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The Effect of Microbial Phytase in Broiler Grower Diets Containing Low Phosphorus, Energy and Protein

Mehmet Bozkurt[1], Metin Çabuk[2]* and Ahmet Alçiçek[3]

[1] Poultry Research Institute, Erbeyli 09600, Aydın-Turkey
[2] Department of Poultry Science, Vocational School of Celal Bayar University, Akhisar 45210, Manisa-Turkey
[3] Department of Animal Science, Agricultural Faculty of Ege University, Bornova 35100, Izmir-Turkey

One thousand two hundred and fifty sexed 21 day-old commercial broilers (Ross-308) were randomly divided into five dietary treatment groups of 250 broilers each. Each treatment group was further sub-divided into five replicates of 50 broilers (25 male and 25 female) per replicate. The treatments groups were control ; low phosphorus ; low phosphorus plus 500 FTU phytase/kg diet ; low phosphorus, energy, protein ; and low phosphorus, energy, protein plus 500 FTU phytase/kg diet. There were significant effects of dietary treatments on body weight, body weight gain, feed intake and feed conversion ratio at 21 to 42 days. The body weight and the body weight gain of the broilers fed the control and low phosphorus plus phytase diet were heavier than other treatment. Feed intake of broilers was not affected by the supplementation of phytase to the low phosphorus diet at 21 to 42 days. Feed conversion ratio of broiler fed on low phosphorus, energy and protein diet plus phytase was significantly better than that of broilers fed on low phosphorus, energy and protein diet. Neither phytase supplementation nor diet nutrient density and dietary phosphorus level had a significant effect on broiler mortality. The percentage of tibia ash and phosphorus was significantly increased by the addition of microbial phytase to low phosphorus, energy and protein diet. This study demonstrates that microbial phytase can compensate for reduced available phosphorus levels, but could not compensate for reduced dietary protein and energy.

Key words : phytase, phosphorus, broiler grower diet

Introduction

The major portion of phosphorus (P) in plant feed ingredients, including corn and soybean is present in the form of phytate, which is largely unavailable in monogastric animals. The interest in the use of microbial feed enzymes such as phytase arises from the need to improve the availability of phytate-bound phosphorus and to reduce the phosphorus levels in effluent from intensive livestock operations. In addition to reducing phosphorus availability, phytates are associated with a number of anti-nutritional effects ; largely because of they can chelate divalent cations such as Ca, Mg, Fe, Zn, Cu, Mn and also can reduce protein availability (Ravindran *et al.,* 2001 ; Bedford and Schulze, 1998). Phytase may significantly improve the utilization of the essential amino acid in broilers fed soybean meal basal diets (Biehl *et al.,* 1997). Diets deficient in P depressed growth rate and feed efficiency (Fernandes *et al.,* 1999 ; Li *et al.,* 2000), but microbial phytase supplementation has been shown to relieve the detrimental effects a phosphorus deficiency on broiler health status. Besides ameliora-

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* To whom correspondings should be addressed :
Tel : +902364126896 Fax : +902364137058 E-mail : metin.cabuk@deu.edu.tr

tive effects on phosphorus deficiency, microbial phytase has been shown to compensate for dietary protein, essential amino acid and metabolizable energy deficiencies to varying degrees (Namkung and Leeson, 1999). The low availability of phosphorus in plant ingredients poses problems both economically and environmentally. Studies have demonstrated that exogenous dietary phytase improves phytate phosphorus utilization and enhanced over all performance in broilers (Huff *et al.,* 1998 ; Atia *et al.,* 2000 ; Waldroup *et al.,* 2000). The majority of studies which show the effect of phytase on broilers have been conducted from 0 to 2 or 0 to 3wks (Sabestian *et a*l., 1996 ; Zhang *et al.,* 1999 and Zyla *et al.,* 2000), few studies have investigated the efficacy of microbial phytase using 3-wk-old broilers (Orban *et al.,* 1999 ; Ravindran *et al.,* 2001).

This study was designed to determine the efficacy of microbial phytase in broiler grower diets containing low phosphorus, energy and protein.

Material and Methods

One thousand two hundred and fifty sexed 21 day-old commercial broilers (Ross-308) were randomly divided into five dietary treatment groups of 250 broilers each. Each treatment group was further sub-divided into five replicates of 50 broilers (25 male and 25 female) per replicate. Commercial brooding and management procedures were followed, and all broilers were fed a typical commercial broiler starter diet for the first 3 weeks of the experiment. The treatment groups were control diet ; a low phosphorus diet ; low phosphorus plus 500 FTU phytase/kg diet ; low phosphorus, energy, protein diet and a low phosphorus, energy and protein plus 500 FTU phytase/kg diet (Table 1).

Criteria used to measure response were body weight, body weight gain, feed consumption, feed conversion ratio, mortality, tibia ash and phosphorus level, fecal ash and phosphorus level. Individual body weight and feed consumption of broilers from all pens were measured at the 21, 35, and 42 days of age. Mortality of each pen was recorded on a daily basis. Feed conversion ratio was adjusted according to the feed consumption of the dead broilers. All diets were provided for *ad-libitium* consumption in mash form and broiler had free access to water. The broilers were housed in floor pens and pine shaving served as litter material. Broilers were exposed to natural day-light and continuous fluorescent lighting at night. When the broilers were 38 days of age, two broilers per pen (one male and one female) were identified with weights closest to the mean body weight of the pen and placed in individual cages and allowed to acclimatize for 3 consecutive days. Broiler had unlimited access to experimental diet and water during the acclimation and excrete collection periods. At 41 and 42 days, feces were collected on aluminum foil for a 48 hours period. The excreta were immediately frozen, freeze-dried, and analyzed for ash and phosphorus content.

At 42 days of age, two broilers per pen (one male and one female), representative of the mean body weight, were killed by cervical dislocation, the right tibia of each broiler was removed and stored in a freezer at −18℃ for bone ash and phosphorus content determination. Tibias were cleaned of adhering tissue, then dried at 105℃ for 24 hours and extracted with ether, dried again and reweighed. The dry fat-free bones were burned in a muffle furnace at 600℃ over night (Orban *et al.,* 1999). The standard techniques of the proximate analysis were used to determine nutrient content of experimental diets (Naumann and Bassler, 1993). Metabolizable energy content of the diets was calculated based on the proximate chemical composition (Anonymous, 1991). The data were analyzed using the General Linear Models procedure of SAS (1985). Significant differences between treatment means were separated using the Duncan's multiple range test.

Result and Discussion

The effects of diet nutrient density, phosphorus deficiency and microbial phytase supplementation on the performance of broiler are shown in Table 2. Reducing total phosphorus level from 0.65% to 0.49% significantly depressed body weight at day 35 and 42, and body weight gain at day 21 to 35 and 21 to 42 compared with control diet. This lower body weight was due to deficiency of phosphorus in broilers fed 0.49% phosphorus level, which was slightly below the recommended level of 0.65% phosphorus for broilers 3 to 6 wk of age (NRC, 1994). This effect of phosphorus deficiency was also reported in broilers 3 to 6 wk of age by Sohail and Roland (1999), Orban *et al.* (1999) and

Fernandes *et al.* (1999). However, phytase supplementation to the grower diet ameliorated this negative effect. Broilers fed 0.49% phosphorus weighted 2006 g as compared with 2054 g for broilers receiving 0.48% phosphorus plus phytase at day 42. Phytase supplementation to low phosphorus diet also improved the body weight gain of broilers at either the 21 to 35 or 21 to 42 day intervals. These results were in agreement with those of Qian *et al.* (1997), Huff *et al.* (1998), Namkung and Leeson (1999) and Zyla *et al.* (2000) which reported that the growth rate and feed conversion ratio of broilers fed the low phosphorus diets containing microbial phytase are comparable with or even better than those obtained for broilers fed the standard phosphorus diets. These results supported the con-

Table 1. Ingredients and chemical composition of the experimental diets (as fed)

Ingredients (kg/1000 kg)	Starter	Finisher				
		Control	Low P	Low P +Phytase	Low P-energy-protein	Low P-energy-protein+phytase
Maize	536.3	606.4	609.2	608.2	635.1	634.1
Soybean meal (0.46 CP)	368.3	294.5	294.1	294.1	279.6	279.6
Sunflower Oil	40.0	39.9	38.9	38.9	27.6	27.6
Fish meal (0.72 CP)	14.5	9.9	10.2	10.2	10.2	10.2
Meat and bone meal (0.32 CP)	—	30.0	30.0	30.0	30.0	30.0
Limestone	18.5	4.2	8.2	8.2	8.1	8.1
Dicalcium phosphate (0.24 Ca, 0.18 P)	13.4	5.7	—	—	—	—
Salt	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin Premix**	2.5	2.5	2.5	2.5	2.5	2.5
Mineral Premix***	1.0	1.0	1.0	1.0	1.0	1.0
Anticoccidial agent	1.0	1.0	1.0	1.0	1.0	1.0
Methionine	1.0	0.9	0.9	0.9	0.9	0.9
Lysine	1.0	1.5	1.5	1.5	1.5	1.5
Phytase (500 000 FTU/kg)†			—	1.0		1.0
Total	1000	1000	1000	1000	1000	1000
Composition, g/kg (analyzed)						
Crude protein	226.0	200.1	198.3	198.1	188.1	188.6
Ether extract	58.0	68.0	67.3	68.1	59.0	54.0
Starch	355.7	387.5	386.8	389.2	392.5	395.0
Sugar	46.3	50.6	50.8	49.4	42.8	46.8
Metabolizable Energy, (Kcal/kg)	3079	3216	3202	3212	3083	3069
Calcium	10.5	9.2	8.8	8.7	8.9	9.0
Total phosphorus	6.7	6.5	4.9	4.8	5.0	4.9
Available phosphorus*	4.7	4.5	3.0	3.0	3.0	3.0
Methionine+cystine*	8.5	7.7	7.7	7.7	7.7	7.7
Lysine*	14.1	12.4	12.4	12.4	12.4	12.4

* Calculated.

** Vitamin premix (IU or mg/kg 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 , 0.75 mg ; D-biotin, 0.075 mg ; choline chloride, 375 mg ; antioxidant, 10 mg.

*** Mineral combination (mg/kg diet) : manganese,80 ; iron, 80 ; zinc, 60 ; copper,8 ; iodine,0.5 ; cobalt, 0.2 ; selenium, 0.15.

† Phytase (Natuphos® 500000 FTU/kg) : One phytase unit FTU/kg unit is defined as the amount that liberates 1 μmol of inorganic P/ min from 0.0015 mol sodium phytate at 37℃ and pH 5.5.

Table 2. Effect of microbial phytase supplementation on the performance of boilers

Parameter	Age, day	Control	Low P	Low P+phytase	Low P-energy-protein	Low P-energy-protein+phytase	s.e.m.	P
Body weight, g	21	610.9	613.3	610.8	612.2	613.9	5.26	0.9973
	35	1545.4^{a}	1480.6^{c}	1522.1^{ab}	1477.7^{c}	1505.9^{bc}	11.57	0.0002
	42	2083.7^{a}	2006.0^{c}	2054.9^{ab}	2002.3^{c}	2020.3^{bc}	15.72	0.0011
Body weight gain, g	21 to 35	934.5^{a}	867.4^{c}	911.4^{ab}	865.5^{c}	891.9^{bc}	13.26	0.0036
	21 to 42	1472.8^{a}	1392.7^{b}	1444.2^{ab}	1390.2^{b}	1406.3^{b}	20.60	0.0366
Feed intake, g	21 to 35	1572.3^{bc}	1570.2^{bc}	1547.0^{c}	1686.5^{a}	1611.0^{abc}	31.42	0.0269
	21 to 42	2864.0^{b}	2865.3^{b}	2825.3^{b}	3031.7^{a}	2854.7^{b}	49.12	0.0467
FCR, g/g	21 to 35	1.68^{c}	1.81^{b}	1.69^{c}	1.94^{a}	1.80^{b}	0.02	0.0001
	21 to 42	1.94^{c}	2.05^{b}	1.95^{c}	2.18^{a}	2.03^{bc}	0.02	0.0001
Liveability, %	21 to 42	98.3	98.3	97.7	96.6	97.7	1.39	0.9464

^{a,b,c} : Means within row with no common superscripts differ significantly ($P<0.05$).

cept that phytase was improving phosphorus availability and phosphorus level can be lowered in broiler grower diets added phytase. Although the 35 and day 42 body weights and body weight gain of broilers fed a low phosphorus, energy and protein plus phytase was comparable with that of low phosphorus plus phytase. There were no significantly differences in feed consumption between control and treatment groups except of low phosphorus, energy and protein group. But, the reducing phosphorus, energy and protein level of the diet increased feed intake per broiler ($P<0.05$).

Phytase supplementation to the low phosphorus diet at 500 FTU/kg improved feed conversion ratio of broilers at both day 35 and day 42 compared with low phosphorus diet without phytase ($P<0.01$). The current study supports the observations of Huff *et al.* (1998), Sohail and Roland (1999) and Ravindran *et al.* (2001) who reported that phytase supplementation to broiler grower diets caused numerical improvements in feed efficiency of broilers fed a phosphorus deficient diet compared to phosphorus adequate diet fed without phytase. Reducing dietary energy and protein density resulted in higher consumption of feed which resulted in a poorer feed efficiency. Phytase supplementation to diet deficient in phosphorus alleviated the phosphorus deficiency effect, and broilers receiving 0.49 and 0.48% phosphorus with phytase performed similar feed conversion ratio to that broilers receiving 0.65% phosphorus. Feed intake and feed efficiency of broilers fed diet containing phytase were also similar to those broilers fed control diet containing dicalcium phosphate. The results indicate that phytase at 500 FTU/kg released phytate phosphorus that was adequately utilized for growth in a similar manner as would phosphorus supplied by dicalcium phosphate. Similar results were observed for the duck, turkey and broiler grower diets respectively (Huff *et al.,* 1998 ; Orban *et al.,* 1999 ; Atia *et al.,* 2000). This result suggests that phytate phosphorus released by phytase was sufficient to meet grower broiler's growth requirements. The effects of microbial phytase supplementation to low phosphorus, energy, protein diet on tibial and fecal characteristics are presented in Table 3.

The percentage of tibia crude ash was significantly increased by the addition of dietary phytase, an observation that agrees with the previous studies dealing with broilers (Sabestian *et al.,* 1996 ; Zyla *et al.,* 2000), Pekin ducks (Orban *et al.,* 1999) and turkeys (Atia *et al.,* 2000). However, as it was reported in some experiments (Fernandes *et al.,* 1999 ; Harter-Dennis and Sterling, 1999), dropping dietary phosphorus level decreased tibia ash, also in the current study. Phytase supplementation to diets increased the content of phosphorus in the tibia compared with diet containing low phosphorus, energy and protein without phytase ($P<0.01$). Such an improvement in ash and phosphorus percentage in tibia was described by Sabestian *et al.* (1996) as a good indication of increased availability of phosphorus from phytase-mineral complex by the action of phytase. The response of tibia charac-

Table 3. The effects of microbial phytase supplementation to low phosphorus, energy, protein diet on tibial and fecal characteristics

Parameter	Control	Low P-energy-protein	Low P-energy-protein+phytase	s.e.m.	P
Tibia crude ash, %	54.09[a]	51.03[c]	53.48[b]	0.19	0.0001
Tibia phosphorus, %	12.16[a]	10.80[b]	11.84[a]	0.12	0.0001
Fecal crude ash, %	11.68[a]	11.51[b]	11.27[c]	0.05	0.0003
Fecal phosphorus, %	1.61[a]	1.28[b]	1.21[b]	0.04	0.0001

[a,b,c] : Means within row with no common superscripts differ significantly ($P<0.05$).

teristics such as tibia ash and phosphorus content to dietary levels of microbial phytase in the present study was similar to previous observations reported in broilers and ducks, in which dietary phytase phosphorus was found to increase tibia ash and phosphorus percentage (Orban *et al.,* 1999 ; Sohail and Roland, 1999). The improvement in phosphorus availability by phytase reduced the amount of inorganic phosphorus added to grower diet of broilers, and reduced significantly the amount of ash excreted in manure ($P<0.01$, Table 3). The report of Simons *et al.* (1990) showed that the availability of phosphorus increased to over 60% and the amount of phosphorus in manure decreased by 50% when microbial phytase was added to low phosphorus diets.

The results of this study suggest that microbial phytase in broiler finisher diet enhanced the availability of phosphorus that supported the growth performance, increased tibia ash and phosphorus content, decreased manure ash and phosphorus level. The results of this study showed that the use of phytase in the grower diet of broilers improved phosphorus availability. The increasing of phosphorus availability may reduce the amount of phosphorus that would be excreted in the manure, thus reducing the environmental pollution potential. However phytase could not compensate for reduced dietary crude protein and energy deficiency even if those were slightly lower than the level of standard control diet. It can be concluded that with 500 FTU of microbial phytase/kg, dietary phosphorus can be reduced to 0.48% in the broiler grower diet without affecting fattening performance and overcame the depression of growth rate observed on the low phosphorus diet.

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