Effect of Various Dietary Fats on Fatty Acid Profile in Duck Liver: Efficient Conversion of Short-chain to Long-chain Omega-3 Fatty Acids

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Abstract

Omega-3 fatty acids, especially long-chain omega-3 fatty acids, have been associated with potential health benefits for chronic disease prevention. Our previous studies found that dietary omega-3 fatty acids could accumulate in the meat and eggs in a duck model. This study was to reveal the effects of various dietary fats on fatty acid profile and conversion of omega-3 fatty acids in duck liver. Female *Shan Partridge Ducks* were randomly assigned to five dietary treatments, each consisting of 6 replicates of 30 birds. The experimental diets substituted the basal diet by 2% of flaxseed oil, rapeseed oil, beef tallow, or fish oil, respectively. In addition, a dose response study was further conducted for flaxseed and fish oil diets at 0.5%, 1%, and 2%, respectively. At the end of the 5-week treatment, fatty acids were extracted from the liver samples and analyzed by GC-FID. As expected, the total omega-3 fatty acids and the ratio of total omega-3/omega-6 significantly increased in both flaxseed and fish oil groups when compared with the control diet. No significant change of total saturated fatty acids or omega-3 fatty acids was found in both rapeseed and beef tallow groups. The dose-response study further indicated that 59-81% of the short-chain omega-3 ALA in flaxseed oil-fed group was efficiently converted to long-chain DHA in the duck liver, whereas 1% of dietary flaxseed oil could produce an equivalent level of DHA as 0.5% of dietary fish oil. The more omega-3 fatty acids, the less omega-6 fatty acids in the duck liver. Taken together, this study showed the fatty acid profiling in the duck liver after various dietary fat consumption, provided insight into a dose response change of omega-3 fatty acids, indicated an efficient conversion of short- to long-chain omega-3 fatty acid, and suggested alternative long-chain omega-3 fatty acid-enriched duck products for human

health benefits.

Keywords: omega-3 fatty acid, duck, liver, dietary fat, health benefits

Introduction

Omega-3 polyunsaturated fatty acids (ω-3 PUFAs), in addition as essential nutrients, have been associated with potential health benefits in chronic disease prevention. There are two types of ω-3 PUFAs, known by short-chain ω-3 PUFAs like ALA (alpha-linolenic acids, C18:3n-3) or long-chain ω-3 PUFAs such as EPA (eicosapentaenoic acid, C20:5n-3) and DHA (docosahexaenoic acid, C22:6n-3). Short-chain ω-3 PUFAs are presented in plant oil such as flaxseed and soybean oil, while long-chain ω-3 PUFAs are usually found in marine products such as fish oil. Although short-chain ω-3 PUFAs are more common and less expensive, the potential health benefits of ω-3 PUFAs have been related to long-chain ω-3 PUFAs only.

Compelling data from epidemiological and interventional studies have demonstrated an inverse correlation between long-chain ω-3 PUFAs and risk of some chronic diseases, including cardiovascular diseases,[1,2] myocardial infarction,[3,4] psoriasis,[5] mental illnesses,[6] cancer,[7,8] and bronchial asthma.[9] Although it is not conclusive, some clinical trials also found long-chain ω-3 PUFAs contributed to a lower risk of cancers, such as colon,[10,11] breast,[12,13] and prostate cancers.[14] Therefore, the 2015 Dietary Guidelines for Americans recommends the consumption of 8 oz. of seafood per week to provide an average of 250 mg/day of long chain omega-3 Fatty acids for health benefits.[15] Moreover, The American Heart Association's Strategic Impact Goal Through 2020 and Beyond recommends at least two servings with 3.5-oz. fish every week to increase EPA and DHA intakes[16], while an adequate intake of ω-6 linoleic acid as 17 g/d for men and 12 g/d for women at the age of 19 to 50 years[17].

Humans can convert short-chain to long-chain omega-3 fatty acids, but the conversion efficiency is limited, usually below 5% in adults[18] or even less than 1% in infants and aging people.[19] When compared with humans, however, waterfowls such as geese have been reported to convert short-chain to long-chain omega-3 fatty acids more efficiently by a series of desaturase and elongase in the liver[20] and subsequently excreted into blood circulation to other tissues.[21] The diverse conversion rates between human and waterfowl have driven scientists to consider that waterfowls may provide an alternate and sustainable source of long-chain ω-3 PUFAs from plant-derived short-chain ALA.[22-24]

Ducks are aquatic birds which have a high rate of lipogenesis to meet their energetic requirements during ancient migratory flight.[25-26] It has been reported, for instance, the percent body fat could be reached as high as 37-42% in Peking ducks and 20-30% in Muscovy ducks.[27] Although duck products are popular with its unique flavor and juicy texture, the high fat content has raised health concerns. While certain species of lean ducks, such as the *Shan Partridge* contains 7.5% of body fat only have been developed, modification of the fat composition in favor of higher ω-3 PUFAs in replace of ω-6 PUFAs and/or saturated fatty acids may provide promising healthy benefits. It has been noted that supplemented diets with vegetable oil and fish oil effectively enhanced ω-3 PUFAs in the products of pork,[28] broilers[29,30] and broiler eggs.[31,32] Dietary ALA was also reported to promote EPA and DHA contents in chicken liver.[33] Furthermore, Chen and Hsu reported an increased trend of EPA, DHA, and total ω-3 PUFAs in duck egg yolks by feeding cod liver oil diet.[34] In addition to storage and transportation of lipids, liver of birds has a very high capacity of lipogenesis[35]. Fatty acids can be synthesis or converted in the liver, and then transported to other tissues such as adipose, cardiac and skeletal muscle. The conversion of fatty acids in the liver and incorporation of them into various tissues are well-established in response to the observed change of fatty acid composition in the relative tissues[36]. Therefore, fatty acid conversion in the liver may provide impact not only on a varied fatty acid composition but also on the meat quality including flavor and muscle color[37]. From an aspect of nutrition value, efficient conversion of short- to long-chain ω-3 PUFAs in the liver may boost the levels of long chain ω-3 PUFAs and thus improve the meat quality. Our previous study found that fish oil and sunflower oil could significantly enhance the levels of EPA and DHA in the leg and chest muscles as well as eggs of *Shan Partridge* duck.[38] However, the effect of various dietary fats on fatty acid profile and the conversion efficacy of ω-3 PUFAs in duck liver, to our knowledge, has yet to be well studied.

The aim of this study was to assess the modification of fatty acid profiles in the liver of *Shan Partridge* duck after feeding various dietary fats, including ALA-enriched flaxseed oil, ω-6 PUFA-enriched rapeseed oil, saturated fatty acid-enriched beef tallow, and EPA/DHA-enriched fish oil. The conversion efficacy of short-chain to long-chain ω-3 PUFAs was further investigated by a dose-response study for flaxseed and fish oil treatment, respectively.

Materials and methods

Animals

Female *Shan Partridge Ducks* of the same genetic background and of comparable body weight at the age of 370 days were housed in the same room with incandescent lighting on 15:9 h light-dark cycle. Feed and water were provided for ad libitum consumption.

Experiment design

Shan Partridge Ducks were randomly assigned into 5 dietary treatment groups including a control group (each group with 6 replicates of 30 birds). The experimental diets substituted the basal diet by 2% of flaxseed oil, rapeseed oil, beef tallow, or fish oil, respectively. Control group was feed with the basal diet. In addition, a dose response study was further conducted for flaxseed and fish oil diets only. Total 7 experimental groups fed various substituted basal diet by 0.5%, 1%, and 2% flaxseed or fish oil respectively, and the control group fed with the basal diet. Each group had 6 replicates. The ingredients and calculated nutrient level of the basal control diet was formulated to meet the nutrient requirements of the National Research Council (Table 1). The measured fatty acid values of the experimental diets in the present study is shown in Table 2. Diets were balanced to similar levels of protein, fat, total energy, and fiber. At the end of the 5-week dietary treatment, ducks were sacrificed and fresh duck livers were stored at -20℃ for further lipid extraction.

Lipid extraction

One gram of the liver sample was grinded and mixed with 2 mL of chloroform/methanol (1:2, v/v in 0.001% Butylated hydroxytoluene), 1 mL of chloroform, and 1 mL of water. The mixture was then centrifuged at 1,000 rpm for 15 min. The lower layer was then collected. The above procedure was repeated twice. All the three lower layers were combined together and evaporated under a stream of N_2 gas. One mL of chloroform was added to the dried tube before stored at -80℃ until further lipid analysis.

Fatty acid analysis

Fatty acid methyl esters were synthesized according to the protocols of the Kansas Lipidomics Research Center. Briefly, each lipid extracted sample was transferred to Teflon-lined screw cap tube. An internal standard, pentadecanoic acid (C15:0), was added to each sample. Derivatization was performed with 1mL of 3 M methanolic hydrochloric acid at 78℃ for 30 min. Then 2 mL of water and 2 mL of hexan:chloroform (4:1, v/v) were added to each tube. The upper phase was collected after vortex and centrifuge. After the above procedure was repeated twice, three upper phase were combined and dried under nitrogen gas, re-dissolved in 200 μL of hexane and transferred into a GC vial with insert.

Fatty acid methyl esters were analyzed using an Agilent 6890N gas chromatography equipped with a programmed temperature vaporization injector, an Agilent 7683 autosampler, and Agilent flame ionization detector (Santa Clara, CA). The GC was fitted with a HP-88 capillary column (100m × 0.25mm × 0.2μm, Agilent, Santa Clara, CA). The injector temperature was operated at 275°C with an injection volume of 1 μL. The detector temperature was set at 260°C. Helium was used as the carrier gas at a flow rate of 1.6 mL/min. The flow rate of air and hydrogen were 400 mL/min and 30 mL/min, respectively. The oven temperature ramp was programmed from an initial value of 150 °C for 1 min to 175 °C at 10 °C/min for 10 min, and then to 210°C at 5 °C/min for 5 min hold, finally to 230 °C at the same speed for 11 min. The total run time per sample is 40.5 min and the sampling rate of the FID was 20 Hz. Fatty acid peaks were identified by comparison of the relative retention times with the Supelco®37 component fatty acid methyl ester mix standards. The content of each fatty acid was calculated based upon the area of each identified peak.

Statistical analysis

Data are expressed as mean ± SD. All the data were analyzed by two-way analysis of variance (ANOVA) and followed by pairwise comparison with Tukey adjustment using SAS 9.2 (SAS Institute Inc., Cary, NC, USA). A value of $P < 0.05$ was considered to be statistically significant.

Results

Fatty acid profile in duck liver

A representative gas chromatography selected from each treatment group was showed in Figure 1. Total 23 fatty acids including the internal standard (peak 2, pentadecanoic acid, 15:0) were identified and analyzed in the duck liver samples, including saturated fatty acids (SFA 14:0, 15:0, 16:0, 17:0, 18:0, 22:0, and 24:0), monounsaturated fatty acids (MUFA 16:1n-10, 16:1n-7, 18:1n-9, 18:1n-7, and 20:1), ω-3 PUFAs (18:3, 18:4, 20:5, 22:5, and 22:6), and ω-6 PUFAs (18:2, 20:2, 20:3, 20:4, 22:4, and 22:5). As shown in Table 3, the contents of fatty acids in duck liver fed various dietary fats for 5 weeks varied. No significant difference of total SFA, total MUFA, total ω-6 PUFAs, or individual ω-6 PUFA 18:2 and 20:2 was found among the treatment groups. Both short-chain ω-3 PUFA ALA (C18:3) and long-chain ω-3 PUFA DHA (C22:6) were significantly abundant in flaxseed oil group, while long-chain ω-3 PUFA

DHA only were considerably found in fish oil group. The highest content of total ω-3 fatty acids was detected in fish oil-fed group, followed by flaxseed oil-fed and rapeseed oil-fed group. The content of arachidonic acid, one of the ω-6 PUFAs (20:4), was significantly lower in both flaxseed oil and fish oil groups when compared with the control. The ratios of total PUFA/SFA and Σn3/Σn6 were significantly higher in the flaxseed oil, rapeseed oil, and fish oil groups than that in the beef tallow or the control groups.

A dose response study

In order to investigate the conversion efficacy of short-chain to long-chain ω-3 fatty acids in the liver, a dose response study using flaxseed oil diet at 0.5%, 1%, and 2% doses versus fish oil diet was conducted. As shown in Table 4, the contents of ALA in the livers of ducks fed various doses of flaxseed oil increased from the basal line of 0.06 to 0.16, 0.36, and 0.65 mg/g fresh weight gradually. Meanwhile, DHA content in flaxseed oil-fed group also increased from 0.29 to 0.72, 1.06, and 1.01 mg/g fresh weight. In fish oil-fed groups, DHA but not EPA content increased significantly from 0.29 to 1.11, 1.76, and 2.08 mg/g fresh weight. On the contrary, the content of ω-6 arachidonic acid (AA, 20:4) decreased in 2% of flaxseed oil- and 1-2% of fish oil-fed groups significantly.

Conversion between ω-3 fatty acids

The effect of short-chain ω-3 ALA-enriched flaxseed oil diet and long-chain ω-3 fatty acids-rich fish oil diet on liver DHA content is shown in Figure 2. DHA in duck liver became predominant in both fish oil and flaxseed oil groups. Among of ω-3 fatty acids, 91%, 92%, and 85% were DHA in the liver of ducks fed various fish oil doses at 0.5%, 1%, and 2%, respectively. Meanwhile, 81%, 73%, and 59% of total ω-3 fatty acids were converted to DHA in the duck liver fed flaxseed oil at 0.5%, 1%, and 2%, respectively. When compared with fish oil group, 1% of flaxseed oil produced an equivalent level of DHA as 0.5% of dietary fish oil.

Ratio of total ω-3/ω-6 fatty acids

As shown in Figure 3, the ratios of Σω3/Σω6 in duck liver gradually increased as the doses of flaxseed oil or fish oil increased. Fish oil group possessed a higher Σω3/Σω6 value than flaxseed oil group at each dose, while a comparable value was observed between 1% of flaxseed oil and 0.5% of fish oil treatment.

Discussion

Fatty acid manipulation via dietary means may provide an effective method to obtain healthy animal products for humans. Our previous studies investigated the effect of dietary fat on fatty acid composition showing that different dietary fats could change ω-3 fatty acid composition in the duck eggs and muscle tissues. However, little information is available about ω-3 fatty acid profile in the liver modified by various dietary fats. Therefore, this present study, to our knowledge, is the first time to examine the modulation of different dietary fats on fatty acid profile and contents in the duck liver.

After 5-week's dietary treatment, all the dietary fats except for beef tallow showed significant modifications of the fatty acid profile and content in the duck liver. Although beef tallow provided more SFA than the control diet, no significant difference was found in 2% beef tallow-fed group, suggesting an effective transport and storage of SFA into non-hepatic tissues such as adipose tissues. Furthermore, the MUFA-enriched rapeseed oil treatment did not affect any fatty acids except for ALA that significantly increased in hepatic tissues.

The most significant modification was observed in the groups fed with either flaxseed oil or fish oil. Ducks fed with flaxseed oil and fish oil diets were found to have much higher total ω-3 PUFA and ω-3/ω-6 ratio than other groups. The ratio of ω-3/ω-6 was achieved as high as 0.28 for flaxseed oil-fed group and 0.36 for fish oil-fed group. Such ratio is much higher than the modern Western diet and is compatible with that of our ancestors about 100-150 years ago.[39] The increase of both ratios in flaxseed oil and fish oil groups directly not only due to the increase of ω-3 fatty acids but also due to the decrease of ω-6 fatty acids, especially for AA. AA is a precursor of the derived eicosanoids such as PGE_2, TXA_2 and LTB_4. The decrease of ω-6 fatty acids like AA may thus reduce risk of platelet aggregation, hemorrhage, and vasoconstriction.[40-41] Some studies also suggested that a lower ratio of ω-3/ω-6 diets suppress inflammation in patients with rheumatoid arthritis,[42,43] and have a beneficial effect on patients with asthma.[44] The ω-3/ω-6 ratio maybe a useful indicator to evaluate the healthy benefits of the functional food products.

It is interesting that duck liver possesses an efficient conversion of all the short-chain ω-3 fatty acids into long-chain DHA. About 60% of ALA in the flaxseed oil was converted to DHA in the duck liver, while 85% of total ω-3 fatty acids, mostly EPA and DHA, in the fish oil was converted to DHA. Such high conversion efficiency may be related to the broad substrate specificity of the duck elongase enzymes that convert the short-chain ω-3 PUFAs to final DHA exceptionally.[23]

The results of dose response study showed that the total ω-3 fatty acids, specifically DHA, and the ratio of ω-3/ω-6 increased as the dose increased in both flaxseed oil and fish oil treatments. It should be noted that 60-81% of the short-chain omega-3 ALA in flaxseed oil-fed group was efficiently converted to long-chain DHA in the duck liver and 1% of dietary flaxseed oil produced an equivalent level of DHA or ω-3/ω-6 ratio as 0.5% of dietary fish oil. Therefore, the ducks fed flaxseed oil could be an alternative source of fish DHA. Considering that the cost of flaxseed oil is much less expensive than fish oil, it appears commercially applicable for flaxseed oil-enriched diet to be used by waterfowl to provide healthy products.

Taken together, this study investigated the effects of various dietary fats on fatty acid profile and contents of ω-3 fatty acids in duck liver. Total ω-3 fatty acids and the ratio of total ω-3/ω-6 significantly increased in both flaxseed oil- and fish oil-fed groups. About 60-81% of the short-chain ω-3 ALA in flaxseed oil-fed group was efficiently converted to long-chain DHA in the duck liver, whereas 1% of dietary flaxseed oil could produce an equivalent level of DHA as 0.5% of dietary fish oil. It is significant that the short-chain ALA was efficiency converted to long-chain DHA in the duck liver, which may provide an alternative DHA-enriched duck products for human health benefits.

Authors' contributions: All authors participated in the review of the manuscript; JS and LL designed the experiments, XC and XD conducted the experiments and performed analysis, and WW and XC wrote the manuscript.

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References

1. Dolecek TA, Granditis G. Dietary polyunsaturated fatty acids and mortality in the Multiple Risk Factor Intervention Trial (MRFIT). *World Rev Nutr Diet* 1991;66:205-16

2. Nestel P. Effects of fish oils and fish on cardiovascular disease. *Curr Atheroscler Rep* 2001;3:68-73

3. Das UN. Beneficial Actions of Polyunsaturated Fatty Acids in Cardiovascular Diseases: But, How and Why? *Curr Nutr Food Sci* 2016;4:2-31

4. von Schacky C, Harris WS. Cardiovascular benefits of omega-3 fatty acids. *Cardiovasc Res* 2007;73:310-5

5. Zulfakar MH, Edwards M, Heard CM. Is there a role for topically delivered eicosapentaenoic acid in the treatment of psoriasis? *Eur J Dermatol* 2007;17:284-91

6. Song C, Zhao S. Omega-3 fatty acid eicosapentaenoic acid. A new treatment for psychiatric and neurodegenerative diseases: a review of clinical investigations. *Expert Opin Investig Drugs* 2007;16:1627-38

7. Calviello G, Serini S, Piccioni E. n-3 polyunsaturated fatty acids and the prevention of colorectal cancer: molecular mechanisms involved. *Curr Med Chem* 2007;14:3059-69

8. Chen YQ, Edwards IJ, Kridel SJ, Thornburg T, Berquin IM. Dietary fat-gene interactions in cancer. *Cancer Metastasis Rev* 2007;26:535-51

9. Reisman J, Schachter HM, Dales RE, Tran K, Kourad K, Barnes D, Sampson M, Morrison A, Gaboury I, Blackman J. Treating asthma with omega-3 fatty acids: where is the evidence? A systematic review. *BMC Complement Altern Med* 2006;6:26

10. Vasudevan A, Yu Y, Banerjee S, Woods J, Farhana L, Rajendra SG, Patel A, Dyson G, Levi E, Maddipati KR, Majumdar AP, Nangia-Makker P. Omega-3 fatty acid is a potential preventive agent for recurrent colon cancer. *Cancer Prev Res* 2014;7:1138-48

11. Cockbain AJ, Toogood GJ, Hull MA. Omega-3 polyunsaturated fatty acids for the treatment and prevention of colorectal cancer. *Gut* 2012;61:135-49

12. Murff HJ, Shu XO, Li H, Yang G, Wu X, Cai H, Wen W, Gao YT, Zheng W. Dietary polyunsaturated fatty acids and breast cancer risk in Chinese women: a prospective cohort study. *Int J Cancer* 2011;128:1434-41

13. Zheng JS, Hu XJ, Zhao YM, Yang J, Li D. Intake of fish and marine n-3 polyunsaturated fatty acids and risk of breast cancer: meta-analysis of data from 21 independent prospective cohort studies. *BMJ* 2013;346:f3706

14. Moreel X, Allaire J, Léger C, Caron A, Labonté MÈ, Lamarche B, Julien P, Desmeules P, Têtu B, Fradet V. Prostatic and dietary omega-3 fatty acids and prostate cancer progression during active surveillance. *Cancer Prev Res* 2014;7:766-76

15. Tagtow A, Rahavi E, Bard S, Stoody EE, Casavale K, Mosher A. Coming together to communicate the 2015-2020 Dietary Guidelines for Americans. *J Acad Nutr Diet* 2016;116:209-12

16. Lloyd-Jones DM, Hong Y, Labarthe D, Mozaffarian D, Appel LJ, Van Horn L, Greenlund K, Daniels S, Nichol G, Tomaselli GF, Arnett DK, Fonarow GC, Ho PM,Lauer MS, Masoudi FA, Robertson RM, Roger V, Schwamm LH, Sorlie P, Yancy CW, Rosamond WD; American Heart Association Strategic Planning Task Force and Statistics Committee. Defining and setting national goals for cardiovascular health promotion and disease reduction: the American Heart Association's strategic Impact Goal through 2020 and beyond. *Circulation* 2010;121:586–613

17. Harris, WS, Mozaffarian, D, Rimm, E, Kris-Etherton P, Rudel LL, Appel LJ, Engler MM, Engler MB, Sacks F. Omega-6 Fatty Acids and Risk for Cardiovascular Disease: A Science Advisory From the American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention. *Circulation* 2009;119:902–7

18. Gerster H. Can adults adequately convert alpha-linolenic acid (18:3n-3) to eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3)? *Int J Vitam Nutr Res* 1998;68:159-73

19. Brenna JT, Salem N, Sinclair AJ, Cunnane SC. alpha-Linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans. *Prostaglandins Leukot Essent Fatty Acids* 2009;80:85-91

20. Mourot J, Guy G, Peiniau P, Hermier D. Effects of overfeeding on lipid synthesis, transport and storage in two breeds of geese differing in their capacity for fatty liver production. *Anim Res* 2006;55:427-442

21. Scorletti E, Byrne CD. Omega-3 fatty acids, hepatic lipid metabolism, and nonalcoholic fatty liver disease. *Annu Rev Nutr* 2013;33:231-48

22. Deckelbaum RJ, Torrejon C. The omega-3 fatty acid nutritional landscape: health benefits and sources. *J Nutr* 2012;142:587S-591S

23. Gregory MK, James MJ. Functional characterization of the duck and turkey fatty acyl elongase enzymes ELOVL5 and ELOVL2. *J Nutr* 2014;144:1234-9

24. Meyer BJ. Are we consuming enough long chain omega-3 polyunsaturated fatty acids for optimal health? *Prostaglandins Leukot Essent Fatty Acids* 2011;85:275-80

25. Guglielmo CG. Move that fatty acid: fuel selection and transport in migratory birds and bats. *Integr Comp Biol* 2010;50:336-45

26. Ben-Hamo M, McCue MD, Khozin-Goldberg I, McWilliams SR, Pinshow B. Ambient temperature and nutritional stress influence fatty acid composition of structural and fuel lipids in Japanese quail (Coturnix japonica) tissues. *Comp. Biochem. Physiol A Mol Integr Physiol* 2013;166:244-50

27. Silversides FG, Crawford RD. Effects of imperfect albinism (sal-s) on growth in a heavy line of chickens. *Poult Sci* 1991;70:6-12

28. Haak L, De Smet S, Fremaut D, Van Walleghem K, Raes K. Fatty acid profile and oxidative stability of pork as influenced by duration and time of dietary linseed or fish oil supplementation. *J Anim Sci* 2008;86:1418-25

29. Crespo N, Esteve-Garcia E. Nutrient and fatty acid deposition in broilers fed different dietary fatty acid profiles. *Poult Sci* 2002;81:1533-42

30. Lopez-Ferrer S, Baucells MD, Barroeta AC, Grashorn MA. n-3 Enrichment of Chicken Meat. 1. Use of Very Long-Chain Fatty Acids in Chicken Diets and Their Influence on Meat Quality: Fish Oil. *Poult Sci* 2001;80:741-52

31. Lemahieu C, Bruneel C, Termote-Verhalle R, Muylaert K, Buyse J, Foubert I. Impact of feed supplementation with different omega-3 rich microalgae species on enrichment of eggs of laying hens. *Food Chem* 2013;141:4051-9

32. Antruejo A, Azcona A, Garcia P., Gallinger C, Rosmini M, Ayerza R, Coates W, Perez CD. Omega-3 enriched egg production: the effect of alpha-linolenic ω-3 fatty acid sources on laying hen performance and yolk lipid content and fatty acid composition. *Br Poult Sci* 2011;52:750-60

33. Chen TF, Hsu JC. Incorporation of n-3 Long-chain Polyunsaturated Fatty Acids into Duck Egg Yolks. *Asian-Australasian J Anim Sci* 2003;16:565-9

34. Liu WM, Lai SJ, Lu LZ, Shi FX, Zhang J, Liu Y, Yu B, Tao ZR, Shen JD, Li GQ, Wang DQ, Li JJ, Tian Y. Effect of dietary fatty acids on serum parameters, fatty acid compositions, and liver histology in Shaoxing laying ducks. *J Zhejiang Univ Sci B* 2011;12:736-43

35. Du M, Ahn DU. Dietary CLA affects lipid metabolism in broiler chicks. *Lipids* 2003;38:505–11

36. Dodson MV, Hausman GJ, Guan L, Du M, Rasmussen TP, Poulos SP, Mir P, Bergen WG, Fernyhough ME, McFarland DC, Rhoads RP, Soret B, Reecy JM, Velleman SG, Jiang Z. Lipid metabolism, adipocyte depot physiology and utilization of meat animals as experimental models for metabolic research. *Int J Biol Sci*

2010;6:691–9

37. Wood JD, Enser M, Fisher AV, Nute GR, Sheard PR, Richardson RI, Hughes SI, Whittington FM. Fat deposition, fatty acid composition and meat quality: A review. *Meat Science* 2008;78:343-58

38. Kartikasari LR, Hughes RJ, Geier MS, Makrides M, Gibson RA. Dietary alpha-linolenic acid enhances omega-3 long chain polyunsaturated fatty acid levels in chicken tissues. *Prostaglandins Leukot Essent Fatty Acids* 2012;87:103-9

39. Simopoulos AP. Essential fatty acids in health and chronic disease. *Am J Clin Nutr* 1999;70:560S-9S

40. Kaur N, Chugh V, Gupta AK. Essential fatty acids as functional components of foods- a review. *J Food Sci Technol* 2014;51:2289-303

41. Le HD, Meisel JA, de Meijer VE, Gura KM, Puder M. The essentiality of arachidonic acid and docosahexaenoic acid. *Prostaglandins Leukot Essent Fatty Acids* 2009;81:165-70

42. Zampelas A, Paschos G, Rallidis L, Yiannakouris N. Linoleic acid to alpha-linolenic acid ratio. From clinical trials to inflammatory markers of coronary artery disease. *World Rev Nutr Diet* 2003;92:92-108

43. Paschos GK, Magkos F, Panagiotakos DB, Votteas V, Zampelas A. Dietary supplementation with flaxseed oil lowers blood pressure in dyslipidaemic patients. *Eur J Clin Nutr* 2007;61:1201-6

44. Broughton KS, Johnson CS, Pace BK, Liebman M, Kleppinger KM. Reduced

asthma symptoms with n-3 fatty acid ingestion are related to 5-series leukotriene production. *Am J Clin Nutr* 1997;65:1011-7

Table 1. Composition and nutrient levels of the basal diet

Ingredients	Content (g/kg)	Nutrient	Content (g/kg)
Maize grain	400	Metabolizable energy	11.2[b]
Wheat	290	Crude protein	16.5
Soybean meal	120	Total phosphorus	0.70
Wheat bran	90	Total calcium	3.35
Calcium hydrophosphate	12	Total lysine	0.79
Stone powder	80	Total methionine	0.40
Salt	3	Ether extract	29.0
Premix[a]	5		

[a] Supplied per kg of diet: vitamin A 1,500 U, cholecalciferol 200 U, vitamin E (DL-α-tocopheryl acetate) 10 U, riboflavin 3.5 mg, pantothenic acid 10 mg, niacin 30 mg, cobalamin 10 μg, choline chloride 1,000 mg, biotin 0.15 mg, folic acid 0.5 mg, thiamine 1.5 mg, pyridoxine 3.0 mg, Fe 80 mg, Zn 40 mg, Mn 60 mg, I 0.18 mg, Cu 8 mg, Se 0.3 mg; [b] Unit: MJ/kg.

Table 2. Measured fatty acids in the experimental diets

Fatty acid*	**Content (g/100g total fatty acids)**				
	Control	**Flaxseed oil**	**Rapeseed oil**	**Beef tallow**	**Fish oil**
SFA	32.61	25.50	26.22	41.94	30.36
MUFA	37.75	33.03	48.50	36.90	45.65
PUFA	29.84	41.59	25.40	20.25	35.12
Total ω3	7.53	20.54	5.66	4.74	15.57
18:3ω3	6.22	19.69	4.83	4.21	4.42
20:5ω3	0.98	0.64	0.63	0.35	6.14
22:6ω3	0.33	0.21	0.20	0.18	5.01
Total ω6	19.34	19.12	17.83	13.25	14.20
18:2ω6	18.77	18.67	17.42	12.93	12.73
20:2ω6	0.11	0.07	0.12	0.07	0.78
20:4ω6	0.46	0.38	0.29	0.25	0.69
PUFA/SFA	0.92	1.63	0.97	0.48	1.16
Σω3/Σω6	0.39	1.07	0.32	0.36	0.77

* SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids.

Table 3. Fatty acid contents in the duck liver fed various dietary fats for 5 weeks*

Fatty acid**	**Content (mg/g fresh weight)**				
	Control	**Flaxseed oil**	**Rapeseed oil**	**Beef tallow**	**Fish oil**
SFA	8.45±1.94	8.90±0.67	9.99±2.45	9.78±0.97	11.13±3.70
MUFA	6.97±2.29	7.88±1.35	12.20±4.90	9.39±1.32	11.34±5.99
PUFA	5.68±0.93^{a}	7.8±0.5^{ab}	8.02±2.22^{ab}	6.33±0.64^{a}	9.62±3.34^{b}
Total ω3	0.36±0.02^{a}	1.69±0.09^{c}	0.95±0.25^{b}	0.33±0.03^{a}	2.45±0.67^{d}
18:3ω3	0.06±0.01^{a}	0.65±0.15^{c}	0.35±0.25^{b}	0.08±0.02^{a}	0.19±0.12^{ab}
20:5ω3	UD	0.04±0.01^{a}	0.03±0.02^{a}	UD	0.18±0.02^{b}
22:6ω3	0.29±0.03^{a}	1.01±0.13^{b}	0.58±0.07^{a}	0.25±0.02^{a}	2.08±0.51^{c}
Total ω6	5.32±0.92	6.08±0.43	7.07±1.98	6.00±0.62	7.16±2.78
18:2ω6	2.50±0.70	3.88±0.61	4.55±2.14	3.29±0.66	5.35±2.67
20:2ω6	0.08±0.02	0.09±0.01	0.11±0.03	0.09±0.02	0.09±0.04
20:4ω6	2.74±0.29^{a}	2.11±0.30^{b}	2.41±0.25^{ab}	2.62±0.21^{a}	1.72±0.26^{c}
PUFA/SFA	0.68±0.06^{a}	0.88±0.07^{b}	0.80±0.09^{b}	0.65±0.02^{a}	0.86±0.05^{b}
Σω3/ Σω6	0.07±0.01^{a}	0.28±0.02^{c}	0.14±0.01^{b}	0.05±0.00^{a}	0.36±0.09^{d}

*Values are expressed as mean ± SD (n=3-6). Means in a raw without a common letter differ, $p <$ 0.05.

**SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids. UD: undetectable.

Table 4. Fatty acid contents of duck liver fed various dose of dietary fats for 5 weeks*

Fatty acid**	**Content (mg/g fresh weight)**						
	Control	**Flaxseed oil**			**Fish oil**		
		0.5%	1%	2%	0.5%	1%	2%
SFA	8.45±1.94	8.38±1.26	9.20±0.73	8.90±0.67	10.28±2.04	9.83±1.10	11.13±3.70
MUFA	6.97±2.29	6.35±2.80	6.89±1.20	7.88±1.35	11.15±3.62	9.21±2.45	11.34±5.99
PUFA	5.68±0.93^{a}	6.70±0.63^{a}	7.82±0.66^{ab}	7.8±0.5^{ab}	7.44±1.46^{ab}	7.96±0.39^{ab}	9.62±3.34^{b}
Total ω3	0.36±0.02^{a}	0.90±0.05^{b}	1.45±0.24^{cd}	1.69±0.09^{cd}	1.22±0.17^{bc}	1.91±0.01^{d}	2.45±0.67^{e}
18:3ω3	0.06±0.01^{a}	0.16±0.05^{a}	0.36±0.15^{b}	0.65±0.15^{c}	0.09±0.03^{a}	0.11±0.02^{a}	0.19±0.12^{a}
20:5ω3	0.00±0.01^{a}	0.01±0.00^{a}	0.03±0.01^{a}	0.04±0.01^{a}	0.01±0.01^{a}	0.05±0.01^{a}	0.18±0.12^{b}
22:6ω3	0.29±0.03^{a}	0.72±0.09^{b}	1.06±0.08^{b}	1.01±0.13^{b}	1.11±0.15^{b}	1.76±0.05^{c}	2.08±0.51^{c}
Total ω6	5.32±0.92	5.81±0.65	6.37±0.43	6.08±0.43	6.22±1.34	6.05±0.40	7.16±2.78
18:2ω6	2.50±0.70^{a}	2.64±1.06^{a}	3.44±0.52^{ab}	3.88±0.61^{ab}	3.71±1.21^{ab}	3.72±0.55^{ab}	5.35±2.67^{b}
20:2ω6	0.08±0.02	0.07±0.01	0.09±0.01	0.09±0.01	0.10±0.03	0.09±0.03	0.09±0.04
20:4ω6	2.74±0.29^{ab}	3.10±0.42^{a}	2.83±0.16^{ab}	2.11±0.30^{cd}	2.42±0.15^{bc}	2.24±0.16^{c}	1.72±0.26^{d}

PUFA/SFA	0.68±0.06^{a}	0.81±0.05^{bc}	0.85±0.03^{c}	0.88±0.07^{c}	0.73±0.06^{ab}	0.82±0.10^{bc}	0.86±0.05^{c}
Σω3/ Σω6	0.07±0.01^{a}	0.16±0.02^{b}	0.23±0.02^{bc}	0.28±0.02^{cd}	0.20±0.03^{b}	0.32±0.02^{de}	0.36±0.09^{e}

* Values are expressed as mean ± SD (n=6). Means in a raw without a common letter differ, $p < 0.05$.

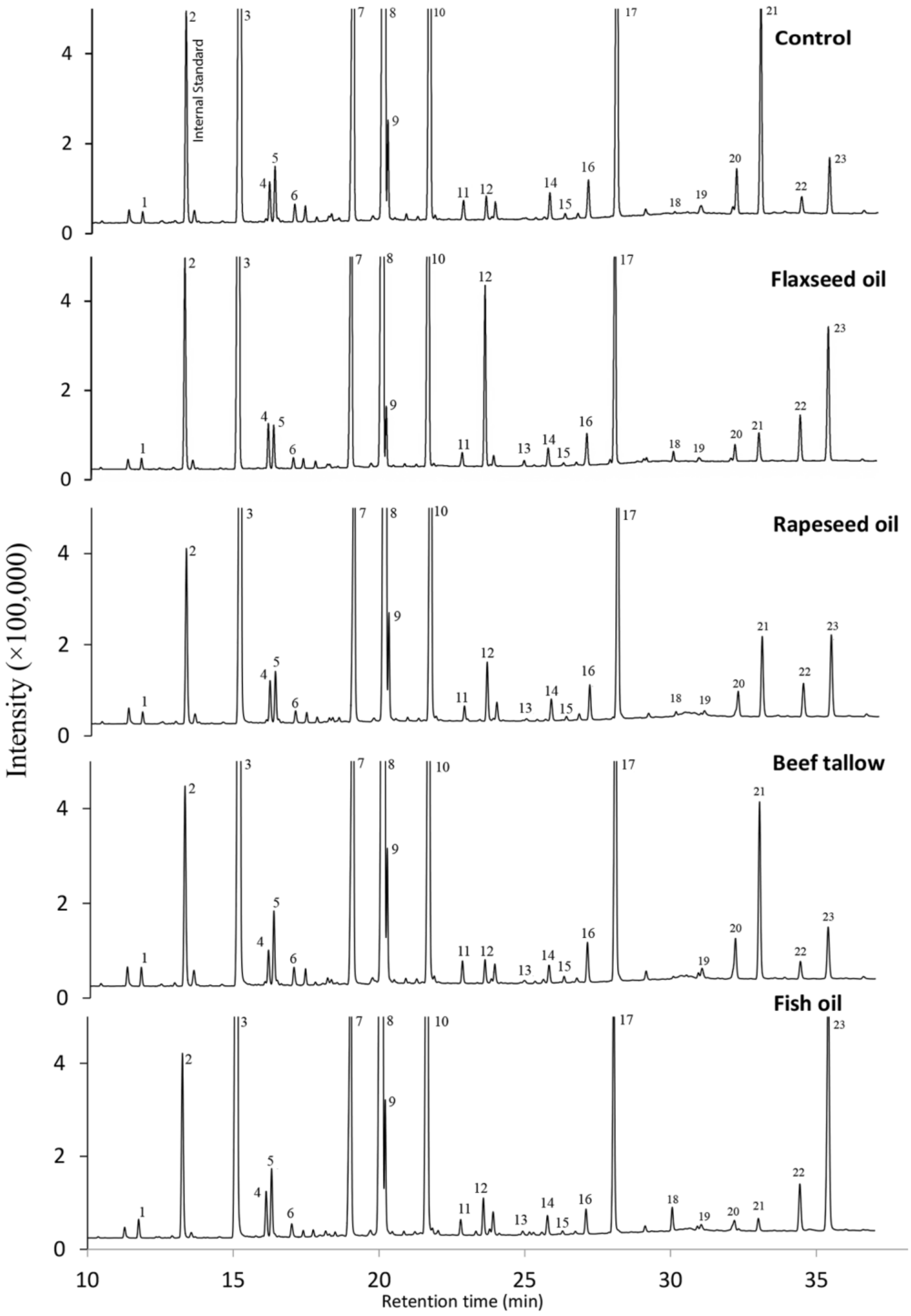
** SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids. Values are expressed as mean ± SD (n=6).

Figure legends：

Figure 1. Representative Gas Chromatography of fatty acid profile in the liver of ducks fed with various dietary fats for 5 weeks. *Shan Partridge Ducks* were randomly assigned into 5 dietary treatments: either the basal diet or 2% of flaxseed oil, rapeseed oil, beef tallow, or fish oil, respectively. At the end of the 5-week treatment, fatty acids in duck liver were analyzed by GC-FID. Totally 23 fatty acids were identified and detected as follows. 1. myristic acid, C14:0; 2. pentadecanoic acid, C15:0 (internal standard); 3. palmitic acid, C16:0; 4. cis-6-hexadecenoic acid, C16:1n-10; 5. palmitoleic acid, C16:1n-7; 6. margaric acid, C17:0; 7. steric acid, C18:0; 8. oleic acid, C18:1n-9; 9. vaccenic acid, C18:1n-7; 10. linoleic acid, C18:2n-6; 11. arachidic acid, C20:0; 12. α-linolenic acid, C18:3n-3; 13. stearidonic acid, C18:4n-3; 14. gondoic acid, C20:1n-9; 15. eicosadienoic acid, C20:2n-6; 16. dihomo-gamma-linolenic acid, C20:3n-6; 17. arachidonic acid, C20:4n-6; 18. eicosapentaenoic acid, C20:5n-3; 19. lignoceric acid, C24:0; 20. adrenic acid, C22:4n-6; 21. docosapentaenoic acid, C22:5n-6 (Osbond acid or all-cis-4,7,10,13,16-docosapentaenoic acid); 22. docosapentaenoic acid, C22:5n-3 (clupanodonic acid or all-cis-7,10,13,16,19-docosapentaenoic acid); 23. docosahexaenoic acid, C22:6n-3.

Figure 2. Dose response of ω-3 fatty acids in the liver of ducks fed with various doses of flaxseed oil or fish oil for 5 weeks. *Shan Partridge Ducks* were randomly assigned into a dose response study by feeding either flaxseed oil or fish oil diets at 0.5%, 1%, and 2%, respectively. At the end of the 5-week treatment, fatty acids in duck liver were analyzed by GC-FID. About 59-81% and 85-92% of total ω-3 fatty acids were converted to DHA in the duck liver fed various doses of flaxseed oil and fish oil, respectively. The dose of 1% flaxseed oil produced an equivalent level of DHA as 0.5% fish oil. Values are expressed as mean ± SD (n=6). Means in a group without a common letter differ, $p < 0.05$.

Figure 3. Dose response of total ω-3/ω-6 ratio in the liver of ducks fed with various disease of flaxseed oil or fish oil for 5 weeks. *Shan Partridge Ducks* were randomly assigned into a dose response study by feeding either flaxseed oil or fish oil diets at 0.5%, 1%, and 2%, respectively. At the end of the 5-week treatment, fatty acids in duck liver were analyzed by GC-FID. The ratios of ω3/ω6 in duck liver gradually increased as the doses of flaxseed oil or fish oil increased. Fish oil group possessed a higher ω3/ω6 value than flaxseed oil group, but a comparable value was observed between 1% of flaxseed oil and 0.5% of fish oil treatment. Values are expressed as mean ± SD (n=6). Means in a group without a common letter differ, $p < 0.05$.



Control
Internal Standard
Flaxseed oil
Rapeseed oil
Beef tallow
Fish oil
Intensity (×100,000)
Retention time (min)
0
2
4
10
15
20
25
30
35
1
2
3
4
5
6
7
8
9
10
11
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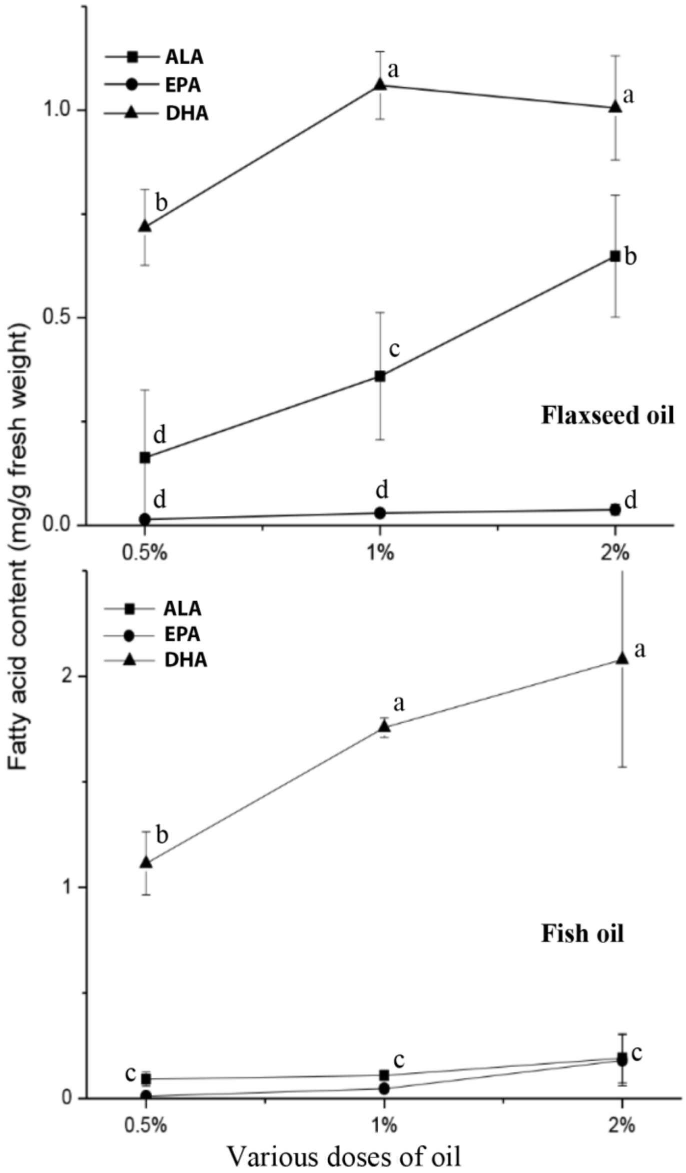
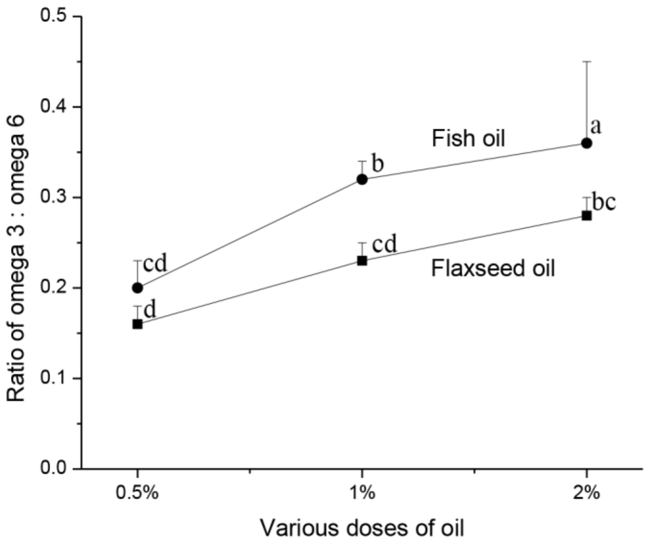

ALA
EPA
DHA
1.0
0.5
0.0
a
a
b
b
c
d
d
d
d
Flaxseed oil
0.5%
1%
2%
Fatty acid content (mg/g fresh weight)
ALA
EPA
DHA
2
1
0
a
a
b
c
c
c
Fish oil
0.5%
1%
2%
Various doses of oil



Ratio of omega 3 : omega 6
0.5
0.4
0.3
0.2
0.1
0.0
0.5%
1%
2%
Various doses of oil
Fish oil
Flaxseed oil
cd
b
a
d
cd
bc