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Effects of Dietary Phytase and Different Levels of Non-Phytate Phosphorus on Some Serum Minerals and Biochemical Parameters in Broiler Chickens

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Abstract: The objectives of this study were to determine the effects of microbial phytase supplementation over a range of Non-Phytate Phosphorus (NPP) on some serum parameters of broiler chickens fed corn-soybean meal diets. The experimental diets were as follows: diet 1) 0.31% NPP during starter and 0.29% NPP during grower and finishing periods without phytase; diet 2) 0.31% NPP during starter and 0.29% NPP during grower and finishing periods plus phytase (a commercially available microbial phytase preparation, Natuphos 1000 was added at 500 phytase units kg^{-1} diet); diet 3) 0.38% NPP during starter and 0.36% NPP without phytase; diet 4) 0.38% NPP during starter and 0.36% NPP during grower and finishing periods plus phytase; diet 5) 0.45% NPP during starter and 0.43% NPP during grower and finishing periods without phytase; diet 6) 0.45% NPP during starter and 0.43% NPP during grower and finishing periods plus phytase. At 21 and 42 day of age, Serum Ca, P, Mg, Albumin and total protein were measured. The results have showed that supplementation broiler diets with commercial phytase increased levels of NPP, P, Albumin and total protein concentrations and decreased serum Mg however, Ca concentration did not change.

Key words: Phytase, NPP, broiler chickens, serum parameters, Albumin, meal diets, Iran

INTRODUCTION

Plant materials are the major constituents of poultry diets. About two-thirds of the Phosforous of plant origin is present as phytic acid in the form of myo-inositol phosphates (Cromwell, 1980). Phosphorus in the phytic acid form is poorly available to monogastric animals because they lack phytase, the enzyme that hydrolyzes phytic acid into inositol and orthophosphate (Peeler, 1972). It is well documented that microbial phytase supplementation improves the availability of phytate-bound P in broiler chickens (Nelson *et al*., 1971; Simons *et al*., 1990; Roberson and Edwards, 1994). However, there is little information about the availability of trace minerals and protein when the broiler diet is supplemented with microbial phytase. Phytate being a strong acid can form various salts with the important minerals such as Ca, Mg, Cu, Zn, Fe and K thus reducing their solubility. Nutritionally more important is the fact that maximum binding of Zn-Ca-Cu-phytate as well as Cu-Ca-phytate occurs at pH 6 wich is the normal pH of the duodenum where maximum absorption of divalent cations takes place (Sebastian *et al*., 1996). When phytic acid is hydrolyzed by microbial phytase it may release all phytate-bound minerals. Microbial phytase improved performance ileal amino acid digestibility of broilers fed a lysine-deficient diet (Ravindran *et al*., 2001). The lack of information and contradictions concerning the efficacy of phytase on the availability of trace minerals and proteins indicate the need for more investigation. Therefore, the objectives of this study were to determine the effects of microbial phytase supplementation over three different levels of Non-Phytate Phosphorus (NPP) on serum Ca, P, Mg, Albumin (Alb) and Total Protein (TP) in broiler chickens fed corn-soybean meal diets.

MATERIALS AND METHODS

A total of 576 day old broiler chickens (Ross 308) were purchased from a commercial hatchery (Varok, Sanandaj, Iran). All birds had *ad libitum* access to water and experimental diets during the experiment. The experimental design was arranged in a 2×3 factorial and coducted in a completely randomized design with three replicate and thirty two birds in each replicate. The experimental diets (Table 1) were as follows: 0.31% NPP during starter and 0.29% NPP during grower and finishing periods without phytase; 0.31% NPP during starter and 0.29% NPP during grower and finishing periods plus phytase (a commercially available microbial phytase preparation, Natuphos 1000[2] was added at 500 phytase units kg^{-1} diet); 0.38% NPP during starter and 0.36% NPP without phytase; 0.38% NPP during starter and

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Table 1: Composition of experimental diets (1-20 days)

	Phytase enzyme (U kg^{-1} diet)					
Ingredients and analysis	Diet 1 (0)	Diet 2 (0)	Diet 3 (0)	Diet 4 (500)	Diet 5 (500)	Diet 6 (500)
None-phytase phosphorus level (%)	0.45	0.38	0.31	0.45	0.38	0.31
Corn (%)	57.83	58.17	58.50	57.83	58.17	58.50
Soydean meal (%)	34.91	34.85	34.78	34.91	34.85	34.78
Fish meal (%)	2.50	2.50	2.50	2.50	2.50	2.50
Corn oil (%)	1.27	1.16	1.05	1.27	1.16	1.05
Calcium carbonate (%)	1.13	1.35	1.57	1.13	1.35	1.57
Dicalcium phosphate (%)	1.33	0.96	0.58	1.33	0.96	0.58
Salt (%)	0.30	0.30	0.30	0.30	0.30	0.30
Mineral premix (%)*	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix (%)**	0.25	0.25	0.25	0.25	0.25	0.25
Methionine (%)	0.17	0.17	0.17	0.17	0.17	0.17
Lysine (%)	0.05	0.06	0.06	0.06	0.06	0.06
Phytase (%)***	0.00	0.00	0.00	0.05	0.05	0.05
Calculated composition**						
ME kcal kg^{-1}	2900.00	2900.00	2900.00	2900.00	2900.00	2900.00
Crude protein (%)	22.00	22.00	22.00	22.00	22.00	22.00
Calcium (%)	0.95	0.95	0.95	0.95	0.95	0.95
Total of phosphorus (%)	0.70	0.63	0.56	0.70	0.63	0.56
Available phosphorus (%)	0.45	0.38	0.31	0.45	0.38	0.31
Methionine (%)	0.53	0.53	0.53	0.53	0.53	0.53
Sodium (%)	0.15	0.15	0.15	0.15	0.15	0.15

*Mineral premix supplied the following per kg of diet: Mn, 40 mg; Zn, 30 mg; Fe, 20 mg; Cu,4 mg; Se, 0.1 mg; I, 0.5 mg. ** Vitamin premix supplied the following per kg of diet: vitamin A (retinyl acetate), 3600 IU; Cholecalciferol, 800 IU; vitamin E, 7200 IU; Choline, 50 mg; vitamin B1, 3 mg; vitamin B_2, 2.6 mg; vitamin B_6, 1.2 mg; vitamin B_{12}, 6 mg; niacin, 8.1 mg; pantothenic acid, 4 mg; biotin, 0.04 mg; folic acid, 0.4 mg; thiamin, 0.5 mg; pyridoxine, 0.5. ***Natuphos, 10000 U g^{-1} F.T.U, BASF Aktiengeseilschaft Strategic Marketing Animal Nutrition, 67056 Ludwigshafen. Germany; ****based on tables of feed composition of NRC

0.36% NPP during grower and finishing periods plus phytase; 0.45% NPP during starter and 0.43% NPP during grower and finishing periods without phytase; 0.45% NPP during starter and 0.43% NPP during grower and finishing periods plus phytase.

Blood samples were obtained by cardiac puncture from three randomly selected chickens in each replicate on day 21 and 42. After collection, the blood samples were allowed to clot at room temperature for approximately 2 h. The blood was centrifuged for 5 min at 643×g. Serum calcium, phosphorus, magnesium, Alb and TP were measured by colorimetric method according to the manufactuer's procedures (ZistChem Diagnostic Kit, Iran). The data were analyzed using the general linear model procedure for analysis of variance. Significant differences among treatment means were separated by Duncan's new multiple range test with a 5% level of probability.

RESULTS AND DISCUSSION

The effect of dietary phytase on the P, Ca, Mg and TP of serum is shown in the Table 2. At 21st day of age there was not a significant difference in the serum P at different levels of NPP and microbial phytic acid. On day 42, broiler chickens which consumed the diet with the lowest level of NPP (the highest level of phytic phospherus) and supplemented with microbial phytase had the same level of serum P. After 21 day of experiment, the concentration of serum Ca was decreased by increasing levels of NPP however, serum Mg, Alb and TP were not affected. Serum Mg was significantly decreased ($p<0.05$) on day 42 by increasing NPP level. There was a significant effect on P concentration by increasing NNP plus phytase on day 42 ($p<0.05$). However, there was not a significant difference in serum Alb and TP concentration of chickens fed with the lowest level of on day 42 were significantly increased by increasing NPP and adding the phytase.

The results of this study show the effect of microbial phytase and different levels of NPP on some serum parameters as indications of nutrient utilization by broiler chickens fed a corn-soybean diet. Phytic acid has been regarded as the primary storage form of phosphate in almost all seeds (Cosgrove, 1966). Approximately 66% of the P in corn and 61% of the P in soybean meal is in the form of phytic acid (Nelson *et al*., 1968). In the present study, the inability of young broiler chickens to utilize phytic acid has been clearly observed in serum P and Alb concentration of chickens fed with the 0.31% NPP during starter and 0.29% NPP during grower and finishing periods without phytase (diet 1) and the same diet which supplemented with microbila phytase (diet 2). Phytase supplementation of the of the diet 4 yieled a significant improvement the serum P, Alb and TP at 42 day as compared with chicks fed diet one (Table 3). Similar observations of improved serum P with phytase supplementation have been reported for broiler chickens (Simons *et al*., 1990; Perney *et al*., 1993;

Table 2: The effect of phytase supplementation and different levels of NPP on serum Ca, Mg, P, Alb and TP on 21 and 42 in broiler chickens fed corn-soybean diets

	Ca (mg dL^{-1})		Mg (mg dL^{-1})		P (mg dL^{-1})		Alb (mg dL^{-1})		TP (mg dL^{-1})	
Diets	21 days	42 days	21 days	45 days	21 days	42 days	21 days	42 days	21 days	42 days
1	11.65[ab]	10.600	2.840	2.510[a]	5.340	3.860[b]	2.120	2.050[b]	4.130	3.720[ab]
2	12.32[a]	10.710	2.450	2.460[a]	5.700	6.590[a]	2.250	2.060[b]	4.180	3.470[b]
3	11.68[ab]	10.870	2.600	2.510[ab]	5.680	5.600[a]	2.090	2.140[a]	4.370	3.880[a]
4	10.97[ab]	10.820	2.460	2.430[b]	5.810	5.790[a]	2.280	2.140[a]	4.390	3.860[a]
5	11.60[ab]	10.840	2.730	2.490[ab]	6.440	6.400[a]	2.230	2.110[ab]	4.340	3.550[ab]
6	10.22[b]	10.970	2.450	2.480[b]	6.100	6.000[a]	2.080	2.100[ab]	4.150	3.770[ab]
SEM	0.79	0.310	0.060	0.001	0.620	0.650	0.070	0.040	0.030	0.040
Source of variation (probabilities)										
Level of NPP	0.015	0.532	0.078	0.049	0.055	0.042	0.714	0.078	0.714	0.078
Phytase	0.052	0.254	0.141	0.019	0.081	0.004	0.284	0.025	0.284	0.025
NPP×Phytase	0.050	0.358	0.198	0.050	0.072	0.036	0.119	0.050	0.119	0.050

[a-c]Means within columns within sex classification, with no common superscript differ significantly ($p<0.05$)

Table 3: Composition of experimental diets (21-42 days)

	Phytase enzyme (U kg^{-1} diet)					
Ingredients and analysis	Diet 1 (0)	Diet 2 (0)	Diet 3 (0)	Diet 4 (500)	Diet 5 (500)	Diet 6 (500)
None-phytase phosphorus level (%)	0.43	0.36	0.29	0.43	0.36	0.29
Corn (%)	61.70	62.04	62.34	61.70	62.04	62.34
Soydean meal (%)	31.25	31.19	31.12	31.25	31.19	31.12
Fish meal (%)	2.00	2.00	2.00	2.00	2.00	2.00
Corn oil (%)	1.53	1.42	1.31	1.53	1.42	1.31
Calcium carbonate (%)	1.08	1.30	1.51	1.08	1.30	1.51
Dicalcium phosphate (%)	1.32	0.95	0.58	1.32	0.95	0.58
Salt (%)	0.28	0.28	0.28	0.28	0.28	0.28
Mineral premix (%)*	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix (%)**	0.25	0.25	0.25	0.25	0.25	0.25
Methionine (%)	0.17	0.17	0.17	0.17	0.17	0.17
Lysine (%)	0.15	0.15	0.15	0.15	0.15	0.15
Phytase (%)***	0.00	0.00	0.00	0.05	0.05	0.05
Calculated composition**						
ME kcal kg^{-1}	2962.00	2962.00	2962.00	2962.00	2962.00	2962.00
Crude protein (%)	20.50	20.50	20.50	20.50	20.50	20.50
Calcium (%)	0.90	0.90	0.90	0.90	0.90	0.90
Total of phosphorus (%)	0.67	0.60	0.53	0.67	0.60	0.53
Available phosphorus (%)	0.43	0.36	0.29	0.43	0.36	0.29
Methionine (%)	0.51	0.51	0.51	0.51	0.51	0.51
Sodium (%)	0.14	0.14	0.14	0.14	0.14	0.14

*Mineral premix supplied the following per kg of diet: Mn, 40 mg; Zn, 30 mg; Fe, 20 mg; Cu,4 mg; Se, 0.1 mg; I, 0.5 mg. ** Vitamin premix supplied the following per kilogram of diet: Vitamin A (retinyl acetate), 3600 IU; Cholecalciferol, 800 IU; vitamin E, 7200 IU; Choline, 50 mg; vitamin B1, 3 mg; vitamin B_2, 2.6 mg; vitamin B_6, 1.2 mg; vitamin B_{12}, 6 mg; niacin, 8.1 mg; pantothenic acid, 4 mg; biotin, 0.04 mg; folic acid, 0.4 mg; thiamin, 0.5 mg; pyridoxine, 0.5. *** Natuphos, 10000 U g^{-1} F.T.U ,BASF Aktiengeseilschaft Strategic Marketing Animal Nutrition, 67056 Ludwigshafen. Germany; ****based on tables of feed composition of NRC

Broz *et al*., 1994; Sebastian *et al*., 1996; Ravindran *et al*., 2001; Peter *et al*., 2000). The improvement in some serum parameters observed in the chicken fed phytase may be due to the release of minerals from the phytate-mineral complex by animals as suggested by Simons *et al*. (1990) or increased availability of protein as suggested by Sebastian *et al*. (1996). Phytate also complexes with proteins making them less soluble (Smith and Rackis, 1957). It has been shown that phytate-protein complexes are less subject to proteolytic digestion than the same protein alone (Hill and Tyler, 1954). It may be possible that phytase liberates proteins from the complex, making them more available to the animal (Sebastian *et al*., 1996). However, further investigations are needed to determine the effect of phytase on availabilities of protein and minerals. The findings might be the result of a combination of improvement in nutrient utilization not only of minerals but also of protein. These results agree with the findings of Simons *et al*. (1990), Perney *et al*. (1993), Broz *et al*. (1994), Sebastian *et al*. (1996), Ravindran *et al*. (2001) and Peter *et al*. (2000). As expected, phytase supplementation increased the serum P by 70 % in chicks that recieved diet four as compared with chicks that received diet one which agrees with results of previous studies dealing with chickens (Simons *et al*., 1990; Broz *et al*., 1994; Sebastian *et al*., 1996) and pigs (Young *et al*., 1993; Lei *et al*., 1994; Mroz *et al*., 1994; Bruce and Sundstol, 1995). The diet one significantly increases the plasma Ca on 21st day. This increasing in Ca was expected because a low NPP diet normally results in an elevated ionized Ca in the serum which depresses the release of Parathyroid Hormone (PTH) thus reabsorption of phosphate and permitting the urinary excretion of additional Ca absorbed from the gut during low NPP diet feeding (Sebastian *et al*., 1996). Although, some small differences in serum Ca was observed on 42 days but in the observation, phytase and different levels of NPP had no significant effect on Ca concentration. This result agrees with the findings of Edwards (1993), Roberson and Edwards (1994) and Murry (1998). Serum Mg was decreased by increasing NPP and adding phytase. This result agrees with Young *et al*. (1993). In contradiction to this observation, some recent studies have shown that phytase had no significant effect on serum Mg (Murry *et al*., 1995).

CONCLUSION

The results of this study indicate that dietary microbial phytase supplementation to a low-NPP corn-soybean diet can improve P and protein utilization as indicated by its effect on serum P, Alb and TP of broiler chickens. The efficacy of phytase particulary in the diet that had least of NPP. The results obtained in this study clearly indicate the importance of reevaluating protein requirements of broiler chickens when the diet is supplemented with phytase enzyme. This study shows that microbial phytase not only reduces the need for inorganic P but also serves to reduce the need for protein in diet.

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