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# Effects of Phytase Supplementation of Low Phosphorous Diets Included Olive Pulp and Date Pits on Productive Performance of Laying Hens, Egg Quality Traits and Some Blood Parameters

**Meysam Afsari[1], Ahmad Mohebbifar[1] and Mehran Torki[1*]**

*[1]Department of Animal Science, Agriculturare Faculty, Razi UniversityImam Avenue, Kermanshah, Iran.*

***Authors' contributions***

*Major contribution for this study came from author MT and he carried out this study with the other assistants. Author AM took part in planning of the study, did the poultry examination, discussed the results and commented on the draft and final manuscript. Author MA took part in data collection. All authors read and approved the final manuscript.*

***Research Article***

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## ABSTRACT

**Aims:** This study was conducted to evaluate effects of phytase (E) supplementation of low phosphorous diets (NPP) included olive pulp (OP) and date pits (DP) on performance of laying hens, egg quality traits, blood parameters and excreta pH of laying hens.
**Study Design:** Data were analyzed based on 2×2×2 factorial arrangements in completely randomized design using GLM procedure of SAS.
**Place and Duration of Study:** The present experiment was done in Animal Science Department of Razi University, Kermanshah, Iran. All procedures used in this study were approved by the Animal Ethics Committee of Razi University and complied with the "Guidelines for the Care and Use of Animals in Research".
**Methodology:** A total number of 288 Lohmann LSL-Lite laying hens was randomly divided in 48 cages (n=6). Based on a 2×2×2 factorial arrangement of treatments, 8 iso-caloric and iso-nitrogenous experimental diets (ME =2720 Kcal/Kg and CP=150 g/Kg) consisting of two

**Corresponding author: Email: torki@razi.ac.ir;*

levels of date pits and olive pulp (0, and 90 g/kg), two levels of dietary non-phytate phosphorus (NPP: 2.6 and 3.3 g/kg diet) and phytase (0 and 150 FTU/kg) were formulated. Hens in every randomly-selected six cages (replicates) allotted to feed on each of 8 experimental diets.

**Results:** Dietary treatment did not have significant effect on egg production (EP) and feed intake (FI). Phytase numerically increased egg mass (EM) compared to control diets. Dietary inclusion of date pits and olive pulp significantly affected feed conversion ratio (FCR). Yolk index and Haugh unit were not significantly affected by dietary treatment (P>0.05). Diet inclusion of olive pulp and date pits numerically decreased eggshell weight and thickness in the first egg sampling (wk 3) in compared to control diet (*P*=.05). In the second egg sampling (wk 7), egg index, yolk index, Haugh unit, egg gravity and eggshell thickness were not significantly affected by dietary treatment. Dietary treatment did not have significant effect on blood parameters except for monocyte, so that a significant interaction between DO, P and E (p=0.01) was detected. Dietary treatment did not have significant effect on body weight changes (BWC) and excreta pH.

**Conclusion:** From the results of the present study, it can be concluded that DP and OP can be included in diet of laying hens up to 9% with no adverse effect on birds' performance. However, diet inclusion of DP and OP has some adverse effects on egg yolk color and eggshell weight.

*Keywords: Olive pulp; date pits; low phosphorous diet; phytase; laying hens; performance; egg qualitytraits; blood parameters.*

## 1. INTRODUCTION

Phosphorus (P) is an essential mineral in all feeds for poultry. Phosphorus is one of the mineral essential for structurally and metabolically growth and production. In the other hand, P is considered as an expensive nutrient that commonly supplemented in poultry diets. It represents the third most expensive nutrients following protein and energy. In poultry diets which are generally formulated based on corn-soybean meal, the major constituents of poultry diets, approximately two-thirds of the total Pits in the form of phytate [1]. An improvement in egg production, weight gain, and feed consumption for hens fed a diet low in non phytate P (NPP) with supplementary phytase was seen when compared to hens fed a low NPP diet without supplemental phytase [2]. There have been many studies conducted on laying hens fed maize-soybean meal indicating that microbial phytase improves performance, bone mineralization, P utilization and digestion of nutrients from NPP-deficient diets [3-6]. It has been shown that supplementing laying hens' diets with microbial phytase results in improved performance [7], particularly when dietary levels of NPP are low [8]. It has been demonstrated that when total P was decreased from 0.67% to 0.53% in broiler diets, the dietapparent metabolisable energy (AME), ileal P, calcium (Ca), protein and amino acid digestibility were increased [9]. Also an increase in diet AME and P digestibility was detected when NPP was decreased from 0.40% to 0.23% in broiler diets [10]. Significant beneficial responses were reported when a microbial phytase was added to chicken and duckling diets based on sorghum and soybean meal [11], so that they reported increased availability of dietary P (by reduced P excretion), the AME of the diet and nitrogen retention. Although it appears that poultry can utilize plant P in a bound form to a limited extent, this is unlikely to be of significance in the young bird [12].

Feed cost is perhaps the most expensive input in poultry production as it constitutes about 70-80% of the real cost of production, especially for intensively reared stocks [13]. Many poultry researchers have attempted to reduce feed costs by using locally available cheaper unconventional feedstuffs. In recent years, agricultural by-products become important feed components in poultry diets in some regions of the world, mainly due partly to the increased competition for the conventional ingredients by humans and the food industries.

Olive pulp (OP) is the remainder of olive cake (the raw material resulting from extraction of olive oil) after the removal of the seed fractions. About 0.3 of cell wall fraction will be removed by sieving [14]. Due to low nutritive value (Including low in energy, digestible proteins and minerals and high crude fiber contents), olive pulp is seldom integrated into poultry feeding. The olive oil industry generates large amounts of by-products that are harmful to the environment. According to the Food and Agriculture Organization of the United Nations [15], 2.7 millions of tons of olive oil are produced annually worldwide, 76% of which are produced in Europe, with Spain (35.2%), Italy (23.1%) and Greece (16.1%) being the highest olive oil producers. Other olive oil producers are Africa (12.5%), Asia (10.5%) and America (0.9%).

The date palm (*Phoenix dactylifera* L.) is considered the most important source of food for both human and animals in arid and semi-arid regions. Dates contain a high percentage of sugars reaching 88% in some varieties [16]. Dates are also rich in mineral salts and vitamins [17]. For the date pits (DP), the percentage of non-reducing sugars is 3.82% and of glucose and fructose is 1.68 and 1.53%, respectively [18]. Date crop plays an important role in the economy and social life in these regions [19]. Based on an experiment in which the effect of using rations containing different levels of whole dates on the growth performance of broiler chicks during the period of 20 to 49 days of age was evaluated [20],no significant difference was detected in feed intake, live weight and feed conversion ratio, when whole dates were used for up to 300 g/kg. Feedstuffs for economical animal production are considerably limited in many regions aroundthe world, where the feedstuff importation becomes necessary. This is becoming even worse due to the use of different crops such as maize in bio-fuel production and the subsequent increase in the price of energy sources in animal rations [21-23]. Based on a research work in which date pits fed to broiler chicks in the amounts of 5-27% of their total ration, date pits or the dietary fat caused no improved chick growth performance [24]. In another study, it was found that date pits treated with sodium hydroxide led to an increase in the rate of *in vitro* digestibility, the treatment making some of the unavailable fiber of the cell wall component [25].

The objectives of the present study were to investigate the effects of phytase supplementation of low phosphorous diets included olive pulp and date pits on the performance, egg quality traits, blood parameters, and excreta pH of laying hens.

## 2. MATERIALS AND METHODS

All procedures used in this experiment were approved by the Animal Ethics Committee of Razi University and complied with the "Guidelines for the Care and Use of Animals in Research".

## 2.1 Birds and Experimental Diets

A total number of 288 Lohmann LSL-Lite hens (56-weeks old) with an average egg production rate of 90.6±4.8% (late laying phase) and 1460±24 g live body weight, were obtained from a commercial supplier and randomly distributed between 48 cages (n=6). The hens were placed in wire-floored cages (0.3 m wide×0.9 m length×0.4 m height) arranged in a single tier within a conventional open-sided house. The cages were located in a windowless and environmentally controlled room with the room temperature kept at 2l-23℃ and the photoperiod set at 16 h of light (incandescent lighting, 10 lux) and 8 h dark. Each cage had a nipple watered. Water was available *ad libitum* throughout the experiment. Feed consumption was measured on a weekly basis. After a week of adaptation, the hens were randomly allocated to one of the 8 experimental diets. Based on a 2×2×2 factorial arrangement of treatments, 8 iso-caloric and iso-nitrogenous experimental diets (ME =2720 Kcal/Kg and CP=150 g/Kg) consisting two levels of date pits and olive pulp (0, and 90 g/kg), two levels of dietary non-phytate phosphorus (2.6 and 3.3 g/kg diet) and phytase (0 and 150 FTU/kg) were formulated and fed to hens with 4 replicates per diet during 7-week trial period. The enzyme used in this experiment, Phyzyme® XP 5000G, is phytase (6-phytase) of bacterial origin (BIOCHEM, activity determined by the manufacturer).The chemical composition (nutrients contents) of used DP was as presented here as well as the footer of the diet table (ME=2000kcal/kg, CP=7.03%, Ether Extract=7.10%, Crude fiber=48.2%, Calcium=0.865%, Available Phosphorous=0.03%).The approximate analysis of the OP used in this study is: dry matter (DM=93%), crude protein (CP= 6.06%), ether extract (EE%= 7.6%), crude fiber (CF%= 48.2), Ash (7.4%), Calcium (Ca%=0.6) and total phosphorous (P%= 0.07). Fatty acid composition (%) of oils from OP used in this study is: total saturated fatty acids (~SFA= 15.01 %), total monounsaturated fatty acids (~MUFA= 71.38%) and total polyunsaturated fatty acids (~PUFA= 9.10%). Feed intake (FI) was recorded on a weekly basis. Feed conversion ratio (FCR) was expressed as kg of feed consumed per kg of egg produced. Egg mass was calculated by multiplying egg weight by egg production. The magnitude of production variables such as feed intake and egg production were adjusted for hen mortalities. Deaths were recorded daily as they occurred.

## 2.2 Egg Quality Traits and Blood Parameters

In weeks 3 and 7, all produced eggs per each dietary group during 3 frequent days were collected to measure egg quality traits. External egg quality characteristics including egg shape index, eggshell thickness, and internal egg quality characteristics including Haugh unit (HU) were monitored in weeks 3 and 7. When determining egg quality characteristics, the sample of eggs was individually weighed at initiation. Afterwards, the egg shape index was calculated by dividing egg length by egg width. Then, the eggs were carefully cracked and the eggshell separated. Haugh unit was calculated using the formula described by Roush [26]. Eggshell weight was expressed as a percentage of the egg weight. After removing the shell membranes manually, eggshell thickness (without inner and outer shell membranes) was measured at three different points (top, middle, and bottom) using a micrometer. An average of three different thickness measurements of an egg was described as eggshell thickness.

At the end of the experiment (wk 7) four hens were randomly selected from each treatment (one hen per replicate) and blood samples were collected from the wing vein into a 5-ml syringe. Part of the blood which had been obtained having been centrifuged (3000×g for 15 min) immediately and serum collected for subsequent analysis, the rest was placed in tubes

with heparin as anticoagulant in order to diacritical counts of white blood cells based on the procedures of [27]. Briefly, two drops of blood were placed on a slide, spin prepared and stained with May-Grünwald-Giemsa stain. All slides were coded and one hundred leukocytes, including granular (heterophils, eosinophils, and basophils) and nongranular (lymphocytes and monocytes) were counted on one slide per each bird, and the heterophil to lymphocyte (H/L) ratio was calculated. Serum triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), and total cholesterol were analyzed using the diagnostic kit (Pars Azmun, Iran), and enzymatic methods.

## 2.3 Collection of Excreta Samples

The excreta were collected on galvanized zinc trays lined with plastic sheets. Dropped feathers, feed particles or foreign materials were removed to prevent contamination. Approximately 200g samples were collected daily. Each of the excreta samples was mixed and homogenized individually. The pH of 1 g of excreta in 10 mL of distilled water was measured using a digital pH meter (model 632 equipped with the electrode 6.0202.000 containing 3 *M* KCl electrolyte; Metrohm, Herisau, Switzerland).

## 2.4 Statistical Analysis

Data were analyzed based on 2×2×2 factorial arrangements in completely randomized design using GLM procedure of SAS [28]. All statements of significance are based a probability of less than.05.The used statistical model was$Y_{ijkl} = \mu + A_j + B_j + C_k + (A.B)_{ij} + (B.C)_{jk} + (A.B.C)_{ijk} + e_{ijkl}$; where $Y_{ijkl}$ = tested parameter of laying fed diets containing graded levels of DP and OP (0, 0 and 90 g/kg), NPP (0.33 and 0.26 g/kg diet) and phytase (E)(0 and 150 FTU/kg), $A_i$ = dietary inclusion of DP and OP (0, 0 and 90 g/kg),$B_j$ = dietary inclusion of NPP (0.33 and 0.26 g/kg diet),$C_k$ = dietary phytase supplementation (0 and 150 FTU/kg),$(A.B)_{ij}$ = interaction between DP and OP and NNP,$(A.K)_{ik}$ = interaction between DP and OP and phytase (E),$(B.C)_{jk}$ = interaction between NPP and phytase (E),$(A.B.K)_{ijk}$ = interaction between DP and OP, NPP and phytase, and $e_{ijkl}$ = random error term.To facilitate the statistical analysis of the data, all of the parameters were keyed in into Microsoft Excel and then transferred to the SAS [28].

**Table 1. Ingredients and composition of experimental diets**

| ***Feed ingredients*** | **g / 100 g diet** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Corn | 55.82 | 55.82 | 55.82 | 55.82 | 46.14 | 46.14 | 46.14 | 46.14 |
| Fish meal | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 |
| Soybean meal | 16.62 | 16.62 | 16.62 | 16.62 | 15.74 | 15.74 | 15.74 | 15.74 |
| Date pits | - | - | - | - | 9 | 9 | 9 | 9 |
| Olive pulp | - | - | - | - | 9 | 9 | 9 | 9 |
| Oil | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 |
| Dicalcium phosphate | 1.29 | 1.29 | 0.93 | 0.93 | 1.33 | 1.33 | 0.97 | 0.97 |
| Lime stone | 8.31 | 8.31 | 8.52 | 8.52 | 7.96 | 7.96 | 8.16 | 8.16 |
| Common salt | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 |
| Phytase | - | + | - | + | - | + | - | + |
| Vit. & Min. Premix[3] | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Sand | 8.14 | 8.11 | 8.29 | 8.26 | 0.96 | 0.93 | 1.11 | 1.08 |
| DL-Methionine | 0.09 | 0.09 | 0.09 | 0.09 | 0.14 | 0.14 | 0.14 | 0.14 |
| *Calculated analyses* | | | | | | | | |
| ME (Kcal/kg) | 2720 | 2720 | 2720 | 2720 | 2720 | 2720 | 2720 | 2720 |
| Crude protein (%) | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 |
| Calcium (%) | 3.67 | 3.67 | 3.67 | 3.67 | 3.67 | 3.67 | 3.67 | 3.67 |
| Available P (%) | 0.33 | 0.33 | 0.26 | 0.26 | 0.33 | 0.33 | 0.26 | 0.26 |
| Lys (%) | 0.82 | 0.82 | 0.82 | 0.82 | 0.82 | 0.82 | 0.82 | 0.82 |
| Met &Cys (%) | 0.62 | 0.62 | 0.62 | 0.62 | 0.62 | 0.62 | 0.62 | 0.62 |

[1]*The chemical composition (nutrients contents) of used date pits: ME= 2000 kcal/kg, Crude protein= 7.03%, Ether Extract= 7.10%, Crude fiber= 48.2%, Calcium= 0.865%, Available Phosphorous= 0.03%.*

[2]*The approximate analysis of the OP used in this study is: dry matter (DM=93%), crude protein (CP= 6.06%), ether extract (EE%= 7.6%), crude fiber (CF%= 48.2), Ash (7.4%), Calcium (Ca%=0.6) and total phosphorous (P%= 0.07). Fatty acid composition (%) of oils from OP used in this study is: total saturated fatty acids (~SFA= 15.01 %), total monounsaturated fatty acids (~MUFA= 71.38%) and total polyunsaturated fatty acids (~PUFA= 9.10%).*

[3]*Mineral mix supplied the following per kg of diet: Cu, 20 mg; Fe, 100 mg; Mn, 100 mg; Se, 0.4 mg.; Zn, 169.4 mg. 'Vitamins mix supplied the following per kg of diet: Vitamin A, 18,000 IU; vitamin D3, 4,000 IU; vitamin E, 36mg; vitarnin K; 4 mg; vitamin B\2, 0.03 mg; thiamine, 1.8 mg; riboflavin, 13.2 mg; pyridoxine, 6 mg; niacin, 60 mg; calcium pantothenate, 20 mg; folic acid, 2 mg; biotin, 0.2 mg; choline chloride, 500 mg*

## 3. RESULTS AND DISCUSSION

### 3.1 Productive Performance and Egg Quality Traitsof Laying Hens

Effects of dietary treatment on feed intake(FI), feed conversion ratio (FCR), egg mass (EM) and hen-day egg production (EP) during the present trial are presented in Table 2. Diet inclusion of date pits and olive pulp increased EM, EP and FCR in compared with the control diet; however, this increase did not statistically significant (*P*=05). Diet inclusion of date pits and olive pulp had significanteffect on FCR in compared with the control group. Studies concerning feeding OP to monogastrics are limited [29]. In general, few studies have been done about the effect of dietary inclusion of DP on laying hens. It has been found that up to 50 % DP in the laying hens' diet has no effect on FCR[30]. It has been also observed that the use of 0, 50, 100, 150 and 200 g/kg OP has no significant effect on EP [31]. Influences of diet OP inclusion on birds' performance would be mostly affected by the approximate analysis of OP, type and age of birds and dietary ingredients.

In the present study,dietary phytase supplementationhad no significant effect on EP, FI and FCR in comparedwiththe control groupexcept for EM. It has been documented that the feed consumption increase in hens fed diets contained 0.1% NPP supplemented with phytase [8,32]. No significant difference was found on feed intake, egg production and egg weight when microbial phytase (300 U/kg) supplemented corn-soybean meal and wheat-soybean meal diets fed Lohmann Brown (22 to 61 week) [33]. Whereas, it was reported that dietary phytase supplementation allow the reduction of NPP content to 0.l2% in diet, without affecting the production performance of laying hens [34]. In hens from 21 to 33 weeks of age, as dietary NPP was decreased from 0.26 to 0.11 % without phytase, FI significantly decreased [4]. In contrast to our finding,the data on mean production performance of laying hens during 60 to 72 weeks of age shown that dietary phytase supplementation decreased hen day egg production [ 6]. Only minor non-significant differences were observed between the 0.33% and 0.26% NPP without phytase supplementation for most of the production parameters (Table 2). The hens fed 0.26% NPP diet without exogenous phytase supplementation had higher EP and EM in compared with the 0.33% NPP treatment, but the differences were not significant. Our results showed that laying hens can maintain optimal health and production when fed a diet containing 0.26% NPP. It has been recommended that white-egg layers require 250 mg of NPP per day per hen if the feed intake is 100 g per day to maintain optimal health and production [35].

**Table 2. Effects of diet inclusion of date pits and olive pulp (0, and 90 g/kg), with two non-phytate phosphorus (NPP) levels (2.6 and 3.3 g/kg diet), supplemented by phytase (0 and 150 FTU/kg)on productive performance of Lohmann LSL-lite laying hens (56-64 WK of age)a**

| Treatments | Feed intake (g/hen/day) | Feed conversion ratio (g feed: g egg) | Hen-day egg production (%) | Egg mass (g/hen/day) |
|---|---|---|---|---|
| Date & Olive (DO) | | | | |
| 0.0 (g/kg diet) | 111±8.10[b] | 2.06±0.22 | 87.3±8.62 | 55.0±6.51 |
| 90 (g/kg diet) | 117±1.93[a] | 2.18±0.11 | 88.3±4.67 | 54.5±2.97 |
| NPP (P) | | | | |
| 3.3 (g/kg diet) | 114±7.44 | 2.17±0.17 | 85.9±7.43 | 52.8±5.24 |
| 2.6 (g/kg diet) | 114±6.12 | 2.07±0.19 | 89.7±5.81 | 56.7±3.97 |
| Phytase (E) | | | | |
| 0.0 ( FTU/kg ) | 113±8.38 | 2.17±0.17 | 85.9±7.43 | 52.8±5.24[b] |
| 150 ( FTU/kg ) | 116±4.22 | 2.07±0.19 | 89.7±5.81 | 56.7±3.97[a] |
| SEM | 1.18 | 0.032 | 1.20 | 0.881 |
| CV | 5.17 | 8.42 | 7.90 | 8.79 |
| *P* values | | | | |
| Date & Olive (DO) | 0.00 | 0.06 | 0.67 | 0.77 |
| NPP (P) | 0.76 | 0.82 | 0.98 | 0.99 |
| Phytase (E) | 0.16 | 0.15 | 0.13 | 0.03 |
| DO×P | 0.96 | 0.82 | 0.70 | 0.86 |
| DO×E | 0.08 | 0.46 | 0.15 | 0.08 |
| P×E | 0.71 | 0.54 | 0.60 | 0.89 |
| DO×P×E | 0.94 | 0.27 | 0.36 | 0.45 |

*aMeans±SD, abMeans within a column showing different superscripts are significantly different (P< 0.05), Duncan's multiple-range test were applied to compare means*

Effects of dietary treatments on egg quality traits for wk 3 (first sampling) and wk 7 (second sampling) are presented in Tables 3 and 4, respectively. Yolk index and Haugh unit were not significantly affected by dietary treatments (P= 0.05). Diet inclusion of olive pulp and date pits decreased eggshell weight and thickness in the first egg sampling (wk 3) in compared with the control diet (P≤ 0.05). There was no interaction between DO, P and E on egg quality traits except for egg index (p=0.04). In second egg sampling (wk 7),egg index, yolk index, Haugh unit, egg gravity and eggshell thickness were not significantly affected by dietary treatments (*P*=05).There was no interaction between DO and P on egg traits except for eggshell weight. These findings were in agreement with previous report in which no significant effect of phytase supplementation on Hugh unit was seen [34].In another report no significant difference in egg specific gravity, regardless of NPP level or phytase inclusion was detected [36].

## 3.2 Blood Parameters

Effects of dietary treatments on white blood cell count as well as plasma level blood parameters are presented in Tables 5 and 6. Dietary treatment did not have significant effect on blood parameters. [37] demonstrated that using 10and 20% of olive cake had no significant effect on TG content but level of 5% reduced plasma cholesterol concentration. A three way interaction between DO, P and E on monocyte (p=0.01) was detected.

## 3.3 Excreta pH and Body Weight Changes

Effects of dietary treatments on the excreta pH and body weight changes are presented in Table 7. Dietary treatments did not have significant effect on body weight changes (BWC) and feces pH.It has been previously found that the body weight of hens fed a diet containing 0.1% NPP was significantly less than that of hens fed the same diet supplemented by phytase [8, 38].

**Table 3.Effects of diet inclusion of date pits and olive pulp (0, and 90 g/kg), with two non-phytate phosphorus (NPP) levels (2.6 and 3.3 g/kg diet), supplemented by phytase (0 and 150 FTU/kg) on egg quality traits (first egg sampling on WK 3 of trial)[a]**

| | Egg quality traits (wk 3) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Treatments** | **Egg index** | **Yolk index** | **Haugh unit** | **Yolk color (Roch)** | **Specific gravity** | **Eggshell weight (gr)** | **Eggshell thickness (mm×10-2)** |
| Date & Olive (DO) | | | | | | | |
| 0.0 (g/kg diet) | 74.4±2.26 | 37.9±1.24 | 67.6±3.73 | 6.58±0.33 | 1.09±0.003[a] | 6.51±0.24[a] | 38.2±1.37[a] |
| 90 (g/kg diet) | 75.0±1.24 | 37.7±3.66 | 67.3±5.63 | 6.42±0.35 | 1.08±0.004[b] | 5.68±0.92[b] | 34.0±4.38[b] |
| NPP (P) | | | | | | | |
| 3.3 (g/kg diet) | 74.8±2.10 | 37.2±3.44 | 68.0±4.87 | 6.46±0.38 | 1.09±0.005 | 6.14±0.70 | 35.9±3.64 |
| 2.6 (g/kg diet) | 74.7±1.55 | 38.4±1.50 | 66.9±4.62 | 6.54±0.32 | 1.09±0.003 | 6.05±0.89 | 36.3±4.12 |
| Phytase (E) | | | | | | | |
| 0.0 ( FTU/kg ) | 74.8±2.10 | 37.2±3.44 | 68.0±4.87 | 6.46±0.38 | 1.09±0.005 | 6.14±0.70 | 35.9±3.64 |
| 150 ( FTU/kg ) | 74.7±1.55 | 38.4±1.50 | 66.9±4.62 | 6.54±0.32 | 1.09±0.003 | 6.05±0.89 | 36.3±4.12 |
| SEM | 0.322 | 0.475 | 0.831 | 0.062 | 0.001 | 0.139 | 0.677 |
| CV | 2.17 | 7.11 | 7.67 | 5.60 | 0.46 | 11.2 | 9.23 |
| *P* values | | | | | | | |
| Date&Olive (DO) | 0.29 | 0.85 | 0.85 | 0.20 | 0.04 | 0.00 | 0.00 |
| NPP (P) | 0.06 | 0.10 | 0.86 | 0.34 | 1.00 | 0.42 | 0.65 |
| Phytase (E) | 0.89 | 0.19 | 0.54 | 0.52 | 0.49 | 0.70 | 0.70 |
| DO×P | 0.06 | 0.27 | 0.62 | 0.34 | 0.49 | 0.12 | 0.35 |
| DO×E | 0.84 | 0.64 | 0.71 | 0.52 | 0.17 | 0.97 | 0.97 |
| P×E | 0.24 | 0.15 | 0.76 | 0.75 | 0.49 | 0.41 | 0.30 |
| DO×P×E | 0.04 | 0.26 | 0.44 | 0.75 | 1.00 | 0.35 | 0.17 |

*[a]Means±SD, [ab]Means within a column showing different superscripts are significantly different (P< 0.05), Duncan's multiple-range test were applied to compare means*

**Table 4. Effects of diet inclusion of date pits and olive pulp (0, and 90 g/kg), with two non-phytate phosphorus (NPP) levels (2.6 and 3.3 g/kg diet), supplemented by phytase(0 and 150 FTU/kg) egg quality traits (second egg sampling on WK7 of trial)[a]**

| | | Egg quality traits (wk 7) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Treatments** | | **Egg index** | **Yolk index** | **Haugh unit** | **Yolk color (Roch)** | **Specific gravity** | **Shell weight (gr)** | **Shell thickness (mm×10-2)** |
| Date & Olive (DO) | | | | | | | | |
| 0.0 (g/kg diet) | | 74.5±2.13 | 39.3±10.5 | 70.4±6.53 | 6.33±0.84[a] | 1.09±0.004 | 5.94±0.31[a] | 36.6±2.04 |
| 90 (g/kg diet) | | 85.0±39.9 | 43.2±1.11 | 66.8±3.67 | 5.88±0.38[b] | 1.09±0.01 | 5.44±0.36[b] | 34.4±3.77 |
| NPP (P) | | | | | | | | |
| 3.3 (g/kg diet) | | 85.1±39.9 | 39.8±10.71 | 68.1±6.29 | 5.83±0.73[b] | 1.09±0.01 | 5.63±0.42 | 36.3±2.20 |
| 2.6 (g/kg diet) | | 74.4±1.77 | 42.6±1.39 | 69.1±4.83 | 6.38±0.53[a] | 1.09±0.01 | 5.75±0.41 | 34.7±3.87 |
| Phytase (E) | | | | | | | | |
| 0.0 ( FTU/kg ) | | 85.1±39.9 | 39.8±10.7 | 68.1±6.29 | 5.83±0.73[b] | 1.09±0.01 | 5.63±0.42 | 36.3±2.20 |
| 150 ( FTU/kg ) | | 74.4±1.77 | 42.6±1.39 | 69.1±4.83 | 6.38±0.53[a] | 1.09±0.01 | 5.75±0.41 | 34.7±3.87 |
| SEM | | 5.00 | 1.35 | 0.979 | 0.12 | 0.001 | 0.073 | 0.56 |
| CV | | 35.4 | 17.9 | 7.44 | 8.77 | 0.55 | 5.37 | 9.07 |
| *P* values | | | | | | | | |
| Date&Olive (DO) | 0.31 | | 0.15 | 0.05 | 0.02 | 1.00 | 0.00 | 0.06 |
| NPP (P) | 0.39 | | 0.28 | 0.15 | 0.02 | 0.25 | 0.47 | 0.70 |
| Phytase (E) | 0.30 | | 0.29 | 0.60 | 0.01 | 1.00 | 0.28 | 0.19 |
| DO×P | 0.33 | | 0.34 | 0.33 | 0.06 | 0.56 | 0.02 | 0.84 |
| DO×E | 0.34 | | 0.25 | 0.44 | 0.51 | 0.56 | 0.82 | 0.65 |
| P×E | 0.31 | | 0.28 | 0.05 | 0.83 | 0.56 | 0.12 | 0.68 |
| DO×P×E | 0.32 | | 0.32 | 0.86 | 0.14 | 0.25 | 0.46 | 0.68 |
| DO P | | | | | | | | |
| 0 0 | | | | | | | 5.77±0.19b | |
| 0 1 | | | | | | | 6.12±0.31a | |
| 9 0 | | | | | | | 5.54±0.31ab | |
| 9 1 | | | | | | | 5.34±0.39c | |
| CV | | | | | | | 5.40 | |
| P values | | | | | | | 0.00 | |

*[a]Means±SD, [ab]Means within a column showing different superscripts are significantly different (P< 0.05), Duncan's multiple-range test were applied to compare means*

**Table 5.Effects of diet inclusion of date pits and olive pulp (0, and 90 g/kg), with two non-phytate phosphorus (NPP) levels (2.6 and 3.3 g/kg diet), supplemented by phytase (0 and 150 FTU/kg)on white blood cell counts (heterophil, lymphocyte, monocyte, eosinophil, basophil and Heterophil to Lymphocyte ratio)[a]**

| **Parameter (%)** | **White blood cell counts (%)** | | | | | |
|---|---|---|---|---|---|---|
| | **H[1]** | **L** | **M** | **E** | **B** | **H/L** |
| Date & Olive (DO) | | | | | | |
| 0.0 (g/kg diet) | 23.3±11.4 | 61.5±25.1 | 1.50±1.67 | 0.63±0.81 | 0.44±0.73 | 0.35±0.20 |
| 90 (g/kg diet) | 31.6±6.69 | 65.9±7.90 | 1.13±1.46 | 0.56±0.73 | 0.75±0.86 | 0.50±0.15 |
| NPP (P) | | | | | | |
| 3.3 (g/kg diet) | 25.7±12.7 | 59.1±24.6 | 1.31±1.54 | 0.63±0.81a | 0.63±0.81 | 0.40±0.23 |
| 2.6 (g/kg diet) | 29.2±6.47 | 68.3±7.17 | 1.31±1.62 | 0.56±0.73b | 0.56±0.81 | 0.44±0.14 |
| Phytase (E) | | | | | | |
| 0.0 ( FTU/kg ) | 25.7±12.7 | 59.1±24.6 | 1.31±1.54 | 0.63±0.81 | 0.63±0.81 | 0.40±0.23 |
| 150 ( FTU/kg ) | 29.2±6.47 | 68.3±7.17 | 1.31±1.62 | 0.56±0.73 | 0.56±0.81 | 0.44±0.14 |
| SEM | 1.79 | 3.26 | 0.27 | 0.13 | 0.14 | 0.034 |
| CV | 25.2 | 12.4 | 93.1 | 101 | 146 | 35.9 |
| **P values** | | | | | | |
| Date & Olive (DO) | 0.14 | 0.28 | 0.07 | 0.77 | 0.32 | 0.17 |
| NPP (P) | 0.48 | 0.53 | 0.17 | 0.00 | 0.84 | 0.68 |
| Phytase (E) | 0.78 | 0.62 | 0.25 | 0.77 | 0.84 | 0.60 |
| DO×P | 0.74 | 0.80 | 0.64 | 0.16 | 0.84 | 0.77 |
| DO×E | 0.93 | 0.84 | 0.25 | 0.05 | 0.55 | 0.99 |
| P×E | 0.38 | 0.62 | 0.82 | 0.01 | 0.55 | 0.50 |
| DO×P×E | 0.33 | 0.18 | 0.01 | 0.39 | 0.84 | 0.26 |

*aMeans±SD, abMeans within a column showing different superscripts are significantly different (P< 0.05), Duncan's multiple-range test were applied to compare means. Heterophil, Lymphocyte, Monocyte, Eosinophil, Basophil, Heterophil to Lymphocyte ratio*

**Table 6. Effects of diet inclusion of date pits and olive pulp (0, and 90 g/kg), with two non-phytate phosphorus (NPP) levels (2.6 and 3.3 g/kg diet), supplemented by phytase (0 and 150 FTU/kg) onserum biochemical metabolites (cholesterol, triglycerides, high density lipoprotein, low density lipoprotein)**

| | Serum biochemical metabolites (mg/dL) | | | |
|---|---|---|---|---|
| **Parameter** | **CHOL(mmol/L)[1]** | **T.G (mg/dL)** | **HDL (mmol/L)** | **LDL(mmol/L)** |
| Date & Olive (DO) | | | | |
| 0.0 (g/kg diet) | 136±74.7 | 1727 ±1025 | 34.4±18.3 | 88.8±47.3 |
| 90 (g/kg diet) | 119±72.5 | 1409±1061 | 32.0±19.9 | 77.6±44.1 |
| NPP (P) | | | | |
| 3.3 (g/kg diet) | 123.06±81.81 | 1548±1137 | 32.7±22.2 | 79.8±50.9 |
| 2.6 (g/kg diet) | 133.19±65.16 | 1589±968 | 33.6±15.6 | 86.6±40.3 |
| Phytase (E) | | | | |
| 0.0 ( FTU/kg ) | 123±81.8 | 1548±1137 | 32.7±22.2 | 79.8±50.9 |
| 150 ( FTU/kg ) | 133±65.1 | 1589±968 | 33.6±15.6 | 86.6±40.3 |
| SEM | 12.8 | 183 | 3.34 | 8.02 |
| CV | 27.5 | 40.4 | 25.2 | 22.1 |
| **P values** | | | | |
| Date & Olive (DO) | 0.63 | 0.54 | 0.74 | 0.34 |
| NPP (P) | 0.78 | 0.92 | 0.67 | 0.96 |
| Phytase (E) | 0.16 | 0.14 | 0.09 | 0.14 |
| DO×P | 0.40 | 0.37 | 0.92 | 0.43 |
| DO×E | 0.10 | 0.06 | 0.09 | 0.15 |
| P×E | 0.89 | 0.99 | 0.43 | 0.72 |
| DO×P×E | 0.39 | 0.31 | 0.84 | 0.44 |

*aMeans±SD, abMeans within a column showing different superscripts are significantly different (P< 0.05), Duncan's multiple-range test were applied to compare means1Cholesterol, Triglycerides, High density lipoprotein cholesterol, Low density lipoprotein cholesterol*

**Table 7. Effects of diet inclusion of date pits and olive pulp (0, and 90 g/kg), with two non-phytate phosphorus (NPP) levels (2.6 and 3.3 g/kg diet), supplemented by phytase (0 and 150 FTU/kg))on excreta pH and body weight changes(BWC)[a]**

| Parameter | Excreta pH | BWC (g) |
|---|---|---|
| Date & Olive (DO) | | |
| 0.0 (g/kg diet) | 7.44±0.41 | 128±78.8 |
| 90 (g/kg diet) | 7.18±0.38 | 94.1±131 |
| NPP (P) | | |
| 3.3 (g/kg diet) | 7.28±0.48 | 104±97.9 |
| 2.6 (g/kg diet) | 7.34±0.34 | 117.7±120 |
| Phytase (E) | | |
| 0.0 ( FTU/kg ) | 7.28±0.48 | 104±97.9 |
| 150 ( FTU/kg ) | 7.34±0.34 | 117.7±120 |
| SEM | 0.072 | 19.1 |
| CV | 5.33 | 99.6 |
| ***P* values** | 0.07 | 0.39 |
| Date & Olive (DO) | | |
| NPP (P) | 0.61 | 0.39 |
| Phytase (E) | 0.67 | 0.74 |
| DO×P | 0.10 | 0.21 |
| DO×E | 0.16 | 0.22 |
| P×E | 0.33 | 0.48 |
| DO×P×E | 0.95 | 0.66 |

*aMeans±SD, abMeans within a column showing different superscripts are significantly different (P< 0.05), Duncan's multiple-range test were applied to compare means*

## 4. CONCLUSION

From the results of the present study, it can be concluded that DP and OP can be included in diet of laying hens up to 9% with no adverse effect on their performance. However, diet inclusion of DP and OP has some adverse effect on egg yolk color and eggshell weight. Dietary phytase and NPP leveldid not have beneficial effect on laying hens' performance and egg quality traits.

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## COMPETING INTERESTS

Major contribution for this study came from M. Torki and he carried out this study with the other assistants. A. Mohebbifar took part in planning of the study, did the poultry examination, discussed the results and commented on the draft and final manuscript. M. Afsari collected the data.

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