Response of broilers to graded levels of microbial phytase added to maize–soyabean-meal-based diets containing three levels of non-phytate phosphorus

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Male 1-d-old broilers (*n* 920) were given 0, 200, 400, 600, 800, 1000 and 1200 U microbial phytase/kg diet in combination with 2·0, 2·7 or 3·4 g non-phytate P (nP)/kg or 4·0, 5·1 or 5·8 g total P (tP)/kg in a 21 d trial to assess the effectiveness of phytase in a maize–soyabean-meal diet. In addition to the above twenty-one diets, a positive control P diet supplied 4·5 g nP/kg, 6·9 g tP/kg and 10 g Ca/kg. The basal diet contained 230 g crude protein/kg, 8·8 g Ca/kg, 4·4 g tP/kg and 2·0 g nP/kg. Defluorinated phosphate and limestone were used to supply P and Ca. A Ca:tP ratio of 2:1 was maintained except in the positive control diet which had a ratio of 1·45:1. Phytase additions linearly increased ($P < 0·01$) body-weight (BW) gain, feed intake, toe ash percentage, and apparent retention (% of intake) or total amount (g/bird) of retained Ca and P, and linearly decreased ($P < 0·01$) P excretion (g/kg of DM intake) at each level of nP with the magnitude of the response inversely related to the level of nP. Above-normal mortality was only observed in the group receiving 2·0 g nP/kg diet without phytase. Adding nP linearly increased ($P < 0·01$) BW gain, feed intake, toe ash percentage, Ca retention, total amount (g/bird) of P retained, and P excretion, and linearly decreased ($P < 0·01$) apparent retention (%) of P. Derived linear and non-linear equations for BW gain and toe ash percentage at the two lower nP levels, 2·0 and 2·7 g/kg, were used to calculate P equivalency values of microbial phytase. The results show that 939 U microbial phytase is equivalent to 1 g P from defluorinated phosphate in broilers fed on maize–soyabean-meal diets. The amount of P released per 100 U phytase decreased as the total amount of phytase increased.

Phytase: Broilers: Phosphorus

It has been well documented that microbial phytase is effective in releasing a significant portion of the phytate P present in maize and soyabean meal and making it available to broilers (Nelson *et al.* 1968; Simons *et al.* 1990; Schoner *et al.* 1991). P excretion by broilers can be reduced when supplemental phytase is included in the diets (Schoner *et al.* 1990, 1991; Simons *et al.* 1990). The amount of P excreted is related to the non-phytate and total P fed and to the level of supplemental phytase. Body-weight (BW) gain and toe ash percentage are sensitive indicators for assessing the efficacy of phytase for broilers and turkey poults fed on soyabean-meal-based semi-purified diets (Denbow *et al.* 1995; Ravindran *et al.* 1995*a*) and for evaluating P availability in broilers and turkey poults fed on maize–soyabean-meal diets (Potter, 1988; Potter *et al.* 1995). Apparent retention (%) or total amount of retention (g/bird) of P and Ca by broilers may also be sensitive indicators for evaluating P availability (Schoner *et al.* 1993). Only limited information is available about the equivalency value of phytase for inorganic P in broilers fed on maize–soyabean-

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meal diets. Schoner *et al.* (1991) reported that 700 U microbial phytase was equivalent to 1 g P in the form of monocalcium phosphate (MCP) when based on P retention of broilers fed on a maize–soyabean-meal diet for 14 d; a value of 762 U phytase was obtained when the calculation was based on crude ash in the body. Schoner *et al.* (1993) using a similar diet reported that 570 U phytase was equivalent to 1 g P as MCP based on BW gain at 14 d and 1050 U phytase was equal to 1 g P when based on P retention. At 40 d, 850 U phytase was equal to 1 g P for both measurements. In each of the above studies (Schoner *et al.* 1991, 1993), only one level of P was used in each of the trials.

The purpose of the present study was: (1) to study the response of broilers to graded levels of microbial phytase added to maize–soyabean-meal-based diets containing three levels of non-phytate P (nP), (2) to evaluate several measurements for their usefulness in predicting the responses to P and phytase additions, and (3) to determine P equivalency values of phytase.

MATERIALS AND METHODS

Peterson × Arbor Acres male broiler chicks (*n* 920) were used in a 3 × 7 factorial arrangement of treatments with four replicates (ten birds/pen) to evaluate the response of broilers to seven levels of phytase (Natuphos®; BASF Corp., 3000 Continental Drive North, Mount Olive, NJ 07828-1234, USA) in combination with three levels of nP. Dietary P levels were formulated at 2·0, 2·7 and 3·4 g nP/kg (or 4·4, 5·1 and 5·8 g total P (tP)/kg) respectively, and each level of P was supplemented with 0, 200, 400, 600, 800, 1000 and 1200 U phytase/kg diet. One unit of phytase activity is defined as the quantity of enzyme that liberates 1 μmol inorganic P/min from 5·1 mM-sodium phytate at pH 5·5 and 37° (Simons *et al.* 1990; Engelen *et al.* 1994). These dietary P levels were formulated below the current National Research Council (NRC) (1994) recommendations to ensure maximum responses with phytase additions. In addition to the twenty-one diets described, a positive control diet was formulated to supply the recommended level of 4·5 g nP/kg or 6·9 g tP/kg.

Composition of the diets is shown in Table 1. Since the nP level of 1·5 g/kg supplied by the maize and soyabean meal was thought to be inadequate to sustain chick life, inorganic P was added (0·5 g/kg) to increase the nP level to 2·0 g/kg; this level of P without phytase, however, was expected to result in some mortality (Potter *et al.* 1995). The desired levels of nP and Ca in the basal diets were achieved by varying the levels of defluorinated phosphate and limestone at the expense of maize. The Ca:tP ratio was maintained at 2:1 in all diets except the positive control diet, which had a ratio of 1·45:1. Since the phytate was supplied only from the maize and soyabean meal, the dietary content of phytate P (2·4 g/kg) was similar in all diets.

The birds were housed in electrically heated, raised wire-floored starting batteries in an environmentally controlled room. The treatments were then randomly assigned to four pens of ten chicks each except the positive control diet which had eight pens of ten chicks each. The diets were fed in a mash form from 1 to 21 d of age. Birds had *ad libitum* access to feed and water at all times. The care and treatment of broilers followed published guidelines (Consortium, 1988). BW and feed intake were recorded on a pen basis at weekly intervals. Records of mortality were also maintained.

During the third week (days 18–20) a total collection of excreta from each pen was carried out. Feed intake and production of excreta were measured quantitatively per pen over the three consecutive days. Excreta from each pen were stored in plastic bags at −20°. After thawing, excreta were dried in an oven at 70° and weighed. Excreta, along with diet samples, were ground to pass a 1 mm sieve. Dry matter was determined according to Association of Official Analytical Chemists (1990) procedures. Following a HNO_3–$HClO_4$

wet digestion, P concentrations were determined photometrically (Association of Official Analytical Chemists, 1990) and Ca contents were determined with an atomic absorption spectrophotometer. Apparent retention values (% of intake or g/bird) for P and Ca during the 3 d were calculated for each dietary treatment.

On day 21, all surviving chicks were killed by cervical dislocation. Toe samples were obtained by severing the middle toe through the joint between the second and third tarsal bones from the distal end. The left middle toes of all chicks within a pen were pooled, and the right middle toes from the same chicks were pooled, yielding two samples of toes per pen. The composite samples were dried to a constant weight at 100° and then ashed in a muffle furnace at 600° for 4 h (Potter, 1988). Toe ash was expressed as a percentage of dry weight.

Data were analysed by the general linear models (GLM) procedure of Statistical Analysis Systems (1990) with pen means as the experimental unit. Linear and quadratic effects of added phytase within each P level and comparisons between dietary P levels without added phytase were tested using orthogonal polynomials.

In order to evaluate further the sensitive indicators for response measurements and to predict the responses of P and phytase levels in broilers, second-order translog equations were derived for the 3 × 7 factorial arrangement of treatments with the model:

$$\ln Y = \alpha_0 + \alpha_1 \ln X_1 + \alpha_2 \ln X_2 + \alpha_3(\ln X_1)^2 + \alpha_4(\ln X_2)^2 + \alpha_5 \ln X_1 \ln X_2,$$

where Y is response measurement, X_1 is nP (%), and X_2 is phytase added (U/kg diet). The second-order translog function was chosen because Driscoll (1994) demonstrated that this function is a flexible functional form that can provide second-order approximations to any underlying function. Non-linear and linear functions were derived for the seven phytase levels at each P level (2·0, 2·7 and 3·4 g nP/kg) and for the four P levels (2·0, 2·7, 3·4 and 4·5 g nP/kg) without phytase added, with the non-linear model: $Y = a(1 - b\mathrm{e}^{-kX})$ and the linear model: $Y = a + bX$, where Y is response measurement; and X is nP (g/kg) or phytase added (U/kg diet). Non-linear and linear equations for nP (no added phytase) and for phytase at the three levels of nP for all measurements were examined for high r^2.

The R^2 values of the second-order translog equations were also examined. Because of high R^2 values and economic importance or ease of obtaining, BW gain and toe ash percentage were used to generate the P equivalency equations. The response equation for nP and the equation for added phytase at each of the two lower levels of nP were set equal and solved (D. M. Denbow, unpublished results). The resulting equations were used to calculate P equivalency values (g/kg) at 250, 500, 750, and 1000 U phytase/kg diet to enable comparisons with other reports in the literature, which often studied the addition of 500 or 1000 U phytase/kg diet. The average of the released P for each of the two P levels was then used to determine the amount of P released expressed as a percentage of phytate P. The P equivalency equation was derived from these four data points for amount of P released. The use of a regression line (equation) from graded levels of P or phytase provides a more accurate means of estimating a response than a single number. Also, the generation of a P equivalency equation from the equations for P and phytase allows for the calculation of the equivalency of phytase for P at any point on the line. Further, the use of mathematical equations allows for the easy incorporation of this information in computer models.

RESULTS

BW gains were increased ($P < 0.01$) by dietary P and phytase additions (Table 2), but a P level × phytase level interaction ($P < 0.01$) was observed. Gains were improved by phytase additions at all P levels; however, the magnitude of the response was greatest at lower rates

Table 1. *Components and calculated composition of basal diets (g/kg)*

	Non-phytate phosphorus (g/kg)			
	2·0*	2·7*	3·4*	4·5†
Components				
Ground yellow maize	578·1	573·9	569·8	572·9
Soyabean meal (485 g CP/kg)	371·0	371·0	371·0	371·0
Stabilized fat	20·0	20·0	20·0	20·0
Limestone	17·9	18·2	18·4	9·2
Defluorinated phosphate‡	3·0	6·9	10·8	16·9
Vitamin premix§	2·0	2·0	2·0	2·0
Trace mineral premix‖	2·0	2·0	2·0	2·0
Salt	4·0	4·0	4·0	4·0
DL-Methionine	2·0	2·0	2·0	2·0
Calculated composition				
CP (N × 6·25)	230·7	230·4	230·0	230·3
Lysine	13·2	13·2	13·2	13·2
Methionine + cystine	9·3	9·3	9·3	9·3
Ca	8·8	10·2	11·6	10·0
Total P	4·4	5·1	5·8	6·9
Non-phytate P	2·0	2·7	3·4	4·5
Ca:total P	2·0	2·0	2·0	1·45

CP, crude protein.

* 0, 200, 400, 600, 800, 1000 and 1200 U phytase (Natuphos®, BASF Corp., Mount Olive, NJ, USA; 5000 U/g)/kg was added to each of the basal diets.

† Positive control diet; no phytase was added.

‡ Fine CDP, Southern Bag Corp., Valdosta, GA 31083, USA.

§ Supplied (per kg diet): retinyl acetate 908 μg, cholecalciferol 66 μg, DL-α-topheryl acetate 26 mg, menadione sodium bisulphite complex 0·75 mg, riboflavin 7·5 mg, D-calcium pantothenate 9·7 mg, niacin 26·4 mg, cyanocobalamin 0·011 mg, choline chloride 1012 mg, D-biotin 0·31 mg, pteroylmonoglutamic acid 3·1 mg, thiamin-HCl 8 mg, pyridoxine-HCl 3·1 mg, ethoxyquin 50 mg, and virginiamycin 2·9 mg.

‖ Supplied per kg diet: Mn 88 mg, Zn 95 mg, Fe 100 mg, Cu 12·5 mg, I 4 mg, and Se 0·6 mg.

of phytase addition for the lower nP levels. At 2·0 g nP/kg, gains improved (linear, $P < 0·01$; quadratic, $P < 0·01$) up to 1000 U phytase/kg diet and then reached a plateau. The response appeared to reach a plateau at 800 U phytase/kg diet for broilers fed on diets containing 2·7 g nP/kg (linear, $P < 0·01$). On the 3·4 g nP/kg diet the response appeared to reach a plateau at 600 U phytase/kg diet ($P < 0·01$).

Total feed intake followed a similar pattern to that of average BW gains (Table 2). The addition of both nP and phytase increased feed intake with the greatest response for phytase at the lowest nP level (2·0 g/kg). Gain/feed (g/kg) was improving by increasing dietary nP level (linear, $P < 0·05$). The addition of phytase improved gain/feed only at 2·0 g nP/kg (linear, $P < 0·01$).

Dietary additions of nP ($P < 0·01$) and phytase ($P < 0·01$) linearly increased toe ash percentage (Table 3). The response to phytase was observed at all levels of nP, although the magnitude of the response seemed to decrease as level of nP increased.

Retention (% of intake and g/bird) of DM, Ca, and P, and excretion (g/kg DM intake) of P during days 18–20 are shown in Tables 4 and 5. Increasing dietary nP (g/kg) level increased (linear, $P < 0·01$) retention (% of intake and g/bird) of DM, Ca and P, and excretion (g/kg DM intake) of P. Increasing nP (g/kg) also increased the total amount (g/bird) of P retained (linear, $P < 0·01$; quadratic, $P < 0·05$), but decreased apparent retention (% of intake) of P (linear, $P < 0·01$, quadratic, $P < 0·05$). Addition of graded

Table 2. *Body-weight gain, feed intake, and gain/feed of broilers fed on maize–soyabean-meal-based diets containing varying amounts of non-phytate phosphorus and supplemental phytase from 1 to 21 d of age**

Phytase added (U/kg diet)	Non-phytate phosphorus (g/kg) 2·0	2·7	3·4	4·5
Body-wt gain (g/bird)†				
0	380‡	511	562§	613
200	487	544	581	
400	508	556	580	
600	524	564	595	
800	548	587	598	
1000	560	585	594	
1200	557	595	612	
Total feed intake (g/bird)†				
0	625‡	835§	906§	963
200	810	873	915	
400	819	876	903	
600	876	924	943	
800	905	929	946	
1000	860	918	933	
1200	872	935	965	
Gain/feed (g/kg)‖				
0	584§	613	620	636
200	604	624	644	
400	622	634	643	
600	599	610	631	
800	606	632	633	
1000	652	638	637	
1200	642	637	636	

* Mean values for four pens of ten chicks each, except for the 4·5 g non-phytate P/kg which are for eight pens of ten chicks each. The root mean square errors (MSE) were 26, 38, and 30 respectively for body-weight gain, total feed intake and gain/feed. The pooled SEM for a single treatment mean is MSE/$\sqrt{n}$.

† Phosphorus linear and quadratic effect ($P < 0·05$); phosphorus × phytase interaction ($P < 0·01$).

‡ Phytase linear and quadratic effect ($P < 0·01$).

§ Phytase linear effect ($P < 0·01$).

‖ Phosphorus linear effect ($P < 0·05$).

levels of phytase increased retention of DM (linear, $P < 0·05$), Ca (linear, $P < 0·01$; quadratic, $P < 0·07$), and P (linear, $P < 0·01$). The amount of P excreted was linearly decreased ($P < 0·01$) as phytase was added. In comparison with the positive control diet (4·5 g nP/kg), P excretion was reduced by 25–54 % with addition of 200–1200 U phytase at various nP levels. The magnitude of the response was greatest for the lowest nP levels (41–54 % at 2·0 g nP/kg; 34–46 % at 2·7 g nP/kg; 25–34 % at 3·4 g nP/kg).

Second-order translog equations of performance, toe ash percentage, apparent retention of DM, P and Ca, and P excretion were generated and are shown in Table 6. The response surfaces obtained from plotting the second-order translog equations for BW gain and toe ash percentage are shown in Fig. 1. All the measurements had high R^2 values, except gain/feed and apparent retention (% of intake) of DM which had low R^2 values. Linear or non-linear response equations were also generated with four nP levels (2·0, 2·7, 3·4 and 4·5 g/kg) without added phytase and with seven phytase levels at each of three nP levels (2·0, 2·7 and 3·4 g/kg) for all the measurements (Table 7). The equations for all the

Table 3. *Percentage ash content of dried toes from broilers fed on maize–soyabean-meal-based diets containing varying amounts of non-phytate phosphorus and supplemental phytase from 1 to 21 d of age**

Phytase added (U/kg diet)	Non-phytate phosphorus (g/kg)†			
	2·0‡	2·7‡	3·4‡	4·5
0	8·2	10·0	11·3	13·3
200	8·7	10·5	11·9	
400	9·3	11·1	11·9	
600	9·8	11·5	11·8	
800	9·9	10·9	12·3	
1000	10·5	11·6	12·2	
1200	11·1	12·6	12·9	

* Mean values for four pens of ten chicks each, except 4·5 g non-phytate P/kg which are for eight pens of ten chicks each. The root mean square error (MSE) was 0·6 and the pooled SEM for a single mean is MSE/$\sqrt{n}$.
† Phosphorus linear ($P < 0.01$) and quadratic ($P < 0.05$) effects.
‡ Phytase linear effect ($P < 0.01$).

Table 4. *Apparent retention of dry matter and calcium during days 18–20 of broilers fed on maize–soyabean-meal-based diets containing varying amounts of non-phytate phosphorus and supplemental phytase**

	Non-phytate P (g/kg)							
	2·0		2·7		3·4		4·5	
Phytase added (U/kg diet)	% of intake	g/bird	% of intake	g/bird	% of intake	g/bird	% of intake	g/bird
Dry matter retention								
0	74·0‡	83·0‡	75·8§	116·5§	75·5§	131·8§	77·5	142·4
200	75·4	111·0	75·2	123·2	76·8	138·1		
400	76·3	116·0	77·5	129·8	76·9	127·9		
600	76·4	117·5	77·1	134·0	77·2	139·2		
800	76·5	127·8	77·9	134·1	77·9	136·4		
1000	77·2	125·0	77·7	133·7	77·5	138·6		
1200	76·2	128·1	77·1	139·4	78·1	144·5		
Calcium retention‖								
0	52·3¶	0·626§	52·3¶	1·007‡	54·1¶	1·312	63·6	1·628
200	51·0	0·810	56·6	1·248	55·7	1·376		
400	50·5	0·756	55·8	1·147	57·4	1·381		
600	53·5	0·863	58·5	1·215	54·2	1·289		
800	53·0	0·918	57·8	1·268	57·5	1·491		
1000	57·1	0·996	61·5	1·334	55·0	1·253		
1200	58·7	1·027	64·0	1·581	62·6	1·643		

* Mean values for four pens of ten chicks, except 4·5 g non-phytate P/kg which are for eight pens of ten chicks each. The root mean square errors (MSE) were 1·6 and 8·7, 3·0 and 0·098 respectively for DM and apparent Ca retention (% of intake and g/bird). The pooled SEM for a single treatment is MSE/$\sqrt{n}$.
† Phosphorus linear effect ($P < 0.01$).
‡ Phytase linear ($P < 0.001$) and quadratic ($P < 0.07$) effects.
§ Phytase linear effect ($P < 0.05$).
‖ Phosphorus linear ($P < 0.01$) and quadratic ($P < 0.01$) effects.
¶ Phytase linear ($P < 0.01$) and quadratic ($P < 0.05$) effects.

Table 5. *Apparent phosphorus retention and phosphorus excretion during days 18–20 of broilers fed on maize–soyabean-meal-based diets containing varying amounts of non-phytate phosphorus and supplemental phytase**

	Non-phytate P (g/kg)							
	2·0		2·7		3·4		4·5	
Phytase added (U/kg diet)	% of intake	g/bird	% of intake	g/bird	% of intake	g/bird	% of intake	g/bird
Phosphorus retention†								
0	58·7‡	0·348‡	54·7‡	0·494‡	52·1‡	0·566‡	54·9	0·888
200	60·6	0·506	60·6	0·619	56·9	0·660		
400	59·7	0·482	60·8	0·606	58·8	0·639		
600	61·8	0·497	63·4	0·653	58·4	0·672		
800	62·3	0·524	64·0	0·677	63·4	0·774		
1000	66·0	0·559	64·8	0·567	63·2	0·787		
1200	67·2	0·599	70·6	0·872	63·4	0·794		
Phosphorus excretion§ (g/kg DM intake)								
0	2·19‡		2·66‡		2·98‡		3·75	
200	2·23		2·46		2·83			
400	2·12		2·37		2·69			
600	2·00		2·17		2·68			
800	2·01		2·18		2·56			
1000	1·78		2·12		2·55			
1200	1·74		2·01		2·48			

* Mean values for four pens of ten chicks each, except 4·5 g non-phytate P/kg which are for eight pens of ten chicks each. The root mean square errors (MSE) were 3·4, 0·06 and 0·36 respectively for apparent P retention (% of intake and g/kg per bird) and P excretion. The SEM for a single treatment is MSE/$\sqrt{n}$.
† Phosphorus linear ($P < 0{\cdot}01$) and quadratic ($P < 0{\cdot}05$) effects.
‡ Phytase linear effect ($P < 0{\cdot}01$).
§ Phosphorus linear effect ($P < 0{\cdot}01$).

measurements with high r^2 values (except apparent P retention) at the two lower nP levels were used to calculate P equivalency values of phytase (Table 8). Data for the highest level of nP (3·4 g/kg) were not used in the estimation of P equivalency values because r^2 values were generally lower and the response to phytase was not as large compared with 2·0 and 2·7 g/kg levels; this would be expected since 3·4 g/kg is much closer to the suggested NRC (1994) level of 4·5 g/kg than 2·0 and 2·7 g/kg. The equation for nP and the equation for added phytase at each of the two lower levels of nP were set equal. For example, the equation for toe ash percentage at 2·0 g nP/kg:

$$21{\cdot}3\,(1-0{\cdot}908\,e^{-0{\cdot}197X_1}) = 8{\cdot}28+0{\cdot}0023X_2,$$
$$X_1 = -5{\cdot}076\ln(0{\cdot}6732-0{\cdot}000119X_2),$$

where X_1 is nP (g/kg) and X_2 is phytase added (U/kg diet). The resulting equations were used to calculate the equivalent P nP (g/kg) at 250, 500, 750, and 1000 U phytase/kg diet. For example, if phytase (X_2) = 500 U/kg diet, then the equivalent nP (X_1) = $-5{\cdot}076\ln(0{\cdot}6732-0{\cdot}000119\times 500)$ and $X_1 = 2{\cdot}50$ g/kg (Table 8). Therefore, released P = equivalent nP − nP in the diet (for example, 2·50 − 2·0 = 0·50 g/kg). BW gain and toe ash percentage were weighted equally and averaged across the two lower nP levels for development of a P equivalency equation: $Y = 1{\cdot}849-1{\cdot}799\,e^{-0{\cdot}0008X}$ (Table 8), where Y is released P (g/kg) and X is phytase activity (U/kg diet). Solution of the equation for 1 g P gave an equivalency

Table 6. *Second-order translog equations of performance, toe ash, apparent retention of dry matter, calcium and phosphorus and phosphorus excretion of broilers fed on maize–soyabean-meal diets*

Item	Coefficients of second-order translog equations* α_0	α_1	α_2	α_3	α_4	α_5	*P* value	R^2
Performance								
Weight gain (g)	5·398	1·034	0·0540	−0·2927	0·0032	−0·0412	0·001	0·84
Feed intake (g)	6·033	0·884	0·0450	−0·2584	0·0024	−0·0352	0·001	0·76
Gain/feed (g/kg)	6·273	0·151	0·0090	−0·0344	0·0009	−0·0060	0·028	0·15
Toe ash (%)	1·280	1·228	0·0295	−0·3761	0·0055	−0·0178	0·001	0·77
Apparent retention (%)								
Dry matter	4·263	0·031	0·0035	0·0054	0·0008	−0·0014	0·001	0·29
Calcium	3·648	0·109	0·0158	0·0341	0·0050	−0·0096	0·001	0·46
Phosphorus	4·018	−0·244	0·0090	0·0821	0·0049	0·0023	0·001	0·62
Apparent retention (g/bird)								
Dry matter	3·516	1·774	0·0594	−0·6493	0·0042	−0·0445	0·001	0·78
Calcium	−2·420	3·260	−1·096	−1·096	0·0089	−0·0478	0·001	0·85
Phosphorus	−1·909	1·112	0·0664	−0·1323	0·0082	−0·0404	0·001	0·79
Excretion of P (g/kg DM intake)	1·058	−0·2100	−0·0160	0·4088	−0·0087	0·0012	0·001	0·88

* Model: $\ln Y = \alpha_0 + \alpha_1 \ln X_1 + \alpha_2 \ln X_2 + \alpha_3(\ln X_1)^2 + \alpha_4(\ln X_2)^2 + \alpha_5 \ln X_1 \ln X_2$; where Y is response measurement, X_1 is non-phytate P (%) and X_2 is phytase added (U/kg diet).

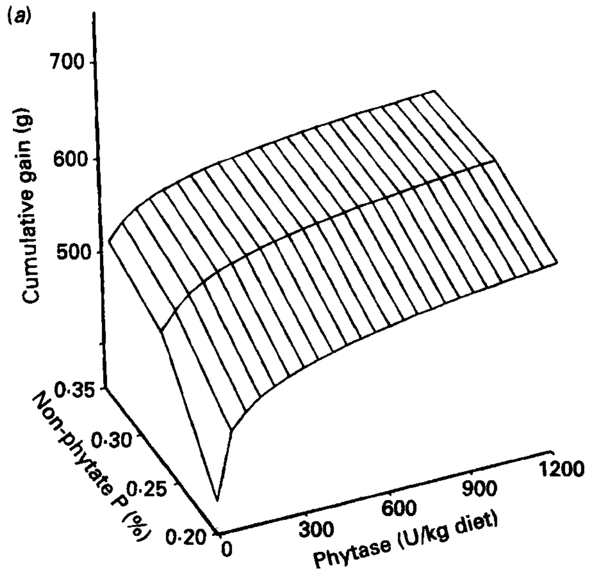
value of 939 U phytase/kg. The proportions of P released from phytate P were 15, 27, 36 and 44 % respectively for 250, 500, 750 and 1000 U phytase/kg diet. The P released (g) per 100 U phytase was decreased as the total amount of phytase increased.

During the 21 d experiment, 14, 0, and 4 of the broilers died from diets containing 2·0, 2·7 and 3·4 g nP/kg. Seven of the fourteen birds that died were from the 2·0 g nP/kg diet without added phytase; the number of deaths declined to normal levels with phytase addition of 200 U/kg diet or more. In the broilers given 2·7 and 3·4 g nP/kg diet, mortality was normal and not influenced by phytase addition. There were no deaths of broilers given 4·5 g nP/kg.

DISCUSSION

The addition of Natuphos® phytase to the maize–soyabean-meal diets improved all the measurements, especially at the lower nP levels when fed to broilers during a 21 d test. These results indicate that microbial phytase is very effective in improving P availability. The maximum growth responses of broilers to phytase level were reduced with increasing levels of dietary nP. The maximum growth responses appeared to occur at 1000, 800 and 600 U phytase/kg diet respectively for 2·0, 2·7 and 3·4 g nP/kg.

The improved growth responses to the addition of phytase were primarily mediated by the increased feed intake. The improvements of phytase on gain/feed were observed only at 2·0 g nP/kg. These results are in agreement with previous findings (Schoner *et al.* 1991; Vogt, 1992; Denbow *et al.* 1995). However, the responses for percentage toe ash and the retention (% of intake and g/bird) of P and Ca of these 1–21-d-old broilers to the supplemental phytase levels did not reach a plateau. It appears that the supplemental phytase may have potential benefits for later growth of broilers which is supported by the findings of Schoner *et al.* (1993) who fed broilers to 40 d. They reported the P equivalency values of phytase at 14 d as 1 g P as MCP = 570 U phytase for BW gain and 1 g P =



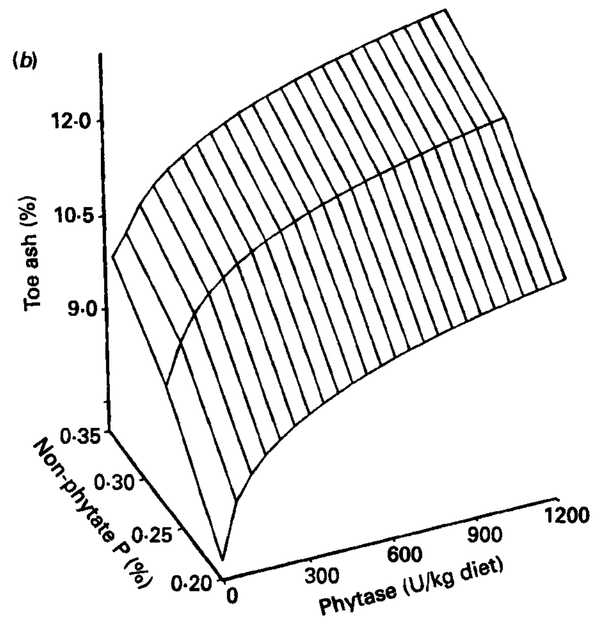


Fig. 1. Body-weight gain (a) and toe ash percentage (b) of broilers fed on maize–soyabean-meal-based diets containing varying amounts of non-phytate phosphorus and supplemental phytase from 1 to 21 d of age. See Table 6 for coefficients of second-order translog equations.

1050 U phytase for P retention. At 40 d, 850 U phytase was equal to 1 g P for both measurements.

P excretion (g/kg DM intake) decreased linearly with increasing amounts of phytase in the present experiment (Table 5). The magnitude of the reduction in P excretion was greater at 2·0 and 2·7 g nP/kg than at 3·4 g nP/kg. This result indicates that microbial phytase provides a means of reducing P pollution in poultry manure. In comparison with the

Table 7. *Non-linear or linear equations of performance, toe ash percentage, apparent retention of dry matter, calcium and phosphorus, and phosphorus excretion for the response of phytase at three levels of non-phytate phosphorus (nP) fed to broilers**

	2·0 g nP/kg		2·7 g nP/kg		3·4 g nP/kg	
Item	Equation	r^2	Equation	r^2	Equation	r^2
Performance						
Weight gain (g)	$Y = 557{\cdot}1\,(1-0{\cdot}312\,e^{-0{\cdot}0036X})$	0·98	$Y = 609{\cdot}0\,(1-0{\cdot}157\,e^{-0{\cdot}0015X})$	0·98	$Y = 622{\cdot}7\,(1-0{\cdot}094\,e^{-0{\cdot}0011X})$	0·88
Feed intake (g)	$Y = 877{\cdot}9\,(1-0{\cdot}255\,e^{-0{\cdot}0051X})$	0·94	$Y = 945{\cdot}5\,(1-0{\cdot}117\,e^{-0{\cdot}0019X})$	0·92	$Y = 902{\cdot}8+0{\cdot}046X$	0·73
Toe ash (%)	$Y = 8{\cdot}28+0{\cdot}0023X$	0·99	$Y = 10{\cdot}10+0{\cdot}0018X$	0·82	$Y = 11{\cdot}42+0{\cdot}0010X$	0·82
Apparent retention (%)						
Dry matter	$Y = 76{\cdot}7\,(1-0{\cdot}036\,e^{-0{\cdot}0041X})$	0·90	$Y = 77{\cdot}8\,(1-0{\cdot}031\,e^{-0{\cdot}0022X})$	0·62	$Y = 78{\cdot}0\,(1-0{\cdot}031\,e^{-0{\cdot}0023X})$	0·91
Calcium	$Y = 50{\cdot}1+0{\cdot}006X$	0·72	$Y = 53{\cdot}0+0{\cdot}008X$	0·90	$Y = 54{\cdot}0+0{\cdot}004X$	0·40
Phosphorus	$Y = 58{\cdot}1+0{\cdot}007X$	0·89	$Y = 56{\cdot}3+0{\cdot}011X$	0·89	$Y = 53{\cdot}9+0{\cdot}009X$	0·87
Apparent retention (g/bird)						
Dry matter	$Y = 96{\cdot}84+0{\cdot}031X$	0·73	$Y = 139{\cdot}5\,(1-0{\cdot}167\,e^{-0{\cdot}0020X})$	0·96	$Y = 131{\cdot}6+0{\cdot}0085X$	0·46
Calcium	$Y = 0{\cdot}671-0{\cdot}0003X$	0·91	$Y = 1{\cdot}041+0{\cdot}0004X$	0·77	$Y = 1{\cdot}30+0{\cdot}0002X$	0·24
Phosphorus	$Y = 0{\cdot}585\,(1-0{\cdot}365\,e^{-0{\cdot}0022X})$	0·82	$Y = 0{\cdot}526+0{\cdot}0002X$	0·51	$Y = 0{\cdot}584+0{\cdot}0002X$	0·90
P excretion (g/kg DM intake)	$Y = 2{\cdot}263-0{\cdot}0004X$	0·91	$Y = 2{\cdot}586-0{\cdot}0005X$	0·93	$Y = 2{\cdot}917-0{\cdot}0004X$	0·94

* Y is response measurement; X is phytase added (U/kg diet).

Table 8. *Calculated equivalency values of phytase for inorganic phosphorus*

Non-phytate P (g/kg)...	2·0				2·7					
Phytase (U/kg diet)...	250	500	750	1000	250	500	750	1000	Equation*	r^2
Equivalent of nP (g/kg)										
BW gain	2·55	2·90	3·10	3·10	3·10	3·40	3·60	3·80	$Y = 625{\cdot}3\,(1-3{\cdot}09\,e^{-1{\cdot}08X})$	0·99
Feed intake	2·65	2·90	3·00	3·10	3·05	3·30	3·55	3·70	$Y = 972{\cdot}6\,(1-3{\cdot}442\,e^{-1{\cdot}17X})$	0·99
Toe ash percentage	2·25	2·50	2·70	3·00	3·00	3·20	3·45	3·70	$Y = 21{\cdot}3\,(1-0{\cdot}908\,e^{-0{\cdot}197X})$	0·99
DM retention (%)	3·40	3·85	4·05	4·15	3·75	4·25	4·55	4·70	$Y = 71{\cdot}8+1{\cdot}234X$	0·87
Ca retention (%)	2·30	2·65	2·95	3·30	3·05	3·55	3·95	4·45	$Y = 41{\cdot}2+4{\cdot}582X$	0·82
DM retention (g/bird)	2·40	2·60	2·85	3·15	3·05	3·35	3·55	3·70	$Y = 146{\cdot}9\,(1-3{\cdot}55\,e^{1{\cdot}05X})$	0·99
Ca retention (g/bird)	2·20	2·35	2·50	2·60	2·90	3·15	3·40	3·60	$Y = 2{\cdot}22\,(1-1{\cdot}60\,e^{-0{\cdot}399X})$	0·99
P retention (g/bird)	2·60	2·85	3·00	3·10	3·15	3·40	3·70	3·90	$Y = -0{\cdot}0888+0{\cdot}210X$	0·97
Mean equivalent of nP (g/kg)†	2·40	2·70	2·90	3·05	3·05	3·30	3·53	3·75		
Released P (g/kg)	0·40	0·70	0·90	1·05	0·35	0·60	0·83	1·05		
Percentage of phytate P‡	16·7	29·2	37·5	43·8	14·6	25·0	34·6	43·8		
Means of the two nP levels										
Released P (g/kg)§					0·38	0·65	0·87	1·05		
Percentage of phytate P‖					15·6	27·1	36·0	43·8		
Released P (g/100 U phytase)¶					0·15	0·13	0·12	0·11		

nP, non-phytate phosphorus; BW, body weight.

* Response equations for nP levels (without phytase), where X is nP (g/kg) and Y is response. See Table 7 for response equations for phytase levels at each of the three levels of nP.

† Only BW gain and toe ash percentage used for calculation of equivalent nP (g/kg).

‡ Phytate P in this diet is 2·4 g/kg (see Table 1).

§ $Y = 1{\cdot}849-1{\cdot}799\,e^{-0{\cdot}0008X}$, r^2 0·99; Y is released P (g/kg) and X is phytase activity (U/kg diet).

‖ $Y = 88{\cdot}17-84{\cdot}91\,e^{-0{\cdot}0007X}$, r^2 0·99; Y is phytate P released (g) and X is phytase activity (U/kg diet).

¶ $Y = \ln Y = -1{\cdot}978+0{\cdot}202\ln X-0{\cdot}034\,(\ln X)^2$, r^2 0·99; Y is released P (g)/100 U phytase and X is phytase activity (U/kg diet).

positive control level of nP (4·5 g/kg), P excretion was reduced by 25–54% with addition of 200–1200 U phytase/kg diet. In terms of the model of Schoner *et al.* (1990) in which one manure unit corresponded to 350 broilers with a P discharge of 55 kg P_2O_5/year, adding phytase could increase the number of broilers per manure unit from 350 to 435 or 525 for the same amount of P excretion. This result confirms the finding of Yi *et al.* (1996).

The results presented here indicate that supplemental phytase improved Ca availability with the increase of apparent retention (% of intake) and total amount of Ca retained (g/bird). These findings are supported by other observations (Schoner *et al.* 1991, 1993; Yi *et al.* 1996). In a broiler study designed to measure the effect of phytase on Ca availability, Schoner *et al.* (1994) reported that 500 U microbial phytase was equivalent to 0·35 g Ca as measured by BW gain and 0·56 g Ca as measured by phalanx ash. Phytic acid can form insoluble salts with Ca^{2+} (Oberleas, 1973; Morris, 1986), potentially rendering Ca unavailable for intestinal absorption. Phytase has the ability to release Ca^{2+} from the insoluble salts and makes Ca available for absorption in broilers.

The results of the present experiment demonstrate that the measurements of BW gain, feed intake, toe ash percentage, apparent retention (% of intake) of Ca and P, total amount retention (g/bird) of DM, Ca and P, and P excretion are sensitive indicators for measuring the efficacy of phytase. BW gain and toe ash percentage were found to be sensitive measurements to evaluate P availability in diets of poultry (Simons *et al.* 1990; Schoner *et al.* 1993; Yi *et al.* 1996). Several tibia, metatarsal and toe measurements, as well as BW gain, were examined in broilers given deficient to adequate levels of P from seven P sources and a dicalcium phosphate dehydrate standard for 3 weeks (Ravindran *et al.* 1995*b*). It was found that BW gain and toe ash percentage were equally or more sensitive for assessment of P availability than tibia ash, and that other measurements, including tibia specific gravity, tibia shear force, toe shear force and metatarsal shear force, were of limited value. Birds are sensitive to dietary P because of their characteristic low P storage and fast growth. Retention (% of intake and g/bird) directly reflected the absorption and utilization of dietary P in the body of broilers. Thus, these measurements are useful in predicting the responses to supplemental P and phytase. Measurement of the total amount of P retained (g/bird) appears to be even better than that of apparent retention (% of intake) of P. This was also observed in the study of Schoner *et al.* (1993). However, because of their economic importance, ease of determination and sensitivity, BW gain and toe ash percentage may be the measurements of choice in many situations.

The results of the present experiment indicate that about 939 U phytase was required to replace 1 g inorganic P as defluorinated phosphate based on equally weighted BW gain and toe ash percentage for equivalent P values averaged across 2·0 and 2·7 g nP/kg (Table 8). This value was obtained using the non-linear equation $Y(\text{g P}) = 1{\cdot}849 - 1{\cdot}799\,e^{-0{\cdot}0008X(\text{U phytase})}$. The amount of P released per 100 U phytase decreased as the total amount of phytase was increased. Yi *et al.* (1996) reported P equivalency values of 1598 U phytase in Expt 1 and 922 U in Expt 2 for broilers fed on a similar soyabean-meal-based diet at 2·7 g nP/kg (4·5 g tP/kg), but they reported a lower P equivalency value (766 U phytase) for broilers fed on a maize–soyabean-meal diet at 2·7 g nP/kg. Denbow *et al.* (1995) reported that released P values were higher at the higher phytase levels for 2·0 g nP/kg (3·8 g tP/kg) compared with 2·7 g nP/kg (4·5 g tP/kg). The P equivalency values of phytase for 1 g P were 609 U and 1133 U respectively for 2·0 and 2·7 g nP/kg. The P equivalency value for the average of the two P levels was 821 U phytase.

Schoner *et al.* (1991) reported that 700 U phytase was equivalent to 1 g P as MCP when P retention data from broilers fed on a maize–soyabean-meal diet for 14 d were used in the calculation; a value of 762 U phytase was obtained when the calculation was based on crude ash in the total body. The diet used by Schoner *et al.* (1991) contained 6 g Ca/kg and

4·5 g tP/kg that included 1 g/kg as MCP. Schoner *et al.* (1993) used a maize–soyabean-meal basal diet (6 g Ca/kg and 3·5 g tP/kg) with no added inorganic P. They reported the P equivalency values of phytase at 14 d as 1 g P as MCP = 570 U phytase for BW gain and 1 g P = 1050 U phytase for P retention. At 40 d, 850 U phytase was equal to 1 g P for both measurements. Simons & Versteegh (1993) suggested for broilers during the first 14 d that 250 U phytase was equivalent to 0·5 g P as MCP. Our interpretation of their data suggests that the response per 250 U phytase decreased as the amount of phytase was increased from 500 to 750 U/kg diet; inorganic P was reduced in the diet as the amount of phytase increased. The difference in P equivalency values may be due to diet type, response indicators, and inorganic P level and source used.

In the calculation of P equivalency values in the present experiment, all the data for birds given 3·4 g nP/kg diet were omitted because of the inconsistency of response measurements (Table 7). The r^2 values for the equations of Ca retention (% of intake and g/bird) were low. Since 3·4 g nP/kg was closer to the optimum level of P (NRC (1994) recommended 4·5 g nP/kg) than were 2·0 and 2·7 g/kg, phytase-released P at 3·4 g nP/kg was less than that at 2·0 and 2·7 g nP/kg. This indicates that the responses of phytase in maize and soyabean meal were influenced by the dietary nP level. This is supported by the findings from the *in vitro* study by Irving & Cosgrove (1974). They found that inorganic orthophosphate was an inhibitor of *Aspergillus ficuum* phytase.

The wide Ca:tP ratio (2:1) used in the present experiment probably reduced the overall response to phytase and inorganic P. Schoner *et al.* (1993) reported that feeding high levels of Ca with a constant low level of tP (3·5 g/kg) reduced the increase in BW gain, feed intake, and P and Ca retention resulting from added phytase. Their Ca:tP ratios were 1·71:1, 2·14:1 and 2·57:1 from the lowest to the highest level of Ca. Similar negative effects of widening the Ca:tP ratio on these response measurements were observed for the lower levels of inorganic P, but not for the higher levels. In our laboratory, Qian *et al.* (1996) found negative effects on response measurements (BW gain, feed intake, gain/feed, toe ash percentage, P and Ca retention) of widening the Ca:tP ratio (1·1:1 to 2:1) at each phytase level (0, 300, 600 or 900 U/kg diet) and each P level (2·7 and 3·6 g nP/kg). The magnitude of the effect of widening the Ca:tP ratio was much larger at the lower P level. For example, widening the Ca:tP ratio from 1·4:1 to 2:1 decreased phytase efficiency by 7·4% for the 2·7 g nP/kg diets and 4·9% for the 3·4 g nP/kg diets.

The results of the present experiment clearly demonstrate that the response of microbial phytase is influenced by the dietary level of nP and that the release of P per unit of phytase decreases as the amount of phytase increases per unit of diet. The optimal amount of microbial phytase to add to a broiler diet will depend on the response of phytase (P equivalent value), the cost of phytase and P, and the disposal cost of P excreted.

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