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EFFECTS OF SUPPLEMENTAL MICROBIAL PHYTASE AND VITAMIN D_3 ON CALCIUM AND PHOSPHORUS UTILIZATION BY GIMMIZAH LAYING HENS FED LOW Ca AND P DIETS

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ABSTRACT

The present study was performed to investigate the effects of feeding low-Ca–P-diets, supplemented with microbial phytase and vitamin D_3, on productive performance, egg quality, egg fertility and hatchability, and some blood constituents of Gimmizah laying hens. Three hundred and thirty, 28-week-old birds were randomly distributed into ten equal groups; each group was divided into 3 replications of 11 birds each (ten females and one male) and housed in floor pens. All birds were kept under the same managerial conditions and fed their respective experimental diets from 28 up to 48 weeks of age. Ten isoenergetic (metabolizable energy of about 2770 kcal/kg)-isonitrogenous (crude protein of about 16.35%) experimental diets were prepared and used. The control diet (Diet 1) was formulated to contain 3.28% Ca and 0.42% available P (AP). Diet 2 (served as a negative control) was the same as the control diet (Diet 1) but had only 75% and 50% of the Ca and AP contents of the control diet, respectively. The remaining eight diets were obtained by supplementing the negative control diet (Diet 2) with two microbial phytase (MP) levels (500 or 1000 U/kg; Diets 3 and 4, respectively), two vitamin D_3 (VD) levels (400 or 800 IU/kg; Diets 5 and 6, respectively), or with different combinations of MP and VD (500 U MP+400 IU VD, 500 U MP+800 IU VD, 1000 U MP+400 IU VD or 1000 U MP+800 IU VD/kg diet; Diets 7, 8, 9 and 10, respectively). Records on body weight, daily feed intake, egg production rate (EPR), egg weight (EW), daily egg mass (DEM) and feed conversion ratio (FCR; g feed: g egg) were maintained. A series of egg quality tests were made at four-week intervals. Egg fertility, hatchablity (% of total eggs) and total embryonic mortality, and egg-weight-loss (%) of hatching eggs were evaluated. At the end of study, some blood plasma parameters [total protein, albumin (A), glubulin (G), A/G ratio, Ca, inorganic P, and activities of transaminases: ALT and AST] and tibia bone length and width, and its Ca and P contents were determined. The results obtained can be summarized as follows:

- Birds fed on Diet 2 (negative control diet; NCD) performed less, but significantly similar to those fed on Diet 1 (control) for EPR, DEM and FCR. On the other hand, birds fed on NCD with supplemental MP, VD or their combinations (Diets 3 to 10) significantly surpassed those of either control or negative control in EPR and DEM with a concomitant improvement in FCR.
- Feeding the negative control diet (NCD) adversely affected eggshell quality (shape index, relative shell weight and shell thickness), interior quality (yolk index and Haugh units), egg fertility, eggshell and tibia Ca contents and plasma concentrations of TP, Ca, and inorganic P compared with the control diet, all other measurements were not affected.
- Supplementing the NCD with MP alone, particularely with the high level of MP (1000 U/kg), offset the negative effects of feeding the NCD and significantly increased EPR, DEM and FCR, and also attained significant improvements in relative yolk weight, yolk index, Haugh units and tibia P content, compared with the control diet.

- Supplementing the NCD with VD alone offset the negative effects of feeding the NCD and slightly improved FCR, egg shape index, compared with the control diet.
- Supplementing the NCD with MP in combination with VD significantly improved EPR, DEM and FCR, and corrected the adverse effects of feeding NCD on the aforementioned criteria.

It could be concluded that adding microbial phytase and/or vitamin D_3 to low-Ca-P-diets can exert a beneficial effect on productive and reproductive performance, and Ca and P utilization by the laying hens.

Keywords: Productive performance, low-Ca-P diet, microbial phytase and/or vitamin D_3 supplementation, laying hens

INTRODUCTION

The majority of P in feed ingredients of plant origin is contained in chemical structures known as phytates (NRC, 1994). Phytate P (PP) is either unavailable or poorly utilized by monogastric animals due to insufficient secretions of endogenous phytases (Nelson, 1967). Thus, the availability of P in foodstuffs of plant origin is generally very low. Bioavailability estimates of P in corn and soybean meal for pigs and poultry range from 10 to 30% (Nelson,1967; Calvert *et al.*,1978). NRC (1994) listed that corn has a total phosphorus (TP) level of 0.28%, whereas non-phytate P (NPP) is only 0.08%; while less than half of the soybean meal P is considered to be available (TP=0.65% and NPP=0.27%). The biological availability of PP (i.e. hydrolysis and absorption) by monogastric animals, including poultry, is influenced by a variety of factors such as dietary levels of Ca, inorganic P and vitamin D_3, age and type of birds, dietary ingredients and feed processing (Ravindran *et al.*, 1995). In addition to reducing the P availability to animals, phytates are associated with a number of antinutritional effects, largely because they can chelate divalent cations such as Ca, Mg, Fe, Zn, Cu and Mn and can reduce dietary protein availability for poultry and pigs (Ravindran *et al.*,1995; Bedford and Schulze,1998). Nowadays, the use of microbial phytase (MP) in poultry diets has been become an effective means for releasing P from phytate, thereby reducing the need for inorganic phosphate supplements in feed formulations and the excessive amounts of P excreted. In broiler chicks, Viveros *et al.* (2002) showed that MP supplementation to low-P diets improved growth performance and utilization of P, Ca, Mg and Zn. Qian *et al* (1997) reported that utilization of phytate P and Ca by the broiler chicks is influenced by dietary Ca: total P ratio, and level of cholecalciferol used. Supplemental dietary phytase has been shown not only to improve P but also Ca utilization in turkeys (Qian *et al.*,1996). However, Vahl *et al.* (1993) used P-deficient layer diets supplemented with monocalcium phosphate (MCP) or 300 U of MP per kg and observed no significant difference between the MP- and MCP-supplemented diets. On the other hand, Simons and Versteegh (1993) fed laying hens on P-deficient diets, and found that all depressions in layer performance could completely be corrected by dietary phytase supplementation. They also concluded that dietary phytase supplementation with a level of 300 U/kg diet was equivalent to 0.10% available phosphorus (AP) in the diet. The efficacy of dietary MP supplementation depends on its

rate of addition, the Ca and PP contents and the dietary Ca:P ratio, obvious benefits have been found in terms of increased availability of phytate–bound minerals and crude protein (Sebastian *et al.*,1998). Also, Lim *et al* (2003) found that with laying hens, the effects of dietary phytase supplementation were significantly modified by the level of Ca and NPP in the diet.

Increasing dietary calcium level has been reported to improve egg production, egg weight and eggshell quality of laying hens (Kuchinski and Harms, 1997). Similarly, Abou-Egla (1995) found that egg production, egg weight and eggshell quality of Rhode Island Red hens were positively affected in response to increasing the dietary calcium level from 3.0 to 4.0 %

Supplementation of vitamin D_3 to laying hens diet resulted in significant improvement in eggshell quality (Soares *et al.*, 1988; Frost *et al.*, 1990); however, they did not observe any significant differences in egg weight, egg production or egg mass. Bell and Marion (1990) and Hashish (1992) found no positive effect of dietary supplementation with 1,25-$(OH)_2$-cholecalciferol on egg weight. Vitamin D_3 is necessary for the bird to absorb, transport and utilize calcium and phosphorus through the intestinal wall. Dietary vitamin D_3 is transported to the liver where it is hydroxylated to 25-hydroxy vitamin D_3 which is further hydroxylated at the C-one position in the kidneys, producing 1,25-dihydroxycholecalciferol [1,25-$(OH)_2$ D_3]. This secosteroid hormone [1,25-$(OH)_2$ D_3] plays a major role in calcium and phosphorus homeostasis. It is considered to be the main physiologically active metabolite of vitamin D_3 needed for the active transport of Ca and inorganic P ions through the cellular membrane in small intestine (Holick and Deluca, 1974). In addition, 1,25-(OH)2 D_3 working in conjunction with parathyroid hormone mobilizes Ca and P from the skeleton when the concentration of Ca ions in the plasma decreases; it may also stimulate the reabsorption of Ca from the kidney tubules (Norman, 1987).

The purpose of the current study was to investigate the effects of feeding low Ca and P diets, supplemented with MP and/or vitamin D_3, on productive performance, egg quality, egg fertility and hatchability, and some blood constituents of Gimmizah laying hens.

MATERIALS AND METHODS

The present study was carried out at El-Gimmizah Poultry Station, Animal Production Researches Institute, Ministry of Agriculture. Three hundred, 28-week-old Gimmizah laying hens and 30 cockerels were randomly distributed into ten equal experimental groups. Each group was divided into 3 replications of 11 birds each (10 females and 1 male) and housed in individual floor pens. All birds were exposed to a daily photoperiod of 16 hr and kept under the same managerial conditions. Feed and fresh water were supplied for *ad libitum* consumption from 28 up to 48 weeks of age. Ten experimental diets were prepared and used. The control diet (Diet 1) was formulated to contain 3.28% Ca and 0.42% available P (AP), provided mainly by ground limestone and dicalcium phosphate (Table 1). Diet 2 (the negative control diet) was the same as diet 1 but had only 75% and 50% of

the Ca and AP contents of the control diet, respectively, and termed as a low-Ca-P diet. The remaining 8 experimental diets were obtained by

Table (1): Composition and chemical analysis of the experimental basal diets

Ingredients	Diet (1) (Control diet)	Diet (2) (Low-Ca-P-diet) (Negative control diet)
Yellow corn	66.65	66.15
Soybean meal, 44 %	23.75	23.10
Wheat bran	00.00	3.80
Limestone	7.50	6.00
Dicalcium phosphate	1.60	0.45
Common salt	0.30	0.30
Vit. & min. mix.*	0.10	0.10
DL-Methionine	0.10	0.10
Total	100	100
Calculated analysis**:		
ME,kcal/kg	2762	2781
Crude protein, %	16.12	16.38
Calcium, %	3.28	2.64
Total phosphorus, %	0.64	0.42
Available phosphorus, %	0.42	0.20
Lysine, %	0.81	0.82
Methionine,%	0.37	0.37
Methionine + cysteine %	0.64	0.65
Determined analysis (Dry matter basis)***:		
Dry matter, %	89.48	88.69
Crude protein, %	17.85	18.35
Crude fiber, %	3.51	3.45
Ether extract, %	3.07	3.17
Ash, %	7.37	7.45
Nitrogen free extract, %	68.20	67.58

*Each 3 kg contains: 12,000,000 IU Vit. A; 2,000,000 IU Vit D_3 10,000 mg Vit. E;1,000mg Vit. K; 1,000mg Vit. B_1; 5,000mg Vit. B_2; 1,500mg Vit B_6; 10mg Vit. B_{12}; 30gm; Niacin,10 gm ; Panatothenic acid, 50mg, Biotin;l,000mg Folic acid;250,000mg choline chloride; 60g manganese; 30g iron; 50g zinc; 40g copper; 30g iodine; 1gm Seleinium and 1g cobalt.

** Calculated according to NRC (1994).

*** Determined according to the methods of A.O.A.C (1980)

supplementing the low-Ca-P diet with two microbial phytase (MP) levels (500 or 1000 U/kg; Diets 3 and 4, respectively), two vitamin D_3 (VD) levels (400 or 800 IU/kg; Diets 5 and 6, respectively), or with different combinations of MP and VD (500 U MP+400 IU VD, 500 U MP+800 IU VD, 1000 U MP+400 IU VD or 1000 U MP+800 IU VD/kg diet; Diets 7, 8, 9 and 10, respectively). The calculated contents of vitamin D_3 in diets 1 and 2 were the same, being about 666 IU/kg diet. All the experimental diets were isoenergetic (metabolizable energy, ME, of about 2779 kcal/kg) and isonitrogenous (crude protein, CP, of about 16.35%) and formulated to meet or slightly exceed the nutrient requirements of laying hens (NRC, 1994), except for Ca and P.

Pullets were weighed at the beginning (28 weeks of age) and at the end (48 weeks of age) of the experimental period. Egg production, egg weight and total egg mass were recorded daily for the whole experimental period. Feed consumption was recorded weekly and then feed conversion (grams of feed:g egg) was determined. Egg quality was examined on a 28-day period basis. In each test, 10 eggs from each dietary treatment were collected, individually weighed and broken out in order to separate their shells, yolks and albumens. Individual weights of yolk, albumen and shell (with membranes) were recorded and calculated as a percent of egg weight. Ca and P contents of eggshells (% of ash weight) were also determined according to AOAC (1980). For evaluating egg fertility and hatchability, three hatches were performed (180 eggs per treatment in each hatch) during the last 6 weeks of the experimental period. The hatching eggs were individually weighed before setting them into the incubator and once again 18 days later, and simultaneously candling of eggs was accomplished. Records on fertile and infertile eggs and the eggs with dead embryos were maintained. Egg-weight-loss during the first 18 days of the incubation period was determined. Egg fertility, hatchability (% of total settable eggs) and total embryonic mortality were computed.

At the end of study, three hens from each treatment were slaughtered in order to take some measurements on the physical (length and width of right tibias) and chemical (right tibia Ca and P contents) characteristics of bones, as infuenced by dietary treatments. The bones were dried, crushed and then ashed at 600°C for 16 hours in a muffle furnace. Bone contents of Ca and P were determined according to AOAC (1980). Blood samples were individually collected during slaughtering in heparinized tubes, then plasma samples were obtained by centrifugating the tubes at 4000 rpm for 15 minutes and stored at –20°C until analysis. Commercial kits were used to measure the blood plasma levels of total protein (TP) (Doumas *et al.*, 1981), albumin (A) (Doumas *et al.*, 1971); then, globulin (G) concentration and albumin/globulin (A/G) ratio were calculated. Levels of plasma Ca (Moorhead and Biggs, 1974) and inorganic P (Goldenberg and Fernandez, 1966) and activities of plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were also determined (Reitman and Frankel, 1957).

Data were analyzed by one-way analysis of variance using the General Linear Model (GLM) procedure of SAS (1994). Significant differences among means were identified by Duncan's multiple range test *(Duncan, 1955)*.

RESULTS AND DISCUSSION

Productive performance of Gimmizah hens

Data on the productive performance of Gimmizah laying hens, fed low-Ca-P-diets, supplemented with microbial phytase (MP), vitamin D_3 (VD) or their combinations from 28 to 48 weeks of age, are shown in Table 2. Even though significant differences were detected among treatments in daily feed intake (DFI), egg production rate (EPR), egg weight (EW), daily egg mass

(DEM) and feed conversion ratio (FCR), a lack of significant variation was observed in final body weight (FBW) and body weight gain (BWG) of experimental birds.

The obtained data clearly showed that birds fed on Diet 2 (negative control) performed less, but significantly equal to those fed on Diet 1 (control), for FBW, BWG, DFI, EPR, EW, DEM and FCR. This response may indicate that Ca and P levels in the negative control diet, investigated herein, were only marginal or adequate for the Gimmizah laying hens.

It is noteworthy, however, that groups of birds fed on Diet 2 with supplemental MP, VD or their combinations (Diets 3 to 10) significantly surpassed those of either the control (on Diet 1) or the negative control (on Diet 2) groups in EPR and DEM with a concomitant improvement in FCR.

Supplementing the negative control diet (Diet 2) with MP at levels of 500 or 1000 U/kg (Diets 3 and 4) significantly improved EPR, DEM and FCR as compared to the control diet (Diet 1). Similarly, supplementing the negative control diet (Diet 2) with VD at levels of 400 or 800 IU/kg (Diets 5 and 6) slightly improved, but not significantly, FCR compared with the control diet (Diet 1). The lack of response to VD supplementation may be attributed to the fact that the negative control diet originally contained about 666 IU VD/kg (based on the calculated analysis), being more than 2 folds the estimated requirement of laying hens for this vitamin (300 IU/kg; NRC, 1994). The dietary combined supplementation with MP and VD (Diets 7, 8, 9 and 10) produced significant improvements in EPR, DEM and FCR as opposed to those of the control group (Diet 1). Although birds fed on the diet supplemented with 1000 U MP+400 IU VD/kg (Diet 9) consumed the least amount of feed they attained the best values of EPR, DEM and FCR compared with those of the control group (Diet 1).

Generally, these results are in good agreement with the findings of Keshavarz (1996) who found that laying performance, measured as egg production, egg weight, egg mass, feed consumption and feed conversion, was not influenced by either dietary levels of Ca (2.8 and 3.8%) or cholecalciferol (2200 or 4400 IU/kg diet). On the other hand, Keshavarz (2003) demonstrated that a high level of supplemental dietary phytase (300 U/kg) was more effective than a low level (150 U/kg) in restoring the performance for egg production of hens fed low-P diets (0.10, 0.15, 0.20 or 0.25% non-phytate P) to the control level. Gordon and Roland (1998) reported that dietary phytase supplementation could completely overcame the adverse effects associated with low dietary P (0.10% non-phytate P) and significantly reduced the impact of low dietary Ca (2.5 or 2.8%) on the laying hen performance.

Parameters of egg quality

Table 3 shows the effects of feeding low-Ca-P-diets, supplemented with MP, VD or their combinations on some exterior and interior parameters of egg quality of Gimmizah laying hens. There were significant differences among dietary treatments in all examined parameters of egg quality.

Table (2): Effects of feeding low-Ca-P-diets supplemented with microbial phytase (MP) and/or vitamin D_3 (VD) on the productive performance of Gimmizah laying hens from 28 to 48 weeks of age.

Treatments	Initial body weight (Kg)	Final body weight (kg)	Body weight gain (kg)	Daily feed intake (g)	Hen-day egg production (%)	Egg weight (g)	Daily egg mass (g)	Feed conversion (g feed/g egg)
Diet 1; (Control)	1.47	1.70	0.23	114.67^{ab}	46.29^{abc}	52.08^{ab}	24.11^{d}	4.76^{ab}
Diet 2; (Low-Ca & P)	1.46	1.66	0.20	116.39^{a}	43.38^{c}	51.27^{b}	22.24^{d}	5.23^{a}
Diet 3; 500U MP/kg	1.53	1.82	0.29	115.00^{ab}	51.09^{bcd}	52.99^{ab}	27.09^{abc}	4.23^{bc}
Diet 4; 1000U MP/kg	1.52	1.82	0.30	115.83^{a}	52.48^{abc}	53.08^{ab}	27.86^{abc}	4.16^{bc}
Diet 5; 400 IU VD/kg	1.49	1.78	0.29	115.80^{a}	49.17^{cd}	52.80^{ab}	26.01^{bcd}	4.50^{b}
Diet 6; 800 IU VD/kg	1.47	1.77	0.30	116.77^{a}	49.69^{cd}	53.00^{ab}	26.35^{bc}	4.44^{b}
Diet 7; 500U MP+400IU VD/kg	1.49	1.81	0.32	106.71^{d}	53.51^{abc}	53.04^{ab}	28.38^{ab}	3.77^{cd}
Diet 8; 500U MP+800IU VD/kg	1.48	1.84	0.36	112.98^{bc}	55.52^{ab}	53.96^{ab}	29.96^{ab}	3.77^{cd}
Diet 9; 1000U MP+400IUVD/kg	1.53	1.82	0.29	105.99^{d}	57.25^{a}	53.15^{a}	30.43^{a}	3.48^{d}
Diet 10; 1000U MP+800IU D/kg	1.45	1.85	0.40	111.42^{c}	56.63^{ab}	53.05^{ab}	30.04^{ab}	3.71^{cd}
Pooled SEM	0.01	0.02	0.02	0.87	1.05	0.22	0.64	0.12
Significance	NS	NS	NS	**	**	*	**	**

a-e : Means in the same column bearing different superscripts differ significantly.
NS: not significant; *P≤0.05; ** P≤0.01
SEM denotes to the standerd error of the means.

Table (3): Effects of feeding low-Ca-P-diets supplemented with microbial phytase (MP) and/or vitamin D_3 (VD) on some exterior and interior parameters[1] of egg quality of Gimmizah laying hens

Treatments	Egg shape Index (%)	Shell weight (%)	Shell thickness (mmx100)	Albumen weight (%)	Yolk weight (%)	Yolk index (%)	Haugh units
Diet 1; (Control)	73.13^{de}	12.62^{bc}	36.25^{ab}	53.82^{c}	31.91^{de}	41.33^{d}	80.11^{e}
Diet 2; (Low-Ca & P)	70.60^{f}	11.88^{d}	33.27^{c}	53.69^{c}	31.39^{e}	39.87^{e}	75.38^{g}
Diet 3; 500U MP/kg	73.80^{cde}	12.93^{ab}	37.05^{a}	54.41^{bc}	32.65^{abcd}	41.80^{cd}	79.60^{e}
Diet 4;1000U MP/kg	74.73^{cd}	12.89^{ab}	36.42^{a}	54.00^{c}	32.85^{abc}	42.47^{bc}	77.60^{f}
Diet 5; 400 IU VD/kg	74.33^{cde}	12.25^{cd}	36.52^{a}	54.22^{bc}	31.78^{de}	40.80^{de}	80.87^{de}
Diet 6; 800 IU VD/kg	75.07^{bc}	12.53^{bc}	34.80^{b}	53.74^{c}	32.16^{bcde}	40.73^{de}	82.18^{cd}
Diet 7; 500U MP+400IU VD/kg	72.87^{e}	12.96^{ab}	37.77^{a}	55.98^{a}	33.21^{ab}	41.60^{cd}	82.65^{c}
Diet 8; 500U MP+800IU VD/kg	77.13^{a}	12.91^{ab}	37.57^{a}	55.31^{ab}	33.35^{a}	41.60^{cd}	83.64^{bc}
Diet 9; 1000U MP+400IUVD/kg	76.47^{ab}	13.29^{a}	37.82$_{a}$	56.05^{a}	33.01^{ab}	43.13^{ab}	84.09^{b}
Diet 10; 1000U MP+800IU D/kg	77.47^{a}	13.14^{a}	37.17$_{a}$	56.34^{a}	32.89^{abc}	43.53^{a}	84.55^{a}
Pooled SEM	0.23	0.06	0.20	0.15	0.12	0.01	0.34
Significance	**	*	*	*	**	**	**

a-e : Means in the same column bearing different superscripts differ significantly.

1: Means represent an avarage of five egg quality tests; *P≤0.05; ** P≤0.01

SEM denotes to the standerd error of the means.

Feeding the negative control diet (Diet 2) adversely affected egg shape index, percent shell weight, shell thickness, yolk index and Haugh units but had no significant effects on the relative weights of yolk and albumen, compared with the control group (Diet 1). Supplementing the negative cotrol diet (Diet 2) with MP at a level of 500 U/kg (Diet 3) produced egg quality mean values equivalent to those of the control group (Diet 1) while the higher level of MP supplementation (1000 U/kg; Diet 4) brought about further significant improvements in the percentage of yolk weight, yolk index and Haugh units, as compared to the control diet (Diet 1). Similarly, supplementing the negative control diet (Diet 2) with VD at a level of 400 IU/kg (Diet 5) produced egg quality values equivalent to those of the control group (Diet 1) while the higher level of VD supplementation (800 IU/kg; Diet 6) caused a further significant improvement in egg shape index compared with the control group (Diet 1). The dietary combined supplementation with MP and VD (particularly Diets 9 and 10) produced additional significant improvements in all parameters studied for egg quality.

These results are in partial agreement with those of Lim *et al.* (2003) who found that feeding the laying hens on a low-Ca diet (3.0% *vs.* 4.0%) resulted in decreased egg specific gravity, eggshell strength, and eggshell thickness during an entire experimental period from 21 to 41 weeks of age. Similarly, Bragg *et al.* (1971) found that the eggshell quality, as measured by shell strength, calcium content of shell and percent shell, was increased with increasing dietary calcium level up to 3.25%, regardless of the level of a supplementary vitamin D_3. In harmony with the present results, Keshavarz (1996) found that egg specific gravity was greater and percentage of cracked eggs was lower for hens fed 3.8% than those fed 2.8% Ca diet; other indicators of shell quality (shell thickness, shell weight, percent shell and shell weight per unit surface area) were not influenced by dietary Ca level. He also observed that eggshell quality; as measured by egg specific gravity and percentage of cracked eggs, was not influenced by supplemental vitamin D_3 level (4400 *vs.* 2200 IU/kg diet). In addition, Gordon and Roland (1998) reported that dietary MP supplementation and increasing dietary Ca level could independently improved the eggshell quality of laying hens.

Blood constituents

Data presented in Table 4 illustrate the effects of feeding low-Ca-P-diets, supplemented with MP, VD or their combinations on blood plasma constituents of 48-week-old Gimmizah laying hens. There were significant differences among treatments in plasma concentrations of total protein (TP), globulin (G), Ca and inorganic P, and activities of plasma transaminases (AST and ALT). Feeding the negative control diet (Diet 2) resulted in significant reductions in plasma TP, Ca and inorganic P compared with those of the control group (on Diet 1), all other measurements were not affected. Supplementing the negative control diet (Diet 2) with MP at levels of 500 or 1000 U/kg (Diets 3 and 4) restored the levels of TP, Ca and inorganic P to the corresponding values of the control group (on Diet 1). Similar responses were observed when the negative control diet (Diet 2) was supplemented with either VD alone (Diets 5 and 6) or in combination with MP (Diets 7, 8, 9 and 10).

Table (4): Effects of feeding low-Ca-P-diets supplemented with microbial phytase (MP) and/or vitamin D_3 (VD) on blood plasma constituents of 48-week-old Gimmizah laying hens

Treatments	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G Ratio	GOT (U/L)	GPT (U/L)	Ca (mg/dl)	P (mg/dl)
Diet 1; (Control)	4.94^{ab}	2.09	2.85^{abcd}	0.74	133.92^{d}	21.09^{b}	17.42^{abc}	5.74^{a}
Diet 2; (Low-Ca & P)	4.00^{c}	1.71	2.29^{d}	0.74	130.39^{d}	21.08^{b}	11.45^{d}	3.61^{b}
Diet 3; 500U MP/kg	5.23^{a}	2.19	3.04^{abc}	0.76	145.40^{cd}	23.44^{ab}	13.91^{cd}	5.31^{a}
Diet 4; 1000U MP/kg	5.18^{a}	2.15	3.03^{abcd}	0.71	146.82^{bcd}	23.80^{ab}	13.68^{cd}	5.03^{ab}
Diet 5; 400 IU VD/kg	4.15^{bc}	1.76	2.39^{cd}	0.73	141.22^{cd}	21.59^{b}	14.82^{bcd}	4.94^{ab}
Diet 6; 800 IU VD/kg	4.25^{bc}	1.81	2.43^{bcd}	0.74	142.63^{cd}	22.06^{b}	16.49^{abc}	5.86^{a}
Diet 7; 500U MP+400IU VD/kg	5.34^{a}	2.28	3.07^{abc}	0.75	147.59^{bc}	24.23^{ab}	17.68^{abc}	6.28^{a}
Diet 8; 500U MP+800IU VD/kg	5.31^{a}	2.21	3.10^{abc}	0.75	148.89^{abc}	24.27^{ab}	19.12^{abc}	6.17^{a}
Diet 9; 1000U MP+400IUVD/kg	5.62a	2.31	3.31^{a}	0.72	149.37^{a}	24.71^{ab}	18.27^{abc}	6.61^{a}
Diet 10; 1000U MP+800IU D/kg	5.46a	2.30	3.16^{ab}	0.75	150.72^{a}	26.16^{a}	21.06^{a}	6.43^{a}
Pooled SEM	0.12	0.06	0.08	00.03	2.62	0.68	0.61	0.20
Significance	*	NS	*	NS	*	*	**	*

a-d : Means in the same column bearing different superscripts differ significantly.
NS: not significant; *P≤0.05; ** P≤0.01
SEM denotes to the standerd error of the means.

Table (5): Effects of feeding low-Ca-P-diets supplemented with microbial phytase (MP) and/or vitamin D_3 (VD) on tibia length and width, and Ca and P contents of tibia bone and eggshell of Gimmizah laying hens

Treatments	Tibia				Egg shell	
	Tibia length (cm)	Tibia Width (mm)	Calcium (%)	Inorganic P (%)	Ca (%)	P (%)
Diet 1; (Control)	11.75	8.00	20.40^{cd}	9.72^{e}	27.36^{bc}	0.30^{c}
Diet 2; (Low-Ca & P)	11.25	7.75	18.50^{e}	9.68^{e}	22.25^{d}	0.28^{c}
Diet 3; 500U MP/kg	11.90	8.00	20.80^{cd}	10.04^{de}	25.96^{cd}	0.30^{c}
Diet 4; 1000U MP/kg	12.00	8.25	21.51^{bc}	10.44^{bcd}	27.02^{bc}	0.32^{bc}
Diet 5; 400 IU VD/kg	11.50	8.00	19.95^{d}	10.18^{cde}	28.01^{bc}	0.30^{c}
Diet 6; 800 IU VD/kg	11.75	8.00	21.17^{cd}	10.57^{bcd}	28.00^{bc}	0.33^{bc}
Diet 7; 500U MP+400IU VD/kg	12.25	8.25	20.79^{cd}	10.62^{abcd}	28.43^{bc}	0.33^{bc}
Diet 8; 500U MP+800IU VD/kg	12.25	8.25	22.36^{ab}	10.68^{abc}	28.82^{bc}	0.38^{bc}
Diet 9; 1000U MP+400IUVD/kg	12.75	8.50	22.46^{ab}	11.00^{ab}	30.92^{a}	0.48^{ab}
Diet 10; 1000U MP+800IU D/kg	12.50	8.50	23.31^{a}	11.19^{a}	31.74^{a}	0.56^{a}
Pooled SEM	0.15	0.09	0.31	0.12	0.77	0.02
Significance	NS	NS	**	**	*	*

a-e : Means in the same column bearing different superscripts differ significantly.
NS: not significant; *P≤0.05; ** P≤0.01
SEM denotes to the standerd error of the means.

These results are in good agreement with the findings of Viveros *et al.* (2002) who found that activities of serum AST, ALT, alkaline phosphatase and lactate dehydrogenase, as well as TP concentration were significantly affected by dietary MP supplementation to low-P diets for broiler chicks. However, Salem *et al.* (2003) observed a positive significant effect of dietary phosphorus level on plasma Ca and P while phytase addition had no effect on plasma Ca and P in broiler chicks. Edwards (2002) found that high dietary levels of vitamin D_3 (110 microgram and 220 microgram/kg diet) increased phytate P utilization, as measured by plasma Ca and P, bone ash and retention of Ca and P in broilers. El Afifi and Abu Taleb (2002) reported that blood calcium level was significantly increased by feeding estradiol plus cholecalciferol in laying Japanese quails.

Bone and egg shell characteristics

Data shown in Table 5 summarize the effects of feeding low-Ca-P-diets, supplemented with MP, VD or their combinations on tibia length and width, and Ca and P contents of tibia bone and eggshell of Gimmizah laying hens. Dietary treatments had significant effects on tibia and eggshell contents of Ca and P, while tibia length and width were not affected. Feeding the low Ca and P diet (Diet 2) significantly reduced Ca contents in tibia bone and eggshell compared with the control diet (Diet 1); P contents, however, were not affected. Adding MP, VD or their combinations could compensate for the adverse effect of feeding the negative control diet (Diet 2) that was exerted on Ca contents of tibia bone and egg shell (Diets 3, 4, 5, 6, 7 and 8). The dietary combined supplementation with MP and VD (particularly Diets 9 and 10) produced additional significant improvements in Ca and P contents of tibia bone and eggshell.

These results are in agreement with those of Salem *et al.* (2003) who found that adding microbial phytase to broiler chick diets significantly increased tibia contents of ash, Ca and P. Working with laying Japanese quails, El Afifi and Abu Taleb (2002) reported that tibia weight of laying Japanese quails was increased by 4% and 12% over that of the control due to feeding estradiol and estradiol plus cholecalciferol, respectively. However, Newman and Leeson (1999) did not observe any improvement in tibia measurements due to cholecalciferol supplementation into layers diets.

Egg fertility and hatchablity

Data on effects of feeding low-Ca-P-diets, supplemented with MP, VD or their combinations on egg fertility, hatchability and embryonic mortality, and egg-weight-loss (%) during incubation of Gimmizah laying hens, are presented in Table 6. With the exception of embryonic mortality, significant differences were observed among dietary treatments in percentages of egg fertility, and hatchability and egg-weight-loss. Feeding the negative control diet (Diet 2) significantly decreased egg fertility compared with that of the control group (Diet 1). Adding MP, VD or their combinations could offset the adverse effect of feeding the negative control diet (Diet 2) that was exerted on egg fertility. This result is in consistent with a conclusion drawn by Um and Paik (1999) that dietary phytase supplementation may influence the utilization of not only phytate P but also that of other nutrients by laying hens.

CONCLUSION

It could be concluded that adding microbial phytase and/or vitamin D3 to low-Ca-P-diets can exert a beneficial effect on productive and reproductive performance, and Ca and P utilization by the laying hens.

Table (6): Effects of feeding low-Ca-P-diets supplemented with microbial phytase (MP) and/or vitamin D_3 (VD) on egg fertility and hatchability, embryonic mortality (EM), and egg-weight-loss of Gimmizah laying hens

Treatments	Fertility (%)	Hatchablity (%)	EM (%)	Egg weight loss (%)
Diet 1; (Control)	86.38^{ab}	80.99^{ab}	6.69	10.32^{abc}
Diet 2; (Low-Ca & P)	82.79^{c}	76.36^{b}	6.81	12.79^{a}
Diet 3; 500U MP/kg	85.67^{b}	81.35^{a}	7.66	10.72^{ab}
Diet 4; 1000U MP/kg	86.22^{ab}	80.71^{ab}	7.11	10.42^{abc}
Diet 5; 400 IU VD/kg	86.52^{ab}	81.27^{a}	5.44	11.04^{ab}
Diet 6; 800 IU VD/kg	86.63^{ab}	81.09^{ab}	7.79	10.30^{abc}
Diet 7; 500U MP+400IU VD/kg	87.25^{ab}	80.98^{ab}	5.42	9.35^{bc}
Diet 8; 500U MP+800IU VD/kg	87.26^{ab}	82.01^{a}	6.95	9.22^{bc}
Diet 9; 1000U MP+400IU VD/kg	88.80^{a}	82.38^{a}	6.07	8.93^{bc}
Diet 10; 1000U MP+800IU VD/kg	88.72^{ab}	83.79^{a}	6.08	8.08^{c}
Pooled SEM	0.49	0.33	0.31	0.33
Significance	*	*	NS	*

a-c : Means in the same column bearing different superscripts differ significantly.
NS: not significant; *P≤0.05
SEM denotes to the standerd error of the means.

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تأثير التدعيم الغذائي بانزيم الفيتيز الميكروبي أو بفيتامين د٣ أوالاثنين معا على الاستفادة من الكالسيوم والفوسفور بواسطة دجاج الجميزة البياض المغذى على علائق ذات محتويات منخفضة من الكالسيوم والفوسفور

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اجريت هذه الدراسة لبحث تأثيرات التغذية على علائق منخفضة في الكالسيوم والفوسفور ومدعمة بانزيم الفيتيز الميكروبي أو بفيتامين د٣ أو كليهما على المظاهر الانتاجية وجودة البيض ونسب خصوبة وفقس البيض وبعض مكونات الدم لدجاج الجميزة البياض. تم استخدام عدد ٣٣٠ طائر (٣٠٠ دجاجة و ٣٠ ديك) عمر ٢٨ أسبوع. تم تقسيم الطيور عشوائيا الى ١٠ مجموعات تجريبية بكل منها ٣٣ طائر (٣٠ دجاجة و٣ ديوك) في ثلاثة مكررات متساوية وتم تسكينها في حظائر أرضية. تم تكوين ١٠ علائق تجريبية متماثلة في الطاقة والبروتين: العليقة الأولى (عليقة المقارنة) احتوت على ٣.٢٨% كالسيوم و٠.٤٢% فوسفور متاح، العليقة الثانية (عليقة المقارنة السالبة) احتوت على ٧٥%، ٥٠% من محتويات عليقة المقارنة من الكالسيوم والفوسفور على الترتيب، تم تكوين الثمانية علائق المتبقية عن طريق تدعيم العليقة الثانية بمستويين من انزيم الفيتيز الميكروبي (٥٠٠ أو ١٠٠٠ وحدة/كجم-العلائق٣، ٤ على الترتيب) أو بمستويين من فيتامين د٣ (٤٠٠ أو ٨٠٠ وحدة دولية/كجم-العلائق ٥، ٦ على الترتيب) أو بتوليفات مختلفة من الانزيم مع فيتامين د٣ (٥٠٠ وحدة من الانزيم+٤٠٠ وحدة دولية من الفيتامين، ٥٠٠ وحدة من الانزيم+٨٠٠ وحدة دولية من الفيتامين، ١٠٠٠وحدة من الانزيم+٤٠٠ وحدة دولية من الفيتامين أو ١٠٠٠وحدة من الانزيم+٨٠٠ وحدة دولية من الفيتامين/كجم عليقة-العلائق ٧، ٨، ٩، ١٠ على الترتيب) وتم تقديمها للمجموعات التجريبية المختلفة من الطيور خلال الفترة من ٢٨ حتى ٤٨ أسبوعا من العمر. تم تحليل عينات ممثلة من العلائق التجريبية لتقدير محتواها الغذائي. تم وزن الطيور فرديا عند بداية ونهاية التجربة مع أخذ قياسات يومية عن كل من انتاج البيض ووزن البيضة وكتلة البيض الكلية وأسبوعية عن كل من استهلاك العلف والتحول الغذائي وذلك طوال الفترة التجريبية.تم أخذ قياسات عن بعض معايير الجودة الخارجية والداخلية للبيض كل ٤ أسابيع خلال الفترة التجريبية. أيضا تم تفريخ ٣ دفعات من بيض المعاملات التجريبية المختلفة خلال الستة أسابيع الأخيرة من التجربة وذلك لتحديد النسب المئوية لكل من الخصوبة والفقس والنفوق الجنيني ونقص الوزن لبيض التفريخ. كما تم اجراء اختبار ذبح لتقدير بعض مكونات بلازما الدم (البروتين الكلي، الألبيومين، الجلوبيولين، نسبة الألبيومين:الجلوبيولين، الكالسيوم والفوسفور غير العضوي، وكذلك نشاط انزيمي الانتين أمينوترانسفيريز وأسبرتيت أمينوترانسفيريز) وتقديرطول وعرض عظمة الساق ومحتوياتها من الكالسيوم والفوسفور. كذلك تم تقدير محتويات فشر بيض المعاملات المختلفة من عنصري الكالسيوم والفوسفور. يمكن تلخيص النتائج المتحصل عليها في الاتي:

- رغم انخفاض المظاهر الانتاجية للطيور التي غذيت على العليقة الناقصة في الكالسيوم والفوسفورالا أنها لم تختلف معنويا مع عليقة الكنترول فى معدل انتاج البيض و كتلة البيض اليومية ومعدل التحويل الغذائي. من ناحية أخرى فان تغذية الطيور على عليقة المقارنة السالبة مع اضافة انزيم الفيتيز أو فيتامين د٣ أو مخلوطهما معا (العلائق من ٣ الى ١٠) حسنت معنويا كلا من معدل انتاج البيض وكتلة البيض اليومية مصحوبة بتحسن معدل التحويل الغذائى مقارنه بعليقة الكنترول وعليقة المقارنة السالبة.
- أدت التغذية على العليقة الناقصة في الكالسيوم والفوسفور الى حدوث نقص معنوي في كل من دليل شكل البيضة، %وزن القشرة، سمك القشرة، معامل الصفار، جودة البياض (وحدات هوف)، خصوبة البيض، محتويات قشرة البيضة وعظمة الساق من الكالسيوم وتركيزات كل من البروتين الكلي والكالسيوم والفوسفور غير العضوي في بلازما الدم بينما لم تتأثر باقي القياسات اذا ما قورنت بعليقة المقارنة (الكنترول).
- نتج عن تدعيم العليقة الناقصة في الكالسيوم والفوسفور بانزيم الفيتيز وخاصة مع المستوى العالي منه (١٠٠٠ وحدة/كجم) ازالة الاثار السلبية سالفة الذكر مع حدوث تحسنا معنويا في كل من معدل انتاج البيض، كتلة البيض اليومية، معامل التحويل الغذائي، %وزن الصفار، معامل الصفار، جودة البياض (وحدات هوف) ومحتوى الفوسفور في عظمة الساق اذا ما قورنت بعليقة المقارنة (الكنترول).
- نتج عن تدعيم العليقة الناقصة في الكالسيوم والفوسفور بفيتامين د٣ ازالة الاثار السلبية سالفة الذكر مع حدوث تحسنا بسيطا في كل من معامل التحويل الغذائي ودليل شكل البيضة اذا ما قورنت بعليقة المقارنة (الكنترول).
- نتج عن تدعيم العليقة الناقصة في الكالسيوم والفوسفور بانزيم الفيتيز +فيتامين د٣ ازالة الاثار السلبية سالفة الذكر مع حدوث تحسنا معنويا في كل من معدل انتاج البيض، كتلة البيض اليومية ومعامل التحويل الغذائي اذا ما قورنت بعليقة المقارنة (الكنترول).

ويمكن أن نستخلص من هذه الدراسة أن تدعيم علائق الدجاج البياض ذات المحتويات المنخفضة من الكالسيوم والفوسفور بانزيم الفيتيز أو بفيتامين د٣ أو بمزيجهما يمكن أن يحقق أثرا ايجابيا على المظاهر الانتاجية و التناسلية من خلال تحسين كفاءة استعمال الكالسيوم والفوسفور في الغذاء.