Effects of Microalgae Oil and Fish Oil on Egg Quality and Fatty Acid Deposition in Egg Yolk: Postprint

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Abstract

This experiment aimed to investigate the effects of dietary supplementation with microalgae oil (MO) and fish oil (FO) on yolk fatty acid deposition and egg quality (fresh eggs and during storage), in order to provide a basis for efficient production of docosahexaenoic acid (DHA)-enriched eggs. A total of 630 Hy-Line Brown laying hens with similar laying rates at 31 weeks of age were selected and randomly allocated into 7 groups with 6 replicates per group and 15 birds per replicate. The control group was fed a basal diet (without additional DHA supplementation), while the experimental groups were supplemented with 1.35, 2.70, and 5.40 mg/g DHA from MO and FO as DHA sources, respectively, with MO supplementation levels of 0.25%, 0.50%, and 1.00%, and FO supplementation levels of 1.08%, 2.17%, and 4.34%. The pre-trial period was 1 week, and the formal trial period was 12 weeks.

The results showed: 1) At the end of the experiment, there were no significant differences in shell strength, shell thickness, egg shape index, or Haugh unit among all groups ($P>0.05$); at week 4, the albumen height in the 1.35 mg/kg DHA group was significantly higher than that in the 2.70 and 5.40 mg/g DHA groups ($P<0.05$); at week 8, compared with the control group, yolk color in all experimental groups was significantly increased ($P<0.05$). 2) After 28 days of storage, there were no significant differences in yolk malondialdehyde (MDA) content among groups ($P>0.05$); as storage time prolonged, yolk MDA content increased significantly ($P<0.05$). 3) After 14 days of storage, the Haugh unit in the MO group was significantly higher than that in the FO group ($P<0.05$); after 14 days of storage, the interaction effect of DHA source and supplementation level on albumen height was significant ($P<0.05$), albumen height in the MO group increased with increasing DHA supplementation level, while albumen height in the FO group showed a trend of first increasing and then

decreasing with increasing DHA supplementation level; after 7 and 28 days of storage, yolk color in the FO group was significantly higher than that in the MO group (P<0.05). 4) Compared with the control group, dietary supplementation with different sources and levels of DHA highly significantly increased yolk DHA, -linolenic acid, eicosapentaenoic acid, monounsaturated fatty acid, and -3 polyunsaturated fatty acid (PUFA) contents (P<0.01), and the FO group was significantly higher than the MO group (P<0.05), and highly significantly decreased the -6 PUFA/ -3 PUFA ratio (P<0.01). The DHA deposition efficiency in eggs highly significantly decreased with increasing DHA supplementation level (P<0.01).

In conclusion, under the conditions of this experiment, at the same DHA supplementation level, FO promoted yolk DHA deposition more effectively than MO, and the DHA deposition efficiency was highest at the supplementation level of 1.35 mg/g.

Full Text

Effects of Microalgae Oil and Fish Oil on Egg Quality and Yolk Fatty Acid Deposition in Laying Hens

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Abstract

This experiment was conducted to investigate the effects of dietary microalgae oil (MO) and fish oil (FO) on yolk fatty acid deposition and egg quality (both fresh and during storage), aiming to provide a basis for efficient production of docosahexaenoic acid (DHA)-enriched eggs. Six hundred and thirty 31-week-old Hy-Line Brown laying hens with similar laying rates were randomly allocated into 7 groups with 6 replicates per group and 15 hens per replicate. The control group was fed a basal diet without supplemental DHA, while experimental groups received the basal diet supplemented with MO or FO as DHA sources at three levels: 1.35, 2.70, and 5.40 mg/g DHA. The MO supplementation rates were 0.25%, 0.50%, and 1.00%, and FO supplementation rates were 1.08%, 2.17%, and 4.34%. The trial consisted of a 1-week pre-period followed by a 12-week formal experimental period. The results showed: 1) At the end of the experiment, no significant differences were observed among groups in eggshell strength, eggshell thickness, egg shape index, or Haugh unit (P>0.05). At week 4, albumen height in the 1.35 mg/g DHA groups was significantly higher than

in the 2.70 and 5.40 mg/g DHA groups (P<0.05). At week 8, yolk color was significantly elevated in all DHA-supplemented groups compared to the control (P<0.05). 2) No significant differences in yolk malondialdehyde (MDA) content were found among groups after 28 days of storage (P>0.05), though MDA content increased significantly with prolonged storage time (P<0.05). 3) After 14 days of storage, Haugh unit was significantly higher in MO groups than in FO groups (P<0.05). The interaction between DHA source and supplementation level significantly affected albumen height at 14 days (P<0.05): in MO groups, albumen height increased with DHA level, while in FO groups it initially increased then decreased. Yolk color in FO groups was significantly higher than in MO groups at both 7 and 28 days of storage (P<0.05). 4) Compared to the control, dietary supplementation with different sources and levels of DHA significantly increased yolk contents of DHA, -linolenic acid, eicosapentaenoic acid, monounsaturated fatty acids, and -3 polyunsaturated fatty acids (PUFA) (P<0.01), with FO groups showing significantly higher values than MO groups (P<0.05). The -6 PUFA/ -3 PUFA ratio was significantly reduced (P<0.01). DHA deposition efficiency in eggs decreased significantly as dietary DHA level increased (P<0.01). In conclusion, under the conditions of this experiment, fish oil was more effective than microalgae oil in promoting DHA deposition in egg yolk at the same DHA supplementation level, with the highest deposition efficiency observed at 1.35 mg/g DHA.

Keywords: microalgae oil; fish oil; DHA; egg quality; deposition efficiency; laying hens

1. Materials and Methods

1.1 Experimental Design and Diets

Six hundred and thirty 31-week-old healthy Hy-Line Brown laying hens with similar laying performance were randomly divided into 7 groups with 6 replicates per group and 15 hens per replicate. The control group received a basal diet without supplemental DHA, while experimental groups were fed the basal diet supplemented with either microalgae oil or fish oil as DHA sources at three levels providing 1.35, 2.70, and 5.40 mg/g DHA. The actual supplementation rates were 0.25%, 0.50%, and 1.00% for MO, and 1.08%, 2.17%, and 4.34% for FO. The measured DHA contents in the diets were 1.1, 2.4, and 4.1 mg/g for MO groups, and 1.1, 2.7, and 4.5 mg/g for FO groups. The MO was purchased from Xiamen Kingdomway Group Co., Ltd., and the FO from Foshan Darma Feed Co., Ltd., with measured DHA contents of 543 mg/g and 125 mg/g, respectively. All experimental diets were formulated to be isonitrogenous and isoenergetic according to the *Feeding Standard of Chicken* (NY/T 33–2004). Diet composition and nutrient levels are presented in Table 2 , and fatty acid composition in Table 3 .

1.2 Animal Management

Hens were housed in three-tier battery cages with 3 hens per cage. Groups were randomly assigned to cage positions to avoid environmental and location effects. Birds had free access to feed and water. Lighting consisted of natural light supplemented with artificial light to provide 16 h of light daily at an intensity of 20 lx. House temperature was maintained at (20±2)°C with relative humidity of 50%-60%. Ventilation combined natural air exchange with longitudinal negative pressure systems. Manure was removed twice daily, and disinfection was performed weekly with routine immunization. Feed was provided three times daily at 08:00, 13:00, and 18:00, and eggs were collected once daily. The experiment included a 1-week pre-period and a 12-week formal experimental period.

1.3 Sample Collection and Measurements

1.3.1 Egg Quality Assessment At the end of weeks 4, 8, and 12, three eggs were randomly collected from each replicate to determine egg quality parameters. Egg weight, albumen height, Haugh unit, and yolk color were measured using a SONOVA Egg Analyzer™ (Orka Technology Ltd.). Eggshell strength was determined with an Egg Force Reader (Orka Technology Ltd.), eggshell thickness with a PEACOCK P-1 gauge (Japan), and egg shape index with an Egg Index Reader (Fujibira Industry Co., Ltd.). For egg component analysis, whole egg, shell, and yolk were weighed separately to calculate proportions.

1.3.2 Egg Quality During Storage At the end of week 12, nine eggs from each replicate (total 378 eggs) were collected, weighed to record fresh egg weight, and stored at 4°C and 65% relative humidity. After 7, 14, and 28 days of storage, water loss rate and egg quality were measured. Yolks were separated, weighed, mixed thoroughly, and stored at -20°C for subsequent analysis.

1.3.3 Yolk Malondialdehyde (MDA) Content Yolk MDA content was determined using the thiobarbituric acid (TBA) method with assay kits purchased from Nanjing Jiancheng Bioengineering Institute. Yolk homogenate was prepared by mixing 0.1 mL yolk sample with 4.5 mL anhydrous ethanol, homogenizing at 60 Hz for 120 s using a sample grinder (Shanghai Jingxin Technology Co., Ltd.), centrifuging at 4,000 r/min for 10 min, and using 0.2 mL of the supernatant for MDA determination.

1.3.4 Fatty Acid Analysis in Diets and Yolk Diet samples were ground to powder, and (100±10) mg was transferred to 15 mL screw-cap glass tubes. Two mL of n-hexane and 1 mL of internal standard solution were added, followed by 4 mL of methanol:chloroacetyl mixture. After mixing, samples were incubated in an 80°C water bath for 2 h. After cooling to room temperature, 5 mL of 7% potassium carbonate was added slowly, vortexed, and centrifuged at 4,000 r/min for 10 min. The upper layer was collected for analysis. Fatty acid

composition was determined using a GC-450 gas chromatograph (Techcomp Scientific Instrument Co., Ltd.) with an Agilent DB-23 column (60 m × 250 μm × 0.25 μm). Helium was used as carrier gas at a constant flow 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. Injection volume was 1.0 μL, with n-hexane as wash solution (3 washes before and after injection).

At the end of week 12, two eggs from each replicate were collected, shelled, and yolks were mixed and freeze-dried. (90±10) mg of yolk powder was used for fatty acid analysis using the same method as for diet samples.

1.3.5 DHA Deposition Efficiency in Eggs DHA deposition efficiency was calculated based on feed-to-egg ratio using the formula:
DHA deposition efficiency (%) = 100 × DHA content in 1 kg eggs / DHA intake to produce 1 kg eggs.
DHA content in 1 kg eggs = 1,000 × yolk index × (1 - yolk freeze-drying water loss rate) × yolk DHA level (mg/g).
DHA intake to produce 1 kg eggs = 1,000 × feed-to-egg ratio × dietary DHA level (mg/g).

1.4 Statistical Analysis

Data were analyzed using the General Linear Model (GLM) procedure in SPSS 19.0 software. Factorial analysis was performed to examine main effects of DHA source and level and their interactions using multivariate ANOVA. Duncan' s multiple range test was used for post-hoc comparisons and correlation analysis. Significance was declared at $P<0.05$ and highly significant at $P<0.01$. Results are expressed as means and standard errors.

2. Results

2.1 Effects of Different DHA Sources and Levels on Egg Quality

As shown in Table 4 , DHA source, level, and their interaction had no significant effects on eggshell thickness or egg shape index ($P>0.05$). At week 4, eggshell strength was significantly affected by the interaction between DHA source and level ($P<0.05$): in MO groups, eggshell strength decreased then increased with dose, while in FO groups it increased then decreased. Haugh unit and albumen height were significantly affected by DHA level at week 4 ($P<0.05$), with the 1.35 mg/kg DHA group significantly higher than the 5.40 mg/g DHA group ($P<0.05$), though no differences were observed at weeks 8 and 12 ($P>0.05$). At week 8, yolk color was significantly higher in all DHA groups compared to the control ($P<0.05$), with no significant effects of DHA source, level, or their interaction ($P>0.05$). Yolk index at week 8 was significantly affected by DHA level ($P<0.05$), with the 2.70 mg/g DHA group highest and significantly greater than the 1.35 mg/g DHA group ($P<0.05$), though no differences were found at

week 12 (P>0.05). Overall, dietary DHA supplementation had no significant effects on most egg quality parameters throughout the experiment, except for Haugh unit, albumen height, yolk color, and yolk index.

2.2 Effects of Different DHA Sources and Levels on Yolk MDA Content During Storage

As shown in Table 5 , yolk MDA content at 7 and 14 days of storage was significantly affected by DHA supplementation level (P<0.05), with the 5.40 mg/g DHA group significantly higher than the 1.35 mg/g DHA group (P<0.05). The highest MDA content was observed in group 7 at 7 days. No significant differences were found among groups at 28 days of storage (P>0.05). Table 6 shows that yolk MDA content increased with DHA supplementation level (P<0.01) and increased extremely significantly with storage time (P<0.01).

2.3 Effects of Different DHA Sources and Levels on Egg Quality During Storage

As shown in Table 5 , DHA source, supplementation level, and their interaction had no significant effects on water loss rate or yolk index at any storage time (P>0.05). Haugh unit at 14 days was significantly affected by DHA source and the interaction effect (P<0.05), with MO groups significantly higher than FO groups (P<0.05), though no differences were observed at 28 days (P>0.05). The interaction between DHA source and level significantly affected albumen height at 14 days (P<0.05): albumen height increased gradually with DHA level in MO groups, while it initially increased then decreased in FO groups. No significant differences in albumen height were found at 28 days (P>0.05). Yolk color in FO groups was significantly higher than in MO groups at both 7 and 28 days of storage (P<0.05), while DHA supplementation level had no significant effect on yolk color (P>0.05).

Table 6 shows that water loss rate, yolk color, and yolk index increased significantly with storage time (P<0.01), while Haugh unit and albumen height decreased significantly (P<0.01). Across the entire storage period, group 3 had significantly higher Haugh unit than group 7 (P<0.05). Group 7 had significantly lower albumen height than the control, group 3, and group 4 (P<0.05), and significantly higher yolk color than the control and group 1 (P<0.05).

2.4 Effects of Different DHA Sources and Levels on Yolk Fatty Acid Content and Composition

As shown in Table 7 , yolk DHA and EPA contents were extremely significantly affected by DHA source, supplementation level, and their interaction (P<0.01). FO groups had significantly higher yolk DHA and EPA contents than MO groups (P<0.05), which increased extremely significantly with supplementation level (P<0.01). Group 7 had the highest yolk DHA and EPA contents, significantly higher than all other groups (P<0.05). Compared to the

control, yolk DHA content increased by 183.01%, 286.10%, 363.32%, 231.66%, 290.35%, and 410.81% in the experimental groups. Yolk ALA content increased extremely significantly with supplementation level ($P<0.01$).

Yolk monounsaturated fatty acid (MUFA) content was extremely significantly affected by DHA source, level, and their interaction ($P<0.01$). MO groups had significantly higher MUFA than FO groups ($P<0.05$), with MUFA content decreasing extremely significantly as supplementation level increased ($P<0.01$). Group 7 had the lowest MUFA content, significantly lower than the control ($P<0.05$). Yolk PUFA content was significantly affected by DHA source, level, and their interaction ($P<0.05$), with MO groups significantly higher than FO groups ($P<0.05$). PUFA content increased extremely significantly with supplementation level ($P<0.01$), with the 5.40 mg/g DHA groups showing 9.28% and 16.79% higher PUFA than the 1.35 and 2.70 mg/g DHA groups, respectively. Group 4 had the highest PUFA content, significantly higher than all other groups ($P<0.05$).

Yolk -3 PUFA content was extremely significantly affected by DHA source, supplementation level, and their interaction ($P<0.01$). FO groups had significantly higher -3 PUFA than MO groups ($P<0.05$), and the 5.40 mg/g DHA groups were significantly higher than the 1.35 and 2.70 mg/g DHA groups ($P<0.05$). Group 7 had the highest -3 PUFA content, 363.80% higher than the control ($P<0.05$), while group 4 showed a 267.71% increase ($P<0.05$). Yolk -6 PUFA content was extremely significantly affected by DHA source ($P<0.01$), with MO groups significantly higher than FO groups ($P<0.05$). Groups 5 and 7 had significantly lower -6 PUFA than the control ($P<0.05$). The -6 PUFA/ -3 PUFA ratio was significantly affected by DHA source, level, and their interaction ($P<0.05$), with FO groups significantly lower than MO groups ($P<0.05$) and decreasing extremely significantly as supplementation level increased ($P<0.01$). Group 7 had the lowest -6 PUFA/ -3 PUFA ratio. All experimental groups had significantly lower ratios than the control ($P<0.05$). As shown in Figure 1 [Figure 1: see original paper] and Figure 2 [Figure 2: see original paper], DHA deposition efficiency in eggs decreased extremely significantly as supplementation level increased ($P<0.01$).

3. Discussion

3.1 Effects of Different DHA Sources and Levels on Egg Quality

Our previous research indicated that 0.5% MO had no significant effect on egg quality. The current study showed that dietary supplementation with 1.35, 2.70, and 5.40 mg/kg DHA from either FO or MO affected some quality parameters at weeks 4 and 8, but not at week 12. At week 4, the high FO inclusion (4.34%) in group 7 may have caused stress, resulting in significantly lower Haugh unit and albumen height in the 5.40 mg/kg DHA groups compared to the 1.35 mg/g DHA groups. However, these differences diminished over time. At week 8, all DHA groups showed significantly higher yolk color than the control, suggesting

that dietary MO and FO supplementation promoted -carotene deposition in yolk, consistent with Lu et al. However, other studies reported that 5% FO had no significant effect on yolk color. Overall, treatments had no significant effects on internal egg quality during peak production, consistent with results from microalgae powder supplementation. Saleh reported that dietary FO at 1.25%, 2.50%, 3.75%, and 5.00% did not affect egg shape index, albumen height, yolk index, or Haugh unit, with only the 3.75% group showing significantly higher eggshell thickness than the control. Other studies reported that FO reduced eggshell thickness and quality compared to soybean oil. Eggshell quality is influenced by dietary calcium, phosphorus, vitamin D3, and trace minerals, particularly manganese, zinc, and copper. Therefore, the effects of dietary DHA on eggshell thickness require further investigation.

3.2 Effects of Different DHA Sources and Levels on Yolk MDA Content During Storage

Dietary unsaturated oils significantly affect biological membrane antioxidant capacity. Supplementing DHA through diet increases membrane DHA content and reduces -6 PUFA content. Studies have shown that dietary flaxseed and FO increased yolk MDA content, while 3% *Schizochytrium* powder did not affect fatty acid oxidation, with no significant differences in oxidation values after 30 days at 4°C compared to the control. Our results showed that yolk MDA content was significantly affected by DHA supplementation level at 7 and 14 days of storage, but no differences were observed among groups at 28 days. However, MDA content increased significantly with storage time in all groups, indicating that longer storage leads to more peroxidation products in yolk.

3.3 Effects of Different DHA Sources and Levels on Egg Quality During Storage

Our results showed no significant differences in Haugh unit or albumen height among groups after 28 days of storage, though both parameters decreased with storage time. Haugh unit, calculated from albumen height and egg weight, is influenced by hen breed, age, and storage time, with higher values indicating fresher eggs with greater albumen height. In this experiment, storage time significantly affected albumen height and Haugh unit, which decreased continuously with prolonged storage, consistent with reported functional relationships between Haugh unit and storage time.

3.4 Effects of Different DHA Sources and Levels on Yolk Fatty Acid Content and Composition

Yolk fatty acid composition is closely related to dietary fatty acid composition, with DHA deposition reaching stability after 14-15 days of feeding. After 4 weeks of feeding, any dietary source rich in -3 PUFA increases yolk -3 PUFA content, with DHA preferentially deposited in yolk. Dietary inclusion of 60 g/kg herring oil (11% EPA and 9% DHA) resulted in 150-200 mg DHA and 40-60 g

EPA per egg. Dietary FO at 1.5% and 3.0% increased yolk DHA to 2.43% and 3.16% of total fatty acids, respectively. Supplementation with 0.5% and 2.0% MO significantly increased yolk DHA content (18 and 31 mg/g), with DHA deposited not only in yolk but also in plasma and tissues. Our study demonstrated that yolk DHA, PUFA, and -3 PUFA contents increased extremely significantly with DHA supplementation level, with group 7 reaching the highest yolk DHA content of 13.23 mg/g and -3 PUFA content of 17.81 mg/g. At the same DHA supplementation level, FO groups showed higher yolk DHA deposition than MO groups, possibly because FO diets contained higher EPA, which could be converted to DHA and deposited in yolk. The results indicate that MO and FO supplementation promoted -3 PUFA deposition while reducing the -6 PUFA/ -3 PUFA ratio, optimizing yolk fatty acid composition.

The DHA in MO is primarily triglyceride-type DHA (TG-DHA) with an absorption efficiency of about 50%. DHA deposition efficiency in eggs tends to decrease as dietary DHA increases. When dietary *Schizochytrium* powder exceeded 2%, yolk DHA content did not continue to increase. Supplementation with 1.5% and 3.0% FO (containing 0.462% and 0.924% -3 PUFA) resulted in yolk -3 PUFA deposition efficiencies of 22% and 18%, respectively, indicating reduced efficiency at higher doses. Microalgae supplementation at 5% and 10% (0.076% and 0.152% -3 PUFA) yielded deposition efficiencies of 25% and 20%. Research suggests that 1.2 mg/g dietary -3 PUFA provides optimal deposition efficiency. Our experiment found the highest DHA deposition efficiency at 1.35 mg/g DHA supplementation, consistent with previous findings.

4. Conclusions

1. Dietary supplementation with MO and FO affected eggshell strength, Haugh unit, albumen height, yolk color, and yolk index at weeks 4 and 8, but had no significant effects on egg quality parameters by week 12.
2. At the same DHA supplementation level, FO promoted greater DHA and EPA deposition in yolk compared to MO, with the highest deposition efficiency achieved at 1.35 mg/g DHA.
3. Increasing DHA supplementation level significantly increased yolk contents of DHA, ALA, EPA, and -3 PUFA, demonstrating that yolk fatty acid content is strongly influenced by dietary fatty acid levels.

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