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Comparison Difference Levels of Phytase Enzyme Supplementation on Laying Hen Performance, Egg Quality and Some Blood Parameters

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ABSTRACT

Eighty-four Hy-line pullets at 19 weeks of age as a commercial egg strain were used and divided into 12 groups (4 treatments×3 replicates). A corn-soybean meal basal diet formulated and served as control. The basal diet was supplemented with three levels of phytase (500, 750 and 1000 FTU kg^{-1} of feed) for treatment groups, two and three, respectively. To evaluate, the effect of phytase supplementation on egg number, egg weight and egg mass egg production, feed consumption, feed conversion ratio and determine minerals. The results show that the addition of phytase increased egg number, overall egg number, feed consumption and feed conversion ratio but the difference not significant. The effects of phytase supplementation on egg quality did not show any significant effect on egg quality measurements excepted egg weight mean shell thickness, shell breaking, shell percentage and Huag unit. Also, it can indicated that phytase supplementation significantly improved calcium (Ca), phosphours (P), magnesium (Mg) and potassium (K) in serum from the present study it can concluded that phytase supplementation at level (1000 FTU kg^{-1} of feed) in period second four months and third four months had significant effect on most of laying hen performance, egg quality and some blood parameters.

Key words: Phytase, corn-soyabean, egg production, egg quality, phosphorus, calcium, magnesium

INTRODUCTION

Phosphorus (P) is an essential mineral in all feeds for poultry. Phosphorus is one of the mineral essential for development structurally metabolically growth and production. Moreover, P is considered as an expensive nutrient that commonly supplemented in poultry diets. It represents the third most expensive nutrients following protein and energy. Birds diets are generally formulated based on corn, soyabean meal, approximately two-thirds of the total P in plants, which are the major constituents of poultry diets, is in the form of phytate (Viveros *et al.*, 2000).

A number of studies have demonstrated that use of microbial phytase supplementation in feeding poultry has the ability to hydrolysis, releasing phytic acid in phosphate form NRC (1994) and adding microbial phytase in laying hen diet improves phytate P utilization and productive performance (Boling *et al.*, 2000a, b; Jalal and Scheideler, 2001; Narahari and Jayaprasad, 2001; Keshavarz, 2003; Lim *et al.*, 2003; Plumstead, 2007). Also increased plasma P but had no effect on plasma Ca or Mn. Plasma Zn concentration was increased only when a high level of AMJC (equivalent to 1,000 U phytase kg^{-1} of feed) was used (Lan *et al.*, 2002).

Also, Francesch *et al.* (2005) and Jalal and Scheideler (2001) saw an improvement in egg production, hen weight gain, feed conversion, egg mass and feed consumption in hens that were fed a diet low in NPP with supplementary phytase when compared to hens fed a low NPP diet without supplemental phytase.

Wu *et al.* (2006) reported that Phyzyme or Natuphos supplementation into diets containing 0.11% nonphytate phosphorus significantly reduced excreta P (approximately 58 and 54%, respectively) with no adverse effect on egg production and egg mass. Plumstead (2007) studied the effects of varying dietary nonphytate phosphorus level with or without added phytase enzyme on performance of broiler breeders from 29 to 64 week of age. And found that eggs per hen housed, hen day egg production (%), fertility (%) and feed per dozen eggs were increased when phytase was added by 500 FTU. Addition of phytase to the 0.15% nonphytate phosphorus diet improved total hen housed egg production to the levels equal or better than the hens fed the 0.35% nonphytate phosphorus diet (Hughes *et al.*, 2008).

Santoyo *et al.* (2009) showed that a feed intake, daily body weight changes, egg production and P excretion were increased by phytase supplementation at levels (3000 and 600 FTU) while on egg weight produced no effect was observed by phytase.

While, Liebert *et al.* (2005) found that no significant differences in feed intake, egg production and egg weight when microbial phytase (300 U kg^{-1}) supplementation to corn-soybean meal and wheat-soybean meal basal diets fed Lohmann Brown (22 to 61 week).

Many researches noted the effect of phytase on egg quality. Cabuk *et al.* (2004) added Phytase (300 FTU kg^{-1}) into the control diet (4.5 g AP kg^{-1}) and the low 3.0 g AP kg^{-1} diet and reported that, there were no significant differences between the treatments in eggshell weight, eggshell thickness, eggshell strength and cracked/broken eggs under high ambient temperature.

Casartelli *et al.* (2005) reported that in the experiment with commercial fed Hi-ssex Brown hens on diets contained different phosphorus sources supplemented with phytase (0, 1000 FTU kg^{-1}) during the periods from 32 to 48 and 48 to 64 week of age (during peak and post peak egg production, respectively). They found that, phytase supplementation improved egg shell quality (specific gravity and shell percentage) during peak of egg production (from 32 to 48 weeks of age). However, phytase supplementation had insignificant effects on egg shell quality during past peak of egg production (from 48 to 64 weeks of age). Similar results were found by Um and Paik (1999) when fed laying hens maize-soy diets with phytase supplementation (500 FTU kg^{-1}) and found no differences were in eggshell strength and Haugh units, only subtle differences in specific gravity and eggshell thickness. On the way, Musapuor *et al.* (2005) reported that dietary phytase caused a significant ($p<0.05$) decrease in plasma alkaline phosphatase activity and excreta phosphorus percentage. Also phytase had no beneficial effect on egg shell quality traits. Panda *et al.* (2005) found that no additional advantage resulted from enhancing the NPP levels beyond 1.8 g kg^{-1} or phytase adding (500 U kg^{-1}) to a diet containing 1.8 g kg^{-1} NPP on shell weight, shell thickness, shell strength and tibia strength but significantly enhanced egg weight, specific gravity and Hugh units.

To improve the economic efficiency and reduce environment phosphorus pollution and at the same time improve the productive performance, this investigation aim to study the effect phytase supplementation on productive performance and mineral utilization of laying hens.

MATERIALS AND METHODS

This experiment was carried out at the Poultry Research Farm, Faculty of Agriculture, South Valley University, Qena, Egypt from 2009 to 2010. Eighty four Hy-line pullets as a commercial

Table 1: Composition and calculated analysis of the experimental diet

Items	(%)
Ingredients	
Yellow corn	60.90
Soybean meal (44% Cp)	21.60
Corn gluten meal (60% Cp)	6.00
Vit and Min. premix*	0.30
Wheat bran	0.45
Dicalcium phosphate	1.36
Calcium carbonate	8.95
Salt	0.40
DL-methionine	0.04
Total	100.00
Calculated analysis	
ME (Kcal Kg^{-1})	2766.00
Crude protein (%)	18.45
Crude fiber (%)	2.68
Crude fat (%)	2.78
Ca (%)	3.87
P available (%)	0.38
Lysine (%)	0.85
Methionine (%)	0.40

*Vitamins and minerals premix provided per kilogram of the diet: Vit A, 1000 IU; D3 2000 ICU; Vit E, 10 mg; Vit K, 1 mg; B1, 10 mg; B2, 5 mg; B6, 1500 mg; B12, 10 mg Pantothenic acid, 10 mg; Nicotinic acid, 30 mg; Folic acid, 1 mg; Biotin, 50 mcg; Chloride, 500 mg; copper, 10 mg; iron, 50 mg; Manganese, 60 mg; Zinc, 50 mg, and Selenium, 0.1 mg

egg strain at 19 weeks of age were used in this experiment. The laying hens were allocated to 12 groups (4 treatments×3 replicates) with 7 hens in each replicate. A corn-soybean meal basal diet formulated and served as control. The basal diet was supplemented with three levels of phytase (500, 750 and 1000 FTU kg^{-1} of feed) for treatment groups one, two and three, respectively. The composition and calculated analysis of experimental control diet illustrated in Table 1. Each replicate was kept in wire cage of 61×55×45 cm for 83 weeks of age in a closed system house using controlled system. Standard commercial management of layer birds was used throughout the experiment. During experimental period hens were kept at 65% relative humidity and 22°C temperature and the photoperiod was 16 h per day and light intensity ranged from 20 to 25 luxes. Feed and water were available *ad libitum*. All hens were kept under similar adequate managerial and hygienic conditions during experimental period.

Egg number, egg weight and egg mass were recorded daily and calculated periodically every 16 weeks. Feed consumption and feed conversion ratio (as g feed/g egg mass) recorded weekly and calculated periodically every 16 weeks. Dead birds were recorded daily throughout the experimental period and expressed as percentages.

At the end of experiment five layers from each treatment weighted and slaughter to take blood samples for analysis to determine posphours (P), potassium (K), magnesium (Mg) and calcium (Ca) concentrations. Egg quality measurements were carried out at 36 week of age. Measurements were taken on total of 20 eggs chosen randomly from each group.

Statistical analysis: Data collected were subjected to ANOVA by applying the general linear models procedure of SAS software (SAS, 1996). Duncan (1955) was used to detect differences among means of different groups for each strain.

RESULTS AND DISCUSSION

From the results in Table 2 it can be observed that, the addition of phytase enzyme at a levels of 500, 750 and 1000 (FTU) kg^{-1} of feed led to an increase in the number of eggs compared to treatment comparison (control). These results are agree with Plumstead (2007) and Boling *et al.* (2000b) who reported that phytase improves phosphorus utilization for laying hen supported optimal egg yield.

The addition of phytase enzyme levels 500, 750 and 1000 (FTU) kg^{-1} of feed during the experimental period illustrated in data of Table 2 shows that the main effect of phytase supplementation on egg weight was not significant. This results just as results of Scott *et al.* (1999) indicated that phytase supplementation had no significant effect on egg weight. Also, Yossef *et al.* (2001) who reported that phytase supplementation was not significant effect on egg weight at the average of 5-period evaluated in that all nonphytate phousphours levels. While presented results are disagreement with findings of Peter (1992) reported that, laying hens fed a diet with phytase

Table 2: Effect of phytase supplementation in the diet of Hy- Line pullets on egg number and egg weight during experimental periods

Treatments	First four months	Second four months	Third four months	Fourth four months	Overall mean
Egg number					
Control	69.10±3.31	73.00±1.30^{b}	60.43±5.17	42.95±2.07	61.37±2.96
T1	69.14±2.54	76.29±3.84^{ab}	63.57±1.15	44.71±2.79	63.42±2.58
T2	71.81±2.53	80.81±1.45^{a}	64.24±1.13	52.90±6.64	67.44±2.93
T3	75.76±1.90	83.33±0.76^{a}	71.05±1.01	61.05±3.18	72.79±1.71
Egg weight					
Control	52.39±3.67	71.15±11.13	61.18±0.85	62.76±0.37	61.86±4.01
T1	50.37±0.62	61.52±3.59	61.95±1.37	60.90±0.88	58.68±1.62
T2	50.51±0.85	58.55±0.39	52.90±6.64	62.65±0.50	56.15±2.09
T3	51.14±0.19	58.15±0.78	62.62±0.75	60.40±1.00	58.08±0.68

Control = Basal diet (un-supplemented); T1 = Control+500 FTU kg^{-1} feed; T2 = Control+750 FTU kg^{-1} feed; T3 = Control+1000 FTU kg^{-1} feed; a and b : Mean values bearing different superscript at the same column in each parameters differ significantly (p>0.05)

Table 3: Effect of phytase supplementation in the diet of Hy-Line pullets on egg mass, feed consumption and feed conversion during experimental periods

Treatments	First four months	Second four months	Third four months	Fourth four months	Overall mean
Egg mass					
Control	3578.71±114.19	5187.57±752.24	3709.00±389.50	2692.00±131.96	3791.82±346.97
T1	3485.33±162.99	4666.19±53.67	3935.38±32.10	2720.71±154.74	3701.90±100.87
T2	3714.05±94.77	4732.29±105.57	3993.90±88.99	3310.00±411.28	3937.56±175.15
T3	3875.05±102.33	4845.52±37.31	4455.71±61.99	3690.00±228.34	4216.57±107.49
Feed consumption					
Control	7387.71±190.09	9681.14±79.74	9539.33±52.27^{ab}	8275.29±263.17	8720.86±146.31
T1	7224.33±197.82	9700.67±374.54	9431.90±64.62^{b}	9668.00±54.85	9006.22±172.95
T2	7542.52±99.42	9354.00±197.14	9858.00±159.17^{a}	9064.52±348.22	8954.76±200.98
T3	7352.10±113.58	9893.62±50.12	9831.62±86.63^{a}	8822.67±284.30	8975.00±133.65
Feed conversion					
Control	3.28±0.75	2.20±0.06	2.71±0.11^{ab}	5.08±1.37	3.31±0.57
T1	5.40±2.03	2.22±0.10	2.46±0.06^{ab}	4.98±1.39	3.76±0.89
T2	4.06±1.36	2.00±0.04	2.92±0.30^{a}	4.96±1.71	3.48±0.85
T3	2.79±0.47	2.08±0.05	2.27±0.06^{b}	3.51±0.60	2.66±0.29

Control = Basal diet (un-supplemented) T1 = Control+500 FTU kg^{-1} feed; T2 = Control+750 FTU kg^{-1} feed; T3 = Control+1000 FTU kg^{-1} feed; a and b: Mean values bearing different superscript at the same column in each parameters different significantly (p>0.05) had significantly higher egg weight than hens fed on the same diet without supplemental phytase (control). The same was found by Ciftci *et al.* (2005) who reported that the highest egg weight was determined in 600 U of microbial phytase kg^{-1} enzyme group (70.29 g) and those following the 300 U of microbial phytase kg^{-1} enzyme group (67.37 g) and control group (64.31 g), respectively ($p<0.01$).

Increasing egg production as a results of Phytase supplementation may be due to that phytate presented in the most ingredients of poultry diet is capable of forming complexes with essential nutrients such as proteins and some in organic cations. The use of phytase led to release these essential nutrients and improving nutritional value of poultry diet which can be positively effect on productive performance of laying hens (Panda *et al.*, 2005). In addition, several studies have examined the influence of microbial phytase on protein digestion and utilization in poultry (Kornegay, 1996; Klis and Versteegh, 1996; Yi *et al.*, 1996; Sebastian *et al.*, 1997). They reported that phytase supplementation poultry diet improved protein digestion and utilization. Moreover, Ravindran *et al.* (1999) and Kies *et al.* (2001) reported that phytase supplementation in poultry diet increased nitrogen retention in broiler chickens. Similar resulted obtained by Klis *et al.* (1996) in layer hens. Moreover, exogenous phytase is effective in improving utilization of phytase to bound mineral in the diet (Biehl *et al.*, 1995). There is evidence to indicate that hydrolysis of phytate by microbial supplementation has improved absorption or retention of calcium, manganese, iron and magnesium in poultry (Qian *et al.*, 1997; Sebastian *et al.*, 1998; Ravindran *et al.*, 2000).

Phytase supplementation by levels (500, 750 and 1000 (FTU) kg^{-1} of feed improved egg mass during the whole laying egg period (Table 3). These results are agreement with Jalal and Scheideler (2001) Who found that, supplementation of phytase in normal, corn-soybean meal diets improved egg mass. Increase egg mass may be due the increase egg production as egg number/hen (Table 2).

As presented in Table 3, the addition of phytase enzyme had insignificant effect on whole period feed consumption. The present results are agreement with Keshavarz (2003) who reported that no significant effect of phytase supplementation (300 U phytase kg^{-1}) on feed consumption. While data in Table 3 illustrated that the addition of Phytase significantly improved the overall mean feed conversion ratios. Improving of feed conversion ratio (feed consumption/egg mass) as result of phytase supplementation in the diet due to significantly increase of egg production and at the same time insignificantly differences in feed consumption among treatments (Table 3). The present results are agreement with Jalal and Scheideler (2001) who found that supplementation of phytase in normal, corn-soybean meal diets improved feed conversion.

Data related to egg quality traits including shell breaking strength, shell thickness, shape index, yolk colour, yolk index, yolk , shell, albumen% and Hugh unit are presented in Table 4. Results showed that Phytase supplementation by levels (1000 FTU kg^{-1} of feed) significantly improved shell breaking strength, while levels (500, 750, 1000 FTU kg^{-1} of feed) of phytase supplemented laying hen diet significantly increased shell thickness, shell% and Haugh Unit. The present results are agreement with Punna and Roland (1999), Narahari and Jayaprasad (2001) and Metwally (2006) they found a beneficial effect of phytase supplementation on shell quality. Increasing shell percentage as a result of phytase supplementation due to. First the improving shell thickness and second to beneficial effect of phytase supplementation on the utilization of phosphorus by poultry (Liebert *et al.*, 2005; Plumstead, 2007). Also, the present result are agreements with Casartelli *et al.* (2005) who studied that the effect of phytase supplementation

Table 4: Effect of phytase supplementation in the diet of Hy-line pullets some of egg quality

Item	Control	T1	T2	T3
Shell breaking strength	2.52±0.19[c]	2.58±0.13[c]	2.68±0.16[b]	2.70±0.15[a]
Shell thickenes	0.33±0.56[c]	0.34±0.53[b]	0.35.±0.66[b]	0.36±0.71[a]
Shap index	77.19±0.59	76.37±0.49	76.49±1.14	77.69±0.34
Yolk colour	8.35±0.15	8.40±0.13	8.05±0.14	8.30±0.13
Yolk index	40.07±0.62	39.50±0.61	40.89±0.73	40.96±0.81
Yolk (%)	28.07±0.57	27.83±0.46	27.52±0.50	27.97±0.29
Shell (%)	11.15±0.15[c]	11.68±0.19[c]	11.94±0.14[b]	12.14±0.17[a]
Albumen (%)	59.79±0.66	60.35±0.38	60.23±0.53	61.33±0.53
Haugh unit	71.13±0.97[b]	74.15±1.45[ab]	71.68±1.09[b]	76.07±1.16[a]

Control = Basal diet (un-supplemented) T1 = Control+500 FTU kg^{-1} feed; T2 = Control+750 FTU kg^{-1} feed; T3 = Control+1000 FTU kg^{-1} feed; a,b and c : Mean values bearing different superscript at the same row in each parameters differ significantly ($p>0.05$)

Table 5: Effect of phytase supplementation in layer diet on calcium (Ca), phosphorus (P), magnesium (Mg) and potassium (K), concentrations (mg 100 mL^{-1}) in serum

Item	Control	T1	T2	T3
Ca	18.23±0.23[c]	18.130±0.35[c]	25.92±0.47[b]	26.00±0.46[a]
P	14.70±0.12[c]	15.100±0.14[c]	16.20±0.19[b]	17.00±0.18[a]
K	16.00±21.13	18.414±0.76	18.55±0.45	18.43±0.53
Mg	2.13±0.75[c]	2.690±0.60[c]	3.00±0.67[b]	38.91±0.564[a]

Control = basal diet (un-supplemented) T1 = Control+500 FTU kg^{-1} feed; T2 = Control+750 FTU kg^{-1} feed; T3 = Control+1000 FTU kg^{-1} feed; a,b and c: Mean values bearing different superscript at the same raw in each parameters different significantly ($p>0.05$)

(0, 1000 FTU kg^{-1}) on egg quality parameters from 32-48 weeks of age and from 48-64 weeks of age and found that, in the first trail, phytase improved shell percentage but, during the post peak period, there were no significant effects of phytase addition. While the present results are disagreements with Jalal and Scheideler (2001) they indicated that there were not significant effects of phytase supplementation in normal, corn-soybean meal diets on dry and wet shell percentage. Also, Panda *et al.* (2005) found that there were no significant effects of phytase supplementation on Hugh units.

Table 5 showed that there are significant effect between the treatments in the concentrations of phosphorus, manganese and calcium is reflected also added Phytase at the level of 1000 FTU kg^{-1} of feed gave the highest results in the concentration of phosphorus, calcium, manganese compared to treatment comparison (control) shows less results while increased was not significant in the case of potassium. These results are well coordinate with those of Attia *et al.* (2001) who reported that a significant increased in plasma Ca was noticed due to phytase addition to diets contained intermediated protein levels and P were greater ($p>0.05$). In contrast Roberson and Edwards (1994), El-deeb *et al.* (2000) and Lan *et al.* (2002) showed that phytase had no significant effect on plasma Ca.

CONCLUSION

From the present study the results conclude that Phytase supplementation can improve layer productive performance, egg quality and mineral utilization.

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