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Effect of Microbial Phytase on Growth Performance and Nutrients Digestibility in Broilers

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Abstract: The effects of microbial phytase supplementation on growth performance, ileal digestibility of protein and ether extract, amount of excreted phosphorus and bone development was investigated in a 3 weeks trial using 192 mixed sex, day-old Ross 508 broiler chicks fed corn-soybean meal diet. The trial was set up according to completely randomized design. Day-old chicks were randomly distributed into three story cage units with wire floor. Thus, each of dietary treatment had 8 replications in which 8 birds were assigned. Three dietary treatments according to available phosphorus (AP) levels were respectively, A) positive control (0.45% AP), B) negative control (0.35% AP) and C) negative control+Phytase (0.35% AP) in diets. Phytase enzyme was added at 0.5 g/kg level into related feeds. Phytase supplementation did not significantly ($P>0.05$) affect bird growth performance Phosphorus and calcium availability were significantly ($P<0.05$) affected by dietary treatments. Supplemental phytase significantly ($P<0.05$) improved ileal digestibility of crude fat, but did not have any effect on toe ash content.

Key words: Broiler, phosphorus, phytase, growth performance, ileal digestibility

Introduction

Phosphorus is an essential element involved in energy metabolism of poultry and necessary for normal appetite, feed intake, feed conversion, bone development, egg shell quality and general health.

Poultry rations are based largely on cereal grains and oilseed meals. Unfortunately, approximately two thirds of the phosphorus in cereal grains and oilseed meals are present in the form of phytic acid and some other minerals such as, Zn, Cu, Co, Fe and Ca, to form phytate phosphorus, which are not available for poultry and most of them are excreted in the litter. Phytate, in its native state, constitutes complexes with various cations, primarily protein, lipids (Cosgrove, 1966) and starch (Thompson and Yoon, 1984). The phytate-protein complexes may reduce the utilization of the proteins and amino acids (Cheryan, 1980). Furthermore, phytate may also form complexes with proteases, such as trypsin and pepsin (Singh and Krikorian, 1982) in the gastrointestinal tract. These complexes may decrease the activity of digestive enzymes with a subsequent decrease in the digestibility of dietary protein and energy (Singh and Krikorian, 1982).

Phytase (E.C.3.1.3.8.), myo-inositol hexaphosphate phosphohydrolase, is the enzyme that releases P from phytate (Gibson and Ullah, 1990). Supplemental microbial phytase has been reported to improve dietary phytate P bioavilability (Biehl *et al*., 1995; Denbow *et al*., 1995; Mitchell and Edwards, 1996; Simons *et al*., 1990). Phytase may also improve the utilization of protein, amino acid and apparent metabolizable energy of the diet supplemented with the enzyme (Ravindran *et al*., 1999).

Phytate dephosphorylation by microbial phytase added to poultry feeds has been shown to increase P digestibility from 35% to around 60%, reduce P content in excreta by 42% (Simons *et al*., 1990) and increase protein digestibility and availability of minerals (Sebastian *et al*., 1997; Yi *et al*., 1996a). It was reported that mineral-phytate complexes might prevent lipid utilization, and by preventing the formation of mineral-phytate complexes, phytase might reduce the degree of soap formation in the gut and enhance the utilization of energy derived from lipids (Ravindran *et al*., 2001). In addition, pollution of the environment with manure nitrogen is also a major argument for improving nitrogen utilization and limiting nitrogen excretion by poultry (Yi *et al*., 1996a). However the influence of phytase supplementation on nutrient digestion and mineral retention have not been fully studied.

Therefore, the aim of the present experiment was to examine the effect of microbial phytase on nutrient digestion with particular interest on fats and mineral retention as well as on the growth performance and toe ash content in broiler chicks fed corn-soybean meal based diets.

Materials and Methods

A total of 192 day-old mixed sex broiler chicks (Ross 508) were used in this experiment. The chemical composition of corn-soybean based experimental diets were analyzed by Wendee proximate analysis methods (Akyildiz, 1984; AOAC, 2000). Chicks were fed with standard starter diet (23% protein; 3050 kcal ME/kg)

according to NRC (1994) recommendations (1).

Day old chicks were individually weighed and randomly allocated to wire floor battery type experimental cages. Dietary treatments were consisted of A) normal Ca and normal available phosphorus (AP) (1.00% Ca, 0.45% AP); B) low level of AP (0.35% AP) and C) low level of AP (0.35% AP) + phytase. In the present study, Ronozyme® P (a phytase preparation obtained from *Peniophora lycii* and supplied by DSM, Istanbul, Turkey) was used as supplemental phytase. Phytase enzyme was added into the test diets at 0.5 g/kg levels (Table 1).

This trial was planned according to completely randomized design. Thus, each of dietary treatments had 8 replications in which 8 birds were assigned. Experimental birds were *ad libitum* intake to test diets and water from feed troughs and nipple drinkers. Experimental cage units were kept in environmentally controlled room. Lighting was controlled by providing 23 hour light and 1 hour dark throughout the 3 weeks experimental period.

Live performances were measured weekly while mortality was recorded daily. During the trial, feed intake and excreta output were measured quantitatively per pen over three consecutive days (Days 18, 19 and 20) to measure the ileal digestibility (Yi *et al.*, 1996a; Qian *et al.*, 1996). The excreta from each pen were collected daily at 09:00 and stored in plastic bags at –20°C for analysis. After thawing, excreta samples were dried in an oven at 70°C. Care was taken to avoid contamination from feathers, scales, and debris. The excreta samples were ground to pass through a 0.5 mm sieve. Randomly chosen one male and one female chicks were killed by cervical dislocation on day 21. The ileum was dissected within 5 min after killing. The ileum was defined as extending from Meckel's diverticulum to a point 40 mm proximal to the ileo-cecal junction. The concentration of crude protein and crude fat were analyzed by Wendee proximate analysis methods (Akyildiz, 1984; AOAC, 2000). Total P and Ca in the samples of ileal contents were determined according to photometric method using molybdo-vanadate procedure and dry ash method, respectively (Akyildiz, 1984; AOAC, 2000). Toe samples were obtained by severing the middle toe through the joint between the second and third tarsal bones from the distal end (Yi *et al.*, 1996a). The left and right middle toes of chicks were pooled. The toe samples were dried at 100°C and then ashed in a muffle furnace at 600°C for 4 h to determine amount of ash. P concentration of toe samples were determined by photometric method using the molybdo-vanadate procedure. Ca concentration of toe samples were analyzed by dry ash method (Akyildiz, 1984; AOAC, 2000).

The collected data was subjected to analysis of variance and the difference between means were analyzed according to Duncan's Multiple Range Test (Soysal, 1998). The mathematical model used was as the following:

$Y_{ij} = \mu + R_i + e_{ij}$

where Y_{ij} is the dependent variable; μ, the overall mean; R_i, the effect of treatment; e_{ij}, the random error.

Results

Mortality during the trial was within acceptable levels (less than 2%) and was not related to dietary treatments. The growth performance of broiler chickens fed the dietary treatments are shown in Table 2. Phytase supplementation did not have significant ($P>0.05$) effect on body weight gain, feed intake and feed conversion ratio. The percentages of excreted nutrients by excreta and ileal digestibility are shown in Table 3 and 4 respectively. Phytase supplementation to broiler diets decreased amount of excreted phosphorus and calcium ($P<0.05$). Phytase supplementation into broiler diets had significant ($P<0.05$) effect on ileal digestibility of ether extract (EE) (Table 4).

The effect of phytase supplementation on the toe dry matter (DM), crude ash (CA), Ca and P contents of 21-day old broilers are shown in Table 5. The analyzed values of toe samples of the birds fed with experimental diets were not significantly different ($P>0.05$).

Discussion

Addition of phytase to corn-soybean meal basal diet did not significantly ($P>0.05$) affect bird performance with respect to body weight, feed intake and feed conversion ratio. These results are in agreement with a number of authors (Ravindran *et al.*, 2001; Kwon *et al.*, 1999; Sohail and Roland, 1999). Similar result was also obtained by Sebastian *et al.* (1997), however, phytase supplementation had no influence on the apparent ileal digestibility in their study.

The effect of phytase supplementation on phosphorus excretion was significant ($P<0.05$). Phytase enzyme hydrolyzed phosphate groups bound to phytate molecules, thereby the amount of retained phosphorus was increased, while the excreted amount of phosphorus was decreased. Apparent availability of phosphorus was higher in phytase supplementation group. Likewise, the percentage of excreted Ca was decreased in the enzyme supplemented group as well. These findings are consistent with those of some other studies (Yi et al., 1996a; Van Der Klis and Versteegh, 1996; Yi *et al.*, 1996b). In addition, phytase supplementation to poultry feeds has been shown to increase P availability from 35% to around 60% and reduced excreted P by 42% (Simons *et al.*, 1990). Phytase enzyme have been reported to increase protein digestibility and availability of minerals as well (Sebastian *et al.*, 1997; Yi *et al.*, 1996a).

Although the ileal digestibility of CP of the group fed with phytase increased, the difference was not significant. However, significant increase in ether extract digestibility

Table 1: Experimental Broiler Starter Diets (0-21 days)

Ingredients (g/kg diet)	A	B	C
Corn	594.11	597.70	596.64
Soybean meal	251.56	250.80	251.03
Fish meal	30.00	30.00	30.00
Corn gluten meal	80.00	80.00	80.00
Dicalcium phosphate	13.73	8.17	8.17
Limestone	11.75	15.64	15.63
Common salt	2.77	2.77	2.77
Vitamin+Mineral*	2.50	2.50	2.50
Vegetable oil	12.91	11.74	12.09
L-Lysine HCl	0.07	0.09	0.08
DL-Methionine	0.60	0.59	0.59
Phytase	-	-	0.50
Total	1000.00	1000.00	1000.00
Calculated values (g/kg diet)			
ME, MJ/kg	12.77	12.77	12.77
Crude protein	23.00	23.00	23.00
Crude fibre	2.63	2.63	2.63
Ether extract	4.42	4.32	4.35
Lysine	1.10	1.10	1.10
Methionine	0.53	0.53	0.53
Met+Cys	0.90	0.90	0.90
Calcium	1.00	1.00	1.00
Available phosphorus	0.45	0.35	0.35
Linoleic acid	2.13	2.06	2.08
Sodium	0.15	0.15	0.15
Chlorine	0.22	0.22	0.22

*Supplied per kilogram of diet: 600 IU vit A, 800 IU vit D_3, 8 mg vit E, 2 mg vit K_3, 1 mg vit B_1, 3 mg vit B_2, 2 mg vit B_6, 8 µg vit B_{12}, 10 mg nichotin amide, 4 mg cal.D.panth., 0.3 mg folic acid, 20 µg D-biotin, 160 mg choline chloride, 32 mg Mn, 16 mg Fe, 24 mg Zn, 2 mg Cu, 800 µg I, 200 µg Co, 60 µg Se, 4 mg antioxcidane, 170.361 mg Ca

Table 2: Effects of Phytase Supplementation on Broiler Performance (0-21 days of age)

	A	B	C	SEM	P-level
Body weight (g)	786.00	796.25	808.25	10.199	0.691
Gain (g)	743.00	753.25	765.25	10.199	0.691
Feed intake (g)	977.63	964.63	979.50	14.368	0.907
Feed/Gain (kg/kg)	1.317	1.281	1.279	0.0112	0.315

and increased retention in Ca and P was observed. Increased P and CP retention by phytase was also observed in previous studies in broilers (Ravindran *et al.*, 2001; Yi *et al.*, 1996b). Chicks fed diets 0.35% AP+ Phytase had significantly ($P<0.05$) higher Ca retention compared with chicks fed 0.45% AP (Table 3). Nelson *et al.* (1968) reported that microbial preparation containing phytase improved utilization of phytate P when supplemented in broiler diets. Phytic acid has the potential to form complexes with protein or cations such as Ca, Mg, Zn and Cu (Morris, 1986; Vohra *et al.*, 1965) which can negatively affect the digestibility of minerals, protein or amino acids (Anderson, 1985; Cosgrove, 1980).

Addition of phytase improved the digestibilities of crude fat in the present study. Ravindran *et al.* (2001) reported that mineral-phytate complexes may contribute to the formation of insoluble metallic soaps in the gastrointestinal tract, which is a constraint on lipid utilization. By preventing the formation of mineral-phytate complexes, phytase may reduce the degree of soap formation in the gut and enhance the utilization of energy derived from lipids (Ravindran *et al.*, 2001). In its native state, phytate is complexed with various cations, protein and lipids (Cosgrove, 1966). Supplemental phytase improved the ileal digestibility of nitrogen and amino acids and apparent metabolizable energy in chicks (Ravindran and Bryden, 1997). Namkung and Leeson (1999) reported that supplemental phytase increased the digestibilities of apparent metabolizable energy and amino acids in broilers.

Although supplemental phytase significantly increased the amount of retained phosphorus, no significant effect on percentage of toe ash was observed (Table 5).

Table 3: Effects of Phytase on Chemical Composition of The Excreta (DM basis), (g/kg)

Treatments	DM	CA	Ca	Total P
A	204.75	32.48[a]	5.85[a]	1.14[a]
B	193.44	28.41[b]	5.28[ab]	1.03[b]
C	182.08	29.05[b]	5.19[b]	1.00[b]
SEM	5.143	0.739	0.133	0.020
P-level	0.203	0.046	0.082	0.065

[a-b] Means within the same column with different superscripts differ significantly (P< 0.05)

Table 4: Effects of Phytase on Ileal Digestibility of Dry Matter, Crude Protein and Ether Extract (DM basis)

Treatments	Ileal digestibility, %		
	Dry Matter	Crude Protein	Ether Extract
A	91.62	76.43	82.94[b]
B	91.49	74.17	76.39[a]
C	91.75	77.30	89.38[c]
SEM	0.185	0.780	2.397
P-level	0.903	0.281	0.003

[a-c]Means within the same column with different superscripts differ significantly (P < 0.05).

Table 5: Effects of Phytase on Toe Ash Values

Treatments	Dry matter g/kg	Crude ash mg/kg	Calcium g/kg	Phos-phorus mg/kg
A	319.53	41.60	44.09	11.49
B	310.36	38.50	41.81	11.68
C	322.89	39.35	44.68	10.31
SEM	3.069	0.654	1.374	0.445
P-level	0.234	0.134	0.685	0.411

Similar results were also obtained by Ravindran *et al.* (2001).

Consequently, the results of the present study demonstrated that phytase supplementation did not affect broiler performance whereas it significantly increased calcium and phosphorus retention in broiler chicks. In addition, significant improvement in ether extract digestibility were detected. Microbial phytase addition to broiler chick diets prevents environmental pollution by excreted phosphorus.

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References

Akyildiz, A.R., 1984. Yemler Bilgisi Laboratuvar Kilavuzu, A.Ü.Z.F. Yayinlari, No: 895, Ankara.

Anderson, P.A., 1985. Interactions between proteins and constituents that affect protein quality. In: Finley, G.W., Hopkins, D.T. (Eds.), Digestibility and amino acid availability in cereals and oilseeds. St. Paul, MN., American Association of Cereal Chemist, pp: 31-46.

Association Of Official Analytical Chemists (AOAC), 2000. Official Method Of Analysis Of The Association Of Analytical Chemists. 17th Rev. Ed., Association of Official Analytical Chemists, Washington, DC.

Biehl, R.R., D.H. Baker and H.F. DeLuca, 1995. 1 alpha-hydroxylated cholecalciferol compounds act additively with microbial phytase to improve phosphorus, zinc and manganese utilization in chicks fed soy-based diet. J. Nutr., 125: 2407-2416.

Cosgrove, D.J., 1980. Inositol Phosphates: Their Chemistry, Biochemistry and Physiology. Elsevier Scientific Publishing Co., New York. NY.

Cheryan, M., 1980. Phytic acid interactions in food systems. CRC Crit. Rev. Food Sci. Nutr., 13: 297-335.

Cosgrove, D.J., 1966. The chemistry and biochemistry of inositol polyphosphates. Rev. Pure Appl. Chem., 16: 209-224.

Denbow, D.M., V. Ravindran, E.T. Kornegay, Z. Yi and M. Hulet, 1995. Improving phosphorus availability in soybean meal for broilers by supplemental phytase. Poult. Sci., 74: 1831-1842.

Gibson, D.M. and A.B.J. Ullah, 1990. Phytase and their actions on phytic acid. In: Morre, D.J., Boss, W.F., Loewus, F.A., (Eds.), Inositol metabolism in plants. New York, Wiley-Liss, Inc., pp: 77-92.

Kwon, K., J.I. Chag, J.C. Ju, M.I. Yoo, K.S. Sohn and Y.W. Choi, 1999. Effects of microbial phytase on performance of broiler chicks fed corn-soy based diets. Korean J. Anim. Sci., 41: 519-526.

Mitchell, R.D. and H.M., Edwards, 1996. Effect of phytase and 1,25-dihydrocholecalciferol on phytate utilization and the quantitative requirement for calcium and phosphorus in young broiler chickens. Poult. Sci., 75: 95-110.

Morris, E.R., 1986. Phytate and dietary mineral bioavailability. In: Graf, E. (Ed.), Phytic acid: Chemistry and Applications. Minneapolis, Pilatus Press, pp: 57-76.

Namkung, H. and S. Leeson, 1999. Effect of phytase enzyme on dietary nitrogen-corrected apparent metabolizable energy and the ileal digestibility of nitrogen and amino acids in broiler chicks. Poult. Sci., 78: 1317-1319.

National Research Council, 1994. Nutrient Requirements of Poultry. 9th Rev. Ed. National Academy Press, Washington, DC.

Nelson, T.S., T.R. Shieh, R.J. Wodzinski and J.H. Wane, 1968. The availability of phytate phosphorus in soybean meal before and after treatment with a mold phytase. Poult. Sci., 47: 1842-1848.

Qian, H., E.T. Kornegay and D.M. Denbow, 1996. Phosphorus equivalence of microbial phytase in turkey diets as influenced by calcium to phosphorus ratios and phosphorus levels. Poult. Sci., 75: 69-81.

Ravindran, V. and W.L. Bryden, 1997. Influence of dietary phytic acid and available phosphorus levels on the response of broilers to supplemental natuphos. Poultry Research foundation of the University Sydney. Report to BASF Australia Ltd., Sydney, Australia.

Ravindran, V., S. Cabahug, G. Ravindran and W.L. Bryden, 1999. Influence of microbial phytase on apparent ileal amino acid digestibility of feedstuffs for broilers. Poult. Sci., 78: 699-706.

Ravindran, V., P.H. Selle, G. Ravindran, P.C.H. Morel, A.K. Kies and W.L. Bryden, 2001. Microbial phytase improves performance, apparent metabolizable energy, and ileal amino acid digestibility of broilers fed a lysine-deficient diet. Poult. Sci., 80: 338-344.

Sebastian, S., S.P. Touchburn, E.R. Chavez and P.C. Lague, 1997. Apparent digestibility of protein and amino acids in broiler chickens fed a corn-soybean diet supplemented with microbial phytase. Poult. Sci., 76: 1760-1769.

Simons, P.C.M., H.A.J. Versteeg, A.W. Jongbloed, P.A. Kemme, P. Slump, K.D. Bos, M.G.E. Wolters, R.F. Beudeker and G.J. Verschoor, 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Br. J. Nutr., 64: 525-540.

Singh, M. and A.D. Krikorian, 1982. Inhibition of trypsin activity in vitro by phytase. J. Agri. Food Chem., 30: 799-800.

Sohail, S.S. and D.A. Roland, 1999. Influence of supplemental phytase on performance of broilers four to 6 weeks of age. Poult. Sci., 78: 550-555.

Soysal, M.I., 1998. Biyometrinin Prensipleri (Istatistik I ve II Ders Notlari). T.U.T.Z.F. Yayin No: 95, Ders Notu: 64, Tekirdag.

Thompson, L.U. and H. Yoon, 1984. Starch digestibility as affected by polyphenols and phytic acid. J. Food Sci., 49: 1228-1229.

Van Der Klis, J.D. and H.A.J. Versteegh, 1996. Phosphorus nutrition in poultry. In: Garnsworthy, P.C., Wiseman, J., Haresign W. (Eds.), Recent Advances in Animal Nutrition. Nottingham University Press, pp: 71-86.

Vohra, P.G., A., Gray and F.H., Kratzer, 1965. Phytic acid-metal complexes. Proc. Soc. Exp. Biol. Med., 120: 447-449.

Yi, Z., E.T. Kornegay, V. Ravindran and D.M. Denbow, 1996a. Improving phytate phosphorus availability in corn and soybean meal for broilers using microbial phytase and calculation of phosphorus equivalency values for phytase. Poult. Sci., 75: 240-249.

Yi, Z., E.T. Kornegay and D.M. Denbow, 1996b. Effect of microbial phytase on nitrogen and amino acid digestibility and nitrogen retention of turkey poults fed corn-soybean meal diets. Poult. Sci., 75: 979-990.