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ROLE OF DRIED PURSLANE LEAVES MEAL AND ESSENTIAL PHOSPHOLIPIDS IN LAYING HEN DIETS IN REDUCING CHOLSTEROL BIOSYNTHESIS

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ABSTRACT

The aim of this study was to explain the cholesterol-lowering mechanism of dietary dried purslane leaves meal and essential phospholipids in egg-yolk and hen muscle tissues. Seventy two Inshas laying hens were randomly divided into 4 groups (3 replicate pens of 6 hens each) housed individually in one cage. Birds were fed from 28 to 40 weeks of age, either the control diet (based on corn-soybean meal) or the control diet with 10% purslane leaves meal with or without 450 mg essential phospholipids (EPL) /kg diet. Feed and water were offered ad libitum, feed intake, feed egg mass ratio, egg number and egg weight were recorded weekly. Birds were injected at 11th week of the experiment with Sheep Red Blood Cells (SRBC's) for immune test. At end of the experiment, sensory evaluation of hard boiled eggs was conducted for teste and flavor evaluation, cholesterol levels in each of egg-yolk, liver breast, thigh muscles and serum were determined. The activity of the rate-limiting enzymes in cholesterol biosynthesis was also determined. Four birds/group were slaughtered for sampling analysis.

Results obtained showed that feeding laying hens on 10% purslane leaves supplemented diet produced the best values of egg production, egg number, egg mass and feed conversion compared with the other dietary treatment groups. Dried purslane leaves, EPL and purslane + EPL diets decreased ($P<0.05$) serum total lipids by 3.05, 6.55 and 9.40% ; serum cholesterol by 16.74, 23.25 and 29.30% and serum triglycerides by 6.72, 8.93 and 14.55%, while, serum high density lipoprotein was significantly ($P<0.05$) increased by 5.35, 6.96 and 8.48%, respectively, and hens fed 10% purslane leaves diet increased ($P<0.05$) serum low density lipoprotein by 3.06% compared with the control group. Moreover, no significant effects were observed on serum AST and ALT as well as taste and flavor of hard boiled eggs at end of the experimental period. Antibody response to SRBC's and leucocytes (WBC's) and lymphocytes counts were increased ($P<0.05$) by feeding the purslane diets. Cholsterol level was decreased ($P<0.05$) by feeding purslane or purslane + ELP diets, in thigh, breast, liver, yolk and serum and this decrease ($P<0.05$) was more pronounced by feeding the purslane + EPL diet.

Supplementation of purslane or EPL to the laying hen diets significantly (P<0.05) decreased the relative weight of liver and oviduct. Abdominal fat percentage of groups while bile volume of gall bladder was significantly (P<0.05) increased compared with the control group. The activity of the rate-limiting enzymes in cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl CoA reductase was suppressed (P<0.05) by feeding purslane or EPL and purslane + EPL diets. Fatty acid synthetase activity was not significantly affected by dietary treatment groups. Both purslane and EPL diets reduced cholesterol -7-α-hydroxylase activity.

This study indicates that purslane and EPL inhibit cholesterol biosynthesis by a similar mechanism.

Key words: Dried purslane leaves meal, essential phospholipids in, laying hen, cholsterol biosynthesis.

INTRODUCTION

Purslane (Portulaca oleracea) is alliaceous plant, widely distributed and used in Egypt, as well as, in all parts of the world as a species and herbal remedy for the prevention and treatment of a variety of diseases. In China, this plant is known inflok medicine as hypotensive and antidiabetic (Meng and Wu, 2008). Purslane is considered as anti atherosclerotic agents, because it has hypolypemic and hypocholesterolemic properties (Chi *et al.*, 1982); antibacterial, antifungal and anti-inflammatory (Chan *et al.*, 2000). Longer-chain omega-3 fatty acids were not detected (Liu *et al.*, 2000). The α-linolenic accounted about 40 and 60% of the total fatty acids content in leaves and seeds, respectively (Liu *et al*., 2000).

The essential phospholipids (EPL) are highly purified phosphatidyl, choline fraction isolated from soybeans; the substance is particularly rich in polyunsaturated fatty acid with linoleic acid (Lekim and Betzin, 1974). Feeding chicks EPL supplemental levels resulted in decreased plasma cholesterol (Pesti and Bakalli, 1996). Addition of 2.5 and 5% linseed oil into the hen diets for 11 weeks gradually reduced ($P < 0.05$) total lipids and cholesterol levels in serum, liver and egg yolk (Qota, 2007). Reduction of plasma and hepatic cholesterol by dietary purslane and EPL has been shown in link between increased plasma cholesterol level and coronary heart disease; researchers focused their attention in studying changes of plasma cholesterol levels (American Heart Association, 2007).

Therefore, this work was to study the cholesterol-lowering effects of pursalne and EPL in the diets on some reproductive traits with special

references to the total lipids and cholesterol concentrations in blood serum and egg yolk of Inshas local laying hens.

MATERIALS AND METHODS

The present study conducted at the Poultry and Rabbit Research Unit, Institute of Efficiency Productivity, Zagazig University, Zagazig, Egypt during the period from January to April, 2010.

A total number of 72 Inches (Siena x Plymouth Rock) laying hens was randomly divided into 4 groups (3 replicate pens of 6 hens each) and housed individually in one cage. Two levels of dried purslane leaves meal (0 and 10%) with two levels of essential phospholipids (0 and 450 mg/kg diet) were investigated in a factorial (2 x 2) arrangement.

Four practical corn-soybean meal diets were formulated to meet nutrient requirements (Table 1). Birds were fed diets contain 0 and 10% dried purslane leaves meal (DPL) with 0 or 450 mg EPL. Feed and water were offered *ad libitum* from 28 to 40 weeks of age as experimental period. Feed intake, feed: egg mass ratio, egg number and egg weight were measured weekly. Sensory evaluation of hard boiled eggs was conducted after 12 wk of the experiment. Thirty five eggs in each group were collected, kept at 5^{o}C for 14 d to facilitate feeling, then boiled for 15 min. and kept in water at round about 35^{o}C to kept them warm until they were served for taste and flavor evaluation (Caston and Lesson, 1990), using twenty-five untrained finalists.

Immunological test was carried out on 8 hens from each group after 11 wk of the experiment. The Sheep Red Blood Cells (SRBC's) were prepared by centrifuging sheep blood then washing 4 times using phosphate buffer saline, pH 7.2), which were used as indicator cells for antibody producing cells. One ml of SRBC's (10% cells/ml) was intraperitonelly injected into each bird.

Seven-days later (Yamamoto and Glick, 1982) blood sample was collected from each bird and collected to separate serum for antibody titration as described by Bachman and Mashaly (1986) and Kai *et al.* (1988). At 40 weeks of age, 15 eggs /group were cracked and 4 birds /group were slaughtered for tissue analysis. At the end of experimental, period, four hens from each group were sacrificed to calculate relative weights of liver, ovary, oviduct, abdominal fat and oviduct length (cm).

Also, bile volume of gall bladder (ml) was measured by tuberculin syringe. Total leucocytes (WBC's) and lymphocytes counts (Wintrobe, 1969) were estimated. Cholesterol concentration in each of egg-yolk, liver, breast and high muscles and serum was extracted and determined according to Folch *et al.* (1957) and Charles and Richmond (1974). For cholsterolemic enzymes activity assay, liver (4 samples/group) tissues were ghopped, suspended in potassium

Table 1: Composition and chemical analysis of the experimental diets.

Ingredients (%)	Control	Purslane leaves		
		10%	0%	10%
		0 mg EPL/kg diet	450 mg EPL/kg diet	450 mg EPL/kg diet
Yellow corn	64.40	60.50	64.40	60.50
Soybean meal 44%	25.38	18.70	25.38	18.70
Portulaca leaves	-	10.00	-	10.00
Wheat bran	1.30	1.80	1.30	1.80
Dicalcuim phosphate	1.40	1.70	1.40	1.70
Limestone	6.82	6.70	6.82	6.70
DL-Methionine	0.10	-	0.10	-
Vit. and Min. premix*	0.30	0.30	0.30	0.30
Salt (NaCl)	0.30	0.30	0.30	0.30
Total	**100**	**100**	**100**	**100**
Crude protein (N x 6.25)	16.65	16.70	16.66	16.69
Calculated values **				
ME (cal/g)	2745	2765	2745	2765
EE	2.70	2.96	2.70	2.96
CF	3.41	3.99	3.41	3.99
Ca	3.01	3.15	3.01	3.15
P	0.39	0.42	0.39	0.42
Lysine	0.91	1.64	0.91	1.64
Methionine	0.40	0.65	0.40	0.65

* Premix contain per 3 kg : Vit A 10000000, Vit D_3 2000 000 IU, Vit E 10000mg, Vit K_3 1000 mg ,Vit B_1 1000 mg, Vit B_2 5000mg, Vit B6 1500 mg, Vit B_{12} 10 mg, Pantothenic acid 10000 mg, Niacin 30000mg, Biotin 50mg, Folic acid 1000mg, Choline 250gm, Selenium 100mg, Copper 4000mg, Iron 30000mg, Manganese 60000mg, Zinc 50000mg, Iodine 1000mg, Cobalt 100mg and $CaCo_3$ to 3000g.

** According to Feed Composition Tables for Animal and Poultry Feedstuffs used in Egypt (2001).

phosphate buffer, pH 7.4 (1:2, wt/vol), homogenized centrifuged twice at 40000 g and the supernatant was centrifuged at 100000 g. The superntan was separated to be assayed for fatty acid synthetase (FAS) activity and the pellets was homogenized with 2 ml homogenizing buffer and sonicated. These procedures were performed at 4^{o}C. Both microsomal fraction and 100000 g supernant were stored at -20^{o}C prior to assay for enzymatic activities. Activity of 3-hydroxy-3-methylglutaryl (HMG)–CoA was determinally by method of Shapiro *et al.* (1974) as modified by Qureshi *et al.* (1982). Also FAS activity was assayed by method of Nepokroeff *et al.* (1975). Cholesterol-7-α-hydroxylase activity was assayed by Carlson *et al.* (1978) method with few modification (Qursshi *et al.*, 1982).

The statistical analysis program was used to analyze data of variance (ANOVA), standard error was calculated using SPSS Version 10 program for windows (SPSS, 2000).

Data obtained from different groups of layers were subjected to factorial analysis of variance (2 x 2) according to the following model:

$$Y_{ijk} = \mu + T_i + L_j + TL_{ij} + e_{ijk}$$

Where; Y_{ijk} = Observation of the tested factor, μ = Overall mean, T_i = The effect of purslane levels (%), i = 0 and 10 %, L_j = The effect of levels the essential phospholipids (EPL) , j = 0 and 450 mg EPL /kg diet, TL_{ij} = The interaction between them and e_{ijk} = Random error.

Significant differences among treatment means were separated by Duncan's New Multiple Range Test (Duncan, 1955) with 5% level of probability.

RESULTS AND DISCUSSION

Fatty acids composition of purslane leaves, essential phospholipids and experimental diets:

Results in Tables (2 and 3) showed that both of purslane leaves and essential phospholipids are rich in total unsaturated fatty acid (TUSFAs). Regarding the total unsaturated fatty acids (TUSFAs), EPL drug had the higher percentage of polyunsaturated fatty acids (lionoleic acid and linolenic acid), followed by purslane leaves. It contained (66.5% and 5.70%) as the total, whereas purslane leaves contained (59.96% and 4.53%), respectively.

Table 2. Fatty acid content (mol %) of purslane leaves meal and essential phospholipids.

Items	No. of carbon atoms	Fatty acids composition	%
Purslane leaves	16:0	Palmitic acid	11.52
	18:0	Stearic acid	3.83
	18:1	Oleic acid	9.45
	18:2	Lenoleic acid	59.96
	18:3	Linolenic acid	4.93
EPL	16:0	Palmitic acid	12.91
	18:0	Stearic acid	4.43
	18:1	Oleic acid	10.53
	18:2	Lenoleic acid	66.50
	18:3	Linolenic acid	5.70

Table 3: Fatty acids composition of experimental diets.

Fatty acids	No. of carbon atoms	Experimental diets (%)			
		Control	10% Purslane leaves	450 mg EPL /kg diet	P+L
Caprylic	8:0	12.77	13.71	14.23	14.91
Caprice	10:0	7.65	8.25	8.52	8.88
Lauric	12:0	1.99	2.15	2.16	2.19
Myristic	14:0	14.95	16.11	16.37	16.49
Myristolic	14:1	17.52	18.88	19.11	19.33
Palmitic	16:0	16.87	18.18	18.20	18.90
Palmitoliec	16:1	0.29	0.31	0.32	0.35
Stearic	18:0	7.74	8.34	8.52	8.73
Oleic	18:1	1.25	1.29	1.31	1.35
Linoleic	18:2	2.72	2.91	2.94	3.03
Linolenic	18:3	1.09	1.13	1.18	1.21
Arachidic	20:0	2.35	2.43	2.51	2.65

The increase in TUSFAs in the experimental diets was found to be mainly due to the increase in TPUSFAs (linoleic acid and linolenic acid). Diets containing purslane leaves and EPL or their combination (purslane leaves + EPL) gave the higher values in this concept as compared to the control diet.

Laying hen performance:

Results in Table (4) revealed a significant ($P<0.05$) increase in egg production (EPR) and egg number (EN) throughout the experimental period due to feeding laying hens on the diet containing purslane leaves meal alone when compared with other dietary treatments.

Table 4. Performance of Inshas laying hens fed dietary purslane leaves meal and EPL drug from 28 to 40 weeks of age.

Treatment groups		Egg/hen/ day (%)	Egg number	Egg weight (g)	Egg mass (g/d)	Feed intake (g/hen/d)	Feed conversion (feed/egg mass)
Purslane Levels (%)	EPL. Levels (mg/kg diet)						
0	0	68.25±^{ab} 1.12	19.16±^{b} 0.29	43.37± 0.32	29.63±^{b} 0.44	111.28±^{ab} 0.88	3.77±^{b} 0.03
10	0	75.06±^{a} 1.12	20.88±^{a} 0.29	41.73± 0.32	31.91±^{a} 0.44	112.16±^{a} 0.88	3.52±^{c} 0.03
0	450	63.80±^{c} 1.12	17.81±^{c} 0.29	43.18± 0.32	27.42±^{c} 0.44	108.30±^{c} 0.88	3.96±^{a} 0.03
10	450	68.28±^{ab} 1.12	19.16±^{b} 0.29	42.88± 0.32	29.30±^{b} 0.44	110.37±^{b} 0.88	3.78±^{b} 0.03

a,b,….c: Means in the same column with different litters are significantly ($P < 0.05$)different .

This may be due to the fact that this plant contains many biologically active compounds acting as a source of many nutrients, especially with the use of isocaloric-isonitrogenous diets which almost provided the layers with the sufficient requirements from the essential unsaturated fatty acids. These findings agreed with the results reported by Rahim and Dogan (2010) who indicated that chickens fed the diet including 10 g /kg or 20 g /kg purslane had a significantly ($P<0.05$) higher egg production as compared to the control. The performance traits recorded by EPL-diet cited herein agree with confirmed the previous results reported by El-Sheikh *et al.,* (2009) who showed that EPL supplementation had no significant effects on egg production percentage. Similar results were also obtained by Hanafy (2006), who reported that injection of EPL at levels of 150 or 300 mg/kg body weight of Gimmizah laying hens had no significant effect on egg production percentage. Also, Aydin *et al.* (2006) showed that addition of 0.25 and 0.50% conjugated linoleic acid in Japanese quail diet did not influence egg production percentage. Moreover, Zhao and Scheidele (1999) stated that dietary linoleic acid had no significant effect on egg production, whereas Bolukbasi and Erhan (2005) observed that sunflower oil and soybean oil negatively influenced egg production of laying hens.

On the other hand, the differences in egg weight (EW) values due to dietary treatments were not significant, while it was significant ($P<0.05$) for egg mass (EM). Layer hens fed the diet including 10% pursalne leaves had significantly ($P<0.05$) higher egg mass values compared to the control group. However, some previous studies reported that inclusion of purslane at the level of 10 g/kg or 20 g/kg in layer diets significantly ($P<0.05$) improved egg weight and egg mass compared to the control (Rahim Aygin and Israfil Dogan, 2010).

Whereas, El-Shiekh *et al.* (2009) reported that EPL drug supplementation had no significant effects on egg weight and egg mass among all experimental periods for laying hens. Also, Meluzi *et al.* (2003) and Schafer *et al.* (2001) indicated insignificant that no significant effect on egg weight when conjugated linoleic acid supplementation in the diet of laying hen. However, An *et al.* (1997) found that the addition of safflower phospholipids to laying hen diets had no significant effect on egg weight. Regarding egg weight decrease with purslane leaves meal diets, may be due to the conversion of cholesterol to prognendone from which all the other steroid hormones are produced (Grodsky, 1977), also it is probable that purslane indirectly alter-egg weight through its inhibitory effect on the cholesterol biosynthesis. Then, decreased the formation of steroid hormones which are involved in the general control of ovarian function either directly specially androgens or indirectly via their effect on the pituitary, as well as, purslane may contain unknown depressant factors, which may be directly involved in reducing egg weight, so, increasing egg number in this respect was expected with decreasing egg weight.

Feed utilization:

Data presented in Table (4) showed that dietary treatments had significant (P<0.05) effect on both feed intake (FI) and feed conversion (FC) of layers. The feed intake was significantly (P<0.05) decreased for hens fed the diet supplemented with EPL (450 mg/kg), while, hens fed 10% dietary purslane leaves recorded the highest (P<0.05) values. The increase in FI may be due to enhanced palatability and physical characteristics of the purslane leaves diets. The average values of feed conversion (FC) showed also that hens fed 10% dietary purslane leaves recorded the best (P<0.05) values. The improvement in feed efficient with dietary purslane leaves meal in this study may be attributed to the more feed consumption and large egg mass of the treatment. Supporting our results, Rahim and Dogan (2010) observed that the diet supplemented with 10 g /kg or 20 g/kg purslane significantly improved feed efficiency compared to the control diet. On the other hand, El-Sheikh et al. (2009) reported that the highest dose of EPL (500 mg/kg diet) in laying hen diets caused a significant (P<0.05) decrease in feed consumption among all experimental periods from 30 to 40 weeks of age compared with other supplementation doses. Also, Meluzi *et al.* (2003) showed that feed consumption of laying hens was significantly lower in groups fed conjugated linoleic acid compared with the control group. Sijben *et al.* (2002) indicated that using three dietary concentrations of linoleic acid with vitamin E in laying hen diets decreased feed consumption. While, El-Shiekh (2005) reported insignificant effect on feed consumption due to feeding laying hens 300 or 1500 mg EPL/kg diet. However, the results reported by Bolukbasi and Erhan (2005) and Schafer *et al.* (2001) showed that the effect of dietary conjugated linoleic acid on feed conversion of laying hens was not significant. Also, Waldroup *et al.* (1986) reported that there was no significant impairment in feed utilization when probucol was added to laying hen diets.

Physiological parameters:

Serum characteristics:

Results of total lipids, cholesterol and triglycerides concentrations and HDL, LDL, AST and ALT activities in serum concentrations of Inshas laying hens fed the dietary treatments are given in Table 5. serum characteristics for all treated groups were significantly (P<0.05) lower than those of the control groups. Serum total lipids decreased by 3.05, 6.55 and 9.40%, serum cholesterol by 16.7, 23.25 and 29.30% and serum triglycerides by 6.72, 8.93 and 14.55%, for groups fed 10% purslane leaves, 450 mg EPL/Kg diet and their combination (purslane + EPL), respectively as compared to the control group. Similar results were obtained by Samuel *et al.* (2011) who found that the diet supplemented with freeze-dried purslane leaves (6 g/day) for 4 weeks reduced (P<0.05) plasma total cholesterol and alter blood lipid metabolism in hypercholsterololemic adults, while plasma triacylglyecerol concentrations

Table 5. Concentration of serum total lipids, cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL) contents and activity of serum (ALT) and (AST) of Inshas laying hens fed dietary purslane leaves meal and EPL drug from 28 to 40 weeks of age.

Treatment groups		Total lipids (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	ALT (IU/l)	AST (IU/l)
Purslane levels (%)	EPL levels (mg/kg diet)							
0	0	1417.14±[a] 2.27	220.15±[a] 0.50	127.76±[a] 0.22	25.57±[d] 0.06	168.93±[b] 0.23	75.29± 0.10	4.81± 0.06
10	0	1373.92±[b] 2.27	183.30±[b] 0.50	119.18±[b] 0.22	26.94±[c] 0.06	174.10±[a] 0.23	75.57± 0.10	4.88± 0.06
0	450	1324.31±[c] 2.27	168.96±[c] 0.50	116.34±[c] 0.22	27.35±[ab] 0.06	165.34±[c] 0.23	75.72± 0.10	4.99± 0.06
10	450	1283.86±[d] 2.27	155.64±[d] 0.50	109.16±[d] 0.22	**27.74±[a]**	169.39±[ab] 0.23	75.87± 0.10	5.01± 0.06

a,b,….d: Means in the same column with different litters are significantly (P<0.05) different.

were not affected by the consumption of purslane supplements. Also, Abdalla Hussein (2010) reported that the rats received either regular diet, high fat diet or high fat diet with additional purslane (150 and 300 mg/kg body weight) for 8 weeks significantly inhibited triglyceride and total cholesterol in a dose dependent manner. These observations are in agreement with previous studies that reported cholesterol lowering effect of purslane (Ezekwe *et al.,* 1995 and Ezekwe *et al.,* 1999).

A recent study with purslane fed rabbits demonstrated a significant reduction in serum total cholesterol was significantly reduced in rabbit fed purslane extract (Movahedian *et al.,* 2007). Ezekwe *et al.* (1995) previously showed that rats fed 10 or 20% purslane for 6-weeks had a significant reduction of plasma total cholesterol by 23.9 and 15.7%, respectively. Plasma triacylglycerol concentrations reduced by 28.4% for rats fed 0 and 17% in rats fed 20% purslane. In a similar study, Ezekwe *et al.* (2004)observed a decreased serum total cholesterol (26.8%) and triacylglcerol (16.2%)in pig fed purslane. The cholesterol lowering effect of purslane may be attributed to the combined effect of omega-3 fatty acids and pectin since purslane is richer in omega-3 fatty and pectin than most commercial vegetables. Simopoulos (1995) found that, *Portulaca oleracea*, has protective factors that influences as lowering effect on blood lipid levels in human and rats. Essential phospholipids (EPL) supplementation had also alowering effect on most serum characteristics, El-Sheikh *et al.* (2009) found that EPL supplementation at 300, 400 and 500 mg/kg diet in Bandarah laying hen diets significantly (P<0.05) decreased serum total lipids by 4.5, 9.6 and 13.5%, serum cholesterol by 26.56, 35.04 and 42.52% serum triglycerides by 7.68, 11.21, 17.83%, respectively. Also, Hanafy

(2006) found that injection of 300 mg EPL/kg body weight of Gimmizah laying hens at 40, 44 and 48 weeks of age significantly ($P<0.05$) decreased serum total lipids, cholesterol and triglycerides compared with the control group. El-Sheikh (2005) indicated that laying hens fed 300 or 1500 mg EPL/kg diet significantly ($P<0.05$) reduced serum cholesterol by 7.7 and 18.0%, serum total lipids by 9.8 and 18.2% and serum triglycerides by 11.7 and 18.6%, respectively compared with the control group after 10 weeks of treatment . Pervious studies have shown that consumption of omega-3 fatty acids derived from fish oil significantly lowered plasma triacylglycerol concentrations and decreased total cholesterol (Laidlaw and Holub, 2003). Vasko *et al.* (2005) indicated that addition of omega-3 polyunsaturated fatty acids from flax and fish oil in laying hen diets significantly decreased serum total lipids.

Besides, results in Table (5) showed that all groups fed purslane leaves, EPL and purslane + EPL diets significantly ($P<0.05$) increased serum HDL by 5.35, 6.96 and 8.48%, respectively, compared with the control group. Also, incorporation of 10% purslane leaves meal in the laying hen diet for 12 wk increased serum LDL by 3.06% compared with the control group, while 450 mg EPL/kg diet decreased LDL by 2.12% compared with the control diet.

Previous studies have shown that consumption of omega-3 fatty acids derived from fish oil significantly decreased LDL-cholesterol by 11% after 28 days (Laidlaw and Holub, 2003). Experimental data from human subjects suggest an association between dietary intake of polyunsaturated fatty acids, in particular omega-3 fatty acids and improved of plasma HDL-cholesterol and decreased LDL-cholesterol (Okuda *et al.*, 2005). Also, rats received either regular diet, high-fat diet or high fat diet with additional purslane (150 and 300 mg/kg body weight) for 8 weeks, purslane, co-administered with a high fat diet, significantly inhibited LDL and HDL-cholesterol in a dose dependent manner (Abdalla Hussein, 2010). The elevation of LDL-cholesterol and free fatty acids values on high fat diet feeding is in agreement with other studies (Ahmed *et al.*, 2007).

Shaomel *et al.* (1995) found that polyunsaturated fatty acids and mono-unsaturated fatty acids significantly decreased total cholesterol and LDL-cholesterol concentrations. El-sayed (2001) found that *Portulaca oleracea* leaves plays a very important role in lowering ($P<0.05$) total lipids and cholesterol in rats. Fenglin *et al.* (2009) mentioned that polysaccharide extracted from *Portulaca oleracea* leaves significant decreased the concentration of blood total cholesterol, triglyceride and modulate the metabolism of blood lipid in diabetes mellitus mice. Hanafy (2006) indicated that injection of 150 or 300 mg EPL/kg body weight significantly ($P<0.01$) increased serum HDL compared with the control group. El-Sheikh (2005) indicated that the addition of 300 and 1500 mg/kg diet of laying hens

significantly ($P < 0.05$) increased serum HDL by 16.6 and 25.9%, respectively as a percentage of the control group after 10 weeks of treatment.

Results in Table (5) indicated no significant differences in serum AST and ALT when laying hens fed diet with 10% purslane leaves, 450 mg EPL kg diet and also with purslane + EPL diet. However, Abdalla Hussein (2010) reported that rats received either regular diet, high fat diet or high fat diet with additional purslane (150 and 300 mg/kg body weight) for 8 weeks. He found that plasma AST and ALT activities were significantly higher for the high fat diet fed group than for the regular diet fed group by 3.85-fold and 2.66-fold, while, purslane ethanolic extract administration significantly supported the observed increases in AST and ALT. Also, plasma AST activity in purslane 150 and 300 mg/kg body weight treated groups also decreased by 67.04% and 76.12%, respectively, as compared to the high fat diet control group. On the other hand, El-Sheikh *et al.* (2009) found that EPL supplementation in laying hen diets had no significant effects on serum AST and ALT during the experimental period. Similar results were reported by Hanafy (2006) and El-Sheikh (2005), who found that EPL did not significantly affect serum AST and ALT levels of laying hens during the whole experimental period.

Egg sensory attributes and immunological status:

Results of taste and flavor reported insignificant differences among groups fed all dietary treatments (Table 6). However, it could be observed that the best results in taste and flavor were obtained by laying hens fed the diet with purslane + EPL or purslane dietary treatment, while those fed the control diet showed the lowest values of the same traits. Moreover, the increase of taste value was about 4.13, 3.94 and 4.69%, while that recorded with flavor value was about 4.20, 3.92 and 4.39%, for hens fed 10% dried purslane leaves, 450 mg EPL/kg diet and both purslane and EPL, respectively compared with the control group. Similar studies have revealed that purslane is a rich source of nutrients like flavonoids, vitamins A, C and E, beta-carotene, and minerals (Xu *et al.*, 2006 ; Xiangl *et al.*, 2005 and Lim and Quah, 2007). Compositional analysis of purslane accessions from various geographical locations around the world indicated high concentrations of total lipids crude protein polyunsaturated FA and other essential nutrients compared to commercialized leafy vegetables in United States (Ezekwe *et al.* 1999). Also, *Portulaca oleracea*, L. is used as a kitchen plant, especially in Mediterranean countries and has recently been considered as a health-protecting vegetable (Simpopoulos and Salem, 1986 and Koch, 1988). Purslane has alemony taste and we can serve it cooked or raw. Purslane is a delicious green for humans and chickens and the highest plant source of omega 3-fatty acids available (American Heart Association, 2007).

Table 6. Taste and flavor of hard boiled eggs and immunological status of Inshas laying hens fed dietary purslane leaves meal and EPL drug from 28 to 40 weeks of age

Treatment groups		Taste[1]	Flavor[1]	Anti-body titer	Leucocytes $10^3/mm^3$	Lymphocytes $10^3/mm^3$
Purslane levels (%)	EPL levels (mg/kg diet)					
0	0	10.66± 0.12	10.70± 0.12	4.87±[b] 0.33	25.04±[b] 0.42	17.05±[b] 0.23
10	0	11.10± 0.12	11.15± 0.12	6.53±[a] 0.33	26.63±[a] 0.44	17.84±[a] 0.22
0	450	11.08± 0.12	11.12± 0.12	4.94±[b] 0.33	25.56±[b] 0.63	17.37±[ab] 0.22
10	450	11.16± 0.13	11.17± 0.13	6.69±[a] 0.33	26.72±[a] 0.63	17.88±[a] 0.23

a and b: Means in the same column with different litters are significantly different (P<0.05).
[1] Range dislike=0, like =15

Moreover, Szymezyk and Pisulewski (2003) indicated that dietary linoleic acid had no significant effect on feed consumption of laying hens. This suggested that the palatability of the diet was not changed by the addition of EPL.

Data presented in Table (6) show that antibody response to SRBC's was increased (P<0.05) by purslane leaves-diet (34.08%) and purslane + EPL diet (37.37%), with no significant differences between these two treatments, compared with the control diet. Both total leucocytes (WBC's) and lymphocytes counts were increased (P<0.05) by feeding purslane diets with or without EPL (6.35-6.71%) and (4.63-4.87%), respectively compared with those fed control diet. No significant effects due to EPL-diet on any of the mentioned immune traits were detected. The present results concerning purslane effect on antibody response confirmed those of El-Sayed (2001) who noticed that rats fed diet contained different levels Portulaca oleracea had significant effect on anti-body titer.

Purslane contains more omega 3-fatty acid than any other leafy vegetable plant. Omega-3 fatty acids aid the body in the production of compounds that affects the immune system and it is contains gluthaione (immune system support) (American Heart Association, 2007).

Tissue cholesterol level:

The most pronounced effects of purslane leaves meal or EPL diets were observed on tissues cholesterol levels (Table 7). Supplementing laying hen diet with either 10% purslane leaves or 450 mg EPL/kg diet for 12 wk caused a sharp decrease (P < 0.05) in tissues cholesterol levels compared with the control diet. The depressive effect of both feed additives was more strong than that resulting from each factor alone.

Table 7. Fresh tissues total cholesterol contents of Inshas laying hens fed dietary purslane leaves meal and EPL drug from 28 to 40 weeks of age.

Treatment groups		Thigh (mg/100 g)	Breast (mg/100 g)	Liver (mg/g)	Yolk (mg/g)	Serum (mg/dl)
Purslane levels (%)	EPL Levels (mg/kg diet)					
0	0	126.31±[a] 0.34	58.32±[a] 2.26	146.15±[a] 0.73	16.59±[a] 0.40	226.41±[a] 0.37
10	0	100.69±[b] 0.34	48.45±[b] 2.26	135.19±[b] 0.73	13.35±[b] 0.40	165.71±[b] 0.37
0	450	99.78±[b] 0.34	45.43±[b] 2.26	129.20±[c] 0.73	12.89±[bc] 0.40	146.82±[c] 0.37
10	450	90.62±[c] 0.34	42.86±[c] 2.26	122.72±[d] 0.73	11.87±[c] 0.37	139.48±[d] 0.37

a, b… c: Means in the same column with different litters are significantly (P<0.05) different.

Cholesterol level of thigh muscle, breast muscle, liver, egg-yolk and serum decreased by about 20.28, 16.92, 7.50, 19.52 and 26.80% for diet supplemented with 10% purslane leaves, 21.00, 22.10, 11.59, 22.30 and 35.15% for diet supplemented with 450 mg EPL/kg diet and 28.25, 26.50, 16.03, 28.45 and 38.39% for diet supplemented with purslane leaves + EPL, compared with control diets, respectively. Regarding the depressive cholesterol effect of purslane leaves cited herein, it confirmed the previous of, Rahim and Dogan (2011) who reported that cholesterol content of egg from the hens fed 0, 10 or 20 g/kg did not differ and was 10.45, 9.51 or 9.51 mg/g dried egg yolk, respectively. Aprikian *et al.* (2003) reported a significant reduction in liver cholesterol and triglycerides when rats were fed a pectin containing diet. Abdalla Hussein (2010) found that liver triglycerides and cholesterol levels in purslane 150 and 300 mg/kg body weight treated groups were markedly decreased by 42.8% and 20.4%, respectively, when compared to the control groups in rats. On the other hand, purslane ethanolic extract was found to significantly suppress increases in liver triglycerides and cholesterol contents, showing apparent anti-obesity actions. The high-fat diet also increased liver fat accumulation and induced fatty liver, but purslane administration lowered fat accumulation. However, some previous studies found that EPL supplementation at levels of 300, 400 and 500 mg/kg diet in laying hen diets, significantly (P<0.05) decreased egg yolk cholesterol contents by 29.71, 32.43 and 37.86%, respectively, compared with the control group (El-Shiekh *et al.*, 2009). These results are consistent with those reported by Hanafy (2006) who found that injection of 300 mg EPL/kg body weight of Gimmizah local hens for ten weeks, decreased liver total lipids and cholesterol by 13.6 and 38.9%, respectively, than control group.

Moreover, El-Sheikh (2005) found that addition of 300 or 1500 mg EPL/kg diet significantly (P<0.05) decreased liver cholesterol by 17.5 and

60.3%, respectively compared with control group, respectively. Various reports have been published on the effect of unsaturated fatty acids on liver total lipids and cholesterol contents, as An *et al.* (1997) who reported that addition of safflower phospholipids in the laying hen diets at 6 weeks of age for seven weeks, significantly decreased liver cholesterol and triglycerides contents in all treated groups as compared with the control group. Bragg *et al.* (1973) indicated that liver lipids and cholesterol were decreased when linoleic acid was provided by soybean or sunflower oil in the laying hen diets.

Changes in serum and liver cholesterol levels are more frequently observed, perhaps because these tissue belong to the fat turnover cholesterol pool (Field *et al.*, 1960 and Chobanian and Hollander, 1962).

The muscle cholesterol pool comprises the slow turnover pool and equilibrates slowly with the plasma cholesterol pool. The muscle cholesterol pool is larger and perhaps less active and it may take a longer feeding period to show a significant reduction in cholesterol levels. Cholesterol levels were found to be much higher in the thigh than in breast muscle. A possible explanation is that cholesterol is usually associated with adipose tissue, which is more abundant in thigh than in breast muscle. Also, thigh muscles have a much greater content of slow-twitch fibers than breast muscles. Slow-twitch fibers have many more mitochondria, their mitochondria are bigger and the metabolic rate was much faster in comparison to fast-twitch fibers. Slow twitch sarcoplasmic reticulum are found to contain two to three times as much cholesterol as fast-twitch sarcoplasmic reticulum in rabbits (Bloch, 1991). The higher cholesterol concentration reduces membrane fluidity (Yeagle, 1989), lowers Ca^{+}-ATPase activity (Madden *et al.*, 1979) and regulates concentration and relaxation rates.

Internal organs:

Results in Table (8) indicate that feeding diet with 10% purslane leaves, 450 mg EPL/kg diet or purslane leaves + EPL had significantly ($P<0.05$) lowered the relative weight of liver and oviduct compared with the control group, while the relative weight of ovary and oviduct length were not significantly influenced by the same treatments. This decrease may be due to the reduction of fat accumulation in these organs.

These results are consistent with those reported by El-Sayed (2001) who found that relative weight of liver of 15% *P.Oleracea*, fed group showed the lowest weight of all treatment groups in rats. Similar observations have been noticed by El-Sheikh *et al.* (2009); Hanafy (2006) and El-Sheikh (2005), they indicated that there were no significant effects on relative weight of ovary and oviduct length due to injection or addition of EPL in the diets of laying hens, whereas the relative weight of liver and oviduct significantly ($P<0.05$) decreased during the whole experimental period.

Table 8. Some internal organs measurements of Inshas laying hens fed dietary purslane leaves meal and EPL drug from 28 to 40 weeks of age.

Treatment groups		Liver wt. (%)	Ovary wt. (%)	Oviduct wt. (%)	Oviduct length (cm)	Bile volume (ml)	Abdominal fat wt. (%)
Purslane levels (%)	EPL Levels (mg/kg diet)						
0	0	2.96±[a] 0.11	0.36±[NS] 0.02	2.71±[a] 0.03	61.94±[NS] 0.18	0.97±[c] 0.02	3.18±[a] 0.02
10	0	2.77±[a] 0.11	0.37± 0.02	2.65±[b] 0.03	61.96± 0.18	1.48±[b] 0.02	3.01±[a] 0.02
0	450	2.59±[bc] 0.11	0.36± 0.02	2.46±[c] 0.03	61.72± 0.18	1.88±[a] 0.02	2.44±[b] 0.01
10	450	2.35±[c] 0.11	0.37± 0.02	2.34±[d] 0.03	61.54± 0.18	1.94±[a] 0.02	2.06±[c] 0.01

a,b,….c: Means in the same column with different litters are significantly (P<0.05) different .

Table (8) also indicates that bile volume of gall bladder for groups fed 10% purslane leaves, 450 mg EPL or a mixture of both was significantly (P<0.05) higher compared with the control group. This observation is in accordance with of El-Sheikh (2009), Hanafy (2006) and El-Sheikh (2005) who found that EPL injection or addition in laying hen diets, significantly (P<0.05) increased bile volume of gall bladder throughout the experimental period compared with the control group.

Moreover, Sim *et al.* (1980) indicated that addition of soya sterols (safflower oil or hydrogenated coconut oil) in the laying hen diets increased feces bile acid excretion. Sim and Bragg (1978) reported that the anti-cholsterolegenic function of plant sterols in laying hen diets is due to an influence on cholesterol catabolism rather than cholesterol absorption, this factor appears to increase the degradation followed by excretion of degraded cholesterol in feces as bile acid and neutral sterol metabolites. On the other hand, data in Table (8) indicated that the groups fed 450 mg EPL/kg diet and purslane leaves + EPL diet recorded significantly (P<0.05) abdominal fat percentage by 23.27 and 35.22%, respectively, compared with the control group, while, 10% purslane leaves diet had no significant effect on abdominal fat percentage compared with the control group.

These observations are in accordance with those of El-Shiekh *et al.* (2009) found that abdominal fat percentage of groups fed 300, 400, and 500 mg EPL/kg diet was significantly (P<0.05) decreased by 8.71, 27.64 and 38.82%, respectively compared with the control group. Also, Hanafy (2006) and El-Sheikh (2005) indicated that injection or addition of EPL in the diets of laying hens significantly (P<0.05) decreased abdominal fat percentage compared with the control group.

Activity of the rate-limiting enzymes in cholesterol biosynthesis:

The HMG-CoA reductase activity was significant by decreased ($P<0.05$) for dietary purslane leaves with or without EPL supplementation (Table 9).

In contrast, fatty acid synthetase activity was not significant affected ($P<0.05$) by dietary EPL with or without purslane leaves meal, while the activity of rate-limiting enzyme in bile acid synthesis, cholesterol-7-α-hydroxylase, was reduced ($P<0.05$) by both purslane leavs, EPL and purslane leaves + EPL diets. The decrease HMG-CoA reductase and cholesterol-7-α-hydroxylase activities was about 19.69 and 55.50%, respectively, in the microsomes of birds fed purslane leaves + EPL diets (Table 9). In this regard, Abdalla Hussein (2010) suggested that purslane ethanolic extract may decrease mRNA expression of acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAs), the rate-limiting enzymes of fatty acid synthesis in the liver, and mRNA expression of sterol regulatory elementbinding protein (SREBP)-1C (Kim *et al.*, 2001), which controls the expression of these enzymes (Guillou, 2008). Also, purslane ethanolic extract effects may be due to increased energy expenditure-related fatty acid synthesis in the liver. However, some of cholesterol lowering drugs have been associated with side effects like elevated liver enzymes, muscle pain and foint aches, nausea, diarrhea, constipation (FDA Medwatch Reporting System, 2003).

Table 9. Hepatic enzyme activities of Inshas laying hens fed dietary purslane leaves meal and EPL drug from 28 to 40 weeks of age.

Treatment groups		HMG-CoA reductase[1]	Fatty acid synthetase[2]	Cholsterol α-hydroxylase[3]
Purslane levels (%)	EPL Levels (mg/kg diet)			
0	0	462.66±0.54[a]	50.63±0.32	1.08±0.02[a]
10	0	390.87±0.54[b]	51.02±0.32	0.61±0.02[b]
0	450	382.39±0.54[c]	50.87±0.32	0.59±0.02[b]
10	450	371.53±0.54[d]	50.96±0.32	0.48±0.02[c]

a,b,….c: Means in the same column with different litters are significantly ($P<0.05$) different

1 HMG CoA reductase = 3 hydroxy-3-methylglutaryl coenzyme -A reductase (picomoles of mevalonic acid synthesized/min/μg microsomol protein).

2 Nanomoles of NADPH oxidized/min/mg of cytosolic fraction.

3 Picomoles of [^{14}C] cholesterol into 7-α-[^{14}C]

Moreover, Sim and Bragg (1978) reported that the anti-cholsterolegenic function of plant sterols in laying hen diets is due to an influence on cholesterol catabolism rather than cholesterol absorption, this factor appears to increase the degradation followed by excretion of degraded cholesterol in feces as bile acid and neutral sterol metabolites. This result is consistent with the hypothesis of substrate availability regulating cholesterol-7-α-hydroxylase activity (Bjorkhem and Akerlund, 1988). Because purslane was confirmed to reduce HMG-CoA

reductase activity and EPL was similarly with FAs activity, we can conclude that the purslane and EPL supplements must retard tissue cholesterol biosynthesis by the same mechanism.

Conclusively, it could be concluded that purslane leaves meal and essential phospholipids (EPL) supplements inhibit cholesterol biosynthesis by the same mechanism.

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دور إضافة مسحوق ورق الرجلة الجاف والدهون الفسفورية الأساسية فى علف الدجاج البياض على تثبيط التخليق الحيوى للكولسترول

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أجريت هذه الدراسة بهدف تحديد آلية كل من مسحوق ورق الرجلة الجاف والدهون الفوسفورية الاساسية فى خفض مستوى الكولستيرول فى بيض ولحوم الدجاج وذلك بإستخدام ٧٢ دجاجة من سلالة انشاص البياضة قسمت عشوائيا الى ٤ مجموعات متماثلة موزعة على ٣ مكررات لكل منها ٦ دجاجات بياضة ، وربيت فى بطاريات فردية لكل مكررة ٦ دجاجات بياضة، وغذيت المجاميع الاربع ، إما على علف الاساسى المكون من الذرة وكسب فول الصويا أو العلف الاساسى مضافاً إليه إما ١٠% مسحوق ورق الرجلة الجاف أو ٤٥٠ ملجم دهون فوسفورية اساسية لكل ١ كجم علف أو الاثنين معا وذلك خلال الفترة من عمر ٢٨ الى ٤٠ اسبوع وتم تسجيل انتاج البيض والعلف المستهلك اسبوعيا ، وتم ايضا حقن الطيور بكرات الدم الحمراء للغنم فى الاسبوع قبل الأخير للتجربة لتقييم المناعة ، وفى الاسبوع الاخير للتجربة قيست صفات النكهة والطعم للبيض المسلوق ، ومستوى الكولسترول فى صفار البيض والكبد والسيرم وعضلات الصدر والفخذ بذبح ٤ طيور من كل مجموعة ، وكذلك تم قياس نشاط الانزيمات المحددة للتخليق الحيوى للكولسترول فى كبد تلك الطيور ، ويمكن تلخيص أهم النتائج كالاتى:

- تحسنت قيم كل من انتاج البيض وعدد البيض وكتلة البيض وكذلك الكفاءة التحويلية عند التغذية على عليقة المحتوية على ١٠% مسحوق ورق الرجلة الجاف بالمقارنة بالمعاملات الغذائية الاخرى.
- اضافة مسحوق ورق الرجلة الجاف والدهون الفوسفورية الاساسية وكذلك الاثنين معا فى غذاء دجاج انشاص البياض ادى الى خفض معنوى للدهون الكلية فى سيرم الدم بقيمة ٣.٠٥ ، ٦.٥٥ ، ٩.٤٠% وكولسترول السيرم بقيمة ١٦.٧٤ ، ٢٣.٢٥ ، ٢٩.٣٠% والجليسريدات الثلاثية فى سيرم الدم بقيمة ٦.٧٢، ٨.٩٣ ، ١٤.٥٥% ، بينما زادت

البروتينات عالية الكثافة فى السيرم بقيمة ٥.٣٥ ، ٦.٩٦ ، ٨.٤٨% على الترتيب كذلك زادت البروتينات منخفضة الكثافة بقيمة ٣.٠٦% فى العليقة المحتوية على ١٠% مسحوق ورق الرجلة الجاف بالمقارنة مع مجموعة الكنترول.

- لم يكن هناك أى تأثير معنوى على مستوى انزيمات الكبد ALT, AST فى سيرم الدم بالاضافة الى عدم وجود تأثير على صفات الطعم والنكهة فى البيض المسلوق وذلك فى الطيور المغذاة بعلف يحتوى على مسحوق الرجلة بدون أو مع الدهون الفوسفورية الاساسية.
- لوحظ زيادة الاستجابة المناعية (عدد كرات الدم البيضاء والليمفاوية فى الدم) بصفة خاصة بالنسبة للدجاج الذى تغذى على العلائق المحتوية على مسحوق الرجلة الجاف.
- كما لوحظ انخفاضا جوهريا أو معنويا لمستوى الكولسترول فى كل من عضلات الصدر والفخذ والكبد وصفار البيض وكذلك فى السيرم للطيور المغذاة بعلف يحتوى على مسحوق ورق الرجلة الجاف مع او بدون اضافة للدهون الفوسفورية الاساسية او كليهما معا.
- أدى اضافة مسحوق ورق الرجلة الجاف والدهون الفوسفورية الاساسية لانخفاض معنوى فى الوزن النسبى للكبد وقناة المبيض بالاضافة الى انخفاض النسبة المئوية لدهن البطن ، بينما ارتفع معنويا حجم الصفراء للحويصلة المرارية بالمقارنة بمجموعة الكنترول.
- أظهرت مجموعة الانزيمات المحددة للتخليق الحيوى للكولستيرول انخفاض فى نشاط انزيم 3-hydroxy-3-methylglutaryl CoA reductase فى الطيور المغذاة بعلف يحتوى على مسحوق ورق الرجلة وحدة أو مع الدهون الفوسفورية الاساسية – فى حين – لم يتأثر نشاط انزيم Fatty acid synthetase بنفس المعاملات الغذائية ، أما نشاط انزيم cholesterol-7-α-hydroxylase فقد انخفض بصورة ملحوظة أيضا عند التغذية على علف يحتوى على مسحوق الرجلة الجاف او الدهون الفوسفورية أو كليهما معا.

من هذه الدراسة يتضح أنه لما كان كل من مسحوق ورق الرجلة الجاف أو الدهون الفوسفورية الاساسية لهما نفس التأثير على الانزيمات المحددة للتخليق الحيوى للكولستيرول ، لذا فان كل منهما يخفض مستوى الكولستيرول فى بيض ولحم الدجاج بنفس الآلية.