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Research article
Ερευνητικό άρθρο

Feeding a definite concentration of eicosapentaenoic and docosahexaenoic fatty acids to laying hens

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ABSTRACT. The experiment designed to study the influences of nutritional eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids (FA) on performance, egg yolk fat characteristics and FA profile in laying hens. From 30 to 36 weeks of age, 180 laying hens were allotted randomly to 2 dietary treatments, each of 6 replicates (15 birds for each replicate). The control diet was supplied with soy oil while the experimental diet was supplied with EPA and DHA to create 2 different ratio of n-6 to n-3 FA (18.8:1 and 5:1, respectively). The egg production % was recorded daily. The eggs were weighed to estimate egg mass. Also, feed consumption was recorded daily and the feed conversion ratio (FCR) was estimated. The FA profile of egg yolk was determined in the last week of the experiment. The dietary EPA and DHA resulted in significantly higher egg production (76.89 versus 67.23%), weightier egg mass (42.46 versus 37. 72 g) and lower FCR (2.49 versus 2.72) than the control. Also, supplying the dietary EPA and DHA was reflected in increasing of total polyunsaturated and n-3 FA in the eggs. Moreover, reducing the ratio of n-6 to n-3 FA to 5:1 decreased egg triglycerides, total cholesterol and cholesterol associated with low density lipoprotein and very low density lipoprotein. In conclusion, supplying EPA and DHA in the diet of laying hens positively influences performance, egg yolk FA profile and cholesterol. Practically, EPA and DHA could be used in laying hen diets to improve their performance and enhance public health of egg consumers.

Keywords: eicosapentaenoic, docosahexaenoic, laying hen, performance, egg yolk.

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INTRODUCTION

Hen eggs are an economic source of protein and fat for humans. But, there is a trouble owing to their cholesterol and fat content. Also, the high n-6 to n-3 FA ratio increases the problem of heart diseases (Simopoulos, 2006). The consumption of EPA and DHA FA is suboptimal, contributing to the problem of heart diseases in humans. The new qualified health claim for n-3 FA is to improve patient outcome measures (Food and Drug Administration, 2013). Animal nutrition has substantial ability to improve FA profile of animal products, reduce cholesterol content and decrease n-6 to n-3 ratio.

EPA and DHA have numerous health values. They are mainly related to cardiovascular diseases, central nervous system, mental health diseases and immune functions (Wassall and Stillwell, 2009; Riediger et al., 2009; Alexander et al., 2017). To our knowledge, the benefits of EPA and DHA for performance and health of laying hens are less investigated. This is the first paper used EPA and DHA with a definite concentration in laying hens diet and declare any deleterious effects of further substances associated with omega 3 FA sources.

Increasing the consumption of n-6 FA alters the FA profile of the phospholipids of cell membranes. Subsequently, increased linoleic and arachidonic acids which might alter gene expression and eicosanoid synthesis toward a pro-inflammatory state (Calder, 2012). Also, increasing n-3 FA consumption would increase these FA in the membrane phospholipids, which are expected to lessen inflammatory responses. So, it will be necessary to decrease the consumption of n-6 FA and increase the consumption of n-3 FA (Cottin et al., 2011).

The previous studies investigated the dietary supplementation with linseeds, linseed oil, algae bio mass, or fish oil. But in this experiment, a mixture of EPA and DHA was used with a definite dose to declare any deleterious substance which could affect hen performance. In this experiment, the design was towards fortification of hen eggs with EPA and DHA and studying its relation to egg yolk FA profile and cholesterol concentration. Therefore, the aim of this experiment was to study the influences of EPA and DHA supplementation on hen performance, lipid characteristics and FA profile in egg yolk.

MATERIALS AND METHODS

Laying hens management and diets

From 30 to 36 weeks of age, 180 Lohman brown laying hens were housed in the poultry experimental facility at the institute of Animal Health and Research, Tanta, Egypt, under standard conditions of lighting (16 h light), environmental temperature (25±2°C) and ventilation. The birds were randomly placed in 12 pens (15 hens per pen) containing deep litter of wood shavings equipped with round drinker, feeder and laying nest. The trial was designed with two dietary treatments and six replicates each (15 hens/replicate). Each diet consisted of constant basal components. In the experimental diet, the EPA and DHA supplement was added to obtain n-6: n-3 of 5:1. According to NRC (1994), the diets were formulated to be isonitrogenous and isocaloric (Table 1). During the experimental period, the feed was given as 110 g/hen/day, water was offered ad libitum.

Performance measurements

All birds were weighed individually at the start and the end of the experiment to determine the change in body weight. The eggs were recorded daily to calculate laying rate (%). The eggs weight and feed intake were determined to calculate feed conversion ratio and egg mass.

Chemical analyses of the eggs yolk

Eighteen eggs from each group were randomly collected. The egg yolk was separated from its albumen using a yolk separator, weighed and homogenized gently. The yolk samples were frozen at – 20 °C until used for analyses of triglycerides, cholesterol and FA profile. The total lipid of the egg yolk was extracted using the chloroform: methanol (2:1) method as explained by Folch et al., 1957. Yolk cholesterol concentration was determined according to Rotenberg and Christensen (1976) using cholesterol reagent and standard (Spinreact, Citra Coloma, Spain). FA profile of the egg yolk was analyzed according to Radwan (1978) using gas chromatograph (Hewlett Packard, 6890). The flame ionization detector and injector temperatures were set at 250 °C and 225 °C, respectively. The temperature was set from 130 °C to 225 °C at a rate 6 °C/min. Helium was used as a carrier

gas at a flow rate of 1 ml/min. A standard FA methyl ester mixture was used to identify all the FA peaks.

Table 1 Physical and chemical composition of the laying hen diets (%)

Ingredient %	Treatments*	
	Control	Omega 3
Corn grain, cracked	72.27	72.27
Soybean meal, without hulls	14.9	14.9
Corn gluten meal	3	3
Di calcium phosphate	0.76	0.76
Limestone	8	8
Salt	0.37	0.37
Methionine	0.03	0.03
Lysine	0.04	0.04
Premix†	0.3	0.3
Soya oil	0.33	-
EPA and DHA supplement‡	_	0.33
Chemical composition		
ME (Kcal/kg)	2910.9	2926
Crude protein %	15.34	15.34
Lysine %	0.69	0.69
Methionine %	0.32	0.32
Crude fat %	3.28	3.2
Linoleic acid %	1.82	1.66
EPA, DHA %	-	0.25
Calcium %	3.3	3.3
Available phosphorus %	0.26	0.26
Sodium %	0.15	0.15
Chloride %	0.27	0.27
Potassium %	0.53	0.53

*Treatments represent the control group which was maintained at n-6: n-3 ratio of 18.8:1 while in the omega 3 group, the ratio was 5:1.
†Provided per kilogram of diet: Retinol, 5500 IU; Cholecalciferol, 1,250 IU; Vitamin E (dl-alpha-tocopherylacetate), 12 IU; menadione, 2.5mg; riboflavin, 6 mg; calcium pantothenate, 8 mg; niacin,15 mg; pyridoxine 2 mg; folic acid, 1 mg; vitamin B12, 7μg; Mn, 50 mg; Zn, 55 mg; Fe 40 mg; Cu, 4 mg; I,2 mg; Co, 0.3 mg; ethoxiquin, 150 mg.
‡EPA and DHA supplement: (Berkley and Jensen) soft gel capsules composed of EPA and DHA 500 mg.

Statistical analysis

Variance of data was analyzed using the ANOVA procedure (Goodnight, 1979) for analysis of variance. Differences due to treatment were considered significant at $P < 0.05$.

RESULTS

Feed intake and laying performance

Feed intake did not significantly differ between the control and the omega 3 groups (Table 2). Subsequently, the intake of total FA was nearly similar (Table 2). But, intake of linoleic and total n-6 FA increased in the control group. While, the intake of EPA, DHA and total n-3 FA increased in the omega 3 group (Table 2). Body weight of hens in both groups remained nearly stable throughout the experiment (Table 2).

Table 2. Effect of dietary eicosapentaenoic and docosahexaenoic fatty acids on feed intake and laying hen performance.

Item	Treatment1		P-value
	Control	Omega 3	
Feed intake, g/d	102.4± 1.51	105.8±1.19	–
FA intake, g/d	3.02±0.04	3.06±0.04	–
Linoleic	1.86±0.12^{a}	1.75±0.07^{b}	*
EPA + DHA	0.0±0.0^{b}	0.27±0.01^{a}	**
Total n-6	1.63±0.1^{a}	1.51±0.06^{b}	*
Total n-3	0.08±0.01^{b}	0.33±0.01^{a}	**
Initial body weight (kg)	1.59±0.03	1.65±0.02	–
Final body weight (kg)	1.62±0.03	1.68±0.08	–
Body weight gain (kg)	0.03±0.04	0.03±0.08	–
Egg production, %	67.23±1.5^{b}	76.89±0.8^{a}	**
Egg weight, g	56.11±2.0	55.22±2.0	–
Egg mass, g	37.72±1.34^{b}	42.46±1.62^{a}	*
Feed conversion ratio	2.72±0.1^{a}	2.49±0.1^{b}	*

*Treatments represent the control group which was maintained at n-6: n-3 ratio of 18.8:1 while in the omega 3 group, the ratio was 5:1. Values±SE with different superscripts within a row represent differences among treatments; *P<0.05 or **P<0.01.

Egg production increased in the omega 3 group and this response was consistent throughout the study (Table 2; Figure 1). Also, FCR decreased in the omega 3 group (Table 2). Throughout the study, hens fed EPA and DHA FA had a consistently weightier egg mass output compared with those in the control group (Table 2).

Fatty acid profile of egg yolk

FA profile of egg yolk was significantly altered by dietary EPA and DHA (Tables 3). The saturated fatty acids (SFA) were decreased, while poly-unsaturated fatty acids (PUFA) were increased (P<0.05). Whereas no significant effect (P>0.05) in the contents of monounsaturated fatty acids (MUFA) (Table 3).

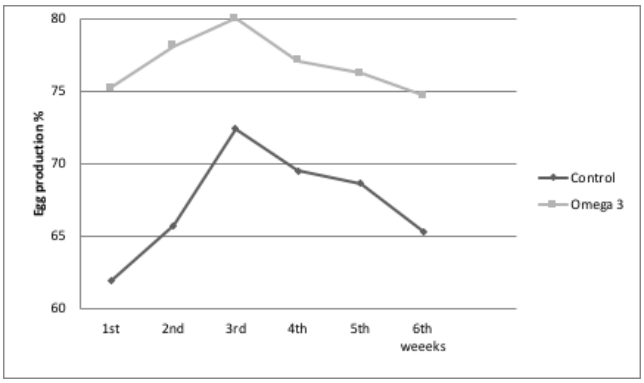
Table 3. Effect of dietary eicosapentaenoic and docosahexaenoic fatty acids on egg yolk fatty acids profile

Fatty acid, %	Treatment*		P-Value
	Control	Omega 3	
Myristic (C14:0)	0.54±0.03	0.51±0.02	–
Petadcaoic (C15:0)	0.38±0.02[a]	0.31±0.02[b]	**
Palmitic (C16:0)	24.38 ±0.49[a]	22.19 ±0.52[b]	**
Palmitoleic (C16:1)	3.57±0.16	4.08±0.1	–
Margaric (C17:0)	0.48±0.04[a]	0.29 ±0.04[b]	**
Stearic fatty acid (C18:0)	16.49±0.31[a]	12.33±0.37[b]	**
Oleic (C18:1)	39.88±0.54	40.22±0.55	–
Elaididic (C18:1 trans2)	0.75±0.04	0.67±0.03	–
Linoleic (C18:2n-6)	10.51±0.28	10.68±0.22	–
Linolenic (C18:3n-3)	0.72 ±0.04[b]	1.93 ±0.13[a]	**
Arachidonic (C20:4n-6)	0.92±0.02[a]	0.85±0.03[b]	*
EPA (C20:5n-3)	0.23±0.03[b]	1.52±0.06[a]	**
DPA (C22:5n-3)	0.46±0.02[b]	1.45±0.07[a]	**
DHA (C22:6n-3)	0.69±0.03[b]	2.97±0.13[a]	**
SFA	42.27±0.62[a]	35.63±0.67[b]	**
MUFA	44.20±0.65	44.97±0.56	–
PUFA	13.53±0.26[b]	19.40±0.28[a]	**
n-6 FA	11.43±0.28	11.53±0.21	–
n-3 FA	2.1±0.04[b]	7.87±0.16[a]	**

*Treatments represent the control group which was maintained at n-6: n-3 ratio of 18.8:1 while in the experimental group, the ratio was 5:1. Values±SE with different superscripts within a row represent differences among treatments; *P<0.05 or **P<0.01. EPA, eicosapentaenoic. DPA, Docosapentaenoic ;DHA, Docosahexaenoic; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

As it was hypothesized that increasing dietary EPA and DHA would increase them in the eggs of hens (Table 3) when compared with the control one. Subsequently, the ratio of n-6 to n-3 FA was decreased (P < 0.05) in egg yolk of the treated group. Although there was no significant effect on egg yolk linoleic acid concentration in both groups. Arachidonic acid was decreased (P<0.05) in omega 3 treated group.

Figure 1. Effect of dietary eicosapentaenic and docosahexaenoic fatty acids on egg production of laying hen.



Egg yolk lipid characteristics

Egg yolk weights of both groups remained unchanged during the experiment (Table 4). There were a significant decrease (P< 0.05) in the levels of egg yolk triglycerides, total cholesterol, VLDL cholesterol and LDL cholesterol in the n-3 FA supplemented group. While HDL cholesterol persisted unchanged in the two groups (Table 4).

DISCUSSION

Feed intake and change in body weight were not significantly different among diets. Similar results were published by Charlotte et al., (2013) who fed laying hens 5% and 10% microalgae supplement contained 1.5% EPA. In other study, feed intake and body weight were not changed in hens fed 2% deodorized menhaden oil, but feed intake only was decreased at a level of 4% (Gonzalez-Esquerra and Leeson, 2000). That was explained by the high metabolizable energy of menhaden oil than vegetable oil. In our study, the diets contained similar metabolizable energy (Table 1). In the current study, the egg production significantly increased (P<0.05) in hens fed diet contained EPA and DHA. Numerical increase (P>0.05 but <0.1) in egg production was

Table 4. Effect of dietary eicosapentaenoic and docosahexaenoic fatty acids on egg yolk lipids characteristics.

Item	Treatment*		P-Value
	Control	Omega 3	
Egg yolk weight (gm)	14.37±0.54	14.04±0.51	–
Triglycerides (mg/egg yolk)	157.6[a]±8.3	130.1[b]±6.3	*
Total cholesterol (mg/egg yolk)	166.1[a]±6.4	146.5[b]±5.6	*
Total cholesterol (mg/g egg yolk)	11.66±0.59	10.5±0.44	–
LDL mg/egg yolk	93.2 [a] ±5.9	78.4 [b]±6.2	–
HDL mg/egg yolk	41.32±1.8	42.02±0.94	–
LDL/HDL ratio	2.33±0.25	1.88±0.17	–
VLDL mg/egg yolk	31.51 [a] ±1.7	26.03 [b]±1.3	*

*Treatments represent the control group which was maintained at n-6: n-3 ratio of 18.8:1 while in the experimental group, the ratio was 5:1. Values±SE with different superscripts within a row represent differences among treatments; *P<0.05 or **P<0.01. LDL: low density lipoprotein. HDL: high density lipoprotein. VLDL: very low density lipoprotein.

reported by Lawlor et al., (2010) for hens fed 4% and 6% fish oil. Other trials in which flaxseeds were fed showed egg production remain unchanged (Bean and Leeson, 2003) or decreased (Aymond and Van Elswyk, 1995). Comparisons the current and the previous results in the literature, our results showed a positive effect of EPA and DHA than the other n-3 FA sources (fish and linseed oils) with respect to feed intake, palatability and egg production as mentioned previously. These differences might be due to the anti-nutritional factors in flaxseed which impair utilization of energy yielding nutrients (Bean and Leeson, 2003). Other factors influence egg production such as hen's strain and age (Scheideler et al., 1998), changes in diet composition (Gonzalez-Esquerra and Leeson, 2001) and feed formulation based on calculated energy value (Ilse et al., 2012).

Laying hens fed EPA and DHA had a weightier egg mass output compared with those fed the control diet. This indicated that supplementation of EPA and DHA increased feed efficiency in hens, probably as a consequence of altered nutrient partitioning by reducing inflammatory response (Komprda, 2012). This hypothesis will require further investigation of the anti-inflammatory effect of EPA and DHA in hens.

These results showed that the diet supplemented with EPA and DHA had no significant influence on egg weight, in agreement with other data (Caston and Leeson, 1990; Aymond and Van Elswyk, 1995; Ferrier et al., 1995; Bean and Leeson, 2003; Charlotte et al., 2013). Some researchers (Caston et al., 1994; Scheideler and Froning, 1996) observed a decrease in egg weight of hens fed flaxseed. Based on the literature, our results have shown the advantage of EPA and DHA over flaxseed as a supplier of n-3 fatty acid for laying hens. Caston et al. (1994) observed reduced metabolizable energy in hens fed diets augmented with flaxseed. Along with anti-nutritional factors in flaxseed which reduce absorption efficiency of energy yielding nutrients (Bean & Leeson, 2003).

Increasing PUFA/SFA ratio in eggs could reduce LDL-cholesterol level in serum of egg consumers (Grundy and Denke, 1990). Also, low SFA reduces development of heart disease and disorders related to blood vessels (Simopoulos, 1997). Concerning to the influences of EPA and DHA on egg yolk FA profile, our results revealed that there were a significant reduction in SFA (C18:0, C16:0, C17:0, C15:0), whereas the contents of MUFA were not altered. Egg yolk from hens fed EPA and DHA contained more UFA than those fed the control diet, indicating that saturation of FA was depressed by dietary EPA and DHA. The results of this experiment were nearly similar to the previous experiment of Cachaldora et al., (2008) who compared effects of various types of fatty acids in hen diets and concluded that fish oils increased EPA and DHA in egg yolk. While, soybean and linseed oil increased LA and ALA, respectively at the expense of monounsaturated fatty acids. As in previous studies (Aymond and Van Elswyk, 1995; Ferrier et al., 1995; Scheideler and Froning, 1996) who have been used flaxseed in hen's diet. They observed that yolk ALA increased proportionally to flaxseed amount. But, content of DHA increased to a smaller extent, not in lined response to flaxseed amount, and content of EPA hardly increased. The results confirms the connection between dietary lipids and egg lipid composition because

hens supplemented with EPA and DHA produced significantly eggs fortified with n-3 FA than those fed the control diet. Subsequently, the n-6/n-3 ratio has been decreased in that eggs. Similar findings have been reported by Yannakopoulos et al., (2005); Yalcyn et al., (2007). Ilse et al., (2012) has reviewed 26 publications concerned with n-3 FA in laying hens and observed that laying hens as well as human have limited ability to convert ALA into DHA or EPA. Previous and current data show the importance of feed supplementation with EPA and DHA to produce eggs fortified with n- 3 FA essential for human.

Hens Supplemented with EPA and DHA showed lower values of arachidonic acid ($P < 0.05$) than those fed the control diet which was consistent with the previous results of Cherian et al., 2007. Hens in the control group consumed more linoleic acid than those supplemented with EPA and DHA. Thus, our results indicated that, there was a positive relation between the consumption of linoleic acid and the denovo synthesis of arachidonic acid. Supplementation of EPA and DHA had a significant reduction of egg yolk triglycerides, total cholesterol, LDL-cholesterol and VLDL-cholesterol. This effect may be due to the influence of n-3 FA in increasing hepatic oxidation (Mashek et al., 2002). Also, it was reported that increasing intake of n-3 FA reduced fat deposition in spite of similar caloric intake which reinforces the influence of n-3 FA in nutrient partitioning (Buckley and Howe, 2010). Increasing consumption of n-3 FA increased concentration of these FA in liver cells, which increased hepatic expression of gluconeogenic enzymes (Bilby et al., 2006 and Do Amaral, 2008). Therefore, alteration of FA profile of hepatic tissue could influence metabolism and synthesis of other nutrients, consequently affect nutrient partitioning and favor egg production of hens. Moreover, Steinhilber, (2005), noticed that hens with high egg production rates have lower egg cholesterol level compared to those with low egg production rate. Ginzberg et al., (2000) found a reduction in blood cholesterol level but were incapable to decrease egg yolk cholesterol after feeding with red algal biomass for only 20 days. They hypothesized that longer feeding red algal biomass might decrease egg cholesterol level.

CONCLUSION

The ratio of n-6 to n-3 of 5 to 1 using EPA and DHA in the diet of laying hens improved egg production and efficiency of feed conversion into egg mass. These results were not on the expense of body weight which indicates that EPA and DHA favor nutrient partitioning for egg production.

The nutritional quality of eggs has been increased with respect to high PUFA/SFA ratio, low n-6/n-3 ratio, low triglycerides and low cholesterol content. Further research should be conducted to evaluate influences of EPA and DHA on hepatic metabolism and gene expression in hens.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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