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RESPONSE OF EGYPTIAN MAMOURAH PULLETS TO DECREASING DIETARY NONPHYTATE PHOSPHORUS LEVEL IN THE ABSENCE OR PRESENCE OF EXOGENOUS MICROBIAL PHYTASE

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ABSTRACT

The present study was carried out to evaluate the effects of feeding low-nonphytate-diets, with and without microbial phytase on the productive and reproductive performance, and egg quality of Mamourah laying hens. Two hundred and forty, 21-week-old Mamourah hens were assigned to eight equal experimental groups of 3 replications of 10 birds each, and housed in individual battery cages. Eight isocaloric (ME; 2700 kcal/kg) and isonitrogenous (16% CP) experimental diets containing graded levels of nonphytate P (NPP; 0.25, 0.225, 0.20 or 0.175%, equivalent to 100, 90, 80 and 70% of NPP level recommended by NRC, 1994 for laying hens) were formulated in the absence or presence of microbial phytase (MP; 500 U/kg diet) and given to the birds, from 21 to 45 weeks of age. At 25 weeks of age and onwards, the hens were artificially inseminated twice a week using freshly-collected undiluted semen from cockerels of the same age and strain, which had been fed the control diet. The criteria of response were change in body weight, productive performance (daily feed and NPP intakes, egg production rate, egg weight, daily egg mass and feed conversion ratio), some egg quality traits (egg components and certain parameters of eggshell and interior quality), reproductive performance (egg fertility, hatchability, embryonic mortality and hatch weight of chicks), certain blood parameters (plasma levels of glucose, total lipids, cholesterol, albumin, total calcium and inorganic P as well as activities of plasma alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase). Ash, Ca and P contents of tibia bone and eggshell were also determined. Regardless of dietary MP supplementation, daily NPP intake and plasma inorganic P concentration were directly related to dietary NPP level, whereas all other criteria were not significantly affected. Dietary supplemental MP, independent of dietary NPP level, significantly ($P \leq 0.01$) improved eggshell quality, as measured by percent egg shell, egg specific gravity and shell weight per unit surface area, and significantly ($P \leq 0.01$) increased the hatch weight of chicks and plasma alkaline phosphatase activity but had no significant effect on all other parameters. No significant NPP level by MP interactions were observed for all criteria measured. It would be concluded that dietary NPP level can be decreased to 0.175% for caged Mamourah laying hens, without adversely affecting their productive and reproductive performance or eggshell quality. Even though the results showed that dietary supplementation with microbial phytase was dispensable; yet as long as it may concern, it appeared to have a slight beneficial effect on eggshell quality.

Keywords: Phosphorus, microbial phytase, laying hens, productive and reproductive performance, eggshell quality

INTRODUCTION

It is well known that the majority of the phosphorus (P) in cereal grains and oilseed meals is bound to phytic acid and that monogastric animals (such as pigs and poultry) do not possess sufficient endogenous phytases, necessary for the utilization of phytate as a source of bioavailable P (Nelson, 1967; Nelson, 1976; Ravindran *et al.*, 1995; Biehl and Baker, 1997). Under normal dietary conditions (*i.e.* plant feed ingredients constitute the major portion in poultry diets), phytate P (PP) is either unavailable to, or poorly utilized by, poultry (Nelson, 1967; Ravindran *et al.*, 1995). The inability, or in adequacy, of poultry to utilize PP causes economic and environmental problems. The low bioavailability of PP necessitates the addition of an inorganic P source which is considered the third most expensive component in diets of monogastric animals. This practice, however, results in an increased excretion of P in animal wastes which may wash into ground water, ponds, lakes and streams and damage the ecosystem, particularly in areas where P loading of land occurs because of heavy fertilization with poultry manure (Ryden *et al.*, 1973).

However, it has been reported that PP utilization by poultry is influenced by a variety of factors such as dietary levels of Calcium, inorganic (or available) P and vitamin D_3, age and genotype of birds, dietary ingredients and feed processing (Ravindran *et al.*, 1995). In addition to reducing the P availability to poultry, phytic acid can form insoluble salts with divalent cations such as Ca, Mg, Fe, Zn, Cu and Mn (Morris, 1986; Ravindran *et al.*, 1995; Bedford and Schulze, 1998). In this regard, Scheideler and Sell (1987) and Van der Klis *et al.* (1994) reported higher PP utilization in laying hens fed diets containing lower calcium levels. In addition, Summers (1995) reported that 0.20% nonphytate (NPP) in corn-soybean meal (CSM) diets was not adequate for optimal performance of laying hens between 32 and 64 weeks of age. In contrast, Boling *et al.* (2000a,b) indicated that 0.15% NPP in CSM diets supported optimal performance for laying hens between 20 and 70 weeks of age. On the other hand, the effectiveness of microbial phytase (MP) supplementation in laying hen diets depends on a variety of factors, mainly dietary levels of calcium and NPP (Van der Klis *et al.*, 1997; Lim *et al.*, 2003) age and strain of laying hens (Boling *et al.*, 2000a; Keshavarz, 2003a,b) and composition of the basal diet (Scott *et al.*, 1999a,b).

Reevaluations of the NPP requirement of the laying hens and the potential of microbial phytase (MP) supplementation to reduce this requirement have been the subject of numerous investigations in recent years. Based on the results of these investigations, diets with 0.15 to 0.20% NPP, in the absence of MP (Keshavarz, 1986a; Gordon and Roland, 1997; Van der Klis *et al.*, 1997; Punna and Roland, 1999; Boling *et al.*, 2000a,b), and diets with 0.10% NPP in the presence of MP (Gordon and Roland, 1998; Boling *et al.*, 2000a,b) have been shown to be sufficient to maintain satisfactory egg production performance during the laying cycle.

To keep step with these recent approaches; bearing in mind the lower egg production rate and egg weight of the Egyptian native hens compared with the egg-type strains, the current study was carried out to investigate the effects of feeding low-NPP-diets, with and without exogenous MP on the productive and reproductive performance, and egg quality of Mamourah laying hens.

MATERIALS AND METHODS

The present study was performed at El-Serw Poultry Research Station, Animal Production Research Institute, Ministry of Agriculture, Egypt. Two hundred and forty, 21-week-old Mamourah laying hens were assigned to eight equal experimental groups of 3 replications of 10 birds each. All birds were kept individually in battery cages set up in an open-sided laying house, exposed to a daily photoperiod of 16 hr and managed similarly. Eight experimental diets were formulated and used. Diet one (which served as a control) contained 0.25% NPP, as suggested by NRC (1994) recommendations for laying hens. Diets 2, 3 and 4 contained NPP levels of 0.225, 0.200 and 0.175% respectively (equivalent to 90, 80 and 70% of the NPP level in the control diet). Diets 5, 6, 7 and 8 contained the same NPP levels as diets 1, 2, 3 and 4, respectively, but supplemented with MP (500 U/kg diet). All the experimental diets were formulated to contain a metabolizable energy (ME) of about 2700 kcal/kg and crude protein (CP) of about 16%. The hens were fed their respective experimental diets (in mash form) from 21 up to 45 weeks of age. In addition, forty 21-week-old Mamourah cockerels were also caged individually, fed the basal (control) diet and kept under the same managerial conditions. All birds had free access to feed and water throughout the experimental period. Composition and chemical analysis of the experimental diets are shown in Table 1.

All hens were weighed at the start (21 weeks of age) and at the end (45 weeks of age) of the experimental period; thus, body weight change (BWC) was calculated. Individual daily records on egg production and egg weight were maintained on a 28-day period basis, for the whole experimental period. Feed intake, nonphytate phosphorus intake and feed conversion ratio (grams of feed consumed: g egg produced) were determined on a replicate group basis. The productive performance of Mamourah laying hens were evaluated in terms of daily feed intake (DFI), daily nonphytate P intake (DNPPI), hen-day egg production rate (EPR), daily egg mass (DEM), egg weight (EW) and feed conversion ratio (FCR) for the entire experimental period. At 25 weeks of age and onwards, the hens were artificially inseminated twice a week using freshly-collected undiluted semen from cockerels of the same age and strain, which had been fed the control diet.

When the birds were 32 weeks of age, two-hundred freshly collected eggs (25 per treatment, collected and examined at two consecutive days) were broken out and used for egg quality measurements. These included egg weight, percentages of egg components (Keshavarz and Nakajima, 1995), egg shape index (ESI), egg shell thickness (EST), egg specific gravity (ESG; Harms *et al.*, 1990) shell weight per unit surface area (SWUSA; Carter, 1975), Haugh units (HU; Haugh, 1937), yolk index (YI) and yolk color score (YCS, by means of the Roche yolk color fan). Shell thickness, as an average of two measures at corresponding positions on the equator of the egg shell, was determined by a special micrometer.

For evaluating the reproductive performance, 3 sets of hatching eggs (1908 eggs; about 79 eggs per treatment in each set) were performed when the birds were 36, 37 and 38 weeks of age. The hatching eggs were collected for five consecutive days in each set. Eggs of each treatment within each set were considered as a replication when these data were subjected to statistical analysis. The eggs were candled two weeks after setting them into the incubator. Records on fertile and infertile eggs and the eggs with dead embryos were maintained. Egg fertility, hatchability (% of fertile and total eggs) and total embryonic mortality were calculated. Weight of healthy hatched chicks was also recorded.

Table 1: Composition and chemical analyses of the experimental diets containing different non-phytate phosphorus (NPP) levels**

Ingredients (%)	Dietary NPP level (%)			
	Control (0.25)	0.225	0.20	0.175
Yellow corn, ground	64.00	64.07	64.10	64.04
Soybean meal, 44% CP	22.60	22.60	22.60	22.60
Wheat bran	4.07	4.07	4.07	4.20
Dicalcium phosphate	0.70	0.56	0.45	0.29
Ground limestone	7.93	8.00	8.08	8.17
Common salt	0.30	0.30	0.30	0.30
Vit.+Min. Premix*	0.30	0.30	0.30	0.30
Dl-Methionine	0.10	0.10	0.10	0.10
Total	100	100	100	100
Calculated analyses; As fed basis (NRC, 1994):				
Metabolizable energy; kcal/kg	2701	2703	2704	2704
Crude protein; %	16.02	16.03	16.03	16.05
Crude fiber, %	3.44	3.44	3.44	3.45
Ether extract; %	2.73	2.74	2.74	2.74
Calcium; %	3.25	3.25	3.25	3.25
Total P; %	0.50	0.478	0.457	0.428
NPP; %	0.25	0.225	0.20	0.175
Lysine; %	0.80	0.80	0.80	0.80
Methionine; %	0.36	0.36	0.36	0.37
Methionine+Cystine; %	0.64	0.64	0.64	0.64
Determined analyses; DM basis (AOAC, 1984):				
Dry matter (DM); %	89.90	90.10	90.05	89.99
Crude protein (CP); %	17.75	17.79	17.70	17.83
Ether extract (EE); %	3.11	3.08	3.13	3.09
Crude fiber (CF); %	3.85	3.83	3.80	3.77
Ash; %	6.68	6.71	6.73	6.74
Nitrogen free extract (NFE); %	68.61	68.59	68.64	68.57
Ca; %	3.60	3.65	3.63	3.66
Total P; %	0.553	0.532	0.500	0.470

*: Each three kilograms contains: Vit. A, 10,000,000 IU; Vit. D_3, 2,000,000 ICU; Vit. E, 10,000 mg; Vit. K_3, 1,000 mg; Vit. B_1, 1,000 mg; Vit. B_2, 5,000 mg; Vit. B_6, 1,500 mg; Vit. B_{12}, 10 mg; Biotin, 50 mg; Choline chloride, 250,000 mg; Pantothenic acid, 10,000 mg; Nicotinic acid, 30,000 mg; Folic acid, 1,000 mg; Mn, 60 g; Zn, 50 g; Fe, 30 g; Cu, 4 g; I, 0.3 g; Se, 0.1 g and Co, 0.1 g.

**: All diets were fed without (-) and with (+) microbial phytase (MP, 500 U/kg diet).

At the end of experiment (45 weeks of age), four hens per treatment were slaughtered within one to two hours of oviposition in order to take some measurements on blood parameters. During slaughtering, blood samples were individually collected in heparinized tubes, and then plasma samples were separated by the centrifugation at 4000 r.p.m. for 15 minutes and stored at -20° C until analysis. Also, the left tibia of each slaughtered hen was removed and cleaned of adhering flesh, dried at 100 °C for 24 hr, crushed and defatted using the Soxhlet extraction apparatus, and dried again prior to ashing at 600 °C overnight. At the same time, 4 eggshells per treatment, from eggs produced by these hens, were oven-dried and ground prior to ashing at 600 °C for 48 hr in a muffle furnace. Egg shell and tibia bone contents of ash, Ca and P were determined according to the methods of Association of Official Analytical Chemists (AOAC, 1984). The experimental diets were also analyzed for dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), ash, calcium and total P by using the official methods (AOAC, 1984). Plasma levels of glucose (Trinder, 1969), total lipids (Frings and Dunn, 1970), total cholesterol (Allain *et al.*, 1974), albumin (Doumas *et al.*, 1971), Ca (Moorehead and Biggs, 1974) and inorganic P (Goldenberg and Fernandez, 1966) and activities of plasma alkaline phosphatase (Kind and King, 1954), and aspartate aminotransferase and alanine aminotransferase (Reitman and Frankel, 1957) were determined, using commercial kits.

A completely randomized design in a 4×2 factorial arrangement of treatments; four dietary levels of NPP (0.25, 0.225, 0.200 and 0.175%) and two levels of MP (0.0 and 500 U/kg diet) supplementation, was used. The statistical processing of data was performed using the Statgraphics Program (Statistical Graphics Corporation, 1991) based on a multifactor analysis of variance, with $P \leq 0.05$ considered to be significant. For each parameter, significant differences among means were separated by using LSD-multiple range test of Quattro Program (Borland International, Inc., 1990).

RESULS AND DISCUSSION

It should be pointed out that dietary nonphytate phosphorus (NPP) level by supplemental microbial phytase (MP) interactions on all criteria investigated were not statistically significant (Tables 2 to 6).

Productive performance

Data on laying performance of Mamourah pullets fed different dietary levels of NPP (0.25, 0.20, 0.225 or 0.175%) with or without MP supplementation, from 21 to 45 weeks of age, are presented in Table 2.

As expected, there was a significant reduction ($P \leq 0.01$) in daily NPP intake of pullets fed the low-NPP diets compared with those of their control counterparts, regardless of MP supplementation. However, there were no significant differences in means of final body weight (FBW), body weight change (BWC), daily feed intake (DFI), hen-day egg production rate (EPR), egg weight (EW), daily egg mass (DEM) or feed conversion ratio (FCR) due to decreasing dietary NPP level from 0.25 to 0.175%, irrespective of MP added. On the other hand, MP supplementation (500 U/kg diet), independent of dietary NPP level, had no significant effect on these aforementioned criteria of laying performance. Apart from supplemental MP, it was observed that although DFI of birds fed the low-NPP diets was approximately similar to that of the control birds the former exhibited insignificantly superior EPR, DEM, and thus FCR to those of the latter (Table 2). This observation may indicate a better utilization of feed and NPP by the birds fed the low-NPP diets compared with their control ones.

Inasmuch as absence of significant differences was observed among dietary treatments in the obtained productive performance of Mamourah pullets of present study, one would speculate therefore that the requirement of this local strain of chickens for NPP during the laying period may has been met at the lowest dietary NPP level (0.175%), with no need to MP supplementation. In this connection, it has been reported that DeKalb Delta (a commercial strain of Single Comb White Leghorn laying hens) does not need more than 159 mg NPP per hen per day, for optimal performance (Boling *et al.*, 2000a,b). Under the conditions of this study and based on the DFI of Mamourah laying hens, the calculated NPP intakes for the different dietary treatments ranged between 255.9 and 179.3 mg/hen/day (Table 2). The level of NPP intake of 179.3 mg/hen/day which is somewhat greater than the value of 159 mg/hen daily, reported by Boling *et al.* (2000a,b), was undoubtedly adequate to support a satisfactory productive performance for Mamourah pullets, under the conditions of the present study.

Table (2): Productive performance of Mamourah laying hens fed different dietary nonphytate P (NPP) levels without or with microbial phytase (MP) supplementation from 21 to 45 weeks of age

Dietary factors	IBW[1] (g)	FBW[2] (g)	BWC[3] (g)	DFI[4] (g/hen)	DNPPI[5] (mg/hen)	DEM[6] (g)	EPR[7] (%)	EW[8] (g)	FCR[9] (g:g)
NPP level (A)									
1 (0.250 %)	1541	1741	202	101.2	253.0^{a}	27.53	57.01	48.29	3.877
2 (0.225 %)	1537	1768	235	102.4	230.4^{b}	29.02	60.07	48.31	3.697
3 (0.200 %)	1541	1779	224	100.8	201.7^{c}	29.39	60.48	48.58	3.556
4 (0.175 %)	1529	1775	245	102.7	179.7^{d}	29.42	60.45	48.67	3.597
Sig. level[9]	NS	NS	NS	NS	**	NS	NS	NS	NS
Pooled SEM[10]	23.5	28.7	24.3	0.58	1.23	0.81	1.59	0.43	0.120
MP added (B)									
1 (0.0)	1536	1746	210	101.4	215.2	28.60	59.46	48.04	3.742
2 (500 U/kg diet)	1538	1786	243	102.2	217.1	29.08	59.55	48.88	3.647
Sig. level[9]	NS	NS	NS	NS	NS	NS	NS	NS	NS
Pooled SEM[10]	16.6	20.3	17.2	0.41	0.87	0.57	1.12	0.30	0.085
AB interaction									
1 (1x1)	1546	1700	167	100.0	250.1	27.04	55.82	48.49	3.927
2 (1x2)	1536	1782	238	102.4	255.9	28.01	58.21	48.09	3.847
3 (2x1)	1537	1769	241	102.4	230.4	29.46	61.68	47.64	3.684
4 (2x2)	1537	1767	229	102.4	230.4	28.58	58.46	48.99	3.710
5 (3x1)	1540	1770	207	100.6	201.2	28.65	59.60	47.90	3.769
6 (3x2)	1541	1788	240	101.1	202.2	30.14	61.36	49.26	3.422
7 (4x1)	1521	1744	224	102.5	179.3	29.25	60.74	48.14	3.587
8 (4x2)	1537	1806	266	102.9	180.0	29.59	60.16	49.19	3.607
Sig. level[9]	NS	NS	NS	NS	NS	NS	NS	NS	NS
Pooled SEM[10]	33.3	40.6	34.4	0.82	1.71	1.14	2.25	0.61	0.169

[1-9]: Refer to means of initial and final body weights, body weight change, daily feed and nonphytate P intakes, daily egg mass, hen-day egg production rate, egg weight and feed conversion ratio, respectively.

[9]: Significance level; NS = not significant; [10]: Pooled SEM refers to standard error of the means.

[a-d]: Means within the same dietary factor and column, for each criterion, bearing different superscripts differ significantly (P≤0.05).

In general, these results are in line with those of Gordon and Roland (1997) who found that decreasing the dietary NPP level from 0.5 to 0.2% gave no adverse effect on laying performance, from 21-38 weeks of age, and concluded that the supplementation of these diets with MP gave no further improvement in laying hens performance. Similar results were also obtained by Punna and Roland (1999) who found that reduction of available P in laying hen (Hy-Line W-36) diets from 0.4 to 0.2% had no effect on feed intake, egg production or egg weight during an experimental period from 21 to 48 weeks of age. and added that supplemental MP (300 U/kg) could completely correct all deficiency symptoms in hens consuming 0.1% available P-diet but had no influence on hens fed diets containing more than 0.2% available P. More recently, Liebert *et al.* (2005) fed laying hens (Lohmann Brown) corn-soybean meal and wheat-soybean meal basal diets (containing 0.12% NPP and 3.1% Ca) supplemented with MP (300 U/kg), from 22 to 61 weeks of age and found that laying performance (feed intake, egg production and egg weight) was not significantly influenced by supplementary MP during the entire experimental period, yet feed conversion ratio was significantly improved. In addition, Panda *et al.* (2005) investigated the effect of feeding diets of varying NPP levels (0.30, 0.24, 0.18 or 0.12%) plus MP supplementation (500 U/kg diet) with the two lowest levels of NPP on production performance of White Leghorn layers from 32 to 48 weeks of age, and they observed no beneficial response due to elevating the dietary NPP levels beyond 0.18% or adding MP to the diet containing 0.18% NPP. Similarly, Snow *et al.* (2005) fed corn-soybean meal diets containing three NPP levels (0.45, 0.14 and 0.10%) to laying hens from 20 to 50 weeks of age and found no significant difference in egg production performance for hens fed the 0.14% NPP-diet compared with those fed the 0.45% NPP-diet.

Egg quality measurements

Certain egg quality traits (measured at 32 weeks of age) of Mamourah laying hens fed different dietary levels of NPP with or without MP supplementation, are shown in Table 3. Apart from the effect of MP supplementation, dietary NPP level had no significant effect on all egg quality traits examined. However, MP supplementation (500 U/kg diet) significantly ($P \leq 0.01$) improved egg shell quality (as measured by shell percent, egg specific gravity and shell weight per unit surface area) while all other egg traits were not significantly affected, regardless of the effect of dietary NPP level. However, it is difficult to interpret such improvement that was observed in eggshell quality; as a result of supplementing the diets with exogenous MP in the present study. Some beneficial effects of dietary supplementation with exogenous MP were reported; including an increased degradation of phytate P and improved Ca and P availability and absorption (Van der Klis *et al.*, 1997); and as a result, an increased retention of these two minerals (Um and Paik, 1999). Consequently, even though one would suggest that the dietary supplementation with MP may account for such improvement which was observed in eggshell quality; particularly during this period of peak egg production (32 weeks of age), the absence of significant differences in ash, Ca and P contents of eggshell (Table 6) among the experimental hen groups fed either diets with or without supplemental MP did not support this approach.

The present results are in partial agreement with the findings of Gordon and Roland (1997) and Punna and Roland (1999) who found that decreasing the NPP level in laying hen diets from 0.5 or 0.4% to 0.2% did not affect egg weights or egg specific gravity, but they reported a positive effect of MP supplementation on eggshell quality when the enzyme was added to a 0.1% NPP diet. Lim *et al.* (2003) fed laying hens (ISA Brown) experimental diets containing two levels of NPP (0.15 and 0.25%) and two levels of MP (0.0 and 300 U/kg diet) from 21-41 weeks of age and found that egg specific gravity was greater in hens fed the 0.15% NPP diets than those fed the 0.25% NPP diets, but MP supplementation significantly decreased the percentage of broken and soft-shell eggs and had no effect on egg specific gravity. In this connection, Panda *et al.* (2005) stated that neither MP supplementation nor decreasing NPP level (from 0.30 to 0.18%) in laying hen diets had a significant effect on some egg quality traits (egg weight, Haugh units, shell weight, shell thickness, shell strength and egg specific gravity).

Table (3): Egg components and some egg quality traits (measured at 32 weeks of age) of Mamourah laying hens fed different dietary nonphytate P (NPP) levels without or with microbial phytase (MP) supplementation

Dietary factors	Egg weight (g)	Egg components (%)			Egg quality traits						
NPP level (A)		Shell	Yolk	Albumen	ESI[1] (%)	ESG[2]	EST[3] (mm)	SWUSA[4] (mg/cm^2)	HU[5]	YI[6] (%)	YCS[7]
1 (0.250 %)	49.03	11.24	28.73	60.03	82.04	1.094	0.37	88.83	85.08	47.08	5.10
2 (0.225 %)	49.16	11.35	28.45	60.20	81.95	1.094	0.38	89.74	82.76	46.94	5.18
3 (0.200 %)	49.01	11.34	28.52	60.14	81.53	1.094	0.37	89.57	84.80	46.96	5.04
4 (0.175 %)	49.76	11.40	28.64	59.96	81.50	1.094	0.38	90.49	85.11	47.43	5.00
Sig. level[8]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Pooled SEM[9]	0.56	0.08	0.26	0.28	0.43	4.505	0.004	0.63	0.79	0.40	0.07
MP added (B)											
1 (0.0)	48.74	11.05[b]	28.54	60.41	81.93	1.092[b]	0.37	87.10[b]	83.78	47.02	5.05
2 (500 U/kg diet)	49.74	11.62[a]	28.63	59.75	81.58	1.096[a]	0.38	92.21[a]	85.09	47.18	5.11
Sig. level[8]	NS	**	NS	NS	NS	**	NS	**	NS	NS	NS
Pooled SEM[9]	0.40	0.06	0.18	0.20	0.31	3.186	0.003	0.45	0.56	0.28	0.05
AB Interaction											
1 (1x1)	49.32	11.05	28.71	60.24	81.79	1.092	0.37	87.44	83.69	46.38	5.04
2 (1x2)	48.75	11.44	28.75	59.81	82.28	1.095	0.37	90.22	86.47	47.80	5.16
3 (2x1)	48.79	11.07	28.56	60.37	82.28	1.093	0.38	87.31	81.22	46.84	5.12
4 (2x2)	49.54	11.62	28.34	60.04	81.62	1.096	0.38	92.17	84.30	47.04	5.24
5 (3x1)	47.78	10.94	28.19	60.87	81.45	1.092	0.36	85.67	85.61	47.48	5.08
6 (3x2)	50.25	11.75	28.84	59.41	81.61	1.096	0.38	93.47	83.98	46.44	5.00
7 (4x1)	49.09	11.13	28.70	60.17	82.19	1.093	87.98	87.98	84.62	47.40	4.96
8 (4x2)	50.44	11.67	28.59	59.74	80.80	1.096	92.99	92.99	85.61	47.46	5.04
Sig. level[8]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Pooled SEM[9]	0.80	0.11	0.36	0.39	0.61	6.372	0.89	0.89	1.11	0.56	0.10

[1-7]: Denote to egg shape index, egg specific gravity, egg shell thickness, shell weight per unit surface area, Haugh units, yolk index and yolk color score, respectively.
[8]: Significance level; NS = not significant; **= significant at P<0.01; [9]: Pooled SEM refers to standard error of the means.
[a-b]: Means in the same dietary factor and column, for each criterion, bearing different superscripts differ significantly (P≤0.05).

Reproductive performance

Egg fertility, hatchability (% of fertile and total eggs), and embryonic mortality as well as chick weight at hatch (measured between 36 and 38 weeks of age) of Mamourah laying hens fed different dietary levels of NPP with or without MP supplementation, are presented in Table 4. With the exception of hatch weight of chicks, analysis of variance proved that none of these parameters was significantly affected by either decreasing dietary NPP level from 0.25 to 0.175% or MP supplementation. However, supplemental MP in the maternal diet led to a significant (P≤0.01) improvement in hatch weight of chicks as compared to the non-supplemented groups, regardless of dietary NPP level.

Table (4): Criteria of reproductive performance (measured between 36 and 38 weeks of age) of Mamourah laying hens fed different dietary nonphytate P (NPP) levels without or with microbial phytase (MP) supplementation

Dietary factors	Egg fertility (%)	Hatchability (% of fertile eggs)	Hatchability (% of total eggs)	Embryonic mortality (%)	Chick weight at hatch (g)
NPP level (A)					
1 (0.250 %)	96.25	86.25	83.03	13.75	35.63
2 (0.225 %)	96.68	88.13	85.17	11.86	36.05
3 (0.200 %)	97.98	87.73	85.95	12.27	35.62
4 (0.175 %)	98.60	89.45	88.19	10.55	35.81
Sig. level[1]	NS	NS	NS	NS	NS
Pooled SEM[2]	0.67	1.67	1.65	1.67	0.18
MP added (B)					
1 (0.0)	96.76	87.07	84.22	12.93	35.38[b]
2 (500 U/kg diet)	97.99	88.71	86.95	11.29	36.18[a]
Sig. level[1]	NS	NS	NS	NS	**
Pooled SEM[2]	0.47	1.18	1.17	1.18	0.13
AB Interaction					
1 (1x1)	96.09	86.41	83.05	13.58	35.27
2 (1x2)	96.41	86.08	83.01	13.92	35.99
3 (2x1)	94.85	89.70	85.06	10.30	35.70
4 (2x2)	98.51	86.57	85.28	13.43	36.39
5 (3x1)	97.47	84.62	82.46	15.38	35.16
6 (3x2)	98.49	90.84	89.43	9.16	36.08
7 (4x1)	98.63	87.55	86.32	12.45	35.38
8 (4x2)	98.56	91.36	90.06	8.64	36.23
Sig. level[1]	NS	NS	NS	NS	NS
Pooled SEM[2]	0.94	2.36	2.34	2.36	0.25

[1]: Significance level; NS = not significant; **= significant at P<0.01; [2]: Pooled SEM refers to standard error of the means.
[a-b]: Means within the same dietary factor and column, for each criterion, bearing different superscripts differ significantly (P≤0.05).

The heavier hatch weight of chicks as a consequence of feeding laying hens on the MP-supplemented diets may be indirectly related to its beneficial effect on egg shell quality (Table 3), resulting in production of chicks with superior skeletal development. In this regard, it has been reported that calcium and phosphorus in the maternal diet are necessary to support normal embryonic bone development and hatchability (Wilson *et al.*, 1980; Wilson, 1997). In an early study, O'Rourke *et al.* (1954) reported that caged laying hens produced eggs which exhibited a depressed hatchability when fed a diet containing 0.19% total phosphorus and when the diet was supplemented to provide 0.18% inorganic and 0.30% total phosphorus the hatchability was significantly improved. Absence of significant differences among means of egg fertility, hatchability and embryonic mortality of Mamourah laying hens, in the present study, is in line with the findings of

Brake (2003), who fed the broiler breeders on experimental diets containing four levels of available P (0.1, 0.2, 0.3 or 0.4%) and two levels of MP (0.0 or 500 U/kg diet) and observed no significant differences among the different dietary treatments in their laying performance, egg fertility or hatchability of fertile eggs. Similar results were also obtained by Godwin *et al.* (2005), who found that decreasing the dietary available P level from 0.55 to 0.17% caused no reductions in the reproductive performance of turkey breeder hens, to 62 weeks of age, but they observed that feeding the phytase-supplemented diets resulted only in significantly fewer hens going out of lay. However, Slaugh *et al.* (1989) reported that egg fertility was significantly declined when dietary available P was reduced from 0.3 to 0.15% for White Orlopp turkey breeder hens.

Blood plasma parameters

Table 5 illustrates blood plasma parameters of 45-week-old Mamourah laying hens fed different dietary NPP levels with or without MP supplementation.

Table (5): Blood plasma parameters of 45-week-old Mamourah laying hens fed different dietary nonphytate P (NPP) levels without or with microbial phytase (MP) supplementation

Dietary factors	GLOC[1] mg/dL	TL[2] g/L	CHOL[3] mg/dL	ALB[4] g/dL	Ca[5] mg/dL	P[6] mg/dL	ALP[7] U/L	ALT[8] U/L	AST[9] U/L
NPP level (A)									
1 (0.250 %)	229	17.24	117	2.27	25.74	7.19[a]	363	32.13	161
2 (0.225 %)	246	21.10	130	2.17	26.31	6.77[b]	348	29.88	147
3 (0.200 %)	233	19.59	120	2.18	26.05	6.47[bc]	390	29.13	153
4 (0.175 %)	243	18.78	113	2.11	26.20	6.02[c]	303	27.88	154
Sig. level[10]	NS	NS	NS	NS	NS	**	NS	NS	NS
Pooled SEM[11]	6.07	0.94	6.72	0.12	0.33	0.11	29.7	1.19	5.41
MP added (B)									
1 (0.0)	233	18.74	118	2.20	26.12	6.72	277[b]	29.81	152
2 (500 U/kg diet)	242	19.62	122	2.16	26.02	6.50	425[a]	29.69	155
Sig. level[10]	NS	NS	NS	NS	NS	NS	**	NS	NS
Pooled SEM[11]	4.29	0.66	4.75	0.08	0.23	0.08	21.0	0.84	3.83
AB Interaction									
1 (1x1)	212	15.21	110	2.42	25.53	7.49	297	32.75	160
2 (1x2)	246	19.26	124	2.13	25.94	6.88	429	31.50	161
3 (2x1)	244	21.05	134	2.17	26.72	6.88	287	29.25	145
4 (2x2)	247	21.15	126	2.17	25.89	6.65	409	30.50	149
5 (3x1)	228	19.91	114	2.10	26.26	6.42	261	28.50	145
6 (3x2)	238	19.27	126	2.26	25.84	6.52	515	29.75	161
7 (4x1)	248	18.77	116	2.11	25.98	6.09	264	28.75	159
8 (4x2)	238	18.80	110	2.10	26.43	5.95	342	27.00	150
Sig. level[10]	NS	NS	NS	NS	NS	NS	NS	NS	NS
Pooled SEM[11]	8.58	1.33	9.50	0.17	0.46	0.15	42.0	1.69	7.65

[1-9]: Refer to means of concentrations of glucose, total lipids, total cholesterol, albumin, total calcium and inorganic phosphorus, and activities of alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase in blood plasma, respectively.

[10]: Significance level; NS = not significant; [11]: Pooled SEM refers to standard error of the means.

[a-d]: Means within the same dietary factor and column, for each criterion, bearing different superscripts differ significantly ($P \leq 0.05$).

With the exception of a significant reduction (P≤0.01) in plasma inorganic P concentration proportionally with decreasing dietary NPP level from 0.25 to 0.175% and a significant increase (P≤0.01) in activity of plasma alkaline phosphatase (ALP) in response to MP supplementation, analysis of variance revealed that none of the examined blood parameters was significantly affected by either dietary NPP level or MP supplementation. Plasma P level, measured in the present study, was directly related to dietary NPP level (Table 5) similarly as reported by Miles *et al.* (1983) and Rao *et al.* (1995). But inconsistently with the present results, Miles *et al.* (1983) and Rao *et al.* (1995) observed an inverse relationship between the high level of dietary P and eggshell quality, while in the present study neither eggshell quality (Table 3) nor eggshell and bone mineralization (Table 6) was affected by dietary NPP level. It should be noted that these authors used wider range of dietary P than that used in the current study. In other words, the highest dietary level of NPP, used herein, was equal to the NRC (1994) recommendation for laying hens, and the lowest one was only 30% less than the recommended level. There are many other contributing factors for the inconsistent responses of laying hens to dietary P; the most relevant include: genotype and age of birds, dietary level of Ca, available P (or NPP) and vitamin D_3, sources of Ca and P, composition of the basal diet, source and level of phytase added and the duration of study (*i.e.* short- or long-term).

Under the conditions of this study, there was no biological importance for the statistically significant increase in plasma ALP activity as a result of feeding the MP-supplemented diets, independent of dietary NPP level, primarily because it coincided with a lack of a significant effect of MP on bone mineralization. Anyway, the mechanism in which dietary MP can enhance plasma ALP activity remains to be ascertained. Apart from the effect of dietary treatments, means of blood parameters of Mamourah laying hens, examined herein, fell within the normal physiological range and agree with those reported by Campbell (2004).

Tibia bone and eggshell components

Data on ash, Ca and P contents of tibia bone and eggshell (measured at 45 weeks of age) of Mamourah laying hens, fed experimental diets containing different NPP levels with or without MP supplementation, are given in Table 6. Statistical analysis of these data clearly showed neither dietary level of NPP nor MP supplementation had an effect on the illustrated components of bone or eggshell. This may suggest that the range of dietary NPP, investigated herein, was not too low to exert a significant effect on those parameters. However, there was no evidence for the existence of a relationship between tibia bone P and plasma inorganic P concentration (Table 5). The insignificant effect of dietary treatments on eggshell and bone ash, Ca and P would suggest that these minerals were normally metabolized for shell formation and maintenance of bone integrity. The current results, however, are in harmony with the observation of Keshavarz (1986b), who detected no significant differences in tibia ash of laying hens due to feeding different available P levels (0.24, 0.44 or 0.64%). Similarly, Slaugh *et al.* (1989) found that femur ash of turkey breeder hens was not significantly affected by feeding varying available P levels (0.15, 0.30, 0.50 or 0.70%).

Recently, Keshavarz (2000) reported that tibia ash of laying hens was not influenced by dietary NPP levels (0.15, 0.20, 0.25, 0.30, 0.35 or 0.40%) or phytase supplementation (300 U/kg diet) from 30 to 42 weeks of age. The present results are also in line with the findings of Hopkins *et al.* (1989), who found that neither tibial bone ash nor its contents of Ca and P of ISA Brown laying hens was affected by feeding for levels of total P (0.34, 0.42, 0.51 and 0.60%), from 20 to 80 weeks of age. On the other hand, means of eggshell ash, Ca and P, reported herein, agree with those published by Atteh and Leeson (1985) for laying hens, regardless of the effect of dietary treatments.

Table (6): Ash, calcium and phosphorus contents of tibia bone and egg shell of 45-week-old Mamourah laying hens fed different dietary nonphytate P (NPP) levels without or with microbial phytase (MP) supplementation

Dietary factors	Tibia bone			Egg shell		
	Ash (%)[3]	Ca (%)[4]	P (%)[4]	Ash (%)[5]	Ca (%)[5]	P (%)[5]
NPP level (A)						
1 (0.250 %)	55.52	33.87	17.56	49.35	38.31	0.086
2 (0.225 %)	55.47	34.21	17.34	48.24	38.24	0.109
3 (0.200 %)	54.77	35.16	17.20	48.45	38.10	0.096
4 (0.175 %)	54.27	34.38	18.00	48.27	38.34	0.096
Sig. level[1]	NS	NS	NS	NS	NS	NS
Pooled SEM[2]	0.78	0.55	0.38	0.66	0.48	0.0096
MP added (B)						
1 (0.0)	55.02	34.63	17.60	48.69	38.57	0.090
2 (500 U/kg diet)	54.99	34.18	17.45	48.47	37.93	0.103
Sig. level[1]	NS	NS	NS	NS	NS	NS
Pooled SEM[2]	0.55	0.39	0.27	0.47	0.34	0.0068
AB interaction						
1 (1x1)	54.81	34.30	17.95	49.25	38.30	0.086
2 (1x2)	56.22	33.44	17.17	49.45	38.33	0.086
3 (2x1)	54.37	34.41	17.18	48.45	38.20	0.098
4 (2x2)	56.57	34.02	17.51	48.04	38.28	0.120
5 (3x1)	55.91	35.40	17.50	48.17	38.50	0.094
6 (3x2)	53.64	34.91	16.90	48.73	37.70	0.098
7 (4x1)	55.01	34.41	17.77	48.88	39.28	0.083
8 (4x2)	53.54	34.35	18.24	47.65	37.40	0.109
Sig. level[1]	NS	NS	NS	NS	NS	NS
Pooled SEM[2]	1.10	0.78	0.54	0.94	0.68	0.0135

[1]: Significance level; NS = not significant.
[2]: Pooled SEM refers to standard error of the means.
[3]: On dry, fat-free basis; [4]: % of bone ash.
[5]: % of dry shell weight.

Conclusion

The results of this study indicated that dietary NPP level can be decreased to 0.175% for caged Mamourah laying hens, without adversely affecting their productive and reproductive performance or eggshell quality. Even though the results showed that dietary supplementation with exogenous microbial phytase was dispensable; yet as long as it may concern, it appeared to have a slight beneficial effect on eggshell quality.

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استجابة دجاج المعمورة المصري للتغذية على علائق ذات مستويات متناقصة من الفوسفور غير الفيتيني المدعمة أو غير المدعمة بانزيم الفيتيز الميكروبي

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أجريت هذه الدراسة بهدف تقييم تأثير تغذية دجاج المعمورة المحلي على علائق ذات مستويات منخفضة من الفوسفور غير الفيتيني المدعمة أو غير المدعمة بانزيم الفيتيز الميكروبي على كفاءتها الإنتاجية والتناسلية وجودة البيض الناتج. تم تقسيم عدد ٢٤٠دجاجة عمرها ٢١أسبوعا عشوائيا إلى ٨ مجموعات تجريبية متساوية بكل منها ٣مكررات متساوية وتحتوي كل مكررة على١٠دجاجات وتم تسكينها في بطاريات ذات أقفاص فردية. تم تكوين ثمانية علائق تجريبية متساوية في محتوياتها من الطاقة القابلة للتمثيل (٢٧٠٠كيلوكالوري/كجم) والبروتين الخام (١٦%) وتحتوي الأربعة الأولى منها على مستويات متدرجة من الفوسفور غير الفيتيني (٠,٢٥أو٠,٢٢٥أو٠,٢٠أو٠,١٧٥% من الغذاء-وهذه المستويات تكافئ ١٠٠أو٩٠أو٨٠أو٧٠% من احتياج دجاج البيض من عنصر الفوسفور في الغذاء تبعا لتوصيات المجلس القومي الأمريكي للبحوث عام١٩٩٤) وتحتوي الأربعة الأخرى على نفس مستويات الفوسفور السابقة الذكر مضافا إليها انزيم الفيتيز الميكروبي (٥٠٠وحدة نشاط/كجم عليقة). وتم تغذية المجموعات التجريبية المختلفة من الطيور على العلائق الخاصة بكل منها حتى نهاية التجربة عند عمر ٤٥أسبوعا. وتم التلقيح الصناعي للدجاج بالسائل المنوي غير المخفف عقب جمعه من الديوك مباشرة مرتان أسبوعيا بدءا من الأسبوع الخامس والعشرين من العمر وحتى نهاية التجربة. وتضمنت معايير الاستجابة للطيور: التغير في وزن الجسم والمظاهر الإنتاجية (الاستهلاك اليومي للغذاء والفوسفور غير الفيتيني ومعدل إنتاج البيض ووزن البيضة وكتلة البيض اليومية ومعامل التحويل الغذائي) وبعض صفات جودة البيض (نسب مكونات البيضة وبعض مقاييس جودة القشرة والجودة الداخلية للبيض) والمظاهر التناسلية (نسب الخصوبة والفقس والنفوق الجنيني للبيض وكذلك وزن الكتاكيت عند الفقس) وبعض معايير بلازما الدم (تركيز كل من الجلوكوز والدهون الكلية والكوليستيرول والألبيومين والكالسيوم والفوسفور غير العضوي وكذلك نشاط إنزيمات الفوسفاتيز القلوي والالنين أمينوترانسفيريز وأسبرتيت أمينوترانسفيريز في البلازما).كما تم تقدير محتويات عظمة الساق وقشرة البيضة من الرماد وعنصري الكالسيوم والفوسفور.

ويمكن تلخيص النتائج المتحصل عليها للفترة التجريبية الكلية فيما يلي: بصرف النظر عن تأثير الإضافة الإنزيمية للغذاء، كان هناك علاقة طردية بين مستوى مستوي الفوسفور غير الفيتيني في الغذاء وكل من المأكول من الفوسفور غير الفيتيني وتركيز الفوسفور غير العضوي في بلازما الدم ولم تتأثر باقي القياسات. كما نتج عن تدعيم العلائق بانزيم الفيتيز الميكروبي تحسنا معنويا في جودة قشرة البيضة (مقاسة في صورة الوزن النسبي للقشرة والثقل النوعي للبيضة ووزن وحدة المساحة من مسطح القشرة) كما حدثت زيادة معنوية في متوسط وزن الكتاكيت عند الفقس وكذلك في نشاط أنزيم الفوسفاتيز القلوي في البلازما-وذلك بغض النظر عن تأثير مستوى الفوسفور غير الفيتيني في الغذاء-ولم تتأثر باقي القياسات. لم يكن للتداخل بين مستوى الفوسفور غير الفيتيني في الغذاء وتدعيم الغذاء بانزيم الفيتيز الميكروبي تأثيرا معنويا على كل الصفات المدروسة.

وعموما فانه بناءا على نتائج هذه الدراسة يمكن التوصية بإمكانية خفض مستوي الفوسفور غير الفيتيني في علائق دجاج المعمورة المحلي البياض (المربى في البطاريات) إلى ٠,١٧٥% دون حدوث تأثيرات سلبية على المظاهر الإنتاجية والتناسلية للدجاج أو جودة البيض الناتج. ورغم أن النتائج أوضحت عدم الحاجة لتدعيم الغذاء بانزيم الفيتيز الميكروبي ففي نفس الوقت يبدو أن له تأثيرا ايجابيا بسيطا على جودة قشرة البيضة.