Effect of a novel phytase on growth performance, apparent metabolizable energy, and the availability of minerals and amino acids in a low-phosphorus corn-soybean meal diet for broilers

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ABSTRACT The addition of microbial phytase to diets for broiler chickens has been shown to improve the availability of phytate P, total P, some other minerals, and amino acids. In this study, the effect of a novel microbial phytase expressed by synthetic genes in *Aspergillus oryzae* on amino acid and mineral availability was assessed. Phytase was incorporated (1,000 and 2,000 U/kg) into low-P corn-soybean meal-based diets for broilers. Broilers received the experimental diets for 3 wk, and excreta were collected from d 18 to 21 for the determination of AME and mineral retention. On the 22nd day, the broilers were killed and the left leg removed and ileal digesta collected. Ileal phytate P and total P absorption, ileal amino acid digestibility, as well as the bone mineral content and bone mineral density were determined. Ileal phytate P absorption and absorbed phytate P content of the low-P corn-soybean meal diet were significantly ($P < 0.05$) higher after dietary inclusion of the novel phytase (49–60% and 65–77% higher, respectively). Apparent ileal total P absorption and apparent total P retention was 12 to 16% and 14 to 19% higher ($P < 0.05$), respectively, after dietary inclusion of phytase. The bone mineral content and bone mineral density in the tibia were 32 to 35% and 19 to 21% higher ($P < 0.05$), respectively, after dietary phytase inclusion. The apparent ileal digestibility of threonine, tyrosine, and histidine increased significantly ($P < 0.05$) by 14, 9, and 7%, respectively, after dietary inclusion of microbial phytase. Overall, the inclusion of a novel microbial phytase into a low-P corn-soybean meal diet for broiler chickens greatly increased phytate P and total P absorption, bone mineral content and density, as well as the digestibility of some amino acids.

Key words: phytase, phytate, broiler, phosphorus, amino acid

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INTRODUCTION

Phytate (inositol hexaphosphate), which is present in many plant-based feedstuffs, is the main phosphorus store in plants (Cosgrove, 1980). Moreover, the bioavailability of phosphorus present in phytate is generally poor (Camden et al., 2001; Rutherfurd et al., 2002). In addition, phytate can complex divalent cations as well as amino acids and proteins, and when present in diets for monogastric animals, can reduce the digestibility and absorption of the latter nutrients.

Phytase is an enzyme that dephosphorylates phytate and falls into 2 main categories: phytases of fungal origin, such as those from *Aspergillus* or *Peniophera* species, or phytases of bacterial origin, such as those from *Escherichia coli*. Both fungal- and bacterial-derived phytases are commonly added to broiler diets and have been shown in some studies to improve the bioavailability of phosphorus (Camden et al., 2001; Rutherfurd et al., 2004a; Cowieson and Adeola, 2005), other minerals (such as calcium, magnesium, potassium, and zinc; Ravindran et al., 2008; Santos et al., 2008; Saima et al., 2009), and amino acids, but particularly threonine (Sebastian et al., 1997; Camden et al., 2001; Rutherfurd et al., 2004a). Although not all studies investigating the impact of dietary phytase supplementation on the bioavailability of minerals other than P and on the bioavailability of amino acids show improvements. For example, for calcium, Um et al. (2000) reported no improvement in availability, whereas for amino acids Camden et al. (2001) reported increases in ileal tyrosine and histidine digestibility with dietary phytase inclusion, but Rutherfurd et al. (2004a) and Zhang et al. (1999) observed no such effect. In addition, and where ileal amino acid digestibility has been found to increase with microbial phytase supplementation, the extent to

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which digestibility increases varies across amino acids, depending on the study. For example, Santos et al. (2008) reported cysteine as the most affected amino acid in one study, whereas threonine appears to be the most affected amino acid in other similar studies (Sebastian et al., 1997; Camden et al., 2001).

There are 2 classes of phytase that have been differentiated based on the first phosphate group in the phytate molecule to undergo phytase attack. The 3-phytases initially attack the carbon in the third position, whereas 6-phytases initially attack the carbon atom in the sixth position. Phytases from different sources and even from the same source can have different pH optima, heat stability, and catalytic properties (Konietzny and Greiner, 2002). Furthermore, when microbial phytases from different sources are included into diets for intensive livestock their efficacy in vivo can vary in comparison to their assayed activity because the activity at pH 2 to 3 (as is present in the stomach) compared with pH 5.5 (the pH at which phytase is routinely assayed) varies across phytases of different origin (Greiner and Bedford, 2010). Recently, a new 6-phytase had been prepared via the expression of synthetic genes in *Aspergillus oryzae* (Aureli et al., 2011). The efficacy of this novel phytase in broilers with respect to growth performance, P and Ca utilization, and tibia strength and tibia ash content has been reported using a low-P corn-soybean meal-based broiler diet (Aureli et al., 2011). The aim of the present study was to examine the effect of dietary inclusion of the same novel 6-phytase on phytate P availability, mineral retention, bone mineral density, and ileal amino acid digestibility in a low-P corn-soybean diet in broiler chickens.

MATERIALS AND METHODS

Growth performance, AME, bone mineral density, toe ash, the ileal phytate P and total phosphorus absorption, apparent fecal mineral retention, and true and apparent ileal amino acid digestibility were determined in broiler chickens receiving either an adequate-P (formulated to contain 0.45% available P) corn-soybean meal diet (positive control) or a low-P (formulated to contain 0.35% available P) corn-soybean meal diet containing either no phytase (negative control) or the novel microbial phytase [Ronozyme HiPhos (GT), Novozymes A/S, Bagavaerd, Denmark] supplemented at 2 dietary concentrations (100 g/t and 200 g/t) representing one times and 2 times the manufacturers recommended inclusion level and resulting in predicted dietary phytase activities of 1,107 U/kg and 2,215 U/kg, respectively. Ronozyme HiPhos (GT) is a 6-phytase that is produced by the expression of synthetic genes that have been incorporated in *Aspergillus oryzae*. The genes are synthesized based on the protein sequence of the phytase enzyme in *Citrobacter brakii* (ATCC 5111; Lichtenberg et al., 2011). The ingredient compositions of the 4 experimental diets are given Table 1 and the calculated nutrient compositions in Table 2. Phytase activity of the microbial phytases was determined before addition to the diets using the method described by the International Organization for Standardization (ISO, 2009). Titanium dioxide was included in each diet as an indigestible marker.

In Vivo Trial

This study was approved by the Animal Ethics Committee, Massey University, Palmerston North, New Zealand. In total, 240 one-day-old Ross male broiler chicks were randomly allocated to one of the 4 experimental diets such that there were 60 birds per diet. The birds were group-housed in wire cages with 6 birds per cage and 10 cages (the experimental unit) per treatment in a controlled room with 20L:4D and at a temperature of 31 ± 2°C on the first day, which was reduced to 21 ±

Table 1. Ingredient composition (g/kg) of the experimental diets

Ingredient	Adequate P	Low P	Low P + phytase (100 g/t)	Low P + phytase (200 g/t)
Phytase	—	—	0.10	0.20
Maize	537.0	539.7	533.9	533.8
Soybean meal	310	310	310	310
Wheat bran	50	50	50	50
Rapeseed meal	30	30	30	30
Soybean oil	40	40	40	40
L-Methionine	1.0	1.0	1.0	1.0
NaCl	4.0	4.0	4.0	4.0
Dicalcium phosphate	7.5	10.5	10.5	10.5
Monocalcium phosphate	6.3	—	—	—
Calcium carbonate	9.2	9.8	9.8	9.8
Vitamin mix[1]	0.50	0.50	0.50	0.50
Mineral mix[1]	1.5	1.5	1.5	1.5
Titanium dioxide	3.0	3.0	3.0	3.0

[1]Vitamin and mineral content of the diets derived from the premixes (Vitec Nutrition Ltd., Auckland, New Zealand) were as follows (supplied per kilogram): Mn, 125.0 mg; Zn, 60.0 mg; Cu, 5.0 mg; Co, 0.3 mg; Fe, 25.0 mg; I, 1.0 mg; choline, 637.5 mg; antioxidants, 100.0 mg; vitamin A (retinylacetate), 10,000 IU; vitamin D (cholecalciferol), 2,400 IU; vitamin E, 66 IU; vitamin K, 4.0 mg; thiamine, 3.0 mg; riboflavin, 12.0 mg; nicotinic acid, 35.0 mg; pantothenic acid, 12.8 mg; pyridoxine, 10.0 mg; vitamin B_{12}, 0.017 mg; folic acid, 5.2 mg; biotin, 0.2 mg. Dicalcium phosphate, monocalcium phosphate, and calcium carbonate were obtained from Denver Stock feeds (Palmerston North, New Zealand).

Table 2. Determined nutrient composition (g/kg) of the experimental diets

Ingredient	Adequate P	Low P	Low P + phytase (100 g/t)	Low P + phytase (200 g/t)
Phytase activity (U/kg)	—	—	1,107	2,215
Crude protein	204	201	198	200
Digestible protein[1]	194	189	187	188
Total P	6.5	5.6	5.7	5.8
Phytate P	3.4	3.2	3.6	3.5
Ca	8.0	8.9	8.7	9.0
Mg	2.1	2.2	2.1	2.2
K	10.3	9.8	10.0	9.4
Na	2.0	1.6	1.6	1.7
S	2.4	2.3	2.3	2.4
Cu (mg/kg)	23.3	26.1	25.9	27.1
Fe (mg/kg)	235	233	219	240
Aspartic acid	23.1	20.7	22.3	23.5
Threonine	6.3	5.9	6.5	5.8
Serine	9.6	8.7	10.3	10.5
Glutamic acid	39.6	37.6	40.6	41.3
Glycine	9.3	8.2	9.6	10.3
Alanine	10.2	9.8	10.4	10.5
Cysteine	3.4	3.3	3.3	3.3
Valine	10.2	9.2	10.6	10.7
Methionine	3.5	3.5	3.3	3.4
Isoleucine	8.4	7.8	8.6	8.6
Leucine	18.5	18.3	19.6	19.8
Tyrosine	5.7	5.3	7.1	7.1
Phenylalanine	10.7	10.2	11.4	11.6
Histidine	3.8	3.6	4.3	3.7
Tryptophan	2.8	2.7	2.6	2.6
Lysine	12.1	10.9	10.9	12.5
Arginine	13.3	12.3	15.2	16.0

[1]Calculated based on true ileal nitrogen digestibility.

2°C by d 21. Water was available at all times. The birds were weighed at the start of the trial and then weekly thereafter. The experimental period for the growth study lasted for 21 d, and during this time and up to 1600 h on d 21, the birds were fed ad libitum. Feed intake was recorded weekly over the experimental period and also over a 4-d period from d 18 to 21 to permit the determination of the apparent energy metabolizability (AEM) and AME of the diets and mineral retention (using the total collection method). Total excreta were collected for each cage from d 18 to 21 of the trial (4-d collection). The excreta were freeze-dried and stored at −20°C before analysis for gross energy and minerals.

On d 22, the broilers were killed by a lethal injection of sodium pentobarbitone (Pentobarb 300, Southern Veterinary Supplies, Christchurch, New Zealand) and the left leg removed to obtain bone and toe samples. The body cavity was then opened and terminal ileal digesta collected as described by Ravindran et al. (1999). Ileal digesta were pooled across all birds housed within the same cage before being freeze-dried and stored at −20°C, ready for chemical analysis. The diets and ileal digesta samples were analyzed for phytate P, total P, amino acids, total nitrogen, and titanium dioxide contents.

Chemical Analysis

Amino acid content was determined as described by Rutherfurd et al. (2012). The total nitrogen content was determined on a LECO analyzer (LECO Corp., St. Joseph, MI) using the Dumas method, and CP was calculated as the total nitrogen content multiplied by 6.25. The titanium dioxide content was determined based on the method of Short et al. (1996) and phytate P was determined using the method of Rutherfurd et al. (2004b). Total P was determined in the diets and digesta after hydrolyzing the samples in an H_2SO_4:HNO_3:H_2O (1:1:1) solution before being determined spectrophotometrically after reaction with ammonium molybdate and amino-naptholsulfonic acid (Rutherfurd et al., 2004b). The mineral content (Na, P, K, Ca, Mg, Fe, Cu, and S) of the diets and excreta were determined using inductively coupled plasma mass spectrometry. Bone mineral content (**BMC**) and bone mineral density (**BMD**) were determined in the left leg bones for each bird using a Hologic Discovery A bone densitometer (Bedford, MA), and toe ash of the left middle toe between the second and third tarsal bones (Wu et al., 2004) was determined gravimetrically after ashing at 500°C using a muffle furnace.

Data and Statistical Analysis

Ileal nutrient flows were calculated as follows (units are mg/kg of DM):

Ileal flow (mg/kg of DM intake; **DMI**) = Ileal content × Diet titanium content/Ileal titanium content.

The amount of phytate P disappearance at the terminal ileum, ileal total P absorption, and true ileal amino acid digestibility was calculated as described by Rutherfurd et al. (2004a).

The apparent mineral retention was calculated as follows:

$$\text{Apparent mineral retention (\%)} = [\text{Mineral}_{(\text{Diet})} \text{ (mg/kg)} \times \text{Feed intake (kg)} - \text{Mineral}_{\text{Excreta}} \text{ (mg/kg)} \times \text{Total excreta (kg)}]/[\text{Mineral}_{\text{Diet}} \text{ (mg/kg)} \times \text{Feed intake (kg)}] \times 100.$$

Apparent retained mineral content of the diets was calculated as follows (units are mg/100 g for the macrominerals and mg/kg for the microminerals):

$$\text{Apparent retained mineral content} = \text{Mineral}_{\text{Diet}} \times \text{Apparent mineral retention (\%)}.$$

Bone mineral density (BMD) was calculated as prescribed by WHO (2003) and is shown as follows:

$$\text{BMD (g/cm}^2) = \text{BMC (g)/Bone surface area of the 2-dimensional scan (cm}^2),$$

where BMC is the bone mineral content.

The AEM was calculated based on the equations of Camden et al. (2001):

$$\text{AEM (\%)} = [\text{Gross energy}_{\text{Diet}} \text{ (kcal/kg)} \times \text{Feed intake (kg)} - \text{Gross energy}_{\text{Excreta}} \text{ (kcal/kg)} \times \text{Total excreta (kg)}]/[\text{Gross energy}_{\text{Diet}} \text{ (kcal/kg)} \times \text{Feed intake (kg)}] \times 100.$$

The AME of the diets was calculated as follows (units are kcal/kg):

$$\text{AME} = \text{Gross energy}_{\text{Diet}} \times \text{AEM (\%)}.$$

Data were analyzed statistically using ANOVA (GLM procedure; SAS Institute, 1999) followed by orthogonal contrast analysis where statistically significant ($P < 0.05$) differences between means were observed.

RESULTS

Growth Performance, AEM, and AME

Over the entire 3-wk period, the feed intake and weight gain were lower ($P < 0.05$) for the birds fed the unsupplemented low-P diet compared with the adequate-P diet (Table 3). Furthermore, there was an increase ($P < 0.01$) in feed intake and weight gain but no effect ($P > 0.05$) on the feed intake to weight gain ratio for the birds fed the phytase (100 g/t)-supplemented low-P diet compared with unsupplemented low-P diet such that performance was equal to that of the adequate-P diet. There was no difference ($P > 0.05$) between supplementing with 100 g/t or 200 g/t of phytase with respect to feed intake or weight gain. For AEM, there was no difference ($P > 0.05$), but AME differed ($P < 0.05$) across treatments, although the differences were small (<2%).

Toe Ash, BMC, and BMD

The toe ash content and all BMC and BMD measurements for the birds fed the low-P diet supplemented with either 100 or 200 g/t of phytase were higher ($P < 0.001$) compared with the unsupplemented low-P diet and were not different ($P > 0.05$) from the birds fed the adequate-P diet (Table 4). In addition, there was no difference ($P > 0.05$) between the 2 phytase-supplemented low-P diets with respect to toe ash content and all BMC and BMD measurements.

Phosphorus

True ileal phytate P absorption and true ileal absorbed phytate P content were not different ($P > 0.05$) between the adequate-P diet and the unsupplemented low-P diet (Table 5). However, dietary supplementation with phytase increased ($P < 0.001$) true ileal phytate P absorption and ileal absorbed phytate P content in comparison not only with the unsupplemented low-P diet but also the adequate-P diet. There was no difference ($P > 0.05$) between the 2 phytase inclusion levels with respect to phytate P absorption or absorbed phytate P content.

Apparent and true ileal total P absorption of the phytase-supplemented low-P diets was higher ($P < 0.01$) compared with the unsupplemented low-P diet for both dietary phytase concentrations. In addition, apparent and true ileal total P absorption were not different ($P > 0.05$) between the phytase-supplemented low-P diet and the adequate-P diet. Apparent total P retention was higher ($P < 0.001$) for the phytase-supplemented low-P diets compared with both the unsupplemented low-P diet and the adequate-P diet.

The apparent and true ileal absorbed total P contents were lower ($P < 0.05$), and apparent total P retention higher ($P < 0.05$), for the unsupplemented low-P diet compared with the adequate-P diet. However, there was no difference ($P > 0.05$) between the apparent and true ileal total P absorption and the apparent retained total P content of the unsupplemented low-P diet and the adequate-P diets.

Apparent and true ileal absorbed total P contents of the phytase-supplemented low-P diets (both dietary phytase concentrations) were higher ($P < 0.001$) than for the unsupplemented low-P diet but not different ($P > 0.05$) from that of the adequate-P diet. Appar-

Table 3. Mean[1] feed intake, weight gain, feed to weight gain ratio, apparent energy metabolizability (AEM), and AME for the broiler chickens fed the experimental diets

	Diet					
Item	Adequate P	Low P	Low P + phytase (100 g/t)	Low P + phytase (200 g/t)	Overall SE	Significance[2]
AEM (%)	75.7	75.2	75.1	75.7	0.29	NS
AME (kcal/kg)	3,127[a]	3,084[ab]	3,080[b]	3,102[ab]	12.7	*
Feed intake (g/d)						
d 1–7	18.4[ab]	17.4[b]	19.0[a]	18.6[ab]	0.32	**
d 8–14	45.5[a]	40.7[b]	45.3[a]	42.7[ab]	0.91	**
d 15–21	75.9	70.3	73.9	74.6	1.50	NS
d 1–21	46.9[a]	43.1[b]	46.3[a]	45.3[ab]	0.65	**
Weight gain (g/d)						
d 1–7	14.0	12.8	13.6	13.4	0.30	NS
d 8–14	32.9[a]	29.1[b]	32.5[a]	31.3[ab]	0.67	***
d 15–21	53.0[a]	49.2[b]	53.2[a]	52.6[ab]	0.98	*
d 1–21	33.3[a]	30.4[b]	33.1[a]	32.4[ab]	0.53	**
Feed to weight gain ratio						
d 1–7	1.32	1.36	1.41	1.39	0.030	NS
d 8–14	1.38	1.40	1.40	1.37	0.024	NS
d 15–21	1.43	1.43	1.39	1.42	0.018	NS
d 1–21	1.41	1.42	1.40	1.40	0.013	NS

[a,b]Means within rows with different superscripts were significantly different.

[1]n = 10.

[2]NS not significant $P > 0.05$.

*$0.05 > P > 0.01$; **$0.01 > P > 0.001$; ***$P < 0.001$.

ent retained total P content was higher ($P < 0.001$) for the phytase-supplemented low-P diets (both dietary phytase concentrations) compared with the unsupplemented low-P and adequate-P diets.

The amount of P present in the excreta was approximately 32% lower for the birds fed the phytase-supplemented low-P diets compared with the birds fed the adequate-P diet.

Mineral Absorption and Retention

In addition to P, the apparent retention and apparent retained content of other minerals was determined for each of the experimental diets (Table 6). There was no difference ($P > 0.05$) between treatments for the apparent retention of K and Fe nor the retained Fe content. When compared with the unsupplemented low-P diet, supplementation with phytase (200 g/t) increased ($P < 0.01$) the apparent retention of Ca, Mg, and S and the apparent retained content of Ca, Mg, Na, and S. Supplementation with 100 g/t of phytase increased ($P < 0.05$) the apparent Ca retention and apparent retained Ca content only, when compared with the unsupplemented low-P diet. There was no difference ($P > 0.05$) between the adequate-P diet and the low-P diet supplemented with 200 g/t phytase with respect to the apparent retention of Mg, Na, and S and the apparent retained content for S. In contrast, the apparent mineral retention of Ca, Cu was 11% and 141% higher, respectively, and the apparent retained mineral content of Ca,

Table 4. Mean[1] toe ash, bone mineral content, and bone mineral density of the tibia, femur, and entire left leg of the broilers receiving the experimental diets

	Diet					
Item	Adequate P	Low P	Low P + phytase (100 g/t)	Low P + phytase (200 g/t)	Overall SE	Significance
Toe ash (g/100 g of DM)	12.6[a]	11.4[b]	12.6[a]	12.8[a]	0.24	***
Bone mineral content (g)						
Whole leg	3.32[a]	2.54[b]	3.14[a]	3.17[a]	0.094	***
Tibia	1.08[a]	0.80[b]	1.08[a]	1.06[a]	0.041	***
Femur	0.84[a]	0.59[b]	0.77[a]	0.79[a]	0.033	***
Bone mineral density (mg/cm^2)						
Whole leg	142[a]	126[b]	144[a]	143[a]	2.8	***
Tibia	218[a]	186[b]	225[a]	221[a]	4.7	***
Femur	208[a]	169[b]	203[a]	209[a]	5.0	***

[a,b]Means within rows with different superscripts were significantly different.

[1]n = 10.

***$P < 0.001$.

Table 5. Mean[1] true ileal phytate P absorption, true ileal absorbed phytate P content, apparent and true[2] ileal total P absorption, apparent and true[2] ileal absorbed total P content, apparent total P retention[3] and apparent retained total P content[3] for the four experimental diets

	Diet					
Item	Adequate P	Low P	Low P + phytase (100 g/t)	Low P + phytase (200 g/t)	Overall SE	Significance
True ileal phytate P absorption (%)	35.7[b]	32.2[b]	51.5[a]	48.0[a]	3.16	***
True ileal absorbed phytate P content (mg/100 g)	123[b]	105[b]	185[a]	172[a]	11.0	***
True ileal total P absorption (%)	64.2[ab]	60.5[b]	66.9[a]	68.7[a]	1.67	**
True ileal absorbed total P content (mg/100 g)	407[a]	339[b]	382[a]	402[a]	9.8	***
Apparent ileal total P absorption (%)	57.8[ab]	53.3[b]	59.9[a]	61.9[a]	1.67	**
Apparent ileal absorbed total P content (mg/100 g)	367[a]	299[b]	342[a]	362[a]	9.8	***
Apparent total P retention (%)	46.9[d]	51.8[c]	58.9[b]	61.4[a]	0.61	***
Apparent retained total P content (mg/100 g)	298[c]	290[c]	336[b]	361[a]	3.6	***

[a,b]Means within rows with different superscripts were significantly different.

[1]n = 10.

[2]Apparent ileal total P absorption values were corrected to true values using endogenous total P flows determined by Rutherfurd et al. (2004a).

[3]Based on dietary total P content and the total P present in the excreta.

**0.01 > P > 0.001; ***P < 0.001.

Mg, and Cu was 25, 15, and 180% higher ($P < 0.05$), respectively, for the 200 g/t phytase-supplemented low-P diet compared with the adequate-P diet. Apparent Na retention and apparent retained Na content were 9% and 27% lower ($P < 0.01$), respectively, for the 200 g/t phytase-supplemented low-P diet compared with the adequate-P diet. The apparent mineral retention of Ca and the apparent retained Ca content was 7% higher ($P < 0.05$) for the 100 g/t phytase-supplemented low-P diet compared with the adequate-P diet. In contrast, the apparent mineral retention of Na was 11% lower ($P < 0.05$) and the apparent retained mineral content for Na, K, and S was 34, 11, and 4% lower ($P < 0.05$) respectively for the 100 g/t phytase-supplemented low-P diet compared with the adequate-P diet.

Amino Acids

Apparent and true ileal CP and amino acid digestibility were determined for each of the 4 dietary treatments (Table 7 and 8). There was no difference ($P > 0.05$) across treatments for apparent or true ileal CP digestibility. However, there was a difference ($P < 0.05$) across treatments for the apparent ileal digestibility of all of the amino acids. The apparent ileal digestibility of most amino acids was not different ($P > 0.05$)

Table 6. Mean[1] apparent mineral (excluding P) retention[2] (%) and apparent retained mineral (excluding P) content[2] for the four experimental diets

	Diet					
Item	Adequate P	Low P	Low P + phytase (100 g/t)	Low P + phytase (200 g/t)	Overall SE	Significance[3]
Apparent mineral retention						
Ca	52.6[b]	50.4[b]	56.6[a]	58.4[a]	0.98	***
Mg	21.6[ab]	21.0[b]	20.1[b]	24.1[a]	0.76	**
Na	61.1[a]	54.8[b]	55.0[b]	56.2[ab]	1.37	**
K	30.9	29.4	28.7	28.2	0.80	NS
S	64.1[ab]	63.5[b]	64.5[ab]	65.8[a]	0.56	*
Cu	6.5[b]	14.6[a]	12.2[ab]	15.7[a]	2.07	**
Fe	18.1	9.4	5.0	10.7	4.61	NS
Apparent retained mineral content						
mg/100 g						
Ca	420.5[c]	447.5[c]	491.1[b]	525.8[a]	8.5	***
Mg	45.0[b]	45.5[b]	42.9[b]	51.9[a]	1.62	**
Na	120.4[a]	85.4[c]	89.6[bc]	95.0[b]	2.29	***
K	318.0[a]	289.3[ab]	285.5[b]	266.3[b]	7.8	**
mg/kg						
S	154.5[a]	146.6[b]	148.3[b]	156.5[a]	1.3	***
Cu	1.5[b]	3.8[a]	3.2[ab]	4.2[a]	0.47	***
Fe	42.6	21.9	10.9	25.7	9.14	NS

[a,b]Means within rows with different superscripts were significantly different.

[1]n = 10.

[2]Based on dietary mineral content and the minerals present in the excreta.

[3]NS not significant $P > 0.05$.

*0.05 > P > 0.01; **0.01 > P > 0.001; ***P < 0.001.

Table 7. Mean[1] apparent ileal amino acid digestibility (%) for the four experimental diets

	Diet					
Item	Adequate P	Low P	Low P + phytase (100 g/t)	Low P + phytase (200 g/t)	Overall SE	Significance[2]
CP	86.3	85.6	85.6	85.4	0.37	NS
Aspartic acid	85.8^{ab}	84.6^{ab}	84.1^{b}	86.1^{a}	0.50	*
Threonine	71.4^{c}	69.7^{c}	82.1^{a}	77.1^{b}	0.79	***
Serine	81.7^{c}	79.6^{b}	83.5^{ab}	84.4^{a}	0.50	***
Glutamic acid	90.1^{b}	89.9^{b}	91.1^{ab}	91.5^{a}	0.35	**
Glycine	80.7^{b}	77.0^{c}	78.8^{bc}	83.7^{a}	0.63	***
Alanine	85.8^{a}	84.9^{ab}	83.1^{b}	86.7^{a}	0.63	**
Cysteine	77.9^{a}	77.8^{a}	75.0^{b}	72.0^{c}	0.69	***
Valine	83.6^{a}	79.8^{b}	83.2^{a}	79.0^{b}	0.60	***
Methionine	88.3^{a}	85.5^{b}	87.3^{a}	87.1^{ab}	0.46	**
Isoleucine	85.0^{c}	83.7^{c}	90.2^{a}	87.1^{b}	0.48	***
Leucine	87.4^{a}	86.7^{a}	88.3^{a}	88.1^{a}	0.44	*
Tyrosine	81.5^{b}	80.4^{b}	87.1^{a}	87.8^{a}	0.64	***
Phenylalanine	87.9^{ab}	87.0^{b}	87.2^{b}	89.1^{a}	0.40	**
Histidine	81.2^{bc}	79.7^{c}	82.8^{b}	87.6^{a}	0.54	***
Tryptophan	83.8^{a}	82.4^{ab}	83.6^{a}	81.2^{b}	0.58	**
Lysine	89.2^{a}	87.4^{b}	89.3^{a}	89.4^{a}	0.39	***
Arginine	88.9^{b}	88.0^{b}	89.0^{ab}	90.7^{a}	0.45	**

$^{a–c}$Means within rows with different superscripts were significantly different.

[1]n = 10.

[2]NS not significant $P > 0.05$.

*$0.05 > P > 0.01$; **$0.01 > P > 0.001$; ***$P < 0.001$.

between the adequate-P and low-P diets; but, serine, glycine, valine, methionine, and lysine were the exceptions, although the actual differences for lysine and serine were small (<3% units).

For aspartic acid, alanine, leucine, and tryptophan, the apparent ileal digestibility of the phytase-supplemented diets (either dietary phytase concentration) was not different ($P > 0.05$) from that of the unsupplemented low-P diet. For threonine, isoleucine, tyrosine, histidine, and lysine, apparent ileal digestibility for the phytase-supplemented diets (both dietary phytase concentrations) was higher ($P > 0.05$) than that for the unsupplemented low-P diet, although the difference was small (<3% units) for lysine. For serine, glutamic

Table 8. Mean[1] true[2] ileal amino acid digestibility (%) for the four experimental diets

	Diet					
Item	Adequate P	Low P	Low P + phytase (100 g/t)	Low P + phytase (200 g/t)	Overall SE	Significance[3]
CP	94.8	94.2	94.3	94.1	0.37	NS
Aspartic acid	90.0^{a}	89.3^{a}	88.4^{a}	90.2^{a}	0.50	*
Threonine	84.6^{c}	84.0^{c}	94.9^{a}	91.6^{b}	0.79	***
Serine	88.9^{bc}	87.5^{c}	90.2^{ab}	90.9^{a}	0.50	***
Glutamic acid	92.9^{a}	92.9^{a}	93.8^{a}	94.2^{a}	0.35	*
Glycine	86.1^{b}	83.1^{c}	84.0^{bc}	88.6^{a}	0.63	***
Alanine	90.4^{a}	89.7^{ab}	87.6^{b}	91.1^{a}	0.63	**
Cysteine	89.7^{a}	89.9^{a}	87.1^{b}	84.2^{c}	0.69	***
Valine	89.6^{a}	86.4^{b}	88.9^{a}	84.7^{b}	0.60	***
Methionine	99.3^{a}	96.5^{b}	98.9^{a}	98.3^{a}	0.46	***
Isoleucine	90.1^{c}	89.2^{c}	95.1^{a}	92.1^{b}	0.48	***
Leucine	90.9	90.3	91.7	91.5	0.44	NS
Tyrosine	87.1^{b}	86.4^{b}	91.6^{a}	92.3^{a}	0.64	***
Phenylalanine	90.7^{ab}	89.9^{b}	89.8^{b}	91.7^{a}	0.40	**
Histidine	91.7^{b}	90.6^{b}	92.5^{b}	98.2^{a}	0.54	***
Tryptophan	90.9^{ab}	89.7^{ab}	91.0^{a}	88.7^{b}	0.58	*
Lysine	92.2^{a}	90.7^{b}	92.7^{a}	92.3^{a}	0.39	**
Arginine	92.0^{ab}	91.5^{b}	91.8^{ab}	93.3^{a}	0.45	*

$^{a–c}$Means within rows with different superscripts were significantly different.

[1]n = 10.

[2]Endogenous flows were determined using the enzyme hydrolyzed protein/ultrafiltration method (Moughan et al., 1990; Butts et al., 1991) reported by Rutherfurd et al. (2004a).

[3]NS not significant $P > 0.05$.

*$0.05 > P > 0.01$; **$0.01 > P > 0.001$; ***$P < 0.001$.

acid, glycine, phenylalanine, and arginine, the low-P diet containing the higher concentration of dietary phytase had a higher ($P < 0.01$) apparent ileal digestibility when compared with the unsupplemented low-P diet, but the low-P diet containing the lower dietary phytase concentration did not. However, the differences were small (<3% units) for glutamic acid, phenylalanine, and arginine. For cysteine, the apparent ileal digestibility of the phytase-supplemented diets (both dietary phytase concentrations) was lower ($P < 0.001$) than that of the unsupplemented low-P diet. For valine, the lower concentration of dietary phytase led to a higher ($P < 0.001$) apparent ileal digestibility when compared with the unsupplemented low-P diet, but the higher dietary phytase concentration did not. The trends observed for the apparent ileal amino acid digestibility values were generally reflected in the true ileal amino acid digestibility values in that the greatest positive effect of phytase on amino acid digestibility was observed for threonine, isoleucine, tyrosine, and histidine, whereas a negative effect was observed for cysteine.

DISCUSSION

Growth Performance, AEM, and AME

Dietary inclusion of a novel microbial phytase into a low-P diet (1,000 to 2,000 U/kg) improved broiler weight gain and feed intake by approximately 8%. Other studies have reported 6 to 11% increases in weight gain for similar phytase-supplemented diets (500 and 1,000 U/kg; Sebastian et al., 1997; Camden et al., 2001; Ravindran et al., 2008). In contrast, greater increases (60–80%) in broiler weight gain have been observed after dietary inclusion of larger amounts of phytase (1,500 and 12,000 U/kg; Shirley and Edwards, 2003), and no change in weight gain was observed after inclusion of 600 U/kg of phytase (Zhang et al., 1999). Aureli et al. (2011) examined the same novel phytase that has been investigated in the present study and reported improvements in weight gain and feed conversion ratio of up to 125% and 37% respectively for broilers fed a low-P corn-soybean meal diet supplemented with between 500 and 2,000 U/kg phytase. These later improvements are considerably greater than those observed in the present study. However, the birds receiving the unsupplemented low-P diet in the study of Aureli et al. (2011) appeared to have a very poor feed conversion ratio (1.988) and very low weight gain, which may explain the large improvements in performance they observed after dietary supplementation with phytase.

Similarly to the present findings, dietary phytase supplementation has been reported to result in either no change (Camden et al., 2001; Ravindran et al., 2008) or a small increase in AME (Shirley and Edwards, 2003; Santos et al., 2008), the latter most likely due to the increased amino acid digestibility.

Toe Ash, BMC, and BMD

In the present study, dietary phytase supplementation increased BMC and BMD by 35% and 24%, respectively, with dietary phytase supplementation such that the BMC and BMD for the phytase-supplemented low-P diet was equal to that for an adequate-P diet. Few studies have examined the effect of dietary microbial phytase on BMD. However, Angel et al. (2006) reported that supplementation of a low-P corn-soybean diet with phytase (600 U/kg of Ronozyme P (CT)) resulted in tibia BMD of 49-d-old broilers similar to that of birds receiving an adequate-P diet.

Phosphorus

Similar to that observed in the present study, Camden et al. (2001) reported an increase in ileal phytate degradability from 22% for an unsupplemented low-P diet to 45 to 53% after phytase supplementation (250–1,000 U/kg). However, much lower ileal phytate P disappearance values (21%) from low-P corn-soybean meal diets has been reported even after phytase supplementation (500–750 U/kg; Rutherfurd et al., 2004a).

The increase in true ileal total P absorption observed in the present study (11–14%) is similar to the 5 to 21% increases in ileal total P absorption after supplementation with 250 to 1,200 U/kg of phytase reported in several studies (Denbow et al., 1998; Um et al., 2000; Camden et al., 2001; Rutherfurd et al., 2004a; Cowieson and Adeola, 2005; Santos et al., 2008). Aureli et al. (2011) reported a much higher increase in total P utilization with supplementation of the low-P corn-soybean meal diet with the 500 to 2,000 U/kg of the same phytase examined in the present study possibly due to a much lower P content in the low-P diet used by Aureli et al. (2011) as compared with that used in the present study.

It is also of note that for each of the phytase levels tested in the present study, the ileal phytate P and total P absorption and ileal absorbed phytate P and total P content did not increase with increasing dietary phytase concentration. This latter finding is consistent with that of Aureli et al. (2011), who showed that with the same phytase and similar diets that apparent total P utilization begins to plateau at a dietary phytase activity of approximately 1,000 U/kg.

The reported impact of dietary phytase on the availability of phytate P varies markedly across studies. This variation is largely due to differences in experimental design, analytical methods, diet composition and processing, and the age and breed of the chickens (Angel et al., 2002). Overall, in the present study, dietary phytase inclusion led to improvements in ileal phytate P and total P absorption that appeared to be similar to observations reported by the majority of other workers, even taking into account the generally higher dietary phytase activity used in the present study in comparison with other reports.

Mineral Absorption and Retention

It has been postulated that dietary microbial phytases can also improve the availability of minerals other than P, particularly divalent cations that can complex phytate in the diet. It is thought that phytate dephosphorylation by phytase occurs mainly in the crop, where the pH is most acidic and phytate-mineral complexes are likely to be the most soluble (Selle et al., 2000). In the present study, dietary inclusion of microbial phytase had no effect on Fe, Cu, and K retention but increased Ca, Mg, and S retention by 16, 15, and 4%, respectively. The effect of dietary microbial phytase on Ca retention observed in the present study was similar to that observed in several other studies (Ravindran et al., 2008; Santos et al., 2008; Saima et al., 2009), but much higher than those observed by other workers (Johnston and Southern, 2000; Um et al., 2000; Cowieson and Adeola, 2005). The improvement in Mg retention observed in the present study was 15% compared with values reported in other studies of 0% (Um et al., 2000) and 111% (Santos et al., 2008). For Fe and Cu, Um et al. (2000) reported a significant increase in the retention of Fe but not Cu after dietary phytase supplementation, whereas in the present study, phytase did not improve the retention of either mineral. Similar to that observed in the present study, Santos et al. (2008) found that dietary inclusion of microbial phytase (500 to 1,000 U/kg) did not affect K retention. In contrast, Ravindran et al. (2008) reported a 34% increase in K retention after dietary inclusion of microbial phytase (500 U/kg). Both in the present study and in the reports of Ravindran et al. (2008), dietary inclusion of microbial phytase had no effect on Na retention.

Overall, dietary inclusion of phytase led to a sizable improvement in the retention of Ca and Mg, but not Fe, K, Na, Cu, or S. It is interesting to note that the relative binding strength of phytate to divalent minerals examined in the present study is Cu > Fe > Ca > Mg and is in the inverse order when compared with the improvement of mineral availability (Weaver and Kannan, 2002). The efficacy of phytase for releasing complexed minerals most likely relates to the relative solubility of the phytate-metal complexes in the gastrointestinal tract, which is in turn a function of the pH (Cheryan, 1980) and the molar ratio of minerals to phytic acid present (Vohra et al., 1965).

Amino Acids

Phytate can reduce the bioavailability of dietary amino acids and does so via several mechanisms. Firstly, phytate can bind free amino acids directly, which is likely to reduce their absorption (Rutherfurd et al., 2004b). Secondly, proteins can also complex with phytate, either by direct interaction between positively charged amino acid side chains and phytate phosphate groups or through divalent cation bridges, reducing the effectiveness of digestive enzymes (Anderson, 1985). Thirdly, phytate can inhibit a range of digestive enzymes, including pepsin and trypsin, further reducing protein digestion (Singh and Krikorian, 1982). The hydrolysis of phytate by supplemented microbial phytase can improve amino acid availability, but the extent will depend on the dietary phytate concentration, the proteins present, and concentration and source of the phytase. In the present study, dietary phytase improved the apparent ileal digestibility of most amino acids, particularly threonine, serine, glycine, isoleucine, tyrosine, and histidine, with increases of 14, 6, 9, 6, 9, and 7%, respectively being observed. Other studies report varying effects of dietary microbial phytase on overall ileal amino acid digestibility of corn-soybean meal broiler diets ranging from essentially no effect (Sebastian et al., 1997; Zhang et al., 1999) and to increases of 2.5% (Ravindran et al., 2008), 2.7% (Camden et al., 2001), 3.2% (Rutherfurd et al., 2004a), and 0.5 to 6% (Santos et al., 2008). Threonine was the most affected amino acid in the present study and in several other reported studies (Sebastian et al., 1997; Camden et al., 2001). In contrast, cysteine (Santos et al., 2008) has also been reported to be the most affected amino acid.

Overall, the main effects of dietary inclusion of a novel 6-phytase were increases in the absorption of phytate P and total P, Ca and Mg, toe ash, BMD, and the digestibility of threonine, tyrosine, and histidine. The novel 6-phytase would appear to perform to a similar or greater degree than most other phytases reported in the literature. Finally, a dietary phytase inclusion level of 200 g/t appears to offer no additional nutritional benefit over that of 100 g/t.

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