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Dietary oils modify lipid molecules and nutritional value of fillet in Nile tilapia: A deep lipidomics analysis☆

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ABSTRACT

The nutritional value of fish fillet can be largely affected by dietary oils. However, little is known about how dietary oils modify lipid molecules in fish fillets. Through biochemical and lipidomics assays, this study demonstrated the molecular characteristics of fillet lipids in Nile tilapia fed with different oils for six weeks. High 18:2n-6 and low 18:3n-3 deposition in phosphoglycerides resulted high 18:2n-6/18:3n-3 ratio in tilapia. Dietary n-3 VLCUFAs intake increased its deposition at sn-1/3 of triglycerides and at sn-2 of phosphatidylcholines. Irrespective of dietary oil, 16:0 was distributed preferentially at the outer positions of glycerol backbone. High 18:2n-6 accumulated at sn-2 position for fish fed with n-3 PUFA-enriched oils. High 18:3n-3 deposited at sn-1/3 in TG, sn-1 in phosphatidylethanolamines, while at sn-2 in phosphatidylcholines. Together, dietary oils change the composition and positional distribution of fatty acids on the glycerol backbone, and change nutritional value of fish for human health.

1. Introduction

Epidemiological and clinical data have revealed a significant association between excessive levels of omega-6 (n-6) long chain polyunsaturated fatty acids (PUFAs) in diets and metabolic and chronic inflammatory disease (Simopoulos, 2008). N-3 PUFAs with beneficial anti-inflammatory properties have been demonstrated to reduce the risk of many diseases, such as stroke, cardiovascular disease, and sudden death (Riediger, Othman, Miyoung, & Moghadasian, 2009). N-3 PUFAs especially EPA (eicosapentaenoic acid), have anti-inflammatory effects by competing with ARA (arachidonic acid) for the cyclooxygenases thereby preventing the negative physiological effects of ARA (Du et al., 2010). Therefore, it is important and necessary for the health of humans to consume appropriate ratios of n-3 PUFAs to n-6 PUFAs in the diet.

Fish has commonly been regarded as the main dietary source of abundant n-3 PUFAs. However, the content of n-6 fatty acids in Nile tilapia (*Oreochromis niloticus)*, the second most cultivated freshwater fish in the world, unlike most fish, is much greater than that of n-3 fatty acids (Lim, Yildirim-Aksoy, & Klesius, 2011). Nile tilapia need n-6 fatty acids in their diet to maximize growth and tend to store large amounts of them in their muscle (Ferreira et al., 2011; Matsushita et al., 2006). Therefore, some nutritionists have questioned the nutritional value of Nile tilapia as a “healthy fish” in the human diet (Ioannis, Bell, Little, Yakupitiyage, & Rakshit, 2006; Weaver et al., 2008). As the global production of Nile tilapia is significant, the potentially negative effects on human health caused by its consumption could be reduced by increasing its content of n-3 fatty acids.

Several studies have previously shown that the fatty acid composition of the fillets of cultivated fish can be modified by dietary oil sources (Bell, Dick, Mcvicar, Sargent, & Thompson, 1993; Bell et al., 2001; Izquierdo et al., 2005), so that of Nile tilapia fillets could also be modified to increase their n-3 PUFAs content. Studies have also shown that the content of n-3 PUFAs in Nile tilapia fillets increased when the fish was fed plant oils enriched with 18:3n-3 (Carbonera et al., 2014; Visentainer, de Souza, Makoto, Hayashi, & Franco, 2005). However, the nutritional value of a lipid depends not only on its fatty acid composition, but also on how fatty acids are arranged on the lipid-glycerol backbone which significantly affects the hydrolysis, absorption and utilization efficiency of fatty acids in humans (Karupaiah & Sundram, 2007; Kubow, 1996). Data from fat absorption studies on humans has shown that the triglyceride (TG) with palmitic acid (PA) located at the

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TG sn-2 position can be more efficiently absorbed, avoiding the formation of fatty soap, than those with PA located at the sn-1 and sn-3 positions (Kubow, 1996). Fish oil (FO) and seal oil are both rich in n-3 PUFAs, but the intramolecular structure of their TGs differs. Other studies have shown that n-3 PUFAs (EPA and DHA, docosahexaenoic acid) are distributed mostly at the sn-1/3 position of seal oil TG and at the sn-2 position of fish oil TG (Aursand, Jørgensen, & Grasdalen, 1995; Christensen, Mortimer, Høy, & Redgrave, 1995). These differences between the locations of n-3 PUFAs in TG in the two oils probably affect the clearance of chylomicrons and the uptake of remnants in the organs of rats (Yoshida et al., 1999). Therefore, the location of fatty acids on the glycerol backbone is important for utilizing fish lipids. Moreover, fish lipid class primarily includes TG, diacylglycerol (DG), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI) (Ruizlopez, Stubhaug, Ipharraguerre, Rimbach, & Menoyo, 2015). The TG, PC and PE are normally the predominant lipid classes with high proportions (> 90%). TG is a major class of neutral lipid as the form of energy storage, while PC and PE are the major classes of polar lipids primarily used as important components of cell membranes, which play crucial roles in the regulation of membrane structure, fluidity and signal transduction (Tocher, 2003). Previous studies have shown that lipid class profile in fish tissues was associated with dietary lipids and the distribution of fatty acids in the lipid species was largely affected by dietary lipids (Bell et al., 1993; Ruizlopez et al., 2015). However, there are few available studies in fish to elucidate the effect of dietary lipids on the retention and the position distribution of fatty acids in different lipid species. Here, we hypothesized that dietary oils would affect glycerol positional distribution of fatty acids in fish, and then affect the nutritional value of fish fillet for consumers. However, little is known about how dietary oils modify the composition, structure and nutritional value of lipids in fish.

Currently, lipidomics has been commonly used in the studies of many metabolic diseases (Wenk, 2005). This technique makes it possible to accurately describe the complete lipid profile, the structures, functions, interactions and dynamics within a cell or tissue, and to identify and quantify thousands of cellular lipid molecular species which differ in their fatty acyl chain lengths and the number of double bonds. However, few studies using lipidomics are available on fish lipids. Therefore, the present study aimed to investigate how different dietary oils influence the lipid composition, and the abundance and structures of lipids in Nile tilapia fillets. Five different dietary oils which were commonly used in aquafeeds, were fed to Nile tilapia for six weeks: palm oil (PO), olive oil (OO), safflower oil (SO), perilla seed oil (PO) and FO, enriched with the same percentage (67.1%) of PA, oleic acid (OA), linoleic acid (LA), linolenic acid (LNA) and n-3 very long chain unsaturated fatty acids (VLCUFAs, sum of EPA and DHA), respectively. The positional distribution of fatty acids (PA, OA, LA, LNA, ARA, EPA and DHA) in the lipids of Nile tilapia muscles (mainly TG, PC and PE) was then be explored using the lipidomics approach. To the best of our knowledge, this is the first study to examine the structures and changes of lipid molecular species in fish fed with different oils. This study will also provide new information for evaluating the nutritional values of cultivated fish, such as Nile tilapia fed with artificial diets.

2. Materials and methods

2.1. Animal experiments

All experiments were approved by the Committee on the Ethics of Animals Experiments of East China Normal University and followed the Guidance of the Care and Usage of Laboratory Animals in China. Nile tilapia was obtained from local fish farm in Guangzhou Province, China and maintained in 15 circular tanks (256 L) in a closed recirculating system containing freshwater. After acclimatization, healthy fish with relatively similar initial body weight (11.1 ± 0.01 g) were distributed randomly into the 15-tanks in triplicates (25 fish per tank). Five oils, including PO, OO, SO, PEO and FO, which contained mainly PA (37.6%), OA (79.7%), LA (79.7%), LNA (67.1%) and n-3 VLCUFAs (EPA and DHA) (83.0%), respectively, were used as the basic dietary oils. Before they were used for diet preparation, different amounts of palmitic acid were added into the five oils to make equal percentages (67.1%) of PA, OA, LA, LNA, EPA and DHA in the corresponding oils. The five mixed oils were chosen to prepare semi-purified diets. The fatty acid compositions of five oils are shown in Supplementary Table 1 and the formulation of the five experimental diets is shown in Supplementary Table 2. During the feeding trial, the fish were fed with the diets twice daily for six weeks. The weights of all fish in each tank were weighed once every 2 weeks and their daily rations were adjusted accordingly. After the trial, fish were euthanized with MS-222 (20 mg/L). Twelve fish per treatment were used for physiological and biochemical analyses. The muscle was dissected without skin and the tissue samples were rapidly frozen in liquid nitrogen and kept at −80 °C until needed for further analysis. In the lipid extraction and the following LC-MS (liquid chromatography-mass spectrometry) analysis, the muscles from twelve fish per group were collected and three fish were mixed as a pooled sample (a total of 4 pooled samples per dietary group, $n = 4$).

2.2. Lipid extraction and separation

The total lipids from muscle was extracted and separated by chloroform/methanol (2/1, v/v), as described previously (Araujo et al., 2010). TG and phospholipids (PL) were separated from the total lipids by thin layer chromatography (TLC). The lipid samples were spotted on Silica Gel TLC plates (200 mm × 200 mm). PL were first separated in a solvent system consisting of methyl acetate/isopropyl acid chloroform/methanol/0.25% KCl (25/25/25/10/9, v/v/v/v) for 100 min. After PL separation, the plates were dried at room temperature for 20 min. Afterwards, the plates were placed in a solvent system composed of isohexane/ether/acetic acid (80/25/1.5, v/v/v) to separate TG for 160 min. TG and PL were stained by iodine. The corresponding bands were scraped off from the plates, and TG and PL bands were extracted by using chloroform and methanol, respectively, and concentrated for further fatty acid analysis as described below.

2.3. Fatty acid methyl esters (FAMEs) preparation and fatty acids composition analysis by gas chromatography (GC)

The fatty acid methyl esters (FAMEs) were prepared according to (Araujo et al., 2010) as following: two mg of the lipid sample and 0.1 mg of 19:0 used as the internal standard were mixed and then converted into soap by saponification with the addition of 0.5 ml methanolic KOH (2 mol/L) at 65 °C for 30 min. Two ml of 14% boron trifluoride-methanol was added for FAMEs derivatization. The mixture was heated at 100 °C for 40 min. Then, 1 ml of hexane (for HPLC, > 95%) and 1 ml of ddH_2O were added and mixed until the mixture was cooled down to the room temperature. The samples were centrifuged at 3000 rpm for 5 min and subsequently the resulting FAMEs from the upper layer were concentrated under nitrogen gas flow.

The FAMEs samples were measured by GC (Shimadzu 2010, Japan). The GC column length (RESTEK, USA) was 100 m with an internal diameter of 0.25 mm and 0.2 um film thickness. High purity nitrogen was used as the carrier gas with the flow rate of 7.8 ml/min. The oven temperature rose from 120 °C to 240 °C at 4 °C/min with a hold time of 30 min at 240 °C. The injector and detector temperatures were set at 250 and 260 °C, respectively. Fatty acids were identified by retention times from standard mixtures (Product number CRM47885; SUPELCO, USA). The concentration of the FAMEs were calculated according to the ratio of different FAMEs peak areas with the internal standard (19:0) peak area. The results of fatty acid composition are expressed as absolute concentration (mg/g muscle).

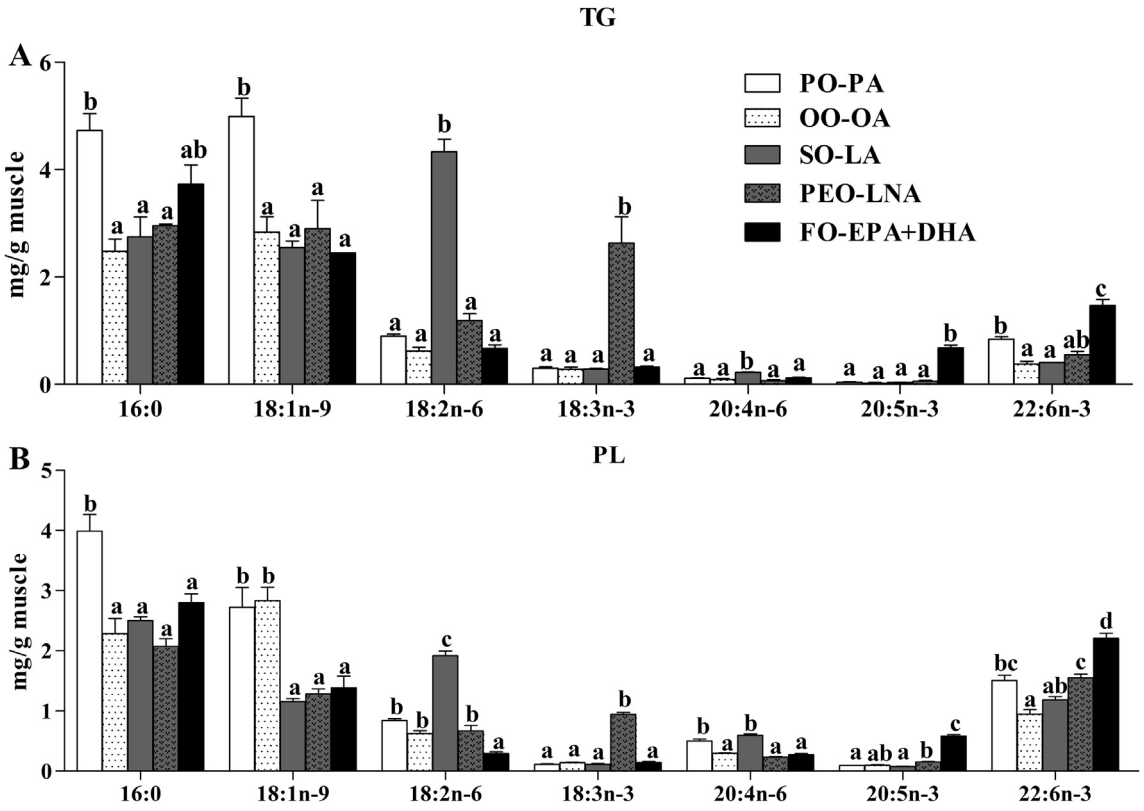


Fig. 1. Effects of different dietary oils on individual fatty acid compositions in Nile tilapia fillets. Results are given as mean ± SEM ($n = 6$). A: Absolute concentration (mg/g muscle) of individual fatty acid in TG; B: Absolute concentration (mg/g muscle) of individual fatty acid in PL. Columns referring to a specific fatty acid having different superscript letters are significantly different ($P < 0.05$).

2.4. Lipid analysis by LC-MS

Lipid were extracted from muscle (about 50 mg) with chloroform/methanol (2/1, v/v). The liquid chromatography/tandem mass spectrometry (LC-MS, Thermo, Ultimate 3000 LC, Orbitrap Elite) was applied as analysis platform. The LC separations were carried out on a reversed phase 100 × 2.1 mm Kinetex C18 column with 1.9 um particles kept at 40 °C. Mobile phase A was acetonitrile/water (60/40, v/v), including 0.1% formic acid and 10 mmol/L ammonium acetate. Mobile phase B was acetonitrile/water (10/90, v/v), including 0.1% formic acid and 10 mmol/L ammonium acetate. Injection volume was 4 μl and automatic injector temperature was 4 °C. Mass spectrometry was performed as the followings: ESI+: Heater temperature 300 °C; Sheath Gas Flow rate, 45arb; Aux Gas Flow Rate, 15 arb; Sweep Gas Flow Rate, 1arb; spray voltage, 3.0 KV (ESI-: 3.2 KV); Capillary Temp, 350 °C; S-Lens RF Level, 30% (ESI-: 60%). The data obtained were extracted and preprocessed with lipid search software (Thermo). They were then normalized and edited into two-dimensional data matrix by excel 2010 software, including LipidIon, class, fatty acid chains (Fatty Acid, FA1, FA2, FA3), CalcMz, IonFormula, retention time (RT) and peak intensity.

2.5. Statistical analyses

The data are expressed as mean ± SEM. The correlation among all lipid molecular species of TG, PC and PE detected by lipidomics were used for principal component analysis (PCA). The top 100 fillet lipid molecule species of TG and PC, which occupied 78%–85% and 99.6%–100% of total TG and PC molecule species, respectively, in five groups were chosen for analysis by heatmap. In the value calculation of the percentage of a specific FA-containing TG, PC and PE molecules, one lipid molecule containing three, two or one specific FA were regarded as one molecule in statistical calculation. One–way analysis of variance (ANOVA) was used to compare all the results. When ANOVA detected showed significant differences, specific comparisons between means were determined by Tukey's multiple test. The position distribution of fatty acids in TG and PL (phosphoglycerides) was determined by using a Student's *t*-test. Treatment effects were considered significant at $P < 0.05$. The statistical package SPSS (version 20.0) was used for all statistical analyses. The heatmap and PCA were performed in Matlab 2014.

3. Results and discussion

3.1. Growth and biochemical composition of muscle

The weight gain (WG), and muscle protein and lipid contents are shown in Supplementary Fig. 1. After six weeks, the WG in the five diet groups were in this order: PEO > SO > OO > FO > PO (Supplementary Fig. 1A). There were no significant differences in WG values between the PEO, SO and OO groups, but all these values were significantly higher than that of the PO group. The WG of the FO group was significantly lower than that of the PEO group, but no significant differences were found between the FO group and the other three groups. In previous studies, PO at < 38.9% in diets did not significantly inhibit the growth of Nile tilapia (Azevedo, Tonini, & Braga, 2013; Ochang, Fagbenro, & Adebayo, 2007). However, Takeuchi et al. reported that a high intake of saturated fatty acids (SFA) was not suitable for *Tilapia nilotica* (Takeuchi, Satoh, & Watanabe, 1983a). In the present study, PO contained more palmitic acid (67.1%) than normal palm oil, therefore the high SFA intake could be the reason for the lower growth in the PO group. Many studies have demonstrated that the tilapia species requires a dietary source of n-6 series fatty acids for maximum growth (Kanazawa, Teshima, Sakamoto, & Awal, 1980; Takeuchi, Satoh, & Watanabe, 1983b). However, some studies have also shown

that tilapia species requires both n-3 and n-6 PUFAs (Chou & Shiau, 1999; Yildirim-Aksoy, Lim, Davis, Shelby, & Klesius, 2007). The present study has indicated that Nile tilapia fed the PEO diet, containing high levels of 18:3n-3, had a similar high weight gain as the fish fed the SO diet, containing high levels of 18:2n-6. Therefore, Nile tilapia could also use 18:3n-3 efficiently for normal growth. In the present study, the muscle protein contents were similar among groups, except that that of the FO group was significantly higher than that of the OO group (Supplementary Fig. 1B). All the groups also had similar contents of muscle lipid (Supplementary Fig. 1C). All these results indicate that the sources of dietary oil can affect the growth performance of Nile tilapia, but would not greatly change the protein and lipid contents of their muscle.

The fatty acid compositions in the muscle of the Nile tilapia fed with different dietary oil sources are shown in Supplementary Table 3. The absolute mass of the seven specific fatty acids studied (16:0, 18:1n-9, 18:2n-6, 18:3n-3, 20:4n-6, 20:5n-3 and 22:6n-3) in total TG and total PL are shown in Fig. 1. Although the muscle lipid content was not markedly affected by the dietary oil sources (Supplementary Fig. 1C), its fatty acid composition was largely influenced by the dietary treatments (Fig. 1 and Supplementary Table 3). In general, the content of a specific FA was positively correlated with its content in the dietary oil. Therefore, in both TG and PL, the highest muscle contents of 16:0, 18:2n-6, 18:3n-3, EPA and DHA were observed in the PO, SO, PEO and FO groups, respectively (Fig. 1). A high content of ARA (20:4n-6) was also observed in the SO group, derived from 18:2n-6 by desaturation and elongation (Kanazawa et al., 1980). However, exceptionally, dietary oleic oil (OO group) did not lead to the greatest content of 18:1n-9 in the TG of the muscle which was observed in the PO group. This suggests that 18:1n-9 was more easily metabolized than other FAs. It also appeared that in the PO diet, 16:0 was the dominant FA component (67.1%), much higher than 18:1n-9 (23.4%), but in the muscles from the PO group, the contents of 16:0 and 18:1n-9 in TG were relatively similar, possibly because, like 18:1n-9, 16:0 is also a good substrate for utilization. In contrast, PUFAs, including 18:2n-6, 18:3n-3 and VLCUFAs, such as EPA and DHA, accumulated more easily in the Nile tilapia fillets. This proves that the content of n-3 PUFAs in the muscles of tilapia species could be increased by using n-3 PUFA-enriched diets, as previously reported (Ferreira et al., 2011). The results of the present study agreed with other studies on Atlantic salmon and rat, where 16:0 and 18:1n-9 were found to be convenient β-oxidation substrates for energy provision in contrast to n-3 VLCUFAs, such as DHA, which were relatively difficult to be β-oxidized (Du, Araujo, Stubhaug, & Frøyland, 2010; Stubhaug, Tocher, Bell, Dick, & Torstensen, 2005). However, although 16:0 is more easily metabolized, it was noticed that PO group obtained the lowest growth performance, suggesting high levels of 16:0 might contribute to lipotoxicity. Recently, it has been found that high dietary 16:0 intake caused liver damage via endoplasmic reticulum stress-induced apoptosis in fish. In addition, 16:0-altered intestinal microbiota composition induced liver damage by increasing endotoxin release and promoting intestinal 16:0 absorption, which further aggravated the lipotoxicity (Ding et al., 2018). Therefore, high PO diet in our study could have induced high lipotoxicity and therefore depressed Nile tilapia growth.

3.2. Composition of lipid classes in muscle

To better evaluate the retention and positional distribution of the seven individual FAs in different lipids and the corresponding glycerol backbone, an LC-MS-based lipidomics approach was applied in the present study. This assay exhibited high stability and reproducibility and identified a large number of lipid molecular species (Supplementary Fig. 2A). The relative amounts and numbers of all lipid classes detected by the lipidomics assay are shown in Table 1 and Supplementary Fig. 2, respectively. Thirteen lipid classes were detected in the muscle (Supplementary Fig. 2B–D). The numbers of lipid classes among five groups were similar, except for the number of TG species in the SO, PEO and FO groups, which were significantly greater than in the PO and OO groups. This could be because the SO, PEO, and FO diets contained more PUFAs than the PO and OO diets, so in turn, this increased the diversity of the TG species. The data on the classes of lipids in the fillets (Table 1) indicated that the total TG percentage in the FO group (70.4%) was significantly higher than that in the PO group (55%). In contrast, the percentages of PC and PE in the FO group (22.7% and 4.73%, respectively) were significantly lower than those in the PO group (36.2% and 6.07%, respectively). The TG, PC and PE percentages of the other groups were more comparable. Table 1 and Supplementary Fig. 2 show that in the muscles of all groups, TG was identified as the predominant lipid class, ranging from 55% to 70%, followed by PC ranging from 23% to 36% then PE ranging from 4.7% to 6.1%. Therefore, the predominant lipid classes, TG, PC and PE, were analyzed more intensively.

3.3. Analysis of the specificity of the TG, PC and PE profiles of muscles using the PCA and heatmap evaluation

The composition of all TG, PC and PE lipid classes in the muscle were analyzed using PCA to discriminate between Nile tilapia fed different dietary oils. The composition of the TG, PC and PE classes from the muscles of Nile tilapia fed the SO, PEO and FO diets could clearly be discriminated (Fig. 2A–C). However, the composition of the TG, PC and PE molecular species in the PO and OO groups always clustered together, showing that feeding PO and OO produced a similar composition of lipid molecules in Nile tilapia muscles. Fig. 1 suggests that both 16:0 and 18:1n-9 are likely to be good metabolizing substrates, so in the PO and OO groups they were mostly metabolized by the fish and caused no large changes in the lipid composition of the muscles.

To further understand the effect of the lipid species in the dietary oils on the composition of the lipid molecular species in the muscles of Nile tilapia, the variation in the TG and PC species identified in the diets and muscles were analyzed using a clustering heatmap (Fig. 3). The composition of the TG and PC species in the diets and muscles are shown in Supplementary Tables 4 and 5. The heatmap directly shows the variation in the TG and PC molecular species in the Nile tilapia muscles before and after the feeding trial. The composition patterns of the TG or PC molecular species in the fish muscles before the feeding trial (Fig. 3A or G, TG or PC) were visually modified after the feeding trial, indicating that the five dietary oils altered the lipid species composition to different extents. A similarity in color between the diet group and the corresponding muscle group (pair group) indicates similar patterns of lipid composition, and also means the composition of lipids in the muscles was largely dependent on the composition of the dietary lipids. On the contrary, a dissimilarity in color means that the muscle lipid composition was independent of the composition of the dietary lipids intake. In the TG lipids (Fig. 3B–F), the degree of similarity between the dietary and muscle lipid molecular species from low to high was: PO < OO < SO < PEO < FO. This indicated that dietary PO and OO intake hardly changed the composition of the TG molecules in the muscle, while the dietary oils enriched with PUFAs (SO and PEO), particularly VLCUFAs (FO), changed the composition of TG molecules in the muscle considerably. This result also confirmed that dietary 16:0 and 18:1n-9 could not be efficiently deposited in Nile tilapia fillets, whereas PUFAs, especially VLCUFAs, such as EPA and DHA, could easily accumulate. Compared with TG, this tendency was not obvious for PC (Fig. 3H–L) and PE (data not shown). This showed that, compared with TG lipids, the molecular composition of the lipids in the PL of Nile tilapia muscle was relatively stable. Therefore, the effects of dietary oils on the TG in Nile tilapia muscle were more significant than on the PL. A previous study has also indicated that dietary fatty acids were mostly incorporated in TG, rather than in PL, because TG is the main form in which dietary fatty acids are deposited (Stubhaug et al., 2005).

Table 1
Lipid composition of experimental diets and Nile tilapia muscles fed on different oils based on lipidomics analysis.

Lipid	Diet (%)					Muscle (%)				
	PO	OO	SO	PEO	FO	PO	OO	SO	PEO	FO
TG	84.3	83.5	85.8	90.0	88.3	55.0 ± 3.30^{a}	61.6 ± 3.65^{ab}	64.0 ± 5.13^{ab}	65.9 ± 3.15^{ab}	70.4 ± 2.15^{b}
PC	8.28	9.66	7.40	5.44	8.21	36.2 ± 3.00^{b}	29.8 ± 3.14^{ab}	28.1 ± 4.53^{ab}	27.7 ± 2.84^{ab}	22.7 ± 1.83^{a}
DG	5.30	4.42	4.93	3.09	1.91	0.902 ± 0.0415^{c}	1.14 ± 0.0458^{d}	0.873 ± 0.0470^{c}	0.606 ± 0.0049^{b}	0.383 ± 0.0104^{a}
LPC	1.01	0.931	0.804	0.614	0.590	0.403 ± 0.0431^{b}	0.398 ± 0.0130^{ab}	0.350 ± 0.0128^{ab}	0.288 ± 0.0289^{a}	0.298 ± 0.0141^{ab}
PE	0.472	0.552	0.431	0.281	0.362	6.07 ± 0.33^{b}	5.56 ± 0.47^{ab}	5.48 ± 0.57^{ab}	4.37 ± 0.28^{a}	4.73 ± 0.27^{a}
SM	0.478	0.639	0.424	0.335	0.445	1.30 ± 0.08	1.38 ± 0.15	1.14 ± 0.14	1.00 ± 0.09	1.35 ± 0.12
CerG1	0.0433	0.0542	0.0388	0.0354	0.0557	0.0046 ± 0.0008	0.0065 ± 0.0026	0.0067 ± 0.0021	0.0041 ± 0.0009	0.0077 ± 0.0011
LPE	0.0390	0.0458	0.0359	0.0240	0.0322	0.0224 ± 0.0025	0.0218 ± 0.0010	0.0179 ± 0.0023	0.0180 ± 0.0014	0.0195 ± 0.0024
So	0.0360	0.0708	0.0356	0.0226	0.0470	0.0514 ± 0.0101^{b}	0.0401 ± 0.0094^{ab}	0.0310 ± 0.0052^{ab}	0.0241 ± 0.0040^{a}	0.0258 ± 0.0026^{a}
Cer	0.0369	0.0438	0.0281	0.0228	0.0415	0.0170 ± 0.0014^{b}	0.0156 ± 0.0011^{b}	0.0128 ± 0.0014^{ab}	0.0104 ± 0.0008^{a}	0.0173 ± 0.0011^{b}
CO	nd	nd	nd	nd	nd	0.0333 ± 0.0050^{ab}	0.0558 ± 0.0122^{b}	0.0288 ± 0.0047^{ab}	0.0162 ± 0.0025^{a}	0.0279 ± 0.0048^{ab}
PI	nd	nd	nd	nd	nd	0.0211 ± 0.0039	0.0247 ± 0.0086	0.0229 ± 0.0051	0.0140 ± 0.0021	0.0116 ± 0.0021
PMe	0.0105	0.0441	0.0107	0.0128	0.0317	nd	nd	nd	nd	nd
PA	0.0079	0.0090	0.0070	0.0002	0.0081	nd	nd	nd	nd	nd
PG	0.0061	0.0107	0.0729	0.162	0.0178	0.0000	0.0000	0.0000	0.0000	0.0000
MGDG	0.0004	0.0008	0.0004	0.0005	0.0003	nd	nd	nd	nd	nd

TG: triglycerides, PC: phosphatidylcholines, DG: diacylglycerols, LPC: lysophosphatidylcholines, PE: phosphatidylethanolamines, SM: sphingomyelins, CerG1: simple G1c series, LPE: lysophosphatidylethanolamines, So: sphingoshines, Cer: ceramides, Co: coenzyme, PI: phosphatidylinositols, PMe: phosphatidylmethanol, PA: phosphatidic acid, PG: phosphatidylglycerol, MGDG: monogalactosyldiacylglycerol. nd: not detected; Values are means ± SEM ($n = 4$) and values with a different letter are significantly different ($P < 0.05$).

3.4. The distribution of key fatty acids in TG, PC and PE of Nile tilapia muscle

To further investigate the distribution of seven key FAs in different lipid molecules, the percentages of TG, PC and PE molecules containing different FAs were calculated and compared. Generally, the percentage of TG molecules containing a specific FA was positively correlated with the content of the corresponding FA in the dietary lipids (Fig. 4A). However, Fig. 1 shows that OO intake did not result in a high accumulation of 18:1n-9. Similarly, OO intake did not cause a specific increase in TG molecules containing 18:1n-9, again indicating that OO could be easily metabolized in TG (Fig. 4A). However, in PE, OO oils significantly increased the percentages of PE molecules containing 18:1n-9 (Fig. 4C). In the muscle of Nile tilapia fed on the PO diet, the accumulation of molecules containing 16:0 and 18:1n-9 was similar in TG (Fig. 4A), but the percentages of the molecules containing 16:0 were higher than those containing 18:1n-9 in PC or PE (Fig. 4B and C), which agreed with the results in Fig. 1A. This indicated that the utilization of FA in TG and PL is different because 16:0 and 18:1n-9 in TG are more easily metabolized than those in PL. TG is the main source of lipid mobilization rather than PL, thus 16:0 and 18:1n-9 in TG would be preferable substrates in FA metabolism. Compared with the other oils, FO intake significantly increased the percentage of TG and PC molecules containing EPA or DHA by > 2 fold (Fig. 4A, B). This suggests that, compared with other FAs, EPA and DHA were preferably deposited. As described earlier, in the calculation of values in Fig. 4, one glycerol-lipid molecule containing three, two or one specific FA was treated as one. Therefore, when the amount of a specific FA is relatively fixed, a higher percentage of lipid molecules containing that FA will lead to it being more widely distributed in more lipid molecules. Therefore, compared with the other groups, the significantly greater amount of TG and PC molecules containing EPA or DHA in the FO group indicated that EPA and DHA were more widely distributed in the TG and PC molecules. Considering the essential functions of EPA and DHA in the functioning of fish cells, this wider distribution could be a basic biological mechanism, a worthwhile topic for further study. DHA was specifically deposited in PE (Fig. 4C), much more than in TG and PC. This agreed with previous studies in mammals that DHA is initially incorporated into PE with lower amounts into PC and that PE is the primary lipid species for DHA deposition (Robinson, Xu, Knoell, Tateno, & Olesiak, 1993; Zerouga, Stillwell, Stone, Powner, & Jenski, 1996). Of note, PE is the second most abundant phospholipid in cells, particularly enriched in mitochondrial inner membranes. In addition to contributing to membrane structure, PE plays important roles in modulating mitochondrial morphology, membrane curvature and fusion (Vance & Tasseva, 2013). It has been reported that the fatty acid type of the acyl

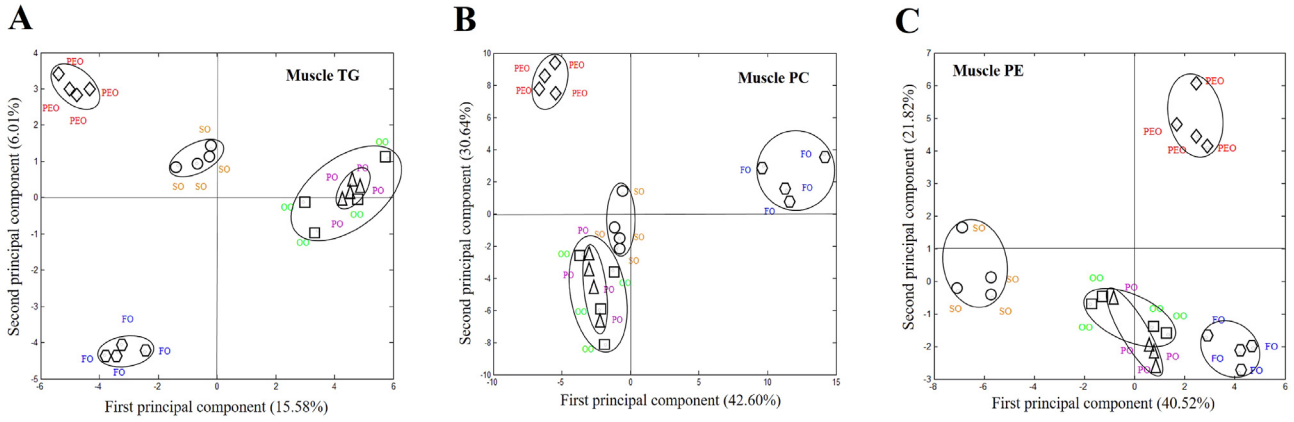


Fig. 2. Principal component analysis (PCA) of the composition of all TG (A), PC (B) and PE (C) species in the fillets of Nile tilapia fed with different dietary oils. △, □, ○, ◊, and ⬡, represent PO, OO, SO, PEO and FO, respectively ($n = 4$).

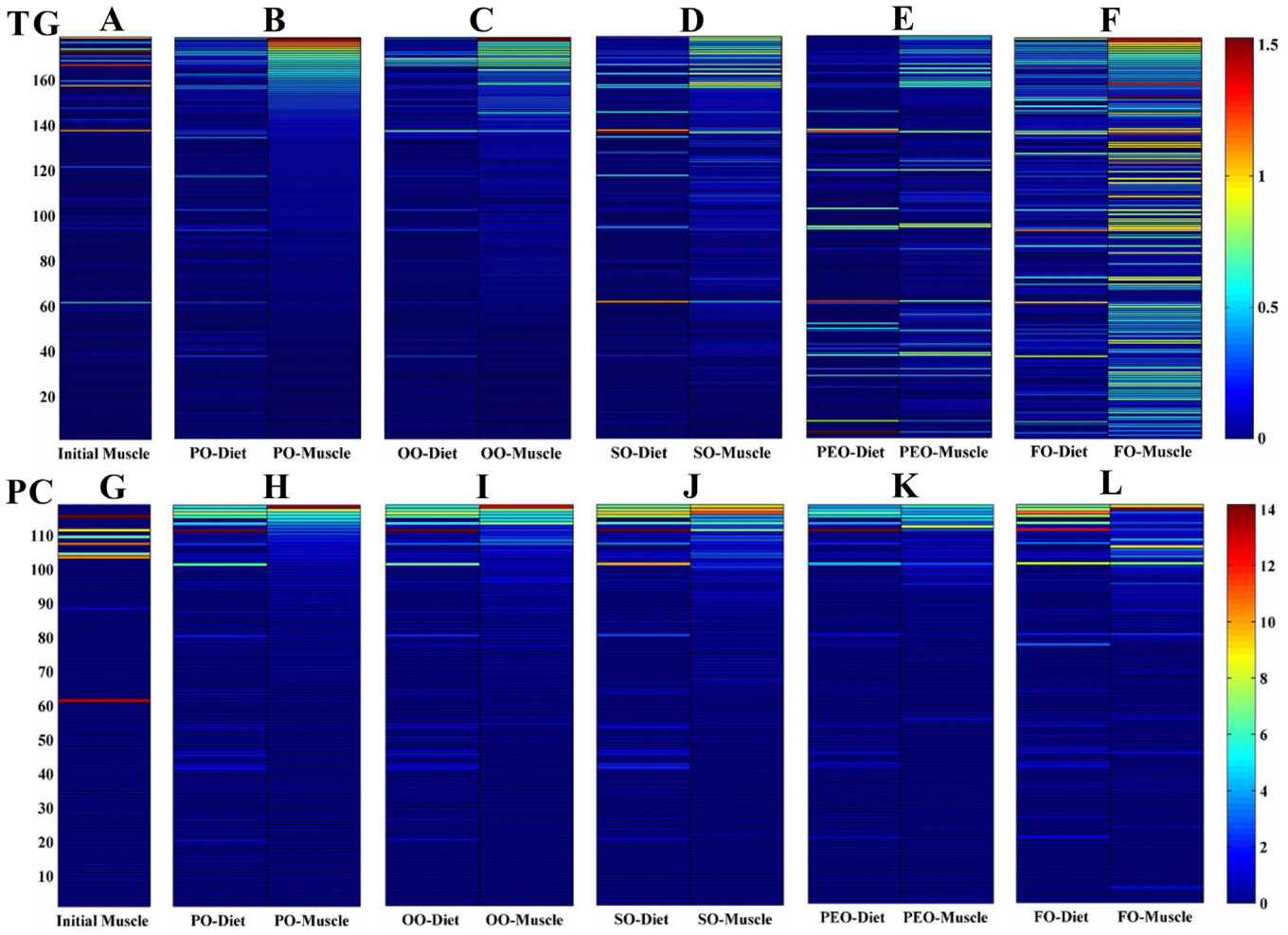


Fig. 3. Clustering heatmap of identified TG (A, B, C, D, E, F) and PC (G, H, I, J, K, L) molecules in initial fish fillets, diets and the corresponding Nile tilapia fillets after dietary feeding. The color of each section is proportional to the content of the identified lipid molecule. The detailed values are listed in Supplementary Table 5.

chains in PE strongly implies unique or specialized roles for PE (Zerouga et al., 1996). In the present study, the preferential incorporation of DHA into PE could contribute to greater stability of phospholipid bilayer in cell membrane. Additionally, DHA in DHA-containing PE could force transfer of cholesterol from the inner leaflet to the outer layer, in order to keep membrane molecular in the right order (Zerouga et al., 1996). Therefore, the specific deposition of DHA in PE could be an important premise for the physiological functions of PE in fish.

3.5. The positional distribution of key fatty acids in TG and PL lipid molecules

The positional distribution of fatty acids in lipid molecules affects the nutritional values of the lipid and also its utilization in metabolism (Kubow, 1996). Therefore, the positional distribution of the seven key FAs in the TG, PC and PE molecules of the Nile tilapia fillets fed with different oils was analyzed comprehensively using the lipidomics assay. Fig. 5 shows the results of each FA positional distribution. The percentage of TG molecules containing 16:0 at the sn-1 and sn-3 positions in the fish muscles was significantly higher than that at the sn-2 position in all the five groups. The same trend was also observed for the PC and PE molecules containing 16:0, with an even lower percentage at the sn-2 position. This indicated that in Nile tilapia muscle, 16:0 was distributed preferentially at the outer positions of the glycerol backbone in all lipid classes (sn-1/3 positions of the TG molecule and sn-1 position of the PL molecule), irrespective of dietary oil sources. This contrasts with the bovine milk fat and lard, which normally contain 16:0 at the sn-2 position (Kubow, 1996). Data from humans has shown that FA are conserved at the sn-2 position throughout the process of digestion and absorption, therefore an excess intake of lipid containing 16:0 at the sn-2 position may increase the risk of atherogenesis. In contrast, FA at the sn-1/3 positions is preferred for pancreatic lipase, which is very easily lipolyzed (Mattson & Volfenhein, 1962). Therefore, the specifically high accumulation of 16:0 in the outer position of the lipid molecules in Nile tilapia muscle indicates its high mobilization potential in the metabolism of Nile tilapia. This also suggests a low risk of atherogenesis for humans after consuming Nile tilapia fillets.

The distribution of 18:1n-9 at the sn-1,3 or sn-2 positions in TG molecules was similar, although some significant differences were detected. For PC molecules, the percentage of those containing 18:1n-9 at the sn-2 position tended to be greater than those at the sn-1,3 positions, especially in the PO and OO groups. However, in PE, a significantly higher amount of 18:1n-9 was specifically deposited at the sn-1 position in all groups. This indicated that the positional distribution of 18:1n-9 in different lipid molecules was affected by dietary oils and also varied between the different lipid classes. However, the biological mechanisms underlying the different distributions of 18:1n-9 in the various lipid classes are still unknown. Considering that 18:1n-9 is considered as a "healthy" FA in human nutrition (Grundy, 1989) and has a low atherogenic risk, the effects of different distributions of 18:1n-9 on nutrient physiology need further investigation.

The muscle of Nile tilapia has the specific deposition of 18:2n-6 and a high ratio of 18:2n-6/18:3n-3 FA as a nutritional characteristic

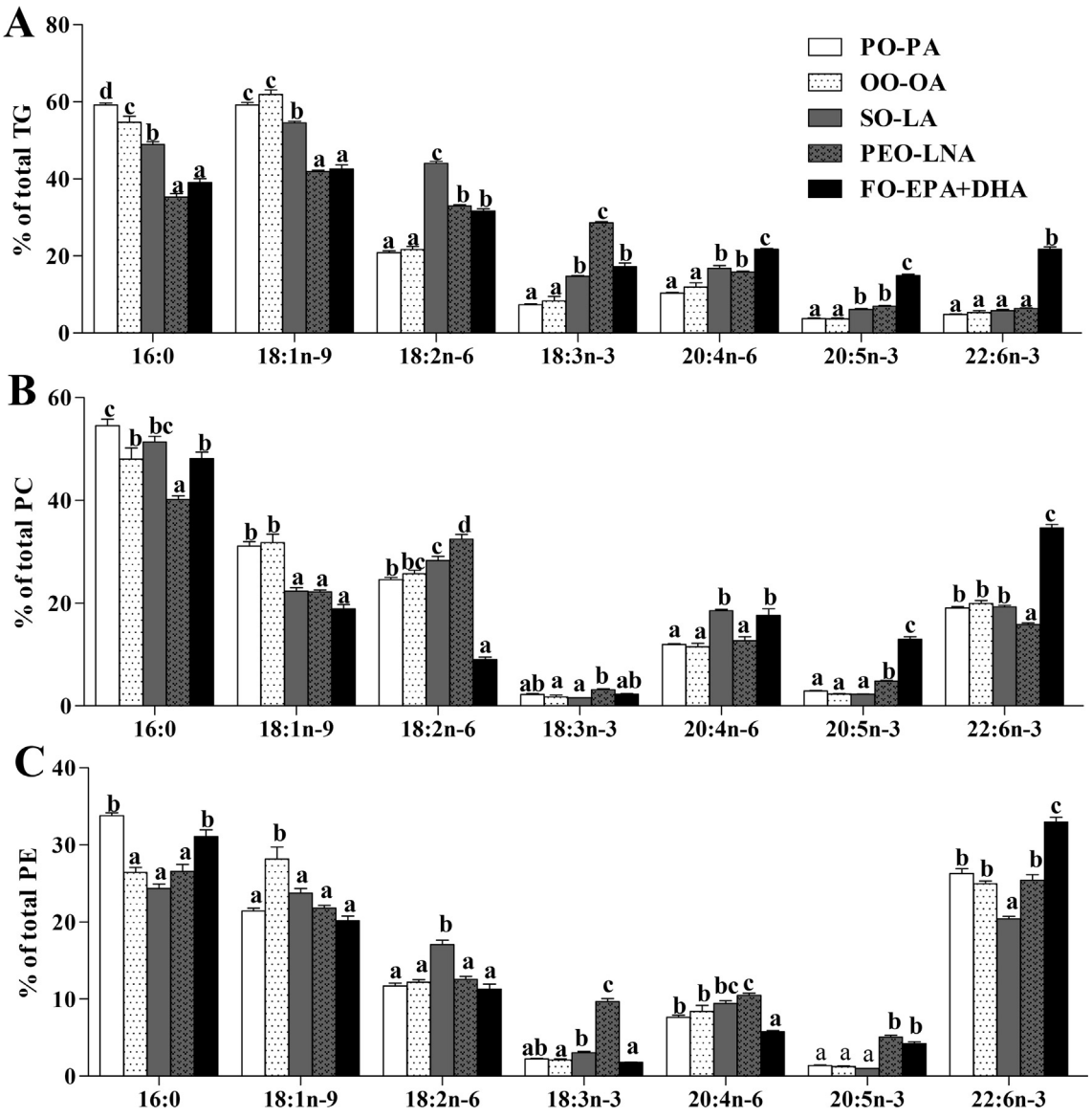


Fig. 4. The distribution of 16:0, 18:1n-9, 18:2n-6, 18:3n-3, 20:4n-6, 20:5n-3 and 22:6n-3 in the TG (A), PC (B) and PE (C) molecules in Nile tilapia fillets. Values are means ± SEM ($n = 4$). Values with different letters are significantly different ($P < 0.05$).

compared with most other fish species (Ferreira et al., 2011; Izquierdo et al., 2005; Ruizlopez et al., 2015). However, the positional distribution of 18:2n-6 in Nile tilapia lipids has not been investigated before. The present study is the first to demonstrate that the positional distribution of 18:2n-6 in TG was affected by dietary oils (Fig. 5C). Moreover, in the PO, OO and SO groups, more 18:2n-6 was found at the sn-1,3 position, but for Nile tilapia fed with oils enriched with n-3 PUFAs (PEO and FO), more 18:2n-6 was deposited at the sn-2 position. Regarding nutritional aspects, 18:2n-6 at the sn-1/3 positions could be more easily released from TG in the fish fillets into the blood circulation system and might increase the risks of inflammation (Naughton, Mathai, Hryciw, & Mcainch, 2016). Therefore, decreasing the amount of 18:2n-6 at the sn-1/3 positions could benefit for human health. However, for PC and PE, 18:2n-6 was specifically deposited at the sn-2 position irrespective of the type of dietary oils. In PC, almost no 18:2n-6 was detected at the sn-1 position. This could be an important reason why Nile tilapia muscle preferably accumulates 18:2n-6, and we also showed PLs are the main lipid class for 18:2n-6 deposition in this fish. As FA at the sn-2 position is relatively stable compared with that at the sn-1/3 positions, and although Nile tilapia muscle specifically accumulates high amounts of 18:2n-6 at the sn-2 position of PL molecules, this might not pose a high risk of inflammation to humans. Fig. 5C shows that FO intake largely reduced the amount of PC molecules containing 18:2n-6 at the sn-2 position, thus showing that feeding Nile tilapia with FO could significantly change the lipid characteristics of the muscle.

Compared with 18:2n-6, the positional distribution of 18:3n-3 was more regular (Fig. 5D). In the TG and PE molecules, 18:3n-3 was specifically deposited at the outer position (sn-1/3 positions in TG and sn-1 position in PE), while in PC molecules, more 18:3n-3 was observed at the sn-2 position. The percentage of TG molecules containing 18:3n-3 was much greater than that of the corresponding PC and PE molecules. In Salmon muscle, higher concentrations of 18:3n-3 tended to accumulate at the sn-2 position (Ruizlopez et al., 2015). This did not agree with the results of the present study and could be related to the different fish species. Given that 18:3n-3 has been shown to play anti-inflammatory roles in humans (Zhao et al., 2004), its specific accumulation at the sn-1 position of TG in Nile tilapia muscle probably indicates that these fillets are a good source of 18:3n-3 for human consumption.

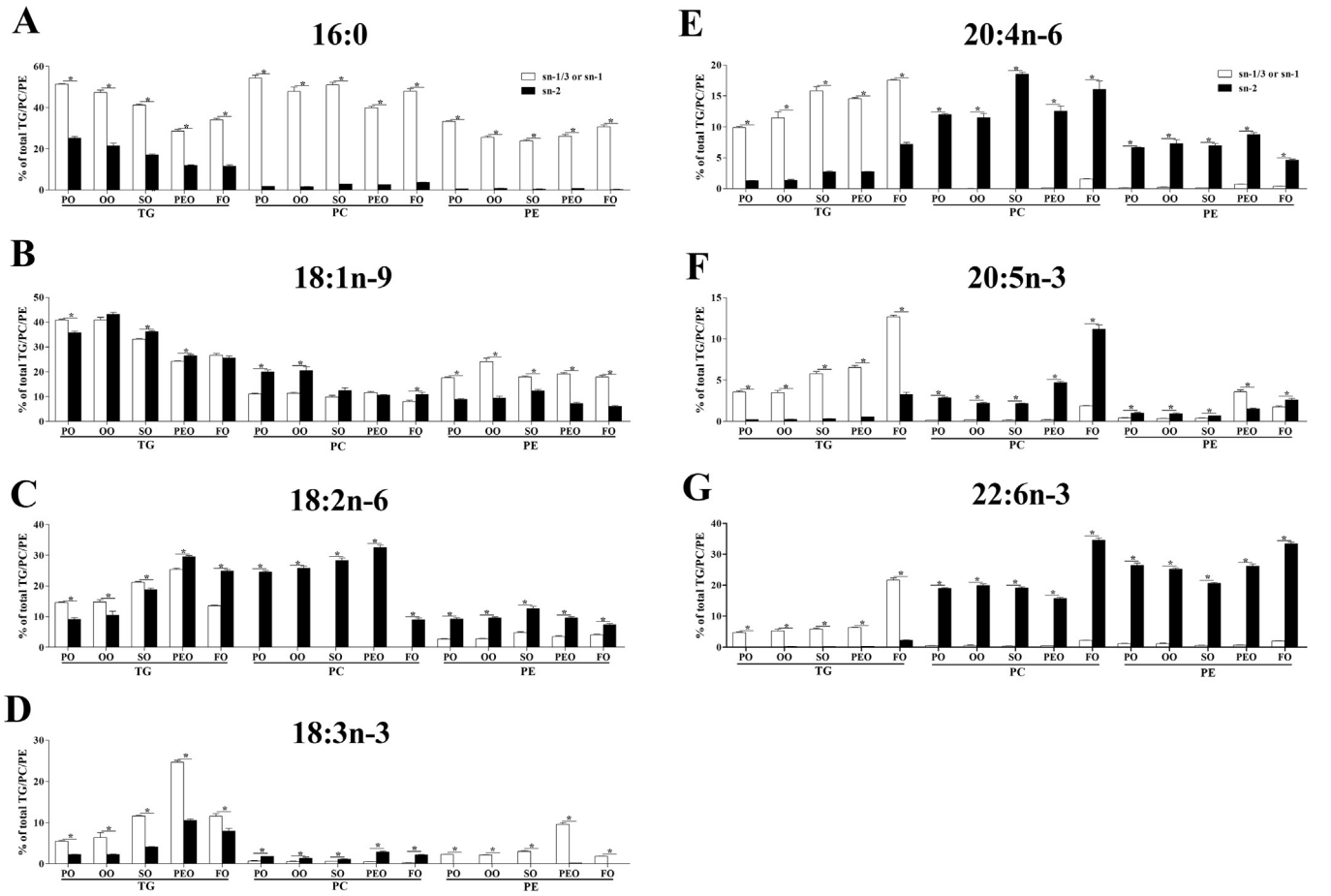


Fig. 5. The positional distribution of 16:0 (A), 18:1n-9 (B), 18:2n-6 (C), 18:3n-3 (D), 20:4n-6 (E), 20:5n-3 (F) and 22:6n-3 (G) in all the TG and PL molecules in Nile tilapia fillets. Values are means ± SEM ($n = 4$). Values with "*" are significantly different between outer position (sn-1/3 in TG or sn-1 in PL) and the inner position (sn-2) ($P < 0.05$).

The positional distribution of VLCUFAs (20:4n-6, 20:5n-3 (EPA) and 22:6n-3 (DHA)) in TG, PC and PE molecules was relatively similar. All three VLCUFAs were preferably deposited at the sn-1/3 positions in TG, and at the sn-2 position in PC and PE, except for the PEO group, where more EPA was deposited at the sn-1 position than at the sn-2 position (Fig. 5F). This mostly agrees with the common generalization that PUFAs are preferentially esterified at the sn-2 position of PL (Ruizlopez et al., 2015; Tocher, Bendiksen, Campbell, & Bell, 2008). In particular, a regiospecific analysis of TG in salmon muscle has shown that dietary DHA bound at the sn-2 position was better retained than DHA bound at the sn-1/3 positions (Ruizlopez et al., 2015). However, seal oil contains mostly EPA and DHA at the sn1/3-positions, in contrast to most fish oils where EPA and DHA accumulate at the sn-2 position (Aursand et al., 1995; Christensen et al., 1995). Therefore, seal oil is regarded as having higher anti-inflammatory effects than fish oil because the EPA and DHA at the sn1/3 positions (seal oil) is more easily released than that at the sn-2 position (fish oil). The positional distribution of VLCUFAs in Nile tilapia muscle could also allow these FAs to be relatively easily released when the fillets are consumed. Fig. 5F and G show that fish oil intake (FO group) generally increased EPA and DHA in TG and PC molecules, thus confirming that dietary fish oil can significantly improve the nutritional value of Nile tilapia fillets. In particular, in fish fed the PEO diet, the amount of PE species containing EPA at the sn-1 position was significantly higher than that at the sn-2 position. Thus it could be hypothesized that the new EPA derived from 18:3n-3 in Nile tilapia fed the PEO diet preferentially esterified at the sn-1 position in PE. The percentages of lipids containing DHA were far greater than those containing EPA, suggesting that DHA is more resistant to lipolysis and tends to be retained in fish fillets, agreeing with the observations in Fig. 1 and Fig. 4.

4. Conclusions

This deep lipidomics analysis of Nile tilapia fed with different oils has mainly indicated that: 1) 16:0 and 18:1n-9 could be used as good substrate sources for metabolism in Nile tilapia; 2) dietary PUFA-enriched oil largely affects the composition of lipid molecules in Nile tilapia fillets; 3) the specifically high accumulation of 18:2n-6 at the sn-2 position in PL and low 18:3n-3 accumulation in PL will provide a high ratio of 18:2n-6/18:3n-3, a nutritional characteristic of Nile tilapia lipids; and 4) the intake of dietary VLCUFAs intake significantly increases its deposition at the sn-1/3 positions in TG and the sn-2 position in PC, and decreases 18:2n-6 at the sn-2 position in PL: this improves the nutritional value of Nile tilapia fillets. To the best of our knowledge, this is the first study reporting how dietary oils can modify the lipid molecules and nutritional value in fish muscle. This study provides novel information to evaluate the nutritional value of Nile tilapia fillets from human health aspects.

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Conflict of interest

All authors declare that there are no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2018.11.020.

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