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Effects of Microbial Phytase on Animal Performance, Amount of Phosphorus Excreted and Blood Parameters in Broiler Fed Low Non-Phytate Phosphorus Diets

[1]N. Tugba Bingol, [1]M. Akif Karsli, [1]D. Bolat, [1]I. Akca and [2]T. Levendoglu
[1]Department of Animal Nutrition, Faculty of Veterinary,
University of Yuzuncu Yil, 65080-Van, Turkey
[2]College of Adilcevaz, University of Bitlis Eren, Adilcevaz, Bitlis, Turkey

Abstract: The aim of the current study was to evaluate the effects of a microbial phytase on broiler performance, mineral retention and mineral excretion in broilers fed corn-soybean meal-barley based diet with low available phosphorus level. A total of 300 one day-old Ross 308 broilers were allotted into 5 treatment groups consisted of 4 subgroups. This basal diet (negative control) was supplemented with enzyme (Rovabio; control). Then, control diet was supplemented with 500 g ton^{-1} microbial phytase (Rovaphos; 500 g phytase), 1000 g ton^{-1} microbial phytase (1000 g phytase) and 1500 g ton^{-1} microbial phytase (1500 g phytase). Body weight of broiler fed low available phosphorus diets supplemented with phytase were significantly higher ($p<0.05$) compared with broilers fed low available phosphorus diet without phytase throughout the experiment starting from second week of experiment. Broilers fed negative control diet had significantly less carcass weights compared with other groups ($p<0.05$). Addition of phytase linearly increased serum P levels and decreased amount of P excreted in feces. It can be concluded that dietary available phosphorus can be reduced up to 30% in broiler diet with 1000 g phytase/ton supplementation without affecting animal performance.

Key words: Broiler, phytase, low non-phytate phosphorus, growth performance, phosphorus excretion

INTRODUCTION

Phosphorus (P) is an essential ingredient in animal and plant production; however too much or too little P can be a problem both for animal production and the environment (Sohail and Roland, 1999). The major portion of phosphorus in diets containing plant ingredients, including corn and soybean is present in the form of phytate, which is largely unavailable in monogastric animals (Bozkurt *et al*., 2006). Inorganic or non-phytate P is therefore added in the feed to meet the demands, which creates an additional cost to poultry producers. Moreover, unavailable phytate P is excreted in the manure and may cause manure to contain more P than plants can use (Sohail and Roland, 1999). Phytase, an enzyme of microbial origin, can increase the availability of phytate P. It has been also reported that phytase can significantly improve the utilization of the essential amino acid in broilers fed soybean meal basal diets (Biehl and Baker, 1997).

It has been reported that phytase additions (from 0, 200, 400, 600, 800, 1000 and up to 1200 U microbial phytase kg diet^{-1}) linearly increased body-weight gain, feed intake, toe ash percentage and apparent retention (% of intake) or total amount (g bird^{-1}) of retained Ca and P and linearly decreased ($p<0.01$) P excretion (g kg^{-1} of DM intake) at each level of none-phytate with the

Corresponding Author: M. Akif Karsli, Department of Animal Nutrition, Faculty of Veterinary, University of Yuzuncu Yil, 65080-Van, Turkey

magnitude of the response inversely related to the level of none-phytate in broiler fed corn soybean based diet (Kornegay *et al*., 1996). Serum concentrations of phosphorus and calcium are also influenced by dietary phosphorus concentrations and the presence or not of exogenous phytase. The inclusion of phytase enzyme in diets with a low concentration of none-phytate phosphorus increases the coefficient of phosphorus retention and reduced the presence of this element in broiler excreta by up to 45% (Juanpere *et al*., 2004). However, contrary to these reports, the addition of dietary phytase to corn-soybean diet containing less phosphorus than the National Research Council (NRC) (1994) recommendation did not improve either body weight gain or feed intake, but it did increase toe and tibia ash and serum inorganic phosphorus in broiler chickens, growing quails and guinea fowl.

The objective of the current study was to evaluate the effects of a microbial phytase on broiler performance, mineral retention and mineral excretion in broilers fed corn-soybean meal-barley based diet with low available phosphorus level.

MATERIALS AND METHODS

Animals and Treatments

A total of 300 one-day old Ross 308 broilers were utilized in this study. Initial weights of each chick were determined at the initiation of experiment. Then, chicks were randomly allotted into 5 treatment groups consisted of 4 subgroups. Each treatment group contained a total of 60 chicks with 15 chicks within each subgroup.

The diets (starter and finisher) used in the experiment were prepared in a private milling company and corn-soybean meal based with considerable amount of barley (14% of DM). Dietary P levels was calculated to provide 70% of required non-phytate P but total P levels were greater than the required levels (NRC, 1994). This basal diet (negative control) was supplemented with enzyme (Rovabio®; control). Then, control diet was supplemented with 500 g ton^{-1} microbial phytase (Rovaphos®; 500 g phytase), 1000 g ton^{-1} microbial phytase (1000 g phytase) and 1500 g ton^{-1} microbial phytase (1500 g phytase). Chemical and botanical compositions of diets were given in Table 1. A total of 5 different diets were prepared by addition of rovabio, 500 g ton^{-1} phytase+rovabio, 1000 g ton^{-1} phytase+rovabio and 1500 g ton^{-1} phytase+rovabio into negative control diet. Chicks were fed these diets as group of 15 chicks within each subgroup and experiment lasted 42 day. Plastic feeders and wagerers were utilized in the experiment. Chicks were maintained on a 23 h constant light schedule and in a heat-controlled room.

Determination of Animal Performance

Individnal live weight gains and feed intakes were determined weekly (initiation, 7, 14, 21, 28, 35 and 42 days of experiment) using a digital scale with a sensitivity of 10 mg. Chicks had free access to fresh feed and water throughout the experiment. Chicks that died were recorded daily. Amount of feed given was recorded daily. Amount of oarts were determined at end of each week. Feed intake per week and feed efficiency were, then, calculated. During the first three days of the 5th and 6th week of trial all excreta were collected from each pen. Excreta were stored in plastic bags at -20°C. Before chemical analysis samples were defrosted, homogenized and dried at 60°C. Then they were weighed and ground to pass a 1 mm sieve. Calcium, total and phytate phosphorus contents were determined by the same chemical methods used in diet analysis.

Determination of Carcass and Internal Organ Weight

Carcass and total internal organ (liver, heart, gizzard and pancreas), intestine and abdominal fat weights were determined at the end of experiment.

Table 1: Botanical and chemical composition of the experimental diets

Ingredients (%)	Starter	Finisher
Maize	36.000	45.000
Soybean meal	38.200	30.010
Barley	14.000	15.000
Dicalcium Phosphate	1.010	0.680
Limestone	1.830	1.830
Sunflower Oil	8.500	6.900
Vitamin-Mineral Premix*	0.270	0.390
Mehionine+Cystine	0.190	0.100
Calculated chemical composition of diets		
Crude Protein (%)	23.000	20.000
Metabolizable Energy (Kcal kg^{-1})	3200.000	3200.000
Calcium (%)	1.000	0.900
Total phosphorus (%)	0.570	0.500
Available phosphorus (%)	0.315	0.245
Chemical composition based on analysis (% of DM)0		
Dry matter	92.830	91.900
Crude Protein	22.670	19.230
Ether extract	8.560	8.350
Crude cellulose	7.690	8.070
Ash	8.310	8.190
Nitrogen free extract	45.600	48.060
Calcium	1.200	0.660
Total phosphorus	0.440	0.420
Metabolizable Energy (Kcal kg^{-1})	3228.000	3187.000

*Vitamin-Mineral premix (IU or mg kg^{-1} diet): Vitamin A: 12000 IU; Vitamin D_3: 1500 IU; Vitamin E: 30 mg; Vitamin K_3: 5 mg; Vitamin B_1: 3 mg; Vitamin B_2: 6 mg; Vitamin B_6 5 mg; Vitamin B_{12} 0.03 mg; Nicotine amid: 40 mg; Calcium-D-pantothenate: 10 mg; Folic acid: 0.075 mg; Choline chloride: 375 mg; Antioxidant: 10 mg; Manganese: 80 mg; Iron: 80 mg; Zinc: 60 mg; Copper: 8 mg; Iodine: 0.5 mg; Cobalt: 0.2 mg; Selenium: 0.15 mg. Phytase: Rovaphos® contains 500 000 FTU kg^{-1} and isolated from Peniophora Iycii. Rovabio® contains Endo-1, 4-β-xylanase: 22,000 visco. Units per g (equivalent to 1,400 AXC units per g) Endo -1, 3 (4) β-glucanase: 2,000 AGL units per g (Trouw Nutrition Turkey)

Blood Sampling

Blood samples were randomly taken from *V. subcutanea* ulnaris of selected 4 chicks from each treatment groups to determine blood glucose, total protein, urea, Ca and P.

Chemical Analysis

Chemical compositions of diets were analyzed according to AOAC (1990). Blood glucose, total protein, urea, Ca and P levels were analyzed using auto-analyzer (Hitachi 912, Boehringer Mannheim). Dietary, bone, feces Ca and P levels were determined according to Combs *et al.* (2003).

Statistical Analysis

Experimental data were subjected to statistical analysis by using GLM of SAS for variance analysis. The differences among means were separated by Duncan t-test (SAS, 2005). All values were presented as means and standard error (SEM). The level of significantly difference was set up at $p<0.05$.

RESULTS AND DISCUSSION

Initial body weights of broilers were similar as a part of experimental design. However, body weight of broiler fed low available phosphorus diets supplemented with phytase were significantly higher ($p<0.05$) compared with broilers fed low available phosphorus diet without phytase throughout the experiment starting from second week of experiment (Table 2). There were no statistical differences on body weight of broilers fed either control or diets containing phytase throughout the experiment

Table 2: Live weight gain (g) of broiler fed different dietary treatment

		Week					
Treatment groups	Initial	1	2	3	4	5	6
Control	67.47	160.03[bc]	341.70[a]	561.25[ab]	885.45[a]	1211.50[a]	1605.58[a]
Negative control	67.57	158.53[c]	317.35[b]	532.53[b]	798.20[b]	1085.88[b]	1327.38[b]
500 g phytase	67.37	168.20[a]	346.28[a]	563.88[ab]	896.85[a]	1276.70[a]	1640.80[a]
1000 g phytase	67.55	165.73[abc]	332.75[ab]	549.93[ab]	871.48[a]	1257.90[a]	1623.88[a]
1500 g phytase	67.58	167.15[ab]	345.43[a]	585.75[a]	912.83[a]	1298.88[a]	1664.05[a]
SEM	0.17	2.27	6.02	11.62	19.18	33.78	62.77

Mean values with different superscripts within a column are significantly different, ($p<0.05$)

Table 3: Daily feed consumption (g) of broiler fed different dietary treatment

		Week				
Treatment groups	Initial	1	2	3	4	5
Control	176.80[a]	562.37[ab]	1162.37[a]	1783.20[a]	2460.46[a]	3204.70[a]
Negative control	147.40[b]	412.55[c]	840.17[b]	1433.63[b]	2105.17[b]	2808.07[b]
500 g phytase	165.64[ab]	587.07[a]	1187.06[a]	1841.16[a]	2558.76[a]	3353.67[a]
1000 g phytase	174.93[a]	557.80[b]	1157.80[a]	1801.47[a]	2523.00[a]	3336.14[a]
1500 g phytase	158.17[ab]	559.17[b]	1159.17[a]	1800.13[a]	2522.99[a]	3347.20[a]
SEM	8.03	8.46	15.09	25.71	41.63	62.45

Mean values with different superscripts within a column are significantly different, ($p<0.05$)

($p>0.05$). However, the highest live weight (1664.05 g) was obtained for the supplementation of 1500 g phytase per ton feed. Many studies (Ahmed *et al*., 2004; Pintar *et al*., 2004; Bozkurt *et al*., 2006) have reported the increases in live weight gain in broilers with increasing concentration of dietary phytase in soybean meal based diets, which are in agreement with the result of this study. Zyla *et al*. (2000) have noted that the growth rate of broilers fed low phosphorus diets containing microbial phytase were comparable with or even better than those fed standard phosphorus diet, supporting the concept that phytase improves not ouly availability of phosphorus (Bozkurt *et al*., 2006) but also the utilization of the essential amino acid in broilers fed soybean meal basal diets (Biehl and Baker, 1997). However, Pizzolante *et al*. (2002) have found that dietary phytase had no effect on live weight gain of broilers. These differences among studies might have resulted from the amount of available phosphorus used and amount of phytase added in diets.

Feed consumption of broilers fed control or diet supplemented with phytase were similar throughout the experiment ($p>0.05$), except second week of experiment. Broilers fed diet containing 500 g phytase/ton had significantly higher feed consumption compared with other diets containing phytase in the second week of experiment ($p<0.05$). Broilers fed negative control had significantly lower feed consumption compared with other groups throughout the experiment ($p<0.05$). Phytase supplementation to low available phosphorus diets significantly increased feed intake ($p<0.05$) compared with low available phosphorus diet without phytase (negative control diet) (Table 3). Similar to the result of this study, Ahmed *et al*. (2004) and Pintar *et al*. (2004) have reported that feed consumption significantly increased in broilers fed diets supplemented with phytase. Bozkurt *et al*. (2006) have noted that feed intake and feed efficiency of broilers fed diet containing phytase were similar to those fed control diet containing dicalcium phosphate, which support the result of the current study. The increases in feed consumption with phytase supplementation might have resulted from increases in digestibility of nutrients and partial cell wall degradation.

Feed efficiency of broilers fed control or diets containing phytase were similar during whole experimental period (Table 4, $p>0.05$). Although broilers fed negative control diet had significantly lower feed efficiency at 2, 3 and 4th weeks of experiment compared with other treatment groups ($p<0.05$), feed efficiencies were similar among all of the treatment groups last two weeks of experiment ($p>0.05$). The feed efficiency results are in agreement with the findings of Bozkurt *et al*. (2006), who

Table 4: Feed efficiency of broiler fed different dietary treatment

		Week				
Treatment groups	Initial	1	2	3	4	5
Control	1.91	2.05[a]	2.36[a]	2.19[a]	2.16	2.09
Negative control	1.62	1.65[b]	1.81[b]	1.96[b]	2.07	2.24
500 g phytase	1.65	2.11[a]	2.39[a]	2.22[a]	2.12	2.14
1000 g phytase	1.79	2.11[a]	2.41[a]	2.24[a]	2.13	2.15
1500 g phytase	1.59	2.02[a]	2.24[a]	2.13[a]	2.05	2.10
SEM	0.10	0.05	0.06	0.04	0.05	0.07

Mean values with different superscripts within a column are significantly different, (p<0.05)

Table 5: Carcass and internal organ weights (g) of broiler fed different dietary treatment

Treatment groups	Live weight	Carcass weight	Empty carcass	Intestine	Internal organs	Abdominal fat
Control	1861.00[a]	1521.17[a]	1309.17[a]	109.17	74.19[a]	23.30
Negative control	1575.00[b]	1308.33[b]	1131.00[b]	101.33	66.52[b]	18.83
500 g phytase	1832.17[a]	1504.50[a]	1293.33[a]	107.02	77.57[a]	18.59
1000 g phytase	1902.83[a]	1582.83[a]	1395.67[a]	100.75	75.37[a]	25.34
1500 g phytase	1853.83[a]	1527.33[a]	1330.50[a]	96.62	74.60[a]	18.51
SEM	62.65	54.94	54.11[a]	6.10	2.45	3.10

Mean values with different superscripts within a column are significantly different, (p<0.05)

Table 6: Percentages of Ca, P in tibia and feces of broiler fed different dietary treatment, (%)

	Tibia		Feces	
Treatment groups	P	Ca	P	Ca
Control	14.03	25.44[a]	1.18[a]	1.35
Negative control	14.21	21.10[b]	1.06[b]	1.24
500 g phytase	13.48	25.15[a]	1.01[bc]	1.04
1000 g phytase	13.93	23.53[a]	0.96[c]	1.07
1500 g phytase	14.40	23.79[a]	0.96[c]	1.47
SEM	0.54	0.74	0.02	0.16

Mean values with different superscripts within a column are significantly different, (p<0.05)

noticed that feed efficiency of broilers fed diet containing phytase were similar to those fed control diet with dicalcium phosphate. However, the lower feed efficiency observed in this experiment with control diet contradicts the result of Bozkurt *et al*. (2006). Reduction in dietary NPP depressed live weight gain and feed intake and increased feed conversion ratio (Singh *et al*., 2003). Similar to present results, some researchers have also noted that even though addition of phytase into diets increased both live weight gain and feed intake, it did not have positive effect on feed efficiency (Huff *et al*., 1998; Brenes *et al*., 2003), which is in agreement with the results of the current study.

Carcass weights of broilers fed control or diets containing phytase were statistically similar (p>0.05), but broilers fed negative control diet had significantly less carcass weights compared with other groups (p<0.05). The highest carcass weight was obtained in broiler fed diet containing 1000 g ton^{-1} pytase (Table 5). A positive correlation between dressed weight and live weight and age has been earlier reported by Howlider and Rose (1989) and Ahmed *et al*. (2004). Dressed weight was a function of live weight, thus, as live weight increased carcass weight also increased accordingly. Preston *et al*. (2000) reported an increased carcass yield with addition of phytase into diet, which coincided with the result of the current study. Weights of intestine and abdominal fat were similar among treatment groups, but weight of internal organs was less in broilers fed negative control compared with other groups. The differences in internal organ weights could be due to the differences in size of broiler in different treatment groups.

The percentages of phosphorus and calcium in tibia increased with the addition of phytase into diet (Table 6, p<0.05). Calcium contents of broiler fed negative control were less compared with those fed either control or diets supplemented with phytase (p<0.05). Similar to this study, Midilli *et al*.

Table 7: Serum glucose, total protein (g dL^{-1}), P, Ca and urea levels (mg dL^{-1}) of broiler fed different dietary treatment

Treatment groups	Glucose	Total protein	Total P	Ca	Urea
Control	196.50	3.10	3.99	12.85^{bc}	4.81^{ab}
Negative control	206.75	2.91	2.63	15.91^{a}	3.75^{b}
500 g phytase	206.00	2.81	3.09	14.86^{ab}	6.42^{a}
1000 g phytase	196.00	2.86	3.32	12.85^{bc}	5.71^{ab}
1500 g phytase	197.50	3.08	4.21	11.30^{c}	6.42^{a}
SEM	8.19	0.15	0.56	0.67	0.61

Mean values with different superscripts within a column are significantly different, ($p<0.05$)

(2003) have reported an increased Ca and P content of tibia with addition of phytase into diet. An increase in ash and phosphorus content of tibia has been described as a good indication of increased availability of phosphorus (Bozkurt *et al*., 2006). The increases in tibia phosphorus and calcium content of broilers fed diets containing phytase clearly indicated that availability of phosphorus increased by the action of phytase in this study. The improvement in phosphorus availability by phytase also significantly reduced amount of phosphorus excreted in manure, which was supported by the results of Pintar *et al*. (2005) ($p<0.05$). However, phytase did not affect amount of calcium excreted in manure ($p>0.05$). Simons *et al*. (1990) have shown that the viability of phosphorus increased over 60% and the amount of phosphorus in manure decreased by 50% with microbial phytase supplementation of low phosphorus diet. Plumstead *et al*. (2007) also showed that phytase inclusion in a broiler breeder laying diet at the expense of all added P from dicalcium phosphate reduced the manure total P and water soluble P concentrations by 42%, which support the result of this study.

Total serum glucose, protein and phosphorus levels were statistically similar among groups (Table 7) ($p>0.05$). However, both total serum protein and phosphorus levels linearly increased with increasing level of phytase in diets. Brenes *et al*. (2003) have reported that serum Ca (4%), P (12%) and total protein (7%) increased with phytase supplementation into broiler diet. Even though increases in serum total P and protein were not significant, they support the results of Brenes *et al*. (2003). While serum calcium levels decreased serum urea level increased with addition of phytase into diet ($p<0.05$).

The results of the current study showed that microbial phytase in broiler diets enhanced the availability of phosphorus. Therefore, the growth performance and carcass yield of broilers increased and phosphorus content of manure decreased with addition of phytase into broiler diet, which supplementation of phytase did not ouly improve animal performance but also reduced potential enviroumental pollution. Thus, it can be concluded that dietary available phosphorus can be reduced to 30% in broiler diet with 1000 g phytase/ton supplementation without affecting animal performance.

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REFERENCES

Ahmed, F., M.S. Rahman, S.U. Ahmed and M.Y. Miah, 2004. Performance of broiler on phytase supplemented soybean meal based diet. Int. J. Poult. Sci., 3: 266-271.

AOAC, 1990. Official Methods of Analysis. 15th Edn., Association of Official Analytical Chemists, Arlington, VA, Washington, USA., pp: 774-784.

Biehl, R.R. and D.H. Baker, 1997. Microbial phytase improves amino acid utilization in young chicks fed diets based on soybean meal but not in diets based on peanut meal. Poult. Sci., 76: 355-360.

Bozkurt, M., M. Cabuk and A. Alcicek, 2006. The effect of microbial phytase in broiler grower diets containing low phosphorus, energy and protein. J. Poult. Sci., 43: 29-34.

Brenes, A., A. Viveros, I. Arija, C. Centeno, M. Pizarro and C. Braro, 2003. The effect of citric acid and microbial phytase on mineral utilization in broiler chicks. Anim. Feed Sci. Technol., 110: 201-219.

Combs, S., B. Hoskins, J. Jarman, J. Kovar, M. Watson, A. Wolf and N. Wolf, 2003. Recommended Methods of Manure Analysis. University of Wisconsin-Extension, USA.

Howlider, M.A.R. and S.P. Rose, 1989. Rearing temperature and the meat yield of broilers. Br. Poult. Sci., 30: 61-67.

Huff, W.E., P.A. Moore, P.W. Waldroup, A.L. Waldroup and J.M. Balog *et al*., 1998. Effect of dietary phytase and high available phosphorus corn on broiler chicken performance. Poult. Sci., 77: 1899-1904.

Juanpere, J., A.M. Perez-Vendrell and J. Brufau, 2004. Effect of microbial phytase on broilers fed barley based diets in the presence or not of endogenous phytase. Anim. Feed Sci. Technol., 115: 265-279.

Korengay, E.T., D.M. Denbow, Z. Yi and V. Ravindran, 1996. Response of broilers to graded levels of microbial phytase added to maize-soybean meal-based diets containing three levels of non-phytate phosphorus. Br. J. Nutr., 75: 839-852.

Midilli, M., O.H. Muglalý, M. Alp, N. Kocabaglý, M.A. Tanor and G.S. Toklu, 2003. The effect of dietary phytase supplementation on the fattening performance and mineral balance of broilers. Turk. J. Vet. Anim. Sci., 27: 751-759.

NRC., 1994. Nutrient Requirement for Poultry. 9th Edn., National Academy Press, Washington DC, USA.

Pintar, J., B. Homen, K. Gazic, D. Grbesa, M. Sikiric and T. Cerny, 2004. Effects of supplemental phytase on performance and tibia ash of broilers fed different cereals based diets. Czech J. Anim. Sci., 49: 542-548.

Pintar, J., B. Homen, K. Gazic, Z. Janjecic, M. Sikiric and T. Cerny, 2005. Effects of supplemental phytase on nutrient excretion and retention in broilers fed different cereal based diets. Czech J. Anim. Sci., 50: 40-46.

Pizzolante, C.C., A.S. Teixeira, C.D. Santos, A.G. Bertechini and E.A. Garcia, 2002. Phytase and calcium levels on performance of broiler chickens. Ciencia-e-Agrotecnologia, 26: 418-425.

Plumstead, P.W., H. Romero-Sanchez, R.O. Maguire, A.G. Gernat and J. Brake, 2007. Effects of phosphorus level and phytase in broiler breeder rearing and laying diets on live performance and phosphorus excretion. Poult. Sci., 86: 225-231.

Preston, C.M., K.J. McCracken and A. McAllister, 2000. Effect of diet form and enzyme supplementation on efficiency and energy utilization of wheat-based diets for broilers. Br. Poult. Sci., 41: 324-331.

SAS, 2005. SAS/STAT Software: Changes and Enhancements. Ver. 6.12, SAS Institute Inc., Cary, North Carolina.

Simons, P.C.M., H.A.J. Versteegh, A.W. Jongbloed, P.A. Kemme and P. Slump *et al*., 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Br. J. Nutr., 64: 525-540.

Singh, P.K., V.K. Khatta, R.S. Thakur, S. Dey and M.L. Sangwan, 2003. Effects of phytase supplementation on the performance of broiler chickens fed maize and wheat based diets with different levels of non-phytate phosphorus. Asian Aust. J. Anim. Sci., 16: 1642-1649.

Sohail, S.S. and D.A. Sr. Roland, 1999. Fabulous phytase: Phytase enzyme proving helpful to poultry producers and enviroument. Highlights Agric. Res.

Zyla, K., J. Koreleski, A. Swiakiwicz Wikiera, M. Kujawaski, J. Piironen and R.R. Lodovx, 2000. Effects of phosphorolytic and cell-wall degrading enzyme on the performance of growing broilers fed wheat-based diets containing different calcium levels. Poult. Sci., 79: 66-76.