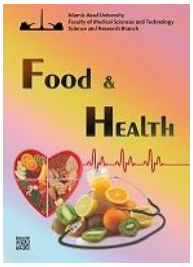

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Laying hens' diet modification with flaxseed and fish oils to enrich egg yolks with omega-3 fatty acids and vitamin D3

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ABSTRACT

Today, food enrichment is one of the most widely used methods in benefiting the food processing industry. The effects of four different dietary groups [basic diet (I), basic diet+flaxseed oil (II), basic diet+non-absorbable fiber+flaxseed oil (III)& basic diet+non-absorbable fiber+fish oil (IV)] were studied on the development of eggs with high content of long-chain omega-3 fatty acids &vitamin D3. To achieve this goal, the egg yolk oil was extracted by the cold extraction method and analyzed by gas chromatography. A total of 24 fatty acid compounds were identified in egg yolk oil, which ~48-52% of contained monounsaturated fatty acids (mainly oleic acid). The diet modifications significantly ($p<0.05$) increased the total content of egg yolk omega-3 fatty acids, and the highest value was observed in the diet (III) (84.2% increase compared to the control). The highest elevations were observed in ALA (3.49%), DPA (0.24%), and DHA (1.21%) fatty acids. Also, the ratio of ω-6/ω-3 fatty acids was ~18.36% in the control group (I), while in the diets II, III, and IV, the ratios decreased to ~3.79 (~80%), ~3.16 (~83%), and ~6.09 (~67%), respectively. Overall, the results indicated that diet (III) & (IV) were the most effective to increase the content of omega-3 fatty acids and Vit-D3 in egg yolk, respectively.

1. Introduction

In recent years, the egg industry has been seeking new ways to increase the nutritional value of eggs. For this reason, and due to increasing demand for this nutritional source, poultry nutrition specialists and experts always had concerns about enhancing the nutritional value (e.g., by increasing the content of omega-3, selenium, Folate, etc.) of this dietary source. The egg is one of the most important food sources for humans that contain high amounts of cholesterol, especially low-density lipoprotein (LDL). Because of its essential role, cholesterol is necessary for the normal functioning of the body; nevertheless, the high levels of LDL in eggs can be harmful to humans' health. Increasing the ratios of dietary omega-3 fatty acids including linolenic acid (LA), α-Linolenic acid (α-LA, C_{18}:3 n-3), Eicosapentaenoic acid (C_{20}:5 n-3), Docosahexaenoic acid (C_{22}:5 n-3), and Docosahexaenoic acid (C_{22}:6 n-3) can reduce the level of harmful lipoproteins in egg (1-3). Omega-3 fatty acids have important roles in preventing cardiovascular diseases, lowering blood cholesterol levels, and anti-inflammatory reactions (4). Various studies have indicated the role of omega-3 fatty acids in suppressing the progress of prostate and breast cancers, improving the immune response, reducing rheumatism, and reducing the mortality risk of coronary heart diseases (4-7). In general, consuming omega-3 and omega-6 fatty acids is essential for health. According to studies, an appropriate ratio of omega-6 to omega-3 fatty acids (ω-6/ω-3) is critical for normal cerebral and retinal activities. Some studies have noted that the appropriate ratio of ω-6/ω-3fatty acids is equal to 5, some have noted 4, and even some researchers have asserted that a ratio of close to 1 is the suitable value. Nevertheless, most researchers consider a ratio of less than 5 (ω-6/ω-3≤5) to be sufficient for enrichment (3, 4, 8). According to the Iranian National Standard (2), the ratio

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of ω-6/ω-3 fatty acids in our diet is unbalanced. The ideal ratio of dietary ω-6/ω-3 fatty acids should range from 1 to a maximum level of 4. However, this ratio varies from 11 to 30 (11≤ω-6/ω-3≤30) in the community. This means that the consumption of omega-6 fatty acids is 30 times higher than omega-3 fatty acids by most individuals. The consumption of either of these two fatty acids alone is not only beneficial but also causes many problems in the human body. Thus, it is necessary to consume these two types of essential fatty acids in a balanced way. In fact, omega-3and omega-6 fatty acids compete with each other in metabolic and enzymatic reactions. In this regard, higher consumption of omega-6 fatty acids prevents omega-3 fatty acids from being metabolized and utilized by the body. Another point is that the biological properties of these two sets of fatty acids are different, as mentioned earlier. Therefore, because we generally receive adequate and even more than necessary dietary omega-6 fatty acids, there is a need to increase the consumption of omega-3 fatty acids (2). Considering the importance of omega-3 fatty acids, it is essential to be properly identified the plant and animal sources of this fatty acid. The most important plant and animal sources of this fatty acid are vegetable oils (such as soybean, sunflower, flaxseed, canola, walnut, hazelnut, and sesame oils, which mainly contain α-LA) and animal oils (such as salmon, rainbow trout, tuna, Esqomari fish and mullet, which mainly contain DHA and EPA) (2). According to studies, adding the sources containing omega-3 fatty acids to the diet of laying hens can be increased the content of these fatty acids in eggs (4). Kim et al. (9) investigated the effects of combining the use of flaxseed oil and microalgae biomass on the production of eggs enriched with omega-3 fatty acids. Their results showed that consuming a diet containing (5% flaxseed oil+10% full-fatted *Staurosira* sp.) for two weeks increased the amounts of α-LA from 18.8 to 499 mg/egg, EPA from 0 to 14.8 mg/egg, and DHA from 42.6 to 120 mg/egg. Lemahieu et al. (10) also assessed the effects of consuming various sources of omega-3 polyunsaturated fatty acids including flaxseed, *Isochrysisgalbana*, fish oil, and DHA Gold supplementation on egg yolk enrichment. The results showed that the highest levels of α-LA, EPA, DPA, and DHA in egg yolk were observed in diets containing flaxseed and fish oils. Also, the level of α-LA increased 49% (9.9 to 19.2 mg/egg) in the egg yolk of the hens fed with a diet containing flaxseed compared to the control. Lemahieu al. (11) studied the effects of four dietary supplements containing omega-3 rich microalgae species (Phaeodactylumtricornutum, Nannochloropsisoculata, Isochrysisgalbana & Chlorella fusca) on egg yolk enrichment, and the highest levels of α-LA, EPA, and DHA were reported in the egg yolks obtained from laying hens fed with the diets containing C.fusca (2.75 g/100 g), I.galbana (3.52 g/100 g), and I.galbana (0.832 g/100 g), respectively. Hosseini Vashan et al. (12) used Kilka fish oil (0, 2 & 4%) to enrich egg yolk of laying hens with omega-3 fatty acids and reported significant elevations ($p<0.05$) in the content of EPA and DHA long-chain fatty acids (323% and 524%, respectively). They also stated that the ratio of ω-6/ω-3 fatty acids in the diets containing 2% and 4% Kilka fish oil decreased to 3.8 and 2.31, respectively (this ratio was 18 in the control group). The aim of this study was to evaluate the feasibility of enriching eggs with omega-3 fatty acids and vitamin D through modifying the diet of laying hens, which was supplemented by certain amounts of flaxseed and fish oils.

2. Materials and methods

2.1. Preparation of raw materials

The sources of omega-3 polyunsaturated fatty acids (PUFAs) including flaxseed oil (plant source) and Kilka fish oil (animal source) were purchased from Noorhan and Tala Dan-e-Shomal companies (Iran), respectively. At first, the PUFAs of these oils were evaluated. Next, the oily part of these sources was methylated according to the method described by Ryckebosch et al. (13). Then based on the method proposed by Lemahieu al. (11), the fatty acid profile was evaluated by gas chromatography (Agilent 5973N, USA). Also, a non-absorbable fiber (ARBOCEL®A300, Germany) with a particle size of 75 μM and a mass density of 0.33 g/mL (Parsian Exir Aria Co., Iran) was added to the diet of laying hens.

2.2. Location, breeding condition, and diet

This study was carried out in a laying hen breeding unit located in Khorasan Razavi province of Iran. The unit was equipped with 3-storey battery cages for breeding (4 hens per cage), a water nipple drinker and gutter feeders, fertilizer collection tape, and environmental control systems. A total of 48 hens (Hy-Line W-36) with the age of 25 weeks and healthy and homogenous appearance (regarding the growth of crown and the distance between the pubic bones and the sternum, and also the distance between the two pubic bones) were selected. The hens were randomly divided into 4 groups of 12 pieces with the same group weight (each group consisted of 3 replications, and each replication included 4 hens). Then the hens were transferred to experimental units (every three adjacent cages were considered as a unit). The feeders and egg locations of the experimental units were separated by barriers to avoid the feed and egg of the units from being mistakenly mixed with adjacent units. The environmental conditions were the same for all the experimental groups during the study. All the hens had free access to drinking water and feed. The hall temperature was kept at about 16-18ºC and controlled by a thermometer during the experiment. The light cycle was implemented with 16 hours of light and 8 hours of dark per day (13). The treatments used in feeding the laying hens were designed as follows and the ingredients and nutrients of the tested diets are given in Table 1:

Group I (control): The laying hens did not receive any additional dietary supplement besides the basic feed during two weeks of breeding.

Group II: The laying hens were fed with (base feed+3% flaxseed oil) and (basic feed+4% flaxseed oil) during the first and the second week of breeding, respectively.

Group III: The laying hens were fed with (base feed+3% flaxseed oil+3% non-absorbable fiber) and (basic feed+4% flaxseed oil+3% non-absorbable fiber) during the first and the second week of breeding, respectively.

Group IV: The laying hens were fed with (base feed+3% fish oil+3% non-absorbable fiber) and (basic feed+4% fish oil+3% non-absorbable fiber) during the first and the second week of breeding, respectively.

During the breeding period of laying hens, from each of the mentioned groups, the necessary eggs were collected and stored at -20°C until the beginning of the next tests.

2.3. Oil extraction from egg yolk

The total oil of egg yolk was extracted at the beginning and the end of the enrichment period using the method of Bruneel et al. (14) with slight modifications. Briefly, an internal standard (Nu-check, C20:0) was added to the egg yolk sample (200-250 mg), and after adding methanol (4.5 mL), the egg yolk sample was homogenized at 8000 rpm for 30 s by a homogenizer (MTOPS, SR30, Korea). Then 9 mL chloroform was added to the mixture which was again homogenized (at 8000 rpm for 60 s). Next, the sample was stirred by centrifugation (HETTICH, EBA200, Germany) at 2800 g for 10 min. The supernatant was separated and the residual oil in the precipitated phase was mixed again with 12 ml of chloroform-methanol solvent (with the ratio of 2:1 v/v, respectively) and extracted. After homogenization and centrifugation at 8000 rpm for 60 s, the supernatant was separated and mixed with the previous sample. The resulting extract was washed with KCl solution (6.2 mL, 0.88%) and after centrifugation, the lower phase was filtered through anhydrous sodium sulfate. The solvents were removed using a rotary evaporator (HEIDOLPH, Germany). After measuring the total oil content by a gravimetric method, the extracted oil was diluted with 2 mL of the chloroform-methanol solvent and stored for further analysis (14).

2.4. Measuring of egg yolk fatty acids profile

The fatty acid profile of egg yolk was evaluated using the method of Lemahieu et al. (11) at the beginning and the end of the enrichment period. At first, the oily part extracted in the previous phase was methylated according to Ryckebosch et al. (13) method. Then, fatty acid methyl esters (FAME) were identified and quantified by gas chromatography (Agilent 5973N, USA).

2.5. Measuring of egg yolk vitamin D

The vitamin D (D_3 or cholecalciferol) of each enriched egg yolk was measured in accordance with the conventional method recognized in the institute of standards and industrial research of Iran (15).

2.6. Statistical analysis

All the chemical analyses (fatty acid profile and vitamin D_3) were measured three times to obtain the average data with acceptable standard deviations. When the standard deviation was higher than the acceptable level, the test was repeated until the average with acceptable standard deviation was obtained for each testing parameter. Software of Statistix (version 8) was used to analyze variances (ANOVA) for the recorded data. The means comparison between different treatments was made using the least significant difference (LSD) test at the confidence level of 95% (16).

3. Results and discussion

In this research, to enrich egg yolk with an omega-3 long-chain fatty acid, the diet of laying hens was modified with containing omega-3 polyunsaturated fatty acids (i.e., flaxseed and fish oils) (Table 1). Before starting the modifying of laying hens' diets, the fatty acid profiles of flaxseed and fish oils were assessed by gas chromatography, which showed that the major fatty acid of flaxseed oil was α-LA (~22.1%). On the other hand, the main profile of fatty acids found in fish oil includes α-LA (~1.1%), stearidonic acid (~1.36%), EPA (~7.1%), DPA (~1.35%), and DHA (~6.76%). The results of these researches were consistent with the findings of Lemahieu et al. (10). The effect of dietary modification of laying hens with different feed groups (I, II, III, and IV) on egg yolk enrichment patterns is presented in Table 2. The results showed that enrichment with group II led to a significant increase ($p<0.05$) in the amount of α-LA (~86%), DPA (~74%), and DHA (~67%) compared to the control sample. Also, the levels of α-LA, DPA and DHA fatty acids for groups III and IV were equal (~87%, ~75% & ~78%) and (~27%, ~65% & ~82%), respectively. As can be seen, the highest increase in omega-3 fatty acids family (i.e., the total of α-LA+DPA+DHA) with a significant difference ($p<0.05$) was observed in group III (~84% increase compared to the control group), which corresponds to the lowest value of ω-6/ω-3 fatty acids ratio (Table 2). Lemahieu et al. (10) assessed the effects of different sources of omega-3 unsaturated fatty acids (flaxseed oil, *Isochrysisgalbana*, fish oil, and DHA Gold) on the enrichment of egg yolk with omega-3fatty acids. The results showed that after the end of the enrichment period, the total amount of omega-3 fatty acids (i.e., the total of fatty acids of α-LA+EPA+DPA+DHA) in the noted sources compared to the control sample (33.9 mg/egg) increased by ~41.3% (57.75 mg/egg), ~60.1% (86.44 mg/egg), ~71% (117.1 mg/egg) and ~68% (106.4 mg/egg), respectively, which is consistent with the results of the present study. According to previous research, α-linoleic acid (α-LA) can be converted to DHA fatty acid in the body of laying hens, but this process is relatively inefficient because a significant amount of ALA enters into the egg yolk. This process is accelerated in the laying hens fed with flaxseed oil (17, 18). In another study, Hosseini Vashan et al. (12) modified the diet of laying hens with Kilka fish oil.

Table 1. The ingredients and nutrients of the supplemented diets used in this study.

Ingredients/nutrients	The ingredients (at 1st & 2nd weeks)/dietary groups		
	Group I	Group I	
INGREDIENTS		**INGREDIENTS**	**INGREDIENTS**
Corn	50	Corn	50	Corn
Soybean meal	20.05	Soybean meal	20.05	Soybean meal
Soy oil	3	Soy oil	3	Soy oil
Flaxseed oil	–	Flaxseed oil	–	Flaxseed oil
Fish oil	–	Fish oil	–	Fish oil
ARBOCEL	–	ARBOCEL	–	ARBOCEL
Wheat bran	5.07	Wheat bran	5.07	Wheat bran
Barley	5.8	Barley	5.8	Barley
Fish powder	5	Fish powder	5	Fish powder
Oyster powder	8.75	Oyster powder	8.75	Oyster powder
Salt	0.17	Salt	0.17	Salt
D-L-Methionine	0.03	D-L-Methionine	0.03	D-L-Methionine
Mineral supplements	0.25	Mineral supplements	0.25	Mineral supplements
Vitamin supplement	0.25	Vitamin supplement	0.25	Vitamin supplement
D-calcium phosphate	1.5	D-calcium phosphate	1.5	D-calcium phosphate
Multienzyme	0.2	Multienzyme	0.2	Multienzyme
CALCULATED NUTRIENTS				
Metabolizable energy (kg/kcal)	2990	Metabolizable energy (kg/kcal)	2990	Metabolizable energy (kg/kcal)
Crude protein	17.2	Crude protein	17.2	Crude protein
Calcium	4.02	Calcium	4.02	Calcium
Phosphorus	0.65	Phosphorus	0.65	Phosphorus
Sodium	0.14	Sodium	0.14	Sodium
Potassium	0.83	Potassium	0.83	Potassium
Chloride	0.19	Chloride	0.19	Chloride
Lysine	1.06	Lysine	1.06	Lysine
Methionine	0.4	Methionine	0.4	Methionine
Methionine+Cysteine	0.45	Methionine+Cysteine	0.45	Methionine+Cysteine
DCAD (meq/kg of diet) (*1)	219.8	DCAD (meq/kg of diet) (*1)	219.8	DCAD (meq/kg of diet) (*1)

(*1) DCAD is equivalent to the dietary cation-anion difference and calculated as below equation.
DCAD (meq/kg of diet) = $[(Na^{+}\times C_{F1})+(K^{+}\times C_{F2})]-(Cl^{-}\times C_{F3})$
Where, C_F is the conversion factor and the subscripts of 1, 2 & 3 are related to the elements of sodium (C_{F1}=435), potassium (C_{F1}=256) & chloride (C_{F1}=282), respectively.
Thus, it can be written:
DCAD = [(0.14×435) +(0.83×256)] −(0.19×282) = +219.8 meq/kg of diet

Their results showed that adding 4% fish oil to the laying hens' diet (during 12 weeks) was able to increase the total amount of omega-3 fatty acids (including α-LA+EPA+DHA) compared to the control sample up to ~98%. According to the results presented in Table 2, it was observed that the type of diet significantly affected the fatty acid profile of egg yolk. Based on the results, in all group diets, the highest amount of fatty acids in egg yolk was related to monounsaturated fatty acids (MUFA). Furthermore, the highest amount of total saturated fatty acids, total MUFAs, and total PUFAs of egg yolk oil with a significant difference ($p<0.05$) were observed in laying hens fed with I, IV & III groups, respectively.

Table 2. The profile of egg yolk fatty acids (average of 2 replicate) during the enrichment period of laying hens for 2 weeks with different diets (groups I, II, III, and IV).

Type of fatty acids	The amount of fatty acids (%)/dietary groups		
	Group I	Group I	
Myristic acid (C14:0)	0.29	Myristic acid (C14:0)	0.29	Myristic acid (C14:0)
Tetradecenoic acid (C14:1)	0.04	Tetradecenoic acid (C14:1)	0.04	Tetradecenoic acid (C14:1)
Pentadecylic acid (15:0)	ND (*1)	Pentadecylic acid (15:0)	ND (*1)	Pentadecylic acid (15:0)
Pentadecanoic acid (C15:1)	ND	Pentadecanoic acid (C15:1)	ND	Pentadecanoic acid (C15:1)
Palmitic acid (C16:0)	22.66	Palmitic acid (C16:0)	22.66	Palmitic acid (C16:0)
Palmitoleic acid (C16:1)	3.76	Palmitoleic acid (C16:1)	3.76	Palmitoleic acid (C16:1)
Margaric acid (C17:0)	0.28	Margaric acid (C17:0)	0.28	Margaric acid (C17:0)
Heptadecanoic acid (C17:1)	0.09	Heptadecanoic acid (C17:1)	0.09	Heptadecanoic acid (C17:1)
Stearic acid (C18:0)	7.62	Stearic acid (C18:0)	7.62	Stearic acid (C18:0)
Oleic acid (C18:1) ω-9	45.33	Oleic acid (C18:1) ω-9	45.33	Oleic acid (C18:1) ω-9
Linoleic acid (C18:2), ω-6	14.29	Linoleic acid (C18:2), ω-6	14.29	Linoleic acid (C18:2), ω-6
Conjugated linoleic acid (CLA)	0.69	Conjugated linoleic acid (CLA)	0.69	Conjugated linoleic acid (CLA)
α-linolenic acid (C18:3), ω-3	0.45[d]	α-linolenic acid (C18:3), ω-3	0.45[d]	α-linolenic acid (C18:3), ω-3
Arachidic acid (C20:0)	0.66	Arachidic acid (C20:0)	0.66	Arachidic acid (C20:0)
Eicosenoic acid (C20:1)	0.19	Eicosenoic acid (C20:1)	0.19	Eicosenoic acid (C20:1)

Table 2. The profile of egg yolk fatty acids (average of 2 replicate) during the enrichment period of laying hens for 2 weeks with different diets (groups I, II, III, and IV).

Type of fatty acids	The amount of fatty acids (%)/dietary groups		
	Group I	Group I	
Arachidonic acid (C20:4), ω-6	0.03	Arachidonic acid (C20:4), ω-6	0.03	Arachidonic acid (C20:4), ω-6
Heneicosylic acid (C21:0)	0.03	Heneicosylic acid (C21:0)	0.03	Heneicosylic acid (C21:0)
Behenic acid (C22:0)	0.16	Behenic acid (C22:0)	0.16	Behenic acid (C22:0)
Erucic acid (C22:1), ω-9	1.04	Erucic acid (C22:1), ω-9	1.04	Erucic acid (C22:1), ω-9
Docosapentaenoic acid or DPA (C22:5), ω-3	0.06[d]	Docosapentaenoic acid or DPA (C22:5), ω-3	0.06[d]	Docosapentaenoic acid or DPA (C22:5), ω-3
Docosahexaenoic acid or DHA (C22:6), ω-3	0.27[d]	Docosahexaenoic acid or DHA (C22:6), ω-3	0.27[d]	Docosahexaenoic acid or DHA (C22:6), ω-3
Tricosylic acid (C23:0)	0.04	Tricosylic acid (C23:0)	0.04	Tricosylic acid (C23:0)
Lignoceric acid (C24:0)	ND	Lignoceric acid (C24:0)	ND	Lignoceric acid (C24:0)
Nervonic acid (C24:1), ω-9	0.12	Nervonic acid (C24:1), ω-9	0.12	Nervonic acid (C24:1), ω-9
Other	1.90	Other	1.90	Other
Total saturated fatty acids	31.74[a]	Total saturated fatty acids	31.74[a]	Total saturated fatty acids
Total monounsaturated fatty acids (ΣMUFA)	50.57[b]	Total monounsaturated fatty acids (ΣMUFA)	50.57[b]	Total monounsaturated fatty acids (ΣMUFA)
Total polyunsaturated fatty acids (ΣPUFA)	15.79[d]	Total polyunsaturated fatty acids (ΣPUFA)	15.79[d]	Total polyunsaturated fatty acids (ΣPUFA)
Total omega-3 fatty acids	0.78[d]	Total omega-3 fatty acids	0.78[d]	Total omega-3 fatty acids
Total omega-6 fatty acids	14.32[c]	Total omega-6 fatty acids	14.32[c]	Total omega-6 fatty acids
The ratio of (ω-6/ω-3) fatty acids	18.36[a]	The ratio of (ω-6/ω-3) fatty acids	18.36[a]	The ratio of (ω-6/ω-3) fatty acids

(*1) ND: Not detected.

The ANOVA results showed that dietary groups of I, II, III, and IV had significant ($p<0.05$) effects on vitamin D_3 content of enriched egg yolks (Fig. 1). The vitamin D_3 content in egg yolks from laying hens fed with group IV was ~84, ~77, and ~74% more than those in groups of I, II, and III, respectively (Fig. 1). Browning and Cowieson (19) stated that the diet of laying hens had a significant ($p<0.01$) influence on increasing the contents of D_3 in egg yolk.

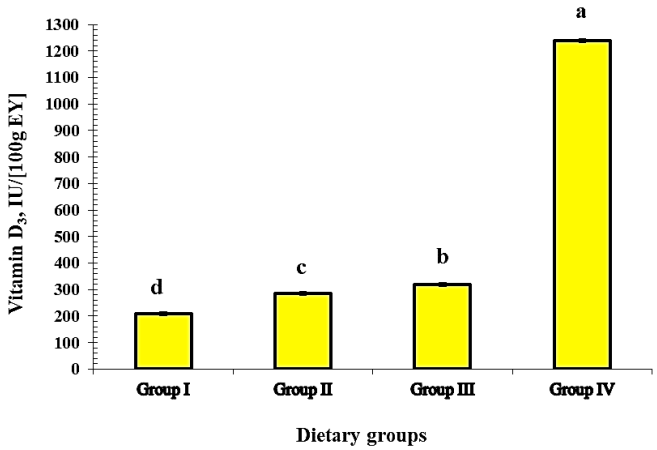

Fig. 1. The mean values (average of 3 replicate) of vitamin D3 content of egg yolks enriched with different dietary groups (I, II, III, & IV).

4. Conclusion

The following remarks were concluded from modifying laying hens' diet with flaxseed and fish oils to enrich egg yolks with omega-3 fatty acids & vitamin D_3: The results of gas chromatographic (GC) evaluation showed that, in four different diets of laying hens (i.e., groups I, II, III and IV), a total of 24 fatty acid compounds were identified in the egg yolk oil, which among these, oleic acid (as a monounsaturated fatty acid) constituted the main fatty acid. According to the findings, the most important omega-3 fatty acids in egg yolk oil were ALA, EPA, and DHA fatty acids, which the highest amount of total fatty acids in egg yolk with a significant difference ($p<0.05$) was related to laying hens fed with group III diet (~84% increase compared to the control group). Modification of the laying hen's diet with group III had the best result in improving the nutrition index of ω-6/ω-3ratio so that the amount of this index in the eggs yolk of hens fed with this group decreased by 83% compared to the control sample, which will have a significant impact on consumers' health. The highest content of vit-D_3 was observed in the eggs yolk of hens fed with group IV. In general, according to the results of this study, it can be concluded that the difference in the enrichment efficiency of egg yolk with omega-3 fatty acids and vit-D_3, can be due to the different bioavailability of omega-3 fatty acids and vit-D_3in different nutritional sources and as well as the diversity of omega-3 fatty acid profiles and vit-D_3in these sources.

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References

1. Lamas A, Anton X, Miranda J, Roca-Saavedra P, Cardelle-Cobas A, Rodriguez JA, et al. Technological development of functional egg products by an addition of n-3 polyunsaturated-fatty-acid-enriched oil. CyTA-*Journal of Food*. 2016;14(2):289-95.
2. Iranian National Standardization Organization (INSO). Milk fat from enriched dairy products-Determination of omega-3 and omega-6 fatty

acid content by gas-liquid chromatography: Tehran: Iranian National Standardization Organization; 2009.
3. Shimizu Y, Arai K, Ise S, Shimasaki H. Dietary fish oil for hens affects the fatty acid composition of egg yolk phospholipids and gives a valuable food with an ideal balance of n-6 and n-3 essential fatty acids for human nutrition. *Journal of Oleo Science*. 2001;50(10):797-803.
4. Hulan H, Proudfoot F, Ackman R, Ratnayake W. Omega-3 fatty acid levels and performance of broiler chickens fed redfish meal or redfish oil. *Canadian Journal of Animal Science*. 1988;68(2):533-47.
5. Okuyama H, Kobayashi T, Watanabe S. Dietary fatty acids—The n-6n-3 balance and chronic elderly diseases. Excess linoleic acid and relative n-3 deficiency syndrome seen in Japan. *Progress in Lipid Research*. 1996;35(4):409-57.
6. Pandalai PK, Pilat MJ, Yamazaki K, Naik H, Pienta KJ. The effects of omega-3 and omega-6 fatty acids on in vitro prostate cancer growth. *Anticancer Research*. 1996;16(2):815-20.
7. Simopoulos AP. Omega-3 fatty acids in health and disease and in growth and development. *The American Journal of Clinical Nutrition*. 1991;54(3):438-63.
8. Simopoulos AP, Robinson J. The omega plan: New York: Harper Collins Pub.; 1998.
9. Kim J, Magnuson A, Tao L, Barcus M, Lei XG. Potential of combining flaxseed oil and microalgal biomass in producing eggs-enriched with n−3 fatty acids for meeting human needs. *Algal Research*. 2016;17:31-7.
10. Lemahieu C, Bruneel C, Ryckebosch E, Muylaert K, Buyse J, Foubert I. Impact of different omega-3 polyunsaturated fatty acid (n-3 PUFA) sources (flaxseed, Isochrysis galbana, fish oil and DHA Gold) on n-3 LC-PUFA enrichment (efficiency) in the egg yolk. *Journal of Functional Foods*. 2015;19:821-7.
11. Lemahieu C, Bruneel C, Termote-Verhalle R, Muylaert K, Buyse J, Foubert I. Impact of feed supplementation with different omega-3 rich microalgae species on enrichment of eggs of laying hens. *Food Chemistry*. 2013;141(4):4051-9.
12. Hosseini Vashan J, Afzali N, Malekane M, Naseri M, Allahresani A. Enrichment of egg yolk with long chain n-3 fatty acids through supplementation of Kilka fish oil to layer diets. *JWSS-Isfahan University of Technology*. 2009;13(47):131-41.
13. Ryckebosch E, Muylaert K, Foubert I. Optimization of an analytical procedure for extraction of lipids from microalgae. *Journal of the American Oil Chemists' Society*. 2012;89(2):189-98.
14. Bruneel C, Lemahieu C, Fraeye I, Ryckebosch E, Muylaert K, Buyse J, et al. Impact of microalgal feed supplementation on omega-3 fatty acid enrichment of hen eggs. *Journal of Functional Foods*. 2013;5(2):897-904.
15. Iranian National Standardization Organization (INSO). Foodstuffs-determination of vitamin D by high performance liquid chromatography-measurement of cholecalciferol (D3) or ergocalciferol (D2): Tehran: Iranian National Standardization Organization; 2011.
16. Zagumny MJ. The SPSS book: A student guide to the statistical package for the social sciences: Indiana: iUniverse; 2001.
17. Bean L, Leeson S. Long-term effects of feeding flaxseed on performance and egg fatty acid composition of brown and white hens. *Poultry Science*. 2003;82(3):388-94.
18. Aymond W, Van Elswyk M. Yolk thiobarbituric acid reactive substances and n-3 fatty acids in response to whole and ground flaxseed. *Poultry Science*. 1995;74(8):1388-94.
19. Browning LC, Cowieson AJ. Vitamin D fortification of eggs for human health. *Journal of the Science of Food and Agriculture*. 2014;94(7):1389-96.