Effects of a new recombinant phytase on performance and mineral utilization of laying ducks fed phosphorus-deficient diets

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Primary Audience: Poultry Nutritionists, Researchers, Commercial Producers

SUMMARY

An experiment was conducted to evaluate the effects of a new phytase supplementation in *Jinding* laying ducks fed different concentrations of non-phytate P (NPP) on production performance, mineral retention, and bone and plasma minerals. A 14-wk experiment was conducted using 200-d-old female laying ducks. A total of 1,000 laying ducks were randomly allocated to 5 treatments and fed 5 diets: a control diet that contained an adequate concentration of NPP (0.45%) and 4 diets that were deficient in NPP (0.38, 0.32, 0.25, and 0.18%, respectively) but supplemented with phytase at 500 U/kg. Decreasing the NPP content from 0.45 to 0.18% in the diets with phytase supplementation had no detrimental effects on performance. However, Cu and Zn retention was significantly lower ($P < 0.05$) for laying ducks consuming the 0.18% NPP diet with phytase supplementation. Likewise, laying ducks fed the 0.18% NPP diet had a significant reduction in bone ash, Ca, P, and Cu contents, and in serum P and Cu ($P < 0.05$). Furthermore, the decrease in NPP content in the diet with phytase supplementation significantly increased ($P < 0.05$) P retention. Therefore, with the supplementation of this novel phytase at 500 U, it is possible to reduce dietary concentrations of NPP to 0.25% and maintain the normal performance of laying ducks.

Key words: phytase, duck, production performance, mineral utilization

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DESCRIPTION OF PROBLEM

Phytate is the major form of P in plants [1]. Approximately 70% of the total P (**TP**) in feed ingredients is in the form of phytate [2, 3]. The inability of poultry to utilize phytate P can be due to a lack of endogenous phytase. In addition, phytate can bind with Ca, Cu, Zn, and other minerals [4]. However, exogenous phytase can help release phytate-bound P [5–7] as well as improve the utilization of other minerals that are bound to plant phytate [8–11]. The efficacy of these improvements was inconsistent among the reports, however, largely because of the types of phytase supplements, dietary concentrations of Ca and P, and bird age [12–15]. Different types of phytase are denatured to different extents when subjected to the temperature and moisture of preconditioning required for pelleting. Furthermore, information regarding the effect of

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exogenous phytase on mineral utilization of laying ducks is largely unavailable. Therefore, manipulations of the P concentration in the diet and adding phytase to improve mineral availability must be validated for their effects on mineral retention and the mineral status of serum and bone. Recently, we have developed a new recombinant phytase product that has shown good efficacy when fed to broiler chickens [16, 17]. The aim of this study was to assess the effect of this novel phytase product on production performance and on utilization of Ca, P, Cu, Zn, and Mg in laying ducks fed low-P diets.

MATERIALS AND METHODS

Enzyme

The phytase product, derived from *Aspergillus niger* phytase, was produced in the Laboratory of Life Sciences (Shandong Agricultural University, Tai-an, P. R. China). An *A. niger* phytase gene from a high extracellular phytase-producing *A. niger* species was cloned and overexpressed in *Pichia pastoris* GS115 by using the secretive expression vector pPICZaA. After cultivation, the active phytase was secreted as a predominantly extracellular protein. The activity of the expressed phytase in fermented broth was 30,000-fold higher than that of native phytase, with a specific activity of 503 U/mg [16]. One unit of phytase is the amount of enzyme that releases 1 μmol of inorganic P/min at pH 5.5 and 37°C.

Experimental Design and Diets

A 14-wk experiment was conducted using 1,000 female *Jinding* laying ducks at 200 d of age [18]. Ducks were allotted to 5 dietary treatments, with 4 replicates of 50 ducks per replicate. The diets were based on corn and soybean meal. The 5 dietary treatments were a diet with an adequate concentration (0.45%, control) of nonphytate P (**NPP**), and diets with reduced concentrations (0.38, 0.32, 0.25, and 0.18%) of NPP but supplemented with 500 U of phytase/kg of DM. Calcium concentration in the diets was set at a 5:1 ratio of Ca:TP (Table 1). A previous study indicated that 500 U of phytase/kg of diet was suitable for broiler chickens on low-NPP diets [17]. In this study, the NPP concentration was decreased and phytase was added at a concentration of 500 U/kg of diet. The enzyme premix, which was analyzed to contain 500 U/g, was added at a level of 0 (control) or 1 g/kg of DM (treatment) to the diet, and the whole content was pelleted at 90°C for 15 s after mixing. Phytase activity was not determined on diets after pelleting.

Birds Feeding and Management

A total of 1,000 female laying ducks, 200 d of age, were housed in 20 pens with floors partially covered with plastic-coated wire over a water drain in an environmentally controlled room. The remaining floor space was covered with litter, and hanging cylindrical feeders were included. The temperature in the room was controlled by ventilation fans. Diets were provided in pellet form for ad libitum intake, and the birds had free access to water under a daily photoperiod of 16L:8D. Laying ducks, feed, and water were checked twice daily throughout the entire experimental period. All laying ducks used in this study were cared for in accordance with local ethical guidelines.

Measurements

Production Performance. Feed consumption was measured weekly throughout the experiment. Egg production and egg weight were recorded daily for calculation of feed conversion.

Determination of Mineral Retention. At 292 d of age, 3 laying ducks with similar BW were selected from each pen (total 12 for each treatment), assigned to metabolic cages equipped with water troughs, and housed in cages for 3 d. The birds were provided with the same experimental diets fed in the pens. Clean stainless steel collection trays were placed under each cage (6 per treatment, each collecting excreta from 2 birds), and excreta from the birds were collected for 72 h. The laying ducks had free access to water during this 72-h period. The samples of excreta were collected in polyethylene bags, weighed, and dried in an oven at 65°C for 48 h. Excreta were mixed thoroughly and frozen at −20°C. Prior to chemical analysis, these samples were ground to pass a 0.5-mm screen and then stored in sealed containers for determination of Ca, P, Mg, Zn, and Cu.

Table 1. Composition (%) and nutrient content of the experimental diets

Item	0.45% NPP,[1] 0 U of phytase/kg	0.38% NPP, 500 U of phytase/kg	0.32% NPP, 500 U of phytase/kg	0.25% NPP, 500 U of phytase/kg	0.18% NPP, 500 U of phytase/kg
Ingredient					
Corn	49.00	48.00	48.00	48.00	48.00
Soybean meal	15.00	15.00	15.00	15.00	15.00
Limestone	8.40	8.25	7.55	6.90	6.20
Wheat red dog	7.56	7.56	7.56	7.56	7.56
Rice bran	0	3.72	4.08	4.83	5.02
Cottonseed meal	4.00	4.00	4.00	4.00	4.00
Corn gluten meal	3.00	3.00	3.00	3.00	3.00
Distillers dried grains with solubles	4.88	2.86	2.86	2.86	2.86
Whole-pressed cottonseed	3.00	3.00	3.00	3.00	3.00
Calcium phosphate	1.80	1.35	0.90	0.45	0.00
Fish meal	1.00	1.00	1.00	1.00	1.00
Soybean oil	1.00	0.90	0.90	0.80	0.80
Zeolite meal	0	0	0.79	1.24	2.20
Salt	0.36	0.36	0.36	0.36	0.36
1% premix[2]	1.00	1.00	1.00	1.00	1.00
Nutrient level, % (calculated)					
ME, kcal/kg	2,583	2,583	2,583	2,583	2,583
CP	18.50	18.50	18.50	18.50	18.50
Ca	3.56	3.41	3.06	2.73	2.39
Total P	0.71	0.67	0.60	0.54	0.48
NPP	0.45	0.38	0.32	0.25	0.18
Ca:total P	5.01	5.09	5.10	5.06	4.98
Lys	0.812	0.827	0.827	0.829	0.829
Met	0.319	0.317	0.318	0.317	0.317

[1]Nonphytate P.

[2]Supplied per kilogram of diet: vitamin A, 12,000 IU; vitamin D_3, 2,400 IU; vitamin E_3, 481 IU; vitamin K_3, 1.68 mg; vitamin B_1, 2.48 mg; vitamin B_2, 9.6 mg; vitamin B_3, 16.2 mg; vitamin B_5, 48 mg; vitamin B_6, 4.41 mg; vitamin B_7, 0.1 mg; vitamin B_{11}, 2.08 mg; vitamin B_{12}, 0.02 mg; Mn, 80 mg; Fe, 40 mg; Zn, 70 mg; Cu, 5 mg; I, 0.5 mg; Se, 0.2 mg.

Mineral Concentration in Blood Plasma and in Bone Ash. At the end of the experiment, 3 birds from each pen (12 for each treatment group) were randomly selected and a blood sample was collected from each duck via cardiac puncture. Blood samples were centrifuged for 10 min at 1,360 × *g* at 4°C. Plasma was collected and frozen until subsequent analysis for Ca, P, Mg, Zn, and Cu. All birds were killed by cervical dislocation, and tibias were collected from the right leg of each duck after bleeding. The tibias were subsequently dried at 105°C for 12 h, extracted with diethyl ether, dried again, and weighed. The dry fat-free bones were ashed in a muffle furnace at 550°C. The ash was used to determine concentrations of Ca, P, Mg, Zn, and Cu.

Chemical Analyses

All chemical analyses were performed in duplicate. Feed and excreta samples were ground to pass a 0.5-mm screen and were ashed as described above for bone samples before the analysis. Calcium, Mg, Zn, and Cu in the ash were determined with an Atomic Absorption Spectrophotometer [19]. Phosphorus was analyzed by the Vanadate colorimetric method [20] with a UV spectrophotometer [21]. Plasma Ca, P, Mg, Zn, and Cu were determined with a Full Automation Biochemistry Analyzer [22].

Calculation and Statistical Analysis

Apparent retention of Ca, P, Mg, Zn, or Cu was calculated using the following equation:

$$\text{Apparent retention (\%)} = [(E1 - E2)/E1] \times 100,$$

where E1 is the amount (mg) of the element (Ca, P, Mg, Zn, or Cu) in the diet that was consumed by each bird during a 72-h period, and E2 is the amount (mg) of the corresponding element (Ca, P, Mg, Zn, or Cu) in excreta collected during

Table 2. Production performance[1] of 200-d-old laying ducks fed normal or low-nonphytate P (NPP) diets supplemented with recombinant phytase at a level of 500 U/kg of DM during the 14-wk feeding experiment

Item	0.45% NPP, 0 U of phytase/kg	0.38% NPP, 500 U of phytase/kg	0.32% NPP, 500 U of phytase/kg	0.25% NPP, 500 U of phytase/kg	0.18% NPP, 500 U of phytase/kg	SEM
Feed consumption, g/d	166	168	167	167	169	2.87
Egg weight, g	73.85	73.85	74.24	73.74	73.69	0.89
Egg production, g/d	70.09	69.72	70.05	69.77	69.64	1.33
Feed conversion,[2] g/g	0.426	0.416	0.420	0.421	0.411	0.012

[1]Means represent 4 pens (50 ducks/pen) per diet.

[2]Feed conversion is grams of egg per gram of feed.

a 72-h period. Ash weight was calculated as a percentage of dry fat-free bone weight and the mineral contents were calculated as the percentage of ash.

Data were analyzed statistically by 1-way ANOVA using PROC MIXED [23]. Values obtained from individual replicate pens were used as units for statistical analysis. Differences among the 5 treatments were determined using least squares means with the PDIFF option and were adjusted with a Tukey's test [23]. In all analyses, significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

Production Performance

The production performance of laying ducks in different treatments is summarized in Table 2. No consistent differences in feed consumption, egg weight, egg production, and feed conversion were observed among treatments during the 14-wk experiment ($P > 0.05$).

This study showed that laying ducks fed 0.18, 0.25, 0.32, and 0.38% NPP-deficient diets supplemented with microbial phytase at 500 U/kg of DM had production performance similar to that of laying ducks fed the diet with adequate NPP. Van der Klis et al. [24] reported that hens consuming low-NPP corn-soybean meal diets demonstrated a depression in egg production. Brown and Hy-line W-36 hens fed low-NPP diets supplemented with phytase at 500 U/kg diet had egg production similar to that of hens fed normal-NPP diets [25, 26]. Boling et al. [27] also demonstrated that in laying chickens, a corn-soybean meal diet containing 0.10% NPP with 300 U of phytase/kg supported optimal egg production from 20 to 70 wk of age. The daily NPP intakes for ducks consuming diets contained 0.45, 0.38, 0.32, 0.25, and 0.18% NPP were 747, 638, 534, 418, and 304 mg/bird, and the corresponding daily TP intakes were 1,179, 1,126, 1,002, 902, and 811 mg/bird, respectively. This suggests that supplementation of phytase may improve the utilization of dietary TP. The reason for this improvement is likely due to P being a nutrient limiting for the laying production of ducks fed diets deficient in NPP. Phytase hydrolyzes phytic acid to orthophosphate inositol, and other phosphoinositol intermediates, making more P available for absorption by laying ducks [28].

Mineral Retention

The effects of NPP concentrations and phytase supplementation on the retention of minerals are presented in Table 3. Data for the main effects indicates that the decrease in NPP content in the diet significantly increased ($P < 0.05$) P retention and had no effect on the retention of Ca and Mg. The Cu and Zn retention of ducks consuming the diet that contained 0.18% NPP was lower ($P < 0.05$) than that in other groups. However, ducks fed the diet containing 0.38% NPP with phytase supplementation had greater ($P < 0.05$) Zn retention compared with ducks fed the diet containing 0.45 or 0.25% NPP.

In the current experiment, the improved P digestibility by adding phytase to the diets is in agreement with the report of Um and Paik [29] in laying hens, Grela et al. [30] and Jongbloed et al. [31] in pigs, and Silversides et al. [32] in broilers. It is possible that when P is limiting, more P is retained in the body to maintain physiological functions, resulting in less P being excreted [33] and P retention increasing. Viveros et

Table 3. Apparent mineral retention (%) of laying ducks fed normal or low nonphytate P (NPP) diets supplemented with recombinant phytase at a level of 500 U/kg of DM[1]

Item[2]	0.45% NPP, 0 U of phytase/kg	0.38% NPP, 500 U of phytase/kg	0.32% NPP, 500 U of phytase/kg	0.25% NPP, 500 U of phytase/kg	0.18% NPP, 500 U of phytase/kg	SEM
Ca	56.33	56.07	56.17	55.11	55.01	1.89
P	53.71[b]	55.09[a]	55.19[a]	55.43[a]	55.25[a]	0.37
Mg	33.88	34.46	35.13	35.08	34.05	1.18
Cu	64.74[a]	62.28[a]	63.21[a]	63.73[a]	57.63[b]	0.93
Zn	21.33[b]	23.81[a]	22.27[ab]	21.72[b]	17.62[c]	0.59

[a–c]Within a row, means without a common superscript letter differ ($P < 0.05$).

[1]Experiment began at the age of 292 d for 72 h.

[2]Means represent 6 replicates (2 birds/replicate) per diet for all these minerals.

al. [34] suggested that although phytase supplementation increased Ca retention, this increase could not reach the level obtained in the normal-P diet. However, Silversides et al. [32] reported that different kinds of phytase had different effects on Ca digestibility. In this study, low dietary NPP with phytase had a low Ca retention, but no significant difference was observed. Viveros et al. [34] found that supplementation of phytase to diets with low NPP prevented the decline of Mg and Zn retention in chickens caused by the low level of dietary NPP. In this study, phytase supplementation to low-P diets tended to increase the retention of Mg, and Zn retention was increased ($P < 0.05$) when phytase was supplemented to the diet with 0.38% NPP as compared with the control, 0.25, and 0.18% NPP diets. These results indicate that the increases in Zn and Mg retention might have been due to the greater availability of Zn and Mg from the phytate-mineral complex. Compared with the normal-NPP diet, the birds fed the 0.18% NPP diet with phytase supplementation had less Cu retention. The reason for the reduced Cu retention with phytase supplementation is not known and further investigation is needed. Overall, this study showed that the retention of minerals is generally improved by adding 500 U of phytase/kg of DM to diets containing 0.38 to 0.25% NPP.

Mineral Concentration in Bone Ash

Tibias had similar ($P > 0.05$) ash contents among ducks fed diets containing 0.45, 0.38, 0.32, and 0.25% NPP, and the same trend was observed for Ca, P, Mg, Cu, and Zn contents of the ash (Table 4). However, laying ducks fed the 0.18% NPP diet with phytase supplementation had lower ($P < 0.05$) ash contents in tibias and lower ($P < 0.05$) Ca, P, and Cu contents in the ash compared with the other groups. All ducks had similar Mg concentrations in their tibias ash, regardless of the treatment. Zinc content in the ash was the highest for ducks fed the 0.38% NPP diet with 500 U of phytase, and was higher ($P < 0.05$) than that of ducks fed the 0.18% NPP diet with phytase supplementation.

Bone ash content has been regarded as an indicator of bone mineralization. In chicks, it has been reported that tibia ash percentages are decreased by a deficiency of Ca and NPP [34, 35] and that phytase addition improves ash percentages [36–38]. In those studies, content of tibia ash was not influenced by the low-NPP (from 0.38 to 0.25%) diets with phytase. This is consistent with the production performance data obtained in this study. This indicates that phytase addition likely reversed the negative effects of dietary NPP deficiency on tibia ash (i.e., improved mineral retention). The improvement in tibia ash might be related to the increase in apparent metabolism of minerals from the phytate-mineral complex with phytase addition. However, laying ducks fed the 0.18% NPP diet with phytase did not reach the level of other treatments. This result suggests that 0.18% NPP in the diet would still be lower than required, even though the supplemented phytase improved utilization. The bone Ca, P, Cu, and Zn contents showed the same trend as bone ash. Ahmad et al. [36] reported that concentrations of Ca and P in the tibia ash were relatively constant and were not greatly affected by phytase supplemen-

Table 4. Tibia ash and mineral content in the ash of laying ducks fed normal or low nonphytate P (NPP) diets supplemented with recombinant phytase at a level of 500 U/kg of DM[1]

Item[2]	0.45% NPP, 0 U of phytase/kg	0.38% NPP, 500 U of phytase/kg	0.32% NPP, 500 U of phytase/kg	0.25% NPP, 500 U of phytase/kg	0.18% NPP, 500 U of phytase/kg	SEM
Ash,[3] %	63.95[a]	64.10[a]	63.21[a]	63.75[a]	61.367[b]	0.54
Ca,[4] %	31.93[a]	31.73[a]	31.65[a]	31.60[a]	28.95[b]	0.43
P, %	15.01[a]	14.85[a]	14.54[a]	14.79[a]	12.35[b]	0.39
Mg, %	0.565	0.65	0.643	0.631	0.593	0.03
Cu, μg/g	8.09[a]	7.66[a]	8.00[a]	8.04[a]	6.64[b]	0.25
Zn, μg/g	467[ab]	483[a]	475[ab]	471[ab]	464[b]	4.91

[a,b]Within a row, means without a common superscript letter differ ($P < 0.05$).

[1]Determination was made on laying ducks sacrificed at the age of 298 d.

[2]Means represent 4 pens (3 birds/pen) per diet for all these minerals.

[3]Ash was calculated as a percentage of dry fat-free bone weight.

[4]Mineral contents were calculated as the percentage of ash.

tation. In this study, similar results were found. Lantzsch et al. [39] observed that phytate could also bind with other minerals, such as Ca, Cu, and Zn, thus making them less available to the animal. In this study, reducing NPP from 0.38 to 0.25% with phytase supplementation improved the bone Mg and Zn contents relative to those of the control group. This might be related to phytase releasing the bound minerals to some degree. Viveros et al. [34] found that bone Ca, P, Mg, and Zn were not impaired when phytase was added to a 0.35% NPP diet at 6 wk. Phytase improved the P digestibility and tibia ash content in some studies, but growth was not always positively affected, depending on the level of P supply [40, 41].

Mineral Concentration in Plasma

Concentrations of plasma minerals (Ca, P, Cu, Zn, and Mg) as affected by dietary treatments are summarized in Table 5. All ducks had similar plasma Ca and Mg concentrations, irrespective of treatments. Concentrations of plasma P and Cu for ducks fed the normal-NPP diet were similar to those of ducks consuming diets of 0.38, 0.32, or 0.25% NPP with phytase supplementation, but were higher ($P < 0.05$) than those of ducks fed the diet with 0.18% NNP with phytase supplementation. However, concentration of Zn in the plasma was higher ($P < 0.05$) for laying ducks fed the 0.38% NPP diet supplemented with 500 U of phytase/kg of DM than that for laying ducks fed the control diet or a diet with 0.18% NPP and phytase.

Serum concentrations of Ca and P are the most important indicators of bird nutritional status of Ca and P. In a diet lacking Ca and P, the bird regulatory mechanism mobilizes the bone Ca and P to maintain normal Ca and P homeostasis, thereby maintaining the normal physiological functions of nerves, muscles, and other tissues. Variations exist among reported studies on the effect of supplementing phytase to low-P diets on serum concentrations of Ca and P. Viveros et al. [34] reported that phytase addition to low-P diets increased P but decreased Ca in the serum of chicks. Lei et al. [42] observed that the increased serum Ca attributable to phytase was positively related to the dietary Ca:TP ratio in pigs. Onyango et al. [43] found that supplementation of the same level of different phytases to a low-P diet resulted in different serum Ca concentrations. In the present study, the ratio of Ca:TP was kept relatively constant (5.03 to 5.07). In this situation, reducing the dietary NPP content from 0.45 to 0.25% did not negatively reduce the bone or plasma concentrations of Ca and P, possibly because phytase was supplemented to the NPP-reduced diets. However, when dietary NPP was reduced to 0.18%, supplementation of phytase was no longer effective and the birds showed reduced bone or plasma concentrations of minerals compared with those fed diets containing 0.25% NPP or more. The similar concentrations of minerals (Ca, P, Mg, and Cu) in the plasma of laying ducks fed diets with 0.38 to 0.25% NPP supplemented with phytase and in the plasma of ducks fed the normal-NPP diet without phytase supplementation observed in

Table 5. Mineral concentration (μmol/mL) in the plasma of laying ducks fed normal diet or low nonphytate P (NPP) diets supplemented with recombinant phytase at a level of 500 U/kg of DM[1]

Item[2]	0.45% NPP, 0 U of phytase/kg	0.38% NPP, 500 U of phytase/kg	0.32% NPP, 500 U of phytase/kg	0.25% NPP, 500 U of phytase/kg	0.18% NPP, 500 U of phytase/kg	SEM
Ca	6.75	6.72	6.72	6.71	6.69	0.32
P	3.14[a]	2.99[a]	2.91[ab]	2.92[ab]	2.64[b]	0.10
Mg	1.66	1.71	1.77	1.80	1.69	0.04
Cu	14.33[a]	14.00[ab]	14.00[ab]	13.67[ab]	11.33[b]	0.82
Zn	76.67[b]	83.00[a]	81.33[ab]	79.00[ab]	76.33[b]	1.55

[a,b]Within a row, means without a common superscript letter differ ($P < 0.05$).

[1]Mineral concentration was determined on laying ducks at the age of 298 d.

[2]Means represent 4 pens (3 birds/pen) per diet for all these minerals.

this study indicate that all ducks had a similar mineral nutritional status. Viveros et al. [34] reported that decreasing dietary NPP by 0.1% and adding 500 U/kg of phytase would increase serum Zn concentration compared with the normal Ca and P diets. Revy et al. [44] also reported that supplementing the basal diet with phytase largely increased serum Zn concentration in pigs. Similarly, in this research, phytase supplementation to the 0.38% NPP diet increased Zn concentration compared with the NPP-adequate diet. The exact mechanism of this effect is not known, but may partly relate to the action of phytase in releasing some minerals from phytic acid complexes upon hydrolysis of this compound [33], thereby increasing the availability of dietary Zn. Further research is required.

CONCLUSIONS AND APPLICATIONS

1. Laying ducks fed low-NPP (from 0.38 to 0.18%) diets supplemented with phytase (500 U/kg) had production performance similar to laying ducks fed a normal-NPP (0.45%) diet.
2. Mineral content in bone and plasma of ducks with supplementation of phytase at a concentration of 500 U/kg to a diet containing 0.18% NPP did not attain the same level as that of ducks fed a diet with 0.45% NPP.
3. The retention of Cu and Zn was significantly decreased for laying ducks consuming the 0.18% NPP diet with phytase supplementation.
4. Laying ducks require at least 0.25% NPP in the diet (418 mg/bird per day) to prevent declining serum P and Cu and reductions in bone ash, Ca, P, and Cu.

REFERENCES AND NOTES

1. Ravindran, V., L. W. Bryden, and E. T. Kornegay. 1995. Phytates: Occurrence, bioavailability and implications in poultry nutrition. Avian Poult. Biol. Rev. 6:125–143.

2. Maenz, D. D. 2001. Enzymatic characteristics of phytases as they relate to their use in animal feeds. Pages 72–76 in Enzymes in Farm Animal Nutrition. M. R. Bedford and G. G. Partridge. CABI Publishing, Wallingford, UK.

3. Kornegay, E. T. 2001. Digestion of phosphorus and other nutrients: The role of phytases and factors influencing their activity. Page 237–271 in Enzymes in Farm Animal Nutrition. M. R. Bedford and G. G. Partridge, ed. CABI Publishing, Wallingford, UK.

4. Lantzsch, H. J., S. E. Scheuermann, and K. H. Menke. 1998. Influence of various phytase sources on the P, Ca and Zn metabolism of young pigs at different dietary Zn levels. J. Anim. Physiol. Anim. Nutr. (Berl.) 60:146–157.

5. Roberson, K. D., and H. M. Edwards. 1994. Effects of 1,25-dihydroxicholecalciferol and phytase on zinc utilization in broiler chicks. Poult. Sci. 73:1312–1326.

6. Kies, A. K. 1999. Phytase—Mode of action. Pages 205–212 in Phytase in Animal Nutrition and Waste Management: A BASF Reference. BASF Corp., Mount Olive, NJ.

7. Selle, P. H., V. Ravindran, R. A. Caldwell, and W. L. Bryden. 2000. Phytate and phytase: Consequences for protein utilization. Nutr. Res. Rev. 13:255–278.

8. Murry, A. C., R. D. Lewis, and H. E. Amos. 1997. The effect of microbial phytase in a pearl millet-soybean meal diet on apparent digestibility and retention of nutrients, serum mineral concentration, and bone mineral density of nursery pigs. J. Anim. Sci. 75:1284–1291.

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

10. Yan, F., J. H. Kersey, and P. W. Waldroup. 2001. Phosphorus requirements of broiler chicks three to six weeks of age as influenced by phytase supplementation. Poult. Sci. 80:455–459.

11. Selle, P. H., and V. Ravindran. 2006. Microbial phytase in poultry nutrition. Anim. Feed Sci. Technol. 135:1–41.

12. Yin, Q. Q., Q. H. Zheng, and X. T. Kang. 2006. Biochemical characteristics of phytases from fungi and the transformed microorganism. Anim. Feed Sci. Technol. 132:341–350.

13. Qian, H., E. T. Kornegay, and D. M. Denbow. 1997. Utilization of phytate phosphorus and calcium as influenced by microbial phytase, cholecalciferol, and the calcium:total phosphorus ratio in broiler diets. Poult. Sci. 76:37–46.

14. Boling, S. D., M. W. Douglas, M. L. Johnson, X. Wang, C. M. Parsons, K. W. Koelkebeck, and R. A. Zimmerman. 2000. The effects of dietary available phosphorus levels and phytase on performance of young and older laying hens. Poult. Sci. 79:224–230.

15. Farrell, D. J., and E. A. Martin. 1998. Strategies to improve the nutritive value of rice bran in poultry diets. III. The addition of inorganic phosphorus and a phytase to ducks diets. Br. Poult. Sci. 39:601–611.

16. Zhao, D. M., M. Wang, X. J. Mu, M. L. Sun, and X. Y. Wang. 2007. Screening, cloning and overexpression of *Aspergillus niger* phytase (phyA) in *Pichia pastoris* with favourable characteristics. Lett. Appl. Microbiol. 45:522–528.

17. Zhou, J. P., Z. B. Yang, W. R. Yang, X. Y. Wang, S. Z. Jiang, and G. G. Zhang. 2008. Effects of a new recombinant phytase on performance and mineral utilization of broilers fed phosphorus deficient diets. J. Appl. Poult. Res. 17:1–9.

18. Liuhe Egg Duck Breeding Farm, Taian Shandong, P. R. China.

19. SP9-400, PYE, Cambridge, UK.

20. Zhang, L. Y. 2002. The assay methods of mineral elements. Pages 131–145 in The Technologies of Feed Analysis and Forage Mass Detection. 2nd ed. L. Y. Zhang, ed. China Agricultural University Publishing, Beijing, China.

21. 7200, Unic, Shanghai, P. R. China.

22. T600-020, RiLi, Tokyo, Japan.

23. SAS Institute. 2001. SAS User's Guide. Version 8.2. SAS Inst. Inc., Cary, NC.

24. Van der Klis, J. D., H. A. J. Versteegh, P. C. M. Simons, and A. K. Kies. 1997. The efficacy of phytase in corn-soybean meal-based diets for laying hens. Poult. Sci. 76:1535–1542.

25. Um, J. S., and I. K. Paik. 1999. Effects of microbial phytase supplementation on egg production, eggshell quality, and mineral retention of laying hens fed different levels of phosphorus. Poult. Sci. 78:75–79.

26. Gordon, R. W., and D. A. Roland. 1997. Performance of commercial laying hens fed various phosphorus levels with and without supplemental phytase. Poult. Sci. 76:1172–1177.

27. Boling, S. D., M. W. Douglas, M. L. Johnson, X. Wang, C. M. Parsons, K. W. Koelkebeck, and R. A. Zimmerman. 2000. The effects of dietary available phosphorus levels and phytase on performance of young and older laying hens. Poult. Sci. 79:224–230.

28. Orban, J. I., O. Adeola, and R. Stroshine. 1999. Microbial phytase in finisher diets of White Pekin ducks: Effects on growth performance, plasma phosphorus concentration, and leg bone characteristics. Poult. Sci. 78:366–377.

29. Um, J. S., and I. K. Paik. 1999. Effects of microbial phytase supplementation on egg production, eggshell quality, and mineral retention of laying hens fed different levels of phosphorus. Poult. Sci. 78:75–79.

30. Grela, E. R., R. Kunek, and A. Lipiec. 2000. The influence of microbial phytase and citric acid in sow feeding on mineral components availability during pregnancy and lactation. Pages 56–60 in Phytase in Animal Nutrition, Proc. Int. Symp. Phytase Anim. Nutr., Lublin, Poland. E. R. Grela, ed. Inst. Anim. Nutr. Agric. Univ., Lublin, Poland.

31. Jongbloed, A. W., J. Th. M. Van Kiepen, P. A. Kemme, and J. Broz. 2004. Efficacy of microbial phytase on mineral digestibility in diets for gestating and lactating sows. Livest. Prod. Sci. 91:143–155.

32. Silversides, F. G., T. A. Scott, and M. R. Bedford. 2004. The effect of phytase enzyme and level on nutrient extraction by broilers. Poult. Sci. 83:985–989.

33. Li, Y. C., D. R. Ledoux, T. L. Veum, V. Raboy, and D. S. Ertl. 2000. Effects of low phytic acid corn on phosphorus utilization, performance, and bone mineralization in broiler chicks. Poult. Sci. 79:1444–1450.

34. Viveros, A., A. Brenes, I. Arija, and C. Centeno. 2002. Effects of microbial phytase supplementation on mineral utilization and serum enzyme activities in broiler chicks fed different levels of phosphorus. Poult. Sci. 81:1172–1183.

35. Leeson, S., H. Namkung, M. Cottrill, and C. W. Forsberg. 2000. Efficacy of new bacterial phytase in poultry diets. Can. J. Anim. Sci. 80:527–528.

36. Ahmad, T., S. Rasool, M. Sarwar, A. Haq, and Z. Hasan. 2000. Effect of microbial phytase produced from a fungus *Aspergillus niger* on bioavailability of phosphorus and calcium in broiler chickens. Anim. Feed Sci. Technol. 83:103–114.

37. Yan, F., J. H. Kersey, and P. W. Waldroup. 2001. Phosphorus requirements of broiler chicks three to six weeks of age as influenced by phytase supplementation. Poult. Sci. 80:455–459.

38. Watson, B. C., J. O. Matthews, L. L. Southern, and J. L. Shelton. 2006. The effects of phytase on growth performance and intestinal transit time of broilers fed nutritionally adequate diets and diets deficient in calcium and phosphorus. Poult. Sci. 85:493–497.

39. Lantzsch, H. J., S. E. Scheuermann, and K. H. Menke. 1988. Influence of various phytate sources on the P, Ca and Zn metabolism of young pigs at different dietary Zn levels. J. Anim. Physiol. Anim. Nutr. (Berl.) 60:146–157.

40. Martin, E. A., J. V. Nolan, Z. Nitsan, and D. J. Farrell. 1998. Strategies to improve the nutritive value of rice bran in poultry diets. IV. Effects of addition of fish meal and a microbial phytase to duckling diets on bird performance and amino acid digestibility. Br. Poult. Sci. 39:612–621.

41. Attia, Y. A. 2003. Performance, carcass characteristics, meat quality and plasma constituents of meat type drakes fed diets containing different levels of lysine with or without a microbial phytase. Archiv Tierernaehr. 57:39–48.

42. Lei, X. G., P. K. Ku, E. R. Millar, M. T. Yokoyama, and D. E. Ullrey. 1994. Calcium level affects the efficacy of supplemental microbial phytase in corn-soybean diets of weanling pigs. J. Anim. Sci. 72:139–143.

43. Onyango, E. M., M. R. Bedford, and O. Adeola. 2003. A comparison of the efficacy of three phytase preparations in broiler chicks. Poult. Sci. 82(Suppl.1):35. (Abstr.)

44. Revy, P. S., C. Jondreville, J. Y. Dourmad, and Y. Nys. 2004. Effect of zinc supplemented as either an organic or an inorganic source and of microbial phytase on zinc and other minerals utilization by weanling pigs. Anim. Feed Sci. Technol. 116:93–112.

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