

1 **Effect of Various Dietary Fats on Fatty Acid Profile in Duck Liver: Efficient**

2 **Conversion of Short-chain to Long-chain Omega-3 Fatty Acids**

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11

## 12 **Abstract**

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

35 health benefits.

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

### 37 **Introduction**

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

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

57 an adequate intake of  $\omega$ -6 linoleic acid as 17 g/d for men and 12 g/d for women at the age  
58 of 19 to 50 years<sup>17</sup>.

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

67 Ducks are aquatic birds which have a high rate of lipogenesis to meet their energetic  
68 requirements during ancient migratory flight.<sup>25-26</sup> It has been reported, for instance, the  
69 percent body fat could be reached as high as 37-42% in Peking ducks and 20-30% in  
70 Muscovy ducks.<sup>27</sup> Although duck products are popular with its unique flavor and juicy  
71 texture, the high fat content has raised health concerns. While certain species of lean  
72 ducks, such as the *Shan Partridge* contains 7.5% of body fat only have been developed,  
73 modification of the fat composition in favor of higher  $\omega$ -3 PUFAs in replace of  $\omega$ -6  
74 PUFAs and/or saturated fatty acids may provide promising healthy benefits. It has been  
75 noted that supplemented diets with vegetable oil and fish oil effectively enhanced  $\omega$ -3  
76 PUFAs in the products of pork,<sup>28</sup> broilers<sup>29,30</sup> and broiler eggs.<sup>31,32</sup> Dietary ALA was also  
77 reported to promote EPA and DHA contents in chicken liver.<sup>33</sup> Furthermore, Chen and  
78 Hsu reported an increased trend of EPA, DHA, and total  $\omega$ -3 PUFAs in duck egg yolks by  
79 feeding cod liver oil diet.<sup>34</sup> In addition to storage and transportation of lipids, liver of

80 birds has a very high capacity of lipogenesis<sup>35</sup>. Fatty acids can be synthesis or converted  
81 in the liver, and then transported to other tissues such as adipose, cardiac and skeletal  
82 muscle. The conversion of fatty acids in the liver and incorporation of them into various  
83 tissues are well-established in response to the observed change of fatty acid composition  
84 in the relative tissues<sup>36</sup>. Therefore, fatty acid conversion in the liver may provide impact  
85 not only on a varied fatty acid composition but also on the meat quality including flavor  
86 and muscle color<sup>37</sup>. From an aspect of nutrition value, efficient conversion of short- to  
87 long-chain  $\omega$ -3 PUFAs in the liver may boost the levels of long chain  $\omega$ -3 PUFAs and  
88 thus improve the meat quality. Our previous study found that fish oil and sunflower oil  
89 could significantly enhance the levels of EPA and DHA in the leg and chest muscles as  
90 well as eggs of *Shan Partridge* duck.<sup>38</sup> However, the effect of various dietary fats on fatty  
91 acid profile and the conversion efficacy of  $\omega$ -3 PUFAs in duck liver, to our knowledge,  
92 has yet to be well studied.

93 The aim of this study was to assess the modification of fatty acid profiles in the liver  
94 of *Shan Partridge* duck after feeding various dietary fats, including ALA-enriched  
95 flaxseed oil,  $\omega$ -6 PUFA-enriched rapeseed oil, saturated fatty acid-enriched beef tallow,  
96 and EPA/DHA-enriched fish oil. The conversion efficacy of short-chain to long-chain  $\omega$ -  
97 3 PUFAs was further investigated by a dose-response study for flaxseed and fish oil  
98 treatment, respectively.

## 99 **Materials and methods**

### 100 **Animals**

101 Female *Shan Partridge Ducks* of the same genetic background and of comparable body

102 weight at the age of 370 days were housed in the same room with incandescent lighting  
103 on 15:9 h light-dark cycle. Feed and water were provided for ad libitum consumption.

104 Experiment design

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

118 Lipid extraction

119 One gram of the liver sample was grinded and mixed with 2 mL of chloroform/methanol  
120 (1:2, v/v in 0.001% Butylated hydroxytoluene), 1 mL of chloroform, and 1 mL of water.  
121 The mixture was then centrifuged at 1,000 rpm for 15 min. The lower layer was then  
122 collected. The above procedure was repeated twice. All the three lower layers were  
123 combined together and evaporated under a stream of N<sub>2</sub> gas. One mL of chloroform was

124 added to the dried tube before stored at -80°C until further lipid analysis.

125 Fatty acid analysis

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

135 Fatty acid methyl esters were analyzed using an Agilent 6890N gas chromatography  
136 equipped with a programmed temperature vaporization injector, an Agilent 7683  
137 autosampler, and Agilent flame ionization detector (Santa Clara, CA). The GC was fitted  
138 with a HP-88 capillary column (100m × 0.25mm × 0.2µm, Agilent, Santa Clara, CA). The  
139 injector temperature was operated at 275°C with an injection volume of 1 µL. The  
140 detector temperature was set at 260°C. Helium was used as the carrier gas at a flow rate  
141 of 1.6 mL/min. The flow rate of air and hydrogen were 400 mL/min and 30 mL/min,  
142 respectively. The oven temperature ramp was programmed from an initial value of  
143 150 °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  
144 min hold, finally to 230 °C at the same speed for 11 min. The total run time per sample is  
145 40.5 min and the sampling rate of the FID was 20 Hz. Fatty acid peaks were identified by

146 comparison of the relative retention times with the Supelco<sup>®</sup>37 component fatty acid  
147 methyl ester mix standards. The content of each fatty acid was calculated based upon the  
148 area of each identified peak.

149 Statistical analysis

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

## 154 **Results**

155 Fatty acid profile in duck liver

156 A representative gas chromatography selected from each treatment group was showed in  
157 Figure 1. Total 23 fatty acids including the internal standard (peak 2, pentadecanoic acid,  
158 15:0) were identified and analyzed in the duck liver samples, including saturated fatty  
159 acids (SFA 14:0, 15:0, 16:0, 17:0, 18:0, 22:0, and 24:0), monounsaturated fatty acids  
160 (MUFA 16:1n-10, 16:1n-7, 18:1n-9, 18:1n-7, and 20:1),  $\omega$ -3 PUFAs (18:3, 18:4, 20:5,  
161 22:5, and 22:6), and  $\omega$ -6 PUFAs (18:2, 20:2, 20:3, 20:4, 22:4, and 22:5). As shown in  
162 Table 3, the contents of fatty acids in duck liver fed various dietary fats for 5 weeks  
163 varied. No significant difference of total SFA, total MUFA, total  
164  $\omega$ -6 PUFAs, or individual  $\omega$ -6 PUFA 18:2 and 20:2 was found among the treatment  
165 groups. Both short-chain  $\omega$ -3 PUFA ALA (C18:3) and long-chain  $\omega$ -3 PUFA DHA  
166 (C22:6) were significantly abundant in flaxseed oil group, while long-chain  $\omega$ -3 PUFA



167 DHA only were considerably found in fish oil group. The highest content of total  $\omega$ -3  
168 fatty acids was detected in fish oil-fed group, followed by flaxseed oil-fed and rapeseed  
169 oil-fed group. The content of arachidonic acid, one of the  $\omega$ -6 PUFAs (20:4), was  
170 significantly lower in both flaxseed oil and fish oil groups when compared with the  
171 control. The ratios of total PUFA/SFA and  $\Sigma n3/\Sigma n6$  were significantly higher in the  
172 flaxseed oil, rapeseed oil, and fish oil groups than that in the beef tallow or the control  
173 groups.

174 A dose response study

175 In order to investigate the conversion efficacy of short-chain to long-chain  $\omega$ -3 fatty acids  
176 in the liver, a dose response study using flaxseed oil diet at 0.5%, 1%, and 2% doses  
177 versus fish oil diet was conducted. As shown in Table 4, the contents of ALA in the livers  
178 of ducks fed various doses of flaxseed oil increased from the basal line of 0.06 to 0.16,  
179 0.36, and 0.65 mg/g fresh weight gradually. Meanwhile, DHA content in flaxseed oil-fed  
180 group also increased from 0.29 to 0.72, 1.06, and 1.01 mg/g fresh weight. In fish oil-fed  
181 groups, DHA but not EPA content increased significantly from 0.29 to 1.11, 1.76, and  
182 2.08 mg/g fresh weight. On the contrary, the content of  $\omega$ -6 arachidonic acid (AA, 20:4)  
183 decreased in 2% of flaxseed oil- and 1-2% of fish oil-fed groups significantly.

184 Conversion between  $\omega$ -3 fatty acids

185 The effect of short-chain  $\omega$ -3 ALA-enriched flaxseed oil diet and long-chain  $\omega$ -3 fatty  
186 acids-rich fish oil diet on liver DHA content is shown in Figure 2. DHA in duck liver  
187 became predominant in both fish oil and flaxseed oil groups. Among of  $\omega$ -3 fatty acids,  
188 91%, 92%, and 85% were DHA in the liver of ducks fed various fish oil doses at 0.5%,

189 1%, and 2%, respectively. Meanwhile, 81%, 73%, and 59% of total  $\omega$ -3 fatty acids were  
190 converted to DHA in the duck liver fed flaxseed oil at 0.5%, 1%, and 2%, respectively.  
191 When compared with fish oil group, 1% of flaxseed oil produced an equivalent level of  
192 DHA as 0.5% of dietary fish oil.

193 Ratio of total  $\omega$ -3/ $\omega$ -6 fatty acids

194 As shown in Figure 3, the ratios of  $\Sigma\omega$ 3/ $\Sigma\omega$ 6 in duck liver gradually increased as the  
195 doses of flaxseed oil or fish oil increased. Fish oil group possessed a higher  $\Sigma\omega$ 3/ $\Sigma\omega$ 6  
196 value than flaxseed oil group at each dose, while a comparable value was observed  
197 between 1% of flaxseed oil and 0.5% of fish oil treatment.

## 198 **Discussion**

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

206 After 5-week's dietary treatment, all the dietary fats except for beef tallow showed  
207 significant modifications of the fatty acid profile and content in the duck liver. Although  
208 beef tallow provided more SFA than the control diet, no significant difference was found  
209 in 2% beef tallow-fed group, suggesting an effective transport and storage of SFA into

210 non-hepatic tissues such as adipose tissues. Furthermore, the MUFA-enriched rapeseed  
211 oil treatment did not affect any fatty acids except for ALA that significantly increased in  
212 hepatic tissues.

213 The most significant modification was observed in the groups fed with either  
214 flaxseed oil or fish oil. Ducks fed with flaxseed oil and fish oil diets were found to have  
215 much higher total  $\omega$ -3 PUFA and  $\omega$ -3/ $\omega$ -6 ratio than other groups. The ratio of  $\omega$ -3/ $\omega$ -6  
216 was achieved as high as 0.28 for flaxseed oil-fed group and 0.36 for fish oil-fed group.  
217 Such ratio is much higher than the modern Western diet and is compatible with that of  
218 our ancestors about 100-150 years ago.<sup>39</sup> The increase of both ratios in flaxseed oil and  
219 fish oil groups directly not only due to the increase of  $\omega$ -3 fatty acids but also due to the  
220 decrease of  $\omega$ -6 fatty acids, especially for AA. AA is a precursor of the derived  
221 eicosanoids such as PGE<sub>2</sub>, TXA<sub>2</sub> and LTB<sub>4</sub>. The decrease of  $\omega$ -6 fatty acids like AA may  
222 thus reduce risk of platelet aggregation, hemorrhage, and vasoconstriction.<sup>40-41</sup> Some  
223 studies also suggested that a lower ratio of  $\omega$ -3/ $\omega$ -6 diets suppress inflammation in  
224 patients with rheumatoid arthritis,<sup>42,43</sup> and have a beneficial effect on patients with  
225 asthma.<sup>44</sup> The  $\omega$ -3/ $\omega$ -6 ratio maybe a useful indicator to evaluate the healthy benefits of  
226 the functional food products.

227 It is interesting that duck liver possesses an efficient conversion of all the short-chain  
228  $\omega$ -3 fatty acids into long-chain DHA. About 60% of ALA in the flaxseed oil was  
229 converted to DHA in the duck liver, while 85% of total  $\omega$ -3 fatty acids, mostly EPA and  
230 DHA, in the fish oil was converted to DHA. Such high conversion efficiency may be  
231 related to the broad substrate specificity of the duck elongase enzymes that convert the  
232 short-chain  $\omega$ -3 PUFAs to final DHA exceptionally.<sup>23</sup>

233 The results of dose response study showed that the total  $\omega$ -3 fatty acids, specifically  
234 DHA, and the ratio of  $\omega$ -3/ $\omega$ -6 increased as the dose increased in both flaxseed oil and  
235 fish oil treatments. It should be noted that 60-81% of the short-chain omega-3 ALA in  
236 flaxseed oil-fed group was efficiently converted to long-chain DHA in the duck liver and  
237 1% of dietary flaxseed oil produced an equivalent level of DHA or  $\omega$ -3/ $\omega$ -6 ratio as 0.5%  
238 of dietary fish oil. Therefore, the ducks fed flaxseed oil could be an alternative source of  
239 fish DHA. Considering that the cost of flaxseed oil is much less expensive than fish oil, it  
240 appears commercially applicable for flaxseed oil-enriched diet to be used by waterfowl to  
241 provide healthy products.

242 Taken together, this study investigated the effects of various dietary fats on fatty acid  
243 profile and contents of  $\omega$ -3 fatty acids in duck liver. Total  $\omega$ -3 fatty acids and the ratio of  
244 total  $\omega$ -3/ $\omega$ -6 significantly increased in both flaxseed oil- and fish oil-fed groups. About  
245 60-81% of the short-chain  $\omega$ -3 ALA in flaxseed oil-fed group was efficiently converted to  
246 long-chain DHA in the duck liver, whereas 1% of dietary flaxseed oil could produce an  
247 equivalent level of DHA as 0.5% of dietary fish oil. It is significant that the short-chain  
248 ALA was efficiency converted to long-chain DHA in the duck liver, which may provide  
249 an alternative DHA-enriched duck products for human health benefits.

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

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256 **References**

257 1. Dolecek TA, Granditis G. Dietary polyunsaturated fatty acids and mortality in the  
258 Multiple Risk Factor Intervention Trial (MRFIT). *World Rev Nutr Diet* 1991;66:205-  
259 16

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

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

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

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

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

271 7. Calviello G, Serini S, Piccioni E. n-3 polyunsaturated fatty acids and the  
272 prevention of colorectal cancer: molecular mechanisms involved. *Curr Med Chem*  
273 2007;14:3059-69

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

- 276 9. Reisman J, Schachter HM, Dales RE, Tran K, Kourad K, Barnes D, Sampson M,  
277 Morrison A, Gaboury I, Blackman J. Treating asthma with omega-3 fatty acids: where  
278 is the evidence? A systematic review. *BMC Complement Altern Med* 2006;6:26
- 279 10. Vasudevan A, Yu Y, Banerjee S, Woods J, Farhana L, Rajendra SG, Patel A,  
280 Dyson G, Levi E, Maddipati KR, Majumdar AP, Nangia-Makker P. Omega-3 fatty  
281 acid is a potential preventive agent for recurrent colon cancer. *Cancer Prev Res*  
282 2014;7:1138-48
- 283 11. Cockbain AJ, Toogood GJ, Hull MA. Omega-3 polyunsaturated fatty acids for the  
284 treatment and prevention of colorectal cancer. *Gut* 2012;61:135-49
- 285 12. Murff HJ, Shu XO, Li H, Yang G, Wu X, Cai H, Wen W, Gao YT, Zheng W.  
286 Dietary polyunsaturated fatty acids and breast cancer risk in Chinese women: a  
287 prospective cohort study. *Int J Cancer* 2011;128:1434-41
- 288 13. Zheng JS, Hu XJ, Zhao YM, Yang J, Li D. Intake of fish and marine n-3  
289 polyunsaturated fatty acids and risk of breast cancer: meta-analysis of data from 21  
290 independent prospective cohort studies. *BMJ* 2013;346:f3706
- 291 14. Moreel X, Allaire J, Léger C, Caron A, Labonté MÈ, Lamarche B, Julien  
292 P, Desmeules P, Têtu B, Fradet V. Prostatic and dietary omega-3 fatty acids and  
293 prostate cancer progression during active surveillance. *Cancer Prev Res* 2014;7:766-  
294 76
- 295 15. Tagtow A, Rahavi E, Bard S, Stoodly EE, Casavale K, Mosher A. Coming together  
296 to communicate the 2015-2020 Dietary Guidelines for Americans. *J Acad Nutr*  
297 *Diet* 2016;116:209-12

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

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

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

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

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

- 320 21. Scorletti E, Byrne CD. Omega-3 fatty acids, hepatic lipid metabolism, and  
321 nonalcoholic fatty liver disease. *Annu Rev Nutr* 2013;33:231-48
- 322 22. Deckelbaum RJ, Torrejon C. The omega-3 fatty acid nutritional landscape: health  
323 benefits and sources. *J Nutr* 2012;142:587S-591S
- 324 23. Gregory MK, James MJ. Functional characterization of the duck and turkey fatty  
325 acyl elongase enzymes ELOVL5 and ELOVL2. *J Nutr* 2014;144:1234-9
- 326 24. Meyer BJ. Are we consuming enough long chain omega-3 polyunsaturated fatty  
327 acids for optimal health? *Prostaglandins Leukot Essent Fatty Acids* 2011;85:275-80
- 328 25. Guglielmo CG. Move that fatty acid: fuel selection and transport in migratory  
329 birds and bats. *Integr Comp Biol* 2010;50:336-45
- 330 26. Ben-Hamo M, McCue MD, Khozin-Goldberg I, McWilliams SR, Pinshow B.  
331 Ambient temperature and nutritional stress influence fatty acid composition of  
332 structural and fuel lipids in Japanese quail (*Coturnix japonica*) tissues. *Comp.*  
333 *Biochem. Physiol A Mol Integr Physiol* 2013;166:244-50
- 334 27. Silversides FG, Crawford RD. Effects of imperfect albinism (sal-s) on growth in a  
335 heavy line of chickens. *Poult Sci* 1991;70:6-12
- 336 28. Haak L, De Smet S, Fremaut D, Van Wallegghem K, Raes K. Fatty acid profile and  
337 oxidative stability of pork as influenced by duration and time of dietary linseed or fish  
338 oil supplementation. *J Anim Sci* 2008;86:1418-25
- 339 29. Crespo N, Esteve-Garcia E. Nutrient and fatty acid deposition in broilers fed  
340 different dietary fatty acid profiles. *Poult Sci* 2002;81:1533-42



- 341 30. Lopez-Ferrer S, Baucells MD, Barroeta AC, Grashorn MA. n-3 Enrichment of  
342 Chicken Meat. 1. Use of Very Long-Chain Fatty Acids in Chicken Diets and Their  
343 Influence on Meat Quality: Fish Oil. *Poult Sci* 2001;80:741-52
- 344 31. Lemahieu C, Bruneel C, Termote-Verhalle R, Muylaert K, Buyse J, Foubert I.  
345 Impact of feed supplementation with different omega-3 rich microalgae species on  
346 enrichment of eggs of laying hens. *Food Chem* 2013;141:4051-9
- 347 32. Antruejo A, Azcona A, Garcia P., Gallinger C, Rosmini M, Ayerza R, Coates  
348 W, Perez CD. Omega-3 enriched egg production: the effect of alpha-linolenic  $\omega$ -3  
349 fatty acid sources on laying hen performance and yolk lipid content and fatty acid  
350 composition. *Br Poult Sci* 2011;52:750-60
- 351 33. Chen TF, Hsu JC. Incorporation of n-3 Long-chain Polyunsaturated Fatty Acids  
352 into Duck Egg Yolks. *Asian-Australasian J Anim Sci* 2003;16:565-9
- 353 34. Liu WM, Lai SJ, Lu LZ, Shi FX, Zhang J, Liu Y, Yu B, Tao ZR, Shen JD, Li  
354 GQ, Wang DQ, Li JJ, Tian Y. Effect of dietary fatty acids on serum parameters, fatty  
355 acid compositions, and liver histology in Shaoxing laying ducks. *J Zhejiang Univ Sci*  
356 *B* 2011;12:736-43
- 357 35. Du M, Ahn DU. Dietary CLA affects lipid metabolism in broiler chicks. *Lipids*  
358 2003;38:505-11
- 359 36. Dodson MV, Hausman GJ, Guan L, Du M, Rasmussen TP, Poulos SP, Mir P,  
360 Bergen WG, Fernyhough ME, McFarland DC, Rhoads RP, Soret B, Reecy JM,  
361 Velleman SG, Jiang Z. Lipid metabolism, adipocyte depot physiology and utilization  
362 of meat animals as experimental models for metabolic research. *Int J Biol Sci*

- 363 2010;6:691–9
- 364 37. Wood JD, Enser M, Fisher AV, Nute GR, Sheard PR, Richardson RI, Hughes SI,  
365 Whittington FM. Fat deposition, fatty acid composition and meat quality: A review.  
366 *Meat Science* 2008;78:343-58
- 367 38. Kartikasari LR, Hughes RJ, Geier MS, Makrides M, Gibson RA. Dietary alpha-  
368 linolenic acid enhances omega-3 long chain polyunsaturated fatty acid levels in  
369 chicken tissues. *Prostaglandins Leukot Essent Fatty Acids* 2012;87:103-9
- 370 39. Simopoulos AP. Essential fatty acids in health and chronic disease. *Am J Clin*  
371 *Nutr* 1999;70:560S-9S
- 372 40. Kaur N, Chugh V, Gupta AK. Essential fatty acids as functional components of  
373 foods- a review. *J Food Sci Technol* 2014;51:2289-303
- 374 41. Le HD, Meisel JA, de Meijer VE, Gura KM, Puder M. The essentiality of  
375 arachidonic acid and docosahexaenoic acid. *Prostaglandins Leukot Essent Fatty Acids*  
376 2009;81:165-70
- 377 42. Zampelas A, Paschos G, Rallidis L, Yiannakouris N. Linoleic acid to alpha-  
378 linolenic acid ratio. From clinical trials to inflammatory markers of coronary artery  
379 disease. *World Rev Nutr Diet* 2003;92:92-108
- 380 43. Paschos GK, Magkos F, Panagiotakos DB, Votteas V, Zampelas A. Dietary  
381 supplementation with flaxseed oil lowers blood pressure in dyslipidaemic patients.  
382 *Eur J Clin Nutr* 2007;61:1201-6
- 383 44. Broughton KS, Johnson CS, Pace BK, Liebman M, Kleppinger KM. Reduced

384 asthma symptoms with n-3 fatty acid ingestion are related to 5-series leukotriene  
385 production. *Am J Clin Nutr* 1997;65:1011-7  
386

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 <sup>b</sup>
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 <sup>a</sup>	5		

<sup>a</sup> Supplied per kg of diet: vitamin A 1,500 U, cholecalciferol 200 U, vitamin E (DL- $\alpha$ -tocopheryl acetate) 10 U, riboflavin 3.5 mg, pantothenic acid 10 mg, niacin 30 mg, cobalamin 10  $\mu$ g, 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; <sup>b</sup> 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
<b>SFA</b>	32.61	25.50	26.22	41.94	30.36
<b>MUFA</b>	37.75	33.03	48.50	36.90	45.65
<b>PUFA</b>	29.84	41.59	25.40	20.25	35.12
<b>Total <math>\omega</math>3</b>	7.53	20.54	5.66	4.74	15.57
<b>18:3<math>\omega</math>3</b>	6.22	19.69	4.83	4.21	4.42
<b>20:5<math>\omega</math>3</b>	0.98	0.64	0.63	0.35	6.14
<b>22:6<math>\omega</math>3</b>	0.33	0.21	0.20	0.18	5.01
<b>Total <math>\omega</math>6</b>	19.34	19.12	17.83	13.25	14.20
<b>18:2<math>\omega</math>6</b>	18.77	18.67	17.42	12.93	12.73
<b>20:2<math>\omega</math>6</b>	0.11	0.07	0.12	0.07	0.78
<b>20:4<math>\omega</math>6</b>	0.46	0.38	0.29	0.25	0.69
<b>PUFA/SFA</b>	0.92	1.63	0.97	0.48	1.16
<b><math>\Sigma\omega</math>3/<math>\Sigma\omega</math>6</b>	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 <sup>a</sup>	7.8±0.5 <sup>ab</sup>	8.02±2.22 <sup>ab</sup>	6.33±0.64 <sup>a</sup>	9.62±3.34 <sup>b</sup>
Total ω3	0.36±0.02 <sup>a</sup>	1.69±0.09 <sup>c</sup>	0.95±0.25 <sup>b</sup>	0.33±0.03 <sup>a</sup>	2.45±0.67 <sup>d</sup>
18:3ω3	0.06±0.01 <sup>a</sup>	0.65±0.15 <sup>c</sup>	0.35±0.25 <sup>b</sup>	0.08±0.02 <sup>a</sup>	0.19±0.12 <sup>ab</sup>
20:5ω3	UD	0.04±0.01 <sup>a</sup>	0.03±0.02 <sup>a</sup>	UD	0.18±0.02 <sup>b</sup>
22:6ω3	0.29±0.03 <sup>a</sup>	1.01±0.13 <sup>b</sup>	0.58±0.07 <sup>a</sup>	0.25±0.02 <sup>a</sup>	2.08±0.51 <sup>c</sup>
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 <sup>a</sup>	2.11±0.30 <sup>b</sup>	2.41±0.25 <sup>ab</sup>	2.62±0.21 <sup>a</sup>	1.72±0.26 <sup>c</sup>
PUFA/SFA	0.68±0.06 <sup>a</sup>	0.88±0.07 <sup>b</sup>	0.80±0.09 <sup>b</sup>	0.65±0.02 <sup>a</sup>	0.86±0.05 <sup>b</sup>
Σω3/ Σω6	0.07±0.01 <sup>a</sup>	0.28±0.02 <sup>c</sup>	0.14±0.01 <sup>b</sup>	0.05±0.00 <sup>a</sup>	0.36±0.09 <sup>d</sup>

\*Values are expressed as mean ± SD (n=3-6). Means in a row 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 <sup>a</sup>	6.70±0.63 <sup>a</sup>	7.82±0.66 <sup>ab</sup>	7.8±0.5 <sup>ab</sup>	7.44±1.46 <sup>ab</sup>	7.96±0.39 <sup>ab</sup>	9.62±3.34 <sup>b</sup>
Total ω3	0.36±0.02 <sup>a</sup>	0.90±0.05 <sup>b</sup>	1.45±0.24 <sup>cd</sup>	1.69±0.09 <sup>cd</sup>	1.22±0.17 <sup>bc</sup>	1.91±0.01 <sup>d</sup>	2.45±0.67 <sup>e</sup>
18:3ω3	0.06±0.01 <sup>a</sup>	0.16±0.05 <sup>a</sup>	0.36±0.15 <sup>b</sup>	0.65±0.15 <sup>c</sup>	0.09±0.03 <sup>a</sup>	0.11±0.02 <sup>a</sup>	0.19±0.12 <sup>a</sup>
20:5ω3	0.00±0.01 <sup>a</sup>	0.01±0.00 <sup>a</sup>	0.03±0.01 <sup>a</sup>	0.04±0.01 <sup>a</sup>	0.01±0.01 <sup>a</sup>	0.05±0.01 <sup>a</sup>	0.18±0.12 <sup>b</sup>
22:6ω3	0.29±0.03 <sup>a</sup>	0.72±0.09 <sup>b</sup>	1.06±0.08 <sup>b</sup>	1.01±0.13 <sup>b</sup>	1.11±0.15 <sup>b</sup>	1.76±0.05 <sup>c</sup>	2.08±0.51 <sup>c</sup>
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 <sup>a</sup>	2.64±1.06 <sup>a</sup>	3.44±0.52 <sup>ab</sup>	3.88±0.61 <sup>ab</sup>	3.71±1.21 <sup>ab</sup>	3.72±0.55 <sup>ab</sup>	5.35±2.67 <sup>b</sup>
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 <sup>ab</sup>	3.10±0.42 <sup>a</sup>	2.83±0.16 <sup>ab</sup>	2.11±0.30 <sup>cd</sup>	2.42±0.15 <sup>bc</sup>	2.24±0.16 <sup>c</sup>	1.72±0.26 <sup>d</sup>

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

\* Values are expressed as mean ± SD (n=6). Means in a row 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.  $\alpha$ -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. docosaheptaenoic acid, C22:6n-3.

**Figure 2. Dose response of  $\omega$ -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  $\omega$ -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  $\pm$  SD (n=6). Means in a group without a common letter differ,  $p < 0.05$ .

**Figure 3. Dose response of total  $\omega$ -3/ $\omega$ -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  $\omega$ 3/ $\omega$ 6 in duck liver gradually increased as the doses of flaxseed oil or fish oil increased. Fish oil group possessed a higher  $\omega$ 3/ $\omega$ 6 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  $\pm$  SD (n=6). Means in a group without a common letter differ,  $p < 0.05$ .







