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Effects of *B*-Mannanase Supplementing of Olive Pulp-Included Diet on Performance of Laying Hens, Egg Quality Characteristics, Humoral and Cellular Immune Response and Blood Parameters

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Abstract: The objective of this investigation was to evaluate performance of laying hens fed olive pulp (OP)-included diets supplemented with a commercial β-mannanase-based enzyme product. A total of 144 laying hens (75-wk old Lohman-LSL Lite) were assigned to feed 6 experimental diets with 4 replicates (cage) and 6 hens per cage. A 3×2 factorial arrangement of treatments including 3 levels of OP (0, 4.5 and 9.0 %) and enzyme (Hemicell® 0 and 0.05%) was employed for 9 wk trial period. Collected data was analyzed based on completely randomized design using GLM procedure of SAS. Diet inclusion of OP and enzyme had no significant effect on overall egg production, egg mass, feed conversion ratio and feed intake ($p>0.05$). Hens fed diet with 9% OP and enzyme had the highest egg weight compared to hens fed other experimental diets ($p<0.05$). Decreased Haugh unit was seen in hens fed diet included 9% OP compared to hens fed other experimental diets ($p<0.05$). Egg shell weight in the birds fed OP-included diets was higher than the birds fed control diet ($p<0.05$). Dietary enzyme supplementation had no significant effect on egg quality characteristics. In conclusion, based on the results of the present experiment OP can be included laying hens diets up to 9 % with no deleterious effects on bird's performance. In addition, dietary OP could improve egg weight.

Key words: Olive Pulp · *B*-Mannanase · Laying Hens · Performance

INTRODUCTION

Feeding agricultural by-products to livestock is a practice as old as the domestication of animals. The main advantages have been less dependency of livestock on grains that can be consumed by humans and the reduction of costs related to waste management. The land area occupied by olive orchards has increased in recent years, largely in response to the worldwide rise in olive oil consumption. Mediterranean countries represent 65% of the world's surface area cultivated in olives [1]. The technologies used for oil manufacturing are very different, so that a wide range of by-products including olive cake, vegetation water, olive leave, olive molasses and olive pulp (OP) are obtained [2] is the remainder of olive cakes (the raw material resulting from extraction of olive oil) after the removal of the seed fractions and can be achieved by sieving the dry olive cake to separate most of the seeds [3]. The utilization of olive by-products as animal feed is undoubtedly a good way of recycling these waste products. Sadeghi1 *et al*., [4].

[4] fed destoned olive cake at 20% to Zel sheep diets. Indeed, the olive mill waste could be of particular interest in broilers chicken for at least two reasons. On the one hand, for its level of residual oil (6.8%), this can constitute a complementary energy source. Secondly, for its particular composition of unsaturated fatty acids (62.4% of oleic acid, 18.2% of linoleic acid, 1.1% of linolenic acid and 2.7% of palmitoleic acid) which could influence the accumulation of fatty acid in the various body compartments during the animal's life and as such could have a certain impact on the quality of meat [5]. 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 [3-6,7] reported that level of OP had no significant effects on visceral organ mass, gastrointestinal tract weight, carcass cuts, carcass composition and dressing percent; however, chicks consuming 100 g OP/kg had the heaviest average live weights. [8] reported that including OP in diets of laying hens up to 9% did not have deleterious effects on bird's productive performance.

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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 [9, 10] also showed the occurrence of the xylan-xyloglucan complexes in the OP cell walls. In addition, [11] extracted glucuronoxylans with a xylose/glucose ratio of 7: 1 from OP. Addition of feed enzymes to improve dietary nutrient utilization has become popular during the last 10 yr. There are growing interests in the potential of other enzyme products to improve performance of poultry provided with corn-soybean meal based diets[12] Hemicell® is a fermentation product of *Bacillus lentus* that has glycosidase enzyme activity. Its active ingredient is mostly β-mannanase, which can hydrolyze β-mannan in feed[13]. β-mannan in ingredients such as guar, soybean meal and sesame meal, is a powerful anti-nutritional factor [12]. β-mannan is a linear polysaccharide composed of repeating β-1-4 mannose and 1-6 galactose and glucose units attached to the β-mannan backbone [13]. Corn-soybean meal based diets are the most popular for laying hens. Because soybean meal contains β-mannan and its derivatives such as β- galactomannan and β-glucomannan, addition of β-mannanase may improve soybean-meal utilization. [14] found that β-mannan significantly decreased egg production, egg weight and feed intake in laying hens. β-mannanase supplementation improved energy utilization of corn soybean layer diets and has potential to reduce the cost of practical laying hen diets containing β-mannan [12]. [13] reported that β-mannanase is capable of increasing egg weight in commercial layers at early stages of production and increasing egg production, particularly delaying the post peak decline in productivity. [15] reported that β-mannanase supplementation would have beneficial effects on performance and immunity in birds fed on corn-soy or corn-soy-dates diets.

This study was conducted to investigate effects of dietary inclusion of OP and enzyme supplementation on laying hens' performance.

MATERIALS AND METHODS

Animals and Diets: Nutrient composition of the OP used in the present trail is presented in Table 1. Based on a 3×2 factorial arrangement of treatments, six iso-caloric and iso-nitrogenous diets including 3 levels of OP (0, 45 and

Table 1: Nutrient composition of olive pulp (dry matter (DM) basis)

DM (%)	CP (%)	ME (kcal/kg)	CF (%)	EE (%)
95	6.06	1600	48.2	7.06

Table 2: Ingredients and nutrient content of the experimental diets

Olive pulp (%)	0.0		4.5		9.0	
Hemicell (%)	0.0	0.05	0.0	0.05	0.0	0.05
Ingredient (%)						
Corn	61.73	61.73	59.43	59.43	57.13	57.13
Soybean meal	21.12	21.12	20.93	20.93	20.74	20.74
Oil	2.23	2.23	2.23	2.23	2.23	2.23
Dicalcium phosphate	0.98	0.98	0.99	0.99	1.01	1.01
Oyster shell	9.10	9.10	9.03	9.03	8.95	8.95
Common salt	0.3	0.3	0.3	0.3	0.3	0.3
Vit & Min premix[a]	0.5	0.5	0.5	0.5	0.5	0.5
DL-Methionine	0.07	0.07	0.08	0.08	0.09	0.09
Sand	3.97	3.92	2.01	1.96	0.06	0.0
Calculated analysis						
Crude protein (%)	14.58	14.58	14.58	14.58	14.58	14.58
Ether extract (%)	4.52	4.52	4.77	4.77	5.03	5.03
Crud fiber (%)	2.84	2.84	4.94	4.94	7.05	7.05
Calcium (%)	3.75	3.75	3.75	3.75	3.75	3.75
Available phosphorus (%)	0.029	0.029	0.029	0.029	0.029	0.029
ME (Kcal/kg)	2720	2720	2720	2720	2720	2720

a vitamin and mineral mixture provides per 2.5 kilogram of diet: vitamin A, 7700,000 IU; vitamin D_3, 3300,000 IU; vitamin E, 6,600 mg; vitamin K_3, 550 mg; thiamine, 2200 mg; riboflavin, 4400 mg; vitamin B_6, 4400 mg; Ca pantothenate, 550 mg; nicotinic acid, 200 mg; folic acid, 110 mg; choline chloride, 275,000 mg; biotin, 55 mg; vitamin B_{12}, 8.8 mg; Trace mineral (milligrams per 2.5 kilogram of diet): Mn, 66000; Zn, 66000; Fe, 33000; Cu, 8800; Se, 300; I, 900

90 gkg^{-1}) and enzyme (Hemicell: 0, 0.5 gkg^{-}) [l]were formulated (Table 2). A total of 144 laying hens (Lohman-LSL Lite) 75 weeks old were randomly divided in 24 cages and assigned to receive one of the six experimental diets with 4 replicates and 6 hens per each replicate. All hens were supplied with feed and water *ad libitum* in 9-week trial period. The diet ingredient and composition is presented in Table 1. Egg production (EP), egg weight (EW) and feed intake (FI) was daily recorded. Egg gravity, shell weight, shell thickness, Haugh Unit and yolk colour was measured at 4 and 8 weeks of experiment.

Antibody Response to Newcastle Disease Virus (NDV): Antibody response to inactivated Newcastle disease virus (NDV) vaccine was used to examine the humoral immunity of chicks. Blood samples were withdrawn from the wing vein on week 4 of experiment to determine antibody (Ab) response. The non-heparinized blood samples (1.5 mL/ chicken, one bird per pen) were placed at 37°C for 2 h, centrifuged (3000×g for 15 min) to separate sera and stored at -20°C until analysis. The sera were applied to hemagglutination inhibition (HI) test to determine Ab to NDV.

Cell-Mediated Immune Response (CBH Responses): Cutaneous basophile hypersensitivity (CBH) response elicited by an intradermal injection of a T-cell mitogen, provided an in vivo assay for cell-mediated immunity. The assay involved injecting each hen intradermally between the third and fourth digits of the right foot with 100 μg of PHA-P in 0.10 *mL* of physiological saline solution. The left foot of each hen was similarly injected with 0.10 *mL* of physiological saline solution to serve as controls. At week 9 of experiment data were obtained on the length (0.1 mm) of the left and right metatarsus (shank) of each hen. A single measurement of skin thickness (0.1mm) was made just prior to and 24 h following the injection with PHA-P. The CBH response was calculated as CBH= PHA-P response, right foot - saline response, left foot; where PHA-P response = (post injection skin thickness, right foot) - (pre injection skin thickness, right foot). Saline response = (post injection skin thickness, left foot) - (pre injection skin thickness, left foot) [16].

White Blood Cell Counts: On day 28 of experimental period, blood samples were withdrawn from the wing vein and were placed in tubes with heparin as anticoagulant. Blood samples were taken from one randomly selected bird from each replicate. Briefly, two drops of blood were placed on a slide, spin prepared and stained with May-Grunwald-Giemsa stain. All slides were coded and white blood cells counted. Relative percentage of the various groups of white blood cells (Heterophile, Lymphocyte, Monocyte, Eosynophil and Basophil) as well as the ratio of heterophiles to lymphocytes per each blood sample was calculated.

Blood Hormones (T3 and T4) and Metabolites: At the end of the experiment, four hens from each treatment were selected at random to collect blood samples via the wing vein and plasma was separated by centrifugation to determine glucose, cholesterol, triglycerides (TG), high density lipoprotein (HDL) cholesterol and low density lipoprotein (LDL). The concentrations of serum thyroxin (T4), triiodothyronine (T3) were measured.

Litter Samples: Litter samples were collected at 4 weeks after feeding of experimental diet. After collection, the samples were dried at 70ºC and analyzed for moisture content and ash content. Litter pH was measured in deionized water (1:4 litters to deionized water) [17].

Statistical Analysis: Data were analyzed by two-way ANOVA for two-factorial experimental design by using general linear model procedure (PROC GLM) of SAS (SAS Institute Inc., Cary, NC). If differences in treatment means were detected by ANOVA, Duncan's multiple range tests was applied to separate means. Statements of statistical significance are based on a probability of ($P \leq 0.05$).

RESULTS AND DISCUSSION

Performance and Egg Characteristics: Effects of dietary OP inclusion and enzyme supplementation on egg production (EP), egg mass (EM), feed conversion ratio (FCR), feed intake (FI) and egg weight (EW) of laying hens are shown in Tables 3 and 4. No significant interaction was observed between dietary OP and enzyme on any performance parameters except egg weight during the 9-wk period. Throughout the experimental period, the use of OP at 45 $g.kg^{-1}$ improved overall EM and FCR but this improvement was not statistically significant. [18] observed that the use of 0, 50, 100, 150 and 200 $g.kg^{-1}$ OP, had no significant effect on EP. [7] observed no significant effect on FI and FCR by using 2.5, 5, 7.5 and 10% of OP in broiler rations. [18] reported that OP at 15% and 20% increased crude fiber concentration and resulted in higher FI, either to meet energy requirements or because OP was more palatable. [12] observed no significant differences in overall average EP and EM by

Table 3: Effects of dietary olive pulp (OP) inclusion and enzyme supplementation on egg production (EP), egg mass (EM) and feed conversion ratio (FCR) of laying hens

		Egg production (%)				Egg mass (g egg/hen/day)				FCR (g:g)			
		Weeks				Weeks				Weeks			
		1-3	4-6	7-9	Overall	1-3	4-6	7-9	Overall	1-3	4-6	7-9	Overall
Olive pulp													
0 (g/kg diet)		86.31	82.74	81.94	83.66	55.46	51.96	52.95	53.46	2.17	2.33	2.28	2.26
45 (g/kg diet)		76.82	82.46	81.55	83.01	51.01	53.20	53.91	54.52	2.02	2.28	2.24	2.21
90 (g/kg diet)		81.45	84.62	77.28	81.12	54.20	54.39	51.01	53.20	2.17	2.21	2.38	2.26
Enzyme													
No enzyme		88.43	84.46	83.33	85.41	57.72	53.44	53.97	55.04	2.08	2.26	2.24	2.18
β-Mannanase		80.09	82.09	77.18	79.79	53.02	52.92	51.27	52.40	2.28	2.28	2.36	2.30
Olive pulp	Enzyme												
0	-	87.30	79.96	83.93	83.73	56.69	50.69	54.56	53.98	2.12	2.39	2.21	2.23
0	+	85.32	85.52	79.96	83.60	54.24	53.23	51.33	52.93	2.22	2.26	2.36	2.28
4.5	-	89.29	83.93	83.93	85.72	58.48	53.07	54.66	55.41	2.05	2.28	2.22	2.17
4.5	+	80.76	80.99	79.17	80.30	54.41	53.34	53.16	53.62	2.24	2.28	2.26	2.25
9	-	88.69	89.49	82.14	86.77	57.98	56.57	52.68	55.74	2.08	2.12	2.30	2.15
9	+	74.21	79.76	72.42	75.47	50.42	52.20	49.34	50.65	2.38	2.31	2.46	2.37
SEM		1.32	1.48	1.69	1.33	0.91	1.07	1.08	0.89	0.04	0.05	0.05	0.04
							P values						
Olive pulp		0.610	0.851	0.554	0.776	0.667	0.721	0.624	0.853	0.067	0.697	0.594	0.711
Enzyme		0.074	0.490	0.125	0.078	0.095	0.833	0.286	0.207	0.154	0.855	0.308	0.237
Interaction		0.381	0.206	0.802	0.338	0.517	0.510	0.943	0.689	0.143	0.528	0.902	0.723

Values of the same column with no common superscripts are significantly different ($P \leq 0.05$)

Table 4: Effects of dietary olive pulp (OP) inclusion and enzyme supplementation on feed intake (FI) and egg weight (EW) of laying hens

		Feed intake (g Feed/hen/day)				Egg weigh (g)			
		Weeks				Weeks			
		1-3	4-6	7-9	Overall	1-3	4-6	7-9	Overall
Olive pulp									
0 (g/kg diet)		119.54	119.50	119.73	119.59	64.32^{b}	62.81	64.66	63.94^{b}
45 (g/kg diet)		119.41	119.40	119.72	119.51	66.38^{a}	64.39	64.15	65.67^{a}
90 (g/kg diet)		119.56	119.64	119.78	119.66	66.62^{a}	64.33	64.19	65.68^{a}
Enzyme									
No enzyme		119.47	119.48	119.75	119.57	65.28	63.22	64.80	64.45^{b}
β-Mannanase		119.53	119.54	119.73	119.60	66.27	64.47	66.53	65.74^{a}
Olive pulp	Enzyme								
0	-	119.53	119.44	119.71	119.56	65.0^{bc}	63.38	65.01	64.50^{bc}
0	+	119.54	119.55	119.74	119.61	63.64^{c}	62.26	63.31	63.38^{c}
4.5	-	119.40	119.38	119.73	119.50	65.50^{abc}	63.05	65.12	64.60^{bc}
4.5	+	119.43	119.43	119.71	119.52	67.26^{ab}	65.73	67.17	66.74^{ab}
9	-	119.49	119.63	119.82	119.65	65.33^{bc}	63.24	64.26	64.25^{c}
9	+	119.62	119.64	119.74	119.67	67.90^{a}	65.43	68.12	67.11^{a}
SEM		0.05	0.06	0.03	0.04	0.33	0.35	0.47	0.32
					P values				
Olive pulp		0.526	0.407	0.718	0.443	0.015	0.138	0.364	0.040
Enzyme		0.611	0.685	0.735	0.744	0.135	0.087	0.092	0.042
Interaction		0.896	0.957	0.791	0.989	0.048	0.077	0.185	0.029

Values of the same column with no common superscripts are significantly different ($P \leq 0.05$)

adding β-mannanase to layer diets. A significant enzyme × OP interaction was observed for 1 to 3 week and overall average egg weight. The highest and lowest EW was seen in the diet including 90 gkg^{-1} OP with enzyme and without enzyme, respectively. Previous researches showed that using 5, 10, 15 and 20% of OP in diet significantly increased EW [12,13-18]. Demonstrated that the supplementation low energy diet with β-mannanase improved EW. OP contains high concentration of polyunsaturated fatty acids, mainly linoleic acid, which is an important factor in egg size and egg weight. [19] who studied on the effects of dietary oil sources on quail's egg quality demonstrated that quails fed diet containing olive oil produced the heaviest eggs [19]. [20] reported that EW was consistently greater with the diet high in linoleic acid. OP also contains high concentration of crude fiber that may be a cause for reduction EW in hens fed diet containing 90 g kg^{-1} OP without enzyme.

Effects of OP and enzyme on egg characteristics are shown in Table 5. Egg gravity, shell thickness and yolk colour were not affected by dietary OP inclusion ($p>0.05$). OP inclusion at 45 and 90 g.kg^{-1} increased shell weight significantly ($p<0.05$) so that the lowest shell weight was seen in control group. OP contains residual unextracted oil that contains high concentration of polyunsaturated fatty acids, mainly linoleic acid. Linoleic acid of OP might increase shell weight. This is in line with[21] who reported that shell weight increased when linoleic acid was the source of diet oil. Dietary inclusion of OP decreased haugh unit. Hens given 90 gkg^{-1} OP diets had significantly ($p<0.05$) lower Haugh unit compared to those given 0.0 and 45 g kg^{-1} OP. It may be a result of increasing EW due to OP feeding. Enzyme supplementation did not significantly ($p>0.05$) affect egg characteristics (Egg gravity, shell thickness, sell weight, haugh unit and yolk colour). [12, 13] also observed no significant effect of β-mannanase on egg gravity.

Antibody Response to NDV, CBH Response and White Blood Cells: Effects of dietary OP inclusion and enzyme supplementation on Ab response against NDV is presented in Table 6. There was no significant effect of OP and enzyme on antibody response to NDV ($p>0.05$). The highest Ab titer was seen in hens fed on 45 g.kg^{-1} OP. [15] reported that dietary supplementation by a β-mannanase-based enzyme significantly improved the broilers' Ab response to NDV. As it is presented in Table 6, cell-mediated immune responses as measured by

Table 5: Effects of dietary olive pulp (OP) inclusion and enzyme supplementation on egg gravity, shell weight, shell thickness, Haugh unit and yolk color

		Egg traits				
		Egg gravity	Shell Weight (gr)	Shell thickness (×10^{-2} mm)	Haugh Unit	Yolk color
Olive pulp						
0 (g/kg diet)		1.081	5.70^{b}	35.73	95.73^{a}	7.71
45 (g/kg diet)		1.083	6.10^{a}	36.28	93.85^{ab}	7.83
90 (g/kg diet)		1.080	6.04^{a}	35.93	92.51^{b}	7.67
Enzyme						
No enzyme		1.080	5.82	35.63	94.18	7.84
β-Mannanase		1.082	6.07	36.33	93.88	7.63
Olive pulp	Enzyme					
0	-	1.080	5.58	34.61	95.69	7.79
0	+	1.082	5.81	36.85	95.77	7.63
4.5	-	1.082	5.98	36.61	93.84	7.96
4.5	+	1.085	6.23	35.96	93.86	7.71
9	-	1.080	5.92	35.66	93.02	7.77
9	+	1.080	6.17	36.19	92.00	7.57
SEM		0.002	0.06	0.31	0.39	0.49
				P values		
Olive pulp		0.317	0.046	0.772	0.025	0.459
Enzyme		0.355	0.080	0.281	0.729	0.789
Interaction		0.600	0.997	0.202	0.848	0.945

Values of the same column with no common superscripts are significantly different ($P \leq 0.05$)

Table 6: Effects of dietary olive pulp (OP) inclusion and enzyme supplementation on antibody response to NDV, white blood cell counts (percentage of total) and cutaneous basophile hypersensitivity (CBH) response

		White blood cell counts							
		NDV	CBH	H	L	M	B	E	H/L
Olive pulp									
0 (g/kg diet)		8.62	4.5	40.75	57.38	0.50	0.50	0.25	0.71
45 (g/kg diet)		10.00	8.75	42.12	56.50	0.50	0.50	0.25	0.77
90 (g/kg diet)		9.00	8.5	40.12	59.12	0.50	0.50	0.00	0.70
Enzyme									
No enzyme		9.17	7.08	41.00	57.58	0.67	0.67	0.33	0.73
β-Mannanase		9.25	7.42	41.00	57.75	0.33	0.33	0.00	0.73
Olive pulp	Enzyme								
0	-	9.25	4.5	39.00	59.25	0.50	1.25	0.00	0.67
0	+	8.00	4.5	42.50	55.50	0.50	1.00	0.50	0.77
4.5	-	9.50	11.5	43.75	54.50	1.00	0.75	0.00	0.81
4.5	+	10.50	6.00	40.50	58.50	0.00	0.50	0.50	0.73
9	-	8.75	5.25	40.25	59.00	0.50	0.25	0.00	0.71
9	+	9.25	11.75	40.00	59.25	0.50	0.25	0.00	0.69
SEM		0.29	2.01	1.19	1.21	0.093	0.097	0.098	0.03
					P values				
Olive pulp		0.207	0.681	0.815	0.716	0.279	0.290	0.352	0.763
Enzyme		0.896	0.940	1.000	0.950	0.194	1.000	0.207	0.983
Interaction		0.327	0.547	0.579	0.502	0.267	0.582	0.573	0.642

H: Heterophile, L: Lymphocytes, M: Monocyte, B: Basophile E: Eosinophils, H/L: Heterophile: Lymphocytes ratio

CBH: Cutaneous basophile hypersensitivity

Values of the same column with no common superscripts are significantly different ($P \leq 0.05$)

Table 7: Effects of dietary olive pulp (OP) inclusion and enzyme supplementation on glucose, cholesterol, triglycerides, HDL, LDL, T_3 and T_4

		Blood serum parameters					Blood serum hormone	
		Glucose (mg/dL)	Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	T_3 (nmol)	T_4 (µg)
Olive pulp								
0 (g/kg diet)		240.25	191.63	1953.8	45.38	64.5	1.30	0.112
45 (g/kg diet)		266.38	200.25	1827.5	47.12	64.62	1.28	0.125
90 (g/kg diet)		252.75	195.00	1937.5	47.12	69.38	1.38	0.138
Enzyme								
No enzyme		259.25	185.00	1680.8	46.25	65.33	1.29	0.117
β-Mannanase		247.00	206.25	2131.7	46.83	67.00	1.34	0.133
Olive pulp	Enzyme							
0	-	253.75	152.50	1232.5^{b}	45.75	57.5^{b}	1.40	0.125
0	+	226.75	230.75	2675.0^{a}	45	71.5^{a}	1.20	0.100
4.5	-	281.50	193.75	1780.0^{b}	48.75	67.75^{ab}	1.25	0.100
4.5	+	251.25	206.75	1875.0^{ab}	45.5	61.5^{ab}	1.30	0.150
9	-	242.50	208.75	2030.0^{ab}	44.25	70.75^{a}	1.22	0.125
9	+	263.00	181.25	1845.0^{ab}	50.00	68.00^{ab}	1.52	0.150
SEM		0.12	0.12	121.03	1.45	1.50	0.06	0.008
				P values				
Olive pulp		0.660	0.611	0.875	0.872	0.266	0.787	0.537
Enzyme		0.603	0.165	0.052	0.855	0.543	0.687	0.367
Interaction		0.614	0.668	0.015	0.497	0.014	0.271	0.250

Values of the same column with no common superscripts are significantly different ($P \leq 0.05$)

Table 8: Effects of dietary olive pulp (OP) inclusion and enzyme supplementation on moisture, pH and ash of litter

		Litter quality		
		Moisture (%)	pH	Ash (%)
Olive pulp				
0 (g/kg diet)		66.48	7.60	33.52
45 (g/kg diet)		68.27	7.38	31.73
90 (g/kg diet)		75.12	7.03	24.88
Enzyme				
No enzyme		69.13	7.25	30.87
β-Mannanase		70.78	7.42	29.22
Olive pulp	Enzyme			
0	-	65.90	7.41	34.10
0	+	67.07	7.79	32.93
4.5	-	65.43	7.33	34.57
4.5	+	71.11	7.41	28.90
9	-	76.07	7.01	23.93
9	+	74.17	7.05	25.83
SEM		1.53	0.09	1.53
			P values	
Olive pulp		0.116	0.092	0.117
Enzyme		0.632	0.408	0.632
Interaction		0.660	0.753	0.660

Values of the same column with no common superscripts are significantly different ($P \leq 0.05$)

cutaneous basophile hypersensitivity (CBH) assay and white blood cell counts were not affected by dietary OP inclusion and enzyme supplementation. [22] also demonstrated that dietary OP inclusion had no significant effect on WBC in fattening rabbits.

Blood Serum Hormones and Metabolites: Effects of dietary OP inclusion and enzyme supplementation on blood parameters (glucose, cholesterol, triglyceride, LDL and HDL) are shown in Table 7. Dietary OP inclusion and enzyme supplementation did not have any significant effect on the blood parameters. There was no significant interaction between diet inclusion of OP and enzyme on glucose, cholesterol and HDL. [23] also demonstrated that using 10 and 20% olive cake in rabbit ration did have no significant effect on serum glucose. Diet supplementation by β-mannanase had no significant effect on concentrations of blood glucose, cholesterol and HDL. It has been previously reported that insulin secretion associated with β-mannan intake in swine [24, 25] and in humans [26]. It appears that β-mannanase may have stimulated insulin secretion or blocked the inhibition function of β-mannan [13]. A significant interaction between enzyme and OP on triglyceride (TG) and LDL content was detected. [27] demonstrated that using 10 and 20% of olive cake had no significant effect on TG content but level of 5% reduced plasma cholesterol concentration.

As it is shown in Table 7, dietary OP inclusion and enzyme supplementation did not have any significant effect on the blood concentrations of T_3 and T_4.

Litter Characteristics: Effects of dietary OP inclusion and enzyme supplementation on litter characteristics are shown in Table 8. Dietary OP inclusion and enzyme supplementation did not have any significant effect on litter characteristics.

CONCLUSIONS

In Conclusion, based on the results of the present experiment OP can be included laying hens diets up to 9 % with no deleterious effects on bird's performance. In addition, dietary OP could improve egg weight.

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