Effects of Microbial Phytase Supplementation to Diets with Low Non-Phytate Phosphorus Levels on the Performance and Bioavailability of Nutrients in Laying Hens

J. S. Um, I. K. Paik[1], M. B. Chang and B. H. Lee[2]
Department of Animal Science, Chung-Ang University, Ansung-Si, Kyonggi-Do, 456-756 Korea

ABSTRACT : An 8 week feeding trial was conducted with 864 ISA Brown laying hens, 48 weeks old, to determine if microbial phytase (Natuphos®) supplementation can reduce non-phytate phosphorus (NPP) level in laying diets. The experiment consisted of four dietary treatments: T1, control diet with 0.26% NPP (0.55% total P) wand 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 parameters. 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). The excretion of P was reduced by the inclusion of 250 U phytase/kg of diet. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 2 : 203-208*)

Key Words : Laying Hens, Phytase, Egg Production, Phosphorus Excretion, Tibia Minerals

INTRODUCTION

Phytate P is a widely occurring complex compound that is a principal source of P in animal feed ingredients of plant origin. About two-thirds of the P of plant origin is present as phytic acid in the form of myo-inositol hexaphosphates (Cromwell, 1980). Because the phytate P does not readily separate into inositol and orthophosphate groups, phosphorus in the phytate form is poorly available to monogastric animals (Peeler, 1972). It has been reported that supplementary microbial phytase improves the bioavailability of dietary phytate P (Simons et al., 1990; Denbow et al., 1995; Ravindran et al., 1995). Phytase also improved N absorption in laying hens and pigs (Van der Klis and Versteegh, 1991; Kornegay and Qian, 1994; Kornegay, 1996).

Phytate can form salts with nutritionally important minerals such as Ca, Mg, Cu, Zn, Fe, and K, thus reducing their solubility (Erdman, 1979). When phytic acid is hydrolyzed by microbial phytase, it may release all phytate-bound minerals (Sebastian et al., 1996). There have been many studies on phytase supplementation. Van der Klis et al. (1996) reported that phosphate or phytase supplementation of layer diets with deficient P level significantly increased weight gain, percentage egg production, egg weight, feed consumption, and tibia weight. 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 the 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%. Due to the poor ability of poultry to utilize phytate P, an increase is required in dietary inorganic P content, thus increasing feed costs and the quantities of P excretion which is of environmental concern (Denbow et al., 1995). The present experiment was 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.

[1] Address reprint request to I. K. Paik.
[2] Department of Food and Nutrition
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MATERIALS AND METHODS

Experimental diet

The four experimental diets based on NRC (1994) standards (table 1) had the same composition except for P contents which were varied by adding different amounts of tricalcium phosphate (TCP). Limestone and acid-washed sand were used to adjust Ca contents and diet volume. Natuphos® (provided by BASF Korea Ltd., Seoul, 100-611, Korea) with 500 U/g microbial phytase product was added to diets at the rate of 250 U/kg.

Feeding regimen

Eight hundred and sixty four ISA-Brown® (Hanil Poultry Farm Co., Ltd., Osan-Si, Kyonggi-Do, 447-130, Korea) laying hens, 48 weeks old, were housed in 3 tier cages with four dietary treatments: T1, control diet containing 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; and T4, 0.11% NPP (0.40% total P) diet with 250 U of phytase/kg. Each treatment consisted of 12 replications of 9 cages (2 birds per cage). Birds were given the experimental diets for 8 weeks. Diets were presented in mash form and water was provided *ad libitum*. The house was provided with programmed lighting and ventilation. The lighting program provided natural and artificial illumination for 16.00 h per day.

Table 1. Formula and composition of experimental diets

	Treatment[3]			
	T1	T2	T3	T4
Ingredients (%)				
Corn, ground	57.37	57.37	57.37	57.37
Soybean meal (44%)	13.63	13.63	13.63	13.63
Wheat bran	10.00	10.00	10.00	10.00
Limestone	9.08	9.32	9.58	9.84
Corn gluten meal	5.00	5.00	5.00	5.00
Animal fat	3.20	3.20	3.20	3.20
Tricalcium phosphate (18% P)	0.90	0.60	0.30	-
Layer premix[1]	0.32	0.32	0.32	0.32
Salt	0.31	0.31	0.31	0.31
Lysine-HCl (78%)	0.09	0.09	0.09	0.09
DL-Methionine (50%)	0.10	0.10	0.10	0.10
Sand	-	0.06	0.10	0.14
Natuphos[2], (500 U/g)	-	+	+	+
Total	100.00	100.00	100.00	100.00
Calculated composition :				
ME (kcal/kg)	2850	2850	2850	2850
Crude protein, %	15.5	15.5	15.5	15.5
Lysine, %	0.70	0.70	0.70	0.70
Methionine+Cystine, %	0.60	0.60	0.60	0.60
Calcium, %	3.75	3.75	3.75	3.75
Non-phytate P, %	0.26	0.21	0.16	0.11
Total P, %	0.55	0.50	0.45	0.40

[1] Provides per kg of diet: vitamin A, 10,000 IU; vitamin D_3, 2,000 IU; vitamin E, 0.25 IU; vitamin K_3, 2 mg; vitamin B_1, 2 mg; vitamin B_2, 6 mg; vitamin B_6, 3 mg; vitamin B12, 10 μg; niacin, 30 mg; pantothenic acid, 10 mg; folic acid, 1 mg; choline, 250 mg; ethoxyquin, 125 mg; I, 0.5 mg; Zn, 50 mg; Mn, 40 mg; Fe, 60 mg; Cu, 10 mg; Se, 0.2 mg.

[2] Provided by BASF Corporation.

[3] T1=control diet of 0.26% NPP with no supplementary phytase; T2=0.21% NPP diet with 250 U phytase/kg of diet; T3=0.16% NPP diet with 250 U phytase/kg of diet; T4=0.11% NPP diet with 250 U phytase/kg of diet.

Parameters of productivity and eggshell quality

Hen-day, hen-housed egg production and egg weights were recorded daily. Feed consumption was measured weekly. Random samples of 25 eggs from each treatment were collected weekly to measure eggshell quality such as specific gravity, eggshell strength, and eggshell thickness. Specific gravity of eggs was determined every week by using salt solutions with 0.005 incremental concentrations in the range from 1.065 to 1.120 (Hempe et al., 1988). Eggshell strength, breaking force from blunt to sharp end in lb/inch^2, was measured by using the Compression Test Cell (Model TC-1) of Texture Test Systems (Model T2100C, Food Technology Corp., Rockville, MD 20852 USA). Shell thickness was the mean value of measurements made at three locations (air cell, medium, and sharp end) with the Dial Pipe Guage (Model 7360, Mitutoyo Corp., Kawasaki, Japan, 213) after determination of eggshell strength.

Retention and excretion of nutrients

After the feeding trial, 9 birds from each treatment were randomly assigned to individual metabolic cages to determine the retention and excretion of dietary nutrients. Excreta were collected for 3 d. Foreign substances (feathers, scurf, etc.) mixed in the collected excreta were removed before drying at 60°C for 48 h and subsequent grinding. Feeds and feces were analyzed by AOAC (1990) procedures for proximate components. The retention of nutrients was calculated by dividing the amount of retained nutrient (ingested nutrient minus excreted nutrient) by the amount of ingested. To determine the concentrations of Ca, P, Mg, Zn, Fe, and Cu in feeds and excreta, samples were dry ashed (AOAC, 1990) and assayed at the specific wavelengths for each element (Ca, 317.933; P, 214,914; Mg, 279.079; Fe, 259.940; Zn, 213.856; and Cu, 324.754 nm) by an ICP (Inductively Coupled Plasma) Emission Spectrometer (Model JY-24, Jobin Yvon, Longjumeau, Cedex, France, 91165).

Tibial bone parameter

After the 56 d feeding trial, fifteen hens per treatment were killed and the left tibiae were removed and dried at 60°C for 72 h. The weight, length, and girth of tibiae were measured before grinding and fat extraction. The dry, fat-free tibiae were analysed for ash and minerals. Bone minerals were assayed as described above.

Statistical analysis

The data obtained from the experiment were analyzed by split block design to allow for the effects of location of cages and week of flaying period. General Linear Models (GLM) procedures (SAS, 1985) was used and significant differences between treatment means were determined at $p<0.05$ using Duncan's new multiple range test (Duncan, 1955).

RESULTS

The results obtained from the feeding trial are shown in table 2. Both hen-day and hen-housed egg production were significantly ($p<0.05$) different among the treatments. T3 showed the highest and T4 the lowest egg production while T1 and T2 showed no significant differences. Significant differences in egg weights ($p<0.05$) showed the same trends as egg production.

Feed consumption and feed conversion was significantly lower ($p<0.05$) with T4 (130.4 g/d); differences between the remaining groups (131.5 to 132.7 g/d) were not significantly different. Feed conversion (feed mass/egg mass) of T3, 2.185, was the lowest ($p<0.05$); those of others (2.216 to 2.226) were not significantly different. Mortalities of T2 and T4 were significantly ($p<0.05$) higher than those of T1 and T3.

Table 2. The productivity of laying hens fed experimental diets from 48 to 55 wk of age

Parameters	Treatments[1]				SEM[2]
	T1	T2	T3	T4	
Egg production (%, hen-day)	90.48^{b}	90.78^{b}	91.77^{a}	89.68^{c}	0.29
Egg production (%, hen-housed)	90.48^{b}	90.17^{b}	91.53^{a}	88.38^{c}	0.28
Egg weight (g)	65.79^{b}	65.81^{ab}	66.01^{a}	65.57^{c}	0.07
Feed consumption (g/hen/d)	131.5^{ab}	132.7^{a}	132.2^{a}	130.4^{b}	0.44
Feed conversion (feed/egg mass)	2.216^{a}	2.226^{a}	2.185^{b}	2.223^{a}	0.0097
Mortality (%)	0.00^{b}	0.36^{a}	0.08^{b}	0.48^{a}	0.098

[1] T1=control diet of 0.26% NPP with no supplementary phytase; T2=0.21% NPP diet with 250 U phytase/kg diet; T3=0.16% NPP diet with 250 U phytase/kg diet; T4=0.11% NPP diet with 250 U phytase/kg diet.

[2] Standard error of the mean.

[a,b,c] Values with different letters in the same row are significantly different ($p<0.05$).

The results of measurements determining eggshell quality are shown in table 3. Eggshell strength, specific gravity of eggs, and eggshell thickness were not significantly different among the treatments. However, the broken egg ratio showed a significant ($p<0.05$) difference, that of T1 being the highest.

Table 3. Shell quality of eggs laid by hens fed experimental diets from 48 to 55 wk of age

Parameters	Treatments[1]				SEM[2]
	T1	T2	T3	T4	
Eggshell strength, PSI (lb/inch^{2})	8.44	8.51	8.72	8.73	0.10
Specific gravity	1.0858	1.0865	1.0859	1.0861	0.0003
Eggshell thickness, mm	372.83	375.86	372.68	375.52	1.77
Broken egg ratio, %	0.80^{a}	0.34^{b}	0.53^{b}	0.32^{b}	0.074

[1] T1=control diet of 0.26% NPP with no supplementary phytase; T2=0.21% NPP diet with 250 U phytase/kg diet; T3=0.16% NPP diet with 250 U phytase/kg diet; T4=0.11% NPP diet with 250 U phytase/kg diet.

[2] Standard error of the mean.

[a,b] Values with different letters in the same row are significantly different ($p<0.05$).

Retentions (%) of major nutrients including minerals are shown in table 4. Retention of fat (ether extract) in T3 was significantly ($p<0.05$) greater than in T2 but not significantly different from T1 and T4. The retentions of dry matter (DM), crude protein (CP), crude fiber, crude ash, and nitrogen free extract (NFE) were not significantly different among the treatments. Differences in the retention of Zn were not significant, but those of Ca, P, Mg, Fe, and Cu were ($p<0.05$). Retentions of Ca, P, Mg, and Cu were greater in the lower NPP plus phytase treatments (T3 and T4) than those of the control (T1) and the higher NPP plus phytase treatment (T2). Retention of Fe in T2 was lower than in T1 and T3, but not significantly different from T4.

Table 4. Retention of nutrients from experimental layer diets[1]

Nutrients	Treatments[2]				SEM[3]
	T1	T2	T3	T4	
Dry matter	75.0	75.0	76.2	76.8	0.90
Crude protein	58.5	62.0	60.6	62.7	2.21
Ether extract	89.8^{ab}	88.8^{b}	91.7^{a}	90.5^{ab}	0.85
Crude fiber	18.3	21.0	27.4	23.5	3.38
Crude ash	48.2	51.6	53.8	49.4	2.94
Nitrogen free extract	88.4	88.2	88.8	88.7	0.50
Calcium	51.2^{b}	53.7^{ab}	62.1^{a}	62.7^{a}	3.20
Phosphorus	25.2^{b}	33.3^{b}	46.2^{a}	44.1^{a}	3.37
Magnesium	19.1^{b}	22.2^{b}	35.3^{a}	29.2^{ab}	3.78
Zinc	21.3	18.8	27.2	24.3	3.06
Iron	36.4^{a}	18.7^{b}	32.7^{a}	29.0^{ab}	3.54
Copper	18.2^{b}	24.9^{b}	39.6^{a}	38.6^{a}	3.55

[1] Retention was calculated by dividing the amount of retained nutrient with the amount of ingested nutrient.

[2] T1=control diet of 0.26% NPP with no supplementary phytase; T2=0.21% NPP diet with 250 U phytase/kg of diet; T3=0.16% NPP diet with 250 U phytase/kg of diet; T4=0.11% NPP diet with 250 U phytase/kg of diet.

[3] Standard error of the mean.

[a,b] Values with different letters in the same row are significantly different ($p<0.05$).

Amounts of fecal N and P excreted from hens are shown in table 5. N excretion was not significantly different among the treatments, but P excretion in the phytase supplemented treatments (T2, T3, and T4) was significantly ($p<0.05$) smaller than that of T1.

Weight, length, and girth of tibiae are shown in table 6. All the variables measured were not significantly different among the treatments.

Contents of ash and minerals of tibiae are shown in table 7. Ash content was not significantly different among the treatments, but the contents of Ca, P, and Mg in tibiae did differ ($p<0.05$). All of those three minerals were highest in T2 followed by T3, and low in T1 and T4. The levels of Zn, Fe, and Cu of tibia showed different trends. Zn was significantly ($p<0.05$) higher in T4 than in T1, but not different from T2 and T3. Fe was not significantly different among the treatments. Cu T1 was significantly ($p<0.05$) higher in T1 than in the other treatment groups.

Table 5. Excretion of fecal N and P from laying hens fed experimental diets

Parameters	Treatments[1]				SEM[2]
	T1	T2	T3	T4	
	(g/bird/d)				
Nitrogen	1.60	1.58	1.63	1.49	0.0876
Phosphorus	0.52[a]	0.46[b]	0.35[c]	0.30[c]	0.0216
	(0.63)	(0.61)	(0.57)	(0.47)	

[1] T1=control diet of 0.26% NPP with no supplementary phytase; T2=0.21% NPP diet with 250 U phytase/kg of diet; T3=0.16% NPP diet with 250 U phytase/kg of diet; T4=0.11% NPP diet with 250 U phytase/kg of diet.
( ) = P intake per hen per day.
[2] Standard error of the mean.
[a,b,c] Values with different letters in the same row are significantly different ($p<0.05$).

Table 6. Tibia weight, length, and girth of laying hens fed experimental diets

Items[1]	Treatments[2]				SEM[3]
	T1	T2	T3	T4	
Weight (g)	6.91	7.09	7.34	6.85	0.219
Length (cm)	11.82	11.78	11.73	11.82	0.086
Girth (cm)	2.28	2.24	2.30	2.27	0.031

[1] Data presented as least square means with body weight at death used as covariate.
[2] T1=control diet of 0.26% NPP with no supplementary phytase; T2=0.21% NPP diet with 250 U phytase/kg of diet; T3=0.16% NPP diet with 250 U phytase/kg of diet; T4=0.11% NPP diet with 250 U phytase/kg of diet.
[3] Standard error of the mean.

Table 7. Content of ash and minerals of tibia of laying hens fed experimental diets

Nutrients[1]	Treatments[2]				SEM[3]
	T1	T2	T3	T4	
Ash (% DM)	37.77	37.00	37.45	38.00	0.44
	(% of tibia)				
Calcium	23.60[bc]	25.08[a]	24.24[b]	23.23[c]	0.26
Phosphorus	11.19[b]	11.89[a]	11.43[b]	11.16[b]	0.12
Mangesium	0.42[c]	0.45[a]	0.44[ab]	0.43[bc]	0.005
	(μg/g of tibia)				
Zinc	213.31[b]	234.33[ab]	237.69[ab]	277.01[a]	17.48
Iron	251.92	237.94	254.55	253.57	11.86
Copper	15.95[a]	10.88[b]	9.16[b]	10.42[b]	1.72

[1] Values from fat free tibia.
[2] T1 = control diet of 0.26% NPP with no supplementary phytase; T2 = 0.21% NPP diet with 250 U phytase/kg of diet; T3 = 0.16% NPP diet with 250 U phytase/kg of diet; T4 = 0.11% NPP diet with 250 U phytase/kg of diet.
[3] Standard error of the mean.
[a,b,c] Values with different letters in the same row are significantly different ($p<0.05$).

DISCUSSION

The level of phosphorus in the laying hen diet is of great concern, economically and environmentally. NRC (1984) recommended 0.32% of NPP for layer diets but revised this downwards (NRC, 1994) to 0.21% and 0.25% of NPP based on 120 g and 100 g/d of feed intake, respectively, or 0.25 g NPP per hen per day. In practical layer diets, however, much higher levels, e.g. 0.35 to 0.45% (Leeson and Summers, 1991) and 0.34 to 0.44% (ISA Brown Inc, 1996), have been recommended.

The results of the present experiment indicate that the dietary level of NPP for hens 48 to 55 weeks of age can be lowered from 0.26% to 0.16% if 250 U of phytase is added per kg of diet. In this experiment, the layer flock had passed peak production and on average consumed over 130 g/d of feed. Therefore, the recommended level of NPP would be less than the 0.21% (0.5% total P) of the NRC (1994). In the present experiment, the laying performance of T3 (0.16% NPP plus 250 U of phytase/kg of diet) equalled or was better than that of T2 (0.21% NPP plus 250 U of phytase/kg of diet). This result implies that T2 provides more than sufficient NPP and that 0.16% of NPP plus 250 U of phytase/kg of diet is adequate for maintenance of egg production by hens of the age in this study. Based on the feed intake and NPP level alone, T2 and T3 were provided with 0.28 g ($132.7\times0.21/100$) and 0.21 g ($132.2\times0.16/100$) of NPP per hen per day, respectively. Additional NPP might have been made available to the birds by supplementary phytase, which made the NPP supply for T3 sufficient for egg production. Supplementation of 200 U of phytase/kg of layer diet was equivalent to 0.6 g of P (Simons et al., 1992) and 250 U of phytase/kg of diet was equivalent to 0.8 g of P from MCP (monocalcium phosphate) per kg of layer diet (Van der Klis et al., 1994). Supplementation of 500 U of phytase/kg of diet was shown to have an effect equivalent to 1 g of NPP (Peter and Jeroch, 1993). Supplementation with a high level of phytase represented a lower equivalence per unit of phytase due to the significant quadratic phytase response in P absorption (Van der Klis and Versteegh, 1996).

The parameters of eggshell quality were not significantly different. However, the broken egg ratio was significantly lower in T4 and T2 than T1. Vandepopuliere and Lyons (1992) reported that at the 0.4% total dietary P level egg specific gravity was improved but egg production and egg weight were reduced. Said et al. (1982) suggested that 0.5% total P, with dicalcium phosphate used as the inorganic source, gave the best results as measured by egg production and eggshell quality. Other researchers suggested 0.4% to 0.5% total P to be adequate for egg production as well as shell quality (Damron et al., 1974; Andrews and Berg, 1977; Hamilton and Sibbald, 1977; Edwards and Suso, 1981). In the present study, total P levels of T1, T2, T3, and T4 were 0.55, 0.50, 0.45, and 0.40%, respectively. T4, the lowest P treatment regardless of

phytase supplementation, showed the lowest broken egg ratio and the highest eggshell strength. A significantly lower broken egg ratio for T2 than for T1 indicates phytase may influence shell strength in some way, such as by improving Ca availability, as well as influencing P metabolism.

Supplementation with microbial phytase improved N retention in broiler chickens (Yi et al., 1996) and in vitro digestibility of vegetable protein (Rutherfurd and Moughan, 1996). In the present study, however, there was no improvement of protein availability in phytase supplemented treatments. Increased retention of Ca, P, Mg and Cu in phytase supplemented treatments was in agreement with the result of Sebastian et al. (1996). Fe retention in T2 was significantly lower than T1, with T3 and T4 not significantly different from T1, and Biehl and Baker (1997) have reported that Fe retention from soybean meal was reduced by supplemental phytase. They suggested that Fe may be bound to phytate differently than are other minerals and that antagonisms of Zn, Cu, and Fe may have influenced the result.

The results of this experiment, however, show that the level of P influences the retention of the microminerals investigated. Excretion of P was reduced by phytase supplementation. Compared to the control T1, the reductions in dietary total P level of T2, T3,innd T4 were 9.1%, 18.2%, and 27.3% while the reductions of P excretion were 11.5%, 32.7%, and 42.3%, respectively. The differences seem to reflect an improvement in P digestibility by phytase supplementation.

The graded decrease in P level and phytase supplementation might have not significantly influenced tibial weight, length, girth, and ash content because bone size (length and girth) had already been established at the time of the experiment. Tibial contents of Ca, P, and Mg in T2 were significantly greater than in T1 but decreased as the dietary NPP level decreased. Tibial Zn was lowest in T1 and increased as the level of NPP in the diets decreased and was significantly greater in T4. The higher level of Zn and the lower level of Cu of tibia in phytase supplemented treatments compared with T1 did not correspond with the results of some other balance trials. Roberson and Edward (1994) reported that dietary phytase supplementation increased tibia Zn concentration but did not improve Zn retention in broiler chicks. Biehl et al. (1995) also found that phytase supplementation considerably improved growth rate and total tibia Zn in chicks. During the accumulation process of minerals, interactions among minerals, especially between Zn and Cu, might have affected the results. Tibiae assay data indicate that lowering NPP level by phytase supplementation did not affect the quality of tibia in this experiment.

Overall, the results indicate that the NPP level in the diet of Brown layers consuming about 130 g/d of feed can be lowered to 0.16% if 250 U of microbial phytase/kg of diet is supplemented. Compared to the control T1 (0.26% NPP without phytase supplementation), egg production and shell quality were as good as if not better, and tibial quality was not adversely affected in T3 (0.16% NPP with phytase supplementation). Also, there was a 32.7% reduction in P excretion.

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