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Comparative Effects of Phytase Derived from *Escherichia coli* and *Aspergillus niger* in Sixty Eight-week-old Laying Hens Fed Corn-soy Diet

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ABSTRACT : Two hundred and sixteen 68-week-old Hy-Line brown laying hens were used in a 6-week feeding trial to compare the efficacy of phytases Optiphos (OPT) and Natuphos (NAT), which were isolated from *Escherichia coli* and *Aspergillus niger*, respectively. Hens were randomly allotted into six treatments with six replications (six layers in three adjacent cages) per treatment according to their initial BW. The hens were then subjected to one of the following dietary treatments: i) Positive Control (PC; available phosphorus (AP) 0.4%); ii) Negative control (NC; AP 0.2%); iii) NAT1 (NC+250 FTU/kg NAT); iv) NAT2 (NC+500 FTU/kg NAT); v) OPT1 (NC+250 FTU/kg OPT); vi) OPT2 (NC+500 FTU/kg OPT). Feed intake, egg production, egg quality, apparent nutrient digestibility and serum P and Ca concentration were evaluated to compare the effect of the two phytases. Feed intake and eggshell thickness were not affected by the treatments. Superior effects ($p<0.05$) of OPT were only observed in egg production and egg weight compared with NAT. Characteristics such as eggshell breaking strength, apparent digestibility of N, Ca and P and serum P concentration were equally increased with the supplementation of both phytases ($p<0.05$), where no significant difference was observed in those characteristics between PC and phytase supplementation at 500 FTU/kg. Equally effective improvements ($p<0.05$) were also observed in egg production and DM digestibility, where no improvements were observed ($p<0.05$) between the PC group and the groups with phytase supplementation at 500 FTU/kg. Equal increases in the serum Ca level were observed when the groups with phytase supplementation were compared to the PC group. Overall, the results of this study suggest that NAT and OPT are equally effective at liberating phytate-bound complexes when included in 0.2% available phosphorus diets for 68-week laying hens; either source of phytase can be fed to commercial 68-week laying hens at 500 FTU/kg to correct the negative effects associated with a 0.2% available phosphorus diet. In conclusion, either source of phytase can be fed to commercial first cycle laying hens at 500 FTU/kg to effectively replace inorganic phosphorus when economically justified. (**Key Words** : Laying Hens, Phytase, Egg Quality, Egg Production, Serum)

INTRODUCTION

Phosphorus has been identified as an essential mineral in the formation of eggshells and the metabolism of laying hens (Sohail and Roland, 2002). Phytate is the primary storage form of phosphorus found in plant feed ingredients; however, phytate P is poorly utilized due to the limited inherent phytase activity in the gastrointestinal tract of non-ruminants (Adeola et al., 2004). Previous studies have shown that supplemental phytase exerts beneficial effects in poultry (Rutherfurd et al., 2004; Francesch et al., 2005). cheaper and more effective sources of phytase would hasten the use of phytase in the poultry industry.

Phytase derived from different sources may differ in biochemical and biophysical properties such as the optimum pH and the ability to resist hydrolysis within the digestive tract, which will affect the ability of the phytase enzyme to function effectively and consistently (Onyango et al., 2005). It is well expected that phytase derived from *Escherichia coli* (ECP) will have superior results when compared to phytase derived from *Aspergillus niger* (ANP) due to differences in their biochemical and biophysical properties (Rodriguez et al., 1999b; Wodzinski and Ullah, 1996). However, the results of practical feeding studies have been inconclusive. For example, Augspurger and Baker (2004) and Rodriguez et al. (1999a) found that ECP had a higher efficacy when compared to ANP in broilers and pigs, whereas similar efficacies of both phytases were observed when they were fed to young chicks (Leeson et al.,

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2000) at the same dosage. To the best of our knowledge, previous studies conducted to evaluate the effects of phytase supplementation on hens have primarily been conducted during various times in the first cycle of laying hens, but limited information exists regarding the effects of phytase supplementation at 68-weeks old fed corn-soy diets. Besides, experiment conducted by Boling et al. (2000) also shown that older hens are more sensitive to P deficiency, which may provide a proper environment to compare this two phtyase.

Therefore, this study was conducted to compare the efficacy of *Escherichia coli*-derived phytase and *Aspergillus niger*-derived phytase on the performance and the digestibility of 68-week laying hens.

MATERIALS AND METHODS

Phytase preparation

One commercial phytase enzymes and an experimental E. coli-derived phtyase (ECP) were used in these experiments. Natuphos (NAT) is a recombinant enzyme from *Aspergillus niger* that is classified as a 3-phytase, with hydrolysis of the phosphate moiety being initiated at the 3-position on the phytate molecule. Phytase Optiphos (OPT) was isolated from *Escherichia coli*, which was expressed and produced through a yeast expression system that has been described previously (Rodriguez et al., 1999a, b). Prior to use in this experiment, the activities of the purified phytases were determined based on the release of inorganic P from sodium phytate in 0.2 M citrate buffer as previously described (Han et al., 1999). One phytase unit (FTU) was defined as the amount of enzyme required to liberate 1μmol of inorganic P per min from 0.0015 mol of sodium phytate at 37°C with a pH of 5.5.

Feeding regimen

A total of two hundred and sixteen 68-week-old Hy-Line brown laying hens (Yang Ji Hatchery, Cheonan, Choongnam, South Korea) were evaluated in 6-week feeding trial. The hens were randomly allotted into one of six treatments according to the individual initial BW, with six replications per treatment. Each replication consisted of three adjacent cages, with two hens per cage. In addition, the replications were equally distributed into the upper and lower cages to minimize the effect of cage level. The hens were housed in a windowless laying house under a 17 h light: 7 h dark photo period at approximately 21°C. There was a 7 d adjustment period prior to the start of the experiment, during which the hens were provided with PC diet. All cages were equipped with nipple drinkers and common trough feeders and feed and water were provided *ad libitum* throughout the experimental period. The animal care protocol used for this experiment was approved by the Animal Welfare Committee of Dankook University.

Experimental design and diets

Diets were formulated to have the same nutrient density, except the levels of P differed (Table 1). Hens were provided with one of the following dietary treatments: i) Positive Control (PC; available phosphorus (AP) 0.4%); ii) Negative Control (NC; AP 0.2%); iii) NAT1 (NC+250 FTU/kg NAT); iv) NAT2 (NC+500 FTU/kg NAT); v) OPT1 (NC+250 FTU/kg OPT); vi) OPT2 (NC+500 FTU/kg OPT). The PC diet was formulated to meet or exceed the NRC (1994) nutrient requirements for laying hens. The NC diet was formulated the same as the PC, except that the AP level was reduced to maximize the response to enzyme supplementation.

Sample and measurement

Parameters of production performance and egg quality : Daily records of egg production and weekly records of feed consumption were kept throughout the experimental period. The egg production was expressed as an average hen-day production, which was calculated from the total number of eggs divided by the number of days and summarized on an average basis. In addition, a total of 30 salable eggs (no shell defects, cracks, or double-yolks) were randomly collected from each treatment at 17:00 h (five eggs per replicate) on a weekly basis. The egg quality of the collected eggs was then determined at 20:00 h on the day of collection. The egg weight was measured using an egg multi tester (Touhoku Rhythm Co. Ltd., Tokyo, Japan). The eggshell breaking strength was evaluated using a model II egg shell force gauge (Robotmation Co., Ltd., Tokyo, Japan). Finally, a dial pipe gauge (Ozaki MFG. Co., Ltd., Japan) was used to measure the egg shell thickness, which was determined based on the average thickness of the rounded end, pointed end, and the middle of the egg, excluding the inner membrane.

Digestibility of nutrients : After the conclusion of the feeding trial, six birds per treatment were randomly chosen for metabolic trials. The selected birds were individually housed in metabolic cages to determine the digestibility of nutrients. Laying hens were fed their respective diets containing chromic oxide (Cr_2O_3) for 4 days prior to the collection period. All excreta of the birds were collected for 3 d. All the fecal samples along with feed samples, were then analyzed according to the AOAC procedures (AOAC, 1990). The mineral contents were assayed using an inductively coupled serum emission spectrometer (Model JY-24, Jobin Yvon, Longjumeau, Cedex, France).

Serum calcium and phosphorus : At the beginning of the experiment, two birds per replicate were randomly selected

Table 1. Ingredient and nutrient content of the experimental diets

Items	Positive control	Negative control	NAT (U/kg)		OPT (U/kg)	
			250	500	250	500
Corn	58.85	59.16	59.14	59.11	59.14	59.11
Soybean meal	27.72	27.68	27.68	27.68	27.68	27.68
Tallow	2.822	2.712	2.712	2.712	2.712	2.712
Tricalcium phosphate[1]	1.84	0.74	0.74	0.74	0.74	0.74
Limestone	8.04	8.98	8.98	8.98	8.98	8.98
Vitamin premix[2]	0.12	0.12	0.12	0.12	0.12	0.12
Mineral premix[3]	0.1	0.1	0.1	0.1	0.1	0.1
Salt	0.31	0.31	0.31	0.31	0.31	0.31
DL-methionine (50%)	0.2	0.2	0.2	0.2	0.2	0.2
Natuphos[4]			0.025	0.05		
Optiphos[4]					0.025	0.05
Total	100	100	100	100	100	100
Calculated composition						
CP (%)	17.00	17.00	17.00	17.00	17.00	17.00
Fat (%)	5.06	4.96	4.96	4.96	4.96	4.96
Ca (%)	3.80	3.80	3.80	3.80	3.80	3.80
Total phosphorus (%)	0.62	0.42	0.42	0.42	0.42	0.42
Available phosphorus (%)	0.40	0.20	0.20	0.20	0.20	0.20
ME (kcal/kg)	2,750	2,750	2,750	2,750	2,750	2,750

[1] Tricalcium phosphate contains 32% calcium and 18% P phosphorus according to the NRC (1994).
[2] Vitamin premix provided (mg/kg diet): 125,000 IU vitamin A; 2,500 IU vitamin D_3; 10 mg vitamin E; 2 mg vitamin K_3; 1 mg vitamin B_1; 5 mg vitamin B_2; 1 mg vitamin B_6; 15 mg vitamin B_{12}; 500 mg folic acid; 35,000 mg niacin; 10,000 mg Ca-Pantothenate and 50 mg biotin.
[3] Mineral premix provided (mg/kg diet): 8 mg Mn; 60 mg Zn; 25 mg Cu; 40 mg Fe; 0.3 mg Co; 1.5 mg I and 0.15 mg Se.
[4] Provided by Easybiosystem, Seoul Feed Ltd., Seoul, Korea.

and 5 ml of blood were collected from their left jugular veins using a sterilized injector. The samples were then transferred into a K_3EDTA vacuum tube (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA). At the end of the experiment, blood was collected from the same laying hens. The blood samples were then used to estimate the serum Ca (AOAC, 1990) and serum P concentrations (Fiske and Subba Row, 1925).

Statistical analysis

All data were evaluated by analysis of variance following the GLM procedure in a completely randomized design. All analyses were conducted using the SAS software program (SAS Institute, 1996). Significant differences among the means of the treatment groups were determined at $p<0.05$ by Duncan's multiple-range test. Orthogonal comparisons were made using polynomial regression to measure the linear and quadratic effects of increasing dietary concentrations of supplemental phytase. According to the results of Duncan's multiple-range test, the effects of phytase supplementation of the NC diets at 250 FTU/kg were obviously lower than the effects of PC treatment; therefore, only differences between the PC group and the NC groups that received phytase supplementation at 500 FTU/kg were evaluated.

RESULTS

Egg production and egg quality

The results of the egg production and egg quality are shown in Table 2. No significant differences were observed in the feed intake among treatments. However, egg production, egg weight, egg shell breaking strength and egg shell thickness were significantly ($p<0.05$) reduced by 11.95%, 2.76%, 8.55%, and 3.47%, respectively, when laying hens fed the NC diet were compared to those that received the PC diet. Additionally, no linear effect in eggshell thickness was observed in response to phytase supplementation when compared to the NC diet, despite increases in thickness of 2.3% and 1.8% when hens were provided the NC diet supplemented with NAT and OPT at 500 FTU/kg, respectively. Conversely, as the level of phytase increased, increased egg production (Linear $p<0.05$) and eggshell breaking strength (Quadratic $p<0.05$) was observed. Moreover, a linear increase in egg weight ($p<0.05$) of 4.8% was observed in response to OPT supplementation, whereas no linear effect was observed in response to NAT supplementation. Furthermore, the effects

Table 2. The effect of supplemental phytase on the production performance and egg quality of 68-week-old laying hens

Dietary treatments	Feed intake (g/d)	Egg production (%)	Egg weight (g)	Eggshell breaking strength (kg/cm^2)	Eggshell thickness (mm)
Positive control[1]	122.67	87.61^{a}	65.88^{a}	3.51^{ab}	38.60^{a}
Negative control[1]	121.39	77.14^{c}	64.06^{b}	3.21^{c}	37.26^{bc}
NAT1[1]	119.00	79.44^{bc}	63.64^{b}	3.34^{bc}	37.00^{c}
NAT2[1]	122.67	80.55^{b}	64.07^{b}	3.48^{ab}	38.13^{ab}
OPT1[1]	119.83	79.68^{bc}	63.39^{b}	3.56^{a}	37.57^{bc}
OPT2[1]	123.00	85.56^{a}	67.17^{a}	3.49^{ab}	37.93^{abc}
SEM[2]	2.499	0.907	0.597	0.067	0.314
	p-value				
Linear (NC, NAT1, NAT2)	0.778	0.020	0.620	0.004	0.070
Quadratic (NC, NAT1, NAT2)	0.456	0.612	0.538	0.966	0.093
Linear (NC, OPT1, OPT2)	0.403	<0.0001	0.0001	0.005	0.090
Quadratic (NC, OPT1, OPT2)	0.189	0.182	0.060	0.020	0.917
Phytase source	0.820	0.007	0.002	0.080	0.544
PC vs. NAT2 and OPT2	0.958	0.003	0.719	0.720	0.145

[1] Positive control (PC), available (P) (0.4%); Negative control (NC), total P 0.4%; NAT1, NC+250 FTU of Natuphos/kg; NAT2, NC+500 FTU of Natuphos/kg; OPT1, NC+250 FTU of Optiphos/kg; OPT2, NC+500 FTU of Optiphos/kg.
[2] Pooled standard error.
[a, b, c] Means in the same column with different superscripts differ ($p<0.05$).

of OPT-supplementation on egg production and egg weight were greater than those of NAT-supplementation ($p<0.05$).

Apparent total tract nutrient digestibility

The digestibilities are outlined in Table 3. The reduction of available P in the NC diet led to significant decreases in the digestibility of DM, N, Ca and P of 18.10%, 14.23%, 15.16% and 29.06%, respectively, when compared to the PC diet ($p<0.05$). However, the negative effects on digestibility were completely attenuated ($p<0.05$) by supplementation of the NC diet with 500 FTU/kg of phytase, with the exception of DM digestibility, which was significantly lower ($p<0.05$) in the NP2 and OP2 treatment groups compared to the PC treatment group. Furthermore, no significant differences were observed between groups supplemented with OPT and NAT.

Table 3. The effect of supplemental phytase on the digestibility and serum P and Ca of 68-week-old laying hens

TRE	DM (%)	N (%)	Ca (%)	P (%)	Serum Ca (mg/dl)	Serum P (mg/dl)
Positive control (PC)[1]	79.93^{a}	70.32^{a}	72.41^{a}	62.63^{a}	34.82^{a}	10.64^{a}
Negative control (NC)[1]	65.44^{d}	60.31^{b}	61.43^{b}	44.43^{c}	29.00^{c}	6.54^{c}
NAT1[1]	70.96^{c}	63.29^{b}	63.30^{b}	51.84^{b}	30.64^{bc}	8.14^{b}
NAT2[1]	73.93^{bc}	69.73^{a}	70.26^{a}	60.93^{a}	32.52^{abc}	9.44^{ab}
OPT1[1]	70.56^{c}	62.45^{b}	65.09^{b}	54.67^{b}	30.48^{bc}	8.20^{b}
OPT2[1]	77.27^{ab}	69.57^{a}	71.68^{a}	61.32^{a}	33.56^{ab}	9.52^{ab}
SEM[2]	1.318	1.738	1.649	1.83	1.113	0.526
	p-value					
Linear (NC, NAT1, NAT2)	0.0003	0.007	0.0005	<0.0001	0.070	0.0004
Quadratic (NC, NAT1, NAT2)	0.678	0.317	0.123	0.649	0.915	0.742
Linear (NC, OPT1, OPT2)	<0.0001	<0.0001	0.0001	<0.0001	0.005	0.009
Quadratic (NC, OPT1, OPT2)	0.202	0.210	0.350	0.415	0.435	0.791
Phytase source	0.276	0.775	0.3406	0.388	0.697	0.895
PC vs. NAT2 and OPT2	0.010	0.750	0.480	0.510	0.210	0.090

[1] Positive control (PC), available P (0.4%); Negative control (NC), total P 0.4%; NAT1, NC+250 FTU of Natuphos/kg; NAT2, NC+500 FTU of Natuphos/kg; OPT1, NC+250 FTU of Optiphos/kg; OPT2, NC+500 FTU of Optiphos/kg.
[2] Pooled standard error.
[a, b, c, d] Means in the same column with different superscripts differ ($p<0.05$).

Serum calcium and phosphorus

The data describing the serum Ca and P concentrations are shown in Table 3. The initial serum Ca and P levels are not presented in the table because of no significant difference in the serum Ca and P. Significant reductions of 16.7% and 38.5% were observed in the serum Ca and P concentrations, respectively, when birds that received the NC treatment were compared to those that received the PC treatment. In addition, the serum P concentrations increased linearly ($p<0.05$) with both forms of phytase when compared those observed in PC treatment. However, the linear effect ($p<0.05$) on serum Ca concentration was only observed in OPT group, whereas only a tendency ($p<0.10$) to increase was observed in the NAT group. No significant differences were observed between phytase sources. In addition, no significant differences were observed between the PC group and groups that received the NC diet supplemented with phytase at 500 FTU/kg.

DISCUSSION

Comparison of PC and NC diets

The beneficial effects of microbial phytase supplementation of P-deficient diets in poultry have been well documented (Adeola et al., 2004; Wu et al., 2006), which suggests that phytase supplementation can release phytate-bound nutrients and consequently improve nutrient utilization. The results of the present experiment appear to support this conclusion, as indicated by significant differences being observed between the PC and NC groups for most of the characteristics studied. These findings suggest that a diet containing 0.20% available phosphorus cannot meet the requirements of the 68-weeks laying hens. However, an experiment conducted by Augspurger et al. (2007) indicated that 0.20% available P is adequate for the older laying hens. This inconsistency suggests that the AP requirement for older laying hens should be revised. However, our objective was to compare phytase sources, not determine or evaluate available phosphorus requirements. The differences between PC and NC that were observed in the current experiment allowed comparison of those two phytases. Most of the negative effects due to P-deficiency in the current experiment were almost attenuated by supplementation of the NC diet with phytase at 500 FTU/kg, which clearly demonstrates that both phytases used in this experiment have the ability to hydrolyze phytate-bound nutrients. Additionally, previous studies have revealed that negatively charged phytate can form insoluble complexes with positively charged proteins at low pH in the gastrointestinal tract (Cheryan, 1980; Reddy et al., 1982). Furthermore, it has been shown that phytic acid can form insoluble salts with minerals such as Ca, Mg, Fe, Zn and Cu (Bedford and Schulze, 1998; Liu et al., 1998), which can then restrict the utilization of nutrient minerals and protein in nonruminant animals.

Comparison of sources

Phytase derived from *Escherichia coli* (ECP) exhibited a broader range of optimal pHs (from 2.5 to 3.5), whereas phytase derived from *Aspergillus niger* (ANP) has a bimodal optimum pH of 2.5 and 5.5. Furthermore, ECP had 25% greater activity at pH 2.5 and 35% less activity at pH 5.5 when compared to ANP (Rodriguez et al., 1999a). A study conducted by Wodzinski and Ullah (1996) also reported that 6-phytases such as ECP would completely dephosphorylate the phytate molecule, whereas 3-phytases such as ANP do not due to their respective initiation sites. The differences in the biochemical and biophysical properties of phytase and the pH of the gut from which the phytate complex was liberated may lead to different levels of nutrients being released in response to different phytases. However, in the current study, the only significant differences observed between groups treated with ECP (Optiphos) and ANP (Natuphos) were egg production and egg weight (Tables 2 and 3). Similar results were found by Stahl et al. (2000) and Sands et al. (2003), who reported that differences in biochemical and biophysical properties were not manifested with different phytases in young pigs and broilers, which was explained as the stomach of the animals used may not have proper pH to show the potential catalytic difference between these two phytase. This may also explain why no significant differences were observed in most of the characteristics investigated in the current experiment. Additionally, previous studies conducted by Carlos and Edwards (1998), Ravindran et al. (1995) and Marounek et al. (2008) also suggested that laying hens utilize phytate P differently as they become older, and also suggested that the gastrointestinal pH or maturation of digestive tract would have changed as chicks become older, which consequently affect the potential phytase actively. Besides, researchers typically only measure phytase at pH 5.5 for dietary formulation, which may also lead to the different phytase activity observed in previous studies, as the pH of gastrointestinal tract from which the phytate complex was liberated is different. So it is appropriate to postulate that the equal effects of the both phytase observed in the current were the results of different gastrointestinal physiological in the older laying hen.

Feed intake

In the current study, although the negative effects of providing 68-week-old laying hens P-deficient diets were quite evident, the severity of P deficiency was not manifested in terms of feed intake. This finding is similar to the results of Hughes et al. (2008), who reported that no

significant effects on feed consumption were observed in response to either different NPP levels or supplemental phytaset of laying hens. However, Payne et al. (2005) and Wu et al. (2006) reported that feed intake increased linearly with the NPP levels in broiler and laying hens, respectively. And the negative effects were subsequently attenuated by phytase supplementation to the lower NPP diets. Despite this finding, the exact reasons for the increase are not known; therefore, further studies should be conducted to evaluate the underline metabolism that how phytase supplementation affect the feed intake.

Egg production

The egg production by laying hens decreased significantly by 11.95% in response to the NC diets. In addition, although supplementation of the NC diet with phytases led to a linear restoration of performance, the production of eggs by hens that received a diet supplemented with phytase at 500 FTU/kg was still significantly lower than that of hens that were fed the PC treatment. The results of the current experiment were partially consistent with those of studies conducted by Lim et al. (2003) and Wu et al. (2006), who found that supplementation of an NPP-deficient diet with phytase at 300 FTU/kg resulted in a significant improvement in egg production. The results of the current study suggest that phytase supplementation at 500 FTU/kg induced a beneficial effect on egg production. This contention was supported by the observed improvement in digestibility and serum concentration in the blood, which are indicative of an increase in available nutrients. However, egg weight responded differently to the different phytases. Specifically, OPT supplementation led to a linear increase in egg weight until the levels observed in the PC treatment were attained, whereas no significant difference in the weight of eggs produced by hens that were fed the NAT diet was observed. Previous studies have also revealed controversial results regarding egg weight in response to phytase supplementation. For example, Silversides et al. (2006) reported that phytase supplementation can enhance the egg production and egg weight of birds, whereas Wu et al. (2006) and Hughes et al. (2008) reported that phytase supplementation had no significant effect on egg weight. The variations in aforementioned studies may have attributed to the relationship between feed intake, egg production and egg weight, which is generally recognized that feed intake is positively associated with egg production while egg weight relates to egg production negatively. However, the reason may not explain the results in herein study, the results observed in current experiment may be due to the highly egg weight observed in the OPT2 treatment, which can attributed to the high phytase activity mostly attenuate the negative effect in the NC diets and partially postpone the depression of laying hen performance at this period. However, the exact reason for the results observed in the present experiment is unknown, further study may be conducted to evaluate the laying hen physiology and performance to phytase supplementation at this age.

Eggshell quality

Eggshell quality is the most important egg quality to be considered in poultry breeding programs. Eggshell quality characteristics, such as shell thickness and shell breaking strength, primarily depend on Ca aggregation into calcium carbonate, organic materials, and trace minerals (Chowdhury and Smith, 2002; Mabe et al., 2003). In general, eggshell quality increases concurrently with the increase in digestibility that occurs in response to phytase supplementation. As expected, phytase supplementation exerted a beneficial effect on eggshell quality in the current experiment. Specifically, the eggshell breaking strength increased in a linear and quadratic manner in response to the addition of ECP and ADP, respectively. These findings are similar to the results of studies conducted by Um and Paik (1999) and Liu et al. (2007). However, no linear effect on eggshell thickness was observed in response to phytase supplementation, even though phytase supplementation at 500 FTU/kg led to an increase in the eggshell thickness to levels comparable to those of eggs produced by hens that received the PC diets, which indicate that supplemental phytase exerted a beneficial effect on eggshell thickness. The reason for the lack of a linear effect was not determined in this study; however, it may have occurred due to the small sample number, because of the large variation among individual eggs or as a result of replication and other systematic experimental errors.

Apparent total tract nutrient digestibility

The results of our metabolic trial indicated that the digestibility of P increased significantly in response to phytase supplementation when compared to the level observed in the NC group. These findings demonstrate that the phytate phosphorus was, to some extent, cleaved by phytase supplementation. Similar results were also observed by Liu et al. (2007), who reported a significant improvement in P digestibility in response to supplementation of the diet of Hy-Line brown layers with microbial phytase. Additionally, the Ca and N digestibility was also increased with the supplementation of the phytase. At first glance, these results support that points that the phytate-bound minerals other than P were released in response to microbial phytase supplementation of the NC diets. However, based on the observation that the digestibility of Ca and N were also negatively impacted by reduced inclusion rate of inorganic phosphorus in NC diet

compare to PC diet, it is presumptuous to state that any increase in digestibility of Ca and N due to phytase supplementation was due to a release of phytate-bound nutrients. However, the effect of phytase on Ca and N digestibility remains unclear, it can be postulated that the previous increase may be more appropriately attributed to simply increased phosphorus absorption as a result of phytase, which is partially in agreement with Martinez-Amezcua et al. (2006), who suggested that it is possible to postulate that the positive effects of phytase on amino acid digestibility were due to alleviating the P deficiency, as Phosphorus is an important and necessary mineral for membrane function and active transporters such as the Na/K ATPase pump that are essential for amino acid absorption. Similarly, to Ca digestibility, it is generally suggested that absorption and retention of Ca is influenced by the ratio of Ca: P, and study conducted by Underwood and Suttle (1999) also suggested that the improvement in skeletal Ca retention is accompanied by improved retention of P because Ca is only well utilized for skeletal growth when P is available at the same time, which may also supported the explanation that increased Ca digestibility is due to increased phosphorus absorption as a result of phytase Additionally, the Ca digestibility and N digestibility observed in this study were increased to levels observed in the PC treatment in response to phytase supplementation at 500 FTU/kg, whereas the digestibility of Ca, P and N was significantly lower in hens that received the NC diet supplemented with phytase at 250 FTU/kg than in those that received the PC diet. These findings indicate that phytase supplemental at 500 FTU/kg is sufficient to induce the hydrolysis of phytate in the 68-week-old of laying hens.

It is well known that the amount of dry matter (also known as dry weight) is a measurement of the mass of a sample that has been completely dried, and includes the weight of proteins, fat, milk, sugars and minerals in the sample. It is in the nature of things that phytase supplementation have positive effects on DM digestibility by inducing improved P, Ca and N digestibility. The results of previous studies have also suggested that phytase supplementation can improve DM digestibility by releasing bound organic nutrients such as protein and starch (Ravindran and Bryden, 1997). The significant difference in DM digestibility that was observed between hens that received the PC treatment and those that received phytase supplementation at 500 FTU/kg in the present study may be explained as follows: i) Other improvements in digestibility that were not investigated in the current experiment may have led to the significant effects observed in the factors evaluated here.

Serum Ca and P

Previous studies have stated that the serum P concentration seems to be less indicative of phytase efficacy than total tract P digestibility and retention of dietary P or the ash percentage of the 10th rib and metacarpals (Yi and Kornegay, 1996; Jongbloed and Mroz, 1999). However, in the current study, the increase in available minerals was also observed in the serum P and Ca of laying hens that were fed the phytase diet, and these values were found to be highly correlated with egg production and digestibility. Similar results were observed in a study conducted by Rama-Rao et al. (1999), who reported that the serum P increased linearly in response to phytase supplementation, but no linear effect was observed on serum Ca, and this may have occurred due to an antagonistic effect of serum Ca and P. In the current study, phytase supplementation led to an increase in serum Ca level to the level observed in the PC group.

CONCLUSION

The results of this study indicated that the negative effects of feeding hens 0.20% available phosphorus were quite evident for most of the characteristics that were investigated. However, supplementation of the diets with phytase at 500 FTU/kg can mostly compensate these negative effects in the current study. In addition, the abilities of NAT and OPT to liberate phytate-bound complexes were similar when they were included in 0.20% available phosphorus diets of laying hens. Taken together, these findings indicate that either source of phytase can be fed to commercial first cycle laying hens at 500 FTU/kg to effectively replace inorganic phosphorus when economically justified.

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