Effects of Dietary Rubber Seed Cake on Fatty Acid Composition of Egg Yolk in Laying Hens (Postprint)

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Abstract

This experiment evaluated the effects of dietary supplementation with different levels of rubber seed cake on egg yolk fatty acid composition and cholesterol content in Hy-Line Brown laying hens. Seventy-two healthy 25-week-old Hy-Line Brown laying hens were selected and randomly allocated into 6 groups with 4 replicates per group and 3 hens per replicate. The control group was fed a basal diet, while diets for treatment groups I–V were supplemented with 5.00%, 10.00%, 15.00%, 20.00%, and 25.00% rubber seed cake, respectively. The experimental period lasted 6 weeks. The results showed that dietary supplementation with different levels of rubber seed cake had no significant effect on saturated fatty acid (SFA) composition in egg yolk ($P>0.05$); however, the contents of linoleic acid (LA), α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) in egg yolk exhibited an increasing trend with increasing dietary rubber seed cake levels, and the DHA content in egg yolk of each treatment group differed significantly from that of the control group ($P<0.05$); with increasing dietary rubber seed cake levels, the contents of polyunsaturated fatty acids (PUFA), n-6 PUFA, and n-3 PUFA in egg yolk exhibited an increasing trend, while the n-6/n-3 PUFA ratio exhibited a decreasing trend; there was no significant difference in egg yolk cholesterol content between each treatment group and the control group ($P>0.05$), but all treatment groups were lower than the control group. It can be concluded that dietary supplementation with different levels of rubber seed cake can achieve a lower n-6/n-3 PUFA ratio and increase the contents of PUFA, n-6 PUFA, and n-3 PUFA in egg yolk.

Full Text

Effects of Dietary Rubberseed Cake on Fatty Acid Composition in Yolk of Laying Hens

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Abstract: This study evaluated the effects of supplementing different levels of rubberseed cake in diets on the fatty acid composition and cholesterol content of egg yolk in Hy-Line Brown laying hens. Seventy-two healthy 25-week-old hens were randomly divided into 6 groups with 4 replicates per group and 3 hens per replicate. The control group received a basal diet, while experimental groups I–V received the basal diet supplemented with 5.00%, 10.00%, 15.00%, 20.00%, and 25.00% rubberseed cake, respectively. The 6-week trial revealed that dietary rubberseed cake levels had no significant effect on saturated fatty acid (SFA) composition in yolk ($P>0.05$). However, yolk contents of linoleic acid (LA), α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) increased with dietary rubberseed cake levels, with all experimental groups showing significantly higher DHA content than the control ($P<0.05$). Yolk polyunsaturated fatty acids (PUFA), n-6 PUFA, and n-3 PUFA contents increased while the n-6/n-3 PUFA ratio decreased with higher dietary rubberseed cake levels. Although cholesterol content did not differ significantly between experimental and control groups ($P>0.05$), all experimental groups exhibited lower values than the control. These findings demonstrate that dietary rubberseed cake supplementation can reduce the n-6/n-3 PUFA ratio while enhancing PUFA, n-6 PUFA, and n-3 PUFA contents in egg yolk.

Keywords: rubberseed cake; laying hens; yolk; polyunsaturated fatty acids

Introduction

Rubberseed, the seed of the rubber tree (*Hevea brasiliensis*), represents a byproduct of rubber cultivation. Fresh rubberseed consists of approximately one-third shell with 25% moisture content, while the kernel is rich in oil with a composition similar to linseed oil. Compared to soybean meal, solvent-extracted and mechanically pressed rubberseed meals contain higher oil residues. The fatty acid profile of rubberseed meal comprises 54.7% PUFA, 25.9% monounsaturated fatty acids (MUFA), and 10.4% SFA, whereas linseed meal contains 67.9% PUFA, 16.4% MUFA, and 9.7% SFA [1]. Rubberseed meal contains 25%–30% crude protein with high levels of essential amino acids in balanced proportions, making it a

high-quality natural tropical protein feed ingredient [2]. Rubberseed cake can partially replace premium protein sources to supplement dietary energy and protein. China currently has approximately 1.4 million acres of seed-bearing rubber trees, yielding 30–45 kg of rubberseed per acre annually, for a national production of 420,000–630,000 tons [3]. By 2008, Yunnan Province alone had 435,800 hectares of rubber plantations [4], with Xishuangbanna Huakun Biotech Co., Ltd. processing over 10,000 tons of rubberseed annually to produce more than 2,000 tons of rubberseed oil and 4,200 tons of rubberseed cake [5]. This substantial resource base indicates significant development potential. At only 2,500 yuan per ton, rubberseed meal is far cheaper than soybean meal, and its widespread adoption in animal feed would reduce regional livestock production costs while providing a valuable supplement to conventional feed ingredients.

Previous research demonstrated that diets containing 8%–24% rubberseed cake caused no clinical toxicity symptoms in broiler chickens, with normal liver function and no pathological changes in liver histology [6]. Another study found that 5%–25% dietary rubberseed cake had no adverse effects on broiler growth, survival rate, daily weight gain, or muscle chemical composition, though feed intake and feed-to-gain ratio were higher than in the control group [7]. However, limited research exists on rubberseed cake application in laying hens, focusing primarily on production performance and egg quality [8]. It is well established that modifying laying hen diets can alter egg fatty acid composition to increase PUFA content, providing PUFA-enriched eggs for human consumption [9-10]. Studies have confirmed the feasibility of producing n-3 PUFA-enriched eggs through dietary manipulation [11-14]. Given that rubberseed is rich in PUFA, this study investigated the effects of different dietary rubberseed cake levels on yolk fatty acid composition and cholesterol content to enhance the value-added utilization of this feed resource.

1.1 Experimental Materials and Animals

Rubberseed cake for this experiment was provided by Xishuangbanna Huakun Biotech Co., Ltd., with proximate nutrient composition shown in Table 1 . Seventy-two healthy 21-week-old Hy-Line Brown laying hens were randomly allocated to 6 groups with 4 replicates per group and 3 hens per replicate. The control group received a basal diet, while experimental groups I–V received the basal diet supplemented with 5.00%, 10.00%, 10.00%, 20.00%, and 25.00% rubberseed cake, respectively. Hens were maintained on their original diets for 3 weeks to ensure adaptation to the environment and stable laying rates across groups before transitioning to experimental diets over 3 days to minimize stress from dietary change. A 1-week pre-trial period preceded the 6-week formal trial beginning at 25 weeks of age.

1.2 Experimental Diets

Experimental diets were formulated according to the *Feeding Standard of Chicken* (NY/T 33–2004) for laying hens, with equalized metabolizable energy and nutrient levels across groups. All ingredients were purchased in a single batch. Diet composition and nutrient levels are presented in Table 2 .

1.3 Animal Management

Hens were housed in the same building in cages (one hen per cage). The facility was disinfected and manure was removed according to standard procedures. Windows were opened each morning before feeding and closed before lights-off to maintain good air quality. Feed was provided daily at 07:00 and 16:00. Natural daylight was supplemented with artificial lighting to maintain a constant 16-hour photoperiod. Ad libitum water and feed access was provided. Eggs were collected daily at 16:30, and house temperature was recorded.

1.4 Analytical Methods

1.4.1 Instruments The analysis utilized an HP-7890 gas chromatograph (USA) equipped with an FID detector and HP INNOWAX capillary column (30 m × 0.25 mm × 0.25 m, temperature range 40–260°C with maximum programmed temperature of 270°C), using high-purity nitrogen as carrier gas and mixed air with high-purity hydrogen as fuel. An HP-1100 high-performance liquid chromatograph (HPLC) (USA) was used with an HP 20RBA×SB-C18 column (5 m, 250 mm × 4.6 mm), mobile phase of acetonitrile-isopropanol (4:1) filtered through 0.45 m organic membrane, flow rate of 1.200 mL/min, column temperature of 35.0°C, detection wavelength of 210 nm, and injection volume of 10 L. Additional equipment included an XW-80A vortex mixer, THZ-82 constant temperature oscillator, CP-214 electronic analytical balance, JI 80-2B centrifuge, DL-180A ultrasonic cleaner, and HH SY21-Ni constant temperature water bath.

1.4.2 Reagents Fatty acid methyl ester standards including methyl laurate, myristate, palmitate, margarate, stearate, oleate, and arachidate were purchased from AccuStandard (USA). Methyl esters of linoleic acid (LA), γ-linolenic acid (GLA), α-linolenic acid (ALA), arachidonic acid (AA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and cholesterol standards were obtained from NU-CHEK PREP (USA). Routine reagents including chromatography-grade acetonitrile, methanol, and isopropanol (USA), as well as n-hexane, methanol, acetyl chloride, and potassium carbonate were used.

1.4.3 Preparation of Standard and Reagent Solutions Standard stock and working solutions were prepared by weighing 100 mg of each fatty acid methyl ester standard, transferring to a 5 mL volumetric flask with multiple

n-hexane washes, and diluting to volume to obtain stock solutions (20 mg/mL), which were sealed and stored at -20°C. Working solutions at concentrations of 5.00, 2.50, 1.25, 0.50, and 0.25 mg/mL were prepared fresh. For cholesterol, 20.2 mg of standard was dissolved in anhydrous ethanol, transferred to a 25 mL volumetric flask, and diluted to obtain a stock solution (1.008 mg/mL). Working solutions at 0.1008, 0.2016, 0.4032, 0.6048, and 0.8064 mg/mL were prepared and filtered through 0.45 m organic membrane before HPLC injection.

1.5 Sample Collection

During weeks 4 and 6, eggs were collected on Fridays and Saturdays. Three eggs per replicate were taken, yolks were separated from albumen, and three yolks were pooled as one sample, mixed, and stored at -20°C for fatty acid analysis. In week 6, four eggs per group were randomly selected for cholesterol determination.

1.6 Sample Preparation

1.6.1 Fatty Acid Methyl Esterification Five grams of yolk sample were freeze-dried for 48 hours. Exactly 0.5000 g of dried yolk was placed in a 20 mL screw-cap tube, mixed with 2 mL n-hexane, and 3 mL of freshly prepared 5% acetyl chloride (prepared by slowly adding 1 part acetyl chloride to 10 parts anhydrous methanol) was added directly to the sample to prevent splashing. The mixture was vortexed slowly for 1 minute, maintaining the reactant level 2–3 cm from the tube bottom, then heated in a 70°C water bath for 2 hours. If solvent evaporated, 2 mL n-hexane was added after cooling to ensure complete methylation. After cooling to room temperature, 5 mL of 6% K_2CO_3 solution and 2 mL n-hexane were added to neutralize the sample. The mixture was shaken at medium speed for 30 seconds and centrifuged at 1,500 rpm for 10 minutes. The upper organic phase (n-hexane) was transferred to a screw-cap tube, dehydrated with 1 g anhydrous sodium sulfate, filtered through 0.45 m organic membrane, and stored for analysis.

1.6.2 Cholesterol Sample Preparation Eggs were broken and albumen removed. Yolks were mixed in a beaker, and 5.0000 g of yolk was diluted with distilled water to 50 mL to obtain a 0.1 g/mL solution. One milliliter of this dilution was transferred to a screw-cap tube, mixed with 1 mL 95% ethanol by vortexing to prevent flocculent precipitation, then extracted with 2.5 mL ether (shaken 30 seconds) followed by 2.5 mL petroleum ether (fully oscillated). After 30 minutes of layer separation, the upper organic phase was transferred to another tube. The extraction was repeated once more, and the combined extracts were evaporated to dryness under nitrogen at 40°C. The residue was dissolved in 2.0 mL anhydrous ethanol, filtered through 0.45 m organic membrane, and analyzed by HPLC.

1.7 Analytical Procedures

1.7.1 Yolk Fatty Acid Determination and Calculation Yolk fatty acids were determined using a modified method of Sukhija et al. [15]. The HP-7890 gas chromatograph (USA) operating conditions were: HP INNOWAX column (30 m × 0.25 mm × 0.25 m) with nitrogen carrier gas; constant column flow plus makeup gas at 23.371 mL/min, hydrogen flow 40 mL/min, air flow 400 mL/min; injector temperature 220°C with splitless injection of 2 L, pressure 433.87 Pa, septum purge flow 3 mL/min; FID detector temperature 280°C. Temperature program: 100°C held for 1 minute, ramped at 15°C/min to 200°C (held 5 minutes), then at 4°C/min to 250°C (held 10 minutes), total time 35.167 minutes. Identification was based on retention time and quantification by external standard method. Standard curves were constructed with peak area (y) versus fatty acid methyl ester concentration (x). Linear equations for each fatty acid methyl ester had correlation coefficients: methyl laurate (R^2=0.9992), myristate (R^2=0.9994), palmitate (R^2=0.9991), margarate (R^2=0.9993), stearate (R^2=0.9994), oleate (R^2=0.9982), LA (R^2=0.9990), GLA (R^2=0.9992), ALA (R^2=0.9993), arachidate (R^2=0.9998), AA (R^2=0.9992), EPA (R^2=0.9991), and DHA (R^2=0.9998).

1.7.2 Chromatograms The mixed standard fatty acid methyl esters eluted in order: methyl laurate (6.439 min), myristate (7.474 min), palmitate (9.275 min), margarate (11.804 min), stearate (13.538 min), oleate (15.581 min), LA (16.912 min), GLA (17.560 min), ALA (18.227 min), arachidate (19.773 min), AA (22.152 min), EPA (23.486 min), and DHA (28.607 min) [Figure 1: see original paper]. The yolk sample chromatogram is shown in Figure 2 [Figure 2: see original paper].

1.7.3 Yolk Cholesterol Determination and Calculation Cholesterol was determined by HPLC using the method of Yang et al. [16]. Identification was based on retention time and quantification by external standard method. A standard curve was constructed with peak area (y) versus cholesterol concentration (x) [Figure 3: see original paper].

1.7.4 Fatty Acid Composition of Rubberseed Cake and Experimental Diets The fatty acid composition of rubberseed cake and experimental diets is presented in Table 3 .

1.8 Statistical Analysis

Data were analyzed using SPSS 17.0 software by one-way ANOVA followed by Duncan' s multiple comparison test. Significance was declared at $P<0.05$. Results are expressed as means ± standard deviation.

Results

2.1 Effects of Dietary Rubberseed Cake Levels on Yolk Fatty Acid Composition

As shown in Table 4 , at week 4, yolk lauric acid content in group I was significantly different from the control ($P<0.05$), while other experimental groups showed no significant differences ($P>0.05$). No significant differences were observed between experimental and control groups for myristic, palmitic, margaric, or arachidic acids ($P>0.05$). Stearic acid content in groups III, IV, and V differed significantly from the control ($P<0.05$), while groups I and II did not ($P>0.05$). Overall, dietary rubberseed cake levels had no significant effect on SFA deposition in yolk ($P>0.05$). Oleic acid content in groups III, IV, and V differed significantly from the control ($P<0.05$), while groups I and II did not ($P>0.05$). LA content in groups IV and V was significantly higher than the control ($P<0.05$) and showed a positive correlation with dietary rubberseed cake level: $y=0.2624x+17.3760$, $R^2=0.938$ ($P=0.006$). No significant differences were observed for GLA content ($P>0.05$). ALA content in all experimental groups differed significantly from the control ($P<0.05$) and correlated positively with dietary level: $y=0.0660x+0.3995$, $R^2=0.992$ ($P<0.001$). AA content in group I differed significantly from the control ($P<0.05$), while other groups did not ($P>0.05$). EPA content in groups III, IV, and V differed significantly from the control ($P<0.05$) and increased with dietary level: $y=0.0020x+0.1079$, $R^2=0.873$ ($P=0.023$). DHA content in all experimental groups differed significantly from the control ($P<0.05$) and increased with dietary level: $y=0.0911x+1.4984$, $R^2=0.983$ ($P<0.001$). SFA and MUFA contents showed no significant changes ($P>0.05$), though group III had slightly higher values. PUFA, n-6 PUFA, and n-3 PUFA contents increased with dietary level: $y=0.4237x+22.7380$, $R^2=0.957$ ($P=0.030$); $y=0.2646x+20.7320$, $R^2=0.917$ ($P=0.010$); and $y=0.1591x+2.0054$, $R^2=0.989$ ($P<0.001$), respectively. The n-6/n-3 PUFA ratio decreased and correlated negatively with dietary level: $y=-0.2430x+9.7929$, $R^2=0.882$ ($P=0.002$).

As shown in Table 5 , at week 6, no significant differences were observed between experimental and control groups for lauric, myristic, palmitic, or arachidic acids ($P>0.05$). Margaric acid content in group I differed significantly from the control ($P<0.05$), while other groups did not ($P>0.05$). Stearic acid content in group II differed significantly from the control ($P<0.05$), while other groups did not ($P>0.05$). Overall, dietary rubberseed cake levels had no significant effect on SFA deposition ($P>0.05$). Oleic acid content in group I differed significantly from the control ($P<0.05$), while other groups did not ($P>0.05$). LA content in all experimental groups differed significantly from the control ($P<0.05$) and increased with dietary level: $y=0.3105x+23.5980$, $R^2=0.924$ ($P=0.008$). No significant differences were observed for GLA content ($P>0.05$). ALA content in groups II–V differed significantly from the control ($P<0.05$) and increased with dietary level: $y=0.0769x+0.5870$, $R^2=0.993$ ($P<0.001$). AA content in group V differed significantly from the control ($P<0.05$), while other

groups did not (P>0.05), and all experimental groups had lower AA content than the control. EPA content showed no significant differences (P>0.05) but increased with dietary level: y=0.0013x+0.1309, R^2=0.891 (P=0.017). DHA content in all experimental groups differed significantly from the control (P<0.05) and increased with dietary level: y=0.0963x+1.8018, R^2=0.986 (P<0.001). SFA and MUFA contents showed no significant changes (P>0.05), though MUFA content in all experimental groups was higher than the control. PUFA, n-6 PUFA, and n-3 PUFA contents increased with dietary level: y=0.4701x+30.4520, R^2=0.953 (P=0.003); y=0.2956x+27.9320, R^2=0.911 (P=0.011); and y=0.1745x+2.5197, R^2=0.991 (P<0.001), respectively. The n-6/n-3 PUFA ratio decreased and correlated negatively with dietary level: y=-0.2502x+10.5670, R^2=0.904 (P=0.013).

2.2 Effects of Dietary Rubberseed Cake Levels on Yolk Cholesterol Content

As shown in Table 6 , no significant differences were observed in yolk cholesterol content between experimental and control groups (P>0.05). However, whole egg cholesterol content was lower in all experimental groups than in the control (P>0.05), with group IV showing the lowest value.

Discussion

This study demonstrated that increasing dietary levels of rubberseed cake, which is rich in LA and ALA, resulted in increased yolk contents of LA, ALA, EPA, and DHA, along with elevated PUFA, n-6 PUFA, and n-3 PUFA levels, while decreasing the n-6/n-3 PUFA ratio. Whitehead [17] reported that dietary fatty acid composition influences yolk fatty acid deposition and composition. Since yolk PUFA and other lipid components reflect dietary content, increasing dietary PUFA enhances their deposition in eggs [18-21]. Bruneel et al. [22] found that dietary microalgae supplementation significantly increased yolk n-3 PUFA content, producing DHA-enriched eggs. Similarly, supplementing diets with ALA-rich sources such as flaxseed and perilla seed increased yolk n-3 PUFA deposition [23-24], consistent with our findings. Dietary n-3 PUFA significantly increases total yolk PUFA content, promotes n-3 PUFA enrichment, and reduces n-6 PUFA content and the n-6/n-3 PUFA ratio [25-26]. In this study, dietary rubberseed cake increased yolk n-6 PUFA content, primarily due to significantly elevated LA (an n-6 PUFA), consistent with Wu et al. [20]. Pardio et al. [27] reported that n-3 PUFA-rich feed ingredients competitively inhibit AA deposition while increasing n-3 PUFA content and reducing the n-6/n-3 PUFA ratio, which our study confirmed.

All experimental groups showed lower AA content than the control (except group IV at week 4), likely due to altered LA:ALA ratios in the diet. Cherian et al. [28] reported that ALA-rich diets reduce egg AA content, while Jiang et

al. [13] suggested that ALA inhibits AA synthesis in hens fed ALA-containing diets. This may occur because linolenic acid and its metabolites can inhibit AA generation and metabolism or displace AA from phospholipids [29], with LA and ALA competing for the same Δ6 and Δ5 desaturase enzymes during carbon chain elongation [30]. Therefore, increased dietary ALA may reduce AA metabolism and deposition [31].

Margaric acid was the most abundant SFA in yolk. Palmitic acid has two metabolic fates: carbon chain elongation to stearic acid or desaturation to palmitoleic acid [31]. Studies on n-3 PUFA-rich ingredients report varying effects on egg SFA content. Caston and Leeson [32] found that dietary flaxseed or fish oil had no significant effect on palmitic acid content, whereas Marshall et al. [33] reported significantly reduced palmitic acid in eggs from hens fed 1.5% menhaden oil. Sims et al. [34] noted that unsaturated fatty acids in the liver inhibit fatty acid synthesis more than SFA. Reduced palmitic acid content may relate to decreased fat synthesis from increased dietary unsaturated fatty acids [31]. Li et al. [35] fed rubberseed oil to macaques and observed significantly reduced SFA (primarily palmitic acid) and increased unsaturated fatty acids, particularly LA with slight ALA increases. Our finding that dietary rubberseed cake had no significant effect on yolk palmitic acid content aligns with Caston et al. [32].

Yolk PUFA content increased with dietary rubberseed cake levels, while MUFA and SFA contents remained relatively unchanged, and cholesterol content showed no significant differences from the control. These results reflect both the fatty acid deposition characteristics of laying hens and the fatty acid composition of rubberseed cake, indicating that yolk fatty acid composition is primarily influenced by dietary PUFA content. Wang [36] reported that dietary flaxseed significantly increased yolk PUFA while reducing MUFA and SFA. Lu et al. [37] found that milk thistle oil significantly increased total SFA and reduced total PUFA compared to soybean oil, though not significantly different from the control diet, while significantly increasing ALA and DHA. Gul et al. [38] reported that 2.0%, 4.0%, and 6.0% dietary canola oil reduced yolk PUFA, significantly increased MUFA, and elevated cholesterol content, with similar results reported by Hoan et al. [39] for sesame oil supplementation at 1.5%, 3.0%, and 4.5%.

Conclusions

1. Dietary supplementation with 5%–25% rubberseed cake had no significant effect on yolk SFA composition, but yolk LA, ALA, EPA, and DHA contents increased with dietary rubberseed cake levels, with all experimental groups showing significantly higher DHA content than the control.
2. Different dietary rubberseed cake levels can achieve a lower n-6/n-3 PUFA ratio while increasing yolk PUFA, n-6 PUFA, and n-3 PUFA contents.

References

[1] Lu ZW. Feed value of rubberseed [J]. Feed Research, 2003(11): 40-41.
[2] Zhao YH, Fan WP, Fan WB. Comprehensive development and utilization of rubberseed [J]. Tropical Agricultural Engineering, 2013, 37(2): 46-49.
[3] Peng Y, Fan WB, Sun J. Research report on comprehensive utilization of rubberseed [J]. China Tropical Agriculture, 2012(4): 6-7.
[4] Liu JZ, Chen JX. Suggestions for sustainable development of Yunnan' s natural rubber industry [J]. China Tropical Agriculture, 2010(2): 16-19.
[5] Hu JF, Du YG. Investigation report on comprehensive processing and utilization of rubberseed by Huakun Company [J]. China Tropical Agriculture, 2012(4): 4-6.
[6] Yang RY, Yang CG. Toxicological study of rubberseed cake in broiler chickens [J]. Journal of Yunnan Agricultural University, 1990, 5(2): 120-124.
[7] Dai ZM, Li QH, Li JP. Feeding effects of rubberseed cake in broiler diets [J]. Yunnan Animal Husbandry and Veterinary Medicine, 1990(1): 4-7.
[8] Song CH, Tang RS, Shi WY. Nutritional evaluation of rubberseed oil cake as laying hen feed [J]. Yunnan Animal Husbandry and Veterinary Medicine, 1989(3): 6-9.
[9] Oliveira DD, Baião NC, Cançado SV, et al. Effects of lipid sources in the diet of laying hens on the fatty acid profiles of egg yolks [J]. Poultry Science, 2010, 89(11): 2484-2490.
[10] Yang CX. Study on biological transformation of n-3 fatty acids and mechanism of n-3 fatty acid-enriched eggs on lipid metabolism [D]. PhD Thesis. Beijing: China Agricultural University, 1997.
[11] Lewis NM, Seburg S, Flanagan NL. Enriched eggs as a source of n-3 polyunsaturated fatty acids for humans [J]. Poultry Science, 2000, 79(7): 971-974.
[12] Baucells MD, Crespo N, Barroeta AC, et al. Incorporation of different polyunsaturated fatty acids into eggs [J]. Poultry Science, 2000, 79(1): 51-59.
[13] Jiang ZR, Ahn DU, Sim JS. Effects of feeding flax and two types of sunflower seeds on fatty acid compositions of yolk lipid classes [J]. Poultry Science, 1991, 70(12): 2467-2475.
[14] Milinsk MC, Murakami AE, Gomes STM, et al. Fatty acid profile of egg yolk lipids from hens fed diets rich in n-3 fatty acids [J]. Food Chemistry, 2003, 83(2): 287-292.
[15] Sukhija PS, Palmquist DL. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces [J]. Journal of Agricultural and Food Chemistry, 1988, 36(6): 1202-1206.
[16] Yang Q, Dai RT. Determination of cholesterol content in different chicken egg varieties by HPLC [J]. Academic Periodical of Farm Products Processing, 2006(8): 71-74.
[17] Whitehead CC. The response of egg weight to the inclusion of different amounts of vegetable oil and linoleic acid in the diet of laying hens [J]. British Poultry Science, 1981, 22(6): 525-532.
[18] Yang F. Animal Nutrition [M]. 2nd ed. Beijing: China Agriculture Press, 2001.

[19] Ye T, Zhao SJ, Jing P. Enrichment of polyunsaturated fatty acids in egg yolk [J]. Food Industry, 2012, 33(6): 90–92.
[20] Wu H, Zhang H, Ding BA, et al. Study on eggs enriched with ω-6 polyunsaturated fatty acids [J]. Sichuan Animal Husbandry and Veterinary Medicine, 2004, 31(5): 29–30.
[21] Yu LH, Liu Y, Li ZB, et al. Effects of deep-sea fish oil on laying performance and egg fatty acid composition in Hua-Feng chickens [J]. China Poultry, 2014, 36(15): 29–32.
[22] Bruneel C, Lemahieu C, Fraeye L, et al. Impact of microalgal feed supplementation on omega-3 fatty acid enrichment of hen eggs [J]. Journal of Functional Foods, 2013, 5(2): 897–904.
[23] Pan XY, Gao YP. Development of functional eggs enriched with n-3 series polyunsaturated fatty acids (PUFA) [J]. Acta Agriculturae Boreali-occidentalis Sinica, 2008, 17(1): 20–26.
[24] Chen JX, Lu J, Xia ZS, et al. Effects of dietary linseed oil levels on yolk lipid composition and lipid metabolism in laying hens [J]. Animal Husbandry and Veterinary Medicine, 2010, 42(6): 40–43.
[25] Herber SM, Van Elswyk ME. Dietary marine algae promotes efficient deposition of n-3 fatty acids for the production of enriched shell eggs [J]. Poultry Science, 1996, 75(12): 1501–1507.
[26] Ye SQ, Zhou AG, Wang ZS. Effects of ω-3 PUFA on polyunsaturated fatty acid composition of egg yolk [J]. China Feed, 2009(3): 24–26.
[27] Pardio VT, Landin LA, Waliszewski KN, et al. The effect of soybean soapstock on the quality parameters and fatty acid composition of the hen egg yolk [J]. Poultry Science, 2005, 84(1): 148–157.
[28] Cherian G, Sim JS. Effect of feeding full fat flax and canola seeds to laying hens on the fatty acid composition of eggs, embryos, and newly hatched chicks [J]. Poultry Science, 1991, 70(4): 917–922.
[29] Mathews CK, Van Holde KE, Ahern KG. Biochemistry [M]. San Francisco: Addison-Wesley Longman, 2000.
[30] Hayek MG, Reinhart GA. Utilization of ω-3 fatty acids in companion animal nutrition [J]. World Review of Nutrition and Dietetics, 1998, 83: 176–185.
[31] Ayerza R, Coates W. Dietary levels of chia: influence on yolk cholesterol, lipid content and fatty acid composition for two strains of hens [J]. Poultry Science, 2000, 79(5): 724–739.
[32] Caston L, Leeson S. Research note: dietary flax and egg composition [J]. Poultry Science, 1990, 69(9): 1617–1620.
[33] Marshall AC, Sams AR, Van Elswyk ME. Oxidative stability and sensory quality of stored eggs from hens fed 1.5% menhaden oil [J]. Journal of Food Science, 1994, 59(3): 561–563.
[34] Sims JS, Qi GH. Designing poultry products using flaxseed [M]//Cunnane SC, Thompson LU. Flaxseed in Human Nutrition. Champaign, IL: AOCS Press, 1995: 315–332.
[35] Li YS, Liu CR, Tang CC, et al. Effects of rubberseed oil on fatty acid composition in macaques [J]. Journal of Hainan University: Natural Science Edition, 1994, 12(4): 338–340.

[36] Wang K. Enrichment of n-3 polyunsaturated fatty acids in egg yolk and tissues and their effects on lipid metabolism in laying hens [D]. PhD Thesis. Beijing: Chinese Academy of Agricultural Sciences, 2000.
[37] Lu J, Qu L, Li SL, et al. Effects of milk thistle oil on laying performance, egg quality and egg fatty acid composition in laying hens [J]. Chinese Journal of Animal Nutrition, 2015, 27(12): 3831-3839.
[38] Gul M, Yoruk MA, Aksu T, et al. The effect of different levels of canola oil on performance, egg shell quality and fatty acid composition of laying hens [J]. International Journal of Poultry Science, 2012, 11(12): 769-776.
[39] Hoan ND, Khoa MA. The effect of different levels of sesame oil on productive performance, egg yolk and blood serum lipid profile in laying hens [J]. Open Journal of Animal Sciences, 2016, 6(1): 85-93.

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