Application of Phytase, Microbial or Plant Origin, to Reduce Phosphorus Excretion in Poultry Production**

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ABSTRACT : In order to prevent pollution from animal waste, the excretion of nutrients should be reduced through proper nutritional management. Among the many nutrients of concern, such as N, P, Cu, Zn and K, P is one of the most concerned nutrients to be managed. Seven feeding trials, three with layers and four with broilers, were conducted to determine if microbial phytase supplementation can reduce non-phytate phosphorus (NPP) level in diets and results in concomitant reductions of P excretion. The results showed that microbial phytase can be successfully used to achieve these purposes. Activity of natural phytase in certain plant feedstuffs is high enough to be considered in feed formulation. Three experiments have been conducted to study the characteristics of plant phytase and its application to feeding of broilers. Selected brands of wheat bran could be successfully used as a source of phytase in broiler feeding. ***(Asian-Aust. J. Anim. Sci. 2003. Vol. 16, No. 1 : 124-135)***

Key Words : Microbial Phytase, Plant Phytase, Wheat Bran, Phosphorus Reduction, Poultry

INTRODUCTION

The animal industry must be environmentally sound to ensure its long-term sustainable growth. Livestock wastes, mostly manure, can be a valuable resource as a fertilizer or soil conditioner. But it can be a potential hazard to environment as well. Environmental concerns relate to water quality, soil degradation, air pollution and rural-urban interface issues. Land application of excessive quantities of nutrients is subject to surface run-off and leaching that may contaminate ground or surface waters. Among the many nutrients of concern, such as nitrogen, phosphorus, copper, zinc and potassium, phosphorus is one of the most concerned nutrients to be managed. Phosphorus (P) from phosphate based detergent and manure enters surface waters and stimulates growth of algae and water plants. Excessive growth of green algae in the lake and red algae in the sea causes great problems. Decomposition of them results in an increased oxygen demand, which may interfere with the well-being of fish and wildlife. According to a 1993 environmental law (Vlaren II), the Netherlands limits manure spreading based on P. To convert the limit of P to number of pigs per unit of land, a factor of 7.1 kg P_2O_5 per growing pig is used. Concessions are given to farmers who use environmentally friendly feeds that have reduced N and P input in their formulation. Such an incentive measure was also adopted in Germany. (Williams and Kelly, 1994). In the Netherlands, the expenses for solving environmental problems are shared by farmers in the form of levies based on the phosphate (P_2O_5) surplus. A farm with 35,000 layers, for example, produces approximately 15.2 ton of surplus phosphate a year. The levies will be 13,700 Dfl for slurry manure and 3,200 Dfl for dry manure (>50% DM).

Major efforts are required to adopt all best available technologies capable of reducing excretion of P from animal industry before further restrictive legislation is enacted to control the problem. There are a number of possible solutions to this problem. The first option of manure management is developing 'an environmentally sound' nutritional management, that is, feeding program and feeds to result in less excreted P that need to be managed. Feedstuffs of plant origin contain adequate levels of P but about two thirds of it is in the form of phytate-P (myoinositol hexabiphosphate) which has a low availability in non-ruminant animals or monogastric animals. This low availability is due to the absence of phytase in the gastrointestinal tract of monogastric animals. Phytase is an example of a specific enzyme, which is used to target phytate to release free P with excellent results in pigs and broilers (Simons et al., 1990; Keteran et al., 1993). There are several options available for increasing the phytase activity of diets – either the diet can be supplemented with phytase of microbial source, or formulated using phytase-rich ingredient of plant origin.

The present paper reports the results of a series of experiments conducted at author's laboratory regarding the nutritional management to control P excretion from broilers and layers. Practical applications of microbial phytase and plant phytase source (wheat and wheat bran) are exemplified.

APPLICATION OF PHYTASE

Phytase and its activity

Phytase have a function to catalyze the hydrolytic phosphate splitting of phytic acid (IP6) to lower inositol phosphate esters (IP5 - IP1) and inorganic phosphate (P*i*). It

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can be divided by origin as followings: a) 3-phytase (myo-inositol hexaphosphate 3-phosphohydrolase, EC 3.1.3.8) from microbes and, b) 6-phytase (myo-inositol hexaphosphate 6-phosphohydrolase, EC 3.1.3.26) from plant. Microbial phytase split the orthophosphate group at the C_3 atom of the inositol ring while plant phytase acts at the C_6 atom. One unit of phytase activity is defined as the amount of enzyme that liberates 1μmol of inorganic phosphorus/min from 5.1 mM sodium phytate at pH 5.5 and 37C (Simons et al., 1990; Engelen et al., 1994). Phytase occurs in many vegetable feeds, e.g. wheat, rye, and barley. Various microorganisms (fungi, yeast and bacteria), including the rumen microbes in ruminants, and soil microbes, are also capable of producing phytases. Many microbial phytase products produced from *Aspergillus ficuum* or *Aspergillus niger* are available for use in feed industry. Plant phytase, especially that of wheat, has been known for many decades. It has been isolated and characterized from several sources including wheat, corn, barley, rice, triticale and beans (Reddy et al., 1982; Pointillart, 1993).

Generally phytase activity is affected by various conditions, such as temperature, incubation time, metal ions, and vitamin D. The optimum pH for plant phytase is about 5.0 (Hill and Tyler, 1954a). At a pH of 2.5 and lower, phytase activity was not detected and irreversibly inactivated. If phytase is not properly protected, it can probably not survive in the strong acid condition of the stomach to resume its activity in the small intestine. At the incubation temperature from 15 to 50°C and a pH of 5.1, plant phytase activity had a linear increases (Hill and Tyler, 1954b).

Practical application of microbial phytase to feed manufacturing experiences difficulty due to thermal denaturation during pelleting or extruding process. Phytase activity declines rapidly above 60°C. Addition of 1% arginine to phytase preparation greatly improved the thermal stability (Ryu and Park, 1998). Efforts are being made to develop thermophillic strains among phytase producing microbes. It was also reported that gene coding for phytase activity in *Aspergillus niger* was engineered in to tobacco seeds. The enzyme was expressed as 1% of the soluble protein in mature seeds that can be supplemented to the feeds. (Pen et al., 1993). Mineral retention is usually increased when phytase or vitamin D_3 [1,25$(OH_2)D_3$] is fed (Edwards, 1993). Supplementation of 1,25-dihydroxy-cholecalciferol [1,25$(OH_2)D_3$] facilitate the function of phytase to improve bioavailability of minerals and major nutrients components (Biehl et al., 1995).

Mode of actions of phytase in animal feeding

Microbial phytase is the enzyme known to split the orthophosphate group from the phytate molecule, but the specific activity of phytase within the body and in the diets of poultry and pigs is very limited. Results from studies with pigs have been shown that microbial phytase might influence the utilization of DM, and other nutrients such as calcium and crude protein (Jongbloed et al., 1992; Lei et al., 1994; Mroz et al., 1994).

It was valid to suggest that molar ratios of dietary phytate to zinc would have some predictive value in assessing the potential hazard of phytate in the diet. Oberleas and Harland (1981) presented data on phytate, zinc, and phytate:zinc molar ratios in a range of foods. With the increased degradation of phytate-P by added exogenous phytases, a better utilization of not only phosphorus but also other minerals such as calcium, iron, and zinc can be expected (Pallauf et al., 1992).

In a study with broiler chickens, Sebastian et al. (1996a) reported that phytase supplementation showed the optimum response for the growth performance (BW gain, feed intake and feed efficiency) at the low Ca diets compared to recommended or high dietary Ca levels. Also, improved phytate-P utilization in pigs was observed at low level of dietary Ca. These findings are ultimately related to dietary Ca:P ratio. Qian et al. (1996) showed that the largest response to dietary Ca:total P(P_t) and supplemental phytase was achieved when the dietary Ca: P_t ratio was low (1.1:1) with dietary phytase supplementation of appreciable level.

Mitchell and Edward (1996a,b) demonstrated that supplementation with 1,25-$(OH)_2D_3$ and microbial phytase has synergistic actions that can greatly enhance the bird's ability to utilize phytate-P over either supplement when used alone. They also suggested that 600U of phytase/kg diet would effectively replace up to 0.1% inorganic phosphorus in corn-soybean meal diets of young broilers without adversely affecting chickens performance. The ability of 1,25-$(OH)_2D_3$ supplementation to make phytate-P more available to the chick confirms studies of Mohammed et al. (1991) and Edwards (1993).

Phytase supplementation also increased plasma P and Cu in chickens (Broz et al., 1994; Mitchell and Edwards, 1996b; Sebastian et al., 1996b) and pigs (Young et al., 1993). At the low Cu in broilers (Sebastian et al., 1996a) and pigs (Lei et al., 1994). It is unknown why plasma Cu was increase whereas Cu retention was reduced by phytase supplementation (Sebastian et al., 1996a). In contrast, Adeola (1996) reported a reduction in serum Cu as absorption of Cu was increased by phytase supplementation. On the other hand, the low P diet increases the plasma Ca for chickens. This increase in Ca may be expected because a low P diet normally causes an elevated ionized Ca in the plasma, which depresses the release of parathyroid hormone (PTH), thus reducing the PTH inhibition on tubular reabsorption of phosphate and permitting the urinary excretion of additional Ca absorbed from the gut during low

P diet feeding (Tayler and Dacke, 1984, Sebastian et al., 1996b). Supplementary phytase at the low calcium diets showed the optimum level of retention of nitrogen, phosphorus, and calcium of broilers (Schoener et al., 1993; Sebastian et al., 1996a) and turkeys (Qian et al., 1996). Yi et al. (1996) suggested that the use of microbial phytase in poultry diets could provide a method for reducing nitrogen excretion

It has been shown that supplementation of a microbial phytase to the rations for growing pigs enabled a reduction in phosphorus excretion by feces and urine up to 50% (Jongbloed, 1989; Nasi, 1991; Kessler and Egli, 1991; Pallauf et al., 1992). Reducing the amount of phosphorus intake is clearly the best way to decrease the excreta P and the reduction in dietary phosphorus must not negatively affect bird performance (Mitchell and Edwards, 1996a).

EXPERIMENTS

Microbial phytase

Layer experiments

It has been reported that hens consuming the low nonphytate P (NPP) diet with supplementary phytase performed as well as the hens fed diets containing higher levels of NPP without supplementary phytase (Gordon and Roland, 1997; Van der Klis and Versteegh, 1996). In a feeding trial with laying hens the effectiveness of microbial phytase in diets based on corn-soya and wheat-soya was tested (Peter and Jeroch, 1993). The supplement of phytase (500 U/kg diet) or inorganic P (0.1% of diet) had a positive effect on the performance of the corn-soya group but no effect on that of the wheat-soya group. The highest breaking strength of the eggshell was recorded with hens that received the phytase supplement in the corn-soya group. Mineralization of the tibia bone was also improved with phytase addition. The response to the level of supplementary phytase was quadratic. Supplementation of 250 U of phytase/kg diet in laying hens was equivalent to 0.8g of P from monocalcium phosphate (MCP) (Van der Klis et al., 1994) while supplementation of 500 U to a corn-soybean meal diet was equivalent to 1g of P (Peter and Jeroch, 1993). Simons et al. (1992) reported that supplementation of diets with 250 U of phytase/kg resulted in the degradation of 62% and 56% of the phytate-P at low and high Ca levels, respectively. Increasing phytase from 250 to 500 U/kg of diet had a further effect on degradation, increasing it by 16% and 11% at respective Ca level. Three layer experiments were conducted to determine if microbial phytase supplementation can reduce non-phytate phosphorus (NPP) level in a practical laying diet and result in concomitant reductions in P excretion. Followings are abstracts of layer feeding trials conducted at author's laboratory.

Layer Exp. 1 : A 20-wk feeding trial (21 to 40 wk of age) was conducted to evaluate the effects of phytase supplementation on egg production, egg quality, nutrient retention, and P excretion of laying hens fed diets containing different levels of P. Nine hundred and sixty ISA brown® hens were randomly allocated to completely randomized block arrangement of four diets: corn-soybean diet (1.4% tricalcium phosphate, TCP) without (T1, control) and with phytase (T2); 0.7% TCP (T3) or 0% TCP (T4) diet with phytase. Dietary microbial phytase (Natuphos®) was added at a level of 500 U/kg. Both hen-day and hen-housed egg production of T2 were significantly ($p<0.05$) higher than other treatments, which were not different among them. Egg weights were also significantly ($p<0.05$) different among treatments, with T2 being the highest. Feed consumption of T2 was significantly ($p<0.05$) higher than other treatments but feed conversion ratio was not significantly different from others. Specific gravity and shell thickness of the eggs were highest in the control (T1) but eggshell strength and broken egg to total egg ratio were not different among treatments. Haugh unit were not different among treatments. Retention of Ca, P, Mg, Fe and Zn were greater ($p<0.05$) in phytase-supplemented groups. There were significant ($p<0.05$) differences in excretion of ash, P and Zn. The excretion of these components was highest in the control, whereas P excretion was significantly lower in T3 and T4 groups. In conclusion, supplementation of the microbial phytase to normal corn-soybean diet improved egg production and can reduce TCP level in the diet without affecting egg production and egg quality. Significant reduction of P excretion can be also achieved. (Um and Paik, 1999)

Layer Exp. 2 : An 8 week feeding trial was conducted with 864 ISA Brown laying hens of 48 wks old to determine if microbial phytase (Natuphos®) supplementation can reduce non-phytate phosphorus (NPP) level in laying diets. Major components of basal were corn, soybean meal and 10% wheat bran. The experiment consisted of four dietary treatments: T1, control diet with 0.26% NPP (0.55% total P) with no supplementary phytase; T2, 0.21% NPP (0.50% total P) diet with 250 U of phytase/kg of diet; T3, 0.16% NPP (0.45% total P) diet with 250 U of phytase/kg of diet; and T4, 0.11% NPP (0.40% total P) diet with 250 U of phytase/kg of diet. T3 showed the highest egg production and egg weight and the lowest feed conversion while T4 gave the lowest egg production and the highest feed conversion and mortality. Daily feed consumption ranged from 130.4 g (T4) to 132.7 g (T2). T1 and T2 were not significantly different in the production parameter. Eggshell strength, egg specific gravity, and eggshell thickness were not significantly different among treatments. However, broken egg ratio was significantly lower in T2 and T4 than in T1. Retentions of Ca, P, Mg and Cu were greater in

phytase-supplemented treatments (T2, T3 and T4) than the control (T1), and those in T3 and T4 were greater than in T2. Excretions of P in phytase-supplemented treatments (T2, T3 and T4) were significantly ($p<0.05$) smaller than in T1 but excretions of N were not significantly different among the treatments. Contents of ash in tibiae were not significantly affected by treatments, but contents of Ca, P, Mg and Zn was increased and that of Cu decreased by phytase supplementation. It is concluded that the NPP concentration in the diet of Brown layers consuming about 130 g/d of feed can be safely lowered from 0.26% (0.55% total P) to 0.16% (0.45% total P) and excretion of P was also reduced by the inclusion of 250 U phytase/kg of diet. (Um et al., 1999)

Layer Exp. 3 : An experiment employing a factorial arrangement of two level (3.0 and 4.0%) of calcium(Ca), two levels (0.15 and 0.25%) of NPP and two levels (0 and 300 U/kg) of a microbial phytase product (Novo Nordisk Corp.) was carried out with 960 ISA Brown layers from 21 to 41 wk of age. Major components of basal diet were corn, soybean meal and 5.6% wheat bran. High NPP level (0.25%) and phytase supplementation increased egg production in the second 10wk period (31-41 weeks). There was a significant interaction between NPP level and phytase for egg production. High NPP (0.25%) and low Ca (3.0%) increased feed intake and significant interaction between levels of NPP and Ca was observed. High NPP (0.25%) improved feed efficiency. Low NPP (0.15%) improved egg specific gravity, eggshell thickness and rate of broken and soft egg production but decreased Haugh unit, whereas low Ca (3.0%) decreased egg specific gravity, eggshell strength, eggshell thickness and increased Haugh unit. Phytase supplementation decreased the rate of broken & soft egg production. High NPP (0.25%) increased fiber availability but decreased Ca availability. High Ca decreased Ca availability while phytase increased availability of DM, fiber and P. High NPP (0.25%) increased retention of P and Fe but also increased excretion of P. High Ca (4.0%) decreased retention of Zn and Fe but increased Ca excretion. Phytase supplementation increased P retention resulting in decrease of P excretion. In conclusion, supplementation of microbial phytase at a level of 300U per kg diet of laying hens can improve egg production, decrease broken and soft egg production rate and P excretion. The level of Ca and NPP significantly modifies the effects of phytase supplementation. (Lim et al., 2002)

Provided phytate P content and plant phytase activity are taken into account, it should be possible to mix layer diets which require minimum amount of supplementary inorganic P with 250 U phytase supplemented (Um et al., 1999) or do not require supplementary inorganic P sources with 500 U phytase supplemented (Um and Paik, 1999). In layers, the degradation of phytate and the absorption of P was slightly decreased by higher amounts of Ca in the diets (4.0% vs 3.0% Ca in feed), nevertheless at both levels the efficacy of phytase addition was satisfactory. Addition of up to 300 units phytase per kg feed for laying hens resulted in a minimal equivalency of 0.3 g MCP P per 100 units phytase. Table 1 shows summary of layer feeding trials.

Broiler experiments

In broiler chickens, phytase supplementation at a level of 1,000 U/kg diet increased the bioavailability of P and Ca by 60% and 26%, respectively (Simons et al., 1990). The beneficial effects of phytase supplementation have been

Table 1. Effects of supplemental phytases on the productivity and P excretion of laying birds

Experiment	Level of NPP[1], %	Supplemental phytase, unit	Performance index		
			Egg production	Feed/egg mass	P excretion
Layer-1	0.37	0	100	100	100
	0.37	500	102.2	99.6	88.5
	0.24	500	100.4	100.4	70.5
	0.12	500	100.4	100.4	59.0
Layer-2	0.27	0	100	100	100
	0.22	250	100.3	100.5	88.5
	0.16	250	101.4	98.6	67.3
	0.11	250	99.1	100.3	57.7
Layer-3	0.25(Ca 4%)	0	100	100	100
	0.25(Ca 4%)	300	103.1	97.4	94.4
	0.25(Ca 3%)	0	102.1	97.0	102.8
	0.25(Ca 3%)	300	104.5	97.0	86.1
	0.15(Ca 4%)	0	97.6	100	86.1
	0.15(Ca 4%)	300	97.2	100.9	72.2
	0.15(Ca 3%)	0	100.6	100	83.3
	0.15(Ca 3%)	300	101.5	99.1	80.6

[1] Nonphytate phosphorus.

illustrated by Zyla and Korelski (1993). The performance of birds fed available P deficient diets was improved by the addition of phytase to the diets. The *in vitro* activity (i.e. ability to dephosphorylate phytate) was also demonstrated, confirming the proposed mode of action of this enzyme. The direct benefits of dietary phytase supplementation on bone mineralization have been shown by Farrel and Martin (Annison and Choct, 1993) who reported that tibial ash deposition was enhanced in birds fed phytase-supplemented diets. Simons and Versteegh (1993) summarized the results of several experiments conducted in Netherlands. A microbial phytase product from *Aspergillus niger* was added to broiler feed with a low inorganic P level. The availability of total P could be increased up to 70%. In comparison with feed with increased levels of inorganic feed phosphates, a significantly larger amount of the P consumed was absorbed. Improved utilization of P decreased its excretion by 40% or more. Growth and feed conversion ratios were comparable with feed to which inorganic feed phosphate was added. In broilers up to 500 units of phytase per kg feed, 250 units phytase was equivalent for P absorption with 0.5 g of P from MCP per kg feed. Followings are abstracts of broiler feeding trials conducted at author's laboratory.

Broiler Exp. 1 : A 5wk feeding experiment was conducted with day-old one thousand broiler chicks (Arbor Acres) to determine the effects of microbial phytase (Natuphos®) supplemented to low NPP corn-soy diet. Five pens of 50 mixed sex birds each were randomly assigned to each of the four dietary treatments: T1, control diet containing normal NPP level; T2, T1-0.1% NPP+600 U of phytase/kg diet; T3, T1-0.2% NPP+600 U of phytase/kg diet; and T4, T1-0.3% NPP+600 U of phytase/kg diet. T1, T2 and T3 showed similar growth rate, feed intake, and feed efficiency, indicating that NPP level in broiler diets could be reduced by approximately 0.2% by the microbial phytase supplementation. But T4 showed significantly ($p<0.05$) lower weight gain than others. The phytase supplementation improved P availability resulting in low P excretion. Weight and girth of metatarsal bone were increased by phytase supplementation at low NPP diet treatments but ash contents were not significantly different. It can be concluded that NPP level of corn-soy broiler diets can be safely lowered by approximately 0.2% by supplementing 600 U of microbial phytase/kg diet. With the adjusted level of NPP and phytase supplementation, P excretion could be reduced by 50%. (Um et al., 2000)

Broiler Exp. 2 : An experiment was conducted with day-old 300 commercial male broiler chicks (Arbor Acres®) to evaluate the efficacy of crude phytase preparations produced from a culture of *Aspergillus ficuum*. The experiment consisted of five dietary treatments; T1, corn-soy control diet with 0.45% NPP for starter period and 0.35% NPP for grower period; T2, control-0.1% NPP; T3, control-0.2% NPP; T4, T3+600 U of crude phytase (broth+cell); and T5, T3+600 U of crude phytase (broth). The body weight gain, feed intake and feed/gain of chickens fed T1 diet were highest ($p<0.01$) among treatments. Body weight gain and feed intake of T4 and T5 were greater than those of T3 but were less than those of T1 and T2. T3 was highest in mortality among treatments. Decreasing the NPP level lowered the availability of DM, crude ash, ether extract, crude fiber, Zn and Fe but supplementation of crude phytase preparation improved the availability of these nutrients as well as those of Ca, P and Cu. Excretion of P and Cu significantly decreased as the NPP level in the diet decreased. Further reduction of P and Cu excretion and reduction of Ca, P, Mg, An, Fe and Cu were achieved by supplementation of crude phytase preparations. The serum concentrations of Ca, P, Mg, Zn, Fe and Cu were significantly increased by crude phytase supplementation. The weight and length of tibia, and contents of Ca, P, Mg and Zn were adversely affected by lowering NPP level but partially recovered by supplementation of crude phytase preparations. In conclusion, lowering NPP level in the broiler diet significantly depressed the performance. Supplementation of crude phytase preparation produced from *Aspergillus ficuum* could partially recover the depression. (Paik et al., 2000)

Broiler Exp. 3 : An experiment was conducted to determine the effects of phytase supplementation to the diets containing different levels of NPP. A 3×2 factorial arrangement of treatments was employed. There were three dietary NPP levels of control (C) (0.45% for starter diet and 0.35% for grower diet) C-0.1% NPP (0.35% for starter diet and 0.25% for grower diet) and C-0.2% NPP (0.25% for starter diet and 0.15% for grower diet) and two levels of phytase (NOVO Nordisk Corp.) (0 and 500 U/kg). Reduced dietary NPP decreased feed intake and weight gain and increased mortality whereas dietary phytase increased feed intake, weight gain and decreased mortality. Supplemental phytase improved availabilities of dry matter, crude fat, ash, P, Zn, Mg and Cu whereas dietary NPP level did not affect availabilities of nutrients except decreased Zn availability and increased Cu availability in reduced NPP diets. Nutrient retention of N, ash, Ca, P, Mg and Zn were linearly decreased as dietary NPP levels reduced but dietary phytase increased their retention. Reduced dietary NPP increased ash excretion but decreased P and Cu excretion while dietary phytase decreased N excretion. Weight, length, girth and contents of ash, Ca, P and Mg of tibia linearly decreased as dietary NPP levels reduced. Dietary phytase increased length and ash content of tibia. It was concluded that dietary phytase could reduce P excretion and alleviate adverse affects caused by feeding low dietary NPP. Effects

of phytase supplementation were greater in the lower NPP diets. (Lim et al., 2001)

Broiler Exp. 4 : An experiment was conducted to measure the effect of crude phytase supplementation on the growing performance, blood concentrations of some minerals and tibia characteristics of broiler chickens. Hatched 240 male broiler chickens (Avian®) were randomly allotted to four treatments. There were six replicates per treatment, and ten chicks per replicate. Treatments consisted of two levels of crude phytase (0 and 600 U/kg) made from *Aspergillus ficuum* and two levels of NPP (0.45 and 0.35% NPP for the starter period, and 0.35 and 0.25% NPP for the grower period), making the experiment 2×2 factorial. The starter period was from hatch to 21d of age, and grower period was from 22 to 35d of age. Feed intake and weight gain of chicks fed diets containing phytase were higher ($p<0.05$) than those of chicks fed diets without phytase. However, no differences were found in feed/gain, mortality, and nutrient availabilities due to the phytase supplementation. Chickens fed diets with low NPP and phytase excreted lower amount of P than did birds fed diets containing normal NPP without phytase. The level of NPP and phytase did not affect the amount of N excretion. The Ca availability was increased by feeding low NPP diet. Dietary phytase increased the availabilities of P and Mg, but decreased those of Fe and Zn. There were interactions between dietary NPP level and phytase addition on mineral availability. Tibia was lighter and shorter in low NPP groups, and heavier in phytase treated groups. The tibial contents of Ca, P and Mg decreased in low NPP treated groups, but increased in phytase treated groups. The ash content of tibia of chickens fed diet with phytase was higher than that of birds fed diets without phytase. These data suggest that the crude phytase supplementation to broiler diets containing low NPP level improves growth performance and mineral availability and reduces fecal P excretion. (Lee et al., 2000)

Plant phytase

It is generally accepted that approximately one third of phosphorus in the plant origin feedstuffs are available to monogastric animals. However, proportion of phytate P of total P varies widely from 12% in tapioca to 83% in wheat bran. And natural phytase content in the feedstuffs also varies widely from almost none in corn to 2395U in wheat bran (Lee et al., 1999). Such differences should be considered in calculating available P content of diets. Three experiments have been conducted in author's laboratory to study the characteristics of plant phytase and it's application to feeding of broilers. Followings are abstracts of experiments.

Plant phytase Exp. 1 : An experiment was conducted to measure the contents of phytate-P, total-P and phytase activity of cereals and cereal by-products. The effects of pH and temperature on the activity of wheat and microbial phytase were compared. Phytate-P content was higher in most cereal by-products than in cereal . Rice bran had the

Table 2. Effects of supplemental phytase on the productivity and P excretion of broiler

Experiment	Level of NPP[1], %		Supplemental	Performance index		
	Starter	Finisher	phytase, unit	Gain	Feed/gain	P excretion
Broiler Exp.1	0.45	0.40	0	100	100	100
	0.34	0.31	600	101.0	98.7	76.2
	0.23	0.22	600	99.3	101.3	54.8
	0.12	0.13	600	96.7	103.3	40.5
Broiler Exp.2	0.45	0.35	0	100	100	100
	0.35	0.25	0	89.4	100.6	84.8
	0.25	0.15	0	60.5	108.7	51.5
	0.25	0.15	600, Phyt-A[2]	82.2	109.3	39.4
	0.25	0.15	600, Phyt-B[3]	78.9	109.3	45.5
Broiler Exp. 3	0.45	0.35	0	100	100	100
	0.45	0.35	500	99.9	100	107.4
	0.35	0.25	0	87.3	102.5	85.2
	0.35	0.25	500	97.0	100.6	70.4
	0.25	0.15	0	57.3	101.2	81.5
	0.25	0.15	500	65.1	108.1	55.6
Broiler Exp.4	0.45	0.35	0	100	100	100
	0.45	0.35	600	100.6	100	92.7
	0.35	0.25	0	92.5	103.0	78.6
	0.35	0.25	600	100.5	101.2	72.9

[1] Nonphytate phosphorus, [2] crude phytase A (soup+cell), [3] crude phytase B (soup).

highest phytate-P (1,201 mg/100 g) followed by defatted rice bran (1,077 mg/100 g), corn gluten feed (896 mg/100 g), wheat bran (742 mg/100 g) and rapeseed meal (535 mg/100 g). The phytate-P contents of other ingredients were lower than 500 mg/100 g. Total-P content was high in defatted rice bran (1,899 mg/100 g), rice bran (1,889 mg/100 g), and rapeseed meal (1,016 mg/100 g) compared to other ingredients. Wheat and wheat bran had the highest phytase activity (1,121.9 and 2,935.1 U/kg) among ingredients tested (Table 3).

Characteristics of wheat phytase and microbial phytase were compared. Both of them showed similar characteristics at varying pH and temperature. The activity of wheat and microbial phytase was gradually increased until pH and temperature reached at 5.5 and 50°C, respectively, then their activity remarkably decreased at pH 6 and 60°C. Wheat phytase was as stable, if not more, as microbial phytase under different pH and temperature (Figure 1 and 2). Considering these characteristics, plant phytase may be as effective as microbial phytase to the animals.

Plant phytase Exp. 2 : This study was conducted to evaluate the efficacy of wheat and wheat bran as the source of phytase in a 5 weeks broiler feeding trial. One thousand day-old broiler chickens (Ross®) were divided into 20 pens of 50 broilers (25 male and 25 female) each. Four pens were randomly arranged to one of the five dietary treatments (Table 4): T1, control diet containing normal NPP level ; T2, T1-0.1% NPP; T3, T2＋600 IU microbial phytase(NOVO®) per kg diet; T4, T2＋600 IU plant phytase from wheat and wheat bran; T5, T2＋600 IU plant phytase from wheat and hydrothermally treated wheat bran. Table 4 shows details of the feed formula of starter diets. Reduction of NPP level by 0.1% (T2) reduced weight gain and feed intake but plant phytase treatments (T4 and T5) recovered the lost performance. Plant phytase treatments showed better performance than the microbial phytase treatment (T3). There was no difference between regular wheat bran treatment (T4) and hydrothermally treated wheat bran treatment (T5). Mortality was highest in low NPP diet (T2) (Table 5). Availability of ether extract and crude ash of grower diet was highest in normal wheat bran diet (T4). Availability of Ca and P of grower diet was highest in T4 followed by T3 and T5. Availability of Mg, Fe and Zn was more drastically improved by phytase treatments (T3, T4 and T5) (Table 6). Excretion of Ca, P, Mg, Fe and Zn was lowest in microbial phytase treatment (T3). Serum level of Ca and Mg was highest in the low NPP treatment (T2). Tibial ash content of T2 and T3 was lower than that of T1, T4 and T5. However, tibial Ca content was higher in T1 and T2 than other treatments. Tibial P and Mg contents were highest in T1. It was concluded that plant

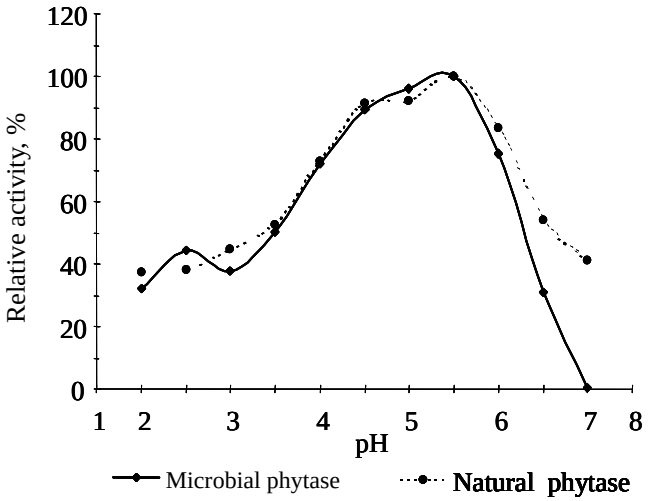

Figure 1. Activity of microbial and plant origin natural phytase at different pH.

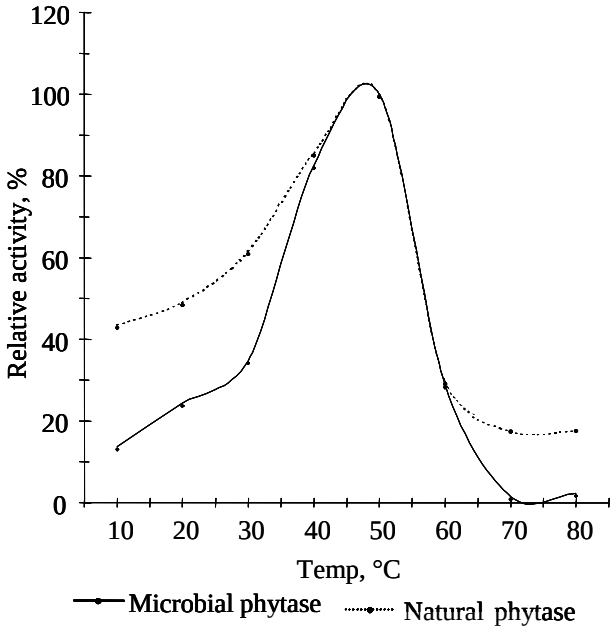

Figure 2. Activity of microbial and plant origin natural phytase at different temperature.

phytase from wheat and wheat bran can be effectively used to improve P utilization of the broilers fed low NPP diets. Plant phytase improved the availability of crude ash and minerals such as Ca, P, Mg, Zn and Fe of the diets. Hydrothermal treatment of wheat bran prior to inclusion in the diet had no beneficial effects (Kim et al., 2002).

Plant phytase Exp. 3 : An in vitro test and a broiler feeding trial have been conducted to test the effect of hydrothermal treatment of wheat bran on phytate-P degradation and its feeding effect in broiler. Hydrothermal treatment of wheat bran was carried out at 55°C with pH

Table 3. Total P, phytate P content and phytase activity of plant origin feedstuffs

Ingredients	Phytate-P mg/100 g	Total-P mg/100 g	Phytate-P % of total P	Phytase activity, U/kg
Corn	60	182	32.7	0.2
Lupin	55	307	17.8	3.2
Tapioca	7	59	11.9	18.8
Wheat	199	295	67.5	1120
Sesame meal	542	816	66.4	3.0
Soybean meal	286	577	49.6	7.5
Cottonseed meal	303	678	44.7	2.4
Coconut meal	204	539	37.8	350
Corn germ meal	32	130	24.4	12.6
Corn gluten meal	287	536	53.5	170
Corn gluten feed	896	1,099	81.5	14.8
Rapeseed meal	535	1,016	52.7	103
Wheat bran	742	893	83.1	2935
Rice bran	1,201	1,886	63.7	-
Rice bran (fat-free)	1,077	1,899	56.7	114

(Lee et al., 1999)

Table 4. Formula and composition of broiler starter diets

Ingredients (%)	Treatments				
	T1	T2	T3	T4	T5
Corn	58.32	58.37	58.37	35.43	35.43
Soybean meal (44% CP)	30.71	31.00	31.00	22.20	22.20
Wheat[1]	-	-	-	15.33	15.33
Wheat bran[2]	-	-	-	10.00	-
Soaked wheat bran	-	-	-	-	10.00
Fish meal (60% CP)	4.00	4.00	4.00	5.00	5.00
Corn gluten	2.15	1.93	1.93	4.88	4.88
Animal fat	2.00	2.00	2.00	4.55	4.55
Limestone	1.08	1.52	1.52	1.54	1.54
Tricalcium phosphate (18% P)	0.93	0.37	0.37	0.27	0.27
Vitamin premix[3]	0.15	0.15	0.15	0.15	0.15
Mineral premix[4]	0.10	0.10	0.10	0.10	0.10
Salt	0.31	0.31	0.31	0.27	0.27
DL-methionine (50%)	0.22	0.22	0.22	0.17	0.17
Lysine-HCl (78%)	0.04	0.03	0.03	0.12	0.12
Microbial phytase[5]	-	-	+	-	-
Total	100.00	100.00	100.00	100.00	100.00
Calculated composition					
ME, kcal/kg	3,000	3,000	3,000	3,000	3,000
Crude protein, %	22.5	22.5	22.5	22.5	22.5
Lysine, %	1.10	1.10	1.10	1.10	1.10
Meth + Cys, %	0.84	0.84	0.84	0.84	0.84
Crude fiber, %	3.18	3.19	3.19	3.66	3.66
Calcium, %	1.00	1.00	1.00	1.00	1.00
Total P, %	0.62	0.52	0.52	0.57	0.57
Available P, %	0.44	0.34	0.34	0.33	0.33
Phytase activity, IU/kg	26.6	26.6	676.6	650.0	650.0

[1] Contained 1,034 units of phytase activity per kilogram.
[2] Contained 4,746 units of phytase activity per kilogram.
[3] Vitamin premix contains the followings per kg: vitamin A, 10,000,000 IU; vitamin D_3, 2,500,000 IU; vitamin E, 25,000 mg; vitamin K_3, 1,700 mg; vitamin B_1, 2.000 mg; vitamin B_2, 5,000 mg; vitamin B_6, 3,000 mg; vitamin B_{12}, 16,000 μg; Niacin, 34,000 mg; Folic acid, 1,000 mg; Biotin, 84,000 μg; Pantothenic acid, 9,000 mg.
[4] Mineral premix contains the followings per mg: Zn, 75,000 mg; Mn, 75,000 mg; Fe, 75,000 mg; Cu, 7,500 mg; I, 1,650 mg; Se, 450 mg; S, 125,000 mg.
[5] Contained 2,500 units of phytase activity per gram of product (NOVO Nordisk Corp).

Table 5. Body weight gain, feed intake, feed/gain and mortality of broiler chickens fed experimental diets from 1 to 35 days

Item	Age (day)	Treatments[1]					SEM
		T1	T2	T3	T4	T5	
Weight gain, g/bird	1-21	703.8^{a}	577.1^{c}	654.5^{b}	683.6^{a}	688.0^{a}	8.89
	22-35	906.9^{a}	712.3^{c}	797.0^{b}	880.7^{a}	904.1^{a}	18.06
	1-35	1610.7^{a}	1289.5^{c}	1451.5^{b}	1564.3^{a}	1592.1^{a}	23.44
Feed intake, g/bird	1-21	973.6^{a}	814.8^{c}	919.5^{b}	951.7^{a}	965.6^{a}	9.42
	22-35	1674.6^{a}	13545.0^{c}	1487.0^{b}	1642.2^{a}	1707.2^{a}	35.69
	1-35	2648.2^{a}	2160.6^{c}	2407.4^{b}	2594.9^{a}	2672.8^{a}	44.01
Feed/gain (g/g)	1-21	1.39	1.41	1.41	1.40	1.40	0.02
	22-35	1.85	1.89	1.87	1.87	1.89	0.02
	1-35	1.65	1.67	1.66	1.66	1.68	0.02
Mortality, %	1-21	2.00	3.50	3.00	1.50	3.50	0.97
	22-35	0.00	3.62	2.06	0.00	1.04	0.92
	1-35	2.00^{b}	7.00^{a}	5.00^{ab}	1.50^{b}	4.50^{ab}	1.27

[1] T1=control diet containing normal NPP level, T2=control diet-0.1% NPP, T3=control diet-0.1% NPP+600 IU microbial phytase, T4=control diet-0.1% NPP+600 IU natural phytase (wheat+wheat bran), T5=control diet-0.1% NPP+600 IU natural phytase (wheat+soaked wheat bran).
[a-c] Values with different superscripts in the same row are significantly different ($p<0.05$).

Table 6. Availability of Ca, P, Mg, Fe and Zn of broiler grower diets

Item (%)	Treatments[1]					SEM
	T1	T2	T3	T4	T5	
Calcium	26.0^{d}	26.7^{d}	46.1^{b}	57.2^{a}	38.9^{c}	1.93
Phosphorus	33.5^{b}	35.1^{b}	47.5^{a}	49.9^{a}	37.0^{b}	2.08
Magnesium	8.2^{b}	13.9^{b}	31.1^{a}	30.7^{a}	26.3^{a}	2.10
Iron	3.36^{d}	3.69^{d}	15.98^{c}	37.64^{a}	23.04^{b}	1.80
Zinc	17.6^{c}	8.0^{d}	29.0^{b}	42.7^{a}	21.3^{b}	1.92

[1] T1=control diet containing normal NPP level, T2=control diet-0.1% NPP, T3=control diet-0.1% NPP+600 IU microbial phytase, T4=control diet-0.1% NPP+600 IU natural phytase(wheat + wheat bran), T5=control diet-0.1% NPP+600 IU natural phytase (wheat+soaked wheat bran).
[a-d] Values with different superscripts in the same row are significantly different ($p<0.05$).

5.5 buffer. Phytate-P content of wheat bran showed quadric decrease as the rate of wheat bran : buffer ratio increased from 1:0.5 to 1:5 (Figure 3 and 4). Phytate-P degradation was not significantly affected by incubation time above 10 min., drying temperature (55°C, 65°C and 75°C) or pH of buffer (5.5 and 7.0). Feeding trial was conducted with 240 sex separated day-old broiler chickens (Ross®). Broilers were randomly housed to 24 cages of 10 birds each. Six cages (3 in each sex) were assigned to 4 treatments (Table 7): control; normal level of non-phytate-P (NPP), LP; low NPP treatment which has 0.1% lower NPP than the control, LPWB; LP with wheat bran which provides 500 IU of plant

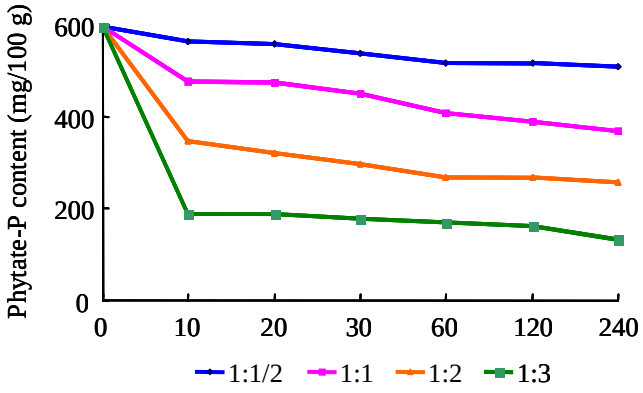

Figure 3. Influence of incubation time and wheat bran : buffer ratio on phytate P content of wheat bran (incubated at 55°C and pH 5.5, and dried at 65°C)

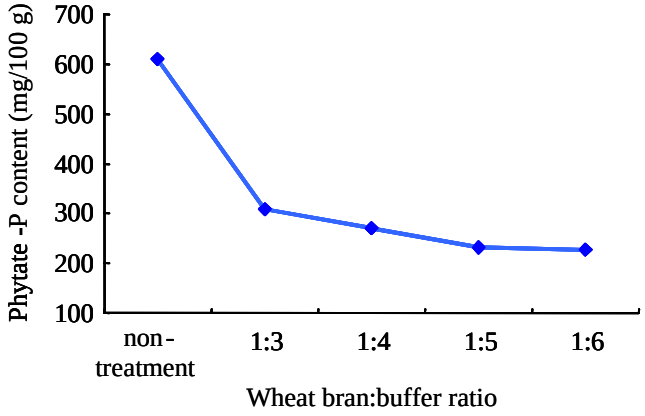

Figure 4. Influence of wheat bran : buffer ratio on the deduction of phytate-P content of wheat bran (incubated at pH 5.5 and 55°C for 1 h and dried at 65°C).

Table 7. Formula and composition of broiler starter diets

Ingredients (%)	Treatments			
	T1	T2	T3	T4
Corn	53.34	53.34	48.25	48.25
SBM-44	32.06	32.06	29.54	29.54
Rice bran	3.00	3.00	-	-
Wheat bran[1]	-	-	10.00	-
Hydrothermally treated wheat bran	-	-	-	10.00
Fish meal-60	2.50	2.50	2.86	2.86
Corn gluten	2.50	2.50	3.00	3.00
Animal fat	3.50	3.50	3.50	3.50
Limestone	1.04	1.59	1.33	1.33
Tricalcium phosphate (18% P)	1.17	0.61	0.66	0.66
Vitamin premix[2]	0.15	0.15	0.15	0.15
Mineral premix[3]	0.10	0.10	0.10	0.10
Salt	0.34	0.34	0.34	0.34
Dl-methionine	0.23	0.23	0.21	0.21
Lysine-HCl	0.07	0.07	0.07	0.07
Total	100.00	100.00	100.00	100.00
Calculated composition				
ME poultry (kcal/kg)	3,000.00	3,000.00	3,000.00	3,000.00
Crude protein, %	22.50	22.50	22.50	22.50
Lysine, %	1.10	1.10	1.10	1.10
Methionine, %	0.60	0.60	0.59	0.59
Methionine & cystine, %	0.84	0.84	0.84	0.84
Crude fiber, %	3.45	3.45	3.59	3.59
Calcium, %	1.03	1.08	1.00	1.00
Total P, %	0.68	0.58	0.57	0.57
Available P, %	0.45	0.35	0.35	0.35
Potassium, %	0.85	0.85	0.85	0.85

[1] Contained 4,746 units of phytase activity per kilogram.

[2] Vitamin premix contains the followings per kg: vitamin A, 10,000,000 IU; vitamin D_3, 2,500,000 IU; vitamin E, 25,000 mg; vitamin K_3, 1,700 mg; vitamin B_1, 2.000 mg; vitamin B_2, 5,000 mg; vitamin B_6, 3,000 mg; vitamin B_{12}, 16,000 μg; Niacin, 34,000 mg; Folic acid, 1,000 mg; Biotin, 84,000 μg; Pantothenic acid, 9,000 mg.

[3] Mineral premix contains the followings per mg: Zn, 75,000 mg; Mn, 75,000 mg; Fe, 75,000 mg; Cu, 7,500 mg; I, 1,650 mg; Se, 450 mg; S, 125,000 mg.

phytase per kg diet, LPHWB; LP with hydrothermally treated wheat bran. Results of feeding trial showed that broilers of LP treatment gained significantly lower than other treatments in starter period (1-21 d) but only male broilers of LP gained significantly lower than the control in grower (22-35 d) and overall period (Table 8). There were no significant differences among the birds of LPWB, LPHWB and control. Feed intake of overall period was not significantly different between LPWB and control but that of LP was lower than LPHWB and that of LPHWB was lower than control. Feed conversion ratio was significantly lower in LPHWB and LP than in control and LPWP. Mortality was highest in LPHWB. Utilizability of crude fat, crude ash and Ca was significantly lower but that of Fe was significantly higher in LP than other treatments.

Table 8. Body weight gain of broiler chickens fed experimental diets from 1 to 35 days (g/bird)

Age (day)	Sex	Treatments[1]				SEM
		T1	T2	T3	T4	
1-21	♂	773.3	704.0	772.6	766.9	23.36
	♀	719.6	678.4	709.7	729.7	17.27
	All	746.5[a]	691.2[b]	741.2[a]	748.3[a]	16.73
22-35	♂	874.8[a]	811.9[b]	863.8[ab]	866.8[ab]	18.04
	♀	782.7	764.9	773.7	790.2	19.64
	All	828.8	788.3	818.8	828.5	21.24
1-35	♂	1,648.2[a]	1,515.9[b]	1,636.5[ab]	1,633.7[ab]	36.69
	♀	1,502.3	1,443.6	1,483.4	1,502.3	23.57
	All	1,575.2	1,496.6	1,559.9	1,576.8	34.15

[1] T1=control diet containing normal nonphytate P (NPP) level, T2= control diet-0.1% NPP, T3=control diet-0.1% NPP+500 IU plant phytase (wheat bran), T4=control diet-0.1% NPP+500 IU plant phytase (hydrothermally treated wheat bran).

[a-b] Values with different superscripts in the same row are different ($p<0.05$).

Table 9. Excretion of Ca, P, Mg, Fe and Zn of broilers fed grower diets

Item	Treatments[1]				SEM
	T1	T2	T3	T4	
	---------- g/bird/d ----------				
Calcium	0.396	0.363	0.346	0.348	0.018
Phosphorus	0.234[a]	0.174[b]	0.168[b]	0.172[b]	0.008
Magnesium	0.108	0.115	0.105	0.102	0.005
	---------- mg/bird/d ----------				
Iron	93.71	91.94	99.16	101.02	4.74
Zinc	48.67	46.52	48.24	48.42	2.12

[1] T1=control diet containing normal nonphytate P (NPP) level, T2= control diet-0.1% NPP, T3=control diet-0.1% NPP+500 IU plant phytase (wheat bran), T4=control diet-0.1% NPP+500 IU plant phytase (hydrothermally treated wheat bran).

[a-b] Values with different superscripts in the same row are different ($p<0.05$).

Utilizability of P, Mg and Zn was higher in LPWB and LPHWB than control and LP. Excretion of P was significantly lower in low NPP treatments than in control (Table 9). Serum Ca level was highest but serum P level was lowest in LP. Tibial crude ash content was high in wheat bran treatments but tibial Ca content was high in control and LP. Tibial P content of LP and LPWB was lower than control. However, Tibial content of Fe was highest in LP. It was concluded that wheat bran, a source of plant phytase, can be used in low NPP broiler diet to prevent the depression of performance. Reduction of P excretion can be achieved concomitantly. Hydrothermal treatment of wheat bran was effective in improving utilizability of some minerals but was not effective in improving performance of broilers (Kim and Paik, 2002).

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