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Original Research

The effect of adding different levels of Evening Primrose Oil (EPO) to laying hens diet on quality traits, fatty acids content, cholesterol and lipid oxidation of the egg yolk

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ABSTRACT:

A research was conducted to evaluate the effect of feeding different levels of Evening Primrose Oil (EPO) on the quality of traits, fatty acids content, cholesterol and the oxidation status indicators of the egg after storage for 14 days. Two hundred forty, thirty one-week-old Isa Brown hens were divided into four treatments (each treatment had three replicates, 20 birds per replicate), fed for 20 weeks divided into five periods included: period 1 (31-34 weeks), period 2 (35-38 weeks), period 3 (39-42 weeks), period 4 (43-46 weeks), and period 5 (47-50 weeks). The treatments included were: T_1 (control) – basal diet without supplementation, T_2 basal diet + 25% of EPO, T_3 basal diet + 50% of EPO, T_4 basal diet + 75% of EPO. The results of this study showed a significant ($P<0.05$) improvement in each of the relative weight of yolk and yolk index in T_2, T_3, and T_4 and for all periods compared to T_1. Furthermore, a significant improvement in the content of egg yolk in proportion of unsaturated fatty acids (linoleic acid and gamma linolenic acid was seen). An increase in gamma linolenic acid with increasing levels of EPO was noted in T_2, T_3, T_4 compared to T_1. Significantly, the proportion of egg cholesterol showed a decrease in T_4 when the addition of EPO was 75%. After 14 days of storage, lipid oxidation indicators which consist of peroxide (PV), Malondialdehyde (MDA), and Free Fatty Acids (FFA) were significantly decreased in T_2, T_3, T_4 compared to T_1. It can be concluded that addition of EPO to laying hen diets improved the unsaturated fatty acid especially linoleic and gamma linolenic acids and enhanced egg quality.

Keywords:

Evening primrose oil, Fatty acids content, Egg yolk, Lipid oxidation.

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INTRODUCTION

Several studies have taken advantage of the synthetic formulation of plant oils in poultry diets, plant oils have used due to their active content such as olive oil, corn oil, soybeans, sunflower and others. Recent studies have focused on the use of therapeutic oils derived from herbaceous plants as feed additives in poultry diets because they have effective ingredients which have contributed to the improvement of the productive and physiological performance of poultry, including flaxseed oil, grape oil and others (Robert *et al*., 2016; Bander, 2017). Another type of therapeutic oil is called Evening Primrose Oil (EPO). This oil is extracted from herbaceous plants belonging to the Oenagracea family and the scientific name of the plant *Oenothera biennis* has several names including the evening star, evening primrose, and *Acacia*. The EPO oil is composed of omega-6 oils and consisted of a group of saturated and unsaturated fatty acids. Saturated fatty acids included palmitic acid (5.5-8%), stearic acid (3%) whereas the unsaturated fatty acids consisted of linoleic acid is (60-80 %) and oleic acid (6-18%) in addition to the unique advantage of EPO oil from the rest of the omica-6 oils, the presence of linoleic acid in the form of Gama-linolenic acid (9-15%) (Kies *et al*., 1989; li, 2005).

Essential fatty acids have unsaturated omega bongs that is important for humans and animals to maintain the natural and functional formation of cellular membranes and enzyme activity (March and MacMillan, 1990; Ibeas and Izquierdo, 1994). Additionally, Producing prostoglands similar to hormones which mediate physiological processes including metabolism (Stillwell *et al*., 2005). Furthermore, the presence of gamma linolenic formula in EPO will ensure the necessary steps in linoleic acid metabolism. In some cases (including stress, age, disease and lack of vitamin B6, zinc and magnesium) a decrease occurs in the rate of activity (Ie, EPO) provides an opportunity to overcome the deficiencies in the enzyme levels to ensure continuity of subsequent steps leading to the manufacture of arachidonic acid and prostoglands (Favati *et al*., 1991; Hathaway, 1999). Studies by Hagerman *et al*. (1998) Moure *et al*. (2001) Wattasinghe and Shahidi (2002) and Zahradnikov *et al*. (2007) found that seeds of the spring primrose plant contain biologically active ingredients that are important in their anti-oxidant role and the free radicals and active forms of active oxygen, hydrogen peroxide, hydroxyl and mono-oxygen, tannins, flavonoids and terpenoids. The EPO oil has also been used in the treatment of many human ailments including skin infections, diabetes, blood pressure, heart, cholesterol reduction, respiratory inflammatory disorders, arthritis and intestinal inflammation (Brzeski *et al*., 1991; Keen *et al*., 1993; Schilcher, 1996; Brown, 1997).

Recent trends in the feeding of domestic poultry and adding of certain percentages of therapeutic essential oils to the poultry diets in addition to improving the health and immune status of birds, they are used to produce high quality chicken meat by improving the quality and ratio of the fatty acids found in them, as well as to produce eggs rich in fatty acids *viz*., omega 3 and omega 6 and to reduce the levels of egg yolk cholesterol as an aid to prevent consumers from diseases (McNaughton *et al*., 1978; Trziszka, 2000 and Liu *et al*., 2010). The production of poultry products classified in the so-called functional foods, which can provide a health benefit beyond the benefit of the traditional nutrients that it contains. The aim of the present study is to investigate the effect of adding different levels of EPO to egg laying chicken diets on the quality of egg yolks, cholesterol level, quality of yolk fatty acids and the ability to maintain egg quality after storage.

Protein concentrate (Laycon-s special) product from Al-Wafi, Netherland with 2125 kcal metabolic energy, 40% crude protein, 5% crude fat, 5% calcium, 2% phosphorous, 80.3% lysine, 85.2% methionine,

Table 1. Chemical composition and diet content of the experiment

S. No	Feed composition	Treatments (%)			
		T_1	T_2	T_3	T_4
1	Corn	55.8	55.55	55.55	55.2
2	Wheat	11.7	11.7	11.40	11.25
3	*EPO (Evening Primrose Oil)	0	0.25	0.50	0.75
4	Soybean meal (48% protein)	18.5	18.5	18.55	18.8
5	Protein concentration	5.0	5.0	5.0	5.0
6	Dicalcium phosphate	1.0	1.0	1.0	1.0
7	Limestone	7.7	7.7	7.7	7.7
8	Salt	0.3	0.3	0.3	0.3
9	Total	100	100	100	100
10					
11	**Chemical composition**	**T_1**	**T_2**	**T_3**	**T_4**
12	Calories (kcal/kg)	2792	2803	2814	2823
13	Crude protein (%)	17	16.9	16.9	17
14	Lysine (%)	0.92	0.92	0.92	0.92
15	Methionine +Cysteine (%)	0.68	0.68	0.68	0.68
16	Linoleic acid	1.29	3.48	1.66	1.83
17	Calcium (%)	3.48	3.48	3.48	3.48
18	Phosphorus (%)	0.38	0.38	0.38	0.38

29.3% methionine + cysteine, 2.2% sodium and 500 mL/kg vitamin E was used in this study. The chemical composition for the diet content was according to NRC (1994).

MATERIAL AND METHODS

This study was conducted at the poultry research station in the department of Livestock Research, department of Agricultural Research, Ministry of Agriculture, Baghdad, Abu Ghraib, from 13th of February 2017 to the 2nd of July 2017 for 20 weeks divided into five stages (each stage 4 weeks). 240 ISA brown layer hen were randomly allotted to four treatments (each treatment 60 birds, three replicates, and 20 birds per replicate) and distributed into wire cages with 12 pins, (per pin was at 3*2 meter length*width) and per pin represent one replicate. Birds were fed with the experimental diets *ad libitum* with free water access. The treatments were: T_1 (control) – basal diet without supplementation; T_2 basal diet + 25% of EPO; T_3 basal diet + 50% of EPO; T_4 basal diet + 75% of EPO. The chemical analysis were done following the guide of Qingdao Yuda Company in the EPO content of fatty acids: palmitic acid 6%, stearic acid 1.8%, oleic acid 6.3%, linoleic acid 72.9%, and gamma linolenic acid 10.2%. Chickens were fed with the different diets (Table 1). Egg yolk cholesterol was measured by taking three eggs of each replicate. The method of Franey and Elias (1968), is followed based on the interaction of cholesterol with ferric chloride and concentrated sulfuric acid, which produces a pink color that was measured using a spectrometer. A sample of 0.1 g of fresh egg yolk was taken and 1.9 mL of ethyl alcohol (95%) was added. After shaking and centrifuging at 3000 rpm for 10 min, 0.5 mL of upper layer and 2 mL of dissolved ferric chloride to ethyl acetate (by mixing 0.1 mL of ferric chloride with 100 mL of ethyl acetate) was added. After that, 2 mL of concentrated sulfuric acid was added and the ingredients were mixed well by shaking the tube and waiting for 10 min until the mixture cools because the reaction leads to high heat production. The samples were read on the spectral scale and at a wavelength of 560 nm. Prior to that, the device was reset using a reagent solution that took the same action steps for the

Table 2. The effect of feeding different levels of Evening Primrose Oil (EPO) on the relative weight of egg yolk of Classic Isa Brown laying hens

S. No	Average relative weight of egg yolk					
	Periods/Weeks					
	1 31-34	2 35-38	3 39-42	4 43-46	5 47-50	Total Period
1	24.81±0.09[b]	24.91±0.12[c]	25.67±0.05[c]	25.66±0.02d	25.86±0.03[b]	25.38±0.02[d]
2	25.65±0.04[a]	25.59±0.03[b]	25.95±0.07[b]	26.06±0.05[c]	26.67±0.18[a]	25.96±0.04$_c$
3	25.77±0.01[a]	26.08±0.03[a]	26.24±0.09[a]	26.56±0.08[a]	26.90±0.07[a]	26.30±0.00[a]
4	25.64±0.10[a]	26.01±0.02[a]	26.27±0.08[a]	26.29±0.07[b]	26.96±0.02[a]	26.17±0.03$_b$
5	*	*	*	*	*	*

[abc] Means with different superscripts within the same column differ significantly (*P< 0.05), ns: P>0.05

samples except adding 0.1 mL of distilled water instead of adding the fat. Standardized cholesterol was also read in the same way as the addition of 0.1 mL of pure cholesterol.

The egg yolk was extracted by sampling the egg yolk after the yolk was separated from the egg by six samples per treatment and placed in the, hexane solvent, in the saxolite to extract fat from egg yolk. Fatty acids in egg yolks were estimated for all T_1, T_2, T_3 and T_4 methods, according to the British standard method 684 published in the IUPAC (1979). The method involved the use of 1 ml of lipid extract to convert free fatty acids into methyl esters (Egan *et al*., 1981) and the sample was put in a test tube size 20 mL. Then, 10 mL of hexane or heptane solvent were added and followed by 0.5 mL of standard methanol methane hydroxide solution, in addition to two molars (14 g KOH + 1 L of methanol) were added. Then the test tube was closed and stirred vigorously for 20 sec. The extract (the upper layer) was pulled from the test tube by a micro syringe (GLC), and then 1 µl was injected into the gas liquid chromatography device, chromatography gas liquid. Lipid oxidation indicators were evaluated in egg yolks after storage of T_1, T_2, T_3 and T_4 for 14 days in the refrigerator at 4° C. Lipid oxidation were measured by estimating the value of Thiobarbituric Acid (TBA) according to the method of Witte *et al*. (1970), a sample of 1 g of egg yolk was mixed with 25 mL of cold solution containing 20% Trichloroacetic Acid (TCA) dissolved in the phosphoric acid with two molars concentration in the naturalization device and homogenized for two min. Then the mixture was transferred to a 50 mL volumetric flask and the size was completed to the mark with distilled water and shacked. After that, 25 mL of the mixture was centrifuged at 3000 rpm for 30 min. Thereafter, samples were filtered after shaking through whatman filter paper No 1 and transferred to a test tube and 5 mL of TBA detector solution (0.005%) was added. Blank solution was prepared by mixing 5 mL of distilled water with 5 mL of TBA reagent solution in a test tubes, then sealed well and were kept in a dark place for 15-16 h at room temperature. After that, the absorption of A for the resulting color was measured at 530 nm using the optical spectrometer and the TBA value was calculated by multiplying the absorption value by factor 5.2.

Method adopted by Pearson and Dustson (1985) was used to estimate the peroxide value (pv), 5 g of fat extracted from the egg yolk was taken with the use of the saxolite and added 30 mL of a mixture containing three parts of the snow acetic acid + two parts of chloroform 1%, (with 0.5 mL saturated potassium iodide, 30 mL distilled water and 1 mL starch) and then the mixture was dissolved with a solution of sodium thiocarbate (0.01) until the disappearance of the blue color. Free

Table 3. The effect of feeding different levels of Evening Primrose Oil (EPO) on the average of egg yolk index of Classic Isa Brown laying hens

S. No	Treatments	Average of egg yolk index					
		Periods / weeks					
		1(31-34)	2 (35-38)	3 (39-42)	4 (43-46)	5 (47-50)	Total period
1	T_1	0.443±0.004^{b}	60.47±0.003^{a}	0.450±0.004^{a}	0.455±0.002^{c}	0.458±0.006^{b}	0.456±0.001^{b}
2	T_2	0.457±0.001^{a}	0.462±0.001^{b}	0.481±0.002^{a}	0.460±0.003^{cb}	0.479±0.001^{a}	0.467±.003^{a}
3	T_3	0.453±0.001^{a}	0.453±0.006^{b}	0.459±0.002^{a}	0.477±0.002^{a}	0.472±0.004^{ab}	0.462±0.002^{ab}
4	T_4	0.451±0.00^{ab}	0.456±0.001^{b}	0.457±0.023^{a}	0.456±0.002^{b}	0.470±0.003^{ab}	0.458±0.001^{b}
5	p	*	*	ns	*	*	*

^{abc} Means with different superscripts within the same column differ significantly (*p < 0.05), ns: p > 0.05

fatty acids were estimated according to method of Pearson and Dustson (1985). A total of 28 g of fat was extracted in a saxolite method and 50 mL of 95% ethyl alcohol was added, then a few drops of phenolphthalein were added, and the mixture was dissolved with a sodium hydroxide solution (0.1) until the solution turns to light pink. All results obtained in this study were analyzed by SAS software (SAS, 2011) in a Completely Randomized Design (CRD) in addition, a comparison between the mean values were done by using Duncan's multiple range tests (1955).

RESULTS AND DISCUSSION

Table 2 indicated that a significant improvement (P<0.05) in the mean relative weight of egg yolk for T_2, T_3, T_4, was detected during all the periods. As well, for the total experiment period (31-50) weeks compared with T_1, which showed a significant decrease (P<0.05) in the mean relative weight of egg yolk. A significant improvement (P<0.05) in the average of the egg yolk index was observed at T_2, T_3 and T_4 for all periods (Table 3). Additionally, a significant improvement in the average of the egg yolk index was noted for the total experimental period, compared to T_1, which had a significant decrease (P<0.05) in the mean of the egg yolk index.

The improvement in the quality of the egg yolk may be attributed to the EPO composition of fatty acids, particularly gamma linolenic fatty acid, which was significantly (P<0.05) focused in egg yolks with the addition of three additive levels (0.25, 0.5, 0.75%) of EPO compared to T_1 (Table 4). This acid is considered a strong anti-oxidant and has high effectiveness in inhibiting free radical activity and inhibition of lipid peroxidation and thus protect liver cell membranes from oxidative damage and to maintain vital functions of the liver in its natural state and this encourages the release of vitellogenin and VLDL and other components of egg yolk from the liver to the yolk through the bloodstream. Moreover, their role as a fatty acid inhibitor and VLDL in maintaining the abundance of essential substances for egg production in blood plasma, such as lipoproteins, the main component of egg yolk (Faria *et al*., 2006). In addition, prostaglandin (PGEF2) also plays a role in the stimulation of steroid hormones (estrogen and progesterone hormone) in addition to its role in the process of ovulation, these hormones play other roles in stimulating the production of vitellogenin fat. The Estrogen hormone, which is released by the ovary and circulates through the blood to the liver, stimulates the synthesis of vitellogenin, very low-density lipoprotein (VLDL), and fatty proteins (Walace, 1985). Furthermore, the estrogen hormone had a role in the production of egg

Table 4. The effect of feeding different levels of evening primrose oil

S. No	Treatments	Average of fatty acids and cholesterol of egg yolk					
		Periods / weeks					
		Palmitic acid	Steric acid	Oleic acid	Linoleic acid	y-Linolenic acid	Cholesterol
1	T_1	0.323±25.57[a]	0.168±8.19[a]	0.058±41.68[a]	0.168±8.19[a]	---------	0.372±13.6[a]
2	T_2	0.272±25.63[a]	0.101±8.08[a]	0.337±41.03[b]	0.081±14.27[c]	0.01±0.307[c]	0.116±12.51[ab]
3	T_3	0.333±25.36[a]	0.238±7.79[a]	0.075±40.56[bc]	0.035±16.18[b]	0.006±0.603[b]	0.357±13.17[a]
4	T_4	0.245±24.82[a]	0.091±7.96[a]	0.064±40.68[c]	0.664±20.47[a]	0.006±0.901[a]	0.426±11.77[b]
5		N.S	N.S	*	*	*	*

(EPO) on the average of fatty acid and cholesterol of egg yolk in Classic Isa Brown laying hens
[abc] Means with different superscripts within the same column differ significantly (*P<0.05), ns: P>0.05

albumin through the egg channel (Brant and Nalbandov, 1956). Hence, the importance of estrogen hormone in the form of egg yolk was observed (Palmer and Bahr, 1985, Walace and Bahr, 1992; Whitehead *et al*., 1993).

Table 4 shows the effect of adding EPO to experimental diets in the form of fatty acid composition of egg yolk. It was noted that no significant differences were observed for both Palmitic acid and citric acid in, T_1, T_2, T_3 and T_4. Oleic acid was significantly (P<0.05) reduced in T_2, T_3 and T_4 with the means of 41.03, 40.56 and 40.29%, respectively, compared to the T_1 (41.68%). However, a significant improvement (P<0.05) in linoleic acid of egg yolk in T_2, T_3 and T_4 was revealed. The recorded means were 14.27, 16.18 and 20.47%, respectively, compared to T_1 which showed a mean of 12.07%. Significantly (P<0.05), the appearance of gamma linolenic acid in egg yolks was noted in T_2, T_3, T_4 with the recorded means of 0.303, 0.013, 0.901% respectively. It was observed that the percentage of gamma linolenic acid in the yolk increased with the addition of EPO compared to the control treatment (T_1) that did not have any appearance of gamma linolenic acid in the egg yolk. The analysis of egg yolk showed a change in the composition of fatty acids as a result of adding EPO to the diet. This result was consistent with Mazzilli *et al*. (2004) who observed that egg yolks from the added treatment levels of EPO could be combined with unsaturated fatty acids. Linoleic acid and gamma-linolenic acid GLA, which contributed to change the composition of fatty acids for egg yolk. It is believed that polyunsaturated fatty acids were the most effective in reducing saturated fatty acids due to reduce the activity of D-9-desaturase enzyme that necessary for oleic acid production. Scheideler and Froning (1996) and Van-Elswyk (1997) demonstrated that addition of sunflower oil containing high levels of linoleic acid and the addition of flaxseed oil, which is considered to be a rich source of linoleic acid has contributed to altering the image of fatty acid by increasing the egg yolk content of linoleic and linolenic acid. Furuse *et al*. (1992) and Ozpinar *et al*. (2003) reported that it was possible to change fatty acids of egg yolks when feeding laying chicken with diets included EPO compared to the control diets. A significant improvement (P<0.05) in GLA acid (which was one of the components of EPO) was detected when the feed intake and digestion and absorption were transferred by blood to reach the liver, which is the main organ for the metabolism of nutrients, including fatty acids, which are transferred to the eggs to accumulate in the egg yolk, meaning that it is possible to change the components of eggs through feed additives which is mainly based on the certain amounts of unsaturated fatty acids, and it is possible to incorporate lipid acid GLA into the fatty acid composition of eggs due to the fatty

Table 5. The effect of feeding different levels of Evening Primrose Oil (EPO) on the antioxidant indicators of egg yolk in Classic Isa Brown laying hens

S. No	Treatments	Lipid oxidation indicators for egg yolk		
		M.D.A mg/kg	P.V mL	F.F.A %
1	T_1	0.017 ± 1.693^{b}	0.100 ± 1.60^{a}	0.020 ± 0.603^{d}
2	T_2	$0.015\pm1.960_{a}$	0.033 ± 0.833^{b}	0.028 ± 0.723^{c}
3	T_3	0.027 ± 1.426^{c}	0.028 ± 0.733^{b}	0.003 ± 0.983^{b}
4	T_4	0.017 ± 1.353^{d}	0.066 ± 0.933^{b}	0.013 ± 1.056^{a}
5	P	*	*	*

^{abc} Means with different superscripts within the same column differ significantly (*P<0.05), ns: P>0.05

acid ratios present in egg yolk are affected by the quality of oil used in the diet (McNaughton *et al*., 1978; Liu *et al*., 2010).

Table 4 revealed the effect of adding different levels of EPO in the experimental diets on the level of egg yolk cholesterol. A significant decrease (P<0.05) in the level of egg yolk cholesterol of T_4 with a mean of 11.77 mg/g compared to T_2 which did not differ significantly from both T_3 and T_1. The low concentration of egg yolk cholesterol may be due to the presence of GLA in EPO. It was notable that fatty acid metabolism and it is regulation occurs in the liver and the GLA acid acts on the liver cells associated with the liver PPRI receptors and their work through genetic modification inhibit the action of the LTU gene responsible for the process of cholesterol synthesis and reduction of fat synthesis (Fan and Chapkin, 1998). Additionally, part of GLA acid was converted into the CLA acid, which has a synergistic role in process reduction of fats and increase the Lipoproteinlipase enzyme (Zhang *et al*., 2007) and egg yolk content are influenced by several factors, including dietary supplements (Washburn and Nix, 1974).

Table 5 detected that a significant decrease (P<0.05) in the value of peroxide in egg yolk was found in T_2, T_3, T_4 with the means of 0.833, 0.733, 0.933 Mg/kg, respectively, compared to T_1 which showed significant (P<0.05) increase in the value of peroxide with a recorded value of 1.60 Mg/kg of egg yolk. The results of Table 6 demonstrated a significant decrease (P<0.05) in the level of MDA in T_2, T_3 and T_4 with the means of 0.075, 0.094, 0.105 mg/kg respectively. An insignificant difference between T_3 and T_2 was noted compared to T_1 that showed a significant increase (P<0.05) in MDA, which reached 0.583 mg/kg.

The percentage of free fatty acids (FFA) for T_4, T_3 were significantly lower (P<0.05) compared with T_1 and T_2, which showed a significant increase in the level of Free fatty acids as it reached 1.693 and 1.960, respectively. The results showed that treatments included EPO have been highly effective as antioxidant properties by reducing the decomposition and release of free fatty acids in egg yolk. This is likely due to the active compounds in EPO that play a role in lipid peroxidation and also promotes the role of glutathione as a defense system and antioxidant (Benatti *et al*., 2004). Several chemical compounds of oil plants also worked as natural antioxidant such as catechin, epicatechin, gallic acid, a-tocopherol (Christie, 1999). Natural antioxidants have a role in fatty acid protection from oxidation and rheumatism by inhibiting lipid peroxidation and inhibiting free radicals that attack the fats, which causes a decrease in the nutritional value of fat, as the role of antioxidants work to prevent the separation of hydrogen atoms from the sets of double bonds in polyunsaturated fatty acids and thus prevent the process of oxidation and lipid peroxidation through the role of antioxidants as a gift for

hydrogen atoms into the free radicals and transform them into a more stable root. They contribute to the removal of free radicals by inhibiting free radical activity and reducing the formation of peroxides, which is an accidental product of the breakdown of hydroperoxides (Soolorattee *et al*., 2005). The decrease of lipid oxidation indicators in egg yolk can be attributed to the content of fatty acid in EPO especially fatty acid Gamma Linolenic Acid (GLA) which acts as an antioxidant effective in reducing the production of free radicals resulting from metabolic reactions within the body in addition to the role of oxidation with Vitamin E that becomes more effective in protecting cells from free radicals, and GLA increases the lifetime of vitamin E in cells (Kanbur *et al*., 2011).

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