

ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF POULTRY SCIENCE

ANSI*net*

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

Egg Production and Calcium-Phosphorus Utilization of Laying Hens Fed Diets Supplemented with Phytase Alone or in Combination with Organic Acid

Mustafa Sari¹, Ahmet G. Önel¹, Mehmet Daskiran² and Özcan Cengiz¹

¹Department of Animal Nutrition and Nutritional Diseases, Veterinary Faculty,
Adnan Menderes University, 09016, Aydın, Turkey

²Johnson & Johnson Corporate Science and Technology, 410 George Street,
New Brunswick, NJ, USA, 08901

Abstract: An experiment was conducted to determine the effects of dietary organic acid and phytase supplementation on performance and calcium and phosphorus utilization in laying hens. Two hundred 23-wk-old Brown Nick layers were randomly allocated into 50 cages (42 x 50 cm) each containing 4 birds. Each dietary treatment had 5 replications and each replication comprised two adjacent cages. Corn-soybean meal based rations were used. Five dietary treatments were formed as followed: (1) a basal ration with no supplemental P (negative control, NC) (2750 kcal ME/kg, 17% CP, 0.34% total P, 3.8% Ca), (2) basal ration supplemented with dicalcium phosphate (1.4%) (positive control, PC), (3) basal ration supplemented with 0.035% phytase (ZY Phytase II-5, NC+P), (4) basal ration supplemented with 1.0% organic acid (Salstop SD, NC+OA) and (5) basal ration supplemented with both 0.035% phytase and 1.0% organic acid (NC+POA). Water and feed were provided for ad libitum consumption and a lighting program of 16 h light : 8 h dark was applied throughout the study. NC treatment resulted in body weights that were lower ($p<0.05$) than those of hens fed diets supplemented with phytase, organic acid, or both. Hens fed diets supplemented with dicalcium phosphate, phytase and organic acid and phytase had higher egg production ($p<0.001$) (91.3, 86.1 and 93.7% respectively) compared to hens fed either basal diet (78.5%) or basal diet with organic acid supplementation (78.6%). Additionally, the unsupplemented or organic acid supplemented diet had lower FI and higher FCR ($p<0.01$). Hen tibia ash and serum calcium levels were not affected by the dietary treatments whereas diets supplemented with dicalcium phosphate, phytase and phytase + organic acid had higher ($p<0.01$) serum inorganic phosphorus. The results of this study indicate that dietary phytase improves P utilization in corn-soybean meal based diets with no supplemental P and acidification of gastrointestinal tract may further improve this utilization.

Key words: Laying hen, organic acid, phytase, egg production and quality, calcium-phosphorus utilization

INTRODUCTION

Phosphorus (P) is an essential mineral for metabolism and egg production of laying hens. In addition to this, with calcium (Ca) it has a major role in the formation and maintenance of bone (Underwood and Suttle, 1999; Waldroup *et al.*, 2000). Furthermore, P is the third most expensive component of poultry diets after energy and amino acids. Plant origin feedstuffs contain a high proportion of poultry feeds because they are cheaper than their animal counterparts. However, approximately two-thirds of P in plant origin feedstuffs is poorly digested because it is in the form of Phytic Acid (PA), which is poorly hydrolyzed by poultry, as they do not have sufficient amounts of phytase enzyme that hydrolyses PA (Bedford, 2000). Because of the low ability of laying hens to hydrolyze PA-bound P, inorganic P sources, which are costly are added to diets for satisfying the P need of birds. This resulted in increased cost of feeding (Selle and Ravindran, 2007). Thus, it is necessary to optimize

the utilization of P by poultry to reduce the cost of feeding. Cereal based poultry diets supplemented with microbial phytase result in increased digestibility and availability of phytate bound P, Ca, zinc and copper. There are a number of factors affecting phytate hydrolysis, such as dietary Ca, inorganic P and vitamin D₃ levels and the age and genotype of birds, there is a wide disagreement concerning the ability of poultry to utilize phytate P. Furthermore, clear benefits have been shown in terms of increased availability of phytate-bound minerals and crude protein and reduced environmental pollution through lower levels of P and nitrogen excretion (Singh, 2008).

Studies subjecting the influence of microbial phytase in the digestive tract of poultry revealed that its maximum activity could be reached at low pH values. Adding Organic Acid (OA) to feed can lower the gastric pH (Garrido *et al.*, 2004). The organic acids like citric acid can reduce the pH of the digesta, which can then result

in increased dissociation between PA and minerals (Maenz *et al.*, 1999) and increased activity of most phytases, which express their optimal activity at low pH (Simon and Igbasan, 2002). Low gastric pH accelerates the conversion of pepsinogen to pepsin, which improves the absorption rate of proteins, amino acids and minerals (Omogbenigun *et al.*, 2003; Youn *et al.*, 2005). In this respect, some researchers studied potential beneficial effects of organic acids (especially citric acid) on the efficacy of microbial phytase preparations in pigs and broiler chickens (Li *et al.*, 1998; Brenes *et al.*, 2003). There is limited report to date of such a study in laying hen.

The efficacy of phytase on performance and Ca and P digestibility in layers fed a corn and soybean-based diet has been well established by Lim *et al.* (2003), Panda *et al.* (2005) and Wu *et al.* (2006). However, there is still little information available on whether combination of phytase with organic acids can improve the availability of phytate P in laying hens. Therefore the objective of the current study was to determine alone or combined effect of dietary OA and microbial phytase supplementation on performance, digestibility of Ca, P and mineralization parameters of tibia bone in layer hens and its effect on efficacy of microbial phytase in corn-soybean diets with low available P level.

MATERIALS AND METHODS

Bird management: A total of 200 Brown Nick layer hens at 23 wk of age were allocated randomly into 5 treatments, with 5 replicates per treatment and 8 layers per replicate. All birds were housed in 3-layered cages (525 cm²/bird) and offered feed and water *ad libitum*. Each dietary treatment had 5 replications and each replication comprised of two adjacent cages (42 x 50 cm). During the experimental period, birds received 16 h/d of manipulated lighting and ventilation at a natural ambient temperature of 20 to 25°C. Egg production and health status of layers were observed as the adjustment period for 1 wk before the experiment commenced. The experimental diets were fed for 20 wk (December to April). Experimental birds were handled with care to avoid unnecessary discomfort and experiment was approved by the Adnan Menderes University, Animal Care and Use Committee.

Experimental diets: The basal diet contained 17.0% CP and 2,750 kcal/kg of ME (Table 1). Other nutrients followed NRC (1994) recommendations. The experiment was performed with 5 dietary treatments: (1) a basal diet with no supplemental P (NC) (2750 kcal ME/kg, 17% CP, 0.34% total P, 3.8% Ca), (2) basal diet supplemented with dicalcium phosphate (1.4%) (PC), (3) basal diet supplemented with 0.035% (500 FYT¹ phytase/kg feed) phytase (ZY Phytase II-5 contains 1500 FYT 6-phytase in each g), (NC+P) (4) basal diet

Table 1: Nutrient composition of basal diet

Ingredients (%)	
Corn	63.65
Soybean meal	25.65
Ground limestone	9.80
Salt (NaCl)	0.35
Vitamin premix*	0.25
Trace mineral premix**	0.20
DL-Methionine	0.10
Chemical composition (Calculated)	
Metabolizable energy, kcal/kg	2750.00
Crude protein, %	17.00
Calcium, %	3.80
Total phosphorus %	0.34
Available P, %	0.11

*Each 2.5 kg of vitamin premix contained; 15 000 000 IU vitamin A, 3 000 000 IU vitamin D₃, 100 000 mg vitamin E, 5 000 mg vitamin K₃, 3 000 mg vitamin B₁, 6 000 mg vitamin B₂, 40 000 mg niacin, 12 000 mg calcium D-pantothenic, 8000 mg vitamin B₆, 30 mg vitamin B₁₂, 1 000 mg folic acid, 150 mg D-biotin, 80.000 mg vitamin C.

**Each 2 kg of mineral premix contained; 100 000 mg manganese, 60 000 mg iron, 60 000 mg zinc, 5 000 mg copper, 300 mg cobalt, 2 000 mg iodine, 150 mg selenium, 400 000 mg colin chloride

supplemented with 1.0% organic acid (Salstop SD), (NC+OA) and (5) basal diet supplemented with both 0.035% phytase and 1.0% organic acid (NC+POA). Adjustment of P_{av} was made by using dicalcium phosphate and the OA was a commercial product, Salstop (Impextraco NV, Wiekevorstsesteenweg 38 - B 2220 Heist-Op-Den-Berg), which contained 54 400 mg propionic acid, 191 542 mg formic acid, 38 100 mg acetic acid, 4 100 mg sorbic acid, 15 300 mg ammonium propionate, 10 200 mg ortofosforic acid, 23 800 mg almond favour in each kg. Treatments 3 and 5 contained 0.035% of phytase (ZY Phytase II-5, Lohmann Animal Health GmbH & Co KG D-27454 Cuxhaven - Germany) in diet. Dicalcium phosphate, phytase and organic acid were supplemented to diets with replacement of corn, soy bean meal and limestone. The feeds were mixed at 14-d intervals to maintain stability.

Measurements

pH value of experimental feeds: Feed samples taken from experimental treatments were homogenized with deionized water after stirred on magnetic mixer for 10 mins. Then pH value of feed samples were determined according to method of Thompson and Hinton (1997) by using pH meter (Mettler Toledo, MP 120).

Productivity: To evaluate productivity, hen-day egg production (number of eggs/number of live birds x 100), hen-housed egg production (number of eggs/number of birds housed x 100), egg weight, soft-shell plus broken eggs, Feed Intake (FI), Feed Conversion Ratio (FCR) and mortality were measured. Egg production was measured every day after egg collection. The mean egg

weight was measured by the weekly average weight of eggs, excluding abnormal eggs (soft-shell plus broken eggs). The FI was measured once per week (FI = supply quantity - remainder). The FCR was calculated by the grams of FI per dozen eggs. Egg production, FI and FCR were calculated according to death bird number. To evaluate egg weight, eggshell weight and thickness, eggs were randomly collected and waited at room temperature for 24 h before measurements. The measurements were taken once per 2 week, 10 times in total (2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 wk). Eggshell thickness was measured using a gauge (Mitutoya, Dial Thickness Gage No: 7360, ± 0.01 mm).

Digestibility trial: At the end of the feeding trial (42th week), 10 birds from each treatments (50 birds totally) were randomly selected and placed into individual cages for digestibility trial. Similar environmental circumstances with feeding trial were provided in digestibility trial. Hens were fasted for 16 h after 2 d of adaptation period. Experimental diets were offered *ad libitum* for 4 d. Hens were again fasted for 16 h to unload the digestive tract. Faeces was collected in every 48 h by using aluminum papers placed on metal trays. Faecal samples were weighed and dried at 60°C and then they were grinded with 0.5 mm pore filter to homogenize. Samples were kept in nylon bags at 4°C before analysis. FI, BW and egg production of hens were recorded daily. Hens with egg production less than 4/7 d were discarded in adaptation and experimental periods. Digestibility of Ca and P were calculated by using differences of their amounts in feed and faeces.

Determination of Ca and P levels in blood serum: Blood samples from *V. Subcutanea Ulnaris* were taken from 10 birds from each treatment followed by digestibility trial. The absorbance of the mixture was read against distilled water at 519 nm with a Pharmacia Ultrospec 2000 spectrophotometer. Serum Ca and P levels were determined by using sepectrophotometry with commercial kits (Randox Laboratories Ltd., Calcium REF CA 590, Inorganic phosphorus REF PH 1016).

Determination of tibia ash: At the end of digestibility trial, hens were killed by cervical dislocation and their left tibia were collected for bone ash determination. Tibia were pooled by replicate pens, autoclaved and all adhering tissue was removed. Bones were dried for 24 h at 100°C, weighed and then dryashed for 24 h in a muffle furnace at 600°C. Ash weight of the tibia was expressed as a percentage of dry bone weight and as milligrams per tibia (Chung and Baker, 1990).

Dry matter, crude ash, total Ca and P (%) in experimental diets, faeces and tibia samples were determined according to the AOAC (2003) procedure. Ca analysis

was performed according to Eppendorph-Microliter method and inorganic P analysis was carried out accordance with the method of Younburg (Ersoy and Baysu, 1981) by using spectrophotometer.

Calculation and statistical analysis: Ca and P excretion (kg/t of feed consumed) determination was based on the analyzed Ca and P content of the faeces and was calculated as:

Ca or P in faeces (%) \times [1 kg of diet - (1 kg of diet \times DM digestibility of diet)]

Data were analyzed as a completely randomized design using the GLM procedures of SAS, 2002 (SAS Inst., Inc., Cary, NC). When a significant F-value for treatment means ($p < 0.05$) was observed in the ANOVA, treatment means were compared with Duncan's multiple range test (Duncan, 1955).

RESULTS

Data for BW are summarized in Table 2. The results of this study suggest that initial BW of laying hens at 23 wk of age was found similar between treatments. BW of birds fed diet containing 0.10% P_{av} (NC) were lower than PC, NC+P and NC+POA birds ($p < 0.05$). Moreover mean BW of birds was not significant among treatments NC and NC+OA with same P_{av} level and PC and NC+OA with different P_{av} level. Mortality results are given in Table 2. On day 42, mortality rate was recorded as 32.5% in treatments with no phytase supplementation and containing 0.10% P_{av} and treatment with OA.

The data for egg performance, FI and FCR are presented in Table 3. In the present study, DCP and phytase addition to NC diet improved egg performance ($p < 0.001$), FI ($p < 0.001$) and FCR ($p < 0.05$) significantly. Egg performance, FI and FCR were found similar in NC and NC+OA treatments with equal P_{av} levels. On the other hand, dietary OA supplementation did not alleviate the performance-depressive effects of low P_{av} level in diets. As seen in Table 3, inclusion of phytase and OA to low P_{av} diet improved egg performance, FI and FCR when compared to NC treatment ($p < 0.001$). Egg performance curve of the treatments are shown in Fig. 1. Egg performance of birds in NC treatment was initially recorded as 58.16%, then reached to pik level of 89.42% and dropped as 71.88-79.29% from 37 to 42 weeks of age. Decrease in egg performance of NC treatment formed an irregular curve in the study.

Mean egg weight, egg shell weight and egg shell thickness are summarized in Table 4. When compared to NC treatment, egg weight was increased significantly with dietary phytase supplementation. Moreover, mean egg shell weight adjusted for egg weight in NC+P were also lower than egg shell weights in NC treatment ($p < 0.05$) (Table 4).

Table 2: Mean Body Weight (BW, g) and mortality rate (%) of treatments in experimental period (n = 25)

Treatments ¹	BW			Mortality		
	23 week	33 week	42 week	23-32 week	33-42 week	23-42 week
NC	1524.60	1647.10 ^b	1649.40 ^b	15.0	20.6	32.5
PC	1516.90	1768.50 ^a	1787.10 ^a	0.0	5.0	5.0
NC+OA	1567.60	1734.90 ^a	1715.00 ^{ab}	12.5	22.9	32.5
NC+P	1537.60	1741.70 ^a	1780.40 ^a	7.5	0.0	7.5
NC+POA	1516.60	1792.50 ^a	1764.20 ^a	0.0	0.0	0.0
SEM	23.97	30.47	30.97			
P	0.5332	0.0127	0.0181			
Contrasts						
NC x NC+OA	NS	*	NS			
PC x NC+OA	NS	NS	NS			
NC x NC+P	NS	*	**			
PC x NC+P	NS	NS	NS			
NC x NC+POA	NS	***	**			
PC x NC+POA	NS	NS	NS			

^{a,b}Means within a treatment and column with different superscripts differ significantly (p<0.05).

NS: Not significant at p>0.05; *p<0.05, **p<0.01, ***p<0.001.

¹Treatments: NC = Negative Control; PC = Positive Control; NC+OA = Negative Control + Organic Acids; NC+P = Negative Control + Phytase; NC+POA = Negative Control + Phytase and Organic Acids

Table 3: Mean egg performance (%) and feed intake (g/day-hen) feed conversion ratio (feed, kg/1 dozen egg) of treatments in experimental period (n = 25)

Treatments ¹	Period								
	23-32 wks			33-42 wks			Overall		
Treatments ¹	Egg performance			Feed intake			Feed conversion ratio		
	23-32 wks	33-42 wks	Overall	23-32 wks	33-42 wks	Overall	23-32 wks	33-42 wks	Overall
NC	75.94 ^c	81.05 ^a	78.50 ^c	102.8 ^b	108.8 ^c	105.8 ^c	2.263 ^a	2.162 ^a	2.212 ^a
PC	89.03 ^{ab}	93.27 ^b	91.29 ^a	114.7 ^a	122.9 ^a	119.1 ^a	2.176 ^b	2.057 ^{bc}	2.112 ^b
NC+OA	72.69 ^c	83.92 ^d	78.59 ^c	102.0 ^b	113.2 ^b	107.9 ^c	2.454 ^a	2.182 ^{ab}	2.283 ^a
NC+P	83.97 ^b	87.93 ^c	86.06 ^b	109.6 ^a	115.2 ^b	112.6 ^b	2.152 ^b	2.046 ^c	2.096 ^b
NC+POA	90.36 ^a	96.72 ^a	93.69 ^a	114.5 ^a	120.4 ^a	117.6 ^a	2.151 ^b	2.013 ^c	2.078 ^b
SEM	1.868	1.027	1.090	1.990	1.370	1.240	0.0445	0.030	0.028
P	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0031	0.0001
Contrasts									
NC x NC+OA	NS	0.0799	NS	0.7827	*	NS	**	NS	0.0730
PC x NC+OA	***	***	***	***	***	***	***	0.0926	***
NC x NC+P	**	***	***	*	**	***	0.0721	**	**
PC x NC+P	0.0511	**	***	0.0747	***	***	NS	NS	NS
NC x NC+POA	**	***	***	*	***	***	NS	**	0.0621
PC x NC+POA	**	**	***	*	***	***	NS	NS	NS

^{a,b,c,d}Means within a treatment and column with different superscripts differ significantly (p<0.05).

NS: Not significant at p>0.05; *p<0.05, **p<0.01, ***p<0.001.

¹Treatments: NC = Negative Control; PC = Positive Control; NC+OA = Negative Control + Organic Acids; NC+P = Negative Control + Phytase; NC+POA = Negative Control + Phytase and Organic Acids

The data for digestibility trial are shown in Table 5. Phytase and/or OA supplementation to diets with 0.1% P_{av} had no significant effect on Ca and P utilization. Furthermore, serum Ca level was not affected from dietary P level or phytase and/or OA supplementation (Table 5). Serum inorganic P level was increased (p<0.01) with dietary phytase supplementation whereas OA addition did not effect. Serum inorganic P level was found similar in PC, NC+P and NC+POA treatments. Bone crude ash and bone Ca-P levels are given in Table 5. Phytase and/or OA supplementation to diets with 0.1% P_{av} did not effect bone ash or bone Ca and P level. Bone ash and P level in NC+OA treatment and P level in

NC+POA treatment was lower when compared to PC treatment (Table 5).

DISCUSSION

The results of the current study indicate that initial BW of laying hens at 23 wk of age was similar among the treatments (Table 2). BW of birds fed corn-soy based diet containing 0.10 % P_{av} (NC) were lower than PC, NC+P and NC+POA birds (p<0.05). This finding is similar with previous studies (Boling *et al.*, 2000b; 2000c; Punna and Roland, 1999; Van Der Klis *et al.*, 1997) which were demonstrated positive effects of dietary phytase supplementation on BW gain of laying

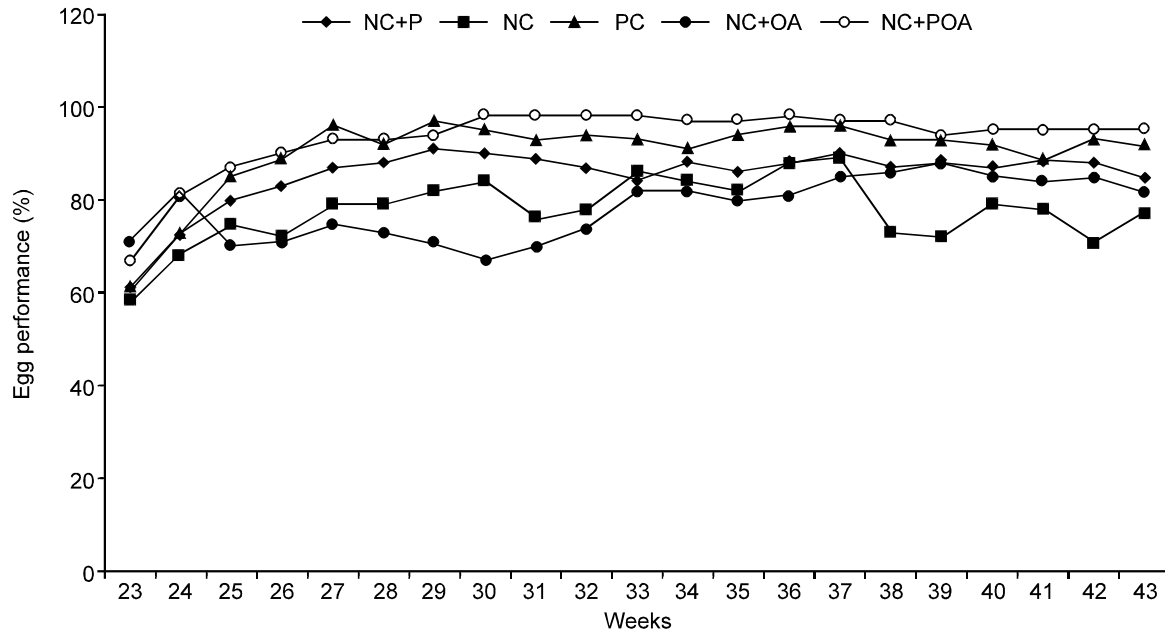


Fig. 1: Egg performance curves of treatments

Table 4: Mean egg weight (g) and egg shell weight (g) egg shell thickness (mm) of treatments in experimental period (n = 25)

Treatment ¹	Egg weight			Egg shell weight			Egg shell thickness		
	23-32 wks	33-42 wks	Overall	23-32 wks	33-42 wks	Overall	23-32 wks	33-42 wks	Overall
NC	60.60 ^{ab}	63.05 ^b	61.70 ^{bc}	6.108 ^a	6.341	6.224 ^a	0.4106	0.4035 ^a	0.4074 ^a
PC	59.92 ^{bc}	64.37 ^a	62.13 ^{ab}	5.942 ^b	6.236	6.086 ^b	0.4044	0.3977 ^{ab}	0.4011 ^{bc}
NC+OA	59.02 ^c	63.51 ^{ab}	61.16 ^c	6.136 ^a	6.347	6.239 ^a	0.4100	0.4018 ^a	0.4061 ^{ab}
NC+P	61.21 ^a	64.62 ^a	62.94 ^a	6.026 ^{ab}	6.238	6.129 ^b	0.4040	0.3969 ^{ab}	0.4004 ^c
NC+POA	59.16 ^c	61.86 ^c	60.51 ^d	5.979 ^b	6.333	6.154 ^{ab}	0.4045	0.3924 ^b	0.3985 ^c
SEM	0.4300	0.0404	0.2990	0.0415	0.4650	0.0314	0.0027	0.0027	0.0019
P	0.0010	0.0001	0.0001	0.0037	0.2319	0.0029	0.2482	0.0413	0.0052
Contrasts									
NC x NC+OA	*	NS	NS	NS	NS	NS	NS	NS	NS
PC x NC+OA	NS	NS	*	**	NS	**	NS	NS	0.0721
NC x NC+P	NS	**	**	NS	NS	*	NS	NS	*
PC x NC+P	*	NS	0.0543	NS	NS	NS	NS	NS	NS
NC x NC+POA	NS	NS	NS	NS	NS	NS	NS	0.0686	*
PC x NC+POA	NS	*	0.0787	*	NS	*	NS	NS	NS

^{a, b, c, d}Means within a treatment and column with different superscripts differ significantly (p<0.05).

NS: Not significant at p>0.05; *p<0.05, **p<0.01.

¹Treatments: NC = Negative Control; PC = Positive Control; NC+OA = Negative Control + Organic Acids; NC+P = Negative Control + Phytase; NC+POA = Negative Control + Phytase and Organic Acids

hens. Similar results for BW were also recorded with dietary phytase addition to diets containing 0.1% P_{av} at 35 wk (Keshavarz, 2003) and 42 wk of age (Keshavarz, 2000).

Mean BW of birds was not significant among treatments NC and NC+OA with same P_{av} level and PC and NC+OA with different P_{av} level. Similarly, Boling *et al.* (2000a) found no significant effect of 1-4% citric acid supplementation in laying hen diets containing 0.1% P_{av}. However, BW of birds fed diets with 0.45% P_{av} was

higher than 0.1% P_{av} or citric acid supplemented treatments. Moreover, it was also suggested that addition of fumaric acid (Vogt *et al.*, 1981) or commercial organic acid blend (Park *et al.*, 2002; Yesilbag, 2003) to diets with sufficient P_{av} had no significant effect on BW. On day 42, mortality rate was recorded as 32.5% in treatments with no phytase supplementation and containing 0.10% P_{av} (NC) and treatment with OA (NC+OA). Mortality in these treatments were started at 30 and 31 wk of age and continued to end of the

Table 5: Ca and P digestibility (%), blood serum (mg/dl) and bone (%) Ca and P levels of treatments (n = 25)

Treatments ^a	Digestibility		Blood serum		Bone		
	Calcium	Phosphorus	Calcium	Phosphorus	Crude ash	Calcium	Phosphorus
NC	53.76	35.91	26.71	4.10 ^b	53.02	14.53	9.03
PC	57.76	25.29	24.46	8.01 ^a	54.73	14.79	9.54
NC+OA	54.93	33.18	27.22	3.77 ^b	52.30	13.82	9.01
NC+P	58.63	38.92	24.67	7.86 ^a	53.11	14.54	9.14
NC+POA	58.24	34.43	25.35	7.99 ^a	52.96	13.81	9.10
SEM	2.616	3.599	2.182	0.894	0.745	0.438	0.147
P	0.6189	0.1499	0.8784	0.0010	0.2253	0.3760	0.0825
Contrasts							
NC x NC+OA	NS	NS	NS	NS	NS	NS	NS
PC x NC+OA	NS	NS	NS	**	*	NS	*
NC x NC+P	NS	NS	NS	**	NS	NS	NS
PC x NC+P	NS	*	NS	NS	NS	NS	0.0561
NC x NC+POA	NS	NS	NS	*	NS	NS	NS
PC x NC+POA	NS	*	NS	NS	*	NS	**

^{a,b,c}Means within a treatment and column with different superscripts differ significantly (p<0.05).

NS: Not significant at p>0.05; *p<0.05, **p<0.01, ***p<0.001.

¹Treatments: NC = Negative Control; PC = Positive Control; NC+OA = Negative Control + Organic Acids; NC+P = Negative Control + Phytase; NC+POA = Negative Control + Phytase and Organic Acids

experimental period. Moreover, dietary phytase supplementation reduced mortality rate significantly (Table 2). This result is in consistent with the previous findings (mortality rates of 12.5, 55, - 22, 24% respectively) of Gordon and Roland (1997), Punna and Roland (1999), Boling *et al.* (2000b), Jalal and Scheideler (2001) and Keshavarz (2003) which was suggested that high mortality rates in low P_{av} treatments (0.10%) ameliorated with dietary phytase supplementation in laying hens. Because of high mortality rate at initial laying period (21 wk) some studies were terminated at 39 (Punna and Roland, 1999) or 42 wk (Boling *et al.*, 2000b) of age.

Dietary DCP and phytase inclusion to NC diet had positive effects on egg performance (p<0.001), FI (p<0.001) and FCR (p<0.05) (Table 3). These results are in agreement with the previous studies which reported that dietary phytase supplementation improve egg performance (Gordon and Roland, 1997; Van Der Klis *et al.*, 1997; Punna and Roland, 1999; Boling *et al.*, 2000b; Boling *et al.*, 2000c; Keshavarz, 2000; Keshavarz, 2003) and FI (Gordon and Roland, 1998; Van Der Klis *et al.*, 1997; Punna and Roland, 1999; Keshavarz, 2003) of hens in early laying period.

Egg performance, FI and FCR were similar in NC and NC+OA treatments with equal P_{av} levels. Dietary OA supplementation did not ameliorate the negative effects of low P_{av} level in diets. Similar to this, Boling *et al.* (2000a) also reported that citric acid supplementation to laying hen diets did not affect egg performance and FCR. This result was confirmed by other researchers (Vogt *et al.*, 1981; Yalcin *et al.*, 2000; Park *et al.*, 2002; Yesilbag, 2003) that dietary OA addition had no additional effect on egg performance, FI and FCR in treatments with sufficient P_{av} level.

Inclusion of phytase and OA improved egg performance, FI and FCR when compared to NC treatment (p<0.001). Besides, similar results were gathered from PC and NC+POA treatments. Dietary phytase or phytase plus OA improved egg performance, FI and FCR. Furthermore, this positive effect was more clear in treatments with phytase plus OA.

Decrease in egg performance of NC treatment formed an irregular reduction curve in the study (Fig. 1). The results for egg performance of NC at 37-42 wks is similar to findings of Punna and Roland (1999) and Van Der Klis *et al.* (1997). The egg performance curve for NC+OA treatment with same P_{av} level was more regular. Difference for egg performance curve between NC and NC+OA treatments might be due to difference in number of birds from same treatment which was caused by sudden deaths or cage layer fatigue like symptoms of hens exposed to P deficiency.

Egg weight was increased significantly with dietary phytase supplementation when compared to NC treatment (Table 4). Similarly, Van Der Klis *et al.* (1997), Gordon and Roland (1998) and Punna and Roland (1999) reported an increase with dietary addition of phytase in egg weight of hens fed diets with 0.1% P_{av} level. On the contrary, it was also suggested that dietary phytase supplementation to diets with low P_{av} level did not affect egg weight of laying hens at early (Gordon and Roland, 1997; Van Der Klis *et al.*, 1997; Carlos and Edwards, 1998), mid (Jalal and Scheideler, 2001) and late (Carlos and Edwards, 1998; Scott *et al.*, 1999; Boling *et al.*, 2000b; Scott *et al.*, 2000) laying periods. In addition, Keshavarz (2000) recorded an increase in egg weight of layer hens fed diets with different P_{av} levels from 54 to 66 wk of age and no effect from 30 to 42 and 42 to 54 wk of age. Our results also show that egg

weight was not affected significantly from dietary OA supplementation and mean egg weight in NC+OA was lower than egg weight recorded in PC treatment ($p<0.05$). This result was confirmed with the findings of Boling *et al.* (2000a) who pointed out that citric acid inclusion did not affect egg weight in treatments had 0.1% P_{av} whereas egg weights were found higher in treatments with 0.45% P_{av} in their study. This might be attributed to increased egg performance in NC+POA treatment which may be resulted in lightweight egg.

Mean egg shell weight adjusted for egg weight in NC+P were recorded lower than egg shell weights in NC treatment ($p<0.05$) (Table 4). On the contrary, phytase supplementation to diets containing 0.1% P_{av} increased (Gordon and Roland, 1998) or did not affect (Scott *et al.*, 2000) mean egg shell weight. It was found that egg shell thickness of NC was lower ($p<0.05$) than egg shell thickness measured in NC+P and NC+POA treatments. OA addition did not affect egg shell thickness among treatments. It was previously indicated that fumaric acid (Vogt *et al.*, 1981), lactic acid (Yalcin *et al.*, 2000) and commercial OA blend supplementation (Yesilbag, 2003) to diets did not affect egg shell thickness, whereas propionic acid addition decreased this variable (Vogt *et al.*, 1981). Nevertheless, there was no significant difference among PC and NC+OA, NC+P, NC+POA treatments. In similar studies, it was also noticed that dietary phytase supplementation to diets with 0.1% P_{av} had no (Van Der Klis *et al.*, 1997; Jalal and Scheideler, 2001; Keshavarz, 2003) or positive (Gordon and Roland, 1997; 1998; Keshavarz, 2000) effect on egg shell quality. Different results for egg shell weight and thickness between treatments might be arise due to low egg performance in NC treatment.

Digestibility trial showed that phytase and/or OA supplementation to diets with 0.1% P_{av} had no significant effect on Ca and P utilization. In parallel to this, Van Der Klis *et al.* (1997) and Carlos and Edwards (1998) also found no significant effect on Ca and P utilization with dietary phytase supplementation to treatments with low P_{av} (0.1%).

Previously, it was suggested that determination of dietary phytase efficacy on Ca and P utilization is difficult while phytase was supplemented to corn-soybean meal based diet containing 0.1% P_{av} (Carlos and Edwards, 1998; Boling *et al.*, 2000b; Keshavarz, 2000; Jalal and Scheideler, 2001; Keshavarz, 2003). On the contrary, Leske and Coon (1999) found a significant improvement on phytate hydrolysis and total P retention with dietary phytase addition to non-phosphorus purified diets composed of different feedstuffs (corn, soy bean meal, non-fat rice bran). Moreover, positive effect of phytase supplementation is demonstrated with the increase in P_{av} of diet (Um and Paik, 1999; Keshavarz, 2000; Jalal and Scheideler, 2001; Keshavarz, 2003; Collazos *et al.*, 2004).

A number of factors including layer breed (Keshavarz, 2003), age/laying period (Leske and Coon, 1999), Ca (Van Der Klis *et al.*, 1997; Leske and Coon, 1999) and P_{av} (Leske and Coon, 1999; Keshavarz, 2003) level of diet, dose and composition of enzyme supplementation (Scheurmann *et al.*, 1988) and usage of purified diets based on corn and soybean meal are critical on phytate hydrolysis, P retention and excretion in laying hens.

Serum Ca level was not affected from dietary P level or phytase and/or OA supplementetion (Table 5). In consistent with this, Carlos and Edwards (1998) noticed that dietary phytase supplementation to 0.1% P_{av} diets did not effect plasma Ca level in different laying periods. On the other hand, Collazos *et al.* (2004) found an increase ($p<0.05$) in plasma Ca level with phytase supplementation to diets containing rice and corn bran (2.58 and 2.56% Ca and 0.35 and 0.34% P_{av} respectively). Serum inorganic P level was increased ($p<0.01$) with dietary phytase supplementation whereas OA addition did not effect. Serum inorganic P level was found similar in PC, NC+P and NC+POA treatments. In parallel to this, phytase supplementation to corn soybean meal based diets with 0.1% P_{av} (Carlos and Edwards, 1998) or rice and corn bran based diets with 0.33 and 0.34% P_{av} (Collazos *et al.*, 2004) increased plasma P level significantly.

Phytase and/or OA supplementation to diets with 0.1% P_{av} did not effect bone ash or bone Ca and P level. Bone ash and P level in NC+OA treatment and P level in NC+POA treatment was lower when compared to PC treatment (Table 5). Several researchers (Gordon and Roland, 1997; Van Der Klis *et al.*, 1997; Carlos and Edwards, 1998; Gordon and Roland, 1998; Punna and Roland, 1999; Boling *et al.*, 2000b; Keshavarz, 2003) demonstrated positive effects on bone quality characteristics with phytase supplementation to low P (0.1% P_{av}) diets. Opposite to this finding, Jalal and Scheideler (2001) found lower bone ash level in groups treated with phytase. They also reported that their result might be caused by phosphorus deficiency which resulted in higher bone phosphorus retention (preventative mechanism). Different results among studies may be attributed to some factors including age and breed of hens, level of P feeding in pre-experimental period. Commonly, it was demonstrated that bone ash (Boling *et al.*, 2000b; Keshavarz, 2000), bone mineral content, bone density (Gordon and Roland, 1997; Punna and Roland, 1999) and bone strength (Gordon and Roland, 1997) was not affected from diets with P_{av} level of diets higher than 0.15% in different laying periods. Similar to our findings, Vogt *et al.* (1981) did not find any significant difference for tibia weight and tibia ash in group fed sufficient P_{av} level diets supplemented with 2% fumaric acid.

On the basis of these results, phytase supplementation to diets containing 0.1% P_{av} have positive effects on

observed variables and combination of phytase with OA in low P_{av} diets increased this effect significantly. More, these findings are similar to that observed with 0.45% P_{av} diets recommended by NRC (1994). Synergetic effect of phytase and OA might be a result of increased phytate hydrolysis by OA inclusion. Further research should focused on clarifying the mechanism of (determination of feed and gastrointestinal pH levels in different part of digestive system, adding different level or composition of phytase, feeding diets with P_{av} level higher than 0.1%) un-expected positive effect of OA and phytase combination. Future work may also provide a more economic ration formulation with prevention of P pollution with excreta. On the other hand, it may also ensure bio-secured production of animal protein with prevention of residues by using natural feed additives such as enzymes and organic acids.

ACKNOWLEDGEMENTS

The financial support of Scientific and Technological Research Institute of Turkey (TUBITAK) to research project (VHAG-1817, 101V108) is acknowledged.

REFERENCES

- AOAC, 2003. Official Methods of Analysis. 17th Edn., Association of Official Analytical Chemist, Washington, DC.
- Bedford, M.R., 2000. Exogenous enzymes in monogastric nutrition-their current value and future benefits. *Anim. Feed Sci. Technol.*, 86: 1-13.
- Boling, S.D., M.W. Douglas, J.L. Snow, C.M. Parsons and D.H. Baker, 2000a. Citric acid does not improve phosphorus utilization in laying hens fed a corn-soybean meal diet. *Poult. Sci.*, 79: 1335-1337.
- Boling, S.D., M.W. Douglas, M.L. Johnson, C.M. Wang, C.M. Parsons, K.W. Koelkebeck and R.A. Zimmermant, 2000b. The effect of dietary available phosphorus levels and phytase on performance of young and older laying hens. *Poult. Sci.*, 79: 224-230.
- Boling, S.D., M.W. Douglas, R.B. Shirley, C.M. Parsons and K.W. Koelkebeck, 2000c. The effect of various dietary levels of phytase and available phosphorus on performance of laying hens. *Poult. Sci.*, 79: 535-538.
- Brenes, A., A. Viveros, I. Arijia, C. Centeno, M. Pizzaro and C. Bravo, 2003. The effect of citric acid and microbial phytase on mineral utilization in broiler chicks. *Anim. Feed Sci. Technol.*, 110: 201-219.
- Carlos, A.B. and H.M. Edwards, 1998. The effects of 1,25-dihydroxycholecalciferol and phytase on the natural phytate phosphorus utilization by laying hens. *Poult. Sci.*, 77: 850-858.
- Chung, T.K. and D.H. Baker, 1990. Phosphorus utilization in chicks fed hydrated sodium calcium aluminosilicate. *J. Anim. Sci.*, 68: 1992-1998.
- Collazos, H., J. Granados and D. Piernagorda, 2004. Phytase supplementation in cereals by-products based diets and effects on production, eggshell quality, calcium and phosphorus in laying hens, XXII World's Poultry Congress, June 8-13, Istanbul, Turkey, Book of Abstracts, 395.
- Duncan, D.B., 1955. Multiple range and multiple F-tests. *Biometrics*, 11: 1-42.
- Eppendorph. Microliter System, Eppendorf Photometrischen Methoden, Medizin, A.V. 300, M.V. Eppendorf Gerateba Methelerit Hinz, GmbH.
- Ersoy, E. and N. Baysu, 1981. *Pratik Biyokimya*, Veteriner Fakultesi Yayinlari: 372, Ankara Üniversitesi Basimevi, Ankara.
- Garrido, M.N., M. Skjervheim, H. Oppegaard and H. Sorum, 2004. Acidified litter benefits the intestinal flora balance of broiler chickens. *Appl. Environ. Microbiol.*, 70: 5208-5213.
- 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.
- Gordon, R.W. and D.A. Roland, 1998. Influence of supplemental phytase on calcium and phosphorus utilization in laying hens. *Poult. Sci.*, 77: 290-294.
- Jalal, M.A. and S.E. Scheideler, 2001. Effect of supplementation of two different sources of phytase on egg production parameters in laying hens and nutrient digestibility. *Poult. Sci.*, 80: 1463-1471.
- Keshavarz, K., 2000. Nonphytate phosphorus requirement of laying hens with and without phytase on a phase feeding program. *Poult. Sci.*, 79: 748-763.
- Keshavarz, K., 2003. The effect of different levels of nonphytate phosphorus with and without phytase on the performance of four strains of laying hens. *Poult. Sci.*, 82: 71-91.
- Leske, K.L. and N.C. Coon, 1999. A bioassay to determine the effect of phytate on phytate phosphorus hydrolysis and total phosphorus retention of feed ingredients as determined with broilers and laying hens. *Poult. Sci.*, 78: 1151-1157.
- Li, D., X. Che, Y. Wang, C. Hong and P.A. Thacker, 1998. Effect of microbial phytase, vitamin D3 and citric acid on growth performance and phosphorus, nitrogen and calcium digestibility in growing swine. *Anim. Feed Sci. Technol.*, 73: 173-186.
- Lim, H.S., H. Namkung and I.K. Paik, 2003. Effects of phytase supplementation on the performance, egg quality and phosphorous excretion of laying hens fed different levels of dietary calcium and nonphytate phosphorous. *Poult. Sci.*, 82: 92-99.
- Maenz, D.D., C.M. Engele-Schaan, R.W. Newkirk and H.L. Classen, 1999. The effect of minerals and mineral chelators on the formation of phytase-resistant and phytase-susceptible forms of phytic acid in solution and in a slurry of canola meal. *Anim. Feed Sci. Technol.*, 81: 177-192.

- NRC, 1994. Nutrient Requirements of Poultry. 9th Rev. Edn., Natl. Acad. Press, Washington, DC.
- Omogbenigun, F.O., C.M. Nyachoti and B.A. Slominski, 2003. The effects of supplementing microbial phytase and organic acids to a corn-soybean based diet fed to early-weaned pigs. J. Anim. Sci., 81: 1806-1813.
- Panda, A.K., S.V.R. Rao, M.V.L.N. Raju and S.K. Bhanja, 2005. Effect of microbial phytase on production performance of White Leghorn layers fed on a diet low in non-phytate phosphorus. Br. Poult. Sci., 46: 464-469.
- Park, J.H., G.H. Park and K.S. Ryu, 2002. Effect of feeding organic acid mixture and yeast culture on performance and egg quality of laying hens. Kor. J. Poult. Sci., 29: 109-115.
- Punna, S. and D.A. Roland, 1999. Influence of supplemental microbial phytase on first laying hens fed phosphorus-deficient diets from day one of age. Poult. Sci., 78: 1407-1411.
- SAS, 2002. SAS User's Guide: Statistics. SAS Inst. Inc., Cary, NC.
- Scheurmann, S.E., H.J. Lantzsich and K.H. Menke, 1988. *In vitro* und *in vivo* untersuchungen zur hydrolyse von phytat iloslichkeit von phytat. J. Anim. Physiol. Anim. Nutr., 60: 55-63.
- Scott, T.A., R. Kampen and F.G. Silversides, 1999. The effect of phosphorus, phytase enzyme and calcium on the performance of layers fed corn-based diets. Poult. Sci., 78: 1742-1749.
- Scott, T.A., R. Kampen and F.G. Silversides, 2000. The effect of phosphorus, phytase enzyme and calcium on the performance of layers fed wheat-based diets. Can. J. Anim. Sci., 80: 183-190.
- Selle, P.H. and V. Ravindran, 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Technol., 35: 1.
- Simon, O. and F. Igbasan, 2002. *In vitro* properties of phytases from various microbial origins. Int. J. Food Sci. Technol., 37: 813-822.
- Singh, P.K., 2008. Significance of phytic acid and supplemental phytase in chicken nutrition: A review. World's Poult. Sci. J., 64: 553-580.
- Thompson, J.L. and M. Hinton, 1997. Antibacterial activity of formic and propionic acids in the diet of hens on salmonellas in the crop. Br. Poult. Sci., 38: 59-65.
- 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.
- Underwood, E.J. and N.F. Suttle, 1999. The mineral nutrition of livestock. 3rd Edn., CABI, Wallingford, Oxon, UK. ISBN: 0-85199-128-9, pp: 624.
- 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.
- Vogt, H.V., S. Matthes and S. Harnisch, 1981. The effect of organic acids in the rations on the performances of broilers and laying hens. Arch. Geflugel., 45: 221-232.
- Waldroup, P.W., J.H. Kersey, E.A. Saleh, C.A. Fritts and F. Yan, 2000. Nonphytate phosphorus requirement and phosphorus excretion of broiler chicks fed diets composed of normal or high available phosphate corn with and without microbial phytase. Poult. Sci., 79: 1451-1459.
- Wu, G., Z. Liu, M.M. Bryant and D.A.S.R. Roland, 2006. Comparison of Natuphos and Phyzyme as phytase sources for commercial layers fed corn-soy diet. Poult. Sci., 85: 64-69.
- Yalcin, S., S. Yalcin, A. Sehu and K. Sarifakiogullari, 2000. Yumurta tavugu rasyonlarında laktik asit kullaniminin bazi yumurta kalite özelliklerine etkisi. Uluslararası Hayvan Besleme Kongresi, Isparta-Türkiye, pp: 600-604.
- Yesilbag, D., 2003. Tüm tane bugday içeren yumurta tavugu rasyonlarında organik asidin kullanımı. Ankara Üniversitesi Sağlık Bilimleri Enstitüsü, Doktora Tezi.
- Youn, B.S., K.T. Nam, K.M. Chang, S.G. Hwang and I.S. Choe, 2005. Effects of wood vinegar addition for meat quality improvement of old layer. Kor. J. Poult. Sci., 32: 101-106.

¹FYT: Amount of enzyme dissolving 1 micromole inorganic phosphate from Na phytate at pH 5.5 and 37°C.