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Olive cake in laying hen diets for modification of yolk lipids.

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ABSTRACT: 1. the possibility of improving egg yolk lipids of laying hens by olive cake (OC) feeding was investigated.
2. Forty-two, 54-week-old, Lohman laying hens were fed for 12 weeks on 3 diets formulated to contain 0 (control), 28.5 or 57g OC/kg, providing 0, 3.8 or 7.5g olive oil /kg diet, respectively.
3. Inclusion of OC in hen diets at 28.5 or 57g/kg decreased (P<o.oo1) plasma cholesterol and triglycerides concentrations compared to the control, without affecting plasma high-density lipoproteins.
4. Olive cake feeding at 28.5 or 57g/kg diet decreased (P<o.oo1) yolk concentrations of total lipids, triglycerides, cholesterol, low-density lipoproteins and Phospholipids.
5. Olive cake at 28.5 or 57g/kg diet decreased yolk concentration of total saturated fatty acids by 37.3 and 38.3%, respectively. Total monounsaturated fatty acids was decreased by 30.1% with OC feeding at 28.5g/kg of the diet, while it was increased by 17.3% with the 57g/kg dietary OC. Olive cake feeding at 28.5 or 57g/kg diet increased yolk concentrations of total polyunsaturated fatty acids (2.8 and 2.6 fold, respectively), n-6 polyunsaturated fatty acids (2.7 and 2.5 fold, respectively), and n-3 polyunsaturated fatty acids (3.1 and 3.0 fold, respectively), resulting in 10.4 and 13.1 %, decreases in the ratio of n-6:n-3 polyunsaturated fatty acids of egg yolk respectively, compared to the control.
6. It is concluded that that OC in concentration up to 57g/kg on diets of laying hen successfully to reduced cholesterol, lipids and improved fatty acids.

INTRODUCTION

Many people limit their consumption of eggs because they associated high cholesterol content with cardiovascular disease (Zeidler, 2002). Composition of fatty acids in the diet of laying hens (Shafey *et al.*, 1999) is known to influence fatty acid profile of the egg yolk. Lipids are synthesized in the liver of a laying hen and transported to the ovary by lipoproteins. Lipoproteins serve as precursors of egg yolk lipid, and plasma very low-density lipoproteins (VLDL) are the major components of egg yolk (Chapman, 1980). Cholesterol is largely synthesized in the liver and like lipids, transported to the growing follicles primarily in the VLDL (McDonald and Shafey, 1989).

The dietary fiber means that fraction of the edible part of plants are resistant to the digestion and absorption in the small intestine, usually with complete or partial fermentation in the large intestine; and reduction in blood cholesterol (Truswell, 1999). Hargis (1988) reported that dietary fiber affects cholesterol metabolism of laying hens by decreasing absorption of cholesterol, binding with bile salts in the intestinal tract, shortening intestinal transit time and increasing fecal sterol excretion.

Olive cake a by-product of olive oil industry- contains a high content of residual unextracted olive oil (rich of oleic, linoleic and linolenic fatty acids) along with high content of crude fiber (300-400g/kg Francisco *et al.*, 1989). Therefore, the objective of this study was to investigate the effects of dietary olive cake on plasma lipids, yolk lipids and fatty acids of laying hens.

MATERIALS AND METHODS

Forty-two, 54-week-old, Lohman laying hens were randomly allotted into three dietary treatment groups each of 14 hens (7 replicates of 2 hens). Diets were formulated to contain 0 (control), 28.5 or 57g OC/kg, providing 0, 3.8 or 7.5g olive oil /kg diet, respectively, (Table 1). All diets were iso-caloric and iso-nitrogenous, covering the nutritional requirements of laying hens (NRC, 1994). Diets, in mash form, and fresh water were supplied *ad libitum*. Birds were housed in cages (2hens/cage) at room temperature and were exposed to 18h light/d. The experiment lasted for 12 weeks. The diets were analyzed for proximate composition (AOAC, 1996). Lipid and fatty acid composition of olive cake were also analyzed (Tables 2 & 3, respectively).

At the end of the experiment, heparinzed blood samples were collected, via wing vein, from 4 hens/ treatment, chosen at random and plasma was separated by centrifugation and kept frozen at -20^{o}C until analysis for cholesterol (Stein, 1986), triglycerides (Scheletter and Nussel, 1975) and high density lipoprotein (HDL) cholesterol (Viikari and Scand, 1976).

Eggs were collected for chemical analysis during the last 3d of experimental period and then weight and cracked; thereafter, yolks were separated. Three samples of 4 pooled yolks for each treatment were freezed and stored at -20^{o}C before the chemical analyses were performed. Samples of pooled yolk and olive cake were analyzed for total lipids (Folch *et al*., 1957), total cholesterol (Shen *et al*., 1982), triglycerides (Lowell *et al*., 1973), high density lipoprotein (HDL) cholesterol (Eckel,1977), low density lipoprotein (LDL) cholesterol (Wieland and Seidel, 1983) and phospholipid (Kates, 1972, Kaur *et al*., 1973).

The lipid extract of pooled yolk samples were pooled in one sample/ treatment. Samples of pooled lipid extract and olive cake were methylated (Vogel, 1975), FAs were separated and identified using a Pye:Unicam gas chromatography (PU 4550) equipped with dual flame ionization detectors and dual channel recorder: The fractionation of fatty acids methyl esters was conducted using a coiled glass column (1.5m x 4mm) packed with polyethelene glycol adipate (PEGA) 10%). The fractionation condition for fatty acids was as those described by Farag *et al*., (1990). Peak identification for fatty acids was conducted by comparing the retention time with that of a standard of known composition. Peak areas were measured by normalization method and the relative proportions of the individual fatty acids were computed using ATI Unicam 4880 data station.

The effects of dietary treatments were examined using analysis of variance for completely randomized design experiments using SAS (1996), while differences among means were evaluated using Duncan's multiple range test (Duncan, 1955).

RESULTS

Inclusion of OC in hen diets at 28.5 or 57g/kg decreased (P<o.oo1) concentrations of plasma cholesterol (by 67.1 and 53.4%, respectively) and triglycerides (by 75.2 and 62.2%, respectively) compared to the control (Table 4). Plasma high-density lipoprotein was not affected by OC feeding.

Olive cake feeding at 28.5 or 57g/kg diet decreased (P<o.oo1) yolk concentrations of total lipids (45.5 and 14.3%, respectively), triglycerides (45.4 and 14.1%, respectively), cholesterol (45.4 and 15.0, % respectively), low-density lipoproteins (45.6 and 14.4%, respectively) and Phospholipids (45.9 and 15.7%, respectively); (Table 5).

Feeding OC at 28.5 or 57g/kg diet decreased concentration of total saturated fatty acids of egg yolk by 37.3 and 38.3%, respectively (Table 6), mainly 18.0 (55.4 and 64.6%, respectively). Total monounsaturated fatty acids was decreased by 30.1% with dietary OC at 28.5g/kg (mainly 16:1, 48.7%, with, 18:1 n-9 only 19.3%), while it was increased by 17.3% with dietary OC at 57g/kg diet (mainly 18:1, 82.8% but 16:1 was decreased about 100%). Olive cake feeding at 28.5 or 57g/kg diet increased yolk concentrations of total polyunsaturated fatty acids (2.8 and 2.6 fold, respectively), total n-6 polyunsaturated fatty acids (2.7 and 2.5 fold, respectively) (mainly 18:2 n-6, 4.7 and 4.3 folds, respectively, but,

20:4 n-6 only 33 and 51%, respectively), and total n-3 polyunsaturated fatty acids (3.1 and 3.0 fold, respectively), (mainly 22:6 n-3, 74.5 and 58.6 folds, respectively, but, 18:3 n-3 only 4.9 and 61.6%, respectively), resulting in decreases in the ratio of n-6:n-3 polyunsaturated fatty acids in egg yolk with 10.4 and 13.1 %, compared to the control.

DISCUSSION

These data show that diets containing olive cake reduced the levels of cholesterol and triglycerides, and had no effect on HDL concentration in laying hen plasma. The hypocholesterolemic effect of olive cake has been reported for other animals (El-Husseiny *et al.*, 1997). Shafey *et al.* (2003) observed that feeding laying hens a diet containing olive oil at 20g/kg increased plasma TG in the VLDL fraction and reduced ($P<0.01$) plasma TG concentration in the LDL plus HDL fraction. Taneja and Rakha (2005) suggested that it is not the low cholesterol content alone but also omega-3 fatty acids present in smart eggs that act synergically to prevent a substantial change in blood lipid profile and impose no serious risk to the health of the consumers. Also, decreases in blood cholesterol levels were reported for chicks fed pectin in their diets (Griminger and Fisher (1967) and hens fed a standard layer diet with added 15% cellulose (Menge *et al.*, 1974).

In this experiment, hens given 28.5 or 57g/kg olive cake showed a decrease in egg yolk lipids when compared with those of hens fed on control. The presence of unsaturated fatty acids increases cholesterol and phospholipids (P) synthesis, while saturated fatty acids had little effect (Hargis, 1988). Shafey *et al.* (2003) found that feeding laying hens a diet containing olive oil at 20g/kg did not affect egg yolk content of lipid and cholesterol. Turk and Barnett (1972) demonstrated that diets of 15% alfalfa, 15% oat hulls, 15% cellulose or 15% pectin lowered egg cholesterol when fed to laying hens. However, Menge *et al.* (1974) showed that yolk triglycerides were not influenced by the presence or absence of dietary cellulose in leghorn pullets' diets.

Fatty acid analysis indicated that decreased saturated fatty acids and increased mono and polyunsaturated fatty acids compared with the control. Leskanich and Noble (1997) reported that increasing levels of linoleic acid in hen diets from different vegetable fat sources resulted in increases in its concentration in yolk lipid. Aydin *et al.*, (2001) found that, olive oil decreased saturated fatty acids and, increased mono unsaturated fatty acids compared with the control. Feeding high dietary concentrations (100g/kg) of olive oil with CLA avoided the problem by restoring the yolk oleic acid concentration. Shafey *et al.* (2003) observed that feeding laying hens a diet containing olive oil at 20g/kg increased ($P<0.05$) yolk oleic acid and oleic : linoleic acid ratio.

Furthermore, there is a clear relationship between the proportions of PUFA in the dietary fat and the lipid yolk deposits. Herber and Van Elswyk (1996) found that supplementing diets of laying hens with marine algae as a source of n-3 fatty acids significantly increased yolk total n-3 fatty acids. Fish oil supplementation of conjugated linoleic acid diets did not reduce yolk firmness as it increased the yolk concentrations of long chain n-3, but decreased those of long chain n-6 polyunsaturated fatty acids (Alvarez *et al.*, 2004b). Moreover, Baucells *et al.* (2000) recorded that, Linseed oil is a rich source of linolenic acid which can be incorporated into the eggs of birds to which it is fed. Carrillo-Dominguez (2005) found that inclusion of red crab meal(RCM) in laying hen rations at levels of 3 and 6% increased the n-3 and n-6 fatty acids content in eggs, while the ratio of n-6 to n-3 FA was 3 times lower with 6% RCM than in the control.

It is concluded that inclusion of OC up to 57g/kg in laying hen diets had useful effects on decreasing plasma cholesterol, triglycerides and HDL, which is associated with production of better quality eggs characterized with great decreases in yolk concentrations of total lipids, cholesterol, LDL, triglycerides and phospholipids with a decrease in the concentration of saturated fatty acids in yolk lipids associated with great increases in concentrations of monounsaturated (n-9) and polyunsaturated (n-6 & n-3) fatty acids resulting

in decreasing the ratio of n-6 : n-3 fatty acids in egg yolk lipids.

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Table (1). Feed ingredients and chemical composition (g/kg) of the experimental diets.

OC g /kg diet	Control	28.5	57
Ingredient g/kg diet			
Yellow corn	570	541.5	513.0
Olive cake[1]	0.00	28.5	57.0
Soybean meal (44% CP)	180	180	180
Rice polish	100	100	100
Fish meal	50	50	50
Bone meal	21.5	21.5	21.5
Limestone	70	70	70
Vitamin-mineral Premix[2]	2.5	2.5	2.5
Sodium chloride	5.0	5.0	5.0
Methionin	1.0	1.0	1.0
Chemical composition g/kg			
Crude protein	176.1	175.3	174.6
Crude fiber	30	49.2	59.5
Ether extract	43.1	58.2	63.9
ME, MJ/kg	11.63	11.60	11.58

[1] 482 g/kg DM crude fiber, analytical value.

[2]Vitamin and mineral premix supplied per kg of diet: retinyl acetate 3.4mg, cholecalciferol 0.075mg , dl-alpha-tocopheryl acetate 10 mg, Vitamin K 2mg, Vitamin B_1 Img, Vitamin B_2 4 mg, Vitamin B_6 1.5 mg, Vitamin B_{12} 0.001 mg, Pantothenic acid 10 mg, Niacin 20 mg, Folic acid 1 mg, Biotin 0.05 mg, Choline Chloride 500 mg, Fe 30 mg, Mn 40 mg, Cu 3 mg, I 3 mg, Cobalt 0.2 mg, Zn 45 mg and Se 0.1 mg.

Table (2). Lipid composition of olive cake.

Item	
Total lipids g/100 g	13.23
Phospholipids g/100g	0.40
Triglyceride g/100 g	12.31
Cholesterol g/100 g	0.00
LDL mg/100g	0.00
HDL mg/100g	0.00

Table (3). Analyzed fatty acid of olive cake (g/kg of total fatty acids).

Fatty acid	
16: 0	94.1
16: 1cis/tr9	8.2
17: 1	0.4
18: 0	26.7
18:1cis9	643.3
18:1Isomere	26.3
18: 2cis9,12	124.0
18: 3cis9,12,15	5.8
20. 0	4.5
20: 1 n-9	3.7
22. 0	2.4
22: 1 n-9	1.3
22: 2 n-6	1.2
22: 3 n-3	4.5
24: 0	3.5
24: 1	7.4
TSFA[1]	131.2
TMUFA[2]	690.6
TPUFA[3]	135.5
n-9	5.0
n-6	1.2
n-3	4.5
n-6 : n-3	0.27

[1]Total saturated fatty acids
[2] Total monounsaturated fatty acids
[3] Total polyunsaturated fatty acids

Table (4) Effect of inclusion of olive cake in laying hen diets on cholesterol, triglycerides and HDL concentration of blood plasma[1]

OC g/kg diet Item	Control	28.5	57
Cholesterol , mg/dl **			
Mean	129.3[a]	42.50[b]	60.25[b]
SE	±11.95	±10.78	±4.25
Triglycerides , mg/d l.**	762.0[a]	189.5[c]	288.0[b]
	±12.43	±27.38	±39.62
HDL, mg/dl *	67.00	61.25	57.50
	±4.49	±3.35	±6.38

[1 a, b, c] Means in the same row with different letters are significantly different *P > 0.05 or.**p<0.001
Means ± SEM; n = 3
Means ± SEM; for 3 samples

Table (5) Effect of inclusion of olive cake in laying hen diets on total lipids, triglycerides, Phospholipids, cholesterol, LDL, and HDL of egg yolk (g/100gyolk).

OC g/kg diet Item	Control	28.5	57
Total lipids, g	15.00[a] ±0.58	8.18[c] ±0.44	12.86[b] ±0.18
Triglyceride, g	9.75 [a] ±0.38	5.32[c] ±0.28	8.37[b] ±0.12
Cholesterol, g	1.35[a] ±0.05	0.74[c] ±0.04	1.15[b] ±0.02
LDL, mg	0.180[a] ±0.006	0.098[c] ±0.005	0.154[b] ±0.002
Phospholipids, g	3.03[a] ±0.10	1.64[c] ±0.09	2.56[b] ±0.04

[a, b, c] Means in the same row with different letters are significantly different ($P < 0.001$).

Table (6) Effect of inclusion of olive cake in laying hen diets on fatty acid of egg yolk (g/kg of total fatty acids).

OC g/kg diet Item	• Control	28.5	57
14:0	13.0	9.8	9.0
16:0	323	254	276
16:1	80.7	41.4	-
18:0	294	131	104
18:1 n-9	145	117	265
18:2 n-6	33.7	193	177
18:3 n-3	24.5	25.7	39.6
20:4 n-6	30.0	39.9	45.3
22:6 n-3	-	74.5	58.6
TSFA[1]	630	395	389
TMUFA[2]	226	158	265
TPUFA[3]	88.2	333	321
n-6	63.7	233	222
n-3	24.5	100	98.2
n-6 : n-3	2.60	2.33	2.26

[1]Total saturated fatty acids
[2] Total monounsaturated fatty acids
[3] Total polyunsaturated fatty acids