Research Article

Biochemical Effect of Olive Cake as Feed Additive on Antioxidants and Molecular Expression of FAS, ANS, ACC in Laying Hens

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Abstract | This research evaluates the biochemical effect of adding 7% olive cake as feed additives on lipid profile, fatty acids, antioxidants concentrations in serum and/or egg yolk and on gene expression of fatty acid synthase and acetyl CoA carboxylase in laying hens. The experiment was applied on 42 Commercial Mandarah strain laying hens which divided equally into control and olive cake (7%) group. Addition of olive cake (7%) led to a significant increase in serum and egg yolk HDL-C and a significant decrease in TAG, cholesterol, LDL-C and VLDL concentrations. SOD, GSH and TAC concentrations were significantly increased in serum and egg yolk in olive cake group, while MDA and NO were significantly decreased. Hens fed 7% olive cake recorded a significant increase in the concentration of oleic, linoleic and linolenic with a significant decrease in stearic in egg yolk. In breast muscle the concentration of oleic acid was significantly increased while saturated concentrations fatty acids were significantly decreased in olive cake group. Gene expression of FAS and ACC was significantly increased in the olive cake group.

Keywords | Olive cake, FAS, ACC, Antioxidants, Fatty acids, Egg yolk

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INTRODUCTION

The industrialization of poultry production and the need for the improvement of nutritional efficiency have accelerated the use of feed additives and growth promoter in animal feeding to increase production while maintaining animals in good health and diseases prevention. Vegetable oils were recommended to be a part of a healthy diet due to their high contents of fatty acids (Mæhre et al., 2014). The distribution and content of fatty acids in vegetable oils differ according to plant sources and technology process used for their production. The use of agro-industrial by-products as alternative feed resources for feeding livestock (Al-Harthi et al., 2011) may be a feasible strategy to reduce feeding cost of nutrition which accounting for up to 70% of total production cost, save traditional feed sources such as grains for human consumption and positively influence the quality of the milk, egg and meat especially in the case of by-products that contain vegetable oils which may contribute to improve the fatty-acid composition and the antioxidant content of meat (Vasta and Luciano, 2011) and also decrease waste management cost (Al-Saffar et al., 2012). Inclusion of olive by-products as animal feed is a good way of recycling these waste products (Sadeghi et al., 2009) but there is a need to formulate optimized rations for different animal uses to avoid metabolic disorders caused by the unbalanced ratio of energy and protein and to limit the tasty factors which might reduce feed intake and then the animal performance. The classic production of olive oil generates olive oil (20%), solid waste and aqueous liquor (50%). The solid waste olive oil cake (OOC) is a combination of olive pulp and stones (Niaounakis and Halvadakis, 2006). The olive oil industry generates large amounts of by-products (olive cake, olive leave, olive molasses and olive pulp) that are harmful to the environment. Annually, important quantities of olive

residue are produced, which are abundant, renewable, low cost raw materials (Jeanne et al., 2013). Utilization of olive by products in animal nutrition can enrich animal products with unsaturated fatty acids and improve animal product quality and enhance the nutritional value of animal products for human consumption (Al-Harthi, 2015). Therefore, the present study was conducted to examine the effects of olive seed meal (olive cake) on lipid profile and antioxidants content of serum and/ or egg yolk of laying hens in addition to its effect on saturated and unsaturated fatty acids concentrations in egg yolk and breast muscle. Gene expression of FAS and ACC which involved in lipid metabolism was also evaluated.

MATERIALS AND METHODS

Diet

The experimental diets were formulated to meet the nutrient requirements for layer hens according to the recommendations of the Commercial Management Guide and were analyzed for proximate composition (AOAC, 2002).

Olive cake preparation

The residual material including pulp and stones after oil extraction by screw press was brought from Al-Arish Governorate, olive squwisser. It transported to the integrated poultry project-Fayoum Governorate then it was distributed on the floor of a big room which had 4 big fans for pulling warm air from outside and pushing it again to outside as ventilation cycle. Olive cake was continuously stirred until completely dried, then stored in bags until used in diet formulation. It was included in diet in a concentration of 7% (Al-Harthi, 2015). The ingredients and chemical composition of diets used in the experiment were recorded in (Tables 1, 2).

Table 1: Chemical composition of the different experimental diets (%).

Chemical composition	Control	Olive cake (7%)
Protein	15.5	13.3
Fat	6.13	3.9
Moisture %	6.2	5.95
Ash	12.14	9.9
Fiber	2.96	5
Carbohydrate	50.1	61.95
Total energy	3440	3410

Laying hens groups and experimental design

The experiment was carried out in El-Fayoum Governorate, in the integrated poultry project. Total 42 commercial Mandarah strain laying hens of 31 weeks old with uniform body weight were assigned to two equal groups with 21 hens per group which divided into 3 replicates. The control group which maintained on standard normal diet and the olive cake group which maintained on standard normal diet supplemented with 7% olive cake along the all period of experiment (12 weeks). The experiment was carried out between 31 and 43 weeks of age (12 weeks experimental period). Hens were housed in cages equipped with trough feeders and nipple drinkers. Feed and water were provided ad-libitum throughout the experimental period. Laying hens were illuminated with 14:10 light dark cycle. The hens were maintained under standard conditions in a farm as per the guidelines of Institutional animal care and use Committee, Beni Suef University for the Purpose of Control and Supervision on Experiments on Animal under the following approval number (021-163).

Table 2: Fatty acids concentrations of olive cake.

Fatty acid	Olive cake (g / 100 g)
Myristic (C 14:0)	0.054
Palmitic(C 16:0)	12.2
Stearic (C 18:0)	1.3
Oleic (C 18:1 n9)	58
Linoleic (C 18:2 n6)	9.1
Linolenic (C 18:3 n3)	0.75
Arachidic (C 20:0)	0.46

Sampling

Blood sample

At the end of the experiment, blood samples were collected via wing vein puncture and serum was separated by centrifugation at 1300 g and divided into several aliquots. Some were kept frozen at -20°C until analysis of lipid profile and antioxidants and others were kept at -80°C for analysis of gene expression of FAS and ACC.

Egg sample

Eggs were collected for biochemical analysis during the last 3 days of experimental period. Egg yolks were separated and divided into two parts. 10 samples of the pooled yolks for each group were freezed and stored at -20°C until analysis of lipid profile and antioxidants contents. Another 10 pooled yolks/each group were used for lipid extraction for fatty acid analysis according to method described by (Farag et al., 1990).

Tissue sample

At the end of the experiment, the birds were slaughtered and dissected to separate breast muscle (pectoral superficial muscle). Total lipids were extracted according to the most commonly used procedure for lipid extraction from animal tissues (Folch et al., 1957) then used for fatty acids analysis.

Biochemical measurements

Serum and yolk samples were analyzed for cholesterol,

triacylglycerol, HDL-C, LDL-C and VLDL concentrations according to methods described by (Lee et al., 2008; Mendez et al., 1986) respectively. Total Antioxidant Capacity, MDA, GSH, No and SOD concentrations of serum and egg yolk were measured according to (Koracevic et al., 2001; Kei, 1978; Beutler et al., 1963; Montgomery and Dymock, 1961; Nishikimi et al., 1972) respectively. All chemical reactions were measured by using of Hitachi spectrophotometry, Model U-2000 (Hitachi Ltd. Tokyo, Japan) and by using of commercial kits which purchased from Biodiagnostics Company, Cairo, Egypt. Fatty acid profiles of egg yolk and breast muscle were analyzed by gas chromatography-mass spectrometry (GC-MS) (Saleh et al., 2012).

Quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis

The mRNA expression of FAS (Fatty acid synthase) and ACC (Acetyl CoA carboxylase) genes were determined in serum of laying hens in different groups by SYBR Green real time PCR according to the manufacturer's protocol (Bustin, 2002). The primer pairs used are shown in Table 9. The housekeeping gene β-actin was utilized as an internal control.

Amplification curves and CT values were determined by the strata gene MX3005P software. To estimate the variation of gene expression on the RNA of the different samples, the CT of each sample was compared with that of the control group according to the "ΔΔCt" method stated by (Yuan et al., 2006).

Statistical analysis

All data were expressed as means ± SEM. Differences between the groups were statically determined by one-way ANOVA followed by Tukey's multiple comparison test using SPSS software version 15.0 and the results were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

Utilization of olive by-products in animal nutrition can enrich animal products with unsaturated fatty acids and improve its quality and nutritional value for human consumption (Al-Harthi, 2015). The classic production of olive oil generates three phases and two wastes which are olive oil (20 %), solid waste and aqueous liquor (50 %). The solid waste olive oil cake (OOC) is a combination of olive pulp and stones (Niaounakis and Halvadakis, 2006). The three-phase extraction with a total or partial removal of seeds allows to obtain an olive cake containing low levels of lignin and remarkable levels of residual oil (Tables 1, 2) and antioxidants, such as tocopherols, retinol and bioactive phenols such as secoiridoids and lignans (Servili et al., 2011).

Polyphenols are bioactive molecules commonly used as antioxidants and antimicrobials in the food industry (Goula and Lazarides, 2015). Major phenolic compounds contained in olive cake are hydroxytyrosol, tyrosol, Dialdehydic form of decarboxymethyl elenolic acid, Verbascoside (Servili et al., 2015), Caffeic acid, p-coumaric acid, vanillic acid, Lutein, acetoxypinoresinol and pinoresinol (Roila et al., 2016). In our experiment using of 7% olive cake as feed additives in laying hens for a period of 12 weeks resulted in a significant improvement of serum and egg yolk lipid profile (Tables 3, 4), this agreed with (Abd El-Samee and Hashish, 2011; Abd El-Galil et al., 2017) who recorded a significant decreased in serum lipid profile with production of better quality eggs characterized with great decreases in yolk concentrations of total lipids, cholesterol, LDL-C and triglycerides as a result of olive cake feeding in laying hens.

Table 3: Effect of olive cake on serum cholesterol, TAG, HDL-C, LDL-C and VLDL concentrations of laying hens in control and olive cake groups.

	Cholesterol (mg/dl)	TAG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL (mg/dl)
Control group	177 ± 3.8[a]	105± 2.9[a]	32.7 ± 1.5[a]	117.8±2.1[a]	21 ± 0.6[a]
Olive cake group	161 ± 0.6[b]	85 ± 0.6[b]	63.7 ± 0.9[b]	82 ± 0.9[b]	18 ± 0.6[b]

Values are represented as mean ± standard error. The different superscript letters mean a significant difference at ($P < 0.05$) between different groups.

Table 4: Effect of olive cake on egg yolk cholesterol, TAG, HDL-C, LDL-C and VLDL concentrations of laying hens in control and olive cake groups.

	Cholesterol (mg/dl)	TAG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL (mg/dl)
Control group	281 ± 4.4 [a]	61 ± 3.1 [a]	58 ± 0.6 [a]	222 ± 6.9 [a]	11.8 ± 0.7 [a]
Olive cake group	228 ± 2.6 [b]	48 ± 1.2 [b]	69 ± 1.8 [b]	150 ± 2.7 [b]	9.6 ± 0.2 [b]

Values are represented as mean ± standard error. The different superscript letters mean a significant difference at ($P< 0.05$) between different groups.

Table 5: Effect of olive cake on serum antioxidants of laying hens in control and olive cake groups.

	MDA (nmol / ml)	NO (µmol / L)	SOD (U/ml)	GSH (mmol/L)	TAC (mM / L)
Control group	12.2 ± 0.8 [a]	6.4 ± 0.2 [a]	2.9 ± 0.2 [a]	24.6 ± 1.6 [a]	731.8 ± 7.1 [a]
Olive cake group	7.4 ± 0.4 [b]	4.5 ± 0.3 [b]	6.5 ± 0.6 [b]	35.8 ± 1.2 [b]	892.8±10.4 [b]

Values are represented as mean ± standard error. The different superscript letters mean a significant difference at (P < 0.05) between different groups.

Table 6: Effect of olive cake on egg yolk antioxidants of laying hens in control and olive cake groups.

	MDA (nmol/g.tissue)	NO (µmol/g.tissue)	SOD (U/g.tissue)	GSH (mmol/g.tisse)	TAC (mM/g.tissue)
Control group	4.6 ± 0.2 [a]	1.9 ± 0.05 [a]	1.5 ± 0.06 [a]	8.1 ± 0.5 [a]	114 ± 4.3 [a]
Olive cake group	2.4 ± 0.1 [b]	0.4 ± 0.04 [b]	3.7 ± 0.2 [b]	21.1 ± 0.9 [b]	294 ± 4.1 [b]

Values are represented as mean ± standard error. The different superscript letters mean a significant difference at (P < 0.05) between different groups.

The hypolipidemic and hypocholesterolemic effect of olive cake may be attributed to several factors such as its crude fiber level as fiber play a role in cholesterol metabolism through its ability to decrease absorption of cholesterol, bind with bile salts in the intestinal tract, shorten the intestinal transit time and increase fecal sterol excretion (Boka et al., 2014), also the residual un extracted olive oil which present in the cake has a high proportions of useful components such as omega groups that also decreased egg yolk lipids (Abd El-Samee and Hashish, 2011). It is also due to its polyunsaturated fatty acids content (El-Hachemi et al., 2007; Dal Bosco et al., 2012) mainly linoleic and linolenic acids (Cayan and Erener, 2015) that reduced the plasma concentration of cholesterol and MUFA such as oleic acid which reduced triglyceride concentration in blood. Olive cake phenolic compounds as flavonoids and tannins have hypocholesterolaemic action (Obied et al., 2005).

Poultry in intensive farming systems are frequently exposed to oxidative stress which can result in damage of the body proteins, lipids and DNA and can lead to reduce performance and health (Lykkesfeldt and Svendsen, 2007). Oxidative stress defense depends on an orchestrated synergism between the exogenous and endogenous antioxidant. The stability of living organism must be maintained by its balance between oxidative and antioxidant defense (Zaidi et al., 2019). Antioxidant enzymes, as well as, non-enzymatic antioxidants are first line of defense against ROS, inducing oxidative damage, in a living organism (Al-Shiekh et al., 2014). MDA is one of free radicals and the most important product of lipid peroxidation therefore frequently used to determine oxidative damage (Jensen et al., 1997). SOD and GST are the major scavenging enzymes that remove toxic free radicals *in vivo* (Yang et al., 2011). Our results recorded a significant increase in the antioxidant parameters (TAC, GSH and SOD) and a significant decrease in oxidative parameters (MDA and NO) in serum and egg yolk of laying hens as a result of addition of 7% olive cake in diet for 12 weeks (Tables 5, 6). The antioxidant effect of olive cake is due to its phenols content (98%) (Suárez et al., 2010). Olive phenolic compounds are recognized, as potentially bioactive and may have antioxidant, anti-cancer, anti-viral, anti-inflammatory, hypolipidemic and hypoglycemic effects (Obied et al., 2005). In particular, hydroxytyrosol the most abundant phenols in olive cake (Araújo et al., 2015) which has the highest number of hydroxyl groups, so it possesses the highest antioxidant and radical scavenger activity (Lee and Lee, 2010; Hayes et al., 2011) and act as a hydrogen donor to inhibit oxidation (Servili et al., 2014). Nevertheless, the presence of tyrosol and hydroxytyrosol sulphate may play a role in the antioxidant capacity of olive cake (Rezar et al., 2015). The source and amount of fatty acids in diet markedly modified the fatty acids composition of egg yolk (Grobas et al., 2001). Inclusion of olive cake in laying hen diet caused a significant decrease in the concentration of saturated fatty acids in yolk lipids associated with great increases in concentrations of monounsaturated especially oleic acid and polyunsaturated (n-6 and n-3) fatty acids (Güçlü et al., 2008). Same results reported by (Zhang et al., 2013; Zhang and Kim, 2014). That agreed with our results (Table 7) as feeding of 7% olive cake for 12 weeks for laying hens resulted in a significant decrease in stearic acid level and a significant increase in oleic, linoleic and linolenic levels in egg yolk. Previous studies (Tereza et al., 2010) reported an increase in oleic acid concentration in breast and thigh muscle of broiler fed olive oil, and these results are in line with our results (Table 8) as saturated fatty acids concentrations (myristic, stearic and palmitic) were significantly decreased in breast muscle in olive cake group and oleic concentration was significantly increased. Unsaturated fatty acids content was improved when higher levels of vegetable oils are included in the diet, which inhibit the delta-9 desaturase enzyme system which is responsible for saturated fatty acids desaturation, thereby converting them into USFA (Chamruspollert and Shell, 1999).

Table 7: Effect of olive cake on fatty acids concentration (g/100gm of total fatty acids) in egg yolk of laying hens in control and olive cake groups.

	Control	Olive cake group
C 14:0 (Myristic)	0.22 ± 0.01 [a]	0.12 ± 0.02 [a]
C 16:0 (Palmitic)	20.6 ± 0.45 [a]	20.2 ± 0.3 [a]
C 18:0 (Stearic)	7.00 ± 0.29 [a]	6.7 ± 0.21 [b]
C 18:1 n-9 (Oleic)	39.33 ± 0.5 [a]	43.5 ± 0.4 [b]
C 18:2 n-6 (Linoleic)	10.41 ± 0.82 [a]	12.48 ± 0.12 [b]
C20 18:3 n-3 (Linolenic)	0.55 ± 0.11 [a]	0.83 ± 0.17 [b]
C 20:0 (Arachidic)	0.2 ± 0.05 [a]	0.2 ± 0.01 [a]

Values are represented as mean ± standard error. The different superscript letters mean a significant difference at ($P < 0.05$) between different groups.

Table 8: Effect of olive cake on fatty acids concentration (g/100gm of total fatty acids) in breast muscle of laying hens in control and olive cake groups.

	Control	Olive cake group
C 14:0 (Myristic)	0.45 ± 0.11 [a]	0.42 ± 0.01 [b]
C 16:0 (Palmitic)	21.3 ± 0.34 [a]	20.8 ± 0.3 [b]
C 18:0 (Stearic)	5.23 ± 0.2 [a]	5.0 ± 0.2 [b]
C 18:1 n-9 (Oleic)	26.1 ± 0.7 [a]	33.2 ± 0.3 [b]
C 18:2 n-6 (Linoleic)	12.3 ± 0.22 [a]	12.0 ± 0.2 [a]
C 18:3 n-3 (Linolenic)	1.07 ± 0.2 [a]	1.05 ± 0.2 [a]
C 20:0 (Arachidic)	0.3 ± 0.03 [a]	0.2 ± 0.01 [b]

Values are represented as mean ± standard error. The different superscript letters mean a significant difference at ($P < 0.05$) between different groups.

Besides its role as an energy source and its effect on membrane lipid composition, dietary fat has remarkable influence on gene expression (Clarke et al., 2002). Liver is the vital organ for lipogenesis in avian species, where 95% of de novo fatty acids synthesis occurs (Griffin et al., 1992). Its capacity for de novo synthesis is also related to the total amount of lipogenic enzymes which in turn is reliant on the levels of messenger RNA which codify these enzymes (Berdanier and Hargrove, 1993). Among the lipogenic enzymes are fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC). Fatty acid acyl synthase is an enzyme complex responsible for acid synthesis with palmitic acid as its final product. Its gene expression is important to observe lipogenesis and the rate of fatty acids synthesis (Sinnett-Smith and Waddington, 1992). ACC catalyzes the irreversible conversion of acetyl-CoA to malonyl-CoA, and plays an essential role in the regulation of fatty acid synthesis and degradation in response to the energy status of the body (Torró-Montell et al., 2019). It has been speculated that in the laying hens the de novo lipogenesis in the liver is genetically high in order to generate yolk (Cheng et al., 2004). That matches our results recorded in (Tables 9, 10) gene expression of FAS and ACC were significantly up regulated in relation to the significant increase in egg number in olive cake group. Olive oil exerts its healthy effects through its components of MUFA and phenolic compounds. In olive oil treated animals a several effects on liver have been described including the increase in the activities of ACC and FAS (Takeuchi et al., 2001).

Table 9: Primers used for quantitative real-time PCR analysis.

Gene	Primer sequence (5'-3')	Reference
ß. Actin	CCACCGCAAATGCTTCTAAAC AAGACTGCTGCTGACACCTTC	Yuan *et al.*, 2006
FAS	AATGGCAGCTTTGGAGGTGT TCTGTTTGGGTGGGAGGTG	Zhou *et al.*, 2016
Acc	CTATCGACACAGCCTGCTCCT CAGAATGTTGACCCCTCCTACC	Zhou *et al.*, 2016

The β-actin gene was utilized as an internal control and was chosen as a reference gene because it is a housekeeping gene.

Table 10: Effect of olive cake on FAS (Fatty acid synthase) and ACC (Acetyl CoA carboxylase) genes expression in serum of laying hens in control and olive cake groups.

	ß. Actin control	FAS		ACC	
Groups	CT	CT	Fold change	CT	Fold change
Control	20.51	22.62	-	21.90	-
Olive cake group	20.87	24.52	0.3439	23.56	0.4061

Values are represented as mean ± standard error. The different superscript letters mean a significant difference at ($P < 0.05$) between different groups.

Results concerning the economic efficiency (Table 11) indicated that the lowest feed cost was recorded in olive cake group as compared to control group which agreed with (Abd El-Galil et al., 2017) as these ingredients are available locally at relatively low prices. Alternative feed ingredients may offer more options for poultry nutritionists to formulate diets and to reduce the cost of poultry production.

Table 11: The effect of feeding different dietary sources during laying period on economic efficiency of egg production from 32-44 weeks of age.

	Control	Olive cake group
Feed consumed/hen(kg)	10.080	10.080
Feed consumed/hen(LE)	70.56	66.11
Egg number /hen(n)	16.6	18.6
Egg produced/hen(LE)	24.9	27.9
Economicefficiency %	35.3	42.2
Egg-feed price ratio	1.6	1.8

Price of one Kg of diet (LE) for different ingredients and price

of eggs (LE) calculated at time of experiment.

CONCLUSIONS AND RECOMMENDATIONS

Our study concluded that using of new and low price local ingredient as olive cake in feeding of laying hens has a beneficial effect on serum and egg yolk lipid profile, fatty acid and antioxidant contents. It also has an effect of lipogenic enzymes as FAS and ACC.

NOVELTY STATEMENT

The novelty of our study is improving the lipid metabolism and antioxidant status in laying hens which will reflect on egg and meat quality and consumers health by the reuse of available, economic and natural antioxidants like olive cake.

AUTHOR'S CONTRIBUTION

Mahmoud Mohamed Arafa and Sahar Omar Mohamed had the original idea for the study and carried out the design. Mohamed Ahmed Kandiel and Omayma A. R. Abozaid participated in study design. Ghada Mohamed Safwat responsible for data analysis, interpreted the data and writing the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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