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EFFECT OF USING DIFFERENT LEVELS OF FISH OIL, LINSEED OIL AND THEIR COMBINATION IN LAYER DIETS ON EGG OMEGA 3 ENRICHMENT

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ABSTRACT

One hundred and five Mandara laying hens, 24 wks of age, were divided into seven experimental treatments to study the effect of dietary levels of fish oil (FO), linseed oil (LO) and their combination as a source of omega 3 on egg yolk omega three polyunsaturated fatty acids concentration on cholesterol and total lipids, and the productive performance and egg quality of laying hens. The experimental treatments were divided into seven treatments with three replicates each. The first treatment group was the control group, the second treatment was given 1% FO, the third treatment was given 2% FO, the fourth treatment was given2% LO, the fifth treatment was given 3% LO, the sixth treatment was given 1% FO + 1% LO and the seventh treatment was given1.5% FO + 1.5% LO. The obtained data revealed the following results: Addition of FO, LO, and LO + FO to the diets did not cause significant effects ($P > 0.05$) on feed intake, feed conversion and egg production traits (egg number, egg weight and egg mass). Adding FO, LO, and their combination insignificantly increased yolk percentage. Saturated fatty acids significantly decreased FO and LO increased compared to the control. Diets containing FO, LO, and their combinations resulted in a significant increase of n-3 polyunsaturated fatty acids and polyunsaturated fatty acid concentrations in eggs compared to the control group. Adding 2% FO, 1% FO + 1% LO and 1.5% FO + 1.5% LO to laying diets decreased ($P<0.05$) egg yolk total cholesterol and egg yolk total lipids comparing to control group. The lowest values of egg yolk total cholesterol and egg yolk total lipids were recorded by 1.5% FO + 1.5% LO compared to other treatments. Using 1% FO only or 1% FO + 1% LO in laying hen diets improved the economical efficiency comparable to the control group. The best feed cost/kg egg was recorded by the group fed diet 1% FO + 1% LO.

Keywords: Fish oil, linseed oil, layers, Omega 3, egg quality.

INTRODUCTION

The egg is naturally poor in linolenic acid (LNA) however, Fish oil is rich in omega 3 polyunsaturated fatty acids (n-3 PUFA), especially eicosapentaenoic (EPA) and docosahexaenoic (DHA), Linseed oil contains only α-LNA in greater amounts, which is the precursor of EPA and DHA. However giving hens a standard feed resulted in poor egg in linolenic acid (LNA), and does not contain eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids (Souza *et al.,* 2008). Moreover, conversion of α-LNA into EPA and DHA in humans is only 5–10% (Schreiner *et al.,* 2004).

The modern human diets don't meet the daily requirement from n-3 polyunsaturated (Meyer *et al.,* 2003). Hen eggs are considered a readily available source of protein worldwide. Eggs are very valuable source of proteins and contain many substances with biological function beyond basic nutrition (Laca *et al.,* 2010). The polyunsaturated fatty acids (PUFA) are among the egg nutrients which are important to human health, particularly the omega 3 polyunsaturated fatty acids. These acids are required for healthy foetal brain, proper development, they assist in preventing and curing heart diseases, hypertension, arthritis, several types of cancer especially prostate and breast cancer and play a vital role in lowering blood viscosity and pressure, plasma triglycerides, reducing circulating cholesterol levels, platelet aggregation, and cardiac arrhythmia (Simopoulos, 2000; Sim, 2006), Moreover they have an obvious improved in sight capacity, high neurological development and intellectual abilities (Bourre, 2005).

British National Foundation recommends females and males to have DHA/ EPA of 1.1 and 1.4 g/day, respectively (Ward and Singh, 2005), so modification of yolk fatty acids (FAs) by feeding hens on fish and linseed oil (hens have a unique ability to deposit dietary lipid into the egg yolk) resulted in significant changes of the fatty acids composition in egg yolk which makes the egg a potential source of polyunsaturated fatty acids (PUFAs) (Škrtić *et al.,*2006; Cachaldora et al.,2006). It should be mentioned that the inclusion of n-3 PUFA promotes a qualitative change in the yolk fatty acids profile and reducing the n-6/n-3 ratio to a more beneficial level with regards to the human nutritional needs (Simopoulos, 1998). Simopoulos 2003, declared that the ratio of n-6/n-3 PUFA in standard eggs was 20: 1 while the approved ratio is actually between 4:1 to 10:1 (Mazalli *et al.,* 2004).

Therefore, the objective of this study is to investigate supplementation of layer diets with different levels of linseed oil and fish oil and their combinations on the n-3 PUFA content in eggs yolk.

MATERIALS AND METHODS

The present study was conducted at Inshas Poultry Research Station, (El-Sharkia Governorate), Animal Production Research Institute, Agriculture Research Center.

Birds and diets

One hundred and five Mandara hens, 24 weeks of age at the beginning of the experiment, were housed individually in cages for 12 weeks experimental period in three subsequent interval periods (four weeks each). Birds were randomly divided into seven treatments, with three replicates of five birds each. One treatment of layers received the basal diet, which assigned as a control treatment, other layers of different treatments were given diets containing different levels of fish oil, linseed oil and their combination as shown in Table 1.

Fish oil was added up to 2% only because supplemented layers diet with more than 2% fish oil had a negative effect on egg weight (Gonzalez-Esquerra and Leeson 2000). Feed and water were provided *ad libitum* during the entire trial. Layers were fed isonitrogenous (16% CP) and is caloric (2700 Kcal ME/Kg) diets. The ingredients composition and calculated analysis of the experimental diets are shown in Table 2. The calculated values of the diets were within the required ranges as listed by Egyptian ministerial decree No. 1498 for local strains feed requirements.

Table 1: Treatments groups used in the experiment.

Groups	Treatments
1	Control diet without supplemental oil
2	1% fish oil containing diet
3	2% fish oil containing diet
4	2% linseed oil containing diet
5	3% linseed oil containing diet
6	1% fish oil + 1% linseed oil containing diet
7	1.5% fish oil + 1.5% linseed oil containing diet

Table 2: Composition of the diets.

Ingredients	Dietary treatments						
	Control	**1%F***	**2% F**	**2%L****	**3% L**	**1%F+ 1%L**	**1.5% F+1.5%L**
Yellow corn	61.67	58.48	55.77	55.57	52.66	55.57	52.76
Soybean meal 44%	16.70	16.40	16.60	16.60	18.20	16.60	18.10
Wheat bran	6.70	9.20	10.90	11.20	12.60	11.20	12.50
Corn gluten 60%	4.70	4.70	4.50	4.40	3.30	4.40	3.40
D.L Methionine	0.01	0.01	0.02	0.02	0.03	0.02	0.03
Calcium carbonate	8.13	8.13	8.13	8.13	8.13	8.13	8.13
Di-Calcium phosphate	1.42	1.41	1.41	1.41	1.41	1.41	1.41
Fish oil	-	1.00	2.00	-	-	1.00	1.50
Linseed oil	-	-	-	2.00	3.00	1.00	1.50
Salt	0.37	0.37	0.37	0.37	0.37	0.37	0.37
Premix ***	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Total	100%	100%	100%	100%	100%	100%	100%
Calculated analysis							
ME kcal/kg diet	2699	2699	2709	2708	2709	2705	2708
CP%	16.02	16.02	16.03	16.00	16.02	16.00	16.03
CF%	3.47	3.65	3.78	3.81	3.99	3.81	3.98
Ca%	3.45	3.45	3.46	3.46	3.46	3.46	3.46
Av .p.%	0.41	0.40	0.41	0.40	0.41	0.40	0.41
Lys. %	0.73	0.73	0.74	0.74	0.78	0.74	0.78
Met. %	0.34	0.34	0.34	0.34	0.34	0.34	0.34
Met.+Cys.%	0.62	0.62	0.62	0.62	0.62	0.62	0.62

***Fish oil **linseed oil ***Supplied per kg of diet: Vit. A, 12000 IU; Vit. D3, 2200 IU; Vit. E, 10 mg; Vit K3, 2 mg; Vit. B1, 1 mg; Vit. B2 5mg; B6 1.5mg; B12 10 mcg; Nicotinic acid 30mg; Folic acid 1mg, Pantothenic acid 10mg; Biotin 50 mcg; Choline 250mg; Copper 10mg; Iron 30mg; Manganse 60mg; Zinc 50mg; Iodine 0.3 mg; Selenium 0.1mg; Cobalt 0.1mg.**

Production parameters and analysis

Eggs were recorded and weighed daily; feed consumption was recorded weekly. The productive traits were calculated as a number of eggs/hen/period for each replicate during the three interval periods (from 24-36 wks of age). Egg production percent, egg weight mean were calculated. Egg mass was calculated by multiplying egg number by egg weight average. Feed conversion was calculated as kg feed consumption divided by Kg egg mass (kg feed\kg egg).

Egg quality analysis

Egg quality was measured at the end of the experiment, in which 12 eggs were randomly chosen from each treatment (3 eggs from each replicate). Eggs were individually broken, shape index, yolk index values were measured according to *Sauter et al. (1951)* in addition to shell, yolk, albumen percentages were calculated according to *(Paganelli et al. 1974).* Egg shape index of the egg was measured using the *Vernier calipers* then calculating egg-shape index as follow:

$$\text{Egg shape index} = \frac{\text{Transversal axis}}{\text{Longitudinal axis}} \times 100$$

Albumen and yolk height were measured with tripod micrometer (in mm). The albumen and yolk indices were then calculated as follows:

$$\text{Albumen Index} = \frac{\text{Albumen height}}{\text{Albumen width}} \times 100$$

$$\text{Yolk Index} = \frac{\text{Yolk height}}{\text{Yolk width}} \times 100$$

Haugh Units was measured according to *Williams (1997).*

Haugh Units= 100x log (H- 1.7 W0.37 +7.57).

Where:

H= the height of thick albumen.

W= the egg weight.

Chemical analysis

At the end of the experiment, three eggs from each treatment were broken for yolk samples. Lipids were extract from yolk according to Folch et al. (1957), while fatty acid composition in yolk extract was determined according to (AOAC, 2000). Egg yolk total cholesterol and total lipids were determined by method of Allain, (1974) and Lee *et al.* **(1996)**, respectively.

Statistical analysis

Statistical analyses of data followed a one- way ANOVA analysis. General Linear Model *(*SAS, 2004*)* was used for detecting the differences among treatment groups. The significant differences among means of treatments were compared by Duncan's multiple-range test (Duncan, 1955*).*

RESULTS AND DISCUSSION

Performance Parameters

Results of Table 3 showed that no significant effect was observed by feeding Mandara laying hens diets containing different levels of fish oil, linseed oil and their combination in feed intake and feed conversion at the overall mean of the experiment. This result is in an agreement with that reported by Schumann *et al.,* 2000 and Fe´ bal *et al.,* 2008. On the other hand, a few researchers mentioned some increase in feed intake of birds receiving n-3 PUFA-enriched diets (Lopez-Ferrer *et al.,* 2001; Talebali and Farzinpour, 2005). Feeding linseed as a source of n- 3 PUFA was reported to increase the feed intake in the laying hens (Ansari *et al.*, 2006).

Table 3: Feed intake and feed conversion of Mandara laying hens as affected by adding the fish oil, linseed oil and their combination during the experimental period (24– 36wk of age).

Treatments	First period 24-28wk of age	Second period 28-32wk of age	Third period 32-36wk of age	Overall mean
Feed intake (kg/hen/period)				
T1	3.200 ^{ab} ±0.122	3.665±0.115	3.681 ^{ab} ±0.110	3.517±0.115
T2	3.348 ^{ab} ±0.084	3.563±0.411	3.463 ^{b} ±0.098	3.458±0.137
T3	3.155 ^{b} ±0.030	3.848±0.517	3.528 ^{b} ±0.153	3.511±0.213
T4	3.548 ^{a} ±0.204	3.665±0.383	3.579 ^{b} ±0.083	3.597±0.159
T5	3.437 ^{a} ±0.019	3.679±0.154	3.687 ^{a} ±0.193	3.601±0.110
T6	3.231 ^{ab} ±0.087	3.551±0.175	3.512 ^{b} ±0.069	3.431±0.094
T7	3.390 ^{ab} ±0.226	3.568±0.256	3.560 ^{b} ±0.020	3.506±0.135
Feed conversion (kg feed/kg egg mass)				
T1	2.445±0.053	2.551±0.047	2.595±0.015	2.530±0.018
T2	2.514±0.050	2.532±0.120	2.504±0.018	2.516±0.041
T3	2.532±0.083	2.467±0.040	2.485±0.030	2.493±0.026
T4	2.554±0.151	2.597±0.024	2.601±0.010	2.584±0.043
T5	2.555±0.065	2.497±0.099	2.515±0.077	2.522±0.052
T6	2.601±0.075	2.606±0.030	2.577±0.023	2.594±0.039
T7	2.531±0.057	2.532±0.158	2.556±0.020	2.540±0.076

[a, b:] Means within each column having no similar letter(s) are significantly different ($P \leq 0.05$)

T1: Control, T2: Fish oil (1%), T3: Fish oil (2%), T4: Linseed oil (2%), T5: Linseed oil (3%) T6: Fish oil (1%)+Linseed oil (1%) and T7: Fish oil (1.5%)+ Linseed oil (1.5%).

Adding fish oil and linseed oil or their combinations as a n-3 PUFA source to layers had no significant effect in egg number among the various treatment groups. In this respect, Goncuglu and Ergun (2004) stated that adding linseed oil at a level of 1, 2, 3 or 4% of the diet had no effect on the laying performance of hens and egg production was similar in laying hens received no linseed oil. Similarly (Carrillo-Dominguez *et al.,* 2005; Ebeid *et al.,* 2008) demonstrated that Egg production in laying hens at all production stages was not affected by different dietary levels of n-3 PUFA. However In some studies, there is an increase in egg production when laying hens fed on diets rich in n-3 PUFA (Augustyn *et al.,* 2006 Silke *et al.,* 2008).

No statistical significant differences were determined among egg weight and egg mass with respect to linseed oil or fish oil and linseed oil + fish oil used in the experiment as indicated in Table 4.

Table 4: Egg number, egg weight and egg mass of Mandara laying hens as affected by adding the fish oil, linseed oil and their combination during the experimental period (24-36wk of age).

Treatments	First period 24-28wk of age	Second period 28-32wk of age	Third period 32-36wk of age	Overall mean
Egg number (hen/period)				
T1	15.6± 1.101	14.733±0.437	14.933±0.240	15.090±0.345
T2	15.667±0.581	15.467±1.670	15.467±0.176	15.533±0.656
T3	16.667±0.266	14.067±1.348	15.33±0.405	15.355±0.663
T4	15.00±0.871	15.40±1.600	15.6±0.305	15.33±0.880
T5	15.267±0.333	14.467±0.995	15.133±0.480	14.956±0.357
T6	16.267±0.290	15.267±0.968	15.467±0.569	15.667±0.601
T7	15.400±1.171	15.133±1.729	15.267±0.066	15.267±0.870
Egg weight(g)				
T1	49.230±0.665	47.325±0.777	47.265±0.685	47.940±0.582
T2	48.019±0.814	47.052±0.936	46.820±0.326	47.297±0.024
T3	48.115±0.634	46.770±1.314	46.090±1.456	46.991±1.039
T4	48.168±0.938	47.045±0.439	46.651±0.918	47.288±0.731
T5	48.699±0.442	47.177±1.092	45.255±1.778	47.041±1.050
T6	49.489±0.231	48.387±0.509	47.540±1.145	48.472±0.604
T7	48.955±0.293	47.607±0.487	47.042±0.236	47.868±0.176
Egg mass (Kg/hen)				
T1	0.766±0.044	0.700±0.009	0.706±0.020	0.723±0.008
T2	0.751±0.016	0.729±0.085	0.724±0.021	0.735±0.035
T3	0.802±0.018	0.661±0.081	0.708±0.040	0.724±0.043
T4	0.723±0.045	0.725±0.077	0.728±0.018	0.725±0.043
T5	0.744±0.022	0.683±0.054	0.686±0.041	0.704±0.032
T6	0.805±0.013	0.738±0.038	0.734±0.020	0.759±0.022
T7	0.753±0.052	0.722±0.089	0.718±0.006	0.731±0.043

T1: Control, T2: Fish oil (1%), T3: Fish oil (2%), T4: Linseed oil (2%), T5: Linseed oil (3%), T6: Fish oil (1%)+Linseed oil (1%) and T7: Fish oil (1.5%)+ Linseed oil (1.5%).

However, 1% linseed oil+1% fish oil diet insignificantly increased egg number, egg weight and egg mass during the studied experimental periods. These results are in an agreement with those obtained by (Schreiner *et al.,* 2004; Carrillo-Dominguez *et al.,* 2005) who stated that egg weight was not influenced by feeding different sources and levels of n-3 PUFA in the diet of laying hens . In addition, feeding on 4% linseed oil had no effect on egg weight of the hens (Celebi and Utlu, 2006). Novak and Scheideler (2001) and Grobas *et al.* (2001) noted that egg weight and egg mass was not affected by feeding 5 or 10% linseed to the hens. Also, Silke *et al.* (2008) found that dietary fats (soybean oil and linseed oil) had no effect on egg weight in laying hens. Linseed oil in the diet of layers did not affect neither egg weight nor egg mass (Raes *et al.,* 2002).

Ebeid *et al.*, (2008) reported that dietary fish oil fortification (as a source of n-3 PUFA) had a negative effect on egg weight. However, no differences were found between control diet and diets with 1.25% and 2.5% fish oil whereas; dietary fish oil supplementation of more than 2.5% resulted in a linear reduction in egg weight. Whereas linseed oil can be added up to 4% without any adverse effect on egg weight (Celebi and Utlu, 2006).

Egg Quality Parameters

The results concerning egg quality among the experimental groups which were fed diets containing fish, linseed oils and their combinations are presented in Table 5. Adding fish oil, linseed oil, and their combination had no significant effect on yolk percentage. Increasing dietary n-3 PUFA content did not bring any significant change in egg yolk weight (Sosin *et al.* ,2006 and Augustyn *et al.,* 2006)). However, increasing linseed oil beyond 4% decreases egg yolk weight as reported by Scheideler and Froning (1996), who suggested that these results were due to the effect of PUFA on the estrogen activity of the hens. . The best insignificant value of shape index, yolk index and Haugh unit was recorded by the group fed I% fish oil +1% linseed oil. Grobas *et al.* (2001) found no change in Haugh Unit due to feeding 4% linseed oil. In general, the measured egg quality parameters in this research were not significantly changed. Ceylan *et al.* (2011) demonstrated that dietary supplementation of fish oil and linseed oil did not affect egg quality and performance parameters of laying hens. Raes *et al.* (2002) stated that inclusion of n-3 PUFA in layers diet did not cause any significant effect on egg shell quality.

Fatty acids profile of egg yolk

Table 6 shows the fatty acid composition of the egg yolk of layers fed fish oil, linseed oil, and their combinations. Saturated fatty acids significantly decreased when fish and linseed oils increased as compared to the control. The highest value of Meristic and Palmitic acid was detected in the eggs of control group comparable to the other treatment groups**.** This finding is in an agreement with results obtained by **Souza *et.al,* 2008.** Fish and linseed oil and their different combinations in layers diet had no significant effect ($P > 0.05$) on the content of oleic acid. There was insignificant decrease in linoleic acid (n-6) content. **Hargis *et al.* (1991) and Van Elswyk *et al.* (1992)** reported that the linoleic acid (n-6) content was reduced in eggs through diets containing menhaden oil.

Table 5: Yolk percentage, Shell percentage, Shape index , Yolk index, Albumin index, Albumin percentage and Haugh units of Mandara laying hens as affected by adding the fish oil, linseed oil and their combination during the experimental period (24-36wk of age).

Treatments	Yolk percentage	Shell percentage	Shape index	Yolk index	Albumin index	Albumin percentage	Haugh unit
T1	31.150± 0.802	13.706 ±0.514	78.186 ±2.287	41.508 ±0.572	8.998 ±0.354	55.144 ±0.485	84.190 ±0.783
T2	31.588± 0.303	13.868±0. 340	77.354±0. 612	41.210 ±1.067	8.530 ±0.337	54.544±0. 617	83.896 ±530
T3	31.296± 1.314	13.378 ±0.459	77.124 ±0.560	40.182 ±1.469	8.64 ±0.303	55.326±1. 545	83.667 ±1.04
T4	32.502± 1.105	13.142 ±0.700	77.04 ±0.972	41.756 ±0.738	8.496 ±0.171	54.356±0. 905	84.804 ±0.591
T5	32.322± 1.266	13.302 ±0.400	77.218 ±0.847	40.190 ±0.775	8.994 ±0.326	54.376±1. 310	84.670 ±0.469
T6	31.420± 0.739	13.802 ±0.663	78.200 ±2.214	41.968 ±1.422	8.640 ±0.297	54.778±1. 150	85.018 ±1.069
T7	31.396± 0.560	13.368 ±0.260	77.054 ±0.652	41.734 ±0.432	8.432 ±0.276	54.812±0. 828	83.452 ±2.360

T1: Control, T2: Fish oil (1%), T3: Fish oil (2%), T4: Linseed oil (2%), T5: Linseed oil (3%), T6: Fish oil (1%)+ Linseed oil (1%) andT7: Fish oil (1.5%)+ Linseed oil (1.5%)

Linseed oil resulted in a significant decrease of arachidonic acid. In this respect, Mazzali *et al.* (2004) indicated that high linolenic acid level limits the synthesis of arachidonic acid from linoleic acid (LA), linolenic acid (LNA) competes with LA for the enzyme *D6 desaturase* for their biosynthesis. However, Milinsk *et al.* (2003) verified that arachidonic acid egg content is not influenced by dietary polyunsaturated fatty acid. Also, Cobos *et al.* (1995) did not find an increase in arachidonic acid content in the eggs of layers fed diets with linseed oil.

Diets containing fish oil, linseed oil, and their combinations resulted in a significant increase of linolenic acid (LNA), Eicosapentaenoic acid (EPA), docosahexanoic acid (DHA), n-3 polyunsaturated fatty acids and polyunsaturated fatty acid concentrations in eggs as compared to the control diet. These results are in accordance with the results of Ceylan *et.al*, (2011) who indicated that increasing level of fish oil and linseed oil inclusion caused more deposition of LNA and DHA ($P<0.01$) in egg yolk, while arachidonic acid deposition was declined ($P<0.05$), monounsaturated fatty acid (MUFA) deposition also was decreased with higher inclusion level of fat, whereas PUFA amount was significantly ($P<0.01$) increased.

Table 6: Egg yolk fatty acid profile of Mandara laying hens as affected by adding the fish oil, linseed oil and their combination.

Items	T1	T2	T3	T4	T5	T6	T7
Myristic (C14:0)	0.67^{a} ±0.01	0.66^{a} ±0.01	0.65^{a} ±0.01	0.63^{a} ±0.01	0.62^{a} ±0.01	0.52^{b} ±0.01	0.51^{b} ±0.00
Palmitic (C16:0)	36.25^{a} ±1.00	35.33^{ab} ±0.98	32.97^{ab} ±0.91	31.85^{c} ±0.88	31.25^{c} ±0.84	31.81^{c} ±0.86	31.23^{c} ±0.86
∑SFA	36.92^{a} ±1.02	35.99^{ab} ±0.99	33.62^{bc} ±0.77	32.48^{c} ±0.84	31.87^{c} ±0.84	32.33^{c} ±0.90	33.73^{bc} ±0.91
Oleic(C18:1n9c)	43.55 ±1.13	44.00 ±1.22	44.12 ±1.08	45.00 ±1.19	45.43 ±1.51	44.18 ±1.23	44.32 ±1.25
Linoleic (C18:2n6c)	20.00 ±0.11	19.30 ±0.50	18.55 ±0.51	18.70 ±0.50	18.60 ±0.48	19.50 ±0.50	19.71 ±0.54
Arachidonic	1.84^{a} ±0.05	1.82^{a} ±0.05	1.62^{bc} ±0.04	1.56^{c} ±0.04	1.52^{c} ±0.04	1.75^{ab} ±0.04	1.80^{a} ±0.05
∑n-6PUFA	21.84 ±0.60	21.12 ±0.58	20.17 ±0.54	20.26 ±0.55	20.12 ±0.54	21.25 ±0.58	21.50 ±0.60
Linolenic (C18:3n6)	0.05^{f} ±0.00	0.11^{ef} ±0.00	0.19^{e} ±0.00	1.60^{b} ±0.04	2.25^{a} ±0.06	0.72^{d} ±0.01	1.38^{c} ±0.04
EPA	0.15^{e} ±0.00	2.21^{a} ±0.05	2.22^{a} ±0.06	0.86^{d} ±0.02	1.07^{c} ±0.03	1.36^{b} ±0.01	1.41^{b} ±0.04
DHA	0.56^{e} ±0.01	1.94^{c} ±0.05	2.93^{a} ±0.08	0.71^{de} ±0.01	0.85^{d} ±0.02	2.62^{b} ±0.06	2.66^{b} ±0.07
∑n-3PUFA	0.76^{e} ±0.02	4.26^{c} ±0.11	5.34^{a} ±0.15	3.17^{d} ±0.08	4.17^{c} ±0.11	4.70^{b} ±0.13	5.45^{a} ±0.14
∑PUFA	22.6^{d} ±0.62	25.38^{abc} ±0.70	25.51^{abc} ±0. 1	23.43^{cd} ±0.65	24.29^{bcd} ±0.67	25.95^{ab} ±0.72	26.95^{a} ±0.75
∑UFA	66.15 ±1.66	69.38 ±2.00	69.63 ±2.02	68.43 ±1.92	69.72 ±2.01	70.13 ±2.03	71.25 ±2.06
Omega 6/3	28.73^{a} ±0.69	4.95^{c} ±0.13	3.77^{e} ±0.10	6.39^{b} ±0.18	4.88^{cd} ±0.14	4.52^{cde} ±0.13	3.94^{de} ±0.11

$^{a, b....}$ **Means within each row have no similar letter(s) are significantly different (P ≤ 0.05).**

T1: Control, T2: Fish oil (1%), T3: Fish oil (2%), T4: Linseed oil (2%), T5: Li7nseed oil (3%),T6: Fish oil (1%)+ Linseed oil (1%) and T7: Fish oil (1.5%)+ Linseed oil (1.5%).
∑SFA=total saturated fatty acid, ∑n-6PUFA=total n-6 polyunsaturated fatty acid, EPA=eicosapentaenoic acid, DHA=docosa hexanoic acid,∑n-3PUFA=total n-3 polyunsaturated fatty acid , ∑PUFA=total polyunsaturated fatty acid and ∑UFA=total unsaturated fatty acid

Huang *.,* 1990, used fish oil in rations at levels of 0, 1, 2, and 3 % and found that EPA and DPA levels in the egg yolk significantly increased. The ration containing fish oil significantly decreased omega-6/omega-3 fatty acid ratio, whereas omega-3 fatty acid increased (Hargis *et al,* 1991). The enrichment of layer diets with marine-fish oils or linseed oil, promotes the rapid incorporation of n-3 fatty acids into the egg yolk (Van Elswyk, 1997).

The egg content of EPA was high (P<0.01) in fish oil diets whereas the LNA was high (P<0.01) in linseed diets as shown in Table 6. When hens were fed diets containing fish oil (Marshall *et al*., 1994), eggs presented high linolenic acid, EPA, and DHA levels, which in turn decreased arachidonic acid egg content (Gao and Charter, 2000).

In brief, total saturated fatty acid SFA content significantly decreased and total n-3 polyunsaturated fatty acids content in egg were significantly greater in all treatments than the control group.

Egg yolk cholesterol and total lipids

Results in Table 7 indicate that, adding 2% fish oil, 1% fish oil + 1% linseed oil and 1.5% fish oil + 1.5% linseed oil to laying diets decreased (P<0.05) egg yolk total cholesterol and egg yolk total lipids compared to control group. The lowest values of egg yolk total cholesterol and egg yolk total lipids were recorded by 1.5% fish oil + 1.5% linseed oil compared with other treatments or control groups. These results are in agreement with the previous findings of Radwan *et al.* (2012) and Saleh (2013) who indicate significant (P<0.05) decrease of yolk cholesterol for birds fed diets containing 3.5% fish oil, as compared to those fed on control diet. Egg cholesterol can be decreased due to reduction of hepatic synthesis as well as increased proximal beta oxidation activity or due to the inhibition of hepatic very low density lipoproteins and density lipoproteins production. Also, Kang *et al.* (2001) noted that reduction in yolk cholesterol by dietary fish oil was due mainly to the inhibition of hepatic low density lipoproteins production. Ouyang *et al.* (2004) noted that when laying hens were fed diet supplemented with 5% fish oil, 5% palm oil and 5% soybean oil, the cholesterol level in yolk of fish oil group was lower than palm oil and control. Moreover, Vasko *et al.* (2005) found that when laying hens were fed three diets supplemented with flax oil and fish oil, concentrations of cholesterol in the egg yolk significantly decreased in the groups with supplementation of flax and fish oil.

Table 7: Egg yolk cholesterol and total lipids of Mandara laying hens as affected by adding the fish oil, linseed oil and their combination.

Items	T1	T2	T3	T4	T5	T6	T7
Egg cholesterol (mg/g)	9.85[a] ±0.780	8.00[abc] ±0.768	6.92[bc] ±0.791	9.51[ab] ±0.783	7.64[abc] ±0.789	7.18[bc] ±0.777	5.59[c] ±0.796
Egg total lipids (mg%)	29.58[a] ±1.263	26.87[ab] ±1.287	24.61[b] ±1.301	28.16[ab] ±1.298	26.37[ab] ±1.231	25.41[b] ±1.257	23.98[b] ±1.248

[a, b….] Means within each row have no similar letter(s) are significantly different (P ≤ 0.05).
T1: Control, T2: Fish oil (1%), T3: Fish oil (2%), T4: Linseed oil (2%), T5: Linseed oil (3%), T6: Fish oil (1%)+ Linseed oil (1%) and T7: Fish oil (1.5%)+ Linseed oil (1.5%).

Economic efficiency

Using 1% fish oil only and 1% fish oil + 1% linseed oil in laying hens diets improved the economical efficiency compared to the control group. The lower feed cost /kg egg was observed with the group fed diet containing 1% fish oil + 1% linseed oil it was being 12.31LE, the relative economical efficiency was 99.35. Therefore, the previously mentioned dietary treatment was more economic than the other treatments as shown in Table 8.

It was clear to note that, oils addition increased generally the price of diets because of the highly price of these oils. However, the prices of these fortified eggs with n-3 PUFA can compensate this cost, where the main objective of this research is obtaining an enriched egg with n-3 PUFA as a solution for some health problems especially cardiovascular diseases.

Table 8: Input- output analysis and economic efficiency of different dietary treatments.

Items	T1	T2	T3	T4	T5	T6	T7
Feed price (LE/Kg)	2.548	2.622	2.694	2.713	2.787	2.723	2.771
Total feed intake/hen (kg)	10.551	10.374	10.533	10.791	10.803	10.293	10.518
Total feed cost/hen (LE)	26.883	27.198	28.37	29.27	30.105	28.026	29.145
Total egg mass/hen (Kg)	2.169	2.205	2.172	2.175	2.112	2.277	2.193
Feed cost/kg egg (LE)	12.39	12.34	13.06	13.46	14.26	12.31	13.290
Relative economic efficiency	100	99.6	105.41	108.64	115.1	99.35	107.26

T1: Control, T2: Fish oil (1%), T3: Fish oil (2%), T4: Linseed oil (2%), T5: Linseed oil (3%), T6: Fish oil (1%)+ Linseed oil (1%) and T7: Fish oil (1.5%)+ Linseed oil (1.5%).

CONCLUSIONS

It can be concluded that fatty acid profile of the egg and egg yolk cholesterol and total lipids can be modified using fish oil and linseed oil or their combinations which resulting in eggs with higher n-3 PUFA and lower total cholesterol and total lipids. Using the aforementioned oils did not cause any significant effect on the performance of layers or in the egg quality.

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تأثير إستخدام مستويات مختلفة من زيت السمك وزيت الكتان وخليطهما فى علائق الدواجن على إغناء البيض بالأوميجا 3

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تم تقسيم عدد 105 دجاجة مندرة (سلالة مستنبطة مصرية) عمر 24 أسبوع الى 7 معاملات لدراسة تأثير إستخدام مستويات مختلفة من زيت السمك والكتان وخليطهما كمصدر للأوميجا 3 على تركيز الأوميجا 3 والأحماض الدهنية الغير مشبعة المتعددة فى البيض وكذلك تركيز الكوليسترول والدهون الكلية فى البيضة والصفات الإنتاجية وجودة البيضة للدجاج البياض. قسمت السبع معاملات المستخدمة فى التجربة الى 3 مكررات وتم تغذية المجموعات السبع كالتالى:

1- المجموعة الأولى:- تم تغذيتها على العليقة الأساسية (مقارنة) بدون أى اضافات.
2- المجموعة الثانية :- تم تغذيتها على العليقة الأساسية مضاف اليها زيت السمك بمعدل 1%.
3- المجموعة الثالثة:- تم تغذيتها على العليقة الأساسية مضاف اليها زيت السمك بمعدل 2%.
4- المجموعة الرابعة:- تم تغذيتها على العليقة الأساسية مضاف اليها زيت الكتان بمعدل 2%.
5- المجموعة الخامسة:- تم تغذيتها على العليقة الأساسية مضاف اليها زيت الكتان بمعدل 3%.
6- المجموعة السادسة:- تم تغذيتها على العليقة الأساسية مضاف اليها زيت السمك بمعدل 1% + زيت الكتان بمعدل 1%.
7- المجموعة السابعة:- تم تغذيتها على العليقة الأساسية مضاف اليها زيت السمك بمعدل 5و1% + زيت الكتان بمعدل 5و1%.

وتتلخص أهم النتائج المتحصل عليها فيما يلى:

- وجد أن استهلاك العلف ، معامل التحويل الغذائى ، وعدد ووزن وكتلة البيض لم يتأثر معنويا بإضافة أى من زيت السمك أو زيت الكتان أو خليطهما الى عليقة الدجاج البياض خلال الفترة الكلية للتجربة (24-36 أسبوع).
- وجد أن النسبة المئوية للصفار زادت زيادة غير معنوية نتيجة التغذية على علائق مضاف اليها كل من زيت السمك و زيت الكتان و خليطهما
- وجد أن محتوى الصفار من الأحماض الدهنية الكلية المشبعة إنخفض معنويا بزيادة نسب زيت السمك والكتان بالمقارنة بمجموعة المقارنة.
- وجد أن محتوى الصفار من مجموع الأحماض الدهنية العديدة الغير مشبعة أوميجا 3 زادت معنويا بالتغذية على علائق مضاف اليها زيت السمك أو زيت الكتان أو خليطهما مقارنة بمجموعة المقارنة وسجلت المجموعتين الثالثة والسابعة أعلى القيم.
- سجلت المجاميع التجريبية المغذاه على زيت السمك منفردا أو مضاف الى زيت الكتان زيادة معنوية فى محتوى الصفار من الأحماض الدهنية الكلية العديدة الغير مشبعة وذلك مقارنة بمجموعة المقارنة.
- سجلت النتائج إنخفاضا معنويا فى محتوى الصفار من الكوليسترول والدهون الكلية نتيجة التغذية على علائق مضاف اليها زيت السمك بمعدل 2% وكذلك بالتغذية على العلائق المحتوية على خليط من زيت السمك مع زيت الكتان وذلك مقارنة بمجموعة المقارنة.
- إستخدام 1% زيت سمك فى العلائق المقدمة للدجاج البياض أو 1% زيت سمك + 1% زيت كتان حسن من الكفاءة الإقتصادية بالمقارنة بمجموعة المقارنة.
- أفضل كفاءة إقتصادية سجلتها المجموعة المحتوية على خليط من زيت السمك والكتان بنسبة 1% + 1%.