Efficacy of phytase and glycanase enzymes on the performance, serum mineral levels and antibody titer against newcastle disease virus (NDV) of broilers fed nutritionally marginal diets

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An experiment was conducted from 1 to 42 day post hatching to determine the effects of a microbial phytase (Natuphos) and glycanase preparations with predominantly xylanase and β-glucanase (Feedzyme) on the performance, serum mineral levels and antibody titer against newcastle disease, of broilers fed nutritionally marginal diets. A completely randomized experimental design was used, and chicks were divided into 5 treatment groups, with 4 replicates per treatment and 10 chicks per replicate. Diets were corn-wheat-soybean meal based with the same nutritional specifications, differing only in the concentration of Ca and nonphytate P (Ca-nPP). Treatments were: (1) diet with the energy of 2900 kcal/kg and 0.63% Ca and 0.28% available P, without enzyme (CTL+); T2) diet with the energy of 2900 kcal/kg and 0.63% Ca and 0.28% available P, without enzyme (CTL−); 3, 4 and 5) diet with the energy of 2900 kcal/kg and lower level of Ca and P (experimental group 2) respectively comprising 600,800,1000 phytase unit/kg of diet from Natuphos and 42,70,98 Xylanase unit/kg and 60,100,140 β-glucanase unit/kg of diet from feedzyme. Antibody titers against Newcastle disease were increased by incremental phytase and xylanase addition in experiments. These finding indicates that broilers consuming a ME, Ca, P-deficient corn-soybean meal-wheat diet can achieve maximum humoral immunity when phytase, xylanase and β-glucanase is supplemented to 1000, 98 and140 unit/kg diet. Performances of chicks fed with low-Ca-nPP diets plus phytase, xylanase and glucanase were comparable to those obtained with the low-Ca-nPP and adequate-Ca-nPP diets. Enzymes supplementation increased plasma Ca level (P > 0.05) but had no significant effect on plasma P level.

Key words: Phytase, glycanase, newcastle disease, performance, immunity, broiler.

INTRODUCTION

Feed ingredients with plant origin are a number of compounds that cannot be digested by monogastric species due to the lack or insufficiency of endogenous enzyme secretions (Ravindran et al., 1999). Examples of such antinutritive components include phytic acid (PA) in wheat (Ravindran et al., 1999). Phytic acid is the major storage form of phosphorus (P) in plant seeds (Ravindran et al., 1999). It contains 28.2% of bound P and represents, on average, 70% of the total P (TP) in the feed ingredients commonly used in poultry diets.
(Maenz, 2001), a form poorly available to poultry. Phytic acid is present in grains and seeds as a mixed salt, phytate, which refers to the phytic acid molecule chelated to mineral cations, proteins, starch, lipids, or both starch and lipids (Selle et al., 2000). It has been suggested that the efficacy and amount of endogenous phytase produced in the gastrointestinal tract (GIT) of poultry is insufficient to hydrolyze phytate to remove these effects. Therefore, phytate may be considered an antinutritional factor because it reduces the digestibility of phytate-chelated nutrients. To counteract the antinutritional effects of phytic acid, various modifications have been proposed. Among these alternatives, one of the most practical and effective methods is the addition of microbial phytase. Supplementation of diets with microbial phytase increases availability of phytate P and Zn in chicks (Ravindran et al., 2000).

Other examples of such antinutritive components include xylans in wheat and β-glucans in barley, Malathi and Devegowda (2001) reported that the level of nonstarch polysaccharides (NSP) is up to 29% in soybean meal and 9% in corn. Xylans are the principal NSP of wheat, and high levels of wheat in poultry diets can increase the viscosity of the gut contents, which impedes the circulation and absorption of nutrients, causing reduced feed intake, Body weight gain (BWG), and feed efficiency (Annison and Choct, 1991). Xylanase is used extensively in wheat-based diets to counteract the effects of NSP in broiler (Bedford and Schulze, 1998). Although it is clear that the performance improvement is related to greater digestion and absorption of nutrients in wheat and barley, the underlying mechanisms causing the improvements in nutrient utilization are not clearly understood (Ravindran, 1999). The degradation of plant, which (1) releases the nutrients encapsulated within the cell and (2) lowers digesta viscosity, thus improving the rate of diffusion among substrates, enzymes, and digestion end products, has been proposed as the main contributing factor (Bedford and Schulze, 1998). Conceivably, xylanase, by releasing the encapsulated nutrients and reducing digesta viscosity, may facilitate the action of phytase on phytic acid complexes and the absorption of liberated nutrients (Ravindran, 1999).

Although production performance and mineral retention are important measures of any dietary changes, plasma and bone mineral concentrations are generally more sensitive than performance factors for evaluating bioavailability of minerals. Both xylanase and phytase cause the release of Ca and P and could therefore disrupt the balance of these minerals (Roland and Gordon, 1996). Due to the lack of information and contradictions among several authors, more comprehensive studies are needed to elucidate the efficacy of phytase in bioavailability of minerals.

However, the extraphosphoric effects of phytate dephosphorylation are not well elucidated and warrant further study because endogenous secretion, the GI tract microflora, and the immune status of the host may be expected to be involved. Therefore, it is of interest to understand the effect of phytase on the health and immune status of broilers. It is speculated that the degradation products from the action of phytase on phytate may regulate immunocyte activity (Vucenik and Shamsuddin, 2006; Bozsik et al., 2007), and this may be particularly true for broilers fed diets containing a high concentration of phytate (Cowleson and Adeola, 2005). The literature reviewed by Kidd (2004) has clearly indicated that subnutrition impairs the bird’s ability to mount an effective response in natural and acquired immunity. Until now, few studies have been conducted to determine the effects of phytase and glycanase supplementation to wheat-based diets on immunity of poultry. Therefore, the objectives of this study were to examine the effects of phytase and glycanase supplementation on performance and serum mineral level and on the immune parameters in broilers fed nutritionally marginal diets.

MATERIALS AND METHODS

A total of 200, 1-day-old male broiler chicks (Ross308) were obtained from a commercial hatchery. The chicks were raised on concrete floor pens covered with 8 cm of clean pine wood shavings, and each pen was equipped with a tube feeder and a automatic waterer. Throughout the study, the birds were brooded following standard temperature regimens, which gradually decreased from 32 to 23°C. Birds were maintained on a 23 L: 1D lighting schedule and allowed to consume feed and water ad libitum. Environmental temperature was controlled according to recommendations Ross’s production manual. A completely randomized experiment was used, and chicks were divided into 5 treatment groups, with 4 replicates per treatment and 10 chicks per replicate. The treatments were: diet 1, adequate level of Ca and nPP (Adq Ca-nPP) as positive control (CTL+); diet 2, reduced levels of Ca and nPP (low Ca-nPP) as negative control (CTL−); diets 3 to 5, diet of CTL- group comprising 600, 800, 1000 unit/kg feed phytase (Natuphos 10000; BASF Group, Ludwigshaf, Germany), 42, 70, 98 unit/kg feed Xylanase (feedzyme 2000; granulate; UK) and 60, 100, 140 unit/kg feed β-glucanase (feedzyme 2000; granulate; UK), respectively. The compositions of the diets are presented in Table 1. According to the producer, the microbial phytase (Natuphos 10000 Granulate) contained 10,000 FTU/g phytase activity. All diets were formulated to provide 2900 kcal of Metabolizable Energy /kg and to meet the amino acid requirements and all other nutrients as suggested by the NRC (1994) for broilers from 0 to 6 week of age (Table 1), differing only in the concentration of dietary Ca and nPP. During the experiment, no antibiotics were administered to broilers neither via feed nor water. The enzymes (Natuphos 10000; BASF Group, Ludwigshaf, Germany and feedzyme 2000; granulate; UK) were added to the diets in powder form and all diets were fed as mash.

Body weight gain (BWG) and feed consumption in each pen were recorded weekly. Mortalities were recorded daily. At 42 days of age, three birds were randomly selected from each pen, and blood samples were obtained from the wing veins for determination of minerals (Ca, P) in plasma. Concentrations of minerals were measured at specific wavelengths for each element (Ca, 317.933; P, 214.914 nm) using an inductively coupled plasma emission spectrometer (Model Optima 3000, Perkin Elmer, Norwalk, CT). Calibrations for the mineral assays were conducted with a series of mixtures containing graded concentrations of standard solutions of

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Table 1. Composition of experimental diets.

| Ingredients (%)          | Starter (0-21 days) | Finisher (22-42 days) |
|--------------------------|---------------------|-----------------------|
|                          | Positive control    | Negative control      | Positive control    | Negative control      |
| wheat                    | 30                  | 30                    | 30                  | 30                    |
| Corn                     | 26.24               | 25.97                 | 34.45               | 35.91                 |
| Corn gluten              | 3.02                | 2.69                  | 0                   | 0                     |
| Wheat bran               | 5.21                | 7                     | 5.1                 | 4.97                  |
| Soybean meal             | 28.52               | 28.40                 | 24.61               | 24.37                 |
| Soybean oil              | 3                   | 3                     | 2.16                | 2.10                  |
| Oyster shell             | 1.62                | 1.17                  | 1.73                | 1.27                  |
| Ca phosphate             | 1.41                | 0.78                  | 1.08                | 0.51                  |
| Salt                     | 0.35                | 0.35                  | 0.24                | 0.24                  |
| Mineral premix           | 0.25                | 0.25                  | 0.25                | 0.25                  |
| Vitamin premix           | 0.25                | 0.25                  | 0.25                | 0.25                  |
| l-lysine HCL             | 0                   | 0                     | 0.05                | 0.05                  |
| dl-Methionine            | 0.13                | 0.14                  | 0.08                | 0.08                  |

| Nutrient composition     |                      |                      |                      |                      |
|--------------------------|----------------------|----------------------|----------------------|----------------------|
| ME (kcal/kg)             | 2900                 | 2900                 | 2900                 | 2900                 |
| Crude protein (%)        | 21                   | 21                   | 18.1                 | 18.1                 |
| Ca (%)                   | 0.9                  | 0.63                 | 0.85                 | 0.59                 |
| Available P (%)          | 0.4                  | 0.28                 | 0.4                  | 0.2250               |
| P (Total) (%)            | 0.6911               | 0.4837               | 0.41                 | 0.41                 |
| Na (%)                   | 0.18                 | 0.18                 | 0.1350               | 0.1350               |
| Lys (%)                  | 0.99                 | 0.99                 | 0.91                 | 0.91                 |
| Met (%)                  | 0.45                 | 0.45                 | 0.3516               | 0.3516               |
| Met + Cys (%)            | 0.81                 | 0.81                 | 0.65                 | 0.65                 |
| Trp (%)                  | 0.239                | 0.2425               | 0.2122               | 0.2114               |
| Thr (%)                  | 0.7182               | 0.7174               | 0.6179               | 0.6175               |

1Calculated from NRC (1994). 2Provides per kilogram of diet: Cu (CuSO4·5H2O), 4.0 mg; I (potassium iodate), 1.0 mg; Fe (ferrous sulfate_7 H2O), 60 mg; Mn (manganese sulfate_H2O), 60 mg; Se (sodium selenite), 0.1 mg; Zn (zinc sulfate_7H2O), 44 mg; and Ca (calcium carbonate), 723 mg. For experiment 3, provides per kilogram of diet: Cu (CuSO4·5H2O), 7.0 mg; I (potassium iodate), 1.0 mg; Fe (ferrous sulfate_7 H2O), 50 mg; Mn (manganese sulfate_H2O), 100 mg; Se (sodium selenite), 0.15 mg; and Zn (zinc sulfate_7H2O), 75 mg. 3For experiment, provides per kilogram of diet: vitamin A (vitamin A palmitate), 4,500 IU; vitamin D3, 450 IU; vitamin E (vitamin E acetate), 50 IU; menadione (menadione sodium bisulfite), 2.4 mg; vitamin B12, 0.02 mg; biotin (D-biotin), 0.6 mg; folacin (folic acid), 6 mg; niacin, 50 mg; Ca-panthenate, 20 mg; pyridoxine (pyridoxine_HCl), 6.4 mg; riboflavin, 15 mg; and thiamin (thiamin_HCl), 15.2 mg. For experiment 3, provides per kilogram of diet: vitamin A (vitamin A palmitate), 8,000 IU; vitamin D3, 3,000 IU; vitamin E (vitamin E acetate), 25 IU; menadione (menadione sodium bisulfite), 1.5 mg; vitamin B12, 0.02 mg; biotin (D-biotin), 0.1 mg; folacin (folic acid), 1 mg; niacin, 50 mg; pantothenic acid, 15 mg; pyridoxine, 4 mg; riboflavin, 10 mg; and thiamin (thiamin(HCl)), 3 mg.

each element. Blood plasma and serum were analyzed for minerals (Ca, P) using an ADVIA 1650 chemistry system of Bayer (Bayer diagnostic, Puteaux, France). Prior to division of chicks (1-day-old) in to various groups, a blood sample was taken from each bird and the titers of maternal antibody against Newcastle disease virus (NDV) were measured by haemagglutination-inhibition (HI) test. At the age of 9 days, all chicks were vaccinated with Hitchner B1 NDV vaccine via eye (ophthalmic) route and bivalent killed vaccine (Newpasol 102, Inactivated W/O Emulsion ND + AI (H9N2) Vaccine, Pasouk Biological Co) by inoculation according of the manufacturer’s recommendation. Blood samples were collected every week from the wing veins of individual chickens in all groups and their sera were separated and inactivated at 56 °C for 30 min and kept at −20°C until analysis for the level of NDV antibody. Serum antibody titer was measured by hemagglutination-inhibition test as described by Alexander et al. (1983) on days 7, 14, 21, 28, 35 and 42.

Statistical analysis

When the chicks reached 42 days of age, the feeding trial was terminated. Data were evaluated with ANOVA for a complete randomized design, using the general linear models procedure of SAS software (SAS Institute, 2004). The treatment means with significant differences were compared using Duncan’s new multiple range tests. All statements of differences were based on significance at P ≤ 0.05.

RESULTS

There were no health problems associated with the use of the enzyme throughout the experiment, and there were no obvious clinical health problems. Mortality was low (<1%) and not treatment associated. No bacterial or viral disease infection was detected.
The effects of Ca and nPP concentrations and enzyme supplementation on feed intake (FI), BWG and feed conversion ratio (FCR) of birds are summarized in Table 2. Birds fed diet with low Calcium and Phosphorus concentration had a lower BWG and poorer FCR than those fed the Adq Ca-nPP diets. The differences, however, were not statistically significant (P>0.05). Supplementation with phytase, Xylanase and β-glucanase increased performances of broiler chicks fed with diets deficient Ca and nPP.

The effects of Ca-nPP concentrations and enzyme supplementation on plasma mineral levels are summarized in Table 2. Ca and P content in plasma decreased as dietary Ca and P level decreased. However, there were no significant differences. Enzyme supplementation to low Ca and P diets increased plasma Ca level (P<0.05). Plasma P level was not significantly affected by the enzyme supplementation, but these food additives increased plasma P level from 4.23 mg/dl with no enzyme to 5.47 mg/dl in enzyme supplementation, but these food additives increased further improvements in anti-NDV antibodies.

| Parameter | Treatment | 1-21 days | 22-42 days | 1-42 days |
|-----------|-----------|-----------|------------|-----------|
| FI (g)    | T1        | 980±25    | 3560.5±56.7| 4540.5±52 |
| BWG (g)   | T1        | 972±32    | 3427.8±48.9| 4400.3±47 |
| FCR (g/g) | T1        | 1.664±0.04| 2.151±0.10 | 2.169±0.06 |
| FI (g)    | T2        | 981±19    | 3604±61.7  | 3658.5±39 |
| BWG (g)   | T2        | 995±21    | 3657.5±70.6| 4652.5±58 |
| FCR (g/g) | T2        | 1.644±0.07| 2.034±0.05 | 1.830±0.07 |
| FI (g)    | T3        | 998±27    | 3690±55.7  | 4688±46  |
| BWG (g)   | T3        |           |            |           |
| FCR (g/g) | T3        |           |            |           |
| FI (g)    | T4        | 995±21    | 2085±68.1  | 9.27±0.12 |
| BWG (g)   | T4        |           |            |           |
| FCR (g/g) | T4        |           |            |           |
| FI (g)    | T5        | 998±27    | 2701.25±33 | 6.95±0.06 |
| BWG (g)   | T5        |           |            |           |
| FCR (g/g) | T5        |           |            |           |

The effects of treatments on antibody production against NDV in broilers from day 7 to day 42 are presented in Table 3. On the 7th day of the study, there was no difference among antibody titers of treatment 5. The addition of enzymes to the diet increased antibody production with differing superscripts are significantly different at P<0.05. The effects of treatments on antibody production against NDV in broilers from day 7 to day 42 are presented in Table 3. On the 7th day of the study, there was no difference among antibody titers of treatment 5.

**Table 3. Effect of Phytase and glycanase Enzymes on NDV antibody titers in broiler chicks from 7 to 42 days of age.**

| Treatment | Antibody titers (day) |
|-----------|-----------------------|
|           | 7th | 14th | 21st | 26th | 26th | 35th | 42nd |
| T1        | 6.95 0.28 | 5.02 0.21 | 4.35 0.27 | 5.30 0.35 | 5.37 0.41 | 5.62 0.37 |
| T2        | 6.55 0.22 | 4.62 0.19 | 3.61 0.19 | 4.37 0.31 | 4.37 0.31 | 5.00 0.37 | 4.75 0.32 |
| T3        | 7.15 0.33 | 5.40 0.35 | 4.55 0.34 | 4.90 0.43 | 4.90 0.43 | 5.87 0.29 | 5.37 0.38 |
| T4        | 7.12 0.29 | 5.20 0.31 | 4.77 0.31 | 5.42 0.21 | 5.42 0.21 | 5.87 0.33 | 6.12 0.31 |
| T5        | 7.22 0.34 | 5.70 0.32 | 5.10 0.33 | 5.60 0.38 | 5.60 0.38 | 7.00 0.49 | 5.87 0.42 |

Values within a column with no common letters differ significantly, each value represents the Means±standard error of the mean for 10 chicks per replicate per treatment; 1, 2Means within a column with differing superscripts are significantly different at P<0.05. 3, 4Means within a column with differing superscripts are significantly different at P<0.01. 5P>0.05.
Antibodies are important biological agents prevalent in the healthy immune repertoire, and they participate in the maintenance of immune homeostasis by exposure to environmental stimulation (Bayry et al., 2005). It has been shown that low levels of humoral antibody may be related to disease susceptibility (Parmentier et al., 2004). Serum hemagglutination inhibition antibody is a valid indicator because it is directly effective against NDV in the humoral immunity of chickens (Maas et al., 2003). In the current study, the addition of enzymes to the diet increased antibody production against NDV in broiler from 14 to 42 days of age. These results demonstrated the positive influence of enzymes on the response to vaccination of the chickens’ immune system. Ingestion of diets containing enzymes resulted in higher titers, in comparison to birds which were fed diets without enzymes, specifically during the weeks in which titers tended to decrease. No significant differences on antibody production against NDV were found between the enzymes -treated birds in experimental period.

In conclusion, phytate is a ubiquitous and potent antinutrient in monogastric diets and exerts a range of physiological, nutritional, and immunological consequences on the host. Compensatory mechanisms are in place to allow normal digestive processes to continue, but these carry a substantial nutritional cost to the animal in terms of energy, and amino acid and mineral requirements associated with synthesis, absorption, catabolism, and autolysis. An understanding of the antinutritive effects of phytate is an important first step in developing improved microbial phytases and in maximizing the potential of currently available phytase technology. Zyla et al. (2000) reported that phytase addition to diets with a low P concentration enhanced the bursa weight of 21-days-old Hubbard broilers. Because the bursa is the source organ for B cells, the development of the bursa may induce the proliferation of B cells. Thus, the growth-promoting effect of phytase may be expressed via both nutrient release and a physiological regulation mechanism. The investigation of innate mucosal humoral immunity by Liu et al. (2008) showed that the levels of SIgA were increased by enzyme supplementation of diets with nonstarch polysaccharide-degrading enzyme preparations significantly increased the anti-NDV titers of chicks. Kettunen and Rautonen (2005) reported that the use of xylanase, amylase, and protease or a combination of the enzymes and betaine enhanced nutrient uptake by intestinal cells and concluded that the concentration of IgA in the digesta contributed to improvements in immune competence.

The effects of low-calcium and phosphorus diets and enzyme supplementation on growth performance are summarized in Tables 3 and 4. As seen in this study, deficiencies of Ca and nPP decreased FI, BWG, and FC in chicks in the entire experimental period. In the current study, the application of enzyme to nutritionally marginal diets improved feed intake, body weight gain, and feed conversion ratio of broilers, results that are in agreement with previous work (Cowieson and Adeola, 2005; Liu et al., 2007). Body weight was probably not a good indicator of the effects produced by reduced-P diets plus enzyme with respect to a control diet. Indeed, Dhandu and Angel (2003) reported that BWG was not a sensitive indicator of mineral sufficiency in broilers. In the growing phase and Overall, broilers fed the adequate Ca and P diet had increased feed intake (P<0.05) compared with those fed the low Calcium and Phosphorus diets. The effect of Adq Ca-nPP diets on the same parameters as above in broilers is shown in Tables 3 and 4. Supplementation with enzyme improved the body weight gain (BWG), and feed conversion ratio (FCR) of birds (P<0.01). The highest BWG was shown by the treatment 5. No statistical differences were measured in FCR in the broilers between treatments4 and 5. These data indicate that enzyme increases Performances of broiler chicks fed with deficient in Ca and nPP in diets. These values agree with the findings of Ahmad et al. (2000). It might be due to changes in the viscosity of the diets or transit time through the digestive tract of the chicken. The growth-promoting effect of P caused by phytase can be partially attributed to the increased concentrations of myo-inositol, the final product of phytate desphosphorylation, and to the release minerals and trace elements from complexes with phytic acid. Similarly, it could also be due to a possible increase of starch digestibility or to an increased availability of protein (Selle et al., 2000).

Phytase is able to liberate phytate-bound P and make more P available to the animals. Studies have reported improvements in performance when phytase was used in chickens (Zyla et al., 2000; Onyango et al., 2005), layers (Silverside et al., 2006), and pigs (Young et al., 1993; Yi et al., 1996; Adeola et al., 1998; Matsui et al., 2000; Jendza et al., 2005). Because phytase acts on the phosphate groups associated with the inositol ring of phytic acid backbone and thus releases P and Ca, it is expected that the use of phytase would result in improved performance of the animal if P and Ca is the nutrient limiting for growth. Adeola and Bedford (2004) compared the efficacy of xylanase addition to high viscosity and
low-viscosity wheat diets and reported that xylanase improved performance to a greater extent in high-viscosity compared with low-viscosity wheat, thus showing the relationship between the potential of a feed stuff to cause digesta viscosity and the beneficial effect that may result from enzyme usage. The beneficial effects of exogenous xylanases on the amino acid digestibility of wheat are in agreement with some reports (Bedford et al., 1998; Hew et al., 1998). The modes of action of xylanases in improving the nutrient digestibility in wheat are discussed in detail elsewhere (Bedford and Schulze, 1998). In this study, phytase and glycanase (xylanase and β-glucanase) was used in Corn-wheat -soybean meal diets that were marginally deficient in both P and Ca. In the current study, addition of enzyme produced a significant improvement in performance as compared to the CTL-, demonstrating that P and Ca was a limiting nutrient in the current study. Data also showed that feed consumption was affected at any stage by addition of enzyme. In contrast, other authors have reported that addition of phytase did not affect feed consumption (Ahmad et al., 2000). Watson et al. (2006) reported that phytase decreased transit time in chicks fed diets deficient in Ca and nPP on Day 1. The results of our study suggest that the increase in BWG in chicks fed diets containing enzymes was due to an increase in feed intake. This increase in feed intake might have been due to a faster transit time in chicks fed diets containing enzymes. The chicks fed enzymes ate more and thus gained more weight, regardless of the adequacy of the diet. As a result, the amounts of enzyme and inorganic P needed in diets could be minimized resulting in reduced cost, lower P excretion levels, and decreased environmental impact.

A plasma level of P is a result of the homeostatic regulation of P, and significant lowering of these levels may be indicative of low body P reserves (Onyango et al., 2004). Compared to the Adq Ca-nPP diets, the birds fed with low calcium and phosphorus diets without enzyme had decreased plasma Ca and P levels at 42 days of age but not significantly. Our results were similar to those obtained by Ravindran et al. (2000) in chickens which indicated that the birds have a greater ability to retain P from diets with lower rather than higher nPP content. A possible explanation could be that the higher content of Ca relative to P in the low P diets caused an increase of intestinal pH and reduced the soluble fraction of minerals or that the decreased retention of minerals was related to bone mobilization to maintain serum P and excretion of excess Ca. It is possible that when P is limiting, more P is retained in the body for maintaining physiological functions, thus resulting in less P being excreted in the waste (Li et al., 2000). Enzymes supplementation to the low- Ca-nPP diets increased Ca (P< 0.05) at 6 week of age. Plasma P level increased by enzyme supplementation but there was no significant effect at 6 week of age. As expected, enzymes supplementation to the low Ca and P diets increased plasma Ca and p values, this agrees with the results of previous studies on chickens (Ravindran et al., 2000). Phytate-bound P liberated by phytase is available in the gut to be absorbed to maintain normal P homeostasis. This result may be due to the fact that phytase supplementation to the low nPP diet increases the Ca content, resulting in an efficient use of this mineral by birds. However, at higher nPP levels, Ca is bonded to phytate, therefore it cannot be fully retained by the bird, which leads to excessive excretion of Ca. It is known that phytase improved P digestibility by catalyzing the phosphate monoester hydrolysis of phytic acid, resulting in the stepwise formation of myoinositol and orthophosphates via inositol pentakisphosphate, up to monophosphates as intermediary or end products resulting in liberation of P (Liu et al., 1998). The xylanase is not known to act in this manner, and its ability to enhance P digestibility and retention probably occurs via a different mechanism. One possible mechanism of how xylanase improves P digestibility could be deduced by taking note of the following. Although a phytate molecule is a strong chelating agent, it is not the only molecule that is responsible for chelation of nutrients. There is evidence that some soluble fibers, including soluble NSP, are responsible as well. The author pointed out that the soluble NSP and phytic acid are in the aleurone cell layer. Parkkonen et al. (1997), using in vitro digestion techniques, reported that xylanase increased the permeability of the aleurone cell wall layer, which is the site of phytate storage. It is possible that xylanase, by improving aleurone layer permeability, enhances the access of endogenous phytase to phytate molecules, hence improving P digestibility and retention. It could be expected that an enzyme that is capable of breaking the NSP layer will also provide easier access to phytate. If phytase alone is used, the ability of the enzyme to act on phytate will be limited by its lack of access to its substrate if phytase is within the NSP matrix. Glycanase (xylanase and β-glucanase) that are able to break down the NSP fraction can facilitate the contact between phytase and phytate. Additionally, some soluble fiber bound P may be released in the presence of glycanase, and this may explain how glycanase is able to increase P digestibility.

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