over four consecutive days for the determination of AME. The excreta were collected daily, dried overnight at 80°C in a force-draft oven and collections from each pen were pooled for analysis. Gross energy of the diet and excreta samples was determined using an adiabatic bomb calorimeter (Gallenkamp Model 16CB110) standardised with benzoic acid. The AME values of the diets were calculated using the following formula. Appropriate corrections were made for differences in moisture content.

$$AME_{diet} = \frac{(\text{Feed intake} \times GE_{diet}) - (\text{Excreta output} \times GE_{excreta})}{\text{Feed intake}}$$

On day 28, all birds were euthanased, digesta contents from the distal half of the ileum were collected and processed as described previously (Ravindran et al., 1999a). Nitrogen (N) contents of diet and ileal digesta samples were analysed using a FP-428 nitrogen determinator (LECO® Corporation, St. Joseph, Michigan, USA) as described by Sweeney (1989). Amino acid concentrations were determined using cation-exchange column chromatographic procedures with post-column derivatisation and fluorimetric detection of amino acids using O-phthaldialdehyde (Ravindran et al., 1999a). Tryptophan contents were determined following alkaline hydrolysis of samples according to the procedures of Ravindran and Bryden (1996). Acid insoluble ash determinations were performed after ashing the samples and treating the ash with boiling 4M hydrochloric acid (Siriwan et al., 1993).

The apparent ileal protein (N×6.25) and amino acid digestibility coefficients were calculated by the following formula using acid insoluble ash as the indigestible marker.

$$\text{Apparent nutrient digestibility (\%)} = \frac{(NT/AIA)_d - (NT/AIA)_i}{(NT/AIA)_d} \times 100$$

where, $(NT/AIA)_d$ = ratio of nutrient and acid insoluble ash in diet, and $(NT/AIA)_i$ = ratio of nutrient and acid insoluble ash in ileal digesta.

Toe samples were also obtained and toe ash contents were determined using the procedures of Potter (1988).

Experiment 2

The objective of this experiment was to investigate interactions between phytase and xylanase supplementation in a conventional wheat-based broiler diet (Table 1) where parameters evaluated included performance, toe ash, AME, gut viscosity, nitrogen retention and pancreatic weight. Male broiler chicks (Cobb), 4-day-old, were randomly assigned to four dietary treatments (nil, 600 FTU/kg phytase, 5,490 EXU/kg xylanase, 510 FTU/kg phytase plus 1,976 EXU/kg xylanase). Liquid forms of the enzymes were used in this experiment. The combined liquid formulation was intended to provide 85% of the individual phytase and 36% of the xylanase activities.

Each of the four treatments consisted of 8 replicates of 6 birds per pen. At 15 and 24 days of age, the birds were weighed and feed intakes determined. From 16 to19 days of age, feed intake and excreta output were measures to determine the AME and apparent N retention. On day 24, two birds nearest the mean cage body weight were selected and euthanased. Samples of toes were taken to determine toe ash contents, intestinal contents for gut viscosity and the pancreas was dissected out and weighed. The N contents of the diet and excreta samples were determined and N retention was calculated using the following formula:

$$\text{N retention} = \frac{(\text{feed intake} \times \text{N content}_{\text{feed}}) - (\text{excreta output} \times \text{N content}_{\text{excreta}})}{(\text{Feed intake} \times \text{N content}_{\text{feed}})} \times 100$$

To determine gut viscosity, intestinal contents from the end of the duodenal loop to the vitelline diverticulum were gently expressed and centrifuged. The viscosity of a 0.5 mL aliquot of the supernatant from the centrifuged digesta samples was determined using a Brookfield viscometer (Model DV-1) with a CP40 cone.

Experiment 3

It was estimated retrospectively that the AME content of wheat used in Experiment 2 was only 13.1 MJ/kg dry matter. This wheat is considered by the feed industry as of inferior quality, as wheat samples with AME of less than 13 MJ/kg are arbitrarily classified as 'low ME' wheats (Mollah et al., 1983). Since the quality of wheat can be expected to influence enzyme responses, the study was repeated using a different wheat which had an estimated AME of 14.8 MJ/kg dry matter. Thus the design and procedures used in Experiments 2 and 3 were identical except that wheat used in Experiment 2 (wheat A) had a lower estimated AME content and a higher analysed phytate concentration than the wheat used in Experiment 3 (wheat B). The analysed phytate-P content of wheats A and B were 2.40 and 1.75 g/kg, respectively. The ostensible difference between the two studies was the feed ingredients used to formulate the diets (Table 1), where wheat quality probably would have been the important differentiating factor.

Feed enzymes

The phytase used (Natuphos® 5000, BASF Aktiengesellschaft, Ludwigshahen, Germany) is a specific phytase feed enzyme produced by a genetically modified isolate of *Aspergillus niger*. Both granular and liquid preparations contained a minimum of 5,000 FTU phytase activity per gram. One phytase unit (FTU) is defined as the amount of enzyme which, at 37°C and pH 5.5, liberates 1.00 micromole of inorganic phosphorus per minute from 0.0051 mole per litre sodium phytate.

Natugrain® Blend (BASF Aktiengesellschaft) was used as the source of xylanase. This feed enzyme has predominantly xylanase activity derived from a genetically modified isolate of *Aspergillus niger* and also contains β-glucanase, cellulase, hemi-cellulase and protease activities generated by a wild strain of *Trichoderma longibrachiatum*. The granular form of the enzyme possessed 55,000 EXU/g and the liquid form contained 36,600 EXU/g endoxylanase activity. One endoxylanase unit (EXU) is defined as the amount of enzyme which, at 40°C and pH 3.5, liberates 4.53 micromole of reducing sugars per minute from xylan, measured as xylose equivalents.

Statistical analysis

The data was statistically assessed by linear regressions and analyses of variance by a general linear models procedure using a SPSS® 8.0 software program (SPSS Inc. Chicago, IL). A one-way ANOVA was used for the three experiments; least significant differences (LSD) were calculated to compare treatments where the effect of treatments was significant at the 5% level of probability.

RESULTS

Experiment 1

Phytase and the phytase plus xylanase combination significantly ($p<0.05$) increased weight gains from 809 to 854 and 868 g/bird, or by 5.6% and 7.3% respectively (Table 2). Xylanase had no influence on gain. All enzyme treatments significantly ($p<0.05$) enhanced feed efficiency, the increments with phytase, xylanase and the combination were 5.4, 3.8 and 7.0% for, respectively. Enzyme supplementation had no effect on feed intake or toe ash contents. The AME content of the diet was influenced ($p<0.05$) by all enzyme treatments. Supplementation with phytase, xylanase and phytase plus xylanase increased AME by 0.59, 0.49 and 0.76 MJ/kg DM, respectively.

The effects of enzyme supplementation on digestibility of amino acids are summarised in Table 2. Of the sixteen amino acids assessed, xylanase increased ($p<0.05$) the ileal digestibility of only serine. Phytase increased ($p<0.05$) the overall digestibility of amino acids by 3.5%, which included significant increases in the digestibility of arginine (4.3%).

Table 2. The effects of phytase and xylanase supplementation, individually and in combination, of a phosphorus-adequate, lysine deficient diet based on wheat and sorghum on the performance, toe ash, apparent metabolisable energy (AME) and ileal amino acid digestibility of broilers[1] (Experiment 1)

Parameter	Control	Phytase	Xylanase	Phytase+xylanase	Pooled SEM
Weight gain, g/bird	809[a]	854[b]	823[a]	868[b]	6.6
Feed intake, g/bird	1506	1499	1476	1497	10.4
Feed per gain, g/g	1.86[a]	1.76[bc]	1.79[b]	1.73[c]	0.012
Toe ash, % dry basis	13.26	12.95	12.93	13.19	0.17
AME, MJ/kg DM	13.79[a]	14.38[bc]	14.28[b]	14.55[c]	0.07
Ileal digestibility, %					
Protein	77.5[a]	80.6[b]	78.1[a]	81.2[b]	0.50
Essential amino acids					
Arginine	81.2[a]	84.7[b]	82.2[a]	85.6[b]	0.51
Histidine	78.9[a]	80.9[ab]	79.7[a]	82.5[b]	0.56
Isoleucine	76.5[a]	78.4[b]	76.2[a]	79.5[b]	0.52
Leucine	76.3[a]	79.2[b]	76.4[a]	79.2[b]	0.50
Lysine	79.0[a]	82.6[b]	79.4[a]	83.0[b]	0.56
Methionine	91.0	91.2	91.0	91.7	0.29
Phenylalanine	77.3[a]	80.6[b]	77.1[a]	80.8[b]	0.49
Threonine	75.0[a]	78.1[b]	74.9[a]	78.5[b]	0.63
Tryptophan	75.8[a]	79.8[b]	76.2[a]	79.5[b]	0.55
Valine	76.5[a]	78.8[b]	76.9[ab]	80.8[c]	0.65
Non-essential amino acids					
Alanine	76.8[a]	79.1[b]	77.2[a]	80.1[b]	0.60
Aspartic acid	78.2[a]	79.8[b]	77.2[a]	80.7[b]	0.45
Glutamic acid	79.9[a]	84.4[b]	80.8[a]	85.6[b]	0.56
Glycine	76.1[a]	78.8[ab]	76.8[a]	80.1[b]	0.92
Serine	74.8[a]	79.4[c]	76.3[a]	79.5[c]	0.51
Tyrosine	76.1[a]	78.1[b]	76.1[a]	78.7[b]	0.60
Overall mean	78.1[a]	80.9[b]	78.4[a]	81.6[b]	0.39

[1] Each mean represents six pens of 10 birds each; diets fed from 7 to 28 days post-hatching.
[a,b,c] Mean values in a row not sharing the same superscript are significantly different ($p<0.05$).

isoleucine (2.5%), leucine (3.8%), lysine (4.6%), phenylalanine (4.3%), threonine (4.1%), tryptophan (5.3%) and valine (3.0%). The combination of phytase plus xylanase significantly increased overall digestibility of amino acids by 4.5% and the digestibility of arginine (5.4%), histidine (4.6%), isoleucine (3.9%), leucine (3.8%), lysine (5.1%), phenylalanine (4.5%), threonine (4.7%), tryptophan (4.9%) and valine (5.6%). The absolute increase in ileal digestibility following supplementation with the combination exceeded the sum of the increases generated by the individual enzymes for alanine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, phenylalanine, threonine, tyrosine and valine. In the case of lysine, the response to the combination equalled the summed response of the two individual enzymes.

For phytase and the combination, the percentage improvements in ileal digestibility were negatively correlated with the inherent digestibility of the amino acids in the control diet. The coefficients of correlation (r) were -0.542 ($p<0.05$) and -0.553 ($p<0.05$) for phytase and the combination, respectively

Experiment 2

The effects of enzyme treatments on growth performance are shown in Table 3. Over the entire feeding period (4-24 days of age) enzyme supplementation increased ($p<0.05$) weight gains, feed intake and feed efficiency. However, it was evident that enzyme supplementation had a more pronounced effect during the early phase of the feeding study. Over the 4-24 days of age, phytase increased gains by 10.6%, but had no effect on feed efficiency. Xylanase increased gains and feed efficiency by 15.1 and 5.7%, respectively. The corresponding increases due to the combination of phytase plus xylanase were 15.4 and 7.0%. Treatments did not have any effect on toe ash contents.

Supplementation of the wheat-based diet with exogenous enzymes increased ($p<0.05$) the AME content and reduced ($p<0.05$) intestinal digesta viscosity (Table 3). Phytase alone caused a small, but significant, reduction in digesta viscosity. Viscosity reductions were more pronounced with xylanase and xylanase plus phytase. Xylanase increased the AME by 1.00 MJ/kg DM and the

Table 3. The effects of phytase and xylanase supplementation, individually and in combination, of a phosphorus-adequate diet based on wheat on the performance, toe ash, apparent metabolisable energy (AME), intestinal digesta viscosity, nitrogen retention and pancreatic weights of broilers[1] (Experiment 2)

Parameter	Control	Phytase	Xylanase	Phytase+xylanase	Pooled SEM
Performance data					
4-15 days of age					
Weight gain, g/bird	244[a]	300[b]	325[b]	324[b]	10.5
Feed intake, g/bird	364[a]	417[b]	413[b]	420[b]	9.4
Feed per gain, g/g	1.53[a]	1.39[b]	1.28[c]	1.30[c]	0.048
16-24 days of age					
Weight gain, g/bird	524[a]	547[ab]	559[b]	560[b]	9.1
Feed intake, g/bird	840	894	879	869	17.0
Feed per gain, g/g	1.61	1.64	1.58	1.55	0.025
4-24 days of age					
Weight gain, g/bird	767[a]	848[b]	883[b]	885[b]	16.1
Feed intake, g/bird	1,204[a]	1,311[b]	1,291[b]	1,290[b]	24.0
Feed per gain, g/g	1.57[a]	1.55[a]	1.48[b]	1.46[b]	0.021
Toe ash, % dry basis	13.51	13.88	13.56	13.07	0.33
AME, MJ/kg DM	13.00[a]	13.28[a]	14.00[b]	13.91[b]	0.14
N retention, %	53.3[a]	58.3[b]	60.2[b]	58.4[b]	0.88
Digesta viscosity, cPs	8.79[c]	6.44[b]	3.28[a]	3.73[a]	0.63
Weight of pancreas, mg/100 g body weight	315[b]	316[b]	265[a]	267[a]	9.5

[1] Each mean represents eight pens of six birds each; diets fed from 4 to 24 days post-hatching.
[a,b,c] Mean values in a row not sharing the same superscript are significantly different ($p<0.05$).

combination by 0.91 MJ/kg DM; whereas phytase numerically increased AME by 0.28 MJ/kg DM. Supplemental enzymes also significantly ($p<0.05$) enhanced apparent nitrogen retention by the birds. Xylanase, phytase plus xylanase and phytase additions increased nitrogen retention by 12.9, 9.6 and 9.5%, respectively. Relative pancreatic weights of birds offered diets supplemented with xylanase and xylanase plus phytase were lower ($p<0.05$) than birds on the control and phytase supplemented diets.

Experiment 3

Over the entire feeding period (4-24 days post-hatch), enzyme supplementation increased weight gains ($p<0.05$) but had no effect on feed intake or feed efficiency (Table 4). Phytase plus xylanase increased gains by 5.6% (906 to 957 g/bird) but, individually, the feed enzymes did not influence the gain. During the early phase, enzyme supplementation significantly enhanced growth rate, feed intake and feed efficiency. However, it is apparent that the growth performance of birds was less responsive to enzyme supplementation in the latter stage of the feeding period.

Phytase plus xylanase and xylanase significantly enhanced ($p<0.05$) AME by 0.67 MJ and 0.58 MJ, respectively (Table 4). All enzyme treatments lowered ($p<0.05$) digesta viscosity, but the reductions with xylanase and phytase plus xylanase were greater than phytase alone. Apparent nitrogen retention was improved ($p<0.05$) only with supplemental xylanase. Phytase plus xylanase and xylanase reduced ($p<0.05$) relative pancreatic weights, whereas phytase had no effect.

DISCUSSION

In all three experiments, the most pronounced improvements in growth performance were associated with the combined supplementation of phytase and xylanase. The enzyme combination increased gains by an average of 8.0% and feed efficiency by 4.7%.

In these experiments, the diets were formulated to contain recommended levels of non-phytate P and their P adequacy was confirmed by the lack of treatment effects on toe ash contents. Thus the improvements in feed efficiency observed with added phytase in Experiment 1 cannot be attributed increased P availability induced by phytase, but rather to enhanced protein and/or energy utilisation. The capacity of phytase to increase the apparent ileal digestibility of amino acids in broiler chickens was demonstrated in Experiment 1. This is consistent with the results from a number of studies where phytase has increased amino acid digestibilities from a range of feed ingredients (Ravindran et al., 1999a) and a variety of diet types (Kornegay, 1996; Kornegay et al., 1999; Namkung and Leeson, 1999; Ravindran et al., 2000; Ravindran et al., 2001; Camden et al., 2001). While the negative effects of phytate on apparent ileal digestibility of amino acids require further elucidation, it appears that the main influence of phytate is to reduce protein digestion by forming phytate-protein complexes. This contention is based on the propensity for phytate to bind with protein under acidic conditions to form binary protein-phytate complexes

Table 4. The effects of phytase and xylanase supplementation, individually and in combination, of a phosphorus-adequate diet based on wheat on the performance, toe ash, apparent metabolisable energy (AME), intestinal digesta viscosity, nitrogen retention and pancreatic weights of broilers[1] (Experiment 3)

Parameter	Control	Phytase	Xylanase	Phytase+xylanase	Pooled SEM
Performance data					
4-15 days of age					
Weight gain, g/bird	343[a]	327[a]	380[b]	390[b]	7.0
Feed intake, g/bird	474[a]	455[a]	504[bc]	523[c]	11.0
Feed per gain, g/g	1.38[ab]	1.39[a]	1.33[b]	1.34[ab]	0.019
16-24 days of age					
Weight gain, g/bird	563	553	556	567	11.4
Feed intake, g/bird	852	850	853	872	16.9
Feed per gain, g/g	1.51	1.54	1.54	1.54	0.022
4-24 days of age					
Weight gain, g/bird	906[ab]	881[a]	935[ab]	957[c]	15.0
Feed intake, g/bird	1326	1305	1358	1394	24.5
Feed per gain, g/g	1.46	1.48	1.45	1.46	0.051
Toe ash, % dry basis	12.58	12.23	12.15	12.06	0.27
AME, MJ/kg DM	14.17[a]	14.14[a]	14.72[b]	14.81[b]	0.11
N retention, %	61.0[ab]	60.4[a]	63.9[c]	62.5[bc]	0.71
Digesta viscosity, cPs	10.21[a]	7.61[b]	3.77[c]	4.66[c]	0.82
Weight of pancreas, mg/100 g body weight	280[ab]	295[a]	261[bc]	252[c]	9.3

[1] Each mean represents eight pens of six birds each: diets fed from 4 to 24 days post-hatching.
[a,b,c] Mean values in a row not sharing the same superscript are significantly different ($p<0.05$).

(Rajendran and Prakash, 1993) in the upper digestive tract and that bound protein is refractory to pepsin digestion (Vaintraub and Bulmaga, 1991). Pepsin has a dual role, this endogenous enzyme initiates protein digestion and the peptide end-products generated by pepsin partially regulate the pancreatic phase of the protein digestive process by triggering the release of enteric hormones, including cholecystokinin and gastrin (Hopfer, 1997). It follows that phytate, by complexing with protein, interferes with both of pepsin's roles. Additionally, complexed dietary protein may be less readily digested in the more alkaline conditions of the small intestine although, in theory, binary protein-phytate complexes do not remain intact once the isoelectric point of protein is exceeded. Phytase, by the prior hydrolysis of phytate, may largely prevent the *de novo* formation of binary protein-phytate complexes in the upper digestive tract and ameliorate the negative influence of phytate on digestibility of dietary protein (Selle et al., 2000).

It has been suggested that phytate may also increase endogenous amino acid losses and that part of the improvements in apparent amino acid digestibility following phytase addition may arise from reduced endogenous losses (Ravindran et al., 1999a). Although there does not appear to be any direct evidence that phytate promotes endogenous amino acid losses, the typical pattern of responses to phytase supplementation is consistent with this possibility.

Xylanase had no effect on the digestibility of amino acids in Experiment 1 and this lack of response may be explained on the basis of the relatively low dietary inclusion of wheat (331 g/kg). Nevertheless, the capacity of xylanase to increase the apparent ileal digestibility of amino acids of wheat-based broiler diets has been previously demonstrated (Hew et al., 1998; Bedford et al., 1998). While the possibility that NSP may directly lower the digestibility of dietary amino acids cannot be dismissed, there is sound evidence that wheat pentosans increase the secretion of endogenous amino acids in broiler chicks (Angkanaporn et al., 1994). Decreased reabsorption of endogenous amino acids will also promote losses (Nyachoti et al., 2000) and it is possible that NSP both increase secretion and decrease reabsorption of endogenous amino acids.

The combination of phytase and xylanase had the most positive impact on amino acid digestibility, to the extent that synergistic increases were recorded in the apparent ileal digestibility of alanine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, phenylalanine, threonine, tyrosine and valine following phytase plus xylanase supplementation. These findings are in agreement with the report by Ravindran et al. (1999b), who noted similar synergistic responses for alanine, arginine, glycine, histidine, leucine, lysine, methionine phenylalanine and tyrosine following the addition of phytase plus xylanase to wheat-casein diets. As previously discussed, it is therefore possible that phytate essentially decreases the digestion of dietary protein and NSP essentially increase losses of endogenous amino acids. Then the simultaneous inclusion of phytase and xylanase in wheat-based diets would have complementary modes of action in enhancing the apparent ileal digestibility of amino acids.

Phytate is concentrated in the aleurone layer of wheat (Ravindran et al., 1995) and it is possible that xylanase, by degrading the cell wall matrix, increases the access of phytase to its substrate. This is supported by the *in vitro* observations of Parkkonen et al. (1997) who found that xylanase increased the access of proteolytic enzymes to substrates in the aleurone layer and it follows that this would also apply to phytase. Interestingly, phytate is associated with soluble fibre components of wheat under *in vitro* conditions. (Frolich et al., 1984; Frolich, 1990), which raises the possibility that phytase and xylanase may reciprocally facilitate substrate access in the gut lumen. Enhanced substrate access and complementary modes of action may explain the more pronounced effects of phytase plus xylanase on amino acid digestibility in wheat-based broiler diets.

Phytase supplementation increased average dietary AME by 0.28, from 13.65 to 13.93 MJ/kg DM, in the three present experiments. While the mechanisms responsible for improved AME from phytase are unclear, that phytase increases AME of poultry diets has been previously reported in a number of studies (Namkung and Leeson, 1999; Ravindran et al., 2000; Ravindran et al., 2001; Camden et al., 2001). Of relevance is that Camden et al. (2001) found that phytase supplementation increased the apparent ileal digestibility of protein, starch and fat of corn-soy broiler diets while degrading phytate at the ileal level. It has been suggested that phytate may directly or indirectly interact with starch (Thompson, 1998) and phytate inhibits α-amylase activity *in vitro* (Kikunaga et al., 1991); so phytase may facilitate starch digestion by ameliorating these effects. Phytase may enhance fat digestion due to the involvement of Ca-phytate complexes in the formation of metallic soaps in the gut lumen which limit energy utilisation from fat, logically, this would be reversed by phytase (Ravindran et al., 2000).

Xylanase increased average dietary AME by 0.68, from 13.65 to 14.33 MJ/kg DM in the three experiments. The capacity of exogenous xylanases to enhance energy utilisation of wheat-based poultry diets is extensively documented (Bedford and Schulz, 1998). Phytase plus xylanase significantly increased dietary AME by 0.77, from 13.65 to 14.42 MJ/kg DM in the same experiments. It is probable that the greater increment in AME in Experiment 2 with xylanase supplementation, individually and in combination with phytase, was due the poorer quality of wheat A (Choct et al., 1995; Choct, 1998).

As anticipated, xylanase, alone and in combination with phytase, reduced gut viscosity. Phytase also reduced gut viscosity to a to a lesser, but significant extent. A similar finding has been previously reported (Jacob et al., 2000), but other phytase preparations have not has this effect (Zyla et al., 1999; 2000). Dietary factors other than soluble arabinoxylans may increase gut viscosity. It has been reported that certain protein fractions of wheat with visco-elastic properties can contribute to higher viscosities (Scheele et al., 1995), so phytase may lower gut viscosity by facilitating the digestion of wheat protein. There is, however, also the possibility that the phytase preparation used contained enzymic 'side-activities' (Farrell and Martin, 1998) and any such xylanase activity may have affected gut viscosity.

The possible inhibition of trypsin activity by phytate has real implications as it would be expected to increase endogenous amino acid losses (Barth et al., 1993; Caine et al., 1998); however, several *in vitro* studies have investigated this possibility with conflicting results. While Singh and Krikorian (1982) and Caldwell (1992) argued that phytate does inhibit trypsin, similar findings were not reported by a number of workers (Kanaya et al., 1976; Inagawa et al., 1987; Reddy et al., 1988; Knuckles et al., 1989; Vaintraub and Bulmaga, 1991). In broiler chicks, trypsin inhibition has been shown to cause pancreatic hypertrophy following the dietary inclusion of rice bran, which intrinsically possesses trypsin inhibitor activity (Kratzer et al., 1974; Kratzer and Payne, 1977). If phytate does inhibit trypsin and increase pancreas size, it follows that phytase, by ameliorating this effect, could reduce pancreatic mass; however, phytase had no effect on relative pancreatic weights. Furthermore, in ducklings, phytase supplementation did not alter pancreatic and small intestinal levels of trypsin (Martin et al., 1998), which suggests phytate was not influencing these indicators of trypsin activity. In contrast, xylanase reduced pancreatic weights by 15.9% and 6.8% in Experiments 2 and 3 respectively. Various plant fibre sources (Schneeman, 1977) and polysaccharides (Ikeda and Kusano, 1983) have been found to inhibit trypsin activity *in vitro*. Moreover, in rats, viscous polysaccharides have been shown to increase secretion of digestive enzymes and pancreatic weights (Ikegami et al., 1990). Thus the findings of the present study are consistent with NSP inhibition of trypsin activity but do not support the proposal that phytate inhibits trypsin activity.

It is instructive to compare the effects of phytase plus xylanase supplementation of diets based on different wheats by pooling the results of Experiments 2 and 3 (Selle et al., 2002). There were interactions between wheat type and enzyme addition for growth rate ($p=0.06$) and feed conversion ($p=0.009$) as greater responses to enzyme supplementation were observed were observed with 'wheat A' in Experiment 2. There was an associated interaction for N retention ($p=0.053$), but not AME ($p=0.302$). The greater growth performance responses seen with 'wheat A' are consistent with its higher levels of phytate and, presumably, NSP; but it is interesting that increased protein utilisation, generated by the enzyme combination, appears to be

responsible for the growth performance interactions.

In conclusion, recent studies have shown that the simultaneous inclusion of phytase and xylanase in wheat-based broiler diets is advantageous (Ravindran et al., 1999; Zyla et al., 1999) and the present data confirms these benefits. Of particular interest is the synergetic nature of the increases in apparent ileal digestibility of several amino acids including histidine, isoleucine, phenylalanine, threonine and valine following supplementation with phytase plus xylanase. These synergistic responses are suggestive of complementary modes of action and/or increased substrate access. Clearly further studies are warranted to define the optimum inclusion rates when phytase and xylanase are used in combination and to clarify their modes of operation when used in tandem.

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