Effects of Flaxseed and Fish Oil on n-3 Polyunsaturated Fatty Acid Content in Egg Yolk and Hepatic Fatty Acid Metabolism (Postprint)

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Date: 2017-11-07T00:00:00+00:00

Abstract

This study aimed to investigate the effects of dietary supplementation of flaxseed and fish oil on n-3 polyunsaturated fatty acid content in egg yolk, liver fatty acid composition, and the expression of genes related to fatty acid synthesis and metabolism. A total of 96 Hy-Line Brown laying hens aged 28 weeks were selected and randomly allocated into 4 groups with 24 hens per group. The control group was fed a basal diet, while the experimental groups were fed the basal diet supplemented with 10% flaxseed, 10% flaxseed + 5% fish oil, and 5% fish oil, respectively. Following a 21-day feeding period, eggs were collected continuously for 7 days, and then 4 hens were randomly selected from each group for slaughter and sample collection. The results showed that, compared with the control group: 1) supplementation with flaxseed and fish oil both significantly increased n-3 polyunsaturated fatty acid content in egg yolk ($P<0.05$), with the highest docosahexaenoic acid (DHA) and eicosapentaenoic acid content observed in the group supplemented with fish oil alone; 2) the proportion of monounsaturated fatty acids in liver was significantly decreased in all experimental groups ($P<0.05$), while the proportion of n-3 polyunsaturated fatty acids in liver was significantly increased ($P<0.05$); 3) supplementation with fish oil alone significantly decreased the hepatic gene expression levels of fatty acid elongase 1, fatty acid elongase 2, and desaturases (fatty acid desaturase 1, fatty acid desaturase 2, and stearoyl-CoA desaturase 1) ($P<0.05$). These results indicate that supplementation with flaxseed or fish oil alone can enrich n-3 polyunsaturated fatty acid deposition in egg yolk; flaxseed supplementation promotes the conversion of -linolenic acid to DHA in the liver, as evidenced by upregulated expression of fatty acid elongase and desaturase genes, while fish oil supplementation shows the opposite effect.

Full Text

Enrichment Effects of Dietary Flaxseed and Fish Oil on n-3 Polyunsaturated Fatty Acid Content in Egg Yolk and Hepatic Fatty Acid Metabolism

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Abstract: This study investigated the effects of dietary flaxseed and fish oil on n-3 polyunsaturated fatty acid (PUFA) content in egg yolk, hepatic fatty acid composition, and the expression of genes involved in fatty acid synthesis and metabolism. Ninety-six 28-week-old Hyline Brown laying hens were randomly allocated into 4 groups (n=24). The control group received a basal diet, while treatment groups were fed the basal diet supplemented with 10% flaxseed, 10% flaxseed + 5% fish oil, or 5% fish oil. After 21 days of feeding, eggs were collected continuously for 7 days, after which 4 hens per group were randomly selected for slaughter and sampling. Compared with the control group: 1) Both flaxseed and fish oil supplementation significantly increased yolk n-3 PUFA content ($P<0.05$), with the highest docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) levels observed in the fish oil-only group; 2) All treatment groups showed significantly reduced hepatic monounsaturated fatty acid proportions ($P<0.05$) and significantly increased hepatic n-3 PUFA proportions ($P<0.05$); 3) Fish oil alone significantly downregulated the expression of fatty acid elongase 1 (ELOVL1), fatty acid elongase 2 (ELOVL2), and desaturases including fatty acid desaturase 1 (FADS1), fatty acid desaturase 2 (FADS2), and stearoyl-CoA desaturase 1 (SCD1) ($P<0.05$). These results demonstrate that dietary flaxseed or fish oil can enrich yolk n-3 PUFA deposition. Flaxseed promotes the hepatic conversion of -linolenic acid to DHA by upregulating elongase and desaturase gene expression, whereas fish oil exhibits the opposite effect.

Keywords: flaxseed; fish oil; n-3 polyunsaturated fatty acid; DHA; egg yolk

n-3 (also known as omega-3) polyunsaturated fatty acids primarily include -linolenic acid (18:3 n-3, ALA), eicosapentaenoic acid (20:5 n-3, EPA), docosapentaenoic acid (22:5 n-3, DPA), and docosahexaenoic acid (22:6 n-3, DHA), which have beneficial effects on human cardiovascular disease, central nervous system disorders, mental health, inflammatory responses, and immune function, with DHA receiving the most attention [1-2]. Reports indicate that egg DHA exists exclusively in phospholipid-bound form [3], which relatively enhances fatty acid oxidative stability and absorption/utilization efficiency [4-6] and can produce special biological activities such as anti-inflammatory, anti-tumor, and cholesterol-lowering effects [7-10]. The deposition of n-3 PUFAs in animals is directly influenced by dietary fatty acid composition, manifested through two

pathways: the stepwise conversion of plant-derived ALA to EPA, DPA, and DHA, and the direct absorption and deposition of long-chain PUFAs such as DHA from the diet. Fish oil, microalgae, and flaxseed represent three major dietary sources of n-3 PUFAs, with fish oil yielding the highest DHA enrichment in eggs, followed by microalgae and then flaxseed. However, fish oil supplementation can cause fishy odor in fresh eggs, affecting storage and flavor quality [11]. Investigating the synergistic and competitive effects among these three n-3 PUFA sources could optimize functional enrichment in production, yet relevant reports remain scarce.

Therefore, this study examined the effects of dietary flaxseed, fish oil, and their combination on n-3 PUFA deposition in egg yolk and hepatic fatty acid metabolism (fatty acid composition, carbon chain elongation, and desaturase gene expression) to provide reference for producing n-3 PUFA-enriched eggs.

1.1 Experimental Design, Animal Husbandry, and Sample Collection

Ninety-six 28-week-old Hyline Brown laying hens were randomly divided into 4 groups (n=24). The control group received a basal diet, while treatment groups received the basal diet supplemented with 10% flaxseed (Trial Group I), 10% flaxseed + 5% fish oil (Trial Group II), or 5% fish oil (Trial Group III). Dietary composition and nutrient levels are presented in Table 1 . After 21 days of feeding, eggs were collected continuously for 7 days for yolk separation and spray-drying to produce yolk powder. At the end of the trial, 4 hens per group were randomly selected for slaughter, and liver tissues were collected, snap-frozen in liquid nitrogen, and stored for fatty acid composition and gene expression analysis.

Table 1 Composition and nutrient levels of experimental diets (air-dry basis) %

Items	Control group	Trial group I	Trial group II	Trial group III
Ingredients				
Corn				
Soybean meal				
Wheat bran				
Limestone				
Premix[1])				
NaCl				
Flaxseed				
Fish oil				
Vitamin E				

Items	Control group	Trial group I	Trial group II	Trial group III
Total Nutrient levels²)				
Crude protein (CP)				
Metabolizable energy (ME) (MJ/kg)				
Calcium (Ca)				
Total phosphorus (TP)				
Non-phytate phosphorus (NPP)				

[1]) The premix provided the following per kg of diet: Cu (as copper sulfate) 5 mg, Zn (as zinc sulfate) 40 mg, Mn (as manganese sulfate) 40 mg, Fe (as iron sulfate) 80 mg, Se (as sodium selenite) 0.2 mg, I (as calcium iodate) 0.35 mg, VA 7,500 IU, VD 2,500 IU, VE 15 IU, VK 2 mg, VB 2 mg, VB 4 mg, VB 4 mg, VB 0.01 mg, calcium pantothenate 5 mg, nicotinic acid 20 mg, folic acid 1 mg, biotin 0.2 mg.
[2]) Nutrient levels were calculated values.

1.2 Fatty Acid Methylation and Gas Chromatography Determination

Fatty acid methylation of tissue freeze-dried powder, egg yolk spray-dried powder, or air-dried feed samples was performed according to the method described by Christie [12]. Briefly, 0.5 g of sample was mixed with 2 mL n-hexane and 5 mL acetyl chloride reagent (acetyl chloride:methanol = 1:8), vortexed thoroughly, and incubated in an 80°C water bath for 2 hours with vortexing every 15 minutes. After cooling to room temperature under running water, 15 mL of 6% Na CO solution was added. Following complete reaction, the mixture was centrifuged at 5,000×g for 5 minutes, and the upper organic phase containing fatty acid methyl esters was collected for fatty acid content and composition analysis.

Gas chromatography was performed using an Agilent 7890A equipped with a flame ionization detector (FID) and an Agilent 19091N-213 capillary column (260°C, 30 m × 320 µm × 0.5 µm). The temperature program was: 180°C for 1 minute, increased to 250°C at 10°C/min, held for 15 minutes, with a 3-minute equilibration and 3-minute post-run. Injection volume was 1 µL, inlet temperature 270°C, and FID temperature 275°C. Carrier gas was high-purity nitrogen at 1.0 mL/min with a split ratio of 20:1. Methyl ester standards for ALA, EPA, DPA, and DHA, as well as a 22-component fatty acid methyl ester mixture, were purchased from Sigma. Absolute contents of the four fatty acids were determined using external standard curves, and peak area normalization was used for fatty acid composition analysis.

1.3 Hepatic Tissue Gene Expression Analysis

Total RNA was extracted using a commercial kit, and RNA concentration and purity were determined using a micro-UV spectrophotometer. First-strand cDNA was synthesized using a ReverTra Ace qPCR RT Kit. Gene-specific primers were designed based on NCBI-published sequences (Table 2).

Real-time quantitative PCR was performed on an ABI StepOne Plus system with the following reaction mixture: SYBR Green Master (ROX) 5 µL, sterile water 3 µL, forward and reverse primers (0.75 µL each), and cDNA 0.5 µL. Thermal cycling conditions were: 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 62°C for 34 s. Gene expression levels were calculated using the ΔΔCt method, with mean values and standard deviations determined from triplicate results.

Table 2 List of genes and primer sequences for real-time quantitative PCR

Genes	Primer sequences (5' -3')	Product size (bp)	Gene accession number
Fatty acid elongase 1 (ELOVL1)	ACCAACGGCAAGGTCAAATC GTTCAACAACAGT- GAGAAACAGCA		NM_001012598.1
Fatty acid elongase 2 (ELOVL2)	GCGAAAGTATCTGTGGTGGAAG GTAGGAAGACTG- GAACATGAGGC		NM_001197308.1

Genes	Primer sequences (5' -3')	Product size (bp)	Gene accession number
Fatty acid desaturase 1 (FADS1)	CCATACACCAGGATGAGGAGG GATGATACTGC-CCAGGAGAACA		XM_421052.5
Fatty acid desaturase 2 (FADS2)	TTGGCTAATGGTTTCATACTTCG TGTTCA-CATCTGGGTCTTTCTTG		NM_001160428.2
Stearoyl-CoA desaturase 1 (SCD1)	CTTTGGTCGGCTCCTTCACT CCAGGCACCCTCA-GATGTTC		DQ645535.1

1.4 Data Processing and Statistical Analysis

All data are expressed as "mean ± standard deviation." Statistical analyses were performed using SPSS 16.0 software. One-way ANOVA followed by Duncan' s multiple comparison test was used to analyze differences among groups. Two-way ANOVA using the General Linear Model was employed to evaluate the main effects of flaxseed, fish oil, and their interaction on relevant parameters.

2 Results and Analysis

2.1 Comparison of Fatty Acid Composition Among Four Experimental Diets

As shown in Table 3 , dietary n-3 PUFA proportions were higher in treatment groups than in the control group. ALA proportions exceeded 5% in Trial Groups I and II, while ALA was not detected in Trial Group III or the control group. EPA proportions in Trial Group I and the control group were similar, approximately 25% of those in Trial Groups II and III. DPA was not detected in any diet, while DHA proportion was highest in Trial Group II, followed by Trial Group III, with Trial Group I and the control group showing similarly low levels.

Table 3 Comparison of fatty acid composition among four experimental diets %

Items	Control group	Trial group I	Trial group II	Trial group III
Saturated fatty acids (SFA)				
Myristic acid (C14:0)				
Palmitic acid (C16:0)				
Stearic acid (C18:0)				
Arachidic acid (C20:0)				
Behenic acid (C22:0)				
Lignoceric acid (C24:0)				
Monounsaturated fatty acids (MUFA)				
Myristoleic acid (C14:1)				
Palmitoleic acid (C16:1)				
Oleic acid (C18:1)				
Eicosenoic acid (C20:1)				
Cetoleic acid (C22:1)				
Tetracosenoic acid (C24:1)				

Items	Control group	Trial group I	Trial group II	Trial group III
n-3 Polyunsaturated fatty acids (n-3 PUFA)				
-Linolenic acid (ALA, C18:3)				
Eicosapentaenoic acid (EPA, C20:5)				
Docosapentaenoic acid (DPA, C22:5)				
Docosahexaenoic acid (DHA, C22:6)				
n-6 Polyunsaturated fatty acids (n-6 PUFA)				
Linoleic acid (C18:2)				
-Linolenic acid (C18:3)				
Arachidonic acid (C20:4)				

Items	Control group	Trial group I	Trial group II	Trial group III
Other PUFAs				
Eicosadienoic acid (C20:2)				
All cis 8,11,14-eicosatrienoic acid (C20:3)				
All cis 11,14,17-eicosatrienoic acid (C20:3)				

ND = not detected (same as Table 5).

2.2 Comparison of n-3 PUFA Contents in Egg Yolk Among Groups

As shown in Table 4 , yolk n-3 PUFA contents in Trial Groups I, II, and III were significantly higher than in the control group ($P<0.05$). Trial Group II showed the highest ALA, DPA, and total n-3 PUFA contents, significantly exceeding all other groups ($P<0.05$). Trial Group III exhibited the highest EPA and DHA contents, significantly higher than other groups ($P<0.05$). Two-way ANOVA revealed that flaxseed, fish oil, and their interaction all had extremely significant effects on yolk n-3 PUFA contents ($P<0.01$).

Table 4 Comparison of n-3 PUFA contents in egg yolk from different groups mg/g

Items	Control group	Trial group I	Trial group II	Trial group III	P-value (two-way ANOVA)		
					Flaxseed	Fish oil	Flaxseed×fish oil
- Linolenic acid (ALA, C18:3)	1.61±0.11	28.72±0.08	29.84±0.11	4.92±0.11			

Items	Control group	Trial group I	Trial group II	Trial group III	P-value (two-way ANOVA)
Docosapentaenoic acid (DPA, C22:5)	0.83±0.13	2.38±0.05	6.82±0.12	4.78±0.10	
Eicosapentaenoic acid (EPA, C20:5)	0.53±0.06	1.89±0.17	1.91±0.08	11.80±0.10	
Docosahexaenoic acid (DHA, C22:6)	3.64±0.18	13.62±0.12	29.28±0.28	37.62±0.13	
Total	6.61±0.30	46.6±0.27	67.85±0.55	59.13±0.34	

In the same row, values with different small letter superscripts differ significantly (P<0.05), while values with the same letter or no letter do not differ significantly (P>0.05). The same notation applies below.

2.3 Comparison of Fatty Acid Composition in Liver Among Groups

As shown in Table 5 , compared with the control group, all treatment groups showed significantly reduced monounsaturated fatty acid proportions (P<0.05) and significantly increased n-3 PUFA proportions (P<0.05). Trial Groups II and III also exhibited significantly reduced n-6 PUFA proportions (P<0.05). C18:3 (including and) fatty acid contents were below detection limits in the control and Trial Group III. C20:4, C20:5, and C22:5 fatty acid contents were below detection limits in the control group. Numerically, Trial Group I showed the highest proportions of C18:2, C20:2, and C20:3 fatty acids, similar to the control group. Trial Group II had the highest proportions of C14:0, C20:0, and C20:5 fatty acids, while Trial Group III showed the highest proportions of C16:0, C20:1, C20:4, C22:5, and C22:6 fatty acids. C18:3 was present only in Trial Groups I and II, with Trial Group I significantly higher than Trial Group II (P<0.05).

Two-way ANOVA showed that flaxseed had significant effects on all fatty acids except C14:0, C18:1, C24:1, C22:6, C18:2, and C20:3 (all cis 8,11,14) (P>0.05). Fish oil had significant effects on all fatty acids except C20:0, C24:0, -C18:3, and C20:2 (P>0.05). The interaction between flaxseed and fish oil had significant effects on hepatic proportions of C18:0, C22:0, C24:0, C16:1, C20:1, C18:3, C22:5, C22:6, C20:4, and C20:3 fatty acids (P<0.05).

Table 5 Comparison of fatty acid composition in liver of hens fed among different groups %

Items	Control group	Trial group I	Trial group II	Trial group III	P-value (two-way ANOVA)		
					Flaxseed oil	Fish oil	Flaxseed×fish oil
Saturated fatty acids (SFA)	44.46±1.17	44.86±1.66	50.59±1.58	49.71±1.48			
Myristic acid (C14:0)	0.39±0.05	0.27±0.12	0.73±0.16	0.75±0.11			
Palmitic acid (C16:0)	27.36±1.20	24.02±1.86	30.31±1.92	32.94±1.37			
Stearic acid (C18:0)	12.18±1.07	16.16±0.65	17.22±0.28	14.40±0.75			
Arachidic acid (C20:0)	0.17±0.01	1.35±0.18	1.90±0.60	0.17±0.02			
Behenic acid (C22:0)	4.27±0.43	2.90±0.28	1.71±0.27	1.31±0.08			
Lignoceric acid (C24:0)	0.09±0.01	0.16±0.03	0.14±0.01	0.14±0.01			
Monounsaturated fatty acids (MUFA)	42.20±2.13	36.74±1.48	29.78±1.48	31.35±1.00			
Myristoleic acid (C14:1)	3.89±0.68	1.55±0.31	1.75±0.31	2.33±0.48			
Palmitoleic acid (C16:1)	37.88±2.47	34.76±1.77	27.50±1.78	27.49±1.14			
Oleic acid (C18:1)	0.19±0.01	0.17±0.03	0.67±0.10	0.80±0.03			
Eicosenoic acid (C20:1)	0.24±0.05	0.27±0.05	0.68±0.06	0.73±0.09			

Items	Control group	Trial group I	Trial group II	Trial group III	P-value (two-way ANOVA)
Cetoleic acid (C22:1)	0.79±0.10	5.01±0.16	9.64±0.68	8.59±1.15	
Tetracosenoic acid (C24:1)	2.79±0.10	2.59±0.15	0.15±0.01	0.88±0.23	
n-3 Polyunsaturated fatty acids (n-3 PUFA)	0.68±0.11	0.21±0.04	0.48±0.11	0.83±0.05	
- Linolenic acid (ALA, C18:3)	0.79±0.10	1.86±0.10	5.88±0.27	7.08±1.03	
Eicosapentaenoic acid (EPA, C20:5)	11.53±1.03	12.29±0.71	9.09±0.48	9.63±0.70	
Docosapentaenoic acid (DPA, C22:5)					
Docosahexaenoic acid (DHA, C22:6)					

Items	Control group	Trial group I	Trial group II	Trial group III	P-value (two-way ANOVA)
n-6 Polyunsaturated fatty acids (n-6 PUFA)	11.53±1.03	11.90±0.72	8.70±0.48	8.79±0.70	
Linoleic acid (C18:2)	0.15±0.02	0.13±0.01			
- Linolenic acid (C18:3)	0.24±0.02	0.52±0.04	0.84±0.06		
Arachidonic acid (C20:4)					
Other polyunsaturated fatty acids	0.23±0.03	0.29±0.02	0.25±0.04	0.23±0.02	
Eicosadienoic acid (C20:2)	0.63±0.07	0.46±0.16	0.44±0.12	0.30±0.06	
All cis 8,11,14-eicosatrienoic acid (C20:3)	0.18±0.02	0.34±0.09	0.23±0.02	0.19±0.02	
All cis 11,14,17-eicosatrienoic acid (C20:3)					

2.4 Comparison of Hepatic Fatty Acid Elongase and Desaturase Gene Expression Levels

As shown in Figure 1 [Figure 1: see original paper], compared with the control group, Trial Group I showed significantly increased expression of ELOVL1, ELOVL2, FADS1, and FADS2 ($P<0.05$), with no significant difference in SCD1 expression ($P>0.05$). Trial Group II exhibited significantly increased FADS1 and FADS2 expression ($P<0.05$), significantly decreased ELOVL2 and SCD1 expression ($P<0.05$), and no significant difference in ELOVL1 expression ($P>0.05$). Trial Group III showed significantly decreased expression of all elongase and desaturase genes ($P<0.05$).

Figure 1 Comparison of gene expression levels of fatty acid elongase and desaturase in liver

Two-way ANOVA revealed that dietary flaxseed and fish oil both had significant effects on ELOVL1, ELOVL2, FADS1, FADS2, and SCD1 expression levels ($P<0.05$). The interaction between flaxseed and fish oil had no significant effect on ELOVL1 and SCD1 expression ($P>0.05$) but significantly affected other gene expression levels ($P<0.05$).

Table 6 Two-way ANOVA of gene expression levels of fatty acid elongase and desaturase

Items	P-value		
	Flaxseed	Fish oil	Flaxseed×fish oil
Fatty acid elongase 1 (ELOVL1)			
Fatty acid elongase 2 (ELOVL2)			
Fatty acid desaturase 1 (FADS1)			
Fatty acid desaturase 2 (FADS2)			
Stearoyl-CoA desaturase 1 (SCD1)			

3.1 Enrichment Effects of Flaxseed and Fish Oil Diets on Yolk n-3 PUFAs

Flaxseed, fish oil, and algae are the primary dietary sources of n-3 PUFAs for enrichment in livestock and poultry products [13]. Flaxseed provides protein, oil, and ALA to poultry diets, and its use in producing n-3 PUFA-enriched eggs has been commercially accepted in many countries, increasing ALA content to 200 mg and DHA content to 90 mg per egg [15-16]. Fish oil, rich in DHA and EPA, can directly enrich these fatty acids in eggs [17-19], but is not widely accepted in many countries due to fishy odor development, with inclusion levels limited to 1.5% and DHA enrichment reaching only 100 mg per egg [20-21]. Even microencapsulated or deodorized fish oil still negatively affects egg quality [22]. Microalgae, particularly heterotrophic strains such as "DHA Gold,"are rich in DHA and can achieve greater enrichment of long-chain n-3 PUFAs; dietary

inclusion of 4.8% "DHA Gold" can increase egg DHA content to over 200 mg while maintaining acceptable flavor [23].

Our results showed that dietary supplementation with 5% fish oil enriched yolk primarily with DHA and EPA (Trial Group III), while 10% flaxseed enriched yolk mainly with ALA and DHA (Trial Group I). The combination of 10% flaxseed + 5% fish oil still enriched yolk primarily with DHA and ALA (Trial Group II), with total n-3 PUFA deposition exceeding that of either single supplementation. Notably, 5% fish oil supplementation increased yolk DHA content by 10-fold, reaching over 200 mg DHA per fresh egg, representing a relatively high level among current studies.

Dietary analysis revealed that while DHA proportions were similar between Trial Group I and the control group, significant differences in DHA deposition indicated that yolk DHA in flaxseed-fed hens originated primarily from in vivo ALA conversion. Although Trial Group II diet had the highest total n-3 PUFA and DHA proportions, yolk DHA enrichment was lower than in Trial Group III, with DHA content intermediate between Trial Groups I and III. Two-way ANOVA demonstrated significant effects of flaxseed, fish oil, and their interaction on all four n-3 PUFAs. This interaction differentially affected individual n-3 PUFAs: it promoted total n-3 PUFA deposition but did not achieve additive effects, and inhibited EPA and DHA deposition, with the combined group showing lower levels than the 5% fish oil group alone.

These findings suggest that different dietary n-3 PUFAs may interact during absorption and conversion, with total deposition still dependent on dietary composition. Cachaldora et al. [24] reported that excessive long-chain n-3 PUFAs can inhibit ALA-to-DHA conversion. Lemahieu et al. [11] noted that different n-3 fatty acids exhibit distinct biological activities, and compositional differences between fish oil and flaxseed lead to different tissue deposition outcomes. It can be hypothesized that increasing DHA content may feedback-inhibit ALA-to-DHA conversion, while enhanced ALA-to-DHA conversion may inhibit direct DHA absorption and deposition. The specific mechanisms warrant further investigation at both absorption and metabolic conversion levels.

3.2 Regulatory Effects of Flaxseed and Fish Oil Diets on Hepatic Fatty Acid Metabolism in Laying Hens

The liver is the primary organ for fatty acid metabolism in poultry. Both fish oil and flaxseed supplementation increased hepatic long-chain n-3 PUFA proportions, with fish oil also increasing saturated fatty acid proportions without affecting flaxseed' s effects. Based on dietary composition, fish oil appears to inhibit de novo synthesis of long-chain unsaturated fatty acids while allowing greater direct absorption of dietary DHA and other n-3 PUFAs. In contrast, flaxseed primarily activates elongase and desaturase gene expression to promote endogenous synthesis of long-chain unsaturated fatty acids, with relatively less direct absorption of dietary long-chain n-3 PUFAs. This pattern aligns with

yolk DHA deposition, indicating that flaxseed and fish oil differentially regulate fatty acid metabolic pathways in laying hens.

As shown in Figure 2 [Figure 2: see original paper], two distinct metabolic pathways exist for n-3 and n-6 unsaturated fatty acids, both requiring elongase and desaturase catalysis [25]. Δ6-desaturase specifically introduces double bonds at positions 6 and 7 of C18 and C24 unsaturated fatty acids, while Δ5-desaturase introduces double bonds at positions 5 and 6 of C20 unsaturated fatty acids, converting C-C single bonds to C=C double bonds to form more unsaturated fatty acids. The elongases ELOVL1 and ELOVL2 catalyze carbon chain elongation of C22 and C20/C22 fatty acids, respectively, to form long-chain unsaturated fatty acids [26-29]. These two pathways differ in substrates and end products: the n-6 pathway uses linoleic acid as substrate and produces DPA as the end product, while the n-3 pathway uses ALA as substrate and produces DHA as the end product, with cross-linking through common intermediates and enzymes.

Figure 2 The metabolism pathways of dietary n-3 and n-6 PUFAs in the liver [29]

In this study, flaxseed, rich in ALA (the n-3 pathway substrate), promoted elongase and desaturase gene expression, while fish oil, containing DHA (the n-3 pathway end product), suppressed these genes. This further confirms that fish oil enriches n-3 PUFAs primarily through direct absorption, whereas flaxseed acts mainly through de novo DHA synthesis. Combined with two-way ANOVA results, fish oil significantly inhibits hepatic fatty acid conversion metabolism, making tissue fatty acid composition more dependent on dietary composition, while flaxseed activates hepatic fatty acid conversion metabolism to promote endogenous n-3 PUFA synthesis.

Conclusions

1. Dietary supplementation with flaxseed or fish oil can enrich yolk n-3 PUFA deposition, with the highest total deposition achieved by combined supplementation of 10% flaxseed and 5% fish oil.
2. Yolk DHA deposition was optimal with 5% dietary fish oil, reaching over 200 mg DHA per fresh egg, representing a relatively high level among comparable studies.
3. Dietary flaxseed promotes hepatic ALA-to-DHA conversion by upregulating elongase and desaturase gene expression, whereas fish oil supplementation shows the opposite effect by downregulating these genes.

References

[1] GOGUS U, SMITH C. n-3 omega fatty acids: a review of current knowledge[J]. International Journal of Food Science and Technology, 2010, 45(3): 417–436.

[2] SIRIWARDHANA N, KALUPAHANA N S, MOUSTAID-MOUSSA N. Health benefits of n-3 polyunsaturated fatty acids: eicosapentaenoic acid and docosahexaenoic acid[J]. Advances in Food and Nutrition Research, 2012, 65: 211-222.

[3] 朱玲娇. 禽蛋磷脂组成及其结构特点分析研究 [D]. 硕士学位论文. 武汉: 华中农业大学, 2013: 82-84.

[4] 秦德元, 张鹏. 酶法合成 EPA/DHA 型卵磷脂 [J]. 中国油脂, 2004, 29(9): 45-47.

[5] SCHARITER J A, PACHUSKI J, FRIED B, et al. Determination of neutral lipids and phospholipids in the cercariae of Schistosoma mansoni by high performance thin layer chromatography[J]. Journal Liquid Chromatography Related Technologies, 2002, 25(10/11): 1615-1622.

[6] STILLWELL W, EHRINGER W, JENSKI L J. Docosahexaenoic acid increases permeability of lipid vesicles and tumor cells[J]. Lipids, 1993, 28(2): 103-108.

[7] SHIROUCHI B, NAGAO K, INOUE N, et al. Effect of dietary omega 3 phosphatidylcholine on obesity-related disorders in obese otsuka long-evans tokushima fatty rats[J]. Journal of Agricultural and Food Chemistry, 2007, 55(17): 7170-7176.

[8] 马琴. 二十二碳六烯酸—磷脂生物活性的研究 [D]. 硕士学位论文. 青岛: 中国海洋大学, 2009: 77.

[9] ISHIGAMORI H, HOSOKAWA M, KOHNO H, et al. Docosahexaenoic acid-containing phosphatidylethanolamine enhances HL-60 cell differentiation by regulation of c-jun and c-myc expression[J]. Molecular and Cellular Biochemistry, 2005, 275(1/2): 127-133.

[10] 杨玉红, 王静凤, 龙腾腾, 等. 磷脂型 DHA 对肿瘤细胞凋亡的影响 [J]. 中国药理学通报, 2011, 27(2): 178-182.

[11] LEMAHIEU C, BRUNEEL C, RYCKEBOSCH E, et al. 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[J]. Journal of Functional Foods, 2015, 19: 821-827.

[12] CHRISTIE W W. Preparation of ester derivatives of fatty acids for chromatographic analysis[M]//CHRISTIE W W. Advances in Lipid Methodology. Dundee: Oily Press, 1993: 69-119.

[13] FRAEYE I, BRUNEEL C, LEMAHIEU C, et al. Dietary enrichment of eggs with omega-3 fatty acids: a review[J]. Food Research International, 2012, 48(2): 961-969.

[14] SURAI P F, SPARKS N H C. Designer eggs: from improvement of egg composition to functional food[J]. Trends in Food Science & Technology, 2001, 12(1): 7-16.

[15] AYMOND W M, VAN ELSWYK M E. Yolk thiobarbituric acid reactive substances and n-3 fatty acids response to whole ground flaxseed[J]. Poultry Science, 1995, 74(8): 1388-1394.

[16] BEAN L D, LEESON S. Long-term effects of feeding flaxseed on performance and egg fatty acid composition of brown and white hens[J]. Poultry Science, 2003, 82(3): 388-394.

[17] BOVET P, FAEH D, MADELEINE G, et al. Decrease in blood triglycerides associated with the consumption of eggs of hens fed diets supplemented with oil[J]. Nutrition, Metabolism, and Cardiovascular Diseases, 2007, 17(4): 280-287.

[18] CACHALDORA P, GARCÍA-REBOLLA P, ALVAREZ C, et al. Effect of type and level of fish oil supplementation on yolk fat composition and n-3 fatty acids retention efficiency in laying hens[J]. British Poultry Science, 2006, 47(1): 43-49.

[19] CARRILLO S, LÓPEZ E, CASAS M M, et al. Potential use of seaweeds in the laying hen ration to improve the quality of n-3 fatty acid enriched eggs[J]. Journal of Applied Phycology, 2008, 20(5): 721-728.

[20] GONZALEZ-ESQUERRA R, LEESON S. Effect of feeding hens regular or deodorized menhaden oil on production parameters, yolk fatty acid profile, and sensory quality of eggs[J]. Poultry Science, 2000, 79(11): 1597-1602.

[21] VAN ELSWYK M E. Comparison of n-3 fatty acid sources in laying hen rations for improvement of whole egg nutritional quality: a review[J]. British Journal of Nutrition, 1997, 78(1): S61-S69.

[22] LAWLOR J B, GAUDETTE N, DICKSON T, et al. Fatty acid profile and sensory characteristics of table eggs from laying hens fed diets containing microencapsulated fish oil[J]. Animal Feed Science and Technology, 2010, 156(3/4): 97-103.

[23] HERBER-MCNEILL S M, VAN ELSWYK M E. Dietary marine algae maintains egg consumer acceptability while enhancing yolk color[J]. Poultry Science, 1998, 77(3): 493-496.

[24] CACHALDORA P, GARCÍA-REBOLLA P, ALVAREZ C, et al. Double enrichment of chicken eggs with conjugated linoleic acid and n-3 fatty acids through dietary fat supplementation[J]. Animal Feed Science and Technology, 2008, 144(3/4): 315-326.

[25] QIU X. Biosynthesis of docosahexaenoic acid (DHA, 22:6-4,7,10,13,16,19): two distinct pathways[J]. Prostaglandins, Leukotrienes and Essential Fatty Acids, 2003, 68(2): 181-186.

[26] JAKOBSSON A, WESTERBERG R, JACOBSSON A. Fatty acid elongases in mammals: their regulation and roles in metabolism[J]. Progress in Lipid Research, 2006, 45(3): 237-249.

[27] 孙晓艳, 林艳丽, 熊福银, 等. 二十碳五烯酸和二十二碳六烯酸的生物合成途径研究进展 [J]. 生物技术通讯, 2012, 23(5): 755-758.

[28] SCHACKMANN M J, OFMAN R, DIJKSTRA I M, et al. Enzymatic characterization of ELOVL1, a key enzyme in very long-chain fatty acid synthesis[J]. Biochimica et Biophysica Acta (BBA) -Molecular and Cell Biology of Lipids, 2015, 1851(2): 231-237.

[29] ROSERO D S, BOYD R D, ODLE J, et al. Optimizing dietary lipid use to improve essential fatty acid status and reproductive performance of the modern lactating sow: a review[J]. Journal of Animal Science and Biotechnology, 2016, 7: 34.

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