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Artigo

The effect of supplementation of diet with different amounts of fish oil and rapeseed oil on performance and egg quality in laying hens

Efeito da suplementação de dieta com diferentes quantidades de óleo de peixe e óleo de colza no desempenho e na qualidade dos ovos em galinhas poedeiras

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Abstract: In this study investigated effect of the different levels of fish and rapeseed oils, selenium, vitamin E and zinc in diet laying hens288 white-line layers were used from 45 weeks of age during 90 days. The performance and quality traits including egg weight, intake of feed, egg laying percentage, egg shell weight, white weight, weight and color of yolk were evaluated during the experiment. The results showed that the effect of adding of these supplements to diet of laying hens on performance (egg weight, egg production and feed intake) was not significant. An insignificant increase in yolk weight and significant egg yolk color were observed in treatments containing 2% fish oil and 2% rapeseed oil at a level of 0.2% selenium. A significant decrease in triglyceride levels was observed in treatments containing higher selenium levels. There was a non significant reduction in its cholesterol levels. The amount of vitamin E and selenium in egg yolk increased significantly with increasing fish oil level in diet. Also, higher levels of selenium plus fish oil in the diet increased the amount of useful fatty acids (omega-3) and reduced some unhelpful saturated and unsaturated fatty acids. This study showed that using organic selenium, vitamin E and zinc in diets, although didn't affect performance of hens, but could reduce the level of cholesterol and triglyceride yolk and increase its peroxidation resistance.

Keywords: Laying hens, Fish oil, Rapeseed oil, performance production traits, Egg quality

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Introduction

Nowadays, the many studies investigate the effects of enrichment of diets by the use of lipids or vitamins on the performance and products of animals and also, the effects of nutrient levels in the food product on human health. Among of all the foods that can be rich, egg has a special place due to its high consumption (BOURRE 2005).

Some of researchers have reported that omega-3 fatty acids are unsaturated and more complex chain of unsaturated fatty acids with multiple bonds such as ducozha-azanoic acid (DHA) which enhance the immune system's strength (MOLFINO et al. 2014).

Alpha-linolenic acid (LNA) has little or no metabolic activity in the human body unless was converted into to DHA (DHA). Ducosupentaenoic acid (DPA) is an intermediate compound that plays a role in the conversion of DHA to EPA. Human ability to convert ALA to EPA and DHA is limited and unpredictable and it decreases with increasing age (MOLFINO et al. 2014).

Essential sources of plant and marine are rich in omega-3 fatty acids but fish oil has a significant amount of three omega-3 fatty acids such as EPA, DPA and DHA, in comparison to other vegetable oil sources such as turnip and flaxseed oil. Therefore, one of the most important sources of omega-3 enrichment is animal products.

Some years ago, when chickens like other domestic animals could use vegetables as food, their eggs and meat contained significant amounts of omega-3 fatty acids (Farrell 1995). It has also shown that wild birds with more concentrations of eggs of omega-3 fatty acids (LESKANICH AND NOBLE 2007).

ezapapentaenoic acid (EPA), ducosupentaenoic acid (DPA), and

Food sources Omega-3 fatty acids can be divided into two categories of plant and animal products. Plant sources of this type of fatty acids contain high amounts of LNA, but other levels of omega-3 fatty acids are low in these categories of food sources. Among the plants that contain these fatty acids are fennel, walnut, soya, canola oil, and some green plants such as some algae (KUMAR et al. 2016).

Animal sources of these fatty acids contain high levels of EPA and DHA fatty acids and low levels of LNA. Among the animal resources, fish are rich in these kinds of fatty acids. Therefore, the eggs of hens fed with certain amounts of fish oil are also a source of nutrients for omega-3 fatty acids for humans. Using a good combination of vegetable oils and fish oil can provide good results in increasing the amount of omega-3 fatty acids in egg yolks. The use of omega-3 fatty acids in vegetable or marine oils along with appropriate levels of vitamins, especially vitamins A and E in poultry diets, has a significant effect on improving health and increasing animal performance. Particularly they greatly increase the amount of omega-3 fatty acid precipitate in meat and concentration of fat-soluble vitamins in blood (BEYNEN 2004; GALEA 2003). Ultimately, omega-3 was used by humans that reduces cardiovascular disease, improves health, that total amounts of omega-3, EPA, DPA, and DHA fatty acids in carcass were significantly increased. Feeding the hens on 8, 4, 0 and 12% of fish powder did not have an effect on mortality, feed intake and body weight. However, with increasing levels of fish powder to diet, EPA, DHA and omega-3 fatty acids increased in eggs. Also, all treatments increased the amount of omega-3 fatty acids in breast meat. In general, adding fish oil to diet increases the ratio of omega-3 to omega-6 in total tissues (HULAN et al. 1988). Al-Sultan (2005) reported that increasing omega-3 level of diet play a protective role in coccidiosis in poultry.

Because of the effect of simultaneous application of different and increases the life longevity of humans (BOURRE 2005).

Hulan et al. (1988) by adding 5% of fish powder to laying hens diet, found amounts of fish oil and rapeseed oil in diet has not been done on the performance products of laying hens and the biochemical quality of eggs, as yet. The present study was designed to evaluate and compare the different amounts of this compound in the laying hens' diet.

Materials and Methods

A total of 288 laying hens at the lying eggs peak were used in the poultry farm of Maragheh Islamic Azad University. Diets include rapeseed and fish oil at a compound level of 4% combined with two moderate and high levels of organic selenium (Selplex Organic Selenium Formation from Saccharomyces cerevisiae) and the same levels of Organic Zinc Bioplex and Vitamin E was prepared (Table 1).

Table 1- used treats in study

	4% Oil	Selplex mg/kg	Bioplex Zn mg/kg	Vit.E mg/kg
Treat1	3%Repeseed+ 1%Fish	0.1	50	50
Treat2	3%Repeseed+ 1%Fish	0.2	50	50
Treat3	2%Repeseed+ 2%Fish	0.1	50	50
Treat4	2%Repeseed+ 2%Fish	0.2	50	50

All diets have been formulated according to NRC (Council 1994) recommendations and have been uniform in terms of energy, protein, fiber, amino acids and minerals (Table 2).

The experiment was conducted using a completely randomized CRD-based design (4 treatments each with four replications) for 90 days.

Table 2- Components and compositions of control and experimental diets

Food components	treat	Control
Corn grain	40.00	50.50
Soybean *Meal*	21.75	22.5
Wheat	24.50	11.00
Starch	0.00	6.00
Oil additives[1]	4.00	0.00
Oyster Powder	8.00	8.00
powder of bone	1.35	1.20
DDL-Methionine	0.20	0.10
Salt	0.20	0.20
Vitamin supplement[2]	0.25	0.25
Mineral supplement[3]	0.25	0.25
	Calculated nutrient compositions (on dry matter)	
Metabolism energy (kcal / kg)	2869	2813
Crude protein (%)	15.78	15.55
Crude fiber (%)	3.15	3.28
Crude fat (%)	5.25	2.43
Calcium (%)	3.40	3.34
Available phosphorus (%)	0.33	0.33
Lysine (%)	0.85	0.80

[1] Rapeseed or Fish oil. [2] Per kilogram of supplied vitamins: 3520000 IU vit.A, 1000000 IU vit. D_3, 4400 IU vit. E, 880 mg vit. K_3 , 738.5 mg vit. B_1, 1600 mg vit. B_2, 3136 mg vit. B_3, 13860 mg vit. B_5, 984.8 mg vit. B_6, 192 mg vit. B_9, 4 mg vit. B_{12}, 60 mg biotin, 80000 mg choline chloride 80000 and 400 mg anti oxidant. [3] Per kilogram of supplied minerals: 25870 mg Zn, 30000 mg Fe,29760 mg Mn, 2400 mg Cu,346.8 mg I 346/8 and 80 mg Se .

Measurement of hen performance traits

The performance traits including egg weight, daily feed intake, feed efficiency and egg percentage were determined during the experiment.

Feed intake was measured every two weeks, and on the last day (end of each period) the remaining food was collected from each unit and the total diet was deducted. Then, the amount of feed consumed (each hen per day) by dividing the amount of feed consumed by that unit of test each hen per day of the same unit of experiment was obtained.

Measurement of egg quality traits

Egg quality traits including egg shell weight, white weight, yolk weight and color, cholesterol, triglyceride, fatty acid content, selenium, vitamin E and zinc (Zn) content of egg yolk were evaluated during the experiment.

The weight of shell was weighed by a precise scale (0.1 mg accuracy) after removing the contents of the egg with a clean paper bag in open air. For measuring the white quality was used of the unit hough. It is the most common way to measure the quality of white egg.

The higher the unit shows the better the quality. The height of white was measured about 1 cm from the edge of the yolk using a special instrument for altimeter. The formula of the unit is as follows:

HU=100Log (H+7.57- 1.7 W0.37)

HU = Haugh unit, H = White height (mm), W = Egg weight (g)

Egg yolk color was determined based on color scale. A device that contains a number of standard colors from bright yellow to red and orange is used for this purpose. After breaking the egg on a flat surface, the color of the egg was determined and the number of the color was recorded for the egg yolk color test.

Analysis of cholesterol, triglyceride, TBA or MDA and fatty acids in egg yolk

At mid and the end of the experiment, selected egg samples (two samples from each replicate) were transferred to the lab and the concentration of triglyceride, yolk cholesterol and fatty acid composition was determined using the Folch et al. (1957) method.

TBA values or thiobarbituric acid based on the formation of malondialdehyde (MDA) in fresh eggs were determined. MDA (the second oxidation product), according to a descriptive TBA method, was measured (using spectrophotometer with some possible variations in the analysis process) by Botsoglou et al. (1994). Yolk fatty acids (C14-24) were analyzed by gas chromatography (1000-GC model manufactured by Dany Italy).

Finally, the traits were analyzed after the normalization test using SAS 9.1 software. A P-value of <0.05 was considered for significant differences among groups and the comparison of means was done by using Duncan's multiple range test.

The statistical model was as follows:

$y_{ij} = \mu + t_i + e_{ij}$

y_{ij}= Observed values of each dependent variable

μ=Overall mean

t_i=Effect of treatment

e_{ij}=Random error

Results and discussion

Effect of dietary rations on the performance of laying hens

The results of this study showed that differences between treatments with control in laying hens of white-lines strain were not significant on performance traits (egg weight, egg production, and feed intake) (P<0.05). Also, the production traits were not affected by the vitamin level or the type of supplemented oil in the diet (P<0.05) (Table 3). These results were confirmed by Meluzzi et al. (2000) who used different oils for enrichment of laying hen eggs (fish oil and fat at 3% level with different levels of vitamin E), and also

Dalle Zotte et al. (2015) feeding the laying hens in a diet containing 3% fatty fish oil. These results are also consistent with a study by Al-Sultan (2005), which showed no change in the production of eggs. Yannakopoulos et al. (2005) did not show any significant effect on the yield of broiler laying hens by studying the effect of dietary enrichment with a set of omega-3 rich herbs. However, Novak and Scheideler (2001) reported that feed intake for hens fed with 10% of flaxseed oil in the diet was significant higher than those that received diets containing soya oil (P<0.05). In contrast, Basmacıoğlu et al. (2003), who used 1.5% fish oil and 4.24% flaxseed oil, reported that food intake was not affected by any of the treatments.

Also, the findings of the present study are consistent with the results of research done by other researchers as (GALOBART et al. 2001a; GALOBART et al. 2001b; GEBERT et al. 1998; SIM et al. 1994).

Table 3- Comparison of performance of laying hens fed by two oil sources and two levels of selenium and the same levels of vitamin E and zinc

Treats Performance traits of hens	1	2	3	4	[2]SEM	P value[1]
Egg weight (g)	66.96	66.38	67.35	68.45	0.21	NS
Feed intake (g/b/d)	121.44	121.55	121.45	121.47	--	NS
Feed efficiency (kg/kg)	2.33	2.34	2.34	2.33	--	NS
Egg production (%)	84.27	84.29	84.36	84.34		NS

[1]p<0.01= **, p<0.05=*, p>0.05= non significant (ns), [2] Standard error of mean of twelve observations from each treatment

Treat1: 3%Repeseed+ 1%Fish + 0.1 mg/kg Selplex + 50 mg/kg vit. E + 50mg/kg Zn (Bioplex)
Treat2: 3%Repeseed+ 1%Fish + 0.2 mg/kg Selplex + 50 mg/kg vit. E + 50mg/kg Zn (Bioplex)
Traet3: 2%Repeseed+ 2%Fish + 0.1 mg/kg Selplex + 50 mg/kg vit. E + 50mg/kg Zn (Bioplex)
Treat4: 2%Repeseed+ 2%Fish + 0.2 mg/kg Selplex + 50 mg/kg vit. E + 50mg/kg Zn (Bioplex)

Effect of diets on qualitative traits of laying hens

Among the qualitative characteristics of eggs, an non significant increase in yolk weight and a significant increase in egg yolk were observed in treatments containing 2% fish oil and 2% rapeseed oil, and especially 0.2% selenium (P<0.05). Qi and Sim (1998), Galobart et al. (2001b), Galobart et al. (2001a) did not observe significant effects on yield and quality traits of eggs by performing similar studies. Meluzzi et al. (2000) showed an insignificant increase in the percentage of yolk, after 2 and 4 weeks, in the hens were fed with diets supplemented with 4% oil (fish + canola) in comparison with the control diet. On the other hand, Van Elswyk et al. (1992) who used menhaden oil and vegetable-animal compound oil, as a food supplement, found significant differences in yolk weight and even egg weight.

These results were also confirmed by VAN ELSWYK (1997). Scheideler and Froning (1996) found a significant decrease in weight and color of egg yolk in the hens fed with 5 to 15% flax oil and 1.5% fish oil, and this was related to the effect of long chain fatty acids on hen estrogen activity. Whitehead et al. (1993) found that the decrease in egg size was associated with a decrease in the plasma estradiol concentration and they concluded that there is a nutritional control of high chain fatty acids on the hormonal metabolism of birds. Yannakopoulos et al. (2005), Al-Sultan (2005) and Basmacıoğlu et al. (2003) observed no significant effect on shell weight and yolk color after feeding hens with different sources of omega-3.

The role of selenium as an antioxidant can be a factor in improving the color of egg yolk. Yolk color is produced by oxycarotinoids known as xanthophyllic pigments, derived from diets, especially corn. Oxycarotenoid pigments are soluble in fat and have a similar pathway to fat through the intestine. These materials lose their pigmentation under the influence of oxidation. Therefore, the color of the yolk seems to be dependent on the antioxidants and the oxycarotenoids' stability in the diet or body (MOHITI-Asli et al. 2010). In a study, Beynen (2004) showed that vitamin E has beneficial effects on the stability of egg yolk in hens fed from vitamin E-rich rations. In the present study, the change in yolk color can be affected by the presence of same levels of vitamin E in the diet to support more antioxidant diets and liver transplants. However, some researchers such as Bourre (2005), reported that the addition of vitamin E to laying hens under stress did not have any effect on egg yolk color.

Egg yolk color is an important factor for the buying and selling of eggs. In the egg of hens which fed with 4% oil (rapeseed and fish oil), especially with a level of 0.2 selenium, 50 mg / kg of vitamin E and zinc, was higher than other groups. Some researchers reported this result is due to the presence of natural factors such as lutein or xanthine (colored substances) that there are in foods such as corn with different percentages, and so, it reported a decrease in the percentage of use of foodstuffs due to the use of oils to provide dietary energy. Today, typical eggs are popular by the addition of artificial pigments to the diet of laying hens.

Therefore, if yolk color is one of the factors of consumer satisfaction, industrial pigments can added to enriched omega-3 or omega-6 diets, enriched eggs are more likely to enter the basket of households. The Haugh unit or white height index in both experiments was not affected by the source of oil and selenium levels (Table 4).

Table 4- Comparison of egg internal quality traits of laying hens fed by two oil sources and two levels of selenium and the same levels of vitamin E and zinc

Treats egg internal quality traits	1	2	3	4	²SEM	P value¹
Albumin weight (%)	62.05	62.30	62.58	62.18	0.10	NS
Yolk weight (%)	28.56	28.40	31.25	31.44	0.58	NS
Shell weight (%)	8.64	8.36	8.88	8.95	0.33	NS
Haugh unit	127.46	126.20	127.33	128.24	0.29	NS
Yolk color	4.95c	5.47b	5.86b	6.35a	0.11	*
Shell height ($mm*10^2$)	29.65	30.11	29.80	30.08	0.23	NS
Shell strength (kg/cm^2)	2.50	2.49	2.53	2.55	0.09	NS
yolk pH	6.04	6.05	6.50	6.70	0.01	NS
albumin pH	8.45	8.40	8.45	8.37	0.03	NS

[1]p<0.01= **, p<0.05=*, p>0.05= non significant (ns), [2] Standard error of mean of twelve observations from each treatment

Treat1: 3%Repeseed+ 1%Fish + 0.1 mg/kg Selplex + 50 mg/kg vit. E + 50mg/kg Zn (Bioplex)
Treat2: 3%Repeseed+ 1%Fish + 0.2 mg/kg Selplex + 50 mg/kg vit. E + 50mg/kg Zn (Bioplex)
Traet3: 2%Repeseed+ 2%Fish + 0.1 mg/kg Selplex + 50 mg/kg vit. E + 50mg/kg Zn (Bioplex)
Treat4: 2%Repeseed+ 2%Fish + 0.2 mg/kg Selplex + 50 mg/kg vit. E + 50mg/kg Zn (Bioplex)

Effect of different treatments on egg yolk biochemistry Cholesterol and triglyceride

In the results of biochemical traits of yolk, a significant decrease in triglyceride levels in 2 and 4 treatments containing higher levels of selenium (0.2% selenium) compared to 1 and 3 (0.1% selenium) treatments in the first week as well as later It was observed from one month of maintenance (P<0.05), while a similar but not significant change was observed in cholesterol levels (P<0.05).

Overall, the cholesterol concentration in the first and second months of storage and the concentration of triglyceride increased significantly in the second month of storage compared to the first month of storage (P<0.05).

Farrell (1995) reports that omega-3 fatty acids (especially the acid eicosapentaenoic acid and decocoa aqueous acid) in fish oil reduce low-density lipoprotein levels (VLDL) in blood. This reduces the free rotation of low-density lipoprotein (LDL) concentrations in the blood, which is usually left in the tissue for fat storage or directly deposits in the arteries and reduces the synthesis of triglycerides in the liver. Bourre and Galea (2006) reported that the health effects of omega-3 and antioxidant sources such as vitamin E, highly regarded by nutritionists because of the health of the bird, as well as a healthier product by it.

Bourre and Galea (2006) showed a decrease in cholesterol level in vitamin A, B12, B2, B5 and phosphorus-rich eggs. Yannakopoulos et al. (2005) reported that the levels of egg cholesterol decreased by omega-3 rich dietary herds compared to controls. Butarbutar (2004) reported the level of cholesterol and triglyceride in the yolk is influenced by changes in their concentration in the blood. Increasing the lower density cholesterol (LDL) and plasma VLDL in comparison with HDL-cholesterol (good cholesterol) in blood increase the level of bad cholesterol and Triglyceride in the yolk or tissue.

Al-Sultan (2005) investigated the effect of fish oil diet on the performance and fat composition of laying hens, and reported that adding 1.5 or 3 percent of fish oil to the diet had a significant effect on the reduction of total fatty acids, triglycerides, cholesterol, lipoprotein Low-density (LDL) and very low-density (VLDL). On the other hand, Rustan et al. (1988) had reported that methyl ester of ezosapentaenoic (EPA) which is one of the most useful omega-3 fatty acids plays a significant biological role in the body and indirectly reduces the concentration of triglycerides in the blood and the tissue or egg yolk. EPA synthesizes and secretes a substance called triacylglycerol by reducing the activity of aceyl coenzyme A which acts as a component of 1 and 2 diasylglycerol. According to the presence of vitamin E as an antioxidant in the diet containing oil protects lipid peroxidation (especially EPA or DHA more prone to oxidation). Therefore, it is another effective factor in increasing the EPA level and decreasing cholesterol or triglyceride concentrations in the yolk.

Fat peroxidation

By adding more selenium to oil, a significant decreased in the level of yolk fat peroxidation was observed in the first week, after one and especially two months ($P < 0.01$) (Table 5).

Thus, increasing malondialdehyde levels in the first week of storage in treatments 3 and 4 containing selenium and more fish oil (unsaturated and therefore more susceptible to peroxidation) and after 1 and 2 months in treatments 1 and 2 containing selenium with Rapeseed oil (3%) was more than other treatments ($P < 0.01$).

Table 5- Mean of biochemical traits of egg yolk of laying hens fed by two oil sources and two levels of selenium and the same levels of vitamin E and zinc (In different periods breeding)

treat	Yolk lipid peroxidation (MDA) (ng/g)			Triglyceride (mg/g yolk)			Cholesterol (mg/g yolk)		
	1week	1mon	2mon	1wek	1mon	2mon	1week	1mon	2mon
1	2.30b	3.41a	5.39a	896.5a	911.7 b	919.5	187.0	190.3	202.0
2	2.09c	3.35b	5.02b	821.5c	854.5 b	881.8	175.5	177.1	195.5
3	3.05a	3.98a	6.18a	900.2a	920.5 a	925.2	194.1	190.5	212.5
4	2.54a	3.86a	5.95a	855.7b	880.3 a	914.5	174.5	178.0	188.5
SEM[2]	0.50	0.35	0.61	16.65	28.56	35.30	35.09	16.22	21.58
P*value*[1]	**	**	**	*	*	**ns**	**ns**	**ns**	**ns**

[1]p<0.01= **, p<0.05=*, p>0.05= non significant (ns), [2] Standard error of mean of twelve observations from each treatment
Treat1: 3%Repeseed+ 1%Fish + 0.1 mg/kg Selplex + 50 mg/kg vit. E + 50mg/kg Zn (Bioplex)
Treat2: 3%Repeseed+ 1%Fish + 0.2 mg/kg Selplex + 50 mg/kg vit. E + 50mg/kg Zn (Bioplex)
Traet3: 2%Repeseed+ 2%Fish + 0.1 mg/kg Selplex + 50 mg/kg vit. E + 50mg/kg Zn (Bioplex)
Treat4: 2%Repeseed+ 2%Fish + 0.2 mg/kg Selplex + 50 mg/kg vit. E + 50mg/kg Zn (Bioplex)

Generally, treatments containing 2% fish oil + 2% rapeseed oil were affected by peroxidation, although they were adequately protected by selenium, zinc or vitamin E. This means that protective and protective additives such as vitamins and elements in the treatments containing 1% fish oil + 3% rapeseed oil had more protection against yolk fat peroxidation.

Galobart et al. (2001b) observed that the feeding of laying hens from a diet containing 5% of a plant-rich source of omega-3 (flax oil) and different levels of vitamin E (0, 50, 100 and 200 mg / kg) decreased egg quality in hens fed diets containing oil and vitamin E (especially 100 and 200 mg per kg) after one and two months storage. Our observations are consistent with the obtained results by other researchers (Galobart et al. 2001b; Gebert et al. 1998; Qi and Sim 1998) that have been working on the enrichment of eggs by sources of unsaturated fatty acids. They also showed that the role of natural antioxidants such as tocopherols (vitamin E), carotenoids (vitamin A), and fosvitin is very important in increasing the resistance of fresh eggs to lipid peroxidation. On the other hand, Das et al. (2001) reported that the structure of the yolk LDL could be affected by the amount of egg resistance to fat peroxidation. They believe that phospholipids and proteins are the two main substances in the apparent structure of the yolk LDL that prevent the entry of oxygen into particle fat. When the eggs are stored, the amount of LHP (lipid hydroperoxide) is about 3.1 to 11.8 times and TBA (thiobarbituric acid) increases by about 2.5 to 3.3 times, and these ratios are dependent on the presence of alpha-tocopherol (vitamin E). Long-term storage of eggs can increase lipid oxidation in two ways: the temperature of the environment during storage, which increases the formation of free radicals, also, the breackdown of the structure of LDLs, which would facilitate the reaction between lipids and oxygen, it increases lipid oxidation.

Levels of selenium, zinc and vitamins A and E of egg yolk

A nonsignificant increase in vitamin A and significant increase in vitamin E was observed in egg yolks of treatments 3 and 4 (fed diets containing 2% fish oil + 2% rapeseed oil). This result is probably due to the presence of more vitamin A and E in fish oil which was used to protect the oxidation.

The level of the Zinc had a significant increase in egg yolk fed from 1 and 2 treatments (containing 1% fish oil + 3% rapeseed). While selenium levels of egg yolk treatment 2 was significantly higher than other groups, especially group 4 (2% fish oil + 2% rapeseed oil). This result is due to the higher levels of antioxidants in the groups containing high omega-3 to protect lipid oxidation.

Long-chain fatty acids 3n- and 6n- in membrane cells are highly susceptible to peroxidation. They increase the need for vitamins and elements with antioxidant and beneficial properties for health such as vitamins A and E, selenium (Se), zinc (Zn), and etc. These elements, in addition to the antioxidant properties that lead to the deposition of most of the omega-3 fatty acids in the tissue or egg yolk, can also be transmitted from the feed to these animal products, such as eggs (EBEID 2011; GALOBART et al. 2001b). In fact, to achieve both of these results: better omega-3 deposition, and the transfer of vitamins and beneficial elements to egg yolks, requires a large number of biological antioxidants in rations containing more levels of 3n and 6n.

One of the arguments that use of vitamin E and zinc (Zn) or other natural antioxidants recommends high power absorption of oxygen free radicals in cells. tocopherols and tocotrienols (vitamin E), ascorbic acid (vitamin C), or carotenoids can be easily reacted with free radicals (HOSSAIN ET AL. 1998).

Selenium, which is the strongest antioxidant after vitamin E and is also the most effective nutrient on the health of the body after omega-3, is known as a unique anticancer of clinical studies (FISININ et al. 2008). Unfortunately selenium deficiency in the diet is known as a global problem in many countries.

The dietary composition of almost all humans includes inadequate levels of selenium. As the presence of omega-3 fatty acids in the body of organisms improves the immune system and reduces inflammation by the production of metabolites (FISININ et al. 2008), selenium also plays an important role in improving the immune system. So that its deficiency in humans is associated with a decrease in the immune system and an increase in susceptibility to various types of diseases (SURAI 2006).

Also, selenium plays an important role in its antioxidant role which increases the amount of omega-3 fatty acids and thus reduces the amount of omega-6 to omega-3 in the product (BOURRE 2005; ZDUŃCZYK et al. 2013).

The results of this study agree with Meluzzi et al. (2000), Galobart et al. (2001a) and Ebeid (2011). Ebeid (2011) fed the laying hens with diet supplemented with two types of omega-3 oils (fish liver oil) and omega-6 (Pumpkin seed oil) at 4% plus vitamin E (0, 30 and 60 Mg / kg). They observed a significant decrease in the TBA of egg yolk in hens fed diets containing fish liver oil and vitamin E compared with other sources of oil plus vitamin E, especially in comparison with the control group. The significant increase in the amount of vitamin E by increasing vitamin E oil to your diet observed. There was a significant increase in vitamin E yolks of hens fed both types of oils with increasing levels of vitamin E in the diet (Table 6).

Table 6- Mean of vit. A, E and Zn, Se of egg yolk of laying hens fed by two oil sources and two levels of selenium and the same levels of vitamin E and zinc

Treat	**Vit. A** IU	**Vit. E** mg	**Zn** mg	**Se** mg
1	380	1.02b	0.84	15.55c
2	394	1.09b	0.88	23.85a
3	416	1.42a	0.73	14.38c
4	422	1.50a	0.79	19.71b
SEM[2]	21.33	0.29	0.19	5.62
P*value*[1]	**ns**	*****	**ns**	*****

[1]p<0.01= **, p<0.05=*, p>0.05= non-significant (ns), [2] Standard error of mean of twelve observations from each treatment

Treat1: 3%Repeseed+ 1%Fish + 0.1 mg/kg Selplex + 50 mg/kg vit. E + 50mg/kg Zn (Bioplex)
Treat2: 3%Repeseed+ 1%Fish + 0.2 mg/kg Selplex + 50 mg/kg vit. E + 50mg/kg Zn (Bioplex)
Traet3: 2%Repeseed+ 2%Fish + 0.1 mg/kg Selplex + 50 mg/kg vit. E + 50mg/kg Zn (Bioplex)
Treat4: 2%Repeseed+ 2%Fish + 0.2 mg/kg Selplex + 50 mg/kg vit. E + 50mg/kg Zn (Bioplex)

The composition of egg yolk fatty acids

The results were analyzed by comparing the mean of egg yolk methyl esters in hens fed from two sources of oil (omega-6 and omega-3) and two levels of selplex at different levels (Table 7). The results of the combination of yolk fatty acids of eggs showed a significant presence of unsaturated methyl esters with one or more bivalent grains in diets containing both oil sources, especially fish oil (P <0.01).

Table 7- Mean of metylesters of egg yolk of laying hens fed by two oil sources and two levels of selenium and the same levels of vitamin E and zinc (% of total metylesters)

treat Metylester	1	2	3	4	SEM[2]	*P value*[1]
C14:0	0.44d	0.57c	1.033a	0.86b	0.11	**
C14:1-n 5	0.27c	0.45a	0.32b	0.35b	0.04	**
C16:0	23.24	23.46	23.80	24.04	3.04	ns
C16:1-n9,n7	2.20c	3.06b	2.004c	3.59a	0.65	**
C18:0	7.57a	5.65c	6.04b	5.64c	0.99	**
C18:1-n	45.04	41.29	42.67	43.86	4.27	ns
C18:1-n9, n7	1.60	1.70	1.57	1.75	0.18	ns
C18:2-n 6 cis	11.38	12.78	13.08	12.64	0.84	ns
C18:3-n 3	0.44c	0.55b	1.06a	1.02a	0.12	**
C20:0	0.084	0.11	0.10	0.09	0.003	ns
C18:3-n 6	0.005	0.014	0.011	0.023	0.002	ns
C18:4-n 3	0.57b	0.91a	0.51b	0.57b	0.02	*
C20:5-n 3 *EPA*	0.62c	0.54c	1.09b	1.22a	0.11	**
C20:1-n 9	0.02	0.02	0.03	0.02	0.004	ns
C22:5-n 3 *DPA*	0.30b	0.36b	0.89a	0.88a	0.15	ns
C22:6-n 3 *DHA*	0.68c	0.72c	1.12b	1.24a	0.31	**
C22:0	0.02	0	0.03	0.01	0.001	ns

[1]p<0.01= **, p<0.05=*, p>0.05= non-significant (ns), [2] Standard error of mean of twelve observations from each treatment

Treat1: 3%Repeseed+ 1%Fish + 0.1 mg/kg Selplex + 50 mg/kg vit. E + 50mg/kg Zn (Bioplex)
Treat2: 3%Repeseed+ 1%Fish + 0.2 mg/kg Selplex + 50 mg/kg vit. E + 50mg/kg Zn (Bioplex)
Traet3: 2%Repeseed+ 2%Fish + 0.1 mg/kg Selplex + 50 mg/kg vit. E + 50mg/kg Zn (Bioplex)
Treat4: 2%Repeseed+ 2%Fish + 0.2 mg/kg Selplex + 50 mg/kg vit. E + 50mg/kg Zn (Bioplex)

The amount of saturated fatty acids in the treatment containing fish oil was higher than that of rapeseed. However, by increasing the percentage of fish oil, their level increased insignificantly.

Adding Selplex to omega-6 and omega-3 rations, although it didn't change the mount of major methyl ester of yolk such as palmitic (C16: 0), oleic (C18: 1n9), linoleic acid (C18: 2n6-Cis), and some minor methyl esters of yolks such as alidic acid (C18: 1-n9, n7), arachidonic acid (C20: 0), acid gama linoleic (C18: 3-n6), isocyanoy acid (C20: 1-n9) and beanic acid (C22: 0). But, it was able to significantly reduce methyl esters; myristolic (C14: 1n-5), stearic acid (C18: 0), and alphaparinaric (C18: 4-n3), and also significantly increased myristic acid (C14: 0), palmitoleic (C16: 1 n-7, n-9), especially in treatment 4 and also alpha-linolenic acid (C18: 3-n 3), eicosapentaenoic acid (C20: 5 n3), and ducozhaacanoic acid (C22: 6 n3), especially in treatments 3 and 4.

These results were consistent with observations by Hossain et al. (1998) and Mazalli et al. (2004), but the effect of adding these oils to laying hens diets was significant on the unsaturated fatty acid content of egg yolk. The addition of fish oil to the diet increased the amount of unsaturated omega-3 chain fatty acids such as EPA, DPA and DHA in eggs, and decreased the omega-6 to omega-3 ratio.

Conclusion

Finding of this study showed that using 0.1 and 0.2 mg / kg organic selenium levels with the same levels of vitamin E and zinc in diets containing omega-6 rich fatty acids and especially Omega-3, although didn't affect performance of hens, but could reduce the level of cholesterol and triglyceride yolk and increase its peroxidation resistance. Particularly, when more level of organic selenium were supplemented with the source of fish oil in the laying hens diets, it increased the amount of beneficial fatty acids (omega-3 chain methyl esters) and reduced some saturated, unsaturated methyl esters with a double bond or Omega-6.

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