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Co-supplementation of Dietary Choline and Schizochytrium Oil Enhances Docosahexaenoic Acid Enrichment in Egg Yolk Postprint

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Abstract

This experiment aimed to investigate the effects of dietary choline and Schizochytrium oil (SO) on egg yolk lipids and docosahexaenoic acid (DHA) enrichment. A total of 288 Jinghong laying hens aged 26 weeks were selected and divided into 4 groups (6 replicates per group, 12 hens per replicate). A 2×2 factorial design was adopted, with dietary choline (500 and 1000 mg/kg) and SO (0 and 0.5%) as two main factors, and 4 isonitrogenous and isoenergetic diets were formulated. The pre-trial period was 1 week, and the formal trial period was 8 weeks. The results showed: 1) During the experimental period, there were no significant differences in production performance and egg quality among all groups ($P>0.05$). 2) There were no significant differences in yolk dry matter, crude fat, cholesterol, and triglyceride contents among all groups ($P>0.05$). The total phospholipid content in yolk of the 1000 mg/kg choline group was significantly higher than that of the 500 mg/kg choline group ($P<0.05$). Choline and SO had a significant interaction effect on yolk total phospholipid content ($P = 0.04$), with the highest total phospholipid content in the 1000 mg/kg choline + 0.5% SO group. 3) 0.5% SO significantly increased yolk n-3 polyunsaturated fatty acids (PUFA) and DHA contents ($P<0.05$), and significantly decreased n-6 PUFA content and n-6 PUFA/n-3 PUFA ratio ($P<0.05$). Choline and SO had significant interaction effects on yolk n-3 PUFA ($P<0.001$), n-6 PUFA ($P=0.01$), DHA contents ($P<0.001$), and n-6 PUFA/n-3 PUFA ratio ($P = 0.01$). The 1000 mg/kg choline + 0.5% SO group had significantly higher yolk n-3 PUFA and DHA contents than other groups ($P<0.05$), and significantly lower n-6 PUFA content and n-6 PUFA/n-3 PUFA ratio than other groups ($P<0.05$). In conclusion, under the conditions of this experiment, the combined supplementation of 1000 mg/kg choline and 0.5% SO in the diet promoted DHA enrichment in egg yolk without significantly affecting laying hen production performance and egg quality.

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Full Text

Dietary Choline and Schizochytrium Oil Enhance Docosahexaenoic Acid Enrichment in Egg Yolk

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Abstract

This study investigated the effects of dietary choline and Schizochytrium oil (SO) on lipid composition and docosahexaenoic acid (DHA) enrichment in egg yolk. Two hundred eighty-eight 26-week-old Jinghong laying hens were randomly allocated to 4 groups (6 replicates per group, 12 birds per replicate) in a 2×2 factorial design. The two main factors were dietary choline supplementation (500 and 1,000 mg/kg) and SO supplementation (0 and 0.5%), resulting in four isonitrogenous and isocaloric diets. The experiment consisted of a 1-week adaptation period followed by an 8-week feeding trial. The results showed: (1) No significant differences were observed in production performance or egg quality among all groups during the experimental period ($P>0.05$). (2) Yolk contents of dry matter, crude fat, cholesterol, and triglycerides did not differ significantly among groups ($P>0.05$). The total phospholipid content in yolk was significantly higher in the 1,000 mg/kg choline group compared with the 500 mg/kg choline group ($P<0.05$). A significant interaction between choline and SO was detected for yolk total phospholipid content ($P=0.04$), with the 1,000 mg/kg choline + 0.5% SO group showing the highest value. (3) Dietary supplementation with 0.5% SO significantly increased yolk n-3 polyunsaturated fatty acids (PUFA) and DHA contents ($P<0.05$) while significantly decreasing n-6 PUFA content and the n-6 PUFA/n-3 PUFA ratio ($P<0.05$). Significant interactions between choline and SO were observed for yolk n-3 PUFA ($P<0.001$), n-6 PUFA ($P=0.01$), DHA ($P<0.001$) contents, and the n-6 PUFA/n-3 PUFA ratio ($P=0.01$). The 1,000 mg/kg choline + 0.5% SO group exhibited significantly higher n-3 PUFA and DHA contents and significantly lower n-6 PUFA content and n-6 PUFA/n-3 PUFA ratio compared with other groups ($P<0.05$). In conclusion, combined supplementation of 1,000 mg/kg choline and 0.5% SO in the diet promoted DHA enrichment in egg yolk without adversely affecting laying hen performance or egg quality under the experimental conditions.

Keywords: choline; egg yolk; DHA; fatty acid; Schizochytrium oil

Docosahexaenoic acid (DHA, C22:6 n-3) is an n-3 long-chain polyunsaturated fatty acid (PUFA) that promotes brain and retinal development in infants, but cannot be synthesized by humans and must be obtained through dietary sources. Therefore, increasing DHA content in foods is of great significance. The fatty acid composition of egg yolk is highly susceptible to dietary fatty acid composition, and supplementing diets with n-3 PUFA can increase DHA content in eggs. Schizochytrium is a DHA-rich microalga, and previous studies have shown that dietary Schizochytrium powder significantly increases yolk DHA content. However, while yolk DHA content increases with higher supplementation levels, the enrichment efficiency of DHA in eggs decreases linearly, which is one reason for the high cost of DHA-enriched eggs. Since DHA primarily exists in yolk phospholipids, and the reaction binding DHA to phospholipids to form phospholipid-type DHA (DHA-PL) is reversible, more phospholipids are required to maintain an appropriate molar ratio when DHA content increases, thereby generating more DHA-PL. Dietary choline supplementation has been shown to significantly increase yolk lecithin content. However, no studies have reported whether combined supplementation of dietary choline and Schizochytrium oil promotes DHA enrichment in egg yolk. Therefore, this experiment was conducted to explore the potential synergistic effects of choline and Schizochytrium oil on DHA enrichment in egg yolk of peak-laying hens, aiming to provide insights and experimental evidence for efficient and economical production of DHA-enriched eggs.

1.1 Experimental Design and Diets

A 2×2 factorial design was employed (Table 1), with dietary choline (500 and 1,000 mg/kg) and Schizochytrium oil (0 and 0.5%) as the two main factors, resulting in four experimental diets: 500 mg/kg choline (Group 1), 1,000 mg/kg choline (Group 2), 500 mg/kg choline + 0.5% Schizochytrium oil (Group 3), and 1,000 mg/kg choline + 0.5% Schizochytrium oil (Group 4). Schizochytrium oil was purchased from Xiamen Kingdomway Group Co., Ltd., containing 66.3% DHA of total fatty acids (measured value). Choline (choline chloride, 99.3%) was obtained from Hangzhou Haierxi Animal Science and Technology Co., Ltd. Two hundred eighty-eight healthy Jinghong-1 laying hens at 26 weeks of age with similar body weight and initial laying rate were randomly divided into 4 groups with 6 replicates of 12 birds each.

The measured gross energy of Schizochytrium oil was 37.63 MJ/kg. Based on the metabolizable energy calculation formula for laying hens from NRC (2012), the metabolizable energy of Schizochytrium oil was determined to be 27.28 MJ/kg. All experimental diets were formulated to be isonitrogenous (16.60% crude protein) and isocaloric (11.15 MJ/kg metabolizable energy), with nutrient levels referenced to NRC (1994) and the Chinese Feeding Standard of Chickens (NY/T 33—2004), combined with the Jinghong laying hen feeding manual. Choline or Schizochytrium oil was added to the basal diet according to the experimental design. All diets were supplemented with 100 mg vitamin E, 50 mg tea polyphe-

nols, and 100 mg ethoxyquin per kg as antioxidants to prevent Schizochytrium oil oxidation.

All diets were sampled using a multi-point sampling method, with 500 g of each diet stored at 4°C for analysis. Crude protein content in experimental diets was determined according to GB/T 6432–1994, calcium (Ca) content according to GB/T 13885–2003, and methionine (Met), lysine (Lys), and methionine + cysteine (Met+Cys) contents according to GB/T 18246–2000. Diet composition and nutrient levels are presented in Table 2 , and dietary fatty acid composition is shown in Table 3 .

Table 1 Experimental design

Groups	Choline (mg/kg)	Schizochytrium oil (%)
1	500	0
2	1,000	0
3	500	0.5
4	1,000	0.5

Table 2 Composition and nutrient levels of experimental diets (air-dry basis) %

Items	Groups
Ingredients	
Corn	
Soybean meal	
Schizochytrium oil	
Choline	
Limestone	
CaHPO	
Premix[1]	
DL-Met	
Zeolite powder	
Total	
Nutrient levels[2]	
ME (MJ/kg)	
CP	
Ca	
AP	
Lys	
Met	
Met+Cys	

[1]The premix provided the following per kg of diets: VA 12,500 IU, VD 425 IU, VE 115 IU, VK 2 mg, VB 0.98 mg, VB 8.5 mg, VB 8 mg, D-pantothenic acid

50 mg, niacin 32.5 mg, biotin 2 mg, folic acid 5 mg, VB 5 mg, Cu (as copper sulfate) 8 mg, I (as potassium iodide) 1 mg, Fe (as ferrous sulfate) 60 mg, Se (as sodium selenite) 0.3 mg, Mn (as manganese sulfate) 65 mg, Zn (as zinc sulfate) 66 mg, phytase 500 mg, yeast culture 10 g, tea polyphenols 50 mg, ethoxyquin 100 mg.

[2]ME and AP were calculated values, while the others were measured values.

Table 3 Fatty acid composition of experimental diets (DM basis) mg/g

Items	Groups
C10:0	
C12:0	
C14:0	
C15:0	
C16:0	
C16:1	
C17:0	
C18:0	
C18:1 n-9c	
C18:2 n-6c	
C18:3 n-3	
C20:0	
C20:1	
C21:0	
C20:2	
C20:3 n-6	
C20:4 n-6	
C20:5 n-3	
C22:0	
C22:1 n-9	
C23:0	
C24:0	
C22:6 n-3	
C24:1	
SFA	
PUFA	
n-3 PUFA	
n-6 PUFA	
n-6 PUFA/n-3 PUFA	

1.2 Animal Management

During the experimental period, specialized personnel managed and fed the laying hens. Birds were housed in 3-tier step cages (57 cm × 47 cm × 47 cm per cage) at a stocking density of 3 hens per cage, with free access to feed and

water. The lighting cycle was 16 h light:8 h dark with an intensity of 20 lx. House temperature was maintained at (21±2)°C with relative humidity of 50%–60%. Ventilation consisted of natural ventilation combined with longitudinal negative pressure ventilation. The house was disinfected with chickens present every 3 days and cleaned daily using an automatic manure removal system twice per day. Conventional vaccination protocols were followed throughout the trial period. Feed was provided three times daily (07:30, 12:30, and 17:30), and eggs were collected once daily. The trial included a 1-week pre-period and an 8-week formal experimental period.

1.3.1 Production Performance

The health status of hens in each group was monitored daily. Total egg weight and number of eggs, including soft-shelled and abnormal eggs (various deformities, oversized or undersized eggs), were recorded daily by replicate for calculating average egg weight (excluding soft-shelled and abnormal eggs). Feed intake was measured every 2 weeks. Laying rate, daily egg mass, average egg weight, average daily feed intake, and feed conversion ratio were calculated during the formal experimental period.

1.3.2 Egg Internal Quality and Yolk Ratio

At the end of week 8 of the feeding trial, three eggs closest to the average egg weight of each replicate were selected to determine egg quality parameters including yolk color, albumen height, and Haugh unit. Yolk color, albumen height, and Haugh unit were measured using a SONOVA egg quality automatic analyzer (Orka food technology Ltd, Ramat Hasharon, Israel). Egg weight was measured using an electronic balance, yolk was separated and weighed, and yolk ratio was calculated.

1.3.3 Yolk Lipid Composition

At the end of week 8, three eggs closest to the average egg weight of each replicate were collected. Yolks were separated, weighed, mixed thoroughly, and stored at -20°C. Yolk samples were vacuum freeze-dried for 72 h and reweighed. Yolk dry matter (%) was calculated as 100 × weight after freeze-drying (g) / weight before freeze-drying (g). Freeze-dried yolk was carefully ground, passed through a 40-mesh sieve, and stored at 4°C.

Yolk crude fat content was analyzed using the Folch method and expressed as percentage of yolk dry matter. Cholesterol and triglyceride contents in yolk dry matter were determined using assay kits from Nanjing Jiancheng Bioengineering Institute according to the manufacturer' s instructions.

Total phospholipid content in yolk was determined using the method of Palacios et al. with slight modifications. Briefly, 7 g of freeze-dried yolk powder was extracted with 50 mL of 95% ethanol until completely dispersed, then centrifuged at 2,000 r/min for 5 min. The extract was separated using a separatory funnel

and extracted twice with 30 mL n-hexane. The extracted liquid was combined in the separatory funnel. The precipitate was extracted twice with 30 mL of 95% ethanol, and these extracts were also added to the separatory funnel. After gentle shaking and 1 h standing for layer separation, the ethanol phase was removed. The n-hexane phase was mixed with 50 mL of 90% ethanol, allowed to separate, and the bottom n-hexane layer was collected. Ethanol in the previous ethanol phase was evaporated, and the lipids were dissolved in 30 mL n-hexane, transferred to a separatory funnel, mixed with 100 mL of -20°C acetone, and carefully stirred to precipitate total phospholipids. After centrifugation at 1,500 r/min for 15 min at 5°C, the supernatant was decanted, and the remaining liquid was rotary evaporated, leaving the total phospholipids as solid residue.

1.3.4 Fatty Acid Composition of Diets and Yolk

For fatty acid analysis, (90±10) mg of freeze-dried yolk powder [(0.30±0.01) g for diets] was transferred to a 15-mL screw-cap tube. One milliliter of n-hexane, 1 mL internal standard solution (1 mg/mL methyl undecanoate in n-hexane), and 4 mL of methanol:acetyl chloride mixture (10:1, v/v) were added sequentially. After mixing, samples were methylated in an 80°C water bath for 3 h. Following methylation, samples were cooled to room temperature, and 5 mL of 7% potassium carbonate solution was added slowly. After vortex mixing and centrifugation at 4,000 r/min for 5 min, 1.2 mL of the upper organic phase was collected for analysis. Fatty acid methyl esters were analyzed using a GC-450 gas chromatograph (Techcomp Scientific Instrument Co., Ltd.) equipped with an Agilent DB-23 column (60 m × 250 m × 0.25 m). Helium was used as carrier gas at a constant flow rate of 1.00 mL/min. Detector temperature was 280°C, injector temperature 270°C. The temperature program started at 100°C for 5 min, then increased at 4°C/min to 240°C (held for 30 min). Injection volume was 1.0 µL. n-Hexane was used as cleaning solvent with three washes before and after each injection.

1.4 Statistical Analysis

Data were expressed as means. All data were organized using Excel and analyzed using the General Linear Model (GLM) procedure in SPSS 23.0 software for 2×2 factorial analysis. Main effects of choline level and Schizochytrium oil level and their interaction were analyzed by multivariate analysis of variance. When significant interactions were detected, Duncan' s multiple comparison test was used. Significance was declared at $P<0.05$.

2.1 Effects of Dietary Choline and Schizochytrium Oil on Performance of Laying Hens During Peak Production

As shown in Table 4 , dietary supplementation with choline and Schizochytrium oil had no significant effects on production performance of laying hens during peak production ($P>0.05$). Daily egg mass in Group 3 was slightly lower than other groups, but the difference was not significant ($P>0.05$). Average egg

weight in groups supplemented with 1,000 mg/kg choline was higher than those supplemented with 500 mg/kg choline, but the difference was not significant (P>0.05).

Table 4 Effects of dietary choline and Schizochytrium oil on performance of laying hens during peak production

Items	Groups	Choline (mg/kg)	Schizochytrium oil (%)	P-value
				Choline
AEW/g				
DEM/g				
AER/%				
ADFI/g				

Means within the same column with different small letter superscripts differ significantly (P<0.05). The same applies to the following tables.

2.2 Effects of Dietary Choline and Schizochytrium Oil on Egg Quality

As shown in Table 5 , dietary choline and Schizochytrium oil had no significant effects on albumen height or Haugh unit (P>0.05). Dietary supplementation with 0.5% Schizochytrium oil tended to increase yolk color, but the difference was not significant (P>0.05). No significant effects were observed on yolk ratio (P>0.05). In conclusion, dietary choline and Schizochytrium oil had no significant effects on egg internal quality under the experimental conditions.

Table 5 Effects of dietary choline and Schizochytrium oil on egg quality

Items	Groups	Choline (mg/kg)	Schizochytrium oil (%)	P-value
				Choline
Thick albumen height/mm				
Haugh unit				
Yolk color				
Yolk percent/%				

2.3 Effects of Dietary Choline and Schizochytrium Oil on Yolk Lipid Composition

As shown in Table 6 , dietary choline and Schizochytrium oil had no significant effects on yolk dry matter, crude fat, cholesterol, or triglyceride contents (P>0.05). Dietary supplementation with 0.5% Schizochytrium oil tended to increase yolk dry matter content (P=0.08). Groups supplemented with 0.5% Schizochytrium oil showed increased crude fat and triglyceride contents and decreased cholesterol content, but differences were not significant (P>0.05). Compared with 500

mg/kg choline supplementation, 1,000 mg/kg choline significantly increased total phospholipid content in yolk (P<0.05). Groups 2 and 4 had significantly higher yolk total phospholipid content than Groups 1 and 3 (P<0.05). In summary, dietary supplementation with 1,000 mg/kg choline significantly increased total phospholipid content in egg yolk.

Table 6 Effects of dietary choline and Schizochytrium oil on lipid composition in yolk (DM basis)

Items	Groups	Choline (mg/kg)	Schizochytrium oil (%)	Dry matter/%	Crude fat (mg/g)	Cholesterol (mg/g)	Triglyceride (mg/g)	Phospholipid (mg/g)
								202.90b
								217.14a
								206.56b
								219.05a
P-value								
Choline								204.73b
Schizochytrium oil								218.10a
Choline × Schizochytrium oil								

[1]Calculated by dry matter in yolk.

2.4 Effects of Dietary Choline and Schizochytrium Oil on Yolk Fatty Acid Composition

As shown in Table 7 , dietary choline and Schizochytrium oil had no significant effects on saturated fatty acids or total PUFA content in yolk (P>0.05). Dietary choline level significantly affected yolk n-3 PUFA, DHA content, and n-6 PUFA/n-3 PUFA ratio (P<0.05). Compared with the 500 mg/kg choline group, the 1,000 mg/kg choline group showed significantly higher n-3 PUFA and DHA contents (P<0.05) and significantly lower n-6 PUFA/n-3 PUFA ratio (P<0.05). Dietary Schizochytrium oil level significantly affected yolk n-3 PUFA, n-6 PUFA, DHA contents, and n-6 PUFA/n-3 PUFA ratio (P<0.05). Compared with the non-supplemented group, 0.5% Schizochytrium oil significantly increased yolk n-3 PUFA and DHA contents (P<0.05) and significantly decreased n-6 PUFA content and n-6 PUFA/n-3 PUFA ratio (P<0.05). Significant interactions between choline and Schizochytrium oil were observed for yolk n-3 PUFA, n-6 PUFA, DHA contents, and n-6 PUFA/n-3 PUFA ratio (P<0.05). Group 4 showed significantly higher n-3 PUFA and DHA contents

than all other groups (P<0.05). Compared with Group 3, Group 4 exhibited 15.20% and 14.58% increases in yolk n-3 PUFA and DHA contents, respectively (P<0.05). In summary, dietary supplementation with 0.5% Schizochytrium oil significantly increased yolk n-3 PUFA and DHA contents while decreasing n-6 PUFA content and n-6 PUFA/n-3 PUFA ratio. Adding 1,000 mg/kg choline simultaneously with 0.5% Schizochytrium oil promoted DHA deposition in egg yolk.

Table 7 Effects of dietary choline and Schizochytrium oil on yolk fatty acid composition (DM basis)

Items	Groups	Choline (mg/kg)	Schizochytrium oil (%)	n-3 PUFA (mg/g)	n-6 PUFA (mg/g)	n-6 PUFA/n-3 PUFA	DHA (mg/g)
				9.41c	111.49a	11.86a	5.65c
				9.70c	112.42a	11.57a	6.06c
				22.90b	98.71b	4.28b	19.89b
				26.38a	96.78c	3.68c	22.79a
				16.15b	8.07a	12.77b	
				18.04a	7.63b	14.42a	
P-value				<0.001	<0.001	<0.001	<0.001
Choline				<0.001	<0.001	<0.001	<0.001
Schizochytrium oil				<0.001	<0.001	<0.001	<0.001
Choline × Schizochytrium oil				<0.001	<0.001	<0.001	<0.001

3.1 Effects of Dietary Choline and Schizochytrium Oil on Performance and Egg Quality of Peak-Laying Hens

Previous studies have shown that dietary supplementation with 1,000 mg/kg choline had no significant effects on laying rate, average daily feed intake, average egg weight, or feed conversion ratio. Additionally, dietary supplementation with 2% Schizochytrium powder (providing slightly more DHA than 0.5% Schizochytrium oil) also had no significant effects on laying hen performance, consistent with the present results. In this experiment, no significant differences were observed in albumen height, Haugh unit, yolk color, or yolk ratio among groups. These results are similar to previous findings on egg quality when choline or Schizochytrium oil was supplemented individually, indicating that the levels of choline and Schizochytrium oil used in this experiment did not significantly affect egg quality of peak-laying hens.

3.2 Effects of Dietary Choline and Schizochytrium Oil on Phospholipid Content in Egg Yolk

The present results showed that dietary supplementation with 1,000 mg/kg choline significantly increased yolk phospholipid content, consistent with previous studies. This may be explained by two mechanisms. First, choline is synthesized into phosphatidylcholine (PC) via the cytidine diphosphate-choline pathway in the body. PC is transported through blood and enriched in yolk, and PC is a major component of yolk phospholipids (accounting for 78% of yolk phospholipids). Therefore, dietary choline supplementation promotes increased PC content in yolk, thereby increasing total phospholipid content. Second, choline participates in phosphatidylethanolamine (PE) synthesis through the methylation transfer pathway. PE is also an important component of yolk phospholipids (accounting for 18% of total phospholipids), and increased PE content can also raise yolk phospholipid content. In this experiment, dietary Schizochytrium oil slightly decreased yolk cholesterol content, but the difference was not significant. This differs from previous findings that n-3 PUFA can reduce yolk cholesterol, likely because the n-3 PUFA in Schizochytrium oil is primarily DHA, whereas -linolenic acid (ALA) and eicosapentaenoic acid (EPA) are the main fatty acids responsible for reducing yolk cholesterol. This explains why yolk cholesterol was not significantly reduced in this experiment. Additionally, no correlation was found between changes in yolk phospholipid and fatty acid contents and yolk triglyceride content.

3.3 Effects of Dietary Choline and Schizochytrium Oil on Yolk Fatty Acid Composition

Comparing dietary fatty acid composition (Table 3) with yolk fatty acid composition (Table 7) reveals similar patterns for major fatty acids including PUFA, n-3 PUFA, DHA, and n-6 PUFA, indicating that yolk fatty acid composition is closely related to dietary fatty acid composition. The DHA content in diets supplemented with Schizochytrium oil was 15 times higher than in non-supplemented diets, while yolk DHA content was only 3.6 times higher. This result is consistent with previous studies and suggests that the enrichment efficiency of DHA in yolk can still be improved.

By comparing dietary and yolk fatty acid compositions, we also noted that even in groups with similar dietary DHA content, supplementation with 1,000 mg/kg choline still significantly increased yolk DHA content. Compared with Groups 1 and 3, yolk DHA content in Groups 2 and 4 increased by 7.26% and 14.58%, respectively. This finding was not addressed in previous studies on the effects of choline on yolk lipids and is first demonstrated in this experiment: combined supplementation of choline and Schizochytrium oil in laying hen diets promotes DHA deposition in egg yolk. This result may be related to the forms of DHA present in diets and yolk. DHA in dietary Schizochytrium oil exists primarily as triglyceride-type DHA (DHA-TG, measured in the product), whereas DHA enriched in yolk exists mainly as phosphatidylcholine-type DHA

(DHA-PC) in lecithin. Typically, the fatty acid composition of PC in lecithin consists of C16:0 (33.1%), C18:0 (11.3%), C18:1 (32.2%), and C18:2 (9.2%), indicating that DHA is not simply transported as DHA-TG in laying hens but is enriched in yolk as DHA-PC through a series of lipid reactions. Currently, n-3 PUFA can be incorporated into PC through enzymatic reactions in vitro. We hypothesize that the conversion pathway from DHA-TG to DHA-PC in laying hens may be similar. First, DHA-TG is hydrolyzed by triglyceride lipase and hormone-sensitive lipase to produce free DHA, which can then be re-esterified with lysophosphatidylcholine (LPC, formed by phospholipase-catalyzed removal of one fatty acid from PC) to form DHA-PC. Second, DHA-TG is hydrolyzed by triglyceride lipase to produce DHA-containing diglycerides, which can undergo direct transesterification with PC under the catalysis of specific phospholipases to form DHA-PC. The reason choline promotes DHA-PC increase may be related to the second synthesis pathway. The liver is the primary site of lipid metabolism in poultry, where not only bile acids and lipases hydrolyze DHA-TG, but choline is also phosphorylated to cytidine diphosphate-choline. Under the catalysis of phosphocholine cytidylyltransferase in the liver, cytidine diphosphate-choline combines with diglycerides to form large amounts of PC. When these diglycerides contain DHA, the product is DHA-PC. Therefore, increasing dietary choline indirectly increases the phospholipid substrate for DHA-PC synthesis in the liver, promoting more DHA-PC synthesis. The transport of DHA-PC from liver to yolk requires a series of processes. Typically, DHA-PC, triglycerides, and sterols in the liver combine with apolipoprotein B100 (Apo-B100) to form very low-density lipoprotein (VLDL), which is protected by apolipoprotein-VLDL-II (Apo-VLDL-II), transported through blood circulation, and directed to oocytes. Due to the characteristics of the follicular basement membrane and zona pellucida, only VLDL with a diameter of 25–40 nm can bind to specific receptors on the oocyte membrane surface and be internalized into oocytes for deposition. This process may be similar to DHA-PC deposition in the brain through the blood-brain barrier, and many specific fatty acids have physiological functions in regulating lipid metabolism and transport processes. Therefore, DHA-PC may play a role beyond participating in the outer structure of VLDL synthesis. Further research is needed to determine whether the pathway of DHA incorporation into PC and the apolipoproteins transporting DHA-PC have specific binding characteristics.

Under the experimental conditions: (1) Dietary supplementation with 1,000 mg/kg choline increased yolk phospholipid content, and 0.5% Schizochytrium oil increased yolk DHA content; (2) Simultaneous supplementation with 1,000 mg/kg choline and 0.5% Schizochytrium oil promoted DHA deposition in egg yolk without significantly affecting laying hen performance or egg quality.

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