Egypt. Poult. Sci. Vol (37) (I): (277-292) (2017) **(1710)**

Egyptian Poultry Science Journal

http://www.epsaegypt.com

ISSN: 1110-5623 (Print) – 2090-0570 (On line)

EFFECT OF DIETARY OLIVE LEAVES EXTRACT (OLEUROPEIN) SUPPLEMENTATION ON PRODUCTIVE, PHYSIOLOGICAL AND IMMUNOLOGICAL PARAMETERS IN BANDARAH CHICKENS 2- DURING PRODUCTION PERIOD

Mona M. Ahmed[1], Amina S. El-Saadany[2], Effat Y. Shreif[1] and Amal M. El-Barbary[2]

[1]Dep. of Poult. Nutr. Res. Anim. Prod. Res. Inst., Agric. Res. Center, Egypt

[2]Dep. of Poult.Breeding Res. Anim. Prod. Res. Inst., Agric. Res. Center, Egypt

Corresponding author: Amina. El-saadany; E-mail: aelsaadany55@yahoo.com

Received:13/02/2017 Accepted:08/03/2017

ABSTRACT: The object of this study was to evaluate the effect of graded levels of olive leaves extract (Oleuropein) supplementation on layer hen performance. One hundred and forty four of Bandarah chickens (24 wks) were randomly distributed to four treatment groups of three replicates (10 hens and 2 cocks per each replicate), group 1 was served as a control group. Group 2, 3 and 4 were fed diets supplemented with 50.0, 100.0 and 150 mg Oleuropein /kg diet, respectively. Birds were reared in floored cages in an open system house under similar conditions of management up to 42 wks of age. Results revealed that egg production, egg mass and feed conversion ratio were significantly ($p<0.05$) improved for the all groups supplied with different levels of oleuropein compared with control group. While egg weight and feed consumption were not affected by supplemental oleuropein. Egg yolk color score was significantly ($p<0.01$) increasing for the groups fed oleuropein diets. The significant highest egg yolk color score was recorded for the group fed diet supplied with the highest level of oleuropein (150 mg/kg diet). Egg yolk cholesterol significantly ($p<0.05$) decreased for oleuropein treatments. Significant decrease was observed in saturated fatty acids of egg yolk in treated groups (100, 150 mg oleuropein). Also, results showed significant increase in yolk omega 3 and 6 fatty acids for the groups supplied with oleuropein. Adding oleuropein to hens diets at any tested levels resulted in significantly ($p<0.01$) increased in plasma protein and globulin. The same result was observed in plasma T3, while blood albumin, glucose concentrations and the activity of AST and ALT enzymes were not significantly differed among the all experimental groups. Blood lipid profile for the groups fed diets supplied with oleuropein was significantly ($p<0.01$) improved compared with the control group. In accordance with hematological parameters, addition of oleuropein at different levels significantly ($p<0.01$) increased white blood cells (WBC) and lymphocytes count while heterophils count and H/L ratio significantly ($p<0.01$) decreased. The improvement of superoxide dismutase (SOD) and total antioxidant capacity (TAC) were significantly ($p<0.01$) for the groups supplied with different levels of oleuropein compared with control group. Decrement of plasma lipid peroxidation based on MDA was significantly ($p<0.001$) in treated groups compared with control group. In conclusion, olive leaves extract (oleuropein) supplied up to 150 mg/kg diet improved the performance, lipid profile, immunological and anti-oxidative statues of Bandarah laying hens.

Key words: Oleuropein, laying hens, yolk fatty acids, immunity, antioxidant.

INTRODUCTION

Evidence of olive use for oil production can be found in historical and sacred texts, such as the Holy Koran, Holy Bible, and the Odyssey (Belarbi, et al., 2011). Wood, fruit, leaves, roots and bark of Olive tree contain oleuropein, a non-toxic secoiridoid, the major phenolic compound in olive leaves is oleuropein and varies from 17% to 23% depending upon the harvesting time of the leaves (Le Toutour, and Guedon, 1992). Also, Ryan et al. (2002) reported that olive leaves extracts are rich in components, oleuropein being the most prominent phenolic compound that may reach concentrations
of 60-90 mg/g of dry matter as well as several flavonoids and biflavonoids (Le Tutour and Guedon, 1992). Erener et al. (2009) found 112.98 mg of olive leaf extract, 34.45 mg of rut in, 16.67 mg of hydroxytyrosol, 7.33 mg of vanillin, 4.68 mg of vanillic acid, 4.42 mg of caffeic acid, 1.68 mg concentration of catechin, and had an antioxidant capacity of EC50 19.54 µl in the olive leaf extract. It has been reported that hydroxytyrosol, a main bioactive metabolite of olive leaf extract, is a strong naturally occurring antioxidant; and elenolic acid, another structural sub unit, has strong antiviral properties (Renis, 1975; Saija et al. 1998).

The bioactivation of polyphenolic compounds in the extract of olive leaf as a free radical scavenger by breaking the free radical chain reaction (Lee and Lee, 2010; Hayes et al., 2011). Moreover, the bioactivation of these compounds may contribute also to their antioxidant activity by preventing metal ion chelation (Endgecombe et al., 2000; Visioli et al., 2002; Hayes et al., 2011). Most of the phenolic compounds in the olive leaf extract have been shown to possess hypocholesterolaemic activities due to lowering the concentrations of serum and hepatic triglyceride and altering the metabolism of cholesterol (Romani et al., 1999).

Several pharmacological properties in oleuropein are including anti-inflammatory (Visioli et al., 1998), antioxidant (Visioli et al., 2000), anti-cancer (Owen et al., 2000), anti-atherogenic (Carluccio et al., 2003), antimicrobial (Tripoli et al., 2005), and antiviral (Fredrickson and Group, 2000), so it use as food supplementation in Mediterranean countries. In addition, oleuropein cardioprotective against acute adriamycin cardiotoxicity (Andreadou et al., 2007), and exhibit anti-ischemic and hypolipidemic activities (Andreadou et al., 2006). Lipid metabolism, activator of protein digestion and inhibitor of triacylglycerol absorption were improving by oleuropein (Polzonetti et al., 2004).

Numerous scientific studies have been investigate the beneficial properties of olive leaf powder, olive leaf extracts and oleuropein and its effect on poultry performance. Coni et al. (2000) showed that using of oleuropein as feed additive in rabbit diets increases the ability of LDL to resist oxidation and reduces the plasma levels of cholesterol. Erener et al. (2009) found that supplemented broiler diets with olive leaf extract caused a greater increase in body weight, and improvement the feed conversion ratio. Sarica and Toptas (2014) showed that the dietary oleuropein supplementation at 150 mg/kg level may be used in quail diets enriched with the polyunsaturated fatty acids and vitamin E as a natural antioxidant. Rezar et al. (2015) conclude that hen performance was not affected by supplementation of olive leaves or their extract. Bahsi et al. (2016) reported that oleuropein, which added with 400 ppm, were improved body weight gain, feed conversion ratio and were not affected on blood glucose, and total, HDL and LDL cholesterol. Also, Christaki et al. (2011 a, b) found that supplemented the diet of Japanese quails with olive leave powder resulted in higher egg production and decreased blood serum triglycerides and cholesterol. El-Damarawy et al. (2013) observed that olive leave powder

supplementation at level 2.0% to Mandarah chicks diets improved performance and most of immunological and biochemical traits. Shafey et al. (2013) concluded that replacement of 15 and 30 g wheat bran/kg with olive leaf in started and finisher broiler diets had no significant effect on performance and carcass characteristics of chickens. Cayan and Erener (2015) noted that when laying hens fed diets with olive leaf powder increased body weight and egg yolk color compared to control values.

The aim of the present study was to investigate the effect of oleuropein (olive leaf extract) supplementation to Bandarah chickens diets on productive performance, lipid profile, egg quality and immune response.

MATERIALS AND METHODS

The present experiment was carried out at El-Sabahia Poultry Research Station, Animal Production Research Institute, Agricultural Research Center, Egypt. One hundred and forty four of Bandarah chickens aged 24 wks were wing-banded, weighed and randomly distributed into four treatment groups of three replicates (10 hens and 2 cocks per each replicate) and were housed in floor pens. Chickens were reared under similar management and hygienic conditions. Feed and water were supplied ad libitum throughout the experimental period which ended at 42 wks of age. Diets were kept isocaloric and isonutrogen and cover nutrient requirements according to Feed Composition Table for Animal and Poultry Feedstuffs in Egypt (2001), Table (1).Oleuropein was extracted from olive leaves by method of Sahin and Bilgin (2012). Chickens in group 1 were fed a basal diet and considered as control group, the other three groups (2, 3 and 4) were fed a basal diet supplemented with 50, 100 and 150 mg oleuropein /kg diet, respectively.

Egg weight (g) and egg number were recorded daily. Egg mass per hen was calculated by multiplying egg number by average egg weight. Feed conversion was calculated by feed consumption divided egg mass (g feed/g egg). 15 eggs were randomly taken for egg quality measurements from each group at 32 and 42 wks of age. Shape index, and yolk index values were measured as following:

Shape index (%) = (egg width/ egg length) x 100

Yolk index (%) = (yolk height/ yolk diameter) x 100

Egg shell thickness was measured (mm) by micrometer without inner membranes.

The height of thick albumen (H) and the egg weight (W) were used to calculate Haugh Units (HU) from the formula of Haughs (1937):

$HU = 100 \log (H + 7.57 - 1.7 W^{0.37})$,

Where H= height of thick albumen, W= weight of egg.

Yolk cholesterol was determined using the extraction procedure of Fisher and Leveille (1957). Weight samples of boiled yolk (approximately 2 gm) where extracted for 1 to 2 hours with 2 parts chloroform and 1 part methanol in 125 ml. The contents were filtered to remove the insoluble materials, and the filtrate was brought to volume by in a 50 ml volumetric flask. A sample of 0.2 ml aliquot was then analyzed for cholesterol by the method of Allain et al. (1974) using commercial kits produced by Sentinel Company.

Preparation of fatty acids methyl esters from total lipids of sample was performed according to the procedure of Radwan (1978). A sample of total lipids (50 mg) was transferred into Screw-Cap vial, and 2 ml benzene and 10 ml 1% H2SO4 in absolute methanol were added. The vial was covered under a stream of nitrogen gas before heating in an oven at 90°C for 90 min. Ten ml of distilled water were added to the cooled vial and the methyl esters in

each vial were extracted with 5 ml of petroleum ether for three times. The three petroleum ether extracts were combined and concentrated to its minimum volume by using stream of nitrogen.

Analysis of fatty acids (at 40 wk of age) was carried out by gas liquid chromatography (GLC) using Shimadzu gas chromatograph (GC-4 cm, PFE). A standard mixture of methyl esters was analyzed under identical conditions prior to running the samples. The retention times of the unknown sample of methyl esters were compared with those of the standard. The proportions of methyl esters were calculated by the triangulation method.

Blood samples were taken from 15 hens per each group at 42 wks of age in heparinized tube. White blood cells (WBCs), different subclasses of WBC's (lymphocytes and heterophils) were measured by using fresh blood.

Plasma was obtained from the blood samples by centrifugation for 15 min. at 3000 rpm and was stored at -20 C^0 until the time of analysis. Low density lipoprotein (LDL), high density lipoprotein (HDL), total cholesterol, plasma total protein, albumin, glucose, triglyceride, alanine aminotransferase (ALT), aspartate aminotransferase (AST) were determined by spectrophotometer. Triiodothyronine hormone (T3) was determined using immunoassay Elisa kit. The different between total protein and albumin concentrations (globulin) was calculated. Total antioxidant capacity (TAC), Malondialdehyde (MDA) and Superoxide dismutase (SOD) activity were determined calorimetrically.

Statistical analysis

Experimental data was analyzed according to SAS program (SAS, 2004) using GLM Procedure. All data were subjected to one way analysis of variance model. Mean differences were tested by Duncan's multiple range (Duncan, 1955).

RESULTS AND DISCUSSION

Productive performance

Table 2 displays the effect of supplementation layer diets with graded levels of oleuropein on egg production percentages (EP %), egg weights (EW), egg mass (EM), feed intake (FI) and feed conversion (FCR). The results illustrated that EP, EM and FCR were significantly ($p<0.05$) improved for the all groups supplied with different levels of oleuropein compared with unsupplied group (control group). However, the all groups supplied with different levels of oleuropein were statistically equals on the previous treats. Supplementation oleuropein with 50, 100 and 150 mg/kg diet significant increased EP by 9.28, 10.36 and 9.93 %, EM 10.38, 10.46 and 10.69% and FCR significant improved by 9.49, 9.99 and 10.38%, respectively compared with control group. Concerning of EW and FI, results indicated that both of them were not significant differences among the groups supplied with different levels of oleuropein and the control group.

Numerous scientific studies have been investigate the beneficial properties of olive leaf powder, olive leaf extracts and oleuropein and its effect on poultry performance. The improvement on hen performance observed in this study may be due to the beneficial effect of olive leaf extracts for improving the nutrients digestibility and intestinal absorption. These results may be attributed to the observation of Aziz et al. (1998) who found that oleuropein and other phenolic compounds completely inhibit the development of Escherichia coli and B. cereus. Also, Sudjana et al. (2009) indicated that oleuropein play a role in regulating the composition of the gastric flora by selectively reducing levels of Campylobacter jejuni, Helicobacter pylori and methicillin-resistant Staphylococcus aureus (MRSA). Moreover, Wenk (2002) reported that the increase in hen performance associated with the

supplementation of poultry diets with plant-origin materials are containing polyphenol. Which Polyphenolic compounds increase the activity of digestive enzymes by decreasing of pathogenic microorganisms that spread in these animals' digestive organs, also preventing the formation of toxins within the feed.

Christaki et al. (2011a) reported that according to the regression analysis of the effect of olive leaves on egg production of Japanese quails showed a significant linear increase with $R^2 = 0.569$, but daily feed intake was not affected. El-Damarawy et al. (2013) showed that olive leave powder significantly increased broiler body weight and feed conversion. Bahsi et al. (2016) indicated that oleuropein, which added to mixed feed of Japanese quails with a dose of 400 ppm improves feed conversion ratio. Inversely, Cayan and Erener (2015**)** indicated that feed intake and the feed conversion ratio not affected by dietary olive leaf powder. Also, they observed no differences between control group and the olive leaf powder consuming group with respect to egg yield and egg weight.

Egg quality traits

Results of table 3 referred that all outer and inner egg quality recorded was not significant affected by supplementation hen diet with graded levels of oleuropein, unless egg yolk color score which was significantly ($p<0.01$) increased for the groups fed diets supplied with different levels of oleuropein. The significant highest egg yolk color score was recorded for the group fed diet supplied with the highest level of oleuropein (150 mg/kg diet). These results are in agreement with Christaki et al. (2011a, b) on Japanese quail and Zangeneh and Torki on laying hens (2011) who reported that olive leaf powder increased the egg yolk color. Likewise, Cayan and Erener **(**2015) found that increasing the level of olive leaf powder in the diet also resulted in a linear increase in egg yolk color. This increase in egg yolk color can be attributed to presence of pigments in olive leaf extract.

Cholesterol and fatty acids of egg yolk

Table 4 presents cholesterol and fatty acids compositions of the yolks from eggs of hens fed diets supplemented with oleuropein. It could be speculated that the oleuropein supplementation significantly ($p<0.05$) decreased yolk cholesterol concentrations by 4.77, 3.34 and 11.67% less than control group, respectively. Whereas, high dose of oleuropein recorded the lowest significantly yolk cholesterol. In the same line, Cayan and Erener (2015) revealed that the dietary supplementation of olive leaf powder at 3% in layer diet led to decrease about 10% egg cholesterol level. In the present study total blood cholesterol significantly decreased by increasing oleuropein level and this decreasing related to decrease of egg yolk cholesterol. According to Patrick and Uzick (2001), oleuropein is known to prevent LDL oxidation and inhibit 3-hydroxy-3-methyglutaryl coenzyme A which plays an important role in cholesterol synthesis. These properties of oleuropein might have contributed to the decrease in yolk cholesterol levels.

Based on the egg yolk fatty acids profile, a significant ($p<0.01$) decrease of saturated fatty acids (SFA) percentage was observed in egg yolks from hens fed diet with 100 and 150 mg oleuropein/kg diet compared to control value. Adding oleuropein to layer diet at different levels did not significantly affect the monounsaturated fatty acids while polyunsaturated fatty acids were significantly ($p<0.01$) increased in treated groups compared with control group. Furthermore, omega-3 fatty acids significantly ($p<0.05$) increased by increasing oleuropein level to reach 9.25, 14.23 and 16.73% above control value,

respectively. Likewise, inclusion oleuropein in layer diet resulted in significantly ($p<0.01$) increased in omega-6 fatty acids by 12.76, 11.75 and 15.55% above control value, respectively. These results are consistent with those previously reported by Ozdemir and Azman (2016) who found that the addition of 80 ppm olive leaf extract to quail diet resulted in increase in polyunsaturated fatty acids and omega-6 fatty acids but monounsaturated fatty acids did not affect by treatments. Moreover, Sarica and Toptas (2014) reported that supplemented with oleuropein at the levels of 150 or 200 mg/kg diet had significantly the highest polyunsaturated fatty acid and omega-3 fatty acid contents in thigh meat of quails. The improvement in omega-3 and 6 fatty acids may be due to activities of desaturase enzymes, which involved in synthesis of omega 3 and 6 fatty acids which referred as essential fatty acids, inhibited by increased synthesis of glucocorticoids under stress. On the other hand, the antioxidant effects of oleuropein might have resulted in the increased levels of polyunsaturated fatty acids.

Biochemical parameters

Table 5 shows the effect of supplementation hens' diets with graded levels of oleuropein on some blood constituents. The improvement of blood total protein, globulin and T_3 concentrations were significantly ($p<0.01$) for the groups supplied with different levels of oleuropein compared with control group. However, the highest significant concentrations of total protein, globulin and T_3 (5.29 g/dl, 3.03 g/dl and 2.30ng/ml, respectively) were detected for the group supplied with the highest level of oleuropein. While, blood albumin and glucose concentrations were not significantly differed among the all experimental groups. Also, liver enzymes (AST, ALT) were in normal levels in treated groups. These results are in agreement with El-Damarawy et al. (2013) who showed that olive leave powder significantly increased broiler plasma total protein and its fractions. Also, Parasei (2014) demonstrated that dietary supplementation with olive leave powder had positive effects on liver enzymes of broiler chickens.

Blood lipid profile for the groups fed diets supplied with different levels of oleuropein was significantly ($p<0.01$) improved compared with the unsupplied group (Table 6). The significant total cholesterol concentration (111.76 mg/dl) was detected for the group supplied with the highest level of oleuropein (150mg/kg diet), followed by those received 100 and 50 mg/kg diet. Also, the significant lowest LDL and triglyceride concentration were detected for the groups supplied with the 100 and 150 mg oleuropein /kg diet, followed by the group supplied with 50 mg/kg diet. However, blood HDL concentration was not significant differed among the all experimental groups.

Most of the phenolic compounds in the olive leaf extract have been shown to possess hypocholesterolaemic activities due to lowering the concentrations of serum and hepatic triglyceride and altering the metabolism of cholesterol (Romani et al., 1999). The mechanism of this hypocholesterolaemic action may be due to the inhibition of dietary cholesterol absorption in the intestine or its production by liver or stimulation of the biliary secretion of cholesterol and cholesterol excretion in the feces (Rezar et al., 2015). Similar results were found by Erener et al. (2009), they observed there were decreases in plasma cholesterol concentration resulted from feeding on olive leaf extract in broilers. Sarica and Topbas (2014) demonstrated that decrement of the concentrations of serum and hepatic triglyceride due to oleuropein which main phenolic compounds in the olive leaf have been shown to possess hypocholesterolemic activities. These compounds are known to provide a protective effect against the oxidation of

low-density lipoproteins (LDL), which are involved in the development of atherosclerosis. The hydroxytyrosol and oleuropein found in olive leaves are known to prevent LDL oxidation and to inhibit 3-hydroxy-3-methyglutaryl coenzyme A – an enzyme that plays an important role in cholesterol synthesis. These properties of hydroxytyrosol and oleuropein might have contributed to the decreases in yolk cholesterol levels (Patrick and Uzick, 2001). According to El-Damarawy et al. (2013), using olive leave powder in broilers' ration resulted in significantly decreased plasma triglycerides, cholesterol, thiobarbituric acid reactive substances (TBARS). The same results reported with Parasei (2014) who reported that feeding broiler chickens with olive leaf powder resulted in significant reductions on blood levels of triglyceride, cholesterol, glucose, LDL, VLDL, HDL and liver enzymes. Cayan and Erener (2015) revolved that diet supplemented with 3% olive leaf powder decreased about 10% egg cholesterol level compared to that of control eggs. Obviously, Zangeneh and Torki (2011) noted that olive leaf did not effect on blood cholesterol level in layers.

Results presented in Table 7 showed the effect of supplementation hen diet with graded levels of oleuropein on some white blood cell characteristics, some antioxidant enzymes and lipid peroxidation. White blood cells counts, lymphocytes percentage and the activity of total antioxidant capacity were statistically equals for the groups supplied with 100 and 150 mg oleuropein/kg diet and all of them were significantly ($p<0.01$) recorded the highest levels compared with the other experimental groups. The heterophils percentage, the ratio between heterophils and lymphocytes (H/L) and malondialdhyde (MDA) concentration were significantly decreased for the groups supplied with different levels of oleuropein compared with unsupplied group. The activity of superoxide dismutase (SOD) was significantly improved for the groups supplied with different levels of oleuropein compared with unsupplied group. The significantly highest SOD activity was detected for the group supplied with the highest level of oleuropein compared with the other experimental groups.

Silva et al., 2006 and Jemai et al., 2008 reported that olive leaf or olive leaf extract are a source of many phytochemicals are considered as potential sources of antioxidant. According to Lee and Lee, 2010; Hayes et al., 2011, they reported that phenolic compounds in the olive leaf extract are considered free radical scavenger by breaking the free radical chain reaction. However result reported in current study are in agreement with El-Damarawy et al. (2013) who stated that adding olive leave powder to broilers' diets resulted in significantly increased white blood cells (WBC's), heamaaglutination inhibition (HI), and superoxide dismutase (SOD), while heterophiles : lymphocytes (H:L) significantly decreased. Also**,** Parasei (2014) demonstrated that dietary supplementation with olive leave powder had positive effects immunity of broiler chickens. In conclusion, addition of oleuropein to layer diets improved performance for Bandarah laying hens and their oxidative status and immune response. Moreover used oleuropein led to reduce plasma lipid concentrations, lipid peroxidation (MDA), and yolk cholesterol and increase omega- 3 and 6 in egg yolk. Therefore using oleuropein in Bandarah laying hens 'ration seems to benefit hen performance, immunological and oxidative status to be promising.

Table (1): Composition* and the nutritive value of the basal diets

Ingredients	%	Calculated Composition	
yellow Corn	63.55	Crude Protein, %	16.50
Soybean M. (CP44%)	25.10	ME, Kcal/kg	2700
Premix**	0.30	Crud fiber, %	2.60
NaC l	0.40	Calcium, %	3.50
Di. Ca. phosphate.	1.45	Available Phosphorus, %	0.40
Limestone	8.10	Ly, %	0.89
Mineral supplementations	1.00	Meth, %	0.36
DL-methionine (Meth)	0.10	Total sulpher amino acids %	0.66
Total	100		

*****As recommendation of Anim. Prod. Res. Inst., Agric Res. Center, Minis.of Agric., (2001).
**Composition of premix in 3 kg is: Vit. A 10,000,000 IU, Vit. D_3 2,000,000; Vit. E 10,000 mg, Vit. K_3 1,000 mg, Vit. B_1 1,000 mg, Vit. B_2 4,000 mg, Vit. B_6 1,500 mg, Vit. B_{12} 10 mg; Niacin 20,000 mg; Pantothenic acid 10,000 mg, Folic acid 1,000 mg, Biotin 50 mg, Choline chloride 500, 000 mg, Cu 3,000 mg, Iodine 300 mg, Fe 30,000 mg; Mn 40,000 mg, Zn 45,000 mg, Selenium 100mg.

Table (2): Effect of dietary Oleuropein supplementation on productive performance

Parameters	Oleuropein levels (mg/kg)				SEM	Sig.
	0	50	100	150		
Egg production (%)	51.16^{b}	55.91^{a}	56.46^{a}	56.24^{a}	0.97	*
Egg weight (g)	50.64	51.16	50.69	50.99	0.28	NS
Egg mass (g/h/d)	25.91^{b}	28.60^{a}	28.62^{a}	28.68^{a}	0.63	*
Feed consumption (g/h/d)	117.50	117.20	116.70	116.30	0.54	NS
Feed conversion ratio (g feed/g egg)	4.53^{a}	4.10^{b}	4.08^{c}	4.06^{c}	0.05	*

a, b, c Means in the same row with different superscripts, differ significantly ($p<0.05$), Sig. = Significance, NS = Not Significant, SEM = standard error mean.

Table (3): Effect of dietary Oleuropein supplementation on egg quality

Parameters	Oleuropein levels (mg/kg)				SEM	Sig.
	0	50	100	150		
Albumen (%)	53.52	54.68	54.83	54.64	0.64	NS
Yolk (%)	34.72	33.30	33.70	33.82	0.42	NS
Shell (%)	11.76	12.02	11.47	11.54	0.20	NS
Shell thickness (mm)	0.34	0.33	0.35	0.35	0.01	NS
Shape index (%)	77.50	75.35	76.92	77.13	0.93	NS
Yolk index (%)	43.77	43.88	45.84	45.31	1.08	NS
Haugh unit (%)	85.51	85.62	86.38	88.57	1.28	NS
Yolk color score	6.40^{d}	7.55^{c}	9.33^{b}	9.64^{a}	0.11	**

a, b, c Means in the same row with different superscripts, differ significantly ($p<0.05$), Sig. = Significance, NS = Not Significant, **($p<0.01$), SEM = standard error mean.

Table (4): Effect of dietary Oleuropein supplementation on cholesterol (mg/g) and fatty acids profile (%) of egg yolk

Parameters	Oleuropein levels (mg/kg diet)				SEM	Sig.
	0	50	100	150		
Cholesterol (mg/g)	18.25[a]	17.38[b]	17.64[b]	16.12[c]	0.218	*
S F A	34.714[a]	34.883[a]	31.615[b]	31.522[b]	0.541	**
M U S F A	27.582	28.631	28.890	29.243	0.960	NS
P U S F A	30.476[b]	33.886[a]	34.404[a]	35.379[a]	0.501	**
C18:2 (n-6)	14.480[b]	16.600[a]	16.416[a]	16.613[a]	0.424	**
C18:3 (n-3)	9.293[c]	10.477[b]	11.110[a]	11.390[a]	0.360	**
C18:4 (n-3)	0.140	0.140	0.143	0.147	0.069	NS
C20:4 (n-6)	2.110[c]	2.110[c]	2.116[b]	2.520[a]	0.152	**
C22:4 (n-6)	0.260[c]	0.293[b]	0.300[b]	0.333[a]	0.067	**
C22:5 (n-3)	0.200	0.210	0.213	0.226	0.064	NS
C22:6 (n-3)	3.993	4.056	4.106	4.150	0.101	NS
n-3	13.626[c]	14.883[b]	15.572[ab]	15.913[a]	0.526	*
n-6	16.850[b]	19.003[a]	18.832[a]	19.466[a]	0.396	**

a, b, c Means in the same row with different superscripts, differ significantly ($p<0.05$), Sig. = Significance, NS = Not Significant, *($p<0.05$), **($p<0.01$), SEM = standard error mean. S F A = saturated fatty acids, MUSFA= monounsaturated fatty acids, PUSFA= polyunsaturated fatty acids, n-3=Omega 3 fatty acids, n-6=Omega 6 fatty acids.

Table (5): Effect of dietary Oleuropein supplementation on blood constituents

Parameters	Oleuropein levels (mg/kg)				SEM	Sig.
	0	50	100	150		
Total protein (g/dl)	3.89^{c}	4.49^{b}	4.73^{b}	5.29^{a}	0.02	**
Albumin (g/dl)	2.14	2.20	2.25	2.26	0.11	NS
Globulin (g/dl)	1.75^{c}	2.29^{b}	2.48^{b}	3.03^{a}	0.03	**
Glucose (mg/dl)	176.87	176.68	175.81	175.91	0.57	NS
AST (U/L)	80.63	81.14	81.02	81.22	0.29	NS
ALT (U/L)	34.17	34.04	33.49	34.19	0.37	NS
T3 (ng/ml)	1.89^{d}	2.06^{c}	2.21^{b}	2.30^{a}	0.01	**

a, b, c Means in the same row with different superscripts, differ significantly ($p<0.05$), Sig. = Significance, NS = Not Significant, **($p<0.01$), SEM = standard error mean. T3=triiodothyronine AST= Aspartate aminotransferase enzyme, ALT= Alanine aminotransferase enzyme,

Table (6): Effect of dietary Oleuropein supplementation on lipid concentrations

Parameters	Oleuropein levels (mg/kg)				SEM	Sig.
	0	50	100	150		
Total cholesterol (mg/dl)	138.74^{a}	122.76^{b}	120.86^{b}	111.76^{c}	1.24	**
HDL cholesterol (mg/dl)	43.56	43.78	44.04	44.10	0.39	NS
LDL cholesterol (mg/dl)	70.48^{a}	65.93^{b}	61.74^{c}	61.51^{c}	0.23	**
Triglyceride (mg/dl)	109.38^{a}	97.28^{b}	89.26^{c}	89.08^{c}	0.35	**

a, b, c Means in the same row with different superscripts, differ significantly ($p<0.05$), Sig. = Significance, NS = Not Significant, **($p<0.01$), SEM = standard error mean. HDL= High density lipoprotein, LDL= Low density lipoprotein.

Table (7): Effect of dietary Oleuropein supplementation on white blood cell characteristics, some antioxidant enzymes and lipid peroxidation

Parameters	Oleuropein levels (mg/kg)				SEM	Sig.
	0	50	100	150		
WBCs (10^3/mm^3)	7.24^c	7.91^b	8.87^a	8.94^a	0.02	**
Lymphocytes (%)	65.23^c	67.75^b	69.62^a	69.75^a	0.13	**
Heterophils (%)	38.90^a	36.03^b	33.97^c	32.53^d	0.05	**
H/L ratio (%)	59.64^a	53.18^b	48.79^c	46.64^d	0.21	**
TAC (mmol/L)	0.63^c	0.86^b	0.98^a	1.01^a	0.01	**
SOD (U/ml)	19.94^c	23.76^b	24.02^b	26.13^a	0.10	**
MDA (µmol/ml)	4.20^a	3.63^b	3.22^c	2.56^d	0.07	***

a, b, c, d Means in the same row with different superscripts, differ significantly ($p<0.05$), Sig. = Significance, ** ($p<0.01$), SEM = standard error mean, *** ($p<0.0001$). WBCs= white blood cells, H/L= Heterophils: Lymphocytes, TAC=total antioxidants capacity, SOD = superoxide dismutase, MDA= malondialdehyde

REFERENCES

Allain, C.C.; Poon, L. S.; Chan, C. S. G.; Richmond, W.; and Fu, P. C., 1974. Enzymatic Determination of Total Serum Cholesterol. Clinical Chemistry April 1974 vol. 20 no. 4: 470-475.

Andreadou, I.; Sigala, F.; Iliodromitis, E. K.; Papaefthimiou, M.; Sigalas, C.; Aligiannis, N.; Savvari,P.; Gorgoulis, V.; Papalabros, E.; and Kremastinos, D. T., 2007. Acute doxorubicin cardiotoxicity is successfully treated with the phytochemical oleuropein through suppression of oxidative and nitrosative stress. J Mol. Cell Cardiol. 42:549–558.

Andreadou, I.; Iliodromitis, E. K.; Mikros, E.; Constantinou, M.; Agalis, A.; Magiatis, P.; Skaltsounis, L.; Kamber, E.; Tsantili- Kakoulidou, A.; and Th Kremastinos, D., 2006. The olive constituent oleuropein exhibits anti-ischemic antioxidative and hypolipidemic effects in anesthetized rabbits. J. Nutri., 136: 2213–2219.

Aziz, N. H.; Farag, S. F.; Mousa, L. A.; and Abo-Zaid, M. A., 1998. Comparative antibacterial and antifungal effects of some phenolic compounds. Microbios.93: 43–54. PMid: 9670554.

Bahsi, M.; Çiftci, M.; Şimsek, Ü. G.; Azman, M. A., Özdemir, G.; Yilmaz, Ö.; and Dalkilic, B., 2016. Effects of

olive leaf extract (oleuropein) on performance, fatty acid levels of breast muscle and some blood parameters in Japanese quail (Coturnix coturnix Japonica) reared in different stocking densities. Ankara Üniv Vet Fak Derg, 63, 61-68.

Belarbi, M.; Bendimerad, S.; Sour, S.; Soualem , Z.; Baghdad , C.; Hmimed , S.; Chemat, F.; and Visioli, F., 2011. Oleaster Oil Positively Modulates Plasma Lipids in Humans. Agric. Food Chem., 59 (16): 8667–8669.

Cayan, H.; and Erener, G., 2015. Effect of olive leaf (Olea europaea) powder on laying hens performance, egg quality and egg yolk cholesterol levels. Asian Australas. J. Anim. Sci., 28:538-543.

Carluccio, M. A.; Siculella, L.; Ancora, M. A.; Massaro, M.; Scoditti, E.; Storelli.; C.; Visioli, F.; Distante, A.; and De Caterina, R., 2003. Olive oil and red wine antioxidant polyphenols inhibit endothelial activation: antiatherogenic properties of mediterranean diet phytochemicals. Arterioscler Thromb Vasc Biol. 23: 622–629.

Christaki, E.; Bonos, E.; and Florou-Paneri, E., 2011a. Effect of dietary supplementation of olive leaves and/or tocopheryl acetate on performance and egg quality of laying Japanese quail (Coturnix japonica). Asian J. Anim. Vet. Adv. 6:1241-1248.

Christaki, E. V.; Bonos, E. M.; and Florou-Paneri, P. C., 2011b. Comparative evaluation of dietary oregano, anise and olive leaves in laying Japanese quails. Brazil. J. Poult. Sci. 13:97-101.

Coni E, Benedetto R, Pasquale M, Masella R, Modesti D, Mattei R, Carline EA., 2000. Protective effect of oleuropein, an olive oil biophenol, on low density lipoprotein oxidizability in rabbits. Lipids. 35: 45–54.

Coni, E.; Bendetto, R.; Pasquale, M.; Masella, R.; Modesti, D.; Mattei, R.; and Carlini, E. A., 2000. Protective effect of oleuropein an olive oil biophenol, on low density lipoprotein oxidizability in rabbits. Lipids, 35:45-54.

Duncan, D. B., 1955. Multiple Rang and Multiple F Test Biometrics. 11: 42.

El-Damrawy, S.Z.; Khalifah, M.M.; and Wesam A. Fares, 2013. Dietary olive leaf and antioxidant status in chickens performance, some physiological treats and immunological response of Mandarah chicks supplemented olive leaves powder in their diets. Egypt. Poult. Sci. Vol, 33:279-287.

Endgecombe, S. C.; Stretch, G. L.; and Hayball, P. J., 2000. Oleuropein, an antioxidant polyphenol from olive oil, is poorly absorbed from isolated perfused rat intestine. Journal of Nutrition 130, 2996– 3002.

Erener, G.; Ocak, N.; Oztürk, E.; Cankaya, S.; and Özkanca, R., 2009. The Effects of olive leaf extract on performance, some blood parameters and cecal microflora of broilers.Food Chemistry 93, 197–204.

Feed composition for animal and poultry feed stuff used in Egypt, 2001. Technical pulletil no. Central lab. For Feed and food; Ministry of Agriculture. Egypt.

Fisher, H.; and Leveille, G. A., 1957. Observation on the cholesterol, linoleic and linolenic acid content of eggs as influenced by dietary fats. J. Nutr. 63: 119- 129.

Fredrickson, W. R. F.; and Group, S. Inc., 2000. Method and Composition for Antiviral Therapy with Olive Leaves. U.S. Patent. 6: 117,884.

Haugh, R. R., 1937. The Haugh unit for measuring egg quality. US Egg Poult. Mag., 43: 552-555

Hayes, J. E.; Allen, P.; Brunton, N.; O'Grady, M. N.; and Kerry, J. P.,

2011. Phenolic composition and in vitro antioxidant capacity of four commercial phytochemical products: olive leaf extract (*Olea europaea* L.) lutein, sesamol and ellagic acid. Food Chemistry 126, 948–955.

Jemai, H.; Bouaziz, M.; Fki, I.; El Feki, A.; and Sayadi, S., 2008. Hypolipidimic and antioxidant activities of oleuropein and its hydrolysis derivative-rich extracts from Chemlali olive leaves. Chemico-Biological Interaction 176, 88–98.

Lee, O. H.; and Lee, B. Y., 2010. Antioxidant and antimicrobial activities of individual and combined phenolics in Olea europaea leaf extract. Bioresour Technology 101: 3751–3754.

Le Tutour, B.; and Guedon, D., 1992. Antioxidative activities of Oleauropea leaves and related phenolic compounds. Phytochem. 31:1173-1178.

Owen, R. W.; Giacosa, A.; Hull, W. E.; Haubner, R.; Würtele, G.; Spiegelhalder, B.; and Bartsch, H., 2000. Olive oil consumption and health: the possible role of antioxidants. Lancet Oncol.1: 107–112.

Ozdemir, A.; and Azman, M. A., 2016. Effects of olive leaf extract and vitamin E supplementation in quail diet on some blood parameters and egg yolk fatty acids composition. Ankara Univ. Vet. Fak. Derg. 63:31-37.

Parsaei, S.; Zahra Amini, and Houchmant, M., 2014. Effect of olive leaf on blood stabilities and humoral immunity response of broiler chickens. International jornal of Advanced Biological and Biomedical research, 2: 741-751.

Patrick, L.; and M. Uzick., 2001. Cardiovascular disease: C-reactive protein and the inflammatory disease paradigm: HMG-CoA reductase inhibitors, alpha-tocopherol, red yeast rice, and olive oil polyphenols. A review of the literature. Altern. Med. Rev. 6:248-271.

Polzonetti, V.; Egidi, D.; Vita, A.; Vincenzetti. S.; and Natalini, P., 2004. Involvement of oleuropein in (some) digestive metabolic pathways. Food Chem, 88, 11–15.

Radwan, S.S., 1978. Coupling of two-dimensional thin layer chromatography with gas chromatography for the quantitative analysis of lipids classes and their constituent fatty acids. J. Chromatog. Sci., 16: 538-542.

Renis, H. E., 1975. Inactivation of myxoviruses by calcium elenolate. Antimicrob. Agents Chemother, **8**, 194–199.

Rezar, V.; Levart, A.; and Salobir, J., 2015. The effect of olive by products and their extracts on antioxidative status of laying hens and oxidative stability of eggs enriched with n-3 fatty acids. Poljoprivreda 21: 216-219.

Romani, A.; Mulinacci, N.; Pinelli, P.; Vincieri, F. F.; and Cimato, A., 1999. Polyphenolic Olea europaea L. Journal of Agriculture and Food Chemistry 47, 964–967.

Ryan, D.; Antolovich, M.; Prenzler, P.; Robards, K.; and Lavee, S., 2002. Biotransformations of phenolic compounds in Olea europea L. Scientia Horticulturae, 92:147–176.

Sahin, S.; and Bilgin, M., 2012. Study on oleuropein extraction from olive tree (*Olea europaea*) leaves by means of SFE: Comparison of water and ethanol as co-solvent. Sep. Sci. Technol., 47:2391-2398.

Saija, A.; Trombetta, D.; Tomaıno, A.; Cascio, R. L. O.; Princi, P.; Uccellabonina, N. F.; and Castelli, F., 1998. In-vitro evaluation of the antioxidant activity and biomembrane interaction of the plant phenols oleuropein and hydroxytyrosol. Int J Pharm 166,123-133.

Sarica, S.; and Toptas, S., 2014. Effects of dietary oleuropein supplementation on growth performance, serum lipid concentrations and lipid oxidation of

Japanese quails. J. Anim. Physiol. Anim. Nutr.http://dx.doi.org/10.1111/jpn.12192.

SAS, 2004. SAS/STAT User's Guide Version 9.1.SAS Inc., Cary, NC.

Shafey, T. M.; Heimler, D.; and Pieeters, L., 2013. In vitro anti-complementary activety of flavonoids from olive (Oleaeuropaea L.) leaves. Pharmazie, 51:765-768.

Silva, S.; Gomes, L.; Leitao, F.; Coelho, A. V.; and Boas, L. V., 2006. Phenolic compounds and antioxidant activity of olea europaea L. fruits and leaves. Food Science and Technology International 12, 385–396.

Sudjana, A. N.; D'Orazio, C.; Ryan, V.; Rasool, N.; Ng, J.; Islam, N.; Riley, T. V.; and Hammer, K. A., 2009. Antimicrobial activity of commercial Olea europaea (olive) leaf extract. Int J Antimicrob Agents. 33: 461–463. .

Tripoli, E.; Giammanco, M.; Tabacchi, G.; Di Majo, D.; Giammanco, S.; and La Guardia, M., 2005. The phenolic composition of olive oil: structure, biological activity, and beneficial Visioli F, Bellosta S, Galli C., 1998. Oleuropein, the bitter principles of olives, enhances nitric oxide production by mouse macrophages. Life Sci. 62: 541–546.

Visioli, F.; Bellosta, S.; and Galli, C., 1998. Oleuropein, the bitter principle of olives, enhances nitric oxide production by mouse macrophages. Life Sci 62: 541-

Visioli, F.; Poli, A.; and Galli, C., 2000. Antioxidant and other biological activities of phenols from olives and olive oil. Med Res Rev. 22: 65–75.

Visioli, F.; Poli, A.; and Gali, C., 2002. Antioxidant and other biological activities of phenols from olives and olive oil. Medicinal Research Review 22, 65–75.

Wenk, C., 2002. Herbs, botanicals and other related substances. WPSA-Bremen. Germany.

Zangeneh, S.; and Torki, M., 2011. 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. Global Vet. 7:391-398.

الملخص العربى

تأثير اضافة مستخلص اوراق الزيتون (الاولوروبيين) على الاداء الانتاجى والفسيولوجى والمناعى فى دجاج البندرة

2- اثناء مرحلة الانتاج

منى محمود احمد[1] ، امينة شعبان السعدنى[2]، عفت يحيى شريف[1]، امال محمود البربرى[2]

[1]قسم بحوث تغذية الدواجن، معهد بحوث الانتاج الحيوانى، مركز البحوث الزراعية، وزارة الزراعة

[2]قسم بحوث تربية الدواجن، معهد بحوث الانتاج الحيوانى، مركز البحوث الزراعية، وزارة الزراعة

تهدف هذه الدراسة الى تقييم تأثير اضافة مستخلص اوراق الزيتون (الاولوروبيين) الى العلف على الاداء الانتاجى والفسيولوجى والمناعى لدجاجات البندرة. استخدم فى هذه الدراسة144 طائر عمر 24 اسبوع وتمت التربية فى بيوت ارضية ووزعت عشوائيا على اربع مجاميع كل مجموعة بها ثلاث مكررات بكل مكررة 10 دجاجة + 2 ديك. واستمرت التجربة حتى عمر 42 اسبوع. استخدمت طيور المجموعة الاولى كمجموعة مقارنة (كنترول) وغذيت على العليقة الاساسية بدون اضافة والثلاث مجموعات التالية غذيت على العليقة الاساسية مضاف اليها االاولوروبيين بمعدل 50 ، 100 و 150ملجم/كجم علف.

اشارت النتائج ان اضافة الاولوروبيين فى علائق الدجاج البياض ادى الى زيادة معنوية فى كل من انتاج البيض - كتلة البيض ولم تؤثر الاضافات على وزن البيض و استهلاك العلف. لوحظ زيادة معنوية فى معدل الكفاءة التحويلية فى المجاميع المعاملة مقارنة بمجموعة الكنترول. اوضحت النتائج ان اضافة مستخلص اوراق الزيتون (الاولوروبيين) الى علف دجاج البندرة البياض لم يؤثر على جودة البيض الداخلية والخارجية بينما زاد معنويا لون الصفار بزيادة مستويات الاولوروبيين . انخفض معنويا كل من الكوليستيرول والاحماض الدهنية المشبعة فى صفار البيض نتيجة لاضافة الاولوروبيين فى علف البياض. اظهرت النتائج زيادة معنوية فى الاحماض الدهنية الغير مشبعة (اوميجا 3 و 6) فى المجاميع المعاملة مقارنة بالكنترول.وجدت زيادة معنوية فى نسبة كل من البروتين الكلى والجلوبيولين فى بلازما الدم فى المجاميع المعاملة مقارنة بالكنترول بينما لم يتاثر الالبيومين والجلوكوز و انزيمات الكبد (امينوترانس امينياز) باضافة الاولوروبيين . واثبتت النتائج حدوث انخفاض معنوى فى كل من الكوليستيرول الكلى والكوليستيرول منخفض الكثافة والدهون الثلاثية بينما لم يتاثر الكوليستيرول عالى الكثافة فى المجاميع المعاملة مقارنة بالكنترول. ومن الملاحظ ايضا انخفاض معنوى فى انزيم المالونداليديهايد نتيجة اضافة الاولوروبيين. زادت معنويا كل من عدد كرات الدم البيضاء والنسبة المئوية لخلايا الليمفوسيت بينما انخفضت النسبة المؤية لخلايا الهيتيروفيلز فى الدجاجات التى غذت على علف مضاف اليه مستخلص اوراق الزيتون (الاولوروبيين) . اوضحت النتائج زيادة معنوية فى انزيمات مضادات الاكسدة فى المجاميع المعاملة مقارنة بالكنترول.

الخلاصة، يمكن ان نوصى بأضافة مستخلص اوراق الزيتون (الاولوروبيين) فى علف دجاج البندرة البياض حتى مستوى 150 ملجم/كجم علف حيث انه يحسن من الاداء الانتاجى وبعض الصفات الفسيولوجية والمناعية للدم ويخفض الكوليستيرول و الاحماض الدهنية المشبعة ويحسن من نسبة اوميجا 3و6 فى صفارالبيض .