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Effects of Dietary Inclusion of Ground Pits of Date Palm (*Phoenix dactylifera*) Supplemented With Enzyme on Productive Performance, Egg Quality Traits and Blood Parameters of Laying Hens

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: This study was carried out to evaluate effects of dietary inclusion of ground pits of date palm (DP) (*Phoenix dactylifera*) supplemented with a bacterial endo-xylanase (Nutrase®) on performance of laying hens, egg quality traits and blood parameters.
Study Design: Data were analyzed based on completely randomized design using GLM procedure of SAS (SAS Institute, 2003).
Place and Duration of Study: All procedures used in this seven-week 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".
Methodology: A total number of 144 Lohmann LSL-Lite hens were randomly divided in 24 cages. Based on a 3×2 factorial arrangement of treatments six iso-caloric and iso-nitrogenous experimental diets (ME =2720 Kcal/Kg and CP=15 g/Kg) including: I-corn-soybean meal-based control-1(C1), II-corn-soybean meal-oil-based control-2 (C2), and III-corn-soybean meal-based diet included (240 g/kg DP) with and without enzyme (0.0 and 0.07 g/kg endo-xylanase)(E), were formulated. Data of feed intake (FI), egg production

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(EP), egg mass (EM) were daily collected, and feed conversion ratio (FCR) was calculated weekly. Egg quality traits were evaluated twice on weeks 3 and 7 of trial. Blood parameters were analyzed on week7 of trial.
Results: Dietary treatments did not have significant effect on EP; EM. Dietary inclusion of DP had significant effect on FI and FCR. Among blood parameters only heterophil percentage was affected by dietary inclusion of DP.
Conclusion: Ground pits of date palm can be included in laying hens' diets up to 24% with no adverse effect on EP; however, in terms of feed efficiency, egg shell weight and shell thickness and yolk color more studies in the future are appreciated.

Keywords: Blood parameters; date pits; egg traits; endo-xylanase; laying hens; performance.

1. INTRODUCTION

Agro-industrial by-products in recent years have become important feed components in poultry diets in some regions of the world mainly due to the increased competition for the conventional ingredients by humans and the food industries. Worldwide, date palm is an important crop in arid and semiarid regions. Date palm can be mostly found between latitudes 10º and 39º north and plays an important role in the economic and social lives in the tropical and subtropical regions [1]. Common crop in Middle East countries are dates where approximately 70 % of the world production is recorded [2]. Between 6-12% of the date fruit is date pits (DP: stones, kernels or seeds) that depending on variety and quality grade. The pits of date palm having advantage of being locally produced in large quantities are cheap and high in energy content [3]. The nutritive value of date pits ranged from 5.64 % to 8.20% for CP, 1.60–9.35% for EE, 9.10–22.0% for CF, 58.5–75.4% for NFE, 38.5–73.1% for NDF, 17.2–35.3% for ADF, 0.19–0.32% for methionine and cystine, and 0.19–0.30% for lysine.ME value ranged from 1.350 to 1.746 kcal/kg diet [4]. Moreover, there are accumulative evidences indicating that cell wall non-starch polysaccharides (NSP) had anti-nutritional activity in many mono-gastric animals [5]. A wide range yield of galactomannan from *P. dactylifera* seeds has been reported based on variations in plant origins and extraction conditions [6]. [7] demonstrated that acid hydrolysis of the date polysaccharides yielded galactose (26.6%) and mannose (71.8%). β-mannan has been found to be deleterious to animal performance, compromising body weight gain (BWG) and feed conversion ratio (FCR) as well as glucose and water absorption [8,9].

NSP are mainly composed of arabinoxylans and β-glucans. These polymers are known for their anti-nutritive effects in poultry. High levels of soluble NSP result in increased gut viscosity. High levels of insoluble NSP result in increased water-holding capacity reduced access for digestive enzymes (nutrient packaging) and increased endogenous secretions. Ultimately, these effects lead to reduced performance and nutrient utilization. They can also result in increased microbial proliferation in the gut and poor litter quality [10-12].

NSP degrading enzymes have been commercially used for 20 years and xylanases are among the most commonly used enzyme activities in feed industry. Most xylanases are specific for the internal β-1, 4 linkages of polymeric xylans and are designated as endoxylanases. Studies have shown that xylanases derived from different micro-organisms exhibit different catalytic properties [13,14] and matching an enzyme activity with a substrate does not always guarantee the efficacy of the enzyme in degrading the substrate [15].

There is a discrepancy among reports about the effect of DP on performance of chickens which led to utilize different techniques to improve the nutritional value of DP. In this regard, [16] reported that including DP at a level of 10% in the finisher diets of broiler chicks significantly improve growth and FCR. [17] Found that adding 10% of acid-treated or untreated DP to broiler starter diets supported growth performance similar to those birds fed corn–soybean diet. [18] Found that adding 0.1% enzyme (a mix of xylanase, alpha amylase, and pectinase) to the starter diets containing 10 % DP significantly increased growth rate, but did not significantly affect broiler performance parameters. The poultry feed industry has greatly increased issues of commercially available exogenous enzymes during the past 15 years. Supplementing exogenous enzymes can improve digestion of nutrients from feedstuffs, thereby decreasing feed costs, improving bird performance and decreasing environmental impact of land applied manure [19,20].

Therefore, this study aims to investigate effects of diet inclusion of ground pits of date palm with or without enzyme on performance, egg quality traits and some blood parameters of laying hens.

2. MATERIALS AND METHODS

All procedures used in this seven-week 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 144 Lohmarm 56-week-old LSL-Lite hens 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. Hens were randomly distributed between 24 cages (n=6), with almost similar production rate and body weight between cages. The hens were placed in wire-floored cages (0.3 m wide×0.4 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. After a week of adaptation, the hens were randomly allocated to one of the six experimental diets. Based on a 3×2 factorial arrangement of treatments, six iso-caloric and iso-nitrogenous (ME =2720 Kcal/Kg and CP=150 g/Kg) experimental diets were formulated (Table 1). Hens in 4 cages (replicates) were assigned to feed on one of the 6 experimental diets including: I-corn-soybean meal-based control-1 diet (C1), II-corn-soybean meal-based control-2 diet with vegetable oil(C2), and III-corn-soybean meal-based diet included 240 g/kg ground date pits) and enzyme (0.0 and 0.07 g/kg) (E). Xylanase (Nutrase®), which was contained standardized activities of at endo-1, 4b-xylanase (Sylaxyme method, pH = 6)، β- Glucanase (β-Glucazyme method, pH = 4.2) and α-Amylase (Amylazyme method, pH = 6) was supplemented to half of experimental groups. Enzyme preparations with fine particle sizes (dicalcium phosphate, salt, lysine, methionine, premix and sand) used in the diets were fully mixed with about 5 kg of ground corn and this was added to the other ingredients, which were mixed in mixer located in the feed processing center. The chemical composition (nutrients contents) of the used date pits was as presented here as well as the footer of the diet table (ME= 2000 kcal/kg, Crude protein= 7.03%, Ether Extract= 7.10%, Crude fiber= 48.2%, Calcium= 0.865%, Available Phosphorous= 0.03%). Water was available *ad libitum* throughout the experiment. Feed consumption was measured on a daily basis.

Table 1. Ingredients and composition of experimental diets

Label	C1		C2		E	
Date pits[1]	0.00	0.00	0.00	0.00	24.00	24.00
Nutrase	0.00	0.07	0.00	0.07	0.00	0.07
Feed ingredients	g / 100 g diet					
Corn	67.11	67.11	54.49	54.49	40.96	40.96
Fish meal	0.0	0.0	4.70	4.70	4.70	4.70
Soybean meal	20.97	20.97	16.58	16.58	15.27	15.27
Date pits[1]	-	-	-	-	24.00	24.00
Soybean oil	-	-	5.00	5.00	5.00	5.00
Dicalcium phosphate	1.17	1.17	1.29	1.29	1.33	1.33
Lime stone	8.78	8.78	8.29	8.29	7.74	7.74
Common salt	0.29	0.29	0.23	0.23	0.23	0.23
Nutrase®	-	0.07	-	0.07	-	0.07
Vit. & Min. Premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
Sand	1.31	1.17	9.08	8.758.94	0.100.11	0.03
DL-Methionine	0.12	0.12	0.09	0.09	0.16	0.16
Total	100	100	100	100	100	100
Calculated analyses						
ME (Kcal/kg)	2720	2720	2720	2720	2720	2720
Crude protein (%)	15.00	15.00	15.00	15.00	15.00	15.00
Calcium (%)	3.67	3.67	3.67	3.67	3.67	3.67
Available P (%)	0.33	0.33	0.33	0.33	0.33	0.33
Lys (%)	0.33	0.33	0.82	0.82	0.75	0.75
Met (%)	0.36	0.36	0.38	0.38	0.41	0.41
Met &Cys (%)	0.62	0.62	0.62	0.62	0.62	0.62

1The 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%. 2The premix contained (/kg of diet): vitamin A, 9,600 IU; cholecalciferol, 3,120 IU; vitamin E, 36 IU;menadione, 2.4 mg; vitamin B12, 0.018 mg; riboflavin, 7.2 mg; pantothenic acid, 14.4 mg; niacin, 60 mg; thiamine, 1.2 mg; pyridoxine, 2.4 mg; folic acid, 0.72 mg; biotin, 0.10 mg; Zn, 100 mg; Fe, 80 mg; Mn, 100 mg; Cu, 12 mg; I, 1 mg; and Se, 0.3 mg.

2.2 Egg Quality Traits and Blood Parameters

Egg quality characteristics were measured twice on wk 3 and 7 of experiment and each time all eggs during three frequent days were used. At the end of the experiment (wk 7), four hens from each treatment (one hen per replicate) were randomly selected 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 [21]. 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 Statistical Analysis

Data were analyzed based on a 3×2 factorial arrangement in completely randomized design using GLM procedure of SAS [22]. All statements of significance are based a probability of less than 0.05. The mean values were compared by Duncan's Multiple Range Test. The statistical model used in this investigation was Yijk = µ + Ai + Bj + (A. B)ij + eijk, where Yijk = tested parameter of laying hens fed diets containing graded levels of DP; Ai =dietary inclusion of DP (0, 0 and 240 g/kg); Bj= dietary inclusion of endo-xylanase (0 and 0.7 g/kg), and (A.B)ij= interaction between DP and endo-xylanase addition, and e_{ijkl} = random error term. Statement of significance were based on P<0.05. To facilitate the statistical analysis of the data, all of the parameters were keyed in into Microsoft Excel and then transferred to the SAS.

3. RESULTS AND DISCUSSION

3.1 Productive Performance and Egg Quality Traits of Laying Hens

The results of productive performance and egg quality traits are presented in Tables 2, 3 and 4. As it is shown in Table 2, there was no interaction between DP and enzyme supplementation on feed intake (FI), feed conversion ratio (FCR),egg production (EP) and egg mass(EM). A significant affect in FCR and FI was detected for layers given the DP inclusion diet (*P*>.05). Dietary inclusion of date pits decreased EP and EM when compared with the control diets; however, this decreased did not statistically significant (*P*>.05).

In general, few studies have been done about the effect of dietary inclusion of DP on laying hens. [23] In their experiment found that up to 50 % DP in the laying hen's diet did not affect FCR. However, our results do agree with those of [24] who reported suppressed productive performance due to dietary inclusion rate of 15 % DP in Fayoumi × Barred Plymouth Rock laying hens. The differences in their results and ours may be partly due to the higher soybean oil level we used for balance of energy among the experimental diets and its subsequent effect on improving nutrient utilization [25, 26].This effect may also depend on breed, physiological condition, and variety of DP. [27, 28] also reported the negative effects of dietary DP level on laying hens' performance. However, this effect is due to physiological condition, and variety of DP. Date pits, which are known to contain high proportions of fiber, were used in feeding laying hens in this study. In the present trail the lack of negative effect of 24 % DP level on FCR compared to control-2 (corn-soybean meal-based diet with vegetable oil) may be due to the increasing level of supplemented oil to balance ME value.

Similarly, enzyme supplementation did not significantly improve productive performance compared to C1 and C2 groups except for EP that enzyme supplementation increased EP when compared with the controls diets (C1 and C2); however, this increase did not statistically significant (93.29%; *P*>.05). Lack of response to enzyme supplementation is not completely unexpected considering the high percentages of DP (24%) and the reduced levels of corn (< 50%) in the diets. In addition, it is now well recognized that response of laying hens consuming cereal based diets to enzyme supplementation is variable, depending on various factors. One possible explanation for this observation might be the variable concentrations of NSP, such as arabinoxylans and β-glucans, in cereals originated from different sources. Moreover, it is also possible that effects of enzyme supplementation in poultry nutrition are less evident in older birds, which is the case when exploiting laying hens for egg production [29].The high rate of ingesta passage in chickens may reduce the hydrolytic capacity of exogenous polysaccharidases, which act at considerable slow rates due to the recalcitrance of their substrates. Finally, although in the experiment reported here the feed enzymes were detected in the digesta, it is possible that the enzyme mixture we used was not the most appropriated for the targeted substrates present in DP. Under these circumstances, the most appropriate enzyme mixtures to degrade ground pits of date palm cell wall polysaccharides remain to be identified.

Effects of adding DP and enzyme to hens' diet on egg quality traits are presented in Tables 3 and 4. In the first egg sampling (wk 3), interaction between diet DP inclusion and enzyme supplementation was not significant, except for shell weight. A significant two-way interaction between DP and enzyme on shell weight was also seen (*P =.05*). Main effects of dietary treatments including DP and enzyme on egg traits were not significant, except for yolk color and shell thickness. However, unexplained decrease in shell thickness was observed due to diet inclusion of DP to the diet without enzyme (Table 3). Diet inclusion of DP decreased yolk color, which is most likely due to high carotenoid content of corn. Egg-yolk pigmentation should also be considered if corn is excluded from the diet. DP contains very low levels of pigments, which will result in practically colorless yolks if fed to layers. The diets therefore need to be supplemented with dietary carotenoids. Enzyme supplementation had no significant effects on yolk color. These results show that the capacity of enzyme supplementation to release pigments from the diets is limited. These results were less evident than those observed by Ciftci et al. [30], who reported a significant improvement in egg yolk color in response to enzyme supplementation on triticale and wheat–triticale-based diets. However, it is possible that pigment bioavailability, as a result of enzyme supplementation, may depend on the type of ingredients present in poultry diets. The results obtained in the present study indicate that enzyme supplementation did not affect egg traits (Table 3).

In second egg sampling (wk7), there was no significant interaction between dietary treatments on egg quality traits in wk 7.Hens fed DP-included diet with no enzyme did have the lowest Haugh unit, egg yolk color, shell weight and shell thickness compared to hens fed the other experimental diets. The results obtained in the present study indicate that enzyme supplementation of diets did not affect egg quality trait; However, unexplained decrease in haugh unit was observed due to enzyme supplementation compared to the diet without enzyme (*P*=.05) (Table 4). In disagreement with our results, Abd El-Rahman et al. [31] reported that diet inclusion of DP up to 30 % have no significant effect on shell thickness.[32] indicated that adding enzymes to the rations promotes feed conversion ratio depending on educed viscosity of the intestine. Moreover, [20] illustrated that xylanase supplement to the wheat based rations did have no significant effect on egg production, although it improved egg weight, albumen height and weight. In addition, some researchers reported that enzyme supplement to the rations had a negative effect on egg quality [33] while other researchers reported that enzyme supplement to the rations had a positive effect on egg quality [34]. Possibly, the referred the differences in the results in the literature might be explained by the effect of factors such as strain and age of the laying hens and the source and levels of enzyme. Olukosi et al. [35] stated that older birds require different doses of enzymes because their physiological needs and digestive capacity differ from those of younger chicks. It may be that the anti-nutritional factors in the diet are better tolerated by older birds with mature digestive systems. A possible reason for the ineffectiveness of the enzyme supplementation on the performance of laying hens may be that older hens were used in the present study. In addition, the choice of the trial period could be considered among the factors responsible for differences obtained in the present experiment. Wyatt and Goodman [36] reported that enzyme supplementation might be beneficial during peak production because the laying hen needs high levels of nutrients to maintain body growth and high egg production.

3.2 Blood Parameters

Tables 5 and 6 provided information on white blood cell count as well as plasma level blood parameters characteristics of the experimental, interaction between diet DP inclusion and enzyme supplementation on white blood cell count and serum biochemical metabolites were not statistically significant. There was no significant effect of enzyme supplementation on white blood cell count and serum biochemical metabolites. In agreement with our finding in broiler case, Masoudi et al. [37] reported diet inclusion of 10, 20 or 30% of DP in broiler diets had no significant effect on blood cholesterol, triglycerides, HDL, LDL and VLDL (P>.05). Dietary inclusion DP significantly decreased the blood count of hetrophils (P =.05).There is no record in the literature presenting the effects of diet inclusion of DP on white blood cell count of laying hens. However, further investigations are needed to clarify the effect of DP on blood parameters in laying hens.

Table 2. Effects of diet inclusion of ground pits of date palm (0 and 24 g/100g), with or without enzyme (Nutrase®[1]) on productive performance of Lohmann LSL-lite laying hens (weeks 58-65 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 Pits (DP)				
0 (g/100g diet)	112.92±3.85[b]	1.96±0.13[b]	93.46±4.53	57.93±2.86
0 (g/100g diet)	114.41±6.24[ab]	1.97±0.09[b]	93.16±4.79	58.43±4.21
24 (g/100g diet)	118.38±1.61[a]	2.13±0.15[a]	91.89±3.65	55.97±3.20
Nutrase® (E)				
0.00 (g/100g diet)	116.54±3.58	2.04±0.18	92.39±4.52	57.52±3.51
0.07 (g/100g diet)	113.93±5.56	1.99±0.11	93.29±4.04	57.37±3.62
Pooled SEM	0.973	0.030	0.861	0.712
P values				
Date Pits (DP)	0.05	0.02	0.74	0.34
Nutrase® (E)	0.15	0.35	0.61	0.92
DP×E	0.45	0.37	0.17	0.24

a Means±SD, abMeans within column (main effects) with different superscripts are significantly different (P<0.05).

1Nutrase® is a unique bacterial endo-xylanase with special properties that considerably improve digestibility of corn and wheat diets for poultry and swine

Table 3. Effects of diet inclusion of ground pits of date palm (0 and 24 g/100g), with or without enzyme (Nutrase®) on egg quality characteristics (first egg sampling on wk 3) [a]

Egg quality traits (wk 3)								
Parameters		**Egg index**	**Yolk index**	**Haugh unit**	**Yolk color (Roch)**	**Specific gravity (unit)**	**Shell weight (g)**	**Shell thickness (mm×10^{-2})**
Date Pits (DP)								
0 (g/100g diet)		74.06±1.94	37.93±1.02	71.32±3.68	6.58±0.39^{a}	1.09±0.00	6.35±0.50^{a}	36.50±1.79^{ab}
0 (g/100g diet)		75.15±1.30	39.40±1.28	72.01±2.84	6.29±0.37^{ab}	1.09±0.00	6.40±0.28^{a}	37.58±2.13^{a}
24 (g/100g diet)		75.09±2.06	38.76±1.81	69.99±4.46	5.92±0.46^{b}	1.09±0.00	5.74±0.20^{b}	35.04±1.49^{b}
Nutrase® (E)								
0.00 (g/100g diet)		74.71±1.59	38.55±1.72	72.17±3.78	6.36±0.36	1.09±0.00	6.28±0.48	36.39±2.10
0.07 (g/100g diet)		74.81±2.04	38.85±1.26	70.04±3.35	6.17±0.58	1.09±0.00	6.05±0.41	36.36±2.08
Pooled SEM		0.366	0.303	0.746	0.983	0.001	0.092	0.417
P values								
Date Pits (DP)		0.45	0.14	0.57	0.01	0.85	0.00	0.04
Nutrase® (E)		0.90	0.61	0.19	0.22	0.36	0.07	0.97
DP× E		0.68	0.27	0.91	0.07	0.09	0.04	0.28
P values								
DP	E							
C	-						6.69±0.46^{a}	
C	+						6.00±0.23^{a}	
C1	-						6.34±0.25^{a}	
C1	+						6.45±0.34^{a}	
24	-						5.80±0.18^{a}	
24	+						1.33±5.68^{a}	
CV							0.53	
P values							0.56	

a Means±SD, abMeans 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 ground pits of date palm (0 and 24 g/100g), with or without enzyme (Nutrase®) on egg quality characteristics (second egg sampling on wk 7)[a]

Egg quality traits (wk 7)							
Parameters	**Egg index**	**Yolk index**	**Haugh unit**	**Yolk color (Roch)**	**Specific gravity (unit)**	**Shell weight (g)**	**Shell thickness (mm×10^{-2})**
Date Pits (DP)							
0 (g/100g diet)	75.14±1.51	43.42±1.31	66.72±2.64^{ab}	6.46±0.30^{ab}	1.09±0.01	5.64±0.33^{ab}	36.04±1.41^{a}
0 (g/100g diet)	74.68±1.97	41.33±5.64	71.55±7.21^{a}	6.62±0.33^{a}	1.09±0.00	5.96±0.41^{a}	35.67±1.56^{a}
24 (g/100g diet)	75.78±1.65	43.67±1.92	65.32±4.80^{b}	6.12±0.35^{b}	1.09±0.00	5.27±0.47^{b}	33.79±2.03^{b}
Nutrase® (E)							
0.00 (g/100g diet)	75.45±1.96	42.45±4.95	70.39±5.89^{a}	6.28±0.44	1.09±0.00	5.67±0.47	35.61±1.80
0.07 (g/100g diet)	74.95±1.45	43.16±1.15	65.33±4.36^{b}	6.53±0.26	1.09±0.00	5.57±0.51	34.72±1.97
Pooled SEM	0.349	0.721	1.161	0.078	0.001	0.098	0.388
P values							
Date Pits (DP)	0.46	0.37	0.04	0.02	0.64	0.01	0.03
Nutrase® (E)	0.50	0.63	0.02	0.07	0.96	0.52	0.21
DP× E	0.46	0.29	0.91	0.59	0.79	0.20	0.47

a *Means±SD, abMeans 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 ground pits of date palm (0 and 24 g/100g), with or withoutenzyme (Nutrase®) on white blood cell counts (heterophil, lymphocyte, monocyte, eosinophil,basophil and Heterophil to Lymphocyte ratio) in laying hens[a]

Parameters (%)	White blood cell counts (%)					
	H[1]	L	M	E	B	H/L
Date Pits (DP)						
0 (g/100g diet)	29.62±7.17[ab]	66.50±9.52	2.25±2.37	0.25±0.71	1.37±1.77	0.47±0.17
0 (g/100g diet)	27.75±6.58[b]	67.25±8.38	2.87±2.47	0.75±0.71	1.62±2.26	0.43±0.15
24 (g/100g diet)	36.12±6.60[a]	60.37±8.02	1.25±1.25	1.12±1.12	1.12±0.99	0.62±0.20
Nutrase® (E)						
0.00 (g/100g diet)	31.83±5.22	64.92±7.13	1.33±1.61	0.50±0.80	1.42±1.88	0.50±0.13
0.07 (g/100g diet)	30.50±9.37	64.50±10.63	2.92±2.27	0.92±1.38	1.33±1.56	0.51±0.24
Pooled SEM	1.521	1.807	0.427	0.229	0.345	0.038
P values						
Date Pits (DP)	0.04	0.24	0.29	0.30	0.87	0.07
Nutrase® (E)	0.61	0.91	0.07	0.36	0.91	0.94
DP× E	0.09	0.19	0.88	0.30	0.83	0.09

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

1Heterophil, Lymphocyte, Monocyte, Eosinophil, Basophil, Heterophil to Lymphocyte ratio

Table 6. Effects of diet inclusion of ground pits of date palm (0 and 24 g/100g), with or withoutenzyme (Nutrase®) on serum biochemical metabolites (cholesterol, triglycerides, high densitylipoprotein, low density lipoprotein) in laying hens[a]

Serum biochemical metabolites (mg/dL)				
Parameters	**CHOL[1]**	**TG**	**HDL**	**LDL**
Date Pits (DP)				
0 (g/100g diet)	171.75± 46.99	2346.25± 813.64	42.37± 9.10	110.87± 23.95
0 (g/100g diet)	154.75± 29.62	2007.50± 656.74	39.00± 6.82	101.12± 17.05
24 (g/100g diet)	148.12± 39.25	1661.25± 652.96	37.37± 8.58	96.25± 22.42
Nutrase® (E)				
0.00 (g/100g diet)	153.67± 27.57	2012.08± 600.69	39.08± 6.33	101.42± 16.06
0.07 (g/100g diet)	162.75± 48.52	1997.92± 881.34	40.08± 9.90	104.08± 26.25
Pooled SEM	7.935	150.570	1.662	4.353
P values				
Date Pits (DP)	0.48	0.20	0.50	0.42
Nutrase® (E)	0.58	0.96	0.78	0.77
DP× E	0.35	0.45	0.46	0.43

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

1Cholesterol, Triglycerides, High density lipoprotein cholesterol, Low density lipoprotein cholesterol

4. CONCLUSION

Date pits can be included in laying hens diets up to 24% with no adverse effect on EP; however, in terms of feed efficiency, egg shell weight and thickness and yolk color, lower percentage of date pits should be inserted to diets. Date pits can be used instead of corn as an energy feedstuff in laying hen diets. However, egg yolk color needs to be considered and pigment supplements will be recommended. Further studies are required to explain the mechanisms by which enzyme supplementation of diets affects egg traits in laying hens.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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