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Full Length Research Paper

Productive performance of laying hens fed wheat-based diets included olive pulp with or without a commercial enzyme product

M. Zarei[1], M. Ehsani[2] and M. Torki[2]*

[1]Member of Young Researchers Club of Islamic Azad University, Aligudarz Branch, Iran.
[2]Department of Animal Science, Agriculture Faculty, Razi University, Imam Avenue, Kermanshah, Iran.

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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. It can be achieved by sieving the dry olive cake to separate most of the seeds. To assess effects of dietary inclusion of OP supplementing with a cocktail commercial enzyme on performance of laying hens and egg quality characteristics, one hundred and twenty 80-week-old Lohmann LSL-Lite hens, with an average laying rate of 80.3 ± 3.8% (late production phase) and 1450 ± 14 g live body weight, were divided in 20 cages (n = 6). Hens in 5 cages (replicates) were randomly assigned to feed on one of the 4 experimental diets. Based on a 2×2 factorial arrangement, 4 iso-caloric and iso-nitrogenous diets (ME =2720 kcal/kg and CP=150 g/kg) including OP (0.0 and 90.0 g/kg) and a commercial cocktail enzyme with mostly xylanase activity (Nutrase®, 0.0 or 0.9 g/kg) were formulated. To determine blood biochemical parameters and differentiable count of white blood cells, one hen per replicate was bled via wing vein on day 35 of trial. To determine egg quality parameters, all eggs during three frequent days were collected on week 4 of trial. Collected data of feed intake (FI), egg production (EP), egg mass (EM) and calculated feed conversion ratio (FCR), blood biochemical parameters and egg quality traits during 6 week trial period was analyzed based on completely randomized design. Hens fed the OP-included diet shown decreased EP compared with hens fed the control diet during week 3 of experimental period (p ≤ 0.05). There was no significant difference between hens fed the OP-included diet and the control diet in terms of FI and EM. In addition, the same trend was observed in terms of enzyme effect on FI and EM. Dietary enzyme supplementation improved FCR compared with hens fed the control diet during week 6 of the experimental period (p ≤ 0.05). Including OP in the diets of hens decreased the blood level of triglycerides (p ≤ 0.05). Including OP in the diets of hens increased the yolk index (p ≤ 0.05). From the results of this investigation, it can be concluded that including OP in diets of laying hens up to 9% would not have deleterious effects on bird's productive performance. In addition, dietary supplementation with a cocktail enzyme with mainly xylanase activity improved FCR in hens.

Key words: Olive pulp, enzyme, laying hens, performance, egg quality characteristics, blood parameters.

INTRODUCTION

The biggest single expense in any system of poultry production is feed, accounting for up to 70% of total production cost. In order to reduce feeding costs, attempts have been made to use agricultural and industrial by-products as feed ingredients. Improved utilization of crop residues and by-products to be used in animal feeding deserves more attention. Examples of crop residues and agricultural by-products in Iran are; cereal bran, citrus pulp, tomato pulp, poultry litter and olive pulp (OP). Olive pulp is the remainder of olive cake (the raw material resulting from extraction of olive oil) after the removal of the seed fractions. OP is considered as a good source of

*Corresponding author. E-mail: torki@razi.ac.ir. Tel: 00989133277266.

Abbreviations: AME, Available energy; **NSP,** non-starch polysaccharides; **OP,** olive pulp; **FI,** feed intake; **EP,** egg production; **EM,** egg mass; **FCR,** feed conversion ratio.

calcium, copper and cobalt but poor in phosphorus, magnesium and sodium and with fair levels of manganese and zinc (Harb, 1986). Due to low nutritive value (low in energy, digestible proteins and minerals and high in lignin), OP is seldom integrated into poultry feeding. In addition, a xyloglucan, one of the non-starch polysaccharides (NSP) which has anti-nutritive effects on monogastrics such as poultry and pigs, from OP has been reported by Gil-Serrano and Tejero-Mateo (1988). Coimbra et al. (1995) also showed the occurrence of the xylan-xyloglucan complexes in the OP cell walls. In addition, Rosa´rio and Domingues (2002) extracted glucuronoxylans with a xylose/glucose ratio of 7: 1 from OP.

Although starch is the predominant carbohydrate in cereals, some of the constituent carbohydrates are water-soluble NSP, which are considered the major antinutritive factors in cereals and other varieties of feed ingredients (Bedford and Classen, 1992; Campbell and Bedford, 1992). The common NSP include β-glucans, arabinoxylans and fructans (Classen and Bedford, 1991). The structures of NSP differ between grains and also between varieties of the same grain (Annison, 1990). The similarity between the types of NSP is usually the presence of β (1 → 4) backbones with or without the presence of β (1 → 3) side linkages (Iji, 1999). The effects of NSP upon feed utilization and poultry productivity have been discussed in many reports. Salih et al. (1991) reported that, NSP reduced feed intake and decreased broiler chicken performance. The most noticeable effect of NSP in poultry diets is an increase in the viscosity of digesta and the excretion of sticky droppings. This is considered to be one of the greatest influences of NSP on broiler chicken productivity (Smits and Annison, 1996) and it is necessary to use exogenous enzymes to overcome these problems.

Poultry naturally produces enzymes to aid the digestion of feed nutrients. However, they do not have enzyme to break down fiber completely and need exogenous enzymes in feed to aid digestion. Enzymes are used mainly to achieve consistency in performance and to reduce formulation costs by easing constraints on the inclusion level of some ingredients, such as wheat and barley. Hence, the supplementation of microbial enzymes in poultry diets is now common practice in many countries where the predominant cereals are wheat or barley. Improving poultry performance by dietary manipulation has been the goal of nutritionists. Enzyme has been reported to alleviate the negative effects of arabinoxylans, present in wheat as the dominant cell-wall constituent, by lowering gut viscosity and increasing protein, fat and starch digestibility (Bedford and Morgan, 1996). Moreover, an improvement in overall protein digestibility of dietary components other than wheat, such as soybean, has also been reported (Bedford and Morgan, 1996). Pan et al. (1998) demonstrated that, commercial enzyme mixtures could promote metabolizable energy particularly utilized in wheat based rations. Many years of intensive research, both in official trials as well as in field trials, have shown that its bacterial origin gives it some very distinctive and valuable characte-ristics, distinguishing it clearly from xylanase. It reaches its highest efficiency in rations with a high inclusion of wheat and wheat by-products. Many years of practical experience have shown that, it also has an important effect on improving the digestibility of other grains, such as barley, corn, rice, rye and sorghum. Improved starch digestibility, for instance, has been reported to account for up to 35% of the improvement in available energy (AME) as a result of xylanase supplementation (Carre et al., 1992). Xylanase has been reported to alleviate the negative effects of arabinoxylans, present in wheat as the dominant cell-wall constituent, by lowering gut viscosity and increasing protein, fat and starch digestibility (Bedford and Morgan, 1996).

The objectives of this study were to investigate the effects of dietary inclusion of OP and enzyme supplementation on performance of the laying hens fed with wheat-based diets.

MATERALS AND METHODS

All procedures used in this six-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". A total number of one hundred and twenty 80-week-old Lohmann LSL-Lite hens with an average egg production rate of 80.3 ± 3.8% (late laying phase) and 1450 ± 14 g live body weight, were obtained from a commercial supplier. After a week of adaptation, the hens were randomly allocated to one of four experimental diets. Hens were semi-randomly distributed between 20 cages (n=6) with almost same average body weight and egg production throughout the cages. The hen distribution was based on the previous production records of birds during one month before selecting them from their original flock and transferring to the experimental cages. Hens in 5 cages (replicates) were assigned to feed on one the 4 experimental diets. Four iso-caloric and iso-nitrogenous diets (ME =2720 kcal/kg and CP=150 g/kg) including OP (0.0 and 90.0 g/kg) and a commercial cocktail enzyme with mostly xylanase activity (Nutrase®, 0.0 or 0.9 g/kg) were formulated (Table 1).

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%). Nutrase® xyla is a bacterial endo-1,4-beta-xylanase, designed for use in pig and poultry rations with a high content of arabinoxylans. Nutrase® xyla is produced by *Bacillus subtilis*, which makes it today, the only EU registered bacterial xylanase preparation available on the market.

The hens were housed in laying cages made from galvanized metal wire which provided approximately 430 cm^2/hen. The cages were located in a windowless and environmentally controlled room with the room temperature kept at 21-23°C and the photoperiod set at 16 h of light (incandescent lighting, 10 lux) and 8 h dark. Each cage had a nipple waterier. Water was available *ad libitum* throughout the experiment. Feed consumption was measured on a weekly basis. To determine blood biochemical parameters and differentiable

Table 1. Ingredients and composition of the experimental diets.

Olive pulp (g /100 g)	0.0		9.0	
Enzyme (g /100 g)	**0.00**	**0.09**	**0.00**	**0.09**
Feed ingredients g / 100 g diet				
Wheat	62.78	62.78	58.06	58.07
Fish meal	5.00	5.00	5.00	5.00
Soybean meal	8.80	8.80	8.78	8.78
Dried tomato pomace	3.50	3.50	3.50	3.50
Oil	5.03	5.03	5.03	5.03
Olive pulp	0.00	0.00	9.00	9.00
Enzyme - Nutrease®	0.00	0.09	0.00	0.09
Dicalcium phosphate	1.64	1.64	1.64	1.64
Limestone	8.07	8.07	8.07	8.07
Common salt	0.16	0.16	0.17	0.17
Vit. and Min. Premix[1]	0.25	0.25	0.25	0.25
Sand	4.38	4.29	0.10	0.00
DL-Methionine	0.15	0.15	0.15	0.15
Calculated analyses				
ME (Kcal/kg)	2720	2720	2720	2720
Crude protein (%)	15	15	15	15
Ether extract (%)	6.65	6.65	6.65	6.65
Crude fiber (%)	4.37	4.37	4.37	4.37
Calcium (%)	3.67	3.67	3.67	3.67
Available P (%)	0.33	0.33	0.33	0.33
Lys (%)	0.68	0.68	0.68	0.68
Met (%)	0.38	0.38	0.38	0.38
Met and Cys (%)	0.61	0.61	0.61	0.61

[1]The vitamin and mineral premix provide the following quantities per kilogram of diet: Vitamin A, 10,000 IU (*all-trans*-retinal); cholecalciferol, 2,000 IU; vitamin E, 20 IU (*α*-tocopheryl); vitamin K3, 3.0 mg; riboflavin, 18.0 mg; niacin, 50 mg; D-calcium pantothenic acid, 24 mg; choline chloride, 450 mg; vitamin B12, 0.02 mg; folic acid, 3.0 mg; manganese, 110 mg; zinc, 100 mg; iron, 60 mg; copper, 10 mg; iodine, 100 mg; selenium, 0.2 mg; antioxidant, 250 mg.

count of white blood cells, one hen per replicate was bled via wing vein on day 35 of trial. To determine egg quality parameters, all eggs during three frequent days were used. Collected data of feed intake (FI), egg production (EP), egg mass (EM) and calculated feed conversion ratio (FCR) during 6 week trial period was analyzed based on a 2×2 factorial arrangement of treatments and completely randomized design using GLM procedure of SAS. All statements of significance are based a probability of less than 0.05. The mean values were compared by Duncan's multiple range test.

RESULTS AND DISCUSSION

Effects of dietary OP inclusion and enzyme supplementation on EP, FI, FCR, EW and EM are presented in Tables 2 to 6, respectively. There was no significant interaction between dietary OP inclusion and enzyme supplementation on the measured productive performance parameters throughout the experimental period ($p > 0.05$). Egg production (%) was not significantly affected by dietary OP inclusion ($p > 0.05$), except for week 3 of trial period. Hens fed with the OP-included diet shown decreased EP compared with hens fed with the control diet during week 3 of experimental period ($p \leq 0.05$); but the overall EP during the whole trail period (weeks 1 to 6) was not significantly affected. There was no significant difference between hens fed with OP-included diet and the control diet in terms of FI (Table 3) and EM (Table 6). In addition, the same trend was observed in terms of enzyme effect on FI and EM. Feed conversion ratio was not significantly affected by dietary OP inclusion ($p > 0.05$). Including OP in diet improved EW on weeks 4 and 6 as well as the overall trial period (weeks 1 to 6). Studies concerning feeding with OP to monogastrics are limited (Tortuero et al., 1989). Several research studies were conducted to investigate the feasibility of utilizing OP in broiler rations. The proportion of OP in its rations is variable. There seems to be a limit between 50 and 100g/kg (Abo Omar, 2000; Rabayaa, 2000). Diets with different levels of fiber showed certain influence on gastro-

Table 2. Effect of dietary inclusion of olive pulp (0 and 90 g/kg) and enzyme (0 and 0.9 g/kg) on egg production (%) of laying hens.

Treatment		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Weeks 1 to 6
Enzyme (g /100 g)								
0.00		67.85	75.71	79.28	84.04	86.42	83.80	79.52
0.09		70.47	72.61	79.52	85.71	86.43	88.33	80.51
Olive pulp (g /100 g)								
0.00		71.42	76.42	84.28[a]	85.95	87.14	85.47	81.78
9.00		66.90	71.90	74.52[b]	83.81	85.71	86.66	78.25
Olive pulp	**Enzyme**							
0.00	0.00	69.52	75.71	83.81	84.76	86.66	82.85	80.55
0.00	0.09	73.33	77.14	84.76	87.14	87.62	88.09	83.01
9.00	0.00	66.19	75.71	74.76	83.33	86.19	84.76	78.49
9.00	0.09	67.61	68.09	74.28	84.28	85.24	88.57	78.01
SEM		5.83	5.36	3.84	3.32	3.16	2.26	3.09
CV		19.91	17.06	11.41	9.24	8.64	6.21	9.14
Source of variation					Probability			
Enzyme		0.676	0.591	0.954	0.641	0.999	0.076	0.765
Olive pulp		0.473	0.435	0.028	0.549	0.674	0.625	0.296
Enzyme × Olive pulp		0.849	0.435	0.862	0.841	0.779	0.770	0.659

a-b Means within a column (within main effects) with no common superscript differ significantly ($p < 0.05$); SEM= standard error of means.

Table 3. Effect of dietary inclusion of olive pulp (0 and 90 g/kg) and enzyme (0 and 0.9 g/kg) on feed intake (FI, g/ hen/ day) of laying hens.

Treatment		Feed intake (g/ hen/ day)						
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Weeks 1 to 6
Enzyme (g /100 g)								
0.00		105.66	116.55	117.72	118.22	116.89	118.13	115.53
0.09		111.25	109.91	113.57	112.79	114.14	115.40	112.84
Olive pulp (g /100 g)								
0.00		110.78	114.97	117.16	114.92	114.49	116.32	114.77
9.00		106.13	111.49	114.13	116.09	116.54	117.21	113.60
Olive pulp	Enzyme							
0.00	0.00	109.95	116.88	119.14	116.45	114.81	117.19	115.74
0.00	0.09	111.62	113.07	115.19	113.40	114.16	115.45	113.81
9.00	0.00	101.38	116.23	116.30	120.00	118.97	119.07	115.33
9.00	0.09	110.88	106.76	111.95	112.19	114.12	115.35	111.87
SEM		4.18	4.00	2.96	2.52	3.30	1.61	2.29
CV		9.11	8.34	6.05	5.16	4.71	3.26	4.75
Source of variation					Probability			
Enzyme		0.224	0.135	0.203	0.058	0.275	0.128	0.284
Olive pulp		0.307	0.422	0.346	0.667	0.410	0.606	0.634
Enzyme × Olive pulp		0.388	0.511	0.949	0.385	0.400	0.569	0.756

SEM, Standard error of means.

Table 4. Effect of dietary inclusion of olive pulp (0 and 90 g/kg) and enzyme (0 and 0.9 g/kg) on feed conversion ratio (FCR: g feed: g egg) of laying hens.

Treatment		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Weeks 1 to 6
Enzyme (g /100 g)								
0.00		2.58	2.49	2.39	2.24	2.16	2.26[a]	2.35
0.09		2.57	2.48	2.30	2.12	2.14	2.11[b]	2.29
Olive pulp (g /100 g)								
0.00		2.57	2.47	2.25	2.16	2.14	2.21	2.30
9.00		2.59	2.51	2.44	2.20	2.17	2.15	2.34
Olive pulp	Enzyme							
0.00	0.00	2.64	2.53	2.31	2.20	2.15	2.31	2.36
0.00	0.09	2.49	2.40	2.18	2.11	2.12	2.12	2.24
9.00	0.00	2.50	2.45	2.46	2.27	2.18	2.21	2.34
9.00	0.09	2.68	2.56	2.42	2.13	2.16	2.10	2.34
SEM		0.16	0.13	0.10	0.08	0.07	0.06	0.07
CV		14.68	12.74	10.23	9.15	8.73	6.57	7.46
Source of variation					Probability			
Enzyme		0.944	0.950	0.419	0.213	0.770	0.035	0.451
Olive pulp		0.907	0.776	0.094	0.629	0.717	0.380	0.596
Enzyme × Olive pulp		0.353	0.414	0.680	0.775	0.934	0.562	0.466

SEM, Standard error of means.

intestinal tract weight, length and content (Abo Omar et al., 1994; Abo Omar, 1995). The different levels of olive cake had no effect on carcass cuts of broilers carcass when fed with OP at levels up to 100 g/kg (Abo Omar and Gavoret, 1995). Rabayaa et al. (2001) who incorporated OP in four of the experimental groups at rates of 2.5, 5, 7.5 and 10% in both starter and finisher feeds to replace similar rates of yellow corn reported that weight gain of chicks was the same in chicks consuming up to 7.5% of OP. However, weight gain of chicks fed the level of 10% OP had the lowest significant weight gain. In addition, similar trends were observed in chicks for F and feed conversion efficiency.

Dietary enzyme supplementation improved FCR compared with hens fed with control diet during week 6 of the experimental period ($p \leq 0.05$). Although enzyme addition decreased FCR during the whole trail period, this effect was significant only on week 6 (Table 4). Enzyme supplementation did not have significant effect on EW (Table 5). Xylans are the principal NSP of wheat and high levels of wheat in poultry diets can increase the viscosity of the gut contents, which impedes the circulation and absorption of nutrients, causing reduced feed intake, BW gain and feed efficiency (Annison and Choct, 1991). Chickens do not produce some enzymes, such as galactosidases (xylanase/β-glucanase); thus, corn-soybean-based diets without supplemented enzymes such as xylanases and pectinases might result in gas accumulation in the gut and diarrhea (Jaroni et al., 1999a; Wu et al., 2005). The endosperm cell wall of wheat is mainly composed of arabinoxylans. The addition of exogenous enzymes to wheat-based diets increases digestibility of nutrients in cockerels (Carré et al., 1990). It may also provide additional dietary energy as well as short chain fatty acids and oligosaccahrides (Iji, 1999). Such phenomena are associated with cellular proliferation and improved gut health (Iji, 1999). Xylanase is used extensively in wheat-based diets to counteract the effects of NSP in broiler (Bedford and Schulze, 1998). In addition, xylanase has been used in combination with other enzyme(s) (xylanase-based cocktail enzyme) in layer diets containing wheat or wheat and barley (Brenes et al., 1993; Pan et al., 1998; Scott et al., 1999, Francesch et al., 1995; Oloffs et al., 1998; Salobir, 1998; Jaroni et al., 1999a,b; Mathlouthi et al., 2002) and broiler diets (Bedford and Schulze, 1998) to counteract the effects of NSP; however, the results obtained with different experimental conditions and diets have been inconclusive. The degree of improvement obtained by adding enzymes to the diet depends on many factors including the level of antinutritive factor in the diet, the spectrum and concentration of enzymes used, the type of animal and the

Table 5. Effect of dietary inclusion of olive pulp (0 and 90 g/kg) and enzyme (0 and 0.9 g/kg) on average egg weight (g).

Treatment		Egg weight (g)						
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Weeks 1 to 6
Enzyme (g /100 g)								
0.00		62.29	62.80	63.07	62.77	62.81	42.53	59.38
0.09		61.96	61.93	62.34	62.15	61.85	42.19	58.74
Olive pulp (g /100 g)								
0.00		61.80	61.86	62.25	61.89[b]	61.72	41.40[b]	58.48[b]
9.00		62.46	62.87	63.16	63.03[a]	62.94	43.32[a]	59.63[a]
Olive pulp	Enzyme							
0.00	0.00	61.95	62.05	62.10	62.00	61.90	41.37	58.56
0.00	0.09	61.64	61.66	62.39	61.78	61.54	41.43	58.41
9.00	0.00	62.63	63.54	64.04	63.55	63.73	43.69	60.19
9.00	0.09	62.28	62.21	62.29	62.52	62.16	42.95	59.07
SEM		0.61	0.68	0.71	0.51	0.72	0.32	0.52
CV		2.35	2.58	2.67	1.93	2.75	1.83	2.07
Source of variation					Probability			
Enzyme		0.620	0.249	0.344	0.266	0.226	0.345	0.260
Olive pulp		0.326	0.176	0.240	0.050	0.130	0.045	0.049
Enzyme × Olive pulp		0.976	0.525	0.194	0.465	0.442	0.266	0.390

SEM, Standard error of means.

age of the animal (young animals tend to respond better to enzymes than older animals), type of gut micro flora present and the physiology of the bird. Older birds, because of the enhanced fermentation capacity of the micro flora in their intestines, have a greater capacity to deal with negative viscosity effects (Choct et al., 1995). Typically, enzymes added to layer feed appear to have little effect on egg mass but improve feed efficiency (Benabdeljelil and Arbaoui, 1994). Mathlouthi et al. (2002) who determined the effects of xylanase on true metabolizable energy values of wheat, barley and wheat bran as well as performance of laying hens fed with wheat-, barley- or wheat bran-based diets reported improved egg mass of layers fed with wheat- or wheat bran-based diets supplemented by xylanase. In addition, xylanase supplementation improved egg production, egg mass and feed conversion ratio of layers fed with low energy diet. It did improve the feed conversion ratio of layers fed with high-energy diet. Xylanase increased the TME values for wheat and barley and did not affect the TME value of wheat bran (Mathlouthi et al., 2002). Senkoylu et al. (2009) who investigated the effects of 30% whole-wheat inclusion in a standard layer diet supplemented with xylanase, on laying performance, digestive organs and ileal mucosa development and reported that xylanase supplementation to whole wheat significantly improved egg production and feed conversion rate compared with the ground wheat and whole wheat fed groups. Pirgozliev et al. (2010) who examined the effect of dietary xylanase on the availability of nutrients for laying hens when fed on wheat-rye-soy-based diets and reported that, the AME and nitrogen metabolisability coefficients of xylanase-supplemented diets were greater than the control diet. In addition, they reported supplementary xylanase significantly improved the coefficients of metabolisability of indispensable, dispensable and total amino acids. Their data suggested that, the use of a xylanase might improve the metabolisability of some nutrients, but that such effects might not always benefit production parameters. Feed intake and feed conversion ratio for egg production were not affected by xylanase (Pirgozliev et al., 2010). Diet supplementation with an enzyme cocktail providing 7 U/g of α-1, 6-galactosidase and 22 U/g of β-1, 4-mannanase significantly improved feed conversion of Lohmann Brown-Lite laying hens (Han et al., 2010).

As it is shown in Table 7, among the blood biochemical parameters (cholesterol, high density lipoprotein, low density lipoprotein and triglycerides), only blood level of triglycerides was affected by dietary OP inclusion. Including OP in the diets of hens decreased the blood level of triglycerides ($p \leq 0.05$); however, there was no significant difference between blood levels of cholesterol, high density lipoprotein and low density lipoprotein in

Table 6. Effect of dietary inclusion of olive pulp (0 and 90 g/kg) and enzyme (0 and 0.9 g/kg) on egg mass (g/ hen/ day) of laying hens.

Treatment		Egg mass (g/ hen/ day)						
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Weeks 1 to 6
Enzyme (g /100 g)								
0.00		42.17	47.60	49.99	53.01	54.29	52.43	49.92
0.09		43.59	44.92	49.64	53.33	53.42	54.72	49.94
Olive pulp (g /100 g)								
0.00		43.98	47.27	52.51	53.47	53.80	52.70	50.62
9.00		41.78	45.26	47.13	52.86	53.91	54.46	49.23
Olive pulp	Enzyme							
0.00	0.00	42.97	47.07	52.11	53.04	53.64	50.90	49.96
0.00	0.09	45.00	47.47	52.90	53.90	53.97	54.50	51.29
9.00	0.00	41.38	48.14	47.88	52.97	54.95	53.97	49.88
9.00	0.09	42.19	42.38	46.38	52.76	52.88	54.95	48.59
SEM		3.52	3.54	2.53	2.09	2.04	1.41	2.02
CV		19.39	18.04	11.98	9.07	8.93	6.19	9.53
Source of variation					Probability			
Enzyme		0.708	0.483	0.895	0.883	0.691	0.142	0.992
Olive pulp		0.562	0.597	0.061	0.781	0.961	0.252	0.522
Enzyme × Olive pulp		0.872	0.420	0.674	0.806	0.584	0.391	0.545

Table 7. Effect of dietary inclusion of olive pulp (0 and 90 g/kg) and enzyme (0 and 0.9 g/kg) on serum biochemical parameters (Cholesterol, High density lipoprotein, Low density lipoprotein and Triglycerides) of laying hens.

Treatment	Blood biochemical parameters			
	CHOL[1] (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	TG (mg/dl)
Enzyme				
0.00	144.63	59.875	61.25	1463.9
0.09	137.88	66.125	58.37	1270.9
Olive pulp				
0.00	156.75	62.62	64.00	1749.9[a]
9.00	125.75	63.37	55.62	984.9[b]
SEM	21.44	8.36	8.28	346.82
CV	30.36	26.55	27.67	50.73
Source of variation		Probability		
Enzyme	0.758	0.469	0.734	0.588
Olive pulp	0.173	0.930	0.331	0.047
Enzyme × Olive pulp	0.810	0.815	0.964	0.776

1-Cholesterol, triglycerides, high density lipoprotein, low density lipoprotein; a-b means within a column (within main effects) with no common superscript differ significantly ($p < 0.05$). SEM= Standard error of means.

Table 8. Effect of dietary inclusion of guar meal (0, 25 and 50 g/kg) and enzyme supplementation (0 and 0.4 g/kg) on differentiable counts of white blood cells (%, heterophil, lymphocyte, monocyte, eosinophil and basophil) of laying hens.

Treatment	Differentiable counts of white blood cells (%)				
	Heterophil	Lymphocyte	Monocyte	Eosinophil	Basophil
Enzyme (g /100 g)					
0.00	28.50	67.37	1.00	0.37	2.75
0.09	25.12	70.87	1.37	0.00	2.62
Olive pulp (g /100 g)					
0.00	23.12	72.50	1.25	0.25	2.87
9.00	30.50	65.75	1.12	0.12	2.50
SEM	3.72	3.83	0.48	0.28	1.05
CV	27.72	11.08	81.53	298.14	78.38
Source of variation			Probability		
Enzyme	0.381	0.378	0.453	0.204	0.907
Olive pulp	0.070	0.103	0.800	0.662	0.728
Enzyme × Olive pulp	0.317	0.216	0.453	0.662	0.563

Table 9. Effect of dietary inclusion of guar meal (0, 25 and 50 g/kg) and enzyme supplementation (0 and 0.4g/kg) on egg quality (egg index, yolk index, Haugh unit, eggshell weight and eggshell thickness) of laying hens.

Treatment	Egg quality characteristics				
	Egg index	Yolk index	Haugh unit	Egg shell weight	Egg shell thickness
Enzyme (g /100 g)					
0.00	74.60	41.83	70.06	6.87	40.00
0.09	74.97	42.03	70.78	6.48	37.40
Olive pulp (g /100 g)					
0.00	74.23	40.56[b]	68.41	6.53	38.60
9.00	75.34	43.29[a]	72.44	6.82	38.80
SEM	1.38	2.39	2.90	0.30	1.78
CV	4.36	5.65	9.73	11.65	10.91
Source of variation			Probability		
Enzyme	0.798	0.853	0.817	0.281	0.187
Olive pulp	0.455	0.020	0.207	0.423	0.917
Enzyme × Olive pulp	0.946	0.092	0.312	0.612	0.131

a-b Means within a column (within main effects) with no common superscript differ significantly ($P < 0.05$); SEM, standard error of means.

hens fed with control and the OP-included diets. There was no significant effect of enzyme supplementation on the analyzed blood biochemical parameters ($p > 0.05$). Differentiable count of white blood cells was not significantly affected by dietary OP inclusion and enzyme supplementation (Table 8). There is no record in the literature presenting the effects of diet inclusion of OP on blood biochemical parameters of laying hens.

As it is shown in Table 9, among the egg quality characteristics (egg index, yolk index, haugh unit, eggshell weight and eggshell thickness); only yolk index was affected by dietary OP inclusion. Including OP in the diets of hens increased the yolk index ($p \leq 0.05$); however, there was no significant difference between the other egg quality traits in hens fed with control and the OP-included diets. There was no significant effect of enzyme supplementation on the analyzed egg quality characteristics ($p > 0.05$). Gunawardana et al. (2009) evaluated the effects of

Rovabio (a natural mixture of enzymes produced by the organism *Penicillium funiculosum*), dietary energy and protein on performance, egg composition, egg solids and egg quality of commercial Leghorns in phase 2 and reported dietary protein significantly increased feed consumption but decreased yolk color. As dietary energy increased, feed consumption decreased and yolk color increased. They also found a significant interaction among dietary protein, energy and Rovabio on egg production, BW, egg mass, feed conversion and yolk solids. Egg weight of hens fed with diets supplemented with enzyme was significantly greater than that of hens fed with diets without enzyme during weeks 3 and 4. However, enzyme did not significantly influence average egg weight (Gunawardana et al., 2009). In the research study by Pirgozliev et al. (2010) the yolk color of the birds receiving xylanase was darker than the yolk of the birds given the control diet. In addition, birds receiving xylanase had a significantly higher weight gain than those fed on the unsupplemented diet. Dirty and cracked eggs in their study were not affected by xylanase (Pirgozliev et al., 2010). The improvement in feed conversion for Lohmann Brown-Lite laying hens fed with enzyme cocktail (providing 7 U/g of α-1, 6-galactosidase and 22 U/g of β-1, 4-mannanase) supplemented diet was accompanied by an increase in albumen weight and percentage and a decrease in the percentage of yolk and the ratio of yolk to albumin in the eggs (Han et al., 2010).

Conclusions

In conclusion, OP can be inserted in laying hens diets up to 9% with no adverse effect on bird's performance. Dietary inclusion of OP would have beneficial effect on laying hens' performance in terms of egg weight, yolk index and blood level of triglycerides. In this investigation, dietary supplementation with a cocktail enzyme with mainly xylanase activity improved FCR in hens.

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