

Comparative effects of dietary microalgae oil and fish oil on fatty acid composition and sensory quality of table eggs

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ABSTRACT This study was conducted to investigate the comparative effects of dietary supplementation with microalgae oil or fish oil on fatty acid composition, sensory quality, and overall acceptability of table eggs. A total of six hundred thirty, 30-week-old, Hy-Line Brown laying hens were allocated to 7 dietary treatments. Layers were fed with a control diet or the control diet supplemented with graded levels of docosahexaenoic acid (DHA; 1.25, 2.50, and 5.00 mg/g feed) derived from microalgae oil or fish oil. The feeding trial lasted 10 wk. Enrichment of eggs with DHA by dietary supplementation with microalgae oil or fish oil enhanced yolk DHA and total n-3 polyunsaturated fatty acid (PUFA) enrichment and reduced n-6-to-n-3 ratio in a dose-response manner, whereas the efficiency decreased ($P < 0.05$). The overall efficiency of DHA incorporation into eggs was similar for the 2 sources ($P > 0.05$). In

comparison with fish oil, dietary microalgae oil supplementation resulted in higher scores for egg flavor and overall acceptability, both of which declined linearly in response to DHA supplementation ($P < 0.05$). Among the aroma and flavor characteristics, fishy aroma and flavor scores increased linearly and quadratically ($P < 0.05$) in response to dietary DHA supplementation, and egg aroma and flavor and milky flavor scores decreased linearly ($P < 0.05$). The results from partial least squares analysis showed that fishy flavor and aroma were closely associated with DHA, α -linolenic acid, and total n-3 PUFA, and oleic acid, arachidonic acid, and dihomo-gamma-linolenic acid were more related to egg aroma and flavor. The results suggested that microalgae oil would be more promising for egg DHA enrichment owing to better sensory quality of the resultant eggs.

Key words: docosahexaenoic acid source, fatty acid, sensory quality, table egg

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INTRODUCTION

Increased public awareness of health and clinical benefits of n-3 polyunsaturated fatty acids (PUFA) has improved consumer acceptance and preference to food products enriched with these desirable fatty acids (Calder and Yaqoob, 2009; Heng et al., 2016; Kaewsutas et al., 2017). Eggs enriched with n-3 PUFA accounted for 10% of the market share of eggs and egg products in the USA (USDA, 2016) and accounted for the largest portion of specialty eggs in Canada (Tamini et al., 2018). Enriched eggs with n-3 long-chain PUFA

(LC-PUFA), especially docosahexaenoic acid (DHA; C22:6n3), may be more promising because the health benefits related to n-3 PUFA are mainly ascribed to n-3 LC-PUFA (Lemahieu et al., 2017; Shahidi and Ambigaipalan, 2018; Ghasemi Fard et al., 2019). Daily consumption of DHA-enriched eggs can help address human dietary insufficiencies, which has been shown to reduce the risk of fatal ischemic heart diseases by raising the blood DHA level (Farrell, 1998; Gillingham et al., 2005) and exert active regulatory roles in brain cognition function (Kaewsutas et al., 2017). Fish oil is a traditional dietary source of DHA and has been widely used to produce n-3 PUFA-enriched eggs. Marine microalgae, the original source of n-3 LC-PUFA in the marine food chain, offer an alternative to current sources of fish oil for the production of DHA-enriched eggs. Microalgae oil, extracted from microalgae biomass, was expected to improve bioaccessibility and thus enhance enrichment efficiency of fatty acids (Nitsan et al., 1999; Lemahieu et al., 2015, 2016). However, there is lack of studies that directly compare the effectiveness of these 2

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dietary sources for yolk enrichment with DHA. Moreover, few comparative studies are available with equal dietary DHA supplementation.

In addition, enrichment of eggs with n-3 PUFA always causes a deterioration of sensory properties, which influence consumer acceptance or preference (Leeson et al., 1998; Hayat et al., 2010; Fraeye et al., 2012). Sensory traits of eggs were previously reported to be negatively influenced by dietary supplementation with fish oil, fish meal, and other dietary n-3 PUFA sources in a dose-dependent manner (Fraeye et al., 2012). The intensity of some undesirable sensory attributes, such as “fishy” and “rancid,” also increased with more n-3 PUFA deposition in yolk (Gonzalez-Esquerria and Leeson, 2000; Lawlor et al., 2010). To avoid the fishy taint, the inclusion of fish oil and fish meal was suggested to be less than 1% and 12%, respectively (Leskanich and Noble, 1997). Yalcin (2017) reviewed the effects of supplemental oil on egg sensory properties and concluded that eggs were acceptable when dietary supplementation from fish oil does not exceed 1.5%. The supplemental levels restricted by the sensory problems would reciprocally limit the maximum enrichment of DHA in eggs (up to approximately 100 mg of DHA per egg; Gonzalez-Esquerria and Leeson, 2000). But for microalgae oil, few studies are available that assess how graded levels of dietary supplementation affect egg sensory characteristics. The information would be valuable for the production of functional eggs, concerning the relationship between DHA enrichment and sensory properties of enriched eggs by application of fish oil and microalgae oil.

Thus, the aim of this study was to investigate the effects of dietary supplementation with graded levels of either fish oil or microalgae oil on fatty acid composition, the sensory profile, and overall acceptability of table eggs. Comparative effects of these 2 dietary DHA sources on egg enrichment with DHA and total n-3 PUFA, sensory quality, and overall acceptability of table eggs were also determined. In addition, further analysis was conducted on the potential relationship between sensory attributes and yolk fatty acid levels. This study will provide valuable information for the production of DHA-enriched eggs to balance n-3 PUFA content in eggs and sensory quality to meet consumer demands and further to find the optimum way for DHA enrichment in eggs.

MATERIALS AND METHODS

Bird Housing and Environment

A total of six hundred thirty, 30-week-old, Hy-Line Brown laying hens were allocated to 3-tier battery cages of 3 laying hens each. The birds were exposed to 16 h of light per day, and the temperature was controlled by supplemental heat and ventilation. Feed and water were supplied *ad libitum* in mash form and by nipple drinkers, respectively. The protocol and use of the

animals in this study were approved by the Animal Care and Use Committee of the Feed Research Institute of the Chinese Academy of Agricultural Sciences.

Experimental Design and Diets

Laying hens were randomly assigned to one of the 7 dietary treatments with 6 replicates of 15 birds each. All hens were fed for 1-week adaption, followed by a 10-week trial period. The control group received the standard diet, referred as the control diet. The other 6 groups received control diet supplemented with graded levels of DHA (1.25, 2.50, and 5.00 mg/g of feed) derived from microalgae oil (Inner Mongolia Kingdomway Pharmaceutical Co., Ltd., Inner Mongolia, China) or fish oil (Damao Feed Co., Ltd., Foshan, China). All diets (Table 1), isoenergetic and isonitrogenous, in this study were formulated to meet or exceed the National Research Council (1994) requirements. Fatty acid composition of microalgae oil, fish oil, and experimental diets is shown in Table 2. Palm oil (Tianjin Long Wei Cereals & Oils Industry Co., Ltd., Tianjin, China) was used as the additional fat source owing to its low PUFA contents. In consideration of high fat contents in diets and the susceptibility of PUFA to oxidation, antioxidants were added to all diets.

Fatty Acid Analysis

At week 10 of the trial period, 3 eggs from each replicate were randomly chosen for fatty acid analysis. Egg yolks were separated from the albumen and homogenized. The mixed yolk samples were freeze-dried using the freeze-drying equipment (FD-12, Beijing Huichengjia Scientific Instrument Factory Co., Ltd., Beijing, China). After that, the samples were weighed. Yolk, microalgae oil, and fish oil samples were stored at -20°C until analysis.

A sample of oil (40–60 mg), yolk (80–120 mg), or feed (250–300 mg) was weighed, and transferred into a 15-mL screw-capped test tube. The samples were mixed with 1 mL of hexane, 1 mL of internal standard (1 mg/mL methyl undecylate–hexane mixture, used for quantification), and 4 mL of methanol–acetylchloride (10:1 by volume) mixture. The mixture was heated in a water bath at 80°C for 3 h. After the mixture cooled to room temperature, 5 mL of 7% potassium carbonate solution was slowly added. The mixture was mixed and centrifuged (4°C , 10 min, 4000 g; Anke GL-20B, Shanghai Anting Scientific Instrument Factory, Shanghai, China). The supernatant was transferred into bottles to be analyzed using the GC-450 gas chromatography system (Techcomp Ltd., Shanghai, China) equipped with a flame ionization detector and a methylpolysiloxane capillary column (DB-23, 50% cyanopropyl-methylpolysiloxane, 60 m \times 0.25 mm diameter and 0.25- μm film thickness; Agilent Technologies, Inc., Santa Clara, CA), with helium as the carrier gas. The time–temperature program used was that the initial oven temperature was 100°C (5 min); the oven temperature was increased at

Table 1. Ingredient and nutrient levels of the experimental diets (air-dried basis).

Items	Supplemental DHA (mg/g)						
	Control	Microalgae oil			Fish oil		
		-	1.25	2.50	5.00	1.25	2.50
Ingredients (%)							
Corn	55.45	57.18	56.55	55.82	55.75	54.88	51.20
Soybean meal	27.47	27.49	27.63	27.44	27.40	27.20	26.61
Wheat bran	1.00	-	-	0.90	1.00	2.00	4.00
Limestone	9.00	9.00	9.00	9.00	9.00	9.00	9.00
NaCl	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DL-Methionine	0.09	0.09	0.09	0.09	0.09	0.09	0.21
Lysine	-	-	-	-	-	-	0.13
CaHPO ₄	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Premix ¹	1.46	1.46	1.46	1.46	1.46	1.46	1.46
Antioxidant ²	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Zeolite powder	-	-	0.24	0.06	0.39	0.07	1.72
DHA supplement form: microalgae oil	-	0.25	0.50	1.00	-	-	-
DHA supplement form: fish oil	-	-	-	-	1.08	2.17	4.34
Palm oil	4.20	3.20	3.20	2.90	2.50	1.80	-
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient level³							
ME (MJ/kg)	11.49	11.49	11.49	11.49	11.49	11.49	11.49
Crude protein (%)	16.81	16.81	16.81	16.81	16.81	16.81	16.81
Calcium (%)	3.37	3.37	3.37	3.37	3.37	3.37	3.37
Available phosphorus (%)	0.33	0.33	0.33	0.33	0.33	0.33	0.33
Methionine (%)	0.36	0.36	0.36	0.36	0.36	0.36	0.44
Lysine (%)	0.89	0.89	0.89	0.89	0.89	0.89	0.89
Methionine + cysteine (%)	0.65	0.65	0.64	0.65	0.65	0.64	0.65

Abbreviations: DHA, docosahexaenoic acid.

¹Premix provided the following per kg of the diet: vitamin A, 12,500 IU; vitamin D₃, 4,125 IU; vitamin E, 15 IU; vitamin K, 2 mg; thiamine, 1 mg; riboflavin, 8.5 mg; calcium pantothenate, 11 mg; niacin, 32.5 mg; pyridoxine, 8 mg; biotin, 0.5 mg; folic acid, 1.25 mg; vitamin B₁₂, 0.02 mg; Mn, 65 mg; I, 1 mg; Fe, 60 mg; Cu, 8 mg; Zn, 66 mg; choline, 1000 mg; phytase, 300 mg; montmorillonite, 1000 mg; yeast culture, 10 g.

²Antioxidants provided the following per kg of the diet: ethoxyquin, 150 mg; vitamin E, 100 mg; tea polyphenols, 50 mg.

³Calculated values.

4°C/min to 240°C (30 min). The total running time was ~40 min. The injector and detector temperature was at 270°C and 280°C, respectively.

Sample Preparation for Sensory Evaluation

At week 10, 3 eggs of similar weight (60 ± 2 g) were randomly collected from each replicate before sensory evaluation and kept at 4°C. The egg samples were placed on a metal rack in a steamer with 2 L of water and cooked for 15 min. The samples were cooled to an external temperature of ~40°C and shelled. The albumen and yolk were separated and put into a sealed

container respectively to preserve their aroma compounds. The containers were kept in a water bath at a constant temperature of 55°C until the samples were assessed. All samples were evaluated within 10 min after cooking. Each sample was divided into several portions and presented to panelists.

Recruitment

A total of 9 panelists (4 males and 5 females, aged between 19 and 30 years) were recruited to participate in this sensory evaluation. The panelists were selected and trained to guarantee that they were capable to

Table 2. Fatty acid composition of microalgae oil, fish oil, and layer diets.

Fatty acid (mg/g)	Supplemental DHA (mg/g)								
	DHA sources		Control	Microalgae oil			Fish oil		
	Microalgae oil	Fish oil		-	1.25	2.50	5.00	1.25	2.50
16:0 (PALM)	243.39	326.47	24.47	19.46	22.39	19.47	18.61	20.60	14.59
16:1 (PALMO)	3.68	139.87	0.14	0.12	0.16	0.19	1.07	2.46	3.82
18:0 (SA)	13.18	28.05	3.08	2.39	2.68	2.29	2.56	3.21	2.80
18:1n9 (OA)	0.80	103.70	28.87	21.94	24.50	20.93	20.61	21.20	20.26
18:2n6 (LA)	1.73	32.33	24.39	21.56	23.06	21.93	21.95	23.09	20.92
20:3n6 (DGLA)	2.95	0.98	0	0.02	0.04	0.07	0.02	0.05	0.08
20:4n6 (AA)	2.47	6.96	0	0.04	0.04	0.07	0.15	0.33	0.50
18:3n3 (ALA)	2.28	15.57	1.44	1.38	1.41	1.35	1.44	1.54	1.50
20:5n3 (EPA)	4.86	66.78	0.02	0.02	0.03	0.06	1.09	2.6	4.51
22:6n3 (DHA)	502.23	116.52	0.03	1.23	2.55	4.95	1.28	2.6	5.08
Total n-6	7.15	40.27	24.39	21.62	23.14	22.07	22.11	23.47	21.51
Total n-3	509.37	199.54	1.49	2.63	3.99	6.36	3.81	6.74	11.09

Abbreviations: AA, arachidonic acid; ALA, α -linolenic acid; DGLA, dihomo-gamma-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; OA, oleic acid; PALM, palmitic acid; PALMO, palmitoleic acid; SA, stearic acid; Total n-6, LA + DGLA + AA; Total n-3, ALA + EPA + DHA.

complete this evaluation. The protocols of sensory evaluation and analysis were ethically approved by the China National Institute of Standardization.

Sensory Evaluation

Before evaluation, all panelists needed to participate in 3 training sessions of 1 h each. Panelists, unknowing the treatment, rated the aroma and flavor of each sample in a random order and reached an agreement on descriptive words of aroma and flavor characteristics. Specific aromas and flavors were obtained from the combining results as per the intensity scales, which are shown in Table 3. Cooked commercial eggs, menhaden, straw, seaweed, milk, sucrose, unsalted butter, salt solution, chicken soup, and citric acid were used as reference to evaluate each sensory attribute.

All samples were prepared as described previously, and the evaluation procedure was conducted in separate workstations in the sensory laboratory of the China National Institute of Standardization. The laboratory was well ventilated, out of direct sunlight, and equipped with artificial lights. The samples coded with 3-digit numbers were presented to panelists, guaranteeing every panelist rated samples from all treatments. Appearance, aroma, flavor, texture, and overall acceptability were assessed using a modified method on a 9-point scale. Then, they rated specific aroma and flavor intensity of the samples on unstructured 15-cm line scales from 0 (low) to 15 (high). Purified water and salt-free crackers were provided to panelists to scrub their tongue and gums between sample testing. Egg evaluations from each treatment were replicated 3 times on 3 separate days in 1 wk.

Statistical Analysis

The present study was designed to be completely randomized, and all data were analyzed using Excel (version 2010, Microsoft Corporation, Redmond, WA) and SAS

(version 9.2, SAS Institute, Cary, NC, 2001). The homogeneity of variances and normality of the data were tested, and then, the Shapiro–Wilk test was used to analyze the normality. Data were analyzed using one-way ANOVA, and means were compared using Duncan's multiple range test. Linear and quadratic effects of DHA supplemental levels in diets were assessed using regression analysis. Orthogonal contrasts were used to compare treatment means between dietary microalgae oil and fish oil sources. Differences were considered statistically significant at $P < 0.05$. Partial least squares analysis (XLSTAT version 2016, Addinsoft Inc., New York, NY) was used to generate a biplot with average values for all the sensory attributes and fatty acids, and the biplot provided a visual presentation of the correlation between specific yolk fatty acids and sensory attributes.

RESULTS AND DISCUSSION

Fatty Acid Composition of Dietary DHA Sources and Enriched Diets

Microalgae oil and fish oil, the major dietary sources of n-3 LC-PUFA for laying hens and especially rich in DHA, contained 60.99% and 10.98% DHA of total fatty acids, respectively (Table 2). Higher levels of fish oil was supplemented in the diets than those of microalgae oil to achieve the same dietary DHA levels (Tables 1 and 2) because the DHA contents of microalgae oil was more than 5 times those of fish oil. But fish oil contained relatively higher levels of eicosapentaenoic acid (EPA; C20:5n3; 6.29% of total fatty acids) than microalgae oil (0.59% of total fatty acids). The contents of EPA and total n-3 PUFA in enriched diets by supplementation of fish oil were therefore higher than those in the microalgae oil diets containing approximately the same levels of DHA (Table 2). In consideration of cost control, application, and sustainability, microalgae oil would be a very promising feed

Table 3. Aroma and flavor definitions and standard products used as reference in training sessions.

Attributes	Definition	Standard products ¹ /amount
Aroma		
Egg	Aroma associated with egg yolk	Blended commercial egg cooked and presented as experimental samples (Charoen Pokphand Group, Grade A, Beijing, China)
Fishy	Aroma associated with menhaden	Raw fresh menhaden fillets (Wu market, Beijing, China)/15 g
Barny	Aroma associated with straw	Straw/5 g
Seaweed	Aroma associated with seaweed	Seaweed (Wu market, strong brand, original flavor)/1 g
Flavor		
Egg	Flavor associated with egg yolk	Blended commercial egg cooked and presented as experimental samples (Grade A, Charoen Pokphand Group, Beijing, China)
Fishy	Flavor associated with menhaden	Raw fresh menhaden fillets (Wu market, Beijing, China)/15 g
Milky	Flavor associated with milk	1.26% low-fat milk (Mengniu brand, Inner Mongolia, China)/5 g
Sweet	Flavor associated with sucrose	Sucrose (Taikoo brand, Taikoo Sugar Inc., Guangzhou, China)/1 g
Buttery	Flavor associated with unsalted butter	Unsalted butter (Enbi Cole brand, JD Supermarket, Christchurch, New Zealand)/5 g
Salty	Flavor associated with salt solution	0.25% salt solution (JD Supermarket, China National Salt Industry Corporation, Xinjiang, China)/5 g
Chicken	Flavor associated with chicken soup	Boiled chicken soup ² /10 g
Rancid	Flavor associated with citric acid	0.02 g citric acid (Shandong Lemon Biochemical Co., Ltd., Shandong, China) in 100 g filtered water

¹Placed in a plate and covered with a lid.

²Cook whole chicken (~1.25 kg; Wu market, Beijing, China) in boiling water (5 L), and stew for half an hour.

supplement to enrich egg with DHA. In addition, higher contents of palmitoleic acid (C16:1) and oleic acid (OA, C18:1n9) were also observed in fish oil. The 2 enriched diets could be expected to have varying effects on egg fatty acid composition and sensory properties owing to differences in fatty acid composition.

Fatty Acid Composition of Enriched Egg Yolks

Enrichment of eggs with DHA by supplementation of microalgae oil or fish oil increased the level of total n-3 PUFA in yolk in a dose-dependent manner and correspondingly reduced the n-6 PUFA-to-n-3 PUFA ratio (from 5.20 to 2.26; Table 4), similar to other dietary n-3 PUFA sources (Ehr et al., 2017; Wen et al., 2019). It is of great meaning to raise n-3 LC-PUFA levels and lower the n-6-to-n-3 ratio in human diets, which conduced to reducing the risk of cardiovascular diseases and improving brain development and function (Pottel et al., 2014). The n-3 fatty acid profile of egg yolk is characterized by the linear and quadratic ($P < 0.001$) increases of DHA contents in response to the increase of dietary DHA supplementation up to 5 mg/g in this study. Compared with control eggs, fortified eggs yielded a 2.70- to 5.06-fold increase in DHA content, which contributed to 78.21–97.21% of the increase in total n-3 PUFA contents (ratio (%) = increase in DHA contents/increase in n-3 PUFA contents \times 100%). The maximum contents of DHA in egg yolks in the present study were 11.89 mg/g and 13.51 mg/g, that is, 197.62 mg and 234.29 mg DHA per egg, respectively, obtained by supplementing the diets with 5 mg/g DHA derived from microalgae oil and fish oil, respectively.

Feeding both DHA source-supplemented diets resulted in linear and quadratic ($P < 0.001$) decreases in contents of dihomo-gamma-linolenic acid (C20:3n6)

and arachidonic acid (AA, C20:4n6) in yolks, which were strongly affected by high PUFA concentration in the diets (Petrović et al., 2012; Wu et al., 2018). The addition of microalgae oil linearly and quadratically ($P < 0.01$) increased linoleic acid (LA, C18:2n6) contents in yolks, whereas its contents were not affected by dietary fish oil supplementation ($P > 0.05$). As a result, total n-6 PUFA contents in yolks were not affected ($P > 0.05$) by dietary microalgae oil supplementation and declined linearly ($P = 0.01$) in response to dietary fish oil supplementation. But the contents of AA in fish oil-enriched diets were almost more than those in microalgae oil-enriched diets (Table 2). The decrease in n-6 PUFA contents (especially AA contents) caused by dietary supplementation of fish oil was reported to be due to the competition of substrates and biosynthesis enzymes between the n-3 and n-6 PUFA synthesis pathways (Jia et al., 2008) and the inhibition of high dietary concentration of n-3 PUFA (Cachaldora et al., 2008). But our results indicated the transfer efficiency of AA and OA in fish oil-enriched diets was relatively lower than in microalgae oil-enriched diets, which has not been reported before and needs further investigation.

The overall efficiency of DHA deposition in the fish oil group (32.95–85.48%) was similar to ($P > 0.05$; Figure 1) that in the microalgae oil group (31.77–78.76%), although the transfer efficiency decreased with the increase of the DHA addition level. Higher efficiency of DHA enrichment was observed in the fish oil group, with dietary DHA supplementation only at 1.25 mg/g ($P < 0.01$). It suggested that both supplemental levels and sources would influence the DHA incorporation efficiency. Similarly, higher enrichment efficiencies of n-3 LC-PUFA (~55%) were observed for the supplementation from fish oil than for supplementation from heterotrophic microalgae (~45%) at a concentration of 120 mg of n-3 PUFA per 100 g of feed

Table 4. Fatty acid composition of egg yolks from laying hens fed with diets supplemented with graded levels of DHA derived from either microalgae oil or fish oil.

Items (mg/g)	Supplemental DHA (mg/g)							SEM	Regression analysis		P-value	
	Control	Microalgae oil			Fish oil				Microalgae oil	Fish oil	ANOVA	Contrast
	-	1.25	2.50	5.00	1.25	2.50	5.00					
16:0 (PALM)	85.23 ^a	84.02 ^{a,b}	83.94 ^{a,b}	85.87 ^a	79.36 ^{b,c}	76.65 ^c	81.20 ^{a,b,c}	0.63	NS	Q	<0.001	<0.001
16:1 (PALMO)	8.58 ^c	7.58 ^d	7.72 ^d	7.29 ^d	9.55 ^b	9.43 ^b	11.54 ^a	0.23	LQ	LQ	<0.001	<0.001
18:0 (SA)	26.58 ^{a,b}	26.93 ^{a,b}	24.74 ^c	25.85 ^{a,b,c}	24.22 ^c	24.72 ^{b,c}	26.76 ^a	0.23	NS	Q	0.001	0.11
18:1n9 (OA)	154.67 ^a	153.61 ^a	144.60 ^b	135.46 ^c	137.92 ^{b,c}	125.46 ^d	120.23 ^d	2.00	LQ	LQ	<0.001	<0.001
18:2n6 (LA)	41.65 ^{a,b}	36.96 ^b	41.90 ^{a,b}	46.09 ^a	37.32 ^b	41.12 ^{a,b}	37.09 ^b	0.68	LQ	NS	<0.001	0.008
20:3n6 (DGLA)	0.45 ^a	0.40 ^{a,b,c}	0.43 ^{a,b}	0.37 ^{b,c}	0.37 ^{b,c}	0.35 ^c	0.29 ^d	0.01	LQ	LQ	<0.001	<0.001
20:4n6 (AA)	6.75 ^a	5.66 ^b	4.69 ^c	3.96 ^d	3.93 ^d	2.96 ^e	1.90 ^f	0.24	LQ	LQ	<0.001	<0.001
18:3n3 (ALA)	0.95 ^d	0.97 ^d	1.15 ^c	1.56 ^b	1.16 ^c	1.64 ^b	2.03 ^a	0.06	LQ	LQ	<0.001	<0.001
20:5n3 (EPA)	0.00 ^f	0.09 ^e	0.12 ^e	0.31 ^d	0.49 ^c	0.96 ^b	1.90 ^a	0.098	LQ	LQ	<0.001	<0.001
22:6n3 (DHA)	2.67 ^f	7.20 ^e	10.02 ^c	11.89 ^b	8.49 ^d	10.08 ^c	13.51 ^a	0.52	LQ	LQ	<0.001	<0.001
Total n-6	48.86 ^{a,b}	43.03 ^{b,c,d}	47.02 ^{a,b,c}	50.42 ^a	41.62 ^{c,d}	44.43 ^{a,b,c,d}	39.28 ^d	0.76	Q	LQ	<0.001	0.001
Total n-3	3.61 ^f	8.27 ^e	11.30 ^c	13.79 ^b	10.14 ^d	12.70 ^b	17.47 ^a	0.64	LQ	LQ	<0.001	<0.001
Ratio n-6/n-3	13.55 ^a	5.20 ^b	4.17 ^c	3.66 ^d	4.12 ^{c,d}	3.50 ^d	2.26 ^e	0.55	LQ	LQ	<0.001	<0.001

^{a-f}Means within a row with no common superscripts differ significantly ($P < 0.05$).

Abbreviations: AA, arachidonic acid; ALA, α -linolenic acid; DGLA, dihomo-gamma-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; L, linear effect; LA, linoleic acid; NS, no significance; OA, oleic acid; PALM, palmitic acid; PALMO, palmitoleic acid; Q, quadratic effect; SA, stearic acid; Total n-6, LA + DGLA + AA; Total n-3, ALA + EPA + DHA.

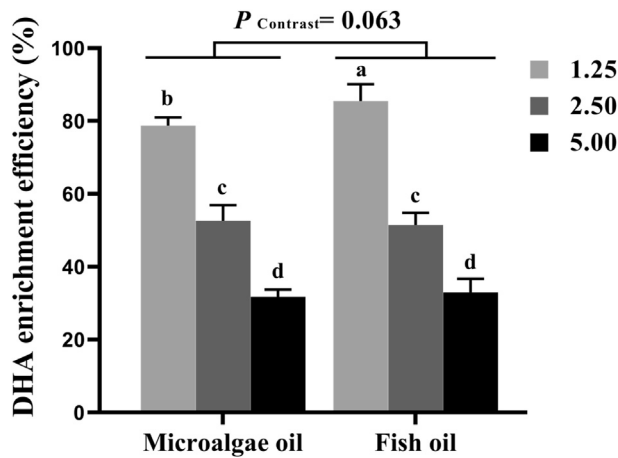


Figure 1. Docosahexaenoic acid (DHA) enrichment efficiency (%) in egg yolk from laying hens fed with diets supplemented with graded levels of DHA derived from microalgae oil or fish oil. The enrichment efficiency was calculated by taking the ratio of the enrichment of DHA in the egg (mg) to the actual DHA intake (mg) and multiplying it with 100%. Error bars represent the standard deviation. ^{a-d} Means with no common superscripts differ significantly ($P < 0.05$).

(Lemahieu et al., 2015). Higher enrichment efficiency was mainly attributed to higher bioaccessibility of n-3 PUFA in fish oil because they were supplemented as oil rather than biomass for microalgae. In fact, DHA in microalgae oil was mainly present in glycolipids and phospholipids and could be absorbed more easily and efficiently, whereas DHA in fish oil was only in neutral lipids (Ryckeboosch et al., 2014). However, fish oil contained relatively higher amounts of EPA than microalgae oil (66.78 vs. 4.86 mg/g, Table 2), which might be largely converted to DHA in yolk, evidenced by a much lesser extent of increase in EPA contents in yolk (Table 4). Even when hens were fed with marine algae rich in EPA and containing no DHA, no substantial increase of EPA was observed in yolk phospholipids, but levels of DHA increased (Fredriksson et al., 2006; Bruneel et al., 2013; Wu et al., 2018), indicating that the chicken has a limitation in direct deposition of EPA in egg yolks. Dietary EPA was reported to be preferentially converted into DHA, and the deposition efficiency of this converted DHA into yolks is only slightly lower than that of dietary DHA into yolks (Poureslami et al., 2012; Neijat et al., 2017). No differences in DHA incorporation efficiency was observed between these two DHA sources with higher DHA supplementation (2.50 and 5.00 mg/g feed; $P > 0.05$; Figure 1), suggesting bioaccessibility of microalgae oil DHA and bioconversion of EPA into DHA may have a similar impact on yolk DHA enrichment.

Recommendation for daily DHA intake is as follows: 100 mg for the infant, 200 mg for pregnant and lactating women (EFSA, 2010), and 250 mg (EPA + DHA) for the adult (FAO, 2008). Docosahexaenoic acid-functional eggs were feasible to help address human dietary insufficiencies. A linear fit between DHA contents in eggs (y, mg/egg) and microalgae oil supplemental level (x, % of diet) was established with the following

equation: $y = 144.78x + 68.84$ ($P < 0.05$; $R^2 = 0.86$). It indicated that feeding hens with $\geq 0.91\%$ of microalgae oil could produce eggs with at least 200 mg DHA. Consuming one DHA-fortified egg (or yolk) daily would almost meet the demands of the infant and pregnant and lactating women and contribute to $\sim 80\%$ of recommended daily intake of EPA and DHA for the adult. On the other hand, dietary supplementation of $\geq 3.38\%$ fish oil would be needed to obtain eggs with more than 200 mg of DHA per egg, according to the following equation: $y = 37.89x + 71.99$ (y, mg DHA per egg; x, % of diet; $P < 0.05$; $R^2 = 0.87$). Nevertheless, there appears to be a tolerable limit for DHA source supplementation in layer diets, in consideration of adverse effects on production performance and health of laying hens (Fraeye et al., 2012). Our previous studies evaluated the biological safety of microalgae oil and fish oil for laying hens (31–42 wk of age) and showed that microalgae oil supplementation in diets at a concentration not more than 2.5% (containing 12.5 mg/g DHA) would not have negative effects on laying performance, egg quality, and layer health (Long, 2018). But inclusion of more than 4.34% fish oil into the diet (containing 5 mg/g DHA) of laying hens was observed to depress performance and impair the function of the liver and kidney (Long, 2018). Therefore, it could be more practicable to fill this nutritional gap with DHA-enriched eggs by dietary microalgae oil supplementation.

Sensory Quality of Table Eggs

Linear declines in flavor ($P = 0.020$, $P = 0.006$, respectively) and overall acceptability ($P = 0.032$, $P = 0.045$, respectively) were observed with increasing dietary DHA supplementation from both microalgae oil and fish oil (Table 5). There was no significant effect of DHA supplemental levels on other sensory properties ($P > 0.05$), indicating that overall acceptability, the integrated sensory item, was mainly affected by flavor characteristics in the current sensory evaluation. The acceptance scores of the resultant eggs from all treatments were higher than or equal to 5 (representing neither preference nor negative attitude of panelists toward the eggs), except the eggs from the fish oil group supplementation of 5.00 mg/g DHA. The biplot (Figure 2) showed the potential relationship between specific fatty acids and sensory attributes. An obvious group separation in the plot suggested the sensory changes caused by dietary oil supplementation.

Dietary enrichment of eggs with n-3 PUFA has been reported to have negative effects on egg sensory characteristics. For example, undesirable off-flavors in the enriched eggs were easily detected when diets were supplemented with high levels of fish meal ($\geq 8\%$; Nash et al., 1996), fish oil ($> 1.5\%$; Gonzalez-Esquerria and Leeson, 2000), microencapsulated fish oil ($\geq 6\%$; Lawlor et al., 2010), or flaxseed ($\geq 10\%$; Leeson et al., 1998) or rapeseed meal ($> 10\%$; Pearson et al., 1979). In the present study, a linear relationship between yolk acceptance score (y) and dietary DHA concentration (mg/g, x) was obtained

Table 5. Sensory properties and overall acceptance of egg yolks from laying hens fed with diets supplemented with graded levels of DHA using microalgae oil or fish oil.

Items ¹	Supplemental DHA (mg/g)							SEM	Regression analysis		P-value	
	Control	Microalgae oil			Fish oil				Microalgae oil	Fish oil	ANOVA	Contrast
	-	1.25	2.50	5.00	1.25	2.50	5.00					
Appearance	5.67	5.44	4.93	5.35	5.49	4.81	5.47	0.15	NS	NS	0.69	0.97
Aroma	5.39	5.40	5.21	4.70	4.95	4.79	4.35	0.13	NS	NS	0.29	0.16
Flavor	5.81 ^{a,b}	6.25 ^a	5.46 ^{a,b}	4.93 ^{a,b}	5.32 ^{a,b}	4.97 ^{a,b}	4.46 ^b	0.14	L	LQ	0.015	0.031
Texture	5.87	6.26	5.34	5.40	5.35	5.06	5.06	0.14	NS	NS	0.23	0.093
Overall acceptance	5.91 ^{a,b}	6.56 ^a	5.46 ^{a,b}	5.31 ^{a,b}	5.56 ^{a,b}	5.00 ^b	4.94 ^b	0.13	L	L	0.026	0.042

^{a-f}Means within a row with no common superscripts differ significantly ($P < 0.05$).

Abbreviations: DHA, docosahexaenoic acid; L, linear effect; NS, no significance; Q, quadratic effect.

¹The scales, a 9-point scale (1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like or dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, and 9 = like extremely), rated the appearance, aroma, taste, texture, and overall acceptance of egg yolks.

(microalgae oil, $y = -0.18x + 6.21$, $P < 0.05$, $R^2 = 0.45$; fish oil, $y = -0.17x + 5.75$, $P < 0.05$, $R^2 = 0.35$). Based on the equation, dietary DHA supplementation from microalgae oil not exceeding 6.64 mg/g and fish oil not exceeding 4.27 mg/g might result in DHA enrichment at 261.11 and 212.56 mg per egg, respectively, maintaining the acceptance scores higher than 5. These data suggested a maximum dietary supplementation of DHA while still obtaining eggs with acceptable sensory properties in commercial production of DHA-enriched eggs. Thus, dietary DHA supplementation from microalgae oil would produce eggs with higher DHA enrichment,

which are still acceptable for humans, than that from fish oil.

Among the aroma and flavor characteristics, fishy aroma and flavor scores increased linearly and quadratically ($P \leq 0.001$; Table 6) in response to increasing dietary DHA supplementation from both microalgae oil and fish oil, whereas egg aroma, egg flavor, and milky flavor scores decreased linearly ($P < 0.05$). The concomitant increase in egg n-3 PUFA contents and fishy flavor and aroma intensity caused by dietary DHA supplementation, as reported previously (Lawlor et al., 2010; Fraeye et al., 2012), indicated that DHA and

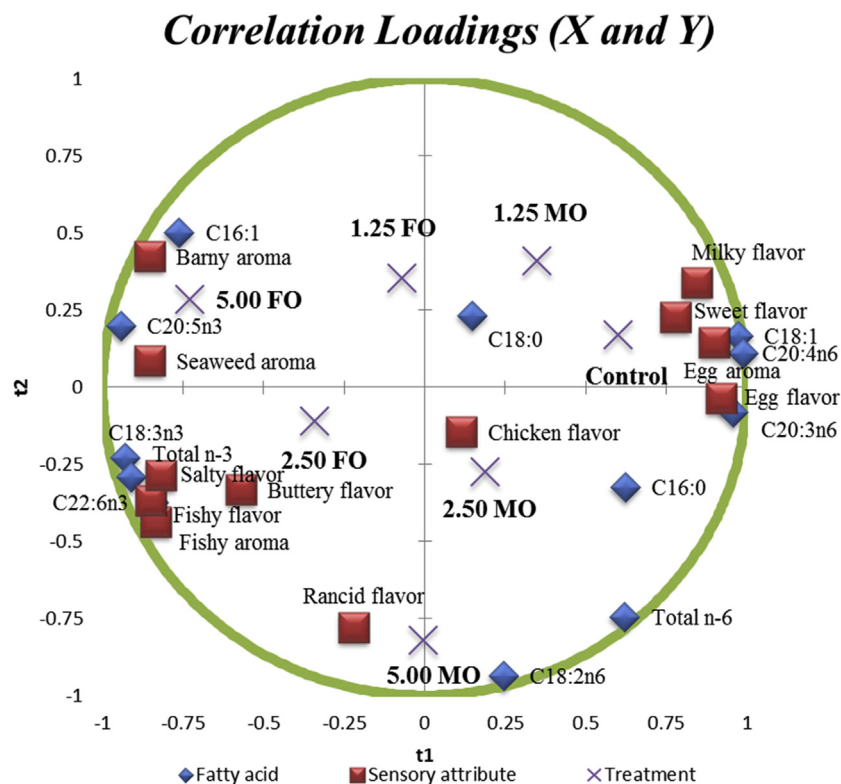


Figure 2. An overview of the potential relationship between sensory attributes and fatty acids of cooked yolk samples. The correlation loadings obtained by partial least squares analysis with yolk fatty acids as X-variables and sensory attributes as Y-variables. Total n-6 = C18:2n6 + C20:3n6 + C20:4n6; Total n-3 = C18:3n3 + C20:5n3 + C22:6n3. 1.25, 2.50, and 5.00 MO or FO denotes treatments supplemented with 1.25, 2.50, and 5.00 mg DHA per gram of diets derived from microalgae oil or fish oil, respectively.

Table 6. Sensory attributes of egg yolks from laying hens fed with diets supplemented with graded levels of DHA using microalgae oil or fish oil.

Items	Supplemental DHA (mg/g)							SEM	Regression analysis		
	Control	Microalgae oil			Fish oil				Microalgae oil	Fish oil	P-value
	-	1.25	2.50	5.00	1.25	2.50	5.00				
Aroma											
Egg	10.15 ^{a,b}	10.59 ^a	8.51 ^{a,b,c}	8.29 ^{a,b,c}	7.45 ^{a,b,c}	6.32 ^c	6.57 ^{b,c}	0.36	L	LQ	0.003
Fishy	0.97 ^c	4.17 ^b	6.27 ^{a,b}	7.70 ^{a,b}	4.21 ^{b,c}	7.31 ^{a,b}	8.63 ^a	0.52	LQ	LQ	0.001
Barry	0.32	0.26	0.38	0.29	1.89	1.42	2.54	0.33	NS	NS	0.94
Seaweed	2.47	2.09	2.45	2.79	3.57	4.33	3.96	0.41	NS	NS	0.85
Flavor											
Egg	11.10 ^a	10.58 ^a	9.83 ^{a,b}	9.53 ^{a,b}	9.93 ^{a,b}	9.24 ^{a,b}	6.23 ^b	0.36	NS	LQ	0.007
Fishy	1.05 ^c	1.90 ^{b,c}	5.12 ^{a,b,c}	5.57 ^{a,b,c}	5.14 ^{a,b,c}	7.47 ^a	6.29 ^{a,b}	0.47	LQ	LQ	<0.001
Milky	6.63	4.93	5.42	2.34	4.05	3.23	2.37	0.53	L	L	0.24
Sweet	1.66	2.67	0.74	1.08	0.75	0.51	0.10	0.27	NS	NS	0.20
Buttery	1.12	0.82	0.89	1.69	1.31	1.05	1.64	0.28	NS	NS	0.98
Salty	1.42	0.78	0.94	2.69	1.72	2.55	3.15	0.38	NS	NS	0.49
Chicken	1.22	0.18	1.00	0.26	0.27	1.73	0.26	0.21	NS	NS	0.27
Rancid	0.34	0.92	0.88	2.59	0.45	2.28	0.55	0.28	L	NS	0.18

^{a-f}Means within a row with no common superscripts differ significantly ($P < 0.05$).

Abbreviations: DHA, docosahexaenoic acid; L, linear effect; NS, no significance; Q, quadratic effect.

other n-3 PUFA contents in yolk may be mainly responsible for the deterioration in aroma and flavor. Many odor-active compounds, such as (Z)-4-heptenal, (E,Z)-2,4-heptadienal, and others, that contributed to fishy flavor or off-flavor from n-3 PUFA during rapid oxidation were identified (Hammer and Schieberle, 2013; Peinado et al., 2016). Our graphical presentation of the correlation loadings (Figure 2) further showed that fishy flavor and aroma were closely related to DHA, α -linolenic acid (ALA), and total n-3 PUFA. α -Linolenic acid and DHA were also characterized by a fishy odor in a pork model experiment conducted to predict the effects of individual fatty acid on flavor (Aaslyng and Schäfer, 2008). The overall profile of fishy oil-enriched eggs was rated with a quite high fishy odor intensity than that of microalgae oil-enriched eggs, which may partly result from the more deposition of DHA and total n-3 PUFA from dietary fish oil supplementation. Besides, the oxidation of DHA, ALA, and EPA generated a set of same odorants but with different concentrations and flavor dilution factors and elicited different overall odor profiles (Hammer and Schieberle, 2013). Thus, these variations in n-3 LC-PUFA composition may be also related to the observed differences in sensory attributes of enriched eggs from dietary microalgae oil and fish oil supplementation.

Interestingly, OA, AA, and dihomo-gamma-linolenic acid were more related to egg aroma and flavor. The control group and the group supplemented with 1.25 mg/g DHA from microalgae oil appeared to be more related to the desirable attributes, including sweet, milky, and egg flavors. The higher AA contents are closely associated with the enhancement of typical chicken meat (Takahashi et al., 2012) and egg flavor (Goldberg et al., 2016; Matsui and Takahashi, 2017). Cerny and Guntz (2004) further proposed that the autoxidation of phospholipid-bound LA and AA would be one of the major factors in determining egg yolk flavor formation. But lower contents of OA and AA in yolk were observed

in eggs from fish oil groups than in those from microalgae oil groups. Thus, DHA enrichment in eggs from different dietary sources and its related change in the overall fatty acid profile should be taken into consideration for egg sensory properties.

In conclusion, enrichment of eggs with DHA by supplementation from microalgae oil or fish oil enhanced contents of DHA and total n-3 PUFA in yolk and reduced the n-6-to-n-3 ratio in a dose-dependent manner, whereas the enrichment efficiency decreased with the increase of the level of DHA addition. An upper limit of the amount of microalgae oil or fish oil in diets should be noted based on the effects on egg flavor, and this upper limit varied with the source of DHA. The maximum dietary supplementation of DHA from microalgae oil and fish oil while still obtaining eggs with acceptable sensory properties was at a concentration of 6.64 mg/g and 4.27 mg/g, and the DHA contents in yolk of obtained eggs could be increased to 261.11 mg and 212.56 mg DHA per egg. Thus, dietary DHA supplementation from microalgae oil would produce eggs with higher DHA enrichment, which are still acceptable for humans, than that from fish oil.

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