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

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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 expression of genes related to fatty acid synthesis and metabolism. Ninety-six 28-week-old Hy-Line Brown laying hens were selected and randomly divided 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. After 21 days of feeding, eggs were collected continuously for 7 days, and then 4 hens were randomly selected from each group for slaughter and sampling. The results showed that, compared with the control group: 1) supplementation of both flaxseed and fish oil significantly increased n-3 polyunsaturated fatty acid content in egg yolk ($P<0.05$), with the highest contents of docosahexaenoic acid (DHA) and eicosapentaenoic acid observed in egg yolk from 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 of 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 of flaxseed or fish oil alone can enrich n-3 polyunsaturated fatty acid deposition in egg yolk; supplementation of flaxseed promotes the conversion of α-linolenic acid to DHA in the liver, as evidenced by upregulated expression of fatty acid elongase and desaturase genes, while supplementation of fish oil shows the opposite effect.

Full Text

Effects of 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 supplementation 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 four groups (n=24 per group): a control group fed a basal diet, and three treatment groups fed the basal diet supplemented with 10% flaxseed, 10% flaxseed + 5% fish oil, or 5% fish oil, respectively. After 21 days of feeding, eggs were collected continuously for 7 days, followed by slaughter sampling of four hens per group. Compared with the control group, dietary supplementation with flaxseed and fish oil 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. All treatment groups exhibited significantly reduced hepatic monounsaturated fatty acid proportions ($P<0.05$) and increased hepatic n-3 PUFA proportions ($P<0.05$). Fish oil supplementation 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 supplementation promotes the hepatic conversion of α-linolenic acid (ALA) to DHA by upregulating elongase and desaturase gene expression, whereas fish oil supplementation produces the opposite effect.

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

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Introduction

n-3 polyunsaturated fatty acids (PUFAs), primarily comprising α-linolenic acid (ALA, 18:3 n-3), eicosapentaenoic acid (EPA, 20:5 n-3), docosapentaenoic acid (DPA, 22:5 n-3), and docosahexaenoic acid (DHA, 22:6 n-3), confer numerous health benefits in humans, including improvements in cardiovascular disease,

central nervous system disorders, mental health conditions, inflammatory responses, and immune function, with DHA receiving particular attention. Notably, egg DHA exists exclusively in phospholipid-bound form, which enhances oxidative stability and absorption efficiency while conferring anti-inflammatory, anti-tumor, and cholesterol-lowering bioactivities. The deposition of n-3 PUFAs in animal tissues is directly influenced by dietary fatty acid composition through two primary mechanisms: the stepwise conversion of plant-derived ALA to EPA, DPA, and DHA, and the direct deposition of long-chain PUFAs from dietary sources. The three main dietary sources of n-3 PUFAs for animal feed are fish oil, microalgae, and flaxseed. While fish oil yields the highest DHA enrichment in eggs, followed by microalgae and then flaxseed, fish oil supplementation can impart fishy off-flavors that compromise egg quality and consumer acceptance. Despite limited research on the synergistic and competitive effects among these different n-3 PUFA sources, understanding these interactions is crucial for optimizing functional enrichment strategies. 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, including fatty acid composition and the expression of genes involved in carbon chain elongation and desaturation, to provide practical guidance for producing n-3 PUFA-enriched eggs.

Materials and Methods

Experimental Design, Animal Husbandry, and Sample Collection

Ninety-six 28-week-old Hyline Brown laying hens were randomly divided into four groups of 24 birds each. The control group received a basal diet, while the 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). The composition and nutrient levels of the experimental diets are presented in Table 1 . Following 21 days of feeding, eggs were collected continuously for 7 days, and yolks were separated, pooled, and spray-dried to produce yolk powder. At the end of the trial, four hens from each 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 analyses.

Fatty Acid Methyl Esterification and Gas Chromatography Analysis

Fatty acid methyl esterification of freeze-dried tissue, spray-dried yolk, or air-dried feed samples was performed according to the method described by Christie. Briefly, 0.5 g of sample was mixed with 2 mL of n-hexane and 5 mL of 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, 15 mL of 6% sodium carbonate 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 composition analysis.

Fatty acid composition was determined using an Agilent 7890A gas chromatograph 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. The injection volume was 1 µL, with injector temperature at 270°C and FID temperature at 275°C. High-purity nitrogen was used as carrier gas 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 target fatty acids were determined using external standard curves, while peak area normalization was used for overall fatty acid composition analysis.

Hepatic Gene Expression Analysis

Total RNA was extracted from liver tissue using a commercial kit, and RNA concentration and purity were measured using a micro-ultraviolet spectrophotometer. First-strand cDNA was synthesized using a ReverTra Ace qPCR RT Kit. Gene-specific primers were designed based on NCBI sequences and are listed in 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. The 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 derived from three replicate analyses.

Data Processing and Statistical Analysis

All data are expressed as means ± 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 measured parameters.

Results

Comparison of Fatty Acid Composition Among Four Experimental Diets

As shown in Table 3 , the n-3 PUFA proportion was higher in all treatment diets compared with the control. ALA content exceeded 5% in Trial Groups I and II but was undetectable in Trial Group III and the control group. EPA

content in Trial Groups I and II was approximately 25% of that in Trial Groups III and control. DPA was not detected in any diet, while DHA content was highest in Trial Group II, followed by Trial Group III, with Trial Group I and control showing similarly low levels.

Comparison of n-3 PUFA Content in Egg Yolk

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

Comparison of Hepatic Fatty Acid Composition

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

Two-way ANOVA indicated that flaxseed significantly affected most fatty acids except C14:0, C18:1, C24:1, C22:6, C18:2, and C20:3 (cis-8,11,14) ($P<0.05$). Fish oil significantly influenced most fatty acids except C20:0, C24:0, γ-C18:3, and C20:2 ($P<0.05$). The interaction between flaxseed and fish oil significantly affected hepatic proportions of C18:0, C22:0, C24:0, C16:1, C20:1, C18:3, C22:5, C22:6, C20:4, and C20:3 ($P<0.05$).

Comparison of Hepatic Fatty Acid Elongase and Desaturase Gene Expression Levels

As illustrated in Figure 1 [Figure 1: see original paper], compared with the control, Trial Group I showed significantly elevated 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 ($P<0.05$) but decreased ELOVL2 and SCD1 expression ($P<0.05$), with no significant change in ELOVL1 ($P>0.05$). Trial Group III showed significantly reduced expression of all elongase and desaturase genes ($P<0.05$).

Two-way ANOVA revealed that dietary flaxseed and fish oil both significantly affected the expression of ELOVL1, ELOVL2, FADS1, FADS2, and SCD1 ($P<0.05$). The interaction between flaxseed and fish oil was not significant for ELOVL1 and SCD1 expression ($P>0.05$) but was significant for the other genes ($P<0.05$) .

Discussion

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

Flaxseed, fish oil, and algae represent the primary dietary sources for enriching n-3 PUFAs in poultry products. Flaxseed provides protein, oil, and ALA for poultry diets, and its use in producing n-3 PUFA-enriched eggs is commercially accepted in many countries, increasing ALA content to 200 mg and DHA to 90 mg per egg. Fish oil, rich in DHA and EPA, directly enriches these long-chain PUFAs but often produces fishy off-flavors that limit its inclusion to less than 1.5% of the diet, resulting in only 100 mg DHA per egg. Even microencapsulated or deodorized fish oil fails to eliminate negative effects on egg quality. Microalgae, particularly heterotrophic strains like "DHA Gold," can achieve greater enrichment, with 4.8% dietary supplementation increasing DHA to over 200 mg per egg while maintaining acceptable flavor profiles.

Our results demonstrate that 5% fish oil supplementation primarily enriched DHA and EPA in yolk (Trial Group III), 10% flaxseed enriched ALA and DHA (Trial Group I), and the combination diet enriched both DHA and ALA (Trial Group II), with total n-3 PUFA deposition exceeding that of either single supplementation. Notably, 5% fish oil increased yolk DHA content 10-fold, reaching over 200 mg per fresh egg—among the highest levels reported. Although Trial Groups I and control had similar dietary DHA proportions, their yolk DHA content differed significantly, indicating that DHA in flaxseed-fed hens originated primarily from endogenous ALA conversion. Conversely, despite Trial Group II having the highest dietary n-3 PUFA and DHA proportions, its yolk DHA enrichment was intermediate between Trial Groups I and III. Two-way ANOVA confirmed significant main effects and interactions of flaxseed and fish oil on all four n-3 PUFAs. The interaction promoted total n-3 PUFA deposition but did not achieve additive effects, while inhibiting EPA and DHA deposition such that the combined group had lower levels than the fish oil-only group.

These findings suggest that different dietary n-3 PUFAs may interact during absorption and metabolism, with deposition outcomes ultimately determined by dietary composition. Previous research indicates that excess long-chain n-3 PUFAs can inhibit ALA-to-DHA conversion, and that varying n-3 fatty acid compositions produce different tissue deposition patterns. We hypothesize that increasing DHA may feedback-inhibit ALA conversion, while enhanced ALA conversion may suppress direct DHA absorption and deposition. The specific

mechanisms warrant further investigation at both absorptive and metabolic levels.

Regulatory Effects on Hepatic Fatty Acid Metabolism

The liver is the primary site of fatty acid metabolism in poultry. Both fish oil and flaxseed increased hepatic long-chain n-3 PUFA proportions, with fish oil also increasing saturated fatty acid proportions. Based on dietary composition, fish oil appears to inhibit de novo synthesis of long-chain unsaturated fatty acids while enabling direct DHA absorption, whereas flaxseed activates elongase and desaturase gene expression to promote endogenous synthesis of long-chain unsaturated fatty acids while limiting direct DHA absorption. This differential regulation aligns with yolk DHA deposition patterns, indicating distinct metabolic pathways for flaxseed and fish oil.

As shown in Figure 2 [Figure 2: see original paper], n-3 and n-6 unsaturated fatty acid metabolic pathways in the liver are catalyzed by elongases and desaturases. Δ6-desaturase introduces double bonds at positions 6 and 7 of C18 and C24 unsaturated fatty acids, Δ5-desaturase acts at positions 5 and 6 of C20 unsaturated fatty acids, and elongases ELOVL1 and ELOVL2 catalyze carbon chain extension of C22 and C20/C22 fatty acids, respectively. These pathways, using linoleic acid (n-6) and ALA (n-3) as substrates, are interconnected through shared intermediates and enzymes.

In this study, flaxseed, rich in ALA, upregulated elongase and desaturase gene expression, while fish oil, containing DHA as the end product, downregulated these genes. This confirms that fish oil enriches n-3 PUFAs primarily through direct absorption, whereas flaxseed acts through de novo synthesis. Two-way ANOVA results further suggest that fish oil significantly suppresses hepatic fatty acid conversion, making tissue composition more dependent on dietary fatty acid profile, while flaxseed activates endogenous conversion pathways to enhance n-3 PUFA synthesis.

Conclusion

1. Dietary supplementation with flaxseed or fish oil enriches yolk n-3 PUFA deposition, with the combination of 10% flaxseed and 5% fish oil producing the highest total n-3 PUFA content.
2. Yolk DHA enrichment is most effective with 5% fish oil supplementation, achieving over 200 mg DHA per fresh egg—among the highest levels reported in comparable studies.
3. Flaxseed supplementation promotes hepatic ALA-to-DHA conversion by upregulating fatty acid elongase and desaturase gene expression, whereas fish oil supplementation inhibits these processes by downregulating the same genes.

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Note: Figure translations are in progress. See original paper for figures.