Reduction of Excretory Phosphorus and Trace Minerals by Dietary Supplementation of Microbial Phytase in Broiler Chicks

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An experiment was conducted to study the effect of microbial phytase on excretory phosphorus (P), copper (Cu), zinc (Zn) and manganese (Mn) in broiler chicks. Male broiler chicks with three replications of 10 chicks each were given free access to the experimental diet from 7 to 21 days of age. The basal diet consisted mainly of corn, soybean meal and defatted rice bran, and contained 0.60% total P, 0.30% non-phytate P, 0.90% calcium, 8.1 mg Cu, 82.3 mg Zn and 95.3 mg Mn per kg of diet, and 0.1% chromium oxide as an indicator. To the basal diet, defluorinated phosphate (0.075 and 0.15% as non-phytate P) or microbial phytase (125, 250 and 500 units/kg of diet) was added. The excreta of each replication were collected for the last three days of the experiment, and the retention ratio and excretion of total P, phytate P, Cu, Zn and Mn were calculated.

The retention ratio of total P, Cu and Zn increased almost linearly with the increase of supplemented phytase. When 500 units/kg phytase were added, total P excretion was reduced by 31%, and the excretion of Cu and Zn was also reduced by 7%, respectively, in comparison with the diet containing 0.45% non-phytate P. However, the excretion of Mn was not affected by the addition of phytase.

Key words : broiler, mineral excretion, microbial phytase, phytate phosphorus, trace minerals

Introduction

More than 50% of phosphorus (P) contained in plant feedstuffs occur as phytate P. It has been well known that phytate P is poorly used by poultry (Japanese Feeding Standard for Poultry, JFSP, 1997). Phytic acid is a strong acid and forms various chelate compounds with important trace minerals such as zinc (Zn), copper (Cu), magnesium (Mg), manganese (Mn), iron (Fe) and potassium (K). These chelate compounds are poorly used by non-ruminant animals such as poultry and swine, because it has low solubility (Oberleas, 1973).

Nelson *et al.* (1971) first demonstrated that the availability of phytate P was improved by the addition of microbial phytase to the chick diets. After that, many investigators have reported that the availability of phytate P, growth performance and

accumulation of bone ash are improved by the addition of phytase (Schörner *et al.*, 1993 ; Broz *et al.*, 1993 ; Perney *et al.*, 1993 ; Roberson *et al.*, 1994 ; Takemasa *et al.*, 1995 ; Mitchell *et al.*, 1996 ; Sevastian *et al.*, 1996 ; and Yi *et al.*, 1996a). In the previous report (Yonemachi *et al.*, 2000), it was confirmed that not only growth performance, tibia ash and plasma inorganic P, but also apparent ME and nitrogen retention of broiler chicks were significantly improved by the addition of phytase to the chick diet.

It was also reported that phytase improved the availability of calcium (Ca), Zn and Cu (Sevastian *et al.*, 1996 ; and Yi *et al.*, 1996b ; and Aoyagi and Baker, 1995). When phytic acid is hydrolyzed by phytase, it is expected that the released phytate-bound minerals are available more effectively for animals and the excretion of these minerals is reduced. However, there is little information about the excretory and retention of phytate P and trace minerals in broiler chicks fed the diets supplemented by microbial phytase. Therefore, in this study, effects of dietary phytase on reduction of excretory total P, phytate P, Zn, Cu and Mn were investigated in broiler chicks.

Materials and methods

1. Design of Experiment

One hundred and eighty male Arbor Acres chicks of 7 days of age were assigned to six dietary treatments. Each treatment consisted of 3 replications of 10 chicks each and chicks were given free access to the experimental diets and water for 14 days.

The excreta of each replication were collected twice a day for the last three days of the experimental period.

2. Diet

The composition of the basal diet (Diet 1) is shown in Table 1. The basal diet consisted mainly of corn, soybean meal and defatted rice bran, and contained 21.4% CP, 3.11 Mcal ME/kg of diet, 0.60% total P, 0.30% non-phytate P, 0.90% Ca, 8.1 mg Cu, 82.3 mg Zn and 95.3 mg Mn per kg of diet, and 0.1% chromium oxide as an indigestible marker. The basal diet met recommended (JSFP, 1997) nutrient requirements except for that of non-phytate P.

Diets 2 and 3 were prepared by adding 0.075 and 0.15% non-phytate P in the form of defluorinated phosphate to the basal diet, respectively. Diets 4, 5 and 6 were supplemented by 125, 250 and 500 units microbial phytase per kg of the basal diet, respectively.

Microbial phytase was obtained by fermentation of non-recombinant *Aspergillus niger* in the laboratory of Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan). The microbial phytase preparation contained 500 units/g phytase. One unit of phytase activity is defined as the amount of enzyme which liberates 1 μ mol inorganic P per min from 0.0051 mol sodium phytate at 37°C and pH 5.5.

3. Analysis

The excreta collected for three days were mixed, dried at 60°C in a ventilated oven, and ground through 1 mm sieve.

Total P and phytate P in excreta and diets were determined by the

Table 1. Composition of the basal diet

Ingredients	(%)
Corn	56.91
Soybean meal (46% CP)	22.50
Defatted rice bran	8.00
Soybean oil	4.00
Casein	5.00
Calcium carbonate	2.20
Defluorinated phosphate	0.36
Sodium chloride	0.40
DL-methionine	0.22
L-lysine-HCl	0.01
Vitamin premix ¹	0.20
Mineral premix ²	0.10
Chromium oxide	0.10
Chemical Analysis	
ME (Mcal/kg) ³	3.11
CP (%)	21.40
Total P (%)	0.60
Non-phytate P (%)	0.30
Ca (%)	0.90
Zn (mg/kg)	82.3
Cu (mg/kg)	8.1
Mn (mg/kg)	95.3

¹ Contains (IU or g/kg premix) : (10⁶ IU) vit. A, 5.0 ; vit. D₃, 1.0 ; (g) vit. E, 0.01 ; vit. K₃, 1 ; thiamin, 1 ; riboflavin, 4 ; pantothenic acid, 15 ; nicotinic acid, 30 ; pyridoxine, 2 ; biotin, 0.1 ; folic acid, 0.5 ; choline, 0.25 ; cyanocobalamin, 0.005 and defatted rice bran to 1 kg.

² Contains (g/kg premix) : Mn, 80 ; Zn, 50 ; Fe, 6 ; Cu, 0.6 ; I, 1 and defatted rice bran to 1 kg.

³ Based on JSFP (1997).

phosphomolybdic acid method (Fiske and Subbarow, 1925). Zn, Cu and Mn in excreta and diets were determined with a flame atomic absorption spectrophotometer (AOAC, 1990). Chromium oxide in excreta and diets was determined by the colorimetric procedure (AOAC, 1990). The retention ratios of total P, phytate P, Zn, Cu and Mn were calculated by the indicator method.

4. Statistical Analysis

The data were analyzed using the General Linear Models procedure for analysis of variance (SAS Institute, 1990). Significant differences among treatment groups were determined by Tukey's multiple range test (Yoshida, 1983). The nonlinear equations [$Y = a(1 - be^{-kx})$] were derived for non-phytate P levels (Diets 1, 2 and 3) and phytase levels (Diets 1, 4, 5 and 6) using the Non-linear Regression procedure of SAS (1990) :

where Y=the retention of total P or phytate P (g/14 days) ; X=percentage of added non-phytate P (percentage) or phytase (units/kg diet), respectively.

Result and Discussion

As shown in Table 2, with increasing dietary non-phytate P added as defluorinated phosphate to Diets 1 to 3, the retention ratio of total P increased significantly and reached a maximum value at 0.45% non-phytate P. However, the retention ratio of phytate P was not affected by increasing dietary non-phytate P.

Furthermore, with increasing dietary non-phytate P, total P excretion increased significantly. This is because the feed intake and the total P intake increased with the non-phytate P addition and P from defluorinated phosphate might not be used completely. De Groote and Huyghebaert (1977) reported that the retention ratio of P from the monobasic calcium phosphate in broiler chicks ranged from 78.1% to 85.5%. Furthermore, the bioavailability of P from defluorinated phosphate is lower than that from monobasic calcium phosphate in the broiler chicks (Coffey *et al.*, 1994). These results show that P excretion increases when defluorinated phosphate are added to satisfy non-phytate P requirement of chicks.

On the other hand, in Diets 4, 5 and 6, the retention of total P and phytate P increased as dietary phytase levels were increased (Table 2).

With increasing dietary non-phytate P or added phytase, the total P retention increased nonlinearly as follows :

Table 2. Effect of dietary non-phytate phosphorus (P) and phytase levels on intake, excretion and retention of total and phytate P in male broiler chicks during 7 to 21 days of age

Diet No.	1	2	3	4	5	6	Pooled
Basal non-phytate P (%)	0.30	0.30	0.30	0.30	0.30	0.30	SE
Added non-phytate P (%)	0	0.075	0.15	0	0	0	
Added phytase (unit/kg)	0	0	0	125	250	500	
Body weight gain (g/14 days)	342 ^d	423 ^{bc}	457 ^a	392 ^c	418 ^c	451 ^{ab}	3
Feed intake (g/14 days)	588 ^d	684 ^{abc}	728 ^a	646 ^c	673 ^{bc}	703 ^{ab}	4
Intake (g/14 days)							
Total P	3.53 ^c	4.62 ^b	5.46 ^a	3.88 ^d	4.04 ^{cd}	4.22 ^c	0.05
Phytate P	1.76 ^d	2.05 ^{bc}	2.18 ^a	1.95 ^c	2.02 ^{bc}	2.11 ^{ab}	0.02
Total P excretion (g/14 days)	2.48 ^c	3.10 ^d	3.54 ^a	2.57 ^c	2.50 ^c	2.43 ^c	0.03
Retention (g/14 days)							
Total P	1.05 ^d	1.52 ^b	1.92 ^a	1.31 ^c	1.54 ^{ab}	1.79 ^a	0.02
Phytate P	0.12 ^d	0.13 ^d	0.13 ^d	0.22 ^c	0.33 ^b	0.52 ^a	0.01
Retention ratio (%)							
Total P	29.7 ^e	32.9 ^d	35.2 ^c	33.7 ^{cd}	38.1 ^b	42.5 ^a	0.30
Phytate P	6.5 ^d	6.1 ^d	5.7 ^d	11.3 ^c	16.4 ^b	24.7 ^a	0.50

Each value is means for 3 replicates with 10 chicks.

a-d Means with different superscripts in the same row are significantly different (P < 0.05).

$$Y = 2.818(1 - 0.63e^{-4.11X_1}), R^2 = 0.98 \text{ for non-phytate P}$$

and

$$Y = 2.138(1 - 0.51e^{-0.0023X_2}), R^2 = 0.99 \text{ for phytase. Where } Y = \text{the retention of total P (g/14 days)}; X_1 = \text{added non-phytate P (percentage)}; X_2 = \text{added phytase (units/kg diet)}.$$

From these two equations, relation between added phytase and non-phytate P was as follows :

$$2.818(1 - 0.63e^{-4.11X_1}) = 2.138(1 - 0.51e^{-0.0023X_2})$$

$$X_1 = -0.2433 \ln (0.3829 + 0.6142e^{-0.0023X_2})$$

For example, when X_2 is 125 units/kg of diet, the equivalent X_1 is 0.040% $[-0.2433 \ln (0.3829 + 0.6142e^{-0.0023 \times 125})]$. This result means that 125 units of phytase/kg of diet is equivalent to 0.040% of non-phytate P for total P retention, which indicates that the P equivalency value of phytase is 312 units for 1 g P.

In the same way, the P equivalency values by added 250 and 500 units of phytase per kg of diet were calculated to be 329 and 377 units for 1 g P, respectively. These results were in agreement with the P equivalency values estimated for performance and accumulation of tibia ash in the previous report (Yonemochi *et al.*, 2000).

Furthermore, the increase of total P retention by added phytase almost corresponded to the increase of phytate P retention (Fig. 1). With increasing added phytase, the feed intake and the total P intake increased. However, the total P excretion decreased with increasing phytase level. When 500 units/kg phytase was added, total P excretion was reduced by 31% in comparison with Diet 3 in which non-phytate P was adjusted to satisfy the requirements of broiler chicks. This reduced

Fig. 1. Effect of dietary nonphytate phosphorus (P) and phytase levels on retention of total P and phytate P in male broiler chicks during 7 to 21 days of age ($\square$ + $\blacksquare$ Total P, $\blacksquare$ Phytate P)

A : $Y_1 = 2.818 (1 - 0.63e^{-4.11 \times 1})$, B : $Y_1 = 2.138 (1 - 0.51e^{-0.0023 \times 2})$, C : $Y_2 = 2.388 (1 - 0.95e^{-0.0004 \times 2})$; where Y_1 =grams/14 days of total P retention ; Y_2 =grams/14 days of phytate P retention ; X_1 =percentage of added non-phytate P ; X_2 =unit/kg of diet of added phytase.

Table 3. Effect of dietary non-phytate phosphorus (P) and phytase levels on intake, excretion and retention of Cu, Zn and Mn in male broiler chicks during 7 to 21 days of age

Diet No.	1	2	3	4	5	6	Pooled
Basal non-phytate P (%)	0.30	0.30	0.30	0.30	0.30	0.30	SE
Added non-phytate P (%)	0	0.075	0.15	0	0	0	
Added phytase (unit/kg)	0	0	0	125	250	500	
Intake (mg/14days)							
Cu	4.8 ^d	5.5 ^{ab}	5.9 ^a	5.2 ^c	5.5 ^{bc}	5.7 ^{ab}	0.1
Zn	48.4 ^d	56.3 ^{abc}	59.9 ^a	53.2 ^c	55.4 ^{bc}	57.9 ^{ab}	0.4
Mn	59.0 ^d	65.2 ^{bc}	69.4 ^a	61.6 ^c	64.1 ^{bc}	67.0 ^b	0.4
Excretion (mg/ 14days)							
Cu	4.6 ^c	5.4 ^{ab}	5.7 ^a	5.0 ^b	5.2 ^{ab}	5.3 ^{ab}	0.1
Zn	45.5 ^c	51.0 ^{ab}	53.5 ^a	48.7 ^b	48.4 ^{bc}	49.7 ^{ab}	0.3
Mn	51.8 ^c	61.8 ^{ab}	64.4 ^a	57.0 ^{bc}	60.3 ^{ab}	62.8 ^a	0.5
Retention ratio (%)							
Cu	2.6 ^e	2.7 ^{de}	3.2 ^{cd}	4.4 ^{bc}	5.5 ^{ab}	6.5 ^a	0.1
Zn	5.9 ^e	9.5 ^{cd}	10.6 ^c	8.5 ^d	12.7 ^b	14.1 ^a	0.1
Mn	7.5	5.2	7.1	7.5	6.0	6.3	0.3

Each value is means for 3 replicates with 10 chicks.

a-d means with different superscript in the same row are significantly different ($P < 0.05$).

Table 4. The contents of Cu, Zn and Mn for mineral premix were added to those contents for the diets 1

	The content of Diet ¹	Source for mineral premix	Source for feedstuffs
Zn (mg/kg)	82.3	50.0 (60.8) ²	32.2 (39.2) ³
Cu (mg/kg)	8.1	0.6 (7.4)	7.5 (92.6)
Mn (mg/kg)	95.3	80.0 (83.9)	15.3 (16.1)

¹ See table 1.

² values in parenthesis are percentage of mineral premix to total in diets.

³ values on parenthesis are percentage of feedstuffs to total in diets.

value of total P excretion agreed with that observed by Takemasa *et al.* (1995).

The retention ratio and excretion of Cu, Zn and Mn are shown in Table 3. With increasing dietary non-phytate P or added phytase, the retention ratio of Cu and Zn increased significantly. Furthermore, the magnitude of responses for retention ratio of Cu and Zn by added phytase was larger than that of non-phytate P. When 500 units/kg phytase was added, the excretion of Cu and Zn was reduced by 7% in comparison with diet 3, respectively. This tendency was in agreement with the results of Sevastian *et al.* (1996) and Yi *et al.* (1996b). However, the retention ratio and excretion of Mn were not affected by increasing dietary levels of non-phytate P or phytase.

Vohra *et al.* (1965) reported that phytate formed complexes with cations in the following descending order of strength : $\text{Cu} > \text{Zn} > \text{Co} > \text{Mn} > \text{Fe} > \text{Ca}$, the amount of liberated Mn by addition of phytase being lower than that of Cu and Zn. In our experiment, the utilization of Mn was not improved by the addition of phytase. In addition to the finding of Vohra *et al.* (1965), this is due to the fact that Mn content derived from feedstuffs was far lower than Cu and Zn. From analytical data, the basal diet contained organic Cu, Zn and Mn derived from feedstuffs, which were 92.6, 39.2 and 16.1% of total content, respectively (Table4).

The present study demonstrated that addition of microbial phytase to the broiler diet could decrease P, Cu and also Zn levels that are added to support the requirements of chicks, reducing environment pollution caused by excretory P, Cu and Zn in broiler chicks.

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