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Metabolic fate of DHA from regio- and stereospecific positions of triacylglycerols in a long-term feeding trial in rats

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ABSTRACT

This study investigated the impact of regio- and stereospecific position of docosahexaenoic acid (DHA) in dietary triacylglycerols (TAGs) on the fatty acid composition of tissues and organs in rats. Four-week feeding with TAGs containing DHA in *sn*-1, 2, or 3 position and palmitic acid in the remaining positions at a daily dosage of 500 mg TAG/kg body weight significantly increased the DHA content in all organs and tissues in rats, except in the brain, where the change in DHA level was not statistically significant. The group fed *sn*-1 DHA showed a significantly higher content of DHA in the plasma TAG than the group fed *sn*-3 DHA. The *sn*-3 DHA group had higher levels of DHA in the visceral fat compared to the *sn*-1, *sn*-2, as well as all other groups. This is the first study showing that DHA from *sn*-1 and *sn*-3 positions of dietary TAGs have differential accumulation in tissues. The new findings improved the current knowledge on the significance of TAG isomeric structure for the bioavailability and metabolic fate of DHA.

1. Introduction

The importance of dietary n-3 long-chain polyunsaturated fatty acids (PUFA) is well documented, including beneficial effects on cardiovascular health (Innes & Calder, 2020), brain development (Petrova et al., 2019; Yamagata, 2017), anti-inflammatory effects (Dawczynski et al., 2018), muscle recovery (Ochi & Tsuchiya, 2018), cancers (Ortea et al., 2018), and mental health (Innes & Calder, 2020). In the human brain and eyes, docosahexaenoic acid (DHA; 22:6*n*-3) is the most abundant n-3 PUFA and plays an important role in cognitive function (Weiser et al., 2016).

The human body is not able to synthesize n-3 PUFA *de novo*. However, *in vivo* conversion of α-linolenic acid (ALA; 18:3*n*-3) through elongation and desaturation to eicosapentaenoic acid (EPA; 20:5*n*-3) can occur to some extent, and small amounts of DHA can be biosynthesized, but this bioconversion rate is very low and influenced by many factors. Thus, DHA can be considered an essential component of a healthy and balanced diet for humans. However, the content of DHA in the diet is low globally (Micha et al., 2014), therefore, it is also common to take food supplements to obtain DHA needed for many biological functions.

The bioavailability of n-3 FAs is affected by different factors such as general health status and other individual factors (Li et al., 2021). Also, chemical forms of n-3 PUFAs affect their uptake; free fatty acids (FFA) have the highest bioavailability, followed by TAG, whereas n-3 PUFA in ethyl esters (EE) is the least bioavailable among the three forms (Cuenoud et al., 2020). Furthermore, the food matrix and especially the presence or absence of other components can affect the bioavailability. It has been shown that the absorption of EPA from fish oil TAGs was significantly improved when co-ingested with a high-fat meal (Lawson & Hughes, 1988). In natural marine oils, DHA is mainly present in TAG form. Recently our group reported that DHA was more oxidatively stable in TAGs than in EEs without α-tocopherol addition. However, with α-tocopherol added the opposite was observed (Ahonen et al., 2022).

In addition to the chemical binding form, the stereospecific position of n-3 long-chain PUFA on the glycerol backbone affects the metabolism.

Abbreviations: ALA, 18:3 (n-3); ARA, 20:4 (n-6); DTA, 22:4 (n-6); DPA, 22:5 (n-3); DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; FAME, fatty acid methyl esters; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TAG, triacylglycerol.

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TAGs undergo enzymatic hydrolysis in the digestive tract, where several stereospecific enzymes contribute to the degradation of TAGs with different degrees of efficiency. Gastric lipase has a strong preference for the *sn*-3 position (Mackie et al. 2020), which releases medium and long-chain FAs in the stomach. Subsequently, the long-chain FAs released in the stomach may influence the activity of pancreatic lipases (Bernback et al., 1990). Many studies have confirmed that pancreatic lipases prioritize cleaving the FAs esterified on the *sn*-1/3 position. This leads to inefficient absorption of cleaved saturated FAs from the primary positions because of the formation of insoluble calcium salts. In contrast, the *sn*-2 monoacylglycerols are absorbed more efficiently (Hunter, 2001). In fish oil, DHA and EPA are located mainly on the *sn*-2 position but in seal oil mainly on the *sn*-1/3 positions (Lee-Chang et al., 2021). Some research has shown that seal oil is more effective than fish oil (a mixture of tuna and sardine oil) in reducing plasma and liver TAG concentrations and arachidonic acid content in liver phospholipids (Ikeda et al., 1998; Yoshida et al., 1996). Yoshinaga et al. (2015) found that dietary EPA esterified in the *sn*-1/3 positions of dietary TAGs is more efficiently retained in liver and epididymis fat than EPA esterified in the *sn*-2 position. The cholesterol-lowering effect was found to be stronger when the DHA was esterified on the *sn*-2 position of TAG. The result of a 12-week hamster feeding trial showed that the value of total n-3 and total PUFA in the brain was the highest in the group fed with structured TAG containing *sn*-2 DHA, compared with the levels in the groups fed with linseed oil, fish oil (commercial fish oil, regardless the *sn*-position of DHA), or fish oil ethyl esters (Bandarra et al., 2016).

Globally DHA intake is low despite its vital role in human physiology and health. To understand the bioavailability of dietary DHA, it is important to not only study the n-3 PUFA levels in the blood but also comprehensively investigate the n-3 PUFA levels in different tissues and organs. Up to now, very little is known about the *in vivo* metabolism of dietary DHA from stereo- and regiospecific TAGs. Previously, our group conducted a short-term (5-day) feeding trial on mildly n-3 FA deficient rats to compare the bioavailability of DHA from enantiopure TAGs and their regioisomeric counterparts (Linderborg et al., 2019). The results indicated that DHA from the middle (*sn*-2) position had a slightly higher bioavailability than those from the *sn*-1/3 positions, but there was no significant difference between the two primary positions (*sn*-1 vs. *sn*-3) (Linderborg et al., 2019). In this study, we aimed to investigate the impact of the regio- and stereospecific position of DHA in dietary TAGs on the bioavailability of this important n-3 PUFA in a long-term feeding trial. The regio- and enantiopure TAGs were synthesized containing DHA located in *sn*-1, 2, or 3 positions, respectively, and palmitic acid esterified in the remaining positions. Before the test feeding the rats were fed with a normal n-3 adequate diet. During the test feeding, the rats received an n-3 deficient diet + DHA containing TAG. Three groups fed with normal n-3 adequate feed, n-3 deficient feed, or n-3 deficient feed with added tripalmitin were included for comparison. The tripalmitin group was included considering the potential impact of additional fat intake and the intake of palmitic acid on DHA absorption and distribution. Based on the literature, dietary intake of long-chain saturated fatty acids such as stearic acid (18:0) and palmitic acid (16:0) may not only influences the absorption efficiency of dietary fat but also has metabolic impacts (Bergstedt et al., 1991; Innis & Dyer, 1997; Lin et al., 2017). The FA composition in plasma, liver, brain, and visceral fat was analyzed at the end of the 4-week feeding period. Even though the metabolism of PUFA is different between humans and rats, rats have been widely considered a useful model for studying DHA metabolism due to their experimental tractability and the possibility of analyzing various tissue samples for the in-depth understanding of the distribution and accumulation of DHA in different organs and tissues. Another important rationale behind using rats was to understand the fundamental metabolism and bioavailability of DHA without the complexities present in humans, such as lifestyle and food intake. To our belief, this is the first published long-term feeding study to investigate the bioavailability of DHA in different tissues and organs using regio- and enantiopure dietary TAGs. The study complements and extends to our earlier short-term feeding trial (Linderborg et al., 2019). The findings of this study improve the current understanding of the impact of the molecular structure of TAGs on the bioavailability and metabolic fate of DHA, an important n-3 PUFA.

2. Material and methods

2.1. Chemicals and reagents

HPLC grade solvents, chloroform, methanol, n-hexane, and diethyl ether were purchased from Merck (Merck Oy, Espoo, Finland). An Elga Purelab Ultra water purification system (Elga LabWater, Woodrige, IL, USA) was used for purifying the water. The acetyl chloride and potassium chloride were bought from Sigma-Aldrich Finland (Helsinki, Finland). The internal standards triheptadecanoin and dinonadecanoylphosphatidylcholine were bought from Larodan AB (Solna, Sweden). The external standards Supelco 37 Component FAME Mix was bought from Supelco (St. Louis, MO, USA) and GLC-566c was from Nu-Check-Prep (Elysian, MN, USA). All the references were at least 98 % purity or higher.

2.2. Synthesis of regio- and enantiopure structured triacylglycerols

DHA (≥95 %) was obtained as an ethyl ester from Pronova Bio-Pharma (Sandefjord, Norway) and hydrolyzed to a free acid (Haraldsson et al., 2000). The structured TAGs were synthesized in three different ways. The TAG with the DHA located in the middle (*sn*-2) position was synthesized based on a two-step chemo-enzymatic route from glycerol involving a highly regioselective immobilized *Candida antarctica* lipase. Pure DHA was introduced to the *sn*-2 position by chemical coupling (Halldorsson et al., 2003). The enantiopure TAG with DHA located in the primary positions (*sn*-1 or *sn*-3) of the glycerol backbone was synthesized in a five-step chemo-enzymatic process from enantiopure (R)- and (S)- solketals as chiral precursors, which was described in detail by Kalpio et al. (2020). Tripalmitin was synthesized according to a previously published method (Haraldsson et al., 2000), by treating the mixture of glycerol and palmitic acid under vacuum conditions at 70–75 °C. With the aid of the immobilized *C. antarctica* lipase, after 48 h the pure product was obtained. All intermediate products and final products were obtained in conditions of high chemical and regioisomeric/enantiomeric purity as characterized by traditional synthetic organic chemistry methods including ^{1}H (400 MHz), ^{13}C NMR, and IR spectrometry analysis, as well as satisfactory high-resolution accurate mass spectrometry. Optical activity data were obtained for all chiral intermediates and products, and excellent enantiomeric purity was established for the DHA-TAG products (Kalpio et al. 2020). The TAGs were stored under nitrogen at −85 °C before use.

2.3. Ethical approval

The protocol of the animal experiment was approved by the Medical Ethics Research Board of the Health Science Centre of Peking University, China (LA2021291).

2.4. Animals and diets

Seventy-two male Sprague-Dawley rats (age 21 ± 2 days) (Beijing Vital River Laboratory Animal Technology Co., Ltd.) were kept for 7 days in isolation at a constant temperature and humidity and on an adaptive feeding of the standard AIN-93G diet (Beijing BioPike Biotechnology Co., Ltd.) (Table 1) which contained soybean oil as a source of (n-3) FA. The environment temperature was 20 °C–23 °C, and the humidity was 50 %–55 %. The rats were individually kept in stainless steel metabolic cages with free access to food and water. After 7 days of adaptive feeding on the standard AIN-93G diet, the rats were

Table 1
Composition of standard AIN-93G diets.

Ingredients	Contents (g/kg)
Corn starch	397
Casein (>85 % protein)	200
Corn dextrin (90–94 % tetrose)	132
Sucrose	100
Oil	70
Fiber	50
Minerals	35.5
Vitamins	10
L-cystinol	3
Choline bitartrate (42 % choline)	2.5
Tert-butylhydroquinone	0.014

randomly divided into six experimental groups, with 12 animals in each group (Table 2). The first group, the normally fed group, referred to as the Control Group, received a standard AIN-93G diet containing soybean oil as a source of n-3 FA. The second group, the n-3 FA deficient group, was fed the AIN-93G diet where soybean oil was replaced by peanut oil naturally low in n-3 FA (Table 3). The other four groups received as the experimental fat tripalmitin, 2,3-dipalmitoyl-1-docosahexaenoyl-*sn*-glycerol (*sn*-1 DHA), 1,3-dipalmitoyl-2-docosahexaenoyl-*sn*-glycerol (*sn*-2 DHA), or 1,2-dipalmitoyl-3-docosahexaenoyl-*sn*-glycerol (*sn*-3 DHA). The last three groups are also referred to as DHA groups.

In Groups 3–6 (Table 2), each rat received a daily dosage of the structured TAG of 500 mg /kg body weight, and the intervention feeding lasted for four weeks. The dosage used in this study was determined based on the dosage used in previous studies (Kaur et al., 2010; Linderborg et al., 2019). The dosage used in Linderborg et al. (2019) was more than 1000 mg/kg body weight and Kaur et al. (2010) used a dosage of around 500–735 mg/kg body weight in a rat trial.

The rats were weighed at the beginning of each week, and the accurate daily dosage was determined weekly based on the weights of the rats.

Each week, the individual doses were divided and stored in nitrogen at −80 °C. α-Tocopherol (100 mg/100 g) was added to the experimental fat before dividing it into individual doses. The experimental fats were melted using a water bath at 40 °C and were embedded in two halves of the feed pellet. A fresh pellet was prepared every night before the feeding and stored in the dark at 4 °C. This pellet was the first food given to the rat every day and was consumed completely before the rest of the feed was given. After consumption of test fat, the rats were provided with food and water *ad libitum*.

2.5. Sample collection

The rats were weighed at the end of the adaptive feeding (baseline) and throughout the dietary intervention at the beginning of each week. At the end of the feeding trial, the rats were sedated by inhaling isoflurane and sacrificed with exsanguination in a fasting state. The heart blood was collected and centrifuged (Zonkia, Hefei, China) for 10 min at 1000 *g* to obtain plasma samples. The organs of the rats including their liver, brain, and visceral fat were collected and weighed. The plasma samples and organs were stored at −80 °C before use.

Table 2
Experiment groups and feeding treatments.

Group	Group name	Treatment feed
1	Normal feed group	AIN-93G[1]
2	n-3 FA deficiency group	AIN-93G containing peanut oil[2]
3	Tripalmitin group	Tripalmitin + AIN-93G containing peanut oil
4	*sn*-1 DHA group	*sn*-1- DHA + AIN-93G containing peanut oil
5	*sn*-2 DHA group	*sn*-2- DHA + AIN-93G containing peanut oil
6	*sn*-3 DHA group	*sn*-3- DHA + AIN-93G containing peanut oil

[1] The standard AIN-93G diet contains soybean oil as a fat resource.
[2] Peanut oil which is naturally deficient in n-3 FA was used to make an n-3 FA deficiency group.

Table 3
Fatty acid composition (% of total fatty acid) of peanut and soybean oils used in the feed.

Fatty acid	Soybean oil	Peanut oil
16:0	10.40	9.61
18:0	4.46	3.54
18:1(n-9)	22.95	49.75
18:2(n-6)	53.30	31.29
18:3(n-3)	7.50	0.11
20:0	0.40	1.24
22:0	0.40	2.39
24:0	0.12	1.04
Others[1]	0.47	1.03

The values are expressed as mean mass percentages of the two replicates.
[1] This category includes 14:0, 16:1(n-7), 20:1(n-9), and 24:1(n-9).

2.6. Lipid extraction and fractionation

Total lipids were extracted from the plasma, liver, brain, and visceral fat with a modified Folch method (Folch et al., 1957). The tissue samples were homogenized with the bead beating technology by using a Tissuelyzer II with the adapter set 2 × 24 (Qiagen, Germany). The beads used were 3 mm steel beads. The internal standards triheptadecanoin and dinonadecanoyl-phosphatidylcholine were added after homogenization. Then, 2 mL MeOH, 2.5 mL $CHCl_3$, and 0.8 mL 0.88 % KCl were added to the sample. The sample was vortexed for 30 s after each addition. After this, the sample was centrifuged (Eppendorf, Hamburg, Germany) at 1000 *g* for 5 min. The lower phase containing the chloroform phase was collected. Another 4 mL $CHCl_3$: MeOH (84:16) was added to the upper phase and vortexed for 30 s. After centrifugation, the lower phase was collected and combined with the former extract. The total lipid sample was evaporated to dryness under a nitrogen flow at 40 °C and dissolved in $CHCl_3$.

The plasma and liver lipids were separated as neutral and polar-rich lipids fractions by solid-phase extraction as described in Fabritius et al., (2020). Based on the method development, the neutral fraction contained mainly TAGs and the polar fraction was mainly PLs. The Sep-Pak Vac silica 1 cc (100 mg) columns or 3 cc (200 mg) columns (Waters, Dublin, Ireland) were conditioned with 6 mL hexane: diethyl ether (1:1). The TAG fraction was eluted with hexane: diethyl ether (1:1), and the PL fraction was eluted with MeOH: $CHCl_3$: H_2O (5:3:2). Both fractions were evaporated to dryness under a nitrogen flow at 40 °C. The TAG and the PL fraction were dissolved in hexane and $CHCl_3$: MeOH (2:1).

2.7. Preparation of fatty acid methyl esters and chromatographic analysis

FA methyl esters (FAME) were formed by the acid-catalyzed method (Christie & Han, 2010). Two mL of acetyl chloride: MeOH (1:10) was added to the lipid sample. After vigorous shaking, the sample was kept at 50 °C overnight to complete the reaction. After cooling down, 2 mL of 1 mol/L K_2CO_3 and 1 mL of hexane were added, and the sample was shaken briefly and centrifuged for 5 min at 1000 *g*. The top layer was collected. The samples were stored in a freezer until analysis with gas chromatography.

The FAMEs were analyzed with a Shimadzu GC-2030 gas chromatograph equipped with an AOC-20i auto-injector and flame ionization detector (Shimadzu Corporation, Kyoto, Japan). The column used was an open tubular DB-23, 50 % cyanopropyl, 50 % methylpolysiloxane, column (60 m × 0.25 mm i.d., liquid film 0.25 μm, Agilent technologies, J.W. Scientific, Santa Clara, CA, USA). The injection mode was splitless and the split was opened 1 min after injection. The injection volume was 0.5 μL. The injection temperature and detector temperatures were 270 °C and 280 °C, respectively. The column oven

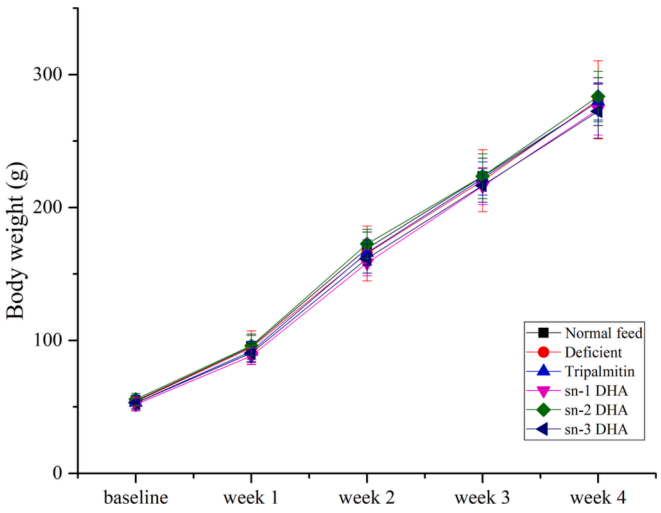

Fig. 1. Bodyweight of the rats before and during the intervention.

temperature was programmed to hold at 130 °C for 1 min, then to rise by 6.5 °C/min to 170 °C, then 2.75 °C/min to 205 °C, which was held for 18 min. Finally, a 30 °C/min decrease was made to 230 °C and held for 2 min. The fatty acids were identified by analysis of FAME 37 and GLC-566c standard mixtures using the same GC-FID method as described for the sample analysis. The quantification of the fatty acids was performed using the internal standard method, with correction factors determined by analysis of fatty acid standard mixtures FAME 37 and GLC-566c.

2.8. Statistical methods

A total of 70 plasma samples out of the initial 72 were included in the FA analysis. In the control group, an insufficient quantity of plasma was obtained from one rat, and another rat in the *sn*-1 group with an extremely low lipid level was excluded (determined as > 2SD outside the mean). The data analysis was performed with IBM SPSS statistics 25.0 software (IBM, Armonk, NY, USA). All data were checked for normality and variance homogeneity and are reported as a mean ± standard deviation (SD). The significance between experimental treatments was tested by a one-way ANOVA followed by Turkey's HSD test. Tamhane's T2 analysis was used when the variance was not homogenous. Statistical significance was considered when the $p < 0.05$.

3. Results and discussion

3.1. Bodyweight and organ weight

The body weights and weights of organs of the rats before (baseline) and during (weeks 1–4) of the intervention phase are presented in Fig. 1. With no difference in the initial body weight among the groups, the weight of the rats increased five to six-fold during the intervention. The rate of body weight gain was the highest during week 2 when the average weight gain was around 70 g. The body weight showed no significant differences between any of the groups because the rats were provided with food and water *ad libitum* after consuming the experimental oil every day. The weight of the fresh organs, such as the heart and lungs, did not show any statistically significant difference between any of the groups (Table 4). The brain weight of the *sn*-2 DHA group had a higher average value compared to the other groups, and the liver weight of the n-3 deficient group had a higher value than the other groups. However, the differences among the groups did not reach statistical significance. These differences were consistent with the results of the brain and liver lipids content presented below.

The dosage of DHA containing TAG in this study was 500 mg/kg body weight of rats. According to the body surface area (BSA) normalization method (Reagan-Shaw et al., 2008), the corresponding human equivalent dosage is 1.6 g DHA per day for a 60 kg adult which is below the highest recommended intake of the Food and Agriculture Organization (FAO, 2010). Besides, many DHA-related human clinical trials used similar or even higher DHA dosages than the human equivalent dosage in this study. For example, Vidgren et al., (1997) used the dosage of 1.68 g DHA per day in TAG form for young healthy males and found that the DHA influenced the linoleic acid metabolism. In Armah's research, 25 healthy males received a fish oil meal which provided 3.2 g DHA per day (Armah et al., 2008), the results indicate DHA in meal can improve postprandial vascular reactivity. Chong et al., (2010) used the dosage of 2.7 g DHA on each meal for healthy men and women to study the effects of n-3 PUFA rich meal on arterial stiffness. The results showed the n-3 PUFA consumption can reduce postprandial arterial stiffness.

3.2. Plasma fatty acid composition

Table 5A and Table 5B show the FA composition of rat plasma TAG (Table 5A) and PL (Table 5B) fractions. The n-3 deficient and the tripalmitin groups showed significantly lower levels of n-3 PUFAs. EPA was not detected in the n-3 deficient and tripalmitin groups in either the TAG or the PL fractions in the plasma. The 22:5(n-3) was not detected in the TAG fractions in the n-3 deficient and tripalmitin groups. Feeding DHA significantly increased the DHA content in three DHA groups both in the TAG and PL fractions of the plasma compared to the tripalmitin and the n-3 deficient groups, as well as the n-3 adequate control group. In the PL fraction of the three DHA groups and the *sn*-1 and *sn*-2 groups of the TAG fraction, the DHA levels were significantly higher than the levels in the normal feed group. Although the *sn*-3 group showed a higher DHA level than the normal feed group, the difference was not significant ($p = 0.592$). Comparing the three groups receiving DHA from the regio- and stereospecific positions of the structured TAGs, the proportion of DHA in plasma TAG of the *sn*-1 group was significantly higher than the value of the *sn*-3 group. Similarly, in a short-term feeding study conducted by our group (Linderborg et al., 2019) the value of DHA in *sn*-1, 2, and 3 groups showed the same trend, the proportion of DHA in the *sn*-3 group being the lowest in both TAG and PL fractions. This might indicate that DHA is less bioavailable from the *sn*-3 than in the *sn*-1 position. Further investigation using prolonged intervention is necessary

Table 4
Weight of rat organs after the intervention phase.

Organs	Groups					
	Normal feed	Deficient	Tripalmitin	*sn*-1 DHA	*sn*-2 DHA	*sn*-3 DHA
Brain (g)	1.67 ± 0.17	1.70 ± 0.19	1.75 ± 0.14	1.74 ± 0.17	1.85 ± 0.15	1.82 ± 0.15
Heart (g)	1.12 ± 0.06	1.16 ± 0.15	1.16 ± 0.14	1.12 ± 0.18	1.11 ± 0.08	1.11 ± 0.16
Lung (g)	1.41 ± 0.25	1.33 ± 0.26	1.39 ± 0.10	1.38 ± 0.14	1.29 ± 0.16	1.30 ± 0.11
Liver (g)	14.84 ± 1.66	15.76 ± 2.11	14.79 ± 1.70	14.24 ± 1.46	14.9 ± 1.30	14.35 ± 1.77
Spleen (g)	0.73 ± 0.11	0.68 ± 0.14	0.76 ± 0.15	0.76 ± 0.11	0.75 ± 0.11	0.76 ± 0.10
Kidney (g)	2.54 ± 0.20	2.44 ± 0.27	2.64 ± 0.23	2.52 ± 0.27	2.41 ± 0.14	2.45 ± 0.20
Testis (g)	2.63 ± 0.16	2.56 ± 0.23	2.61 ± 0.17	2.58 ± 0.19	2.68 ± 0.16	2.54 ± 0.20

Table 5A
The proportion of fatty acids (weight percentage of total fatty acids) in the rat plasma TAG fraction of the different intervention groups.

Fatty acids	Normal feed	Deficiency	Tripalmitin	*sn*-1 DHA	*sn*-2 DHA	*sn*-3 DHA
14:0	0.85 ± 0.23	0.80 ± 0.18	0.86 ± 0.14	0.79 ± 0.24	0.80 ± 0.15	0.78 ± 0.17
15:0	0.15 ± 0.03	0.16 ± 0.02	0.17 ± 0.08	0.17 ± 0.04	0.15 ± 0.03	0.16 ± 0.02
15:1	1.28 ± 0.85	0.81 ± 0.56	1.56 ± 0.84	1.32 ± 0.83	1.24 ± 0.50	1.32 ± 0.60
16:0	20.7 ± 2.56^{b}	23.48 ± 2.67^{a}	21.6 ± 1.94^{ab}	21.29 ± 1.48^{ab}	22.28 ± 1.88^{ab}	21.8 ± 1.71^{ab}
16:1(n-7)	2.69 ± 0.80	3.23 ± 1.12	2.39 ± 0.95	2.38 ± 0.86	2.95 ± 1.02	2.69 ± 1.06
18:0	6.22 ± 0.64	5.51 ± 1.19	6.42 ± 1.65	5.77 ± 0.88	6.01 ± 0.75	6.12 ± 1.06
18:1(n-9)	15.69 ± 2.28^{b}	27.40 ± 3.57^{a}	23.13 ± 4.86^{a}	25.9 ± 3.14^{a}	27.16 ± 3.01^{a}	25.45 ± 3.11^{a}
18:1(n-7)	2.35 ± 0.48	2.61 ± 0.57	2.28 ± 0.82	2.53 ± 0.66	2.87 ± 0.76	2.68 ± 0.74
18:2(n-6)	20.36 ± 2.30^{a}	13.67 ± 1.69^{bc}	12.27 ± 1.45^{c}	14.96 ± 1.07^{b}	13.12 ± 1.11^{bc}	13.71 ± 1.68^{bc}
18:3(n-6)	0.51 ± 0.09	0.47 ± 0.07	0.53 ± 0.17	0.46 ± 0.07	0.50 ± 0.05	0.52 ± 0.09
18:3(n-3)	1.55 ± 0.30^{a}	0.09 ± 0.10^{b}	0.07 ± 0.03^{b}	0.07 ± 0.01^{b}	0.05 ± 0.01^{b}	0.06 ± 0.01^{b}
20:0	0.12 ± 0.05^{b}	0.22 ± 0.07^{a}	0.17 ± 0.03^{ab}	0.15 ± 0.04^{b}	0.16 ± 0.06^{ab}	0.18 ± 0.05^{ab}
20:1(n-9)	0.20 ± 0.04^{c}	0.40 ± 0.04^{a}	0.31 ± 0.09^{b}	0.37 ± 0.08^{ab}	0.38 ± 0.06^{ab}	0.36 ± 0.05^{ab}
20:2(n-6)	0.33 ± 0.07^{ab}	0.40 ± 0.08^{ab}	0.41 ± 0.11^{a}	0.31 ± 0.08^{b}	0.31 ± 0.06^{ab}	0.32 ± 0.10^{ab}
20:3(n-6)	0.51 ± 0.12^{a}	0.31 ± 0.07^{b}	0.32 ± 0.07^{b}	0.47 ± 0.03^{a}	0.49 ± 0.09^{a}	0.44 ± 0.07^{a}
20:4(n-6)	22.75 ± 5.79^{ab}	18.49 ± 4.23^{b}	25.12 ± 5.55^{a}	18.96 ± 3.55^{b}	17.75 ± 3.58^{b}	19.86 ± 3.98^{ab}
20:3(n-3)	0.07 ± 0.02^{ab}	0.05 ± 0.01^{bc}	0.08 ± 0.03^{a}	0.06 ± 0.01^{bc}	0.04 ± 0.01^{c}	0.06 ± 0.02^{abc}
20:5(n-3)	0.66 ± 0.16^{a}	nd[1]	nd	0.22 ± 0.08^{b}	0.23 ± 0.09^{b}	0.21 ± 0.11^{b}
22:0	0.06 ± 0.02^{c}	0.16 ± 0.05^{a}	0.11 ± 0.03^{b}	0.13 ± 0.03^{ab}	0.13 ± 0.06^{ab}	0.15 ± 0.02^{ab}
22:2(n-6)	0.06 ± 0.01^{ab}	0.05 ± 0.02^{b}	0.08 ± 0.04^{a}	0.06 ± 0.01^{ab}	0.05 ± 0.01^{b}	0.06 ± 0.01^{ab}
22:4(n-6)	0.35 ± 0.08^{a}	0.29 ± 0.15^{ab}	0.34 ± 0.21^{a}	0.19 ± 0.04^{b}	0.17 ± 0.06^{b}	0.17 ± 0.04^{b}
22:5(n-6)	0.24 ± 0.05^{c}	0.92 ± 0.42^{b}	1.25 ± 0.37^{a}	0.15 ± 0.06^{c}	0.15 ± 0.05^{c}	0.17 ± 0.07^{c}
22:5(n-3)	0.26 ± 0.08^{a}	nd	nd	0.08 ± 0.04^{b}	0.07 ± 0.03^{b}	0.06 ± 0.05^{b}
24:0	0.16 ± 0.07^{b}	0.29 ± 0.08^{a}	0.28 ± 0.08^{a}	0.25 ± 0.07^{ab}	0.26 ± 0.09^{a}	0.30 ± 0.07^{a}
22:6(n-3)	1.87 ± 0.48^{c}	0.19 ± 0.06^{d}	0.23 ± 0.09^{d}	2.96 ± 0.73^{a}	2.65 ± 0.52^{ab}	2.37 ± 0.57^{bc}
total SFA	28.27 ± 2.36	30.62 ± 3.18	29.62 ± 2.51	28.55 ± 1.10	29.80 ± 1.45	29.49 ± 1.95
total MUFA	22.21 ± 2.98^{b}	34.46 ± 4.47^{a}	29.68 ± 5.99^{a}	32.50 ± 3.52^{a}	34.61 ± 3.50^{a}	32.50 ± 4.04^{a}
total PUFA	49.52 ± 5.05^{a}	34.92 ± 5.37^{b}	40.70 ± 5.44^{b}	39.12 ± 3.76^{b}	35.78 ± 4.35^{b}	38.25 ± 4.31^{b}
total n-3	4.41 ± 0.60^{a}	0.33 ± 0.15^{c}	0.37 ± 0.13^{c}	3.39 ± 0.83^{b}	3.05 ± 0.60^{b}	2.76 ± 0.71^{b}
total n-6	45.11 ± 4.90^{a}	34.59 ± 5.24^{bc}	40.32 ± 5.33^{ab}	35.57 ± 3.72^{bc}	32.54 ± 4.16^{c}	35.25 ± 3.81^{bc}

Values are mean ± SD, n = 12 except for the normal feed group (n = 11) and *sn*-1 DHA group (n = 11). Values with different superscripts in the same row differ statistically significantly ($p < 0.05$).
[1] Not detected.

to confirm the findings. There was no significant difference between *sn*-1, 2, and 3 groups in the plasma levels of any other FAs. The palmitic acid content of the tripalmitin group did not show a significant difference compared to the other groups and the additional intake of palmitic acid in the DHA groups did not show a significant impact on the FA composition of the tissue and organs studied as compared to the normal feed and deficiency groups. This is likely due to the low absorption efficiency of palmitic acid from the *sn*-1/3 positions (Hunter, 2001) or the highly effective counterbalance of palmitic acid by regulation of endogenous biosynthesis via *de novo* lipogenesis (Song et al., 2017).

The 18:3(n-3) (ALA) and 18:2(n-6) (LA) contents in the TAG fraction were significantly higher in the normal feed group than in the other groups. The high value of ALA can explain the significantly high content of DHA and EPA in the normal feed group compared with the deficiency and tripalmitin groups because of the endogenous conversion of ALA to DHA and EPA (Domenichiello et al., 2017; Neff et al., 2011). The high content of LA and ALA in soybean oil provides a logical explanation for the high content of EPA in the normal feed group as compared to the DHA groups. The higher level of ALA in the control group also reflected the FA profile of the oil used in the feed with a clear difference between soybean and peanut oil (Table 3). This resulted in significant differences in the percentage of 18:1(n-9), LA, and ALA in plasma TAGs between the normal feed group and the other groups. The long-chain SFA did not show an obvious difference except 22:0 in the TAG fraction of the plasma. Considering the low levels of n-3 PUFA in peanut oil, the EPA content in the DHA groups may have been partly attributed to the *retro*-conversion of DHA via a minor metabolic pathway (Vossen et al., 2017).

In plasma PLs, the 20:4(n-6) (ARA) is the most abundant PUFA. The level of ARA was the highest in the tripalmitin group, which showed statistically significant differences compared to the n-3 deficient group in the TAG fraction and the *sn*-1/*sn*-2 groups in both the TAG and the PL fractions of the plasma. In the PL fraction, feeding with n-3 deficient feed resulted in a 10-fold increase in the levels of 22:5(n-6) [DPA(n-6)] in the deficiency and the tripalmitin groups (4.55 and 5.57 % respectively, of total FAs) compared to the normal feed and the DHA groups (0.48–0.63 %). The proportion of 22:4(n-6) was increased by around 2-fold in the PL fraction of the deficiency and tripalmitin groups fractions compared to other groups. The increase in these n-6 PUFAs may have been the result of a compensatory response to the n-3 deficient diet to maintain the fluidity of cell membranes and the pool of PUFAs as the precursors of eicosanoids as mediators of the inflammation process (Igarashi et al., 2012).

3.3. Liver fatty acid composition

There was no significant difference in the total lipid content of TAG fractions between any groups. However, in the PL fraction, the deficiency group showed the lowest value of total lipid content and there was a significant difference between the *sn*-2 and *sn*-3 groups (data not shown). Table 6 shows the FA composition of rat liver in lipids TAG (Table 6A) and PL (Table 6B) fractions. Four-week feeding with n-3 deficient feed significantly decreased the percentage of all the n-3 PUFAs in the n-3 deficiency and the tripalmitin groups compared to the normal feed group. Feeding DHA increased the proportion of EPA and DHA in both the TAG and PL fractions of the liver lipids compared to the levels in the deficiency and tripalmitin groups, but there was no significant difference between the three DHA groups in the content of DHA or any other FAs. In the TAG fraction, the DHA level in the *sn*-1 group is significantly higher than in the normal feed group, whereas no statistically significant difference was found between the *sn*-2 or *sn*-3 DHA groups and the normal feed group. This was consistent with our findings in the plasma lipid TAG showing that the level of DHA in the plasma lipid TAG was the highest in the *sn*-1 group among the three DHA-fed groups. In the PL fraction, the percentages of EPA and DHA in the three DHA groups were all significantly higher than the levels in the normal feed group. In the study conducted by Yoshinaga (Yoshinaga

Table 5B
The proportion of fatty acids (weight percentage of total fatty acids) in the rat plasma PL fraction of the different intervention groups.

Fatty acids	Normal feed	Deficiency	Tripalmitin	*sn*-1 DHA	*sn*-2 DHA	*sn*-3 DHA
14:0	0.35 ± 0.04^{a}	0.32 ± 0.03^{ab}	0.32 ± 0.03^{ab}	0.28 ± 0.04^{b}	0.29 ± 0.05^{b}	0.29 ± 0.06^{b}
16:0	24.21 ± 1.54	23.98 ± 1.42	24.97 ± 1.56	23.97 ± 1.36	24.08 ± 1.11	24.13 ± 1.63
16:1(n-7)	0.51 ± 0.12	0.60 ± 0.17	0.53 ± 0.14	0.49 ± 0.17	0.64 ± 0.19	0.55 ± 0.20
18:0	23.62 ± 1.41	22.94 ± 1.67	22.53 ± 1.66	22.49 ± 1.19	22.23 ± 1.96	22.01 ± 1.33
18:1(n-9)	4.28 ± 0.43^{b}	7.42 ± 0.66^{a}	7.42 ± 0.78^{a}	7.02 ± 0.47^{a}	7.40 ± 0.48^{a}	7.09 ± 0.52^{a}
18:1(n-7)	1.92 ± 0.40	1.71 ± 0.32	1.95 ± 0.57	2.00 ± 0.31	2.19 ± 0.38	2.07 ± 0.40
18:2(n-6)	16.27 ± 1.92^{a}	14.99 ± 1.11^{ab}	12.65 ± 2.16^{c}	14.46 ± 1.17^{abc}	13.48 ± 1.25^{bc}	13.93 ± 1.40^{bc}
20:0	0.15 ± 0.02^{b}	0.18 ± 0.02^{ab}	0.19 ± 0.03^{a}	0.17 ± 0.02^{ab}	0.16 ± 0.03^{ab}	0.17 ± 0.02^{ab}
20:1(n-9)	0.13 ± 0.03^{b}	0.22 ± 0.06^{a}	0.26 ± 0.06^{a}	0.27 ± 0.05^{a}	0.25 ± 0.05^{a}	0.23 ± 0.04^{a}
20:2(n-6)	0.42 ± 0.06^{b}	0.51 ± 0.08^{ab}	0.54 ± 0.11^{a}	0.56 ± 0.08^{a}	0.59 ± 0.09^{a}	0.58 ± 0.07^{a}
20:3(n-6)	1.24 ± 0.40^{bc}	1.23 ± 0.35^{c}	1.04 ± 0.35^{c}	1.71 ± 0.22^{a}	2.00 ± 0.40^{a}	1.66 ± 0.21^{ab}
20:4(n-6)	16.97 ± 2.00^{ab}	16.98 ± 1.06^{ab}	17.41 ± 2.21^{a}	15.13 ± 1.14^{b}	15.24 ± 1.21^{b}	16.27 ± 1.58^{ab}
20:5(n-3)	0.22 ± 0.06^{a}	0.08 ± 0.01^{c}	0.10 ± 0.02^{bc}	0.13 ± 0.05^{bc}	0.14 ± 0.04^{b}	0.12 ± 0.04^{bc}
22:0	0.25 ± 0.02^{c}	0.31 ± 0.06^{ab}	0.34 ± 0.06^{a}	0.31 ± 0.03^{ab}	0.28 ± 0.04^{bc}	0.28 ± 0.04^{bc}
22:2(n-6)	0.28 ± 0.04^{a}	0.13 ± 0.03^{b}	0.15 ± 0.05^{b}	0.13 ± 0.03^{b}	0.13 ± 0.02^{b}	0.12 ± 0.01^{b}
22:4(n-6)	0.39 ± 0.04^{b}	0.70 ± 0.05^{a}	0.71 ± 0.11^{a}	0.25 ± 0.03^{c}	0.28 ± 0.02^{c}	0.25 ± 0.05^{c}
22:5(n-6)	0.63 ± 0.11^{c}	4.55 ± 0.68^{b}	5.57 ± 0.77^{a}	0.48 ± 0.12^{c}	0.54 ± 0.12^{c}	0.63 ± 0.33^{c}
22:5(n-3)	0.45 ± 0.09^{a}	0.10 ± 0.03^{b}	0.09 ± 0.02^{b}	0.12 ± 0.03^{b}	0.13 ± 0.04^{b}	0.12 ± 0.03^{b}
24:0	0.93 ± 0.15^{c}	1.17 ± 0.22^{ab}	1.32 ± 0.23^{a}	1.16 ± 0.11^{ab}	1.05 ± 0.16^{bc}	1.07 ± 0.15^{bc}
22:6(n-3)	5.75 ± 0.68^{b}	1.17 ± 0.21^{c}	1.13 ± 0.19^{c}	8.01 ± 0.71^{a}	8.20 ± 0.93^{a}	7.72 ± 0.83^{a}
24:1(n-9)	0.72 ± 0.08^{a}	0.46 ± 0.09^{b}	0.50 ± 0.11^{b}	0.54 ± 0.09^{b}	0.44 ± 0.11^{b}	0.45 ± 0.07^{b}
total SFA	49.67 ± 1.28^{ab}	49.02 ± 0.69^{ab}	49.81 ± 1.93^{a}	48.54 ± 1.04^{ab}	48.22 ± 1.44^{ab}	48.08 ± 1.16^{b}
total MUFA	7.73 ± 0.60^{b}	10.54 ± 0.84^{a}	10.79 ± 0.96^{a}	10.46 ± 0.77^{a}	11.04 ± 0.82^{a}	10.52 ± 0.83^{a}
total PUFA	43.09 ± 0.93^{a}	41.50 ± 0.71^{bc}	40.63 ± 1.17^{c}	42.03 ± 0.82^{ab}	41.65 ± 0.95^{bc}	42.35 ± 1.20^{ab}
total n-3	6.43 ± 0.62^{b}	1.35 ± 0.23^{c}	1.32 ± 0.20^{c}	8.26 ± 0.72^{a}	8.48 ± 0.95^{a}	7.96 ± 0.83^{a}
total n-6	36.18 ± 0.80^{b}	39.08 ± 0.81^{a}	38.07 ± 1.24^{a}	32.73 ± 0.85^{c}	32.26 ± 1.04^{c}	33.43 ± 1.05^{c}

Values are mean ± SD, n = 12 except for the normal feed group (n = 11) and *sn*-1 DHA group (n = 11). Values marked with different superscripts in the same row differ statistically significantly ($p < 0.05$).

et al., 2015), they compared the effects of TAG positional distribution of DHA in mice; the results showed that feeding DHA located in the *sn*-2 position of TAG resulted in a higher accumulation of DHA in the liver, but there was no significant difference between the *sn*-1 and *sn*-3 positions. Ikeda (Ikeda et al., 1998) compared the effects of seal oil (DHA preferentially in *sn*-1/*sn*-3 position) and squid oil (DHA predominantly in *sn*-2 position) on rats, reporting no significant difference between the seal oil and squid oil groups in the level of DHA in the phosphatidylcholine and phosphatidylethanolamine fractions in the liver. However, in liver TAG, the proportion of DHA in the squid oil group was significantly higher than the value of the seal oil group.

The FA composition of the liver lipids closely reflects the FA profiles of the diets. This phenomenon is known in mono-gastric animals (Dugan et al., 2015). The values of 18:1(n-9), 22:0, and 24:0 in the normal feed group were significantly lower than the values of the other groups, where the values of 18:2(n-6) and ALA were significantly higher. Similar

Table 6A
The proportion of fatty acids (weight percentage of total fatty acids) in the rat liver TAG fraction of different intervention groups.

Fatty acids	Normal feed	Deficiency	Tripalmitin	*sn*-1 DHA	*sn*-2 DHA	*sn*-3 DHA
14:0	1.07 ± 0.27	1.08 ± 0.22	0.89 ± 0.10	0.91 ± 0.15	0.94 ± 0.13	0.87 ± 0.12
16:0	31.83 ± 4.06	34.29 ± 4.25	31.20 ± 1.86	30.53 ± 2.72	32.47 ± 2.35	31.57 ± 2.67
16:1(n-7)	4.66 ± 1.73	4.91 ± 1.45	4.01 ± 1.13	3.69 ± 1.35	4.51 ± 0.81	4.39 ± 1.43
18:0	3.35 ± 0.29	3.18 ± 0.40	3.25 ± 0.41	3.72 ± 0.45	3.69 ± 0.50	3.22 ± 0.38
18:1(n-9)	28.73 ± 1.77^{b}	40.53 ± 2.65^{a}	42.41 ± 1.33^{a}	40.6 ± 2.04^{a}	40.98 ± 1.97^{a}	41.46 ± 1.38^{a}
18:1(n-7)	4.32 ± 0.48	3.84 ± 0.72	4.28 ± 0.81	3.81 ± 0.76	3.93 ± 0.54	4.13 ± 0.74
18:2(n-6)	19.11 ± 5.09^{a}	8.29 ± 2.70^{b}	9.17 ± 2.03^{b}	10.60 ± 2.56^{b}	8.31 ± 1.57^{b}	9.56 ± 2.44^{b}
18:3(n-6)	0.80 ± 0.33^{a}	0.51 ± 0.12^{b}	0.61 ± 0.15^{ab}	0.63 ± 0.18^{ab}	0.56 ± 0.17^{ab}	0.56 ± 0.11^{ab}
18:3(n-3)	1.28 ± 0.36^{a}	0.06 ± 0.08^{b}	0.04 ± 0.01^{b}	0.06 ± 0.03^{b}	0.05 ± 0.02^{b}	0.05 ± 0.01^{b}
20:0	0.06 ± 0.05	0.06 ± 0.01	0.07 ± 0.03	0.07 ± 0.02	0.07 ± 0.02	0.06 ± 0.01
20:1(n-9)	0.21 ± 0.06^{b}	0.22 ± 0.08^{ab}	0.29 ± 0.08^{ab}	0.33 ± 0.10^{a}	0.24 ± 0.07^{ab}	0.26 ± 0.08^{ab}
20:2(n-6)	0.32 ± 0.12^{a}	0.20 ± 0.10^{b}	0.24 ± 0.06^{ab}	0.23 ± 0.08^{ab}	0.17 ± 0.05^{b}	0.18 ± 0.05^{b}
20:3(n-6)	0.24 ± 0.08^{a}	0.12 ± 0.05^{c}	0.12 ± 0.02^{c}	0.21 ± 0.06^{ab}	0.19 ± 0.06^{abc}	0.16 ± 0.04^{bc}
20:4(n-6)	2.32 ± 0.58^{ab}	1.92 ± 0.58^{b}	2.43 ± 0.37^{ab}	2.72 ± 0.55^{a}	2.39 ± 0.66^{ab}	2.19 ± 0.45^{ab}
20:5(n-3)	0.12 ± 0.05^{a}	nd[1]	nd	0.05 ± 0.04^{b}	0.05 ± 0.02^{b}	0.04 ± 0.03^{b}
22:0	0.03 ± 0.01^{b}	0.05 ± 0.02^{a}	0.06 ± 0.03^{a}	0.05 ± 0.01^{a}	0.05 ± 0.02^{a}	0.05 ± 0.02^{a}
22:2(n-6)	0.03 ± 0.02	0.03 ± 0.02	0.04 ± 0.02	0.04 ± 0.03	0.07 ± 0.04	0.05 ± 0.03
22:4(n-6)	0.31 ± 0.16^{a}	0.17 ± 0.15^{b}	0.19 ± 0.07^{ab}	0.13 ± 0.06^{b}	0.09 ± 0.04^{b}	0.10 ± 0.03^{b}
22:5(n-6)	0.17 ± 0.09^{b}	0.41 ± 0.24^{a}	0.55 ± 0.20^{a}	0.08 ± 0.04^{b}	0.08 ± 0.06^{b}	0.07 ± 0.02^{b}
22:5(n-3)	0.19 ± 0.08^{a}	nd	nd	0.07 ± 0.05^{b}	0.04 ± 0.02^{b}	0.04 ± 0.01^{b}
24:0	0.03 ± 0.01^{b}	0.06 ± 0.02^{a}	0.07 ± 0.03^{a}	0.06 ± 0.01^{a}	0.06 ± 0.02^{a}	0.06 ± 0.01^{a}
22:6(n-3)	0.81 ± 0.32^{b}	0.07 ± 0.03^{c}	0.08 ± 0.01^{c}	1.41 ± 0.59^{a}	1.09 ± 0.45^{ab}	0.96 ± 0.30^{ab}
total SFA	36.37 ± 4.36	38.72 ± 4.53	35.53 ± 1.79	35.34 ± 2.59	37.28 ± 2.20	35.83 ± 2.57
total MUFA	37.92 ± 2.96^{b}	49.50 ± 1.99^{a}	50.98 ± 1.38^{a}	48.43 ± 2.37^{a}	49.66 ± 1.58^{a}	50.24 ± 1.31^{a}
total PUFA	25.55 ± 6.83^{a}	11.85 ± 3.83^{b}	13.55 ± 2.61^{b}	16.22 ± 3.94^{b}	13.09 ± 2.68^{b}	13.96 ± 3.18^{b}
total n-3	2.39 ± 0.78^{a}	0.14 ± 0.10^{c}	0.12 ± 0.02^{c}	1.59 ± 0.67^{b}	1.22 ± 0.48^{b}	1.08 ± 0.34^{b}
total n-6	23.31 ± 6.16^{a}	11.65 ± 3.75^{b}	13.36 ± 2.57^{b}	14.64 ± 3.40^{b}	11.85 ± 2.25^{b}	12.86 ± 2.92^{b}

Values are mean ± SD, n = 12. Values with different superscripts in the same row differ significantly ($p < 0.05$).
[1] Not detected.

Table 6B
The proportion of fatty acids (weight percentage of total fatty acids) in the rat liver PL fraction of different intervention groups.

Fatty acids	Normal feed	Deficiency	Tripalmitin	*sn*-1 DHA	*sn*-2 DHA	*sn*-3 DHA
14:0	0.19 ± 0.05^{ab}	0.22 ± 0.06^{a}	0.17 ± 0.04^{ab}	0.16 ± 0.03^{b}	0.18 ± 0.03^{ab}	0.18 ± 0.04^{ab}
16:0	16.84 ± 1.03	17.07 ± 1.23	17.04 ± 0.90	17.42 ± 0.74	17.14 ± 1.08	17.57 ± 0.67
16:1(n-7)	0.83 ± 0.27	1.05 ± 0.33	0.79 ± 0.24	0.73 ± 0.25	0.91 ± 0.19	0.86 ± 0.30
18:0	22.97 ± 1.27^{a}	23.15 ± 1.36^{a}	22.35 ± 1.26^{ab}	21.67 ± 0.76^{ab}	21.71 ± 1.42^{ab}	21.31 ± 0.85^{b}
18:1(n-9)	3.30 ± 0.39^{b}	5.62 ± 0.41^{a}	5.70 ± 0.35^{a}	5.32 ± 0.29^{a}	5.49 ± 0.26^{a}	5.49 ± 0.30^{a}
18:1(n-7)	2.74 ± 0.42	2.66 ± 0.42	3.05 ± 0.63	2.77 ± 0.47	3.11 ± 0.39	2.98 ± 0.48
18:2(n-6)	11.27 ± 1.26^{a}	8.82 ± 0.55^{c}	7.98 ± 1.11^{c}	10.1 ± 0.89^{ab}	9.06 ± 0.79^{bc}	9.22 ± 0.97^{bc}
18:3(n-3)	0.12 ± 0.02^{a}	0.02 ± 0.01^{b}	0.02 ± 0.00^{b}	0.02 ± 0.01^{b}	0.02 ± 0.01^{b}	0.02 ± 0.00^{b}
20:0	0.09 ± 0.01^{b}	0.11 ± 0.02^{a}	0.12 ± 0.02^{a}	0.11 ± 0.01^{a}	0.11 ± 0.02^{a}	0.11 ± 0.02^{a}
20:1(n-9)	0.12 ± 0.02^{c}	0.19 ± 0.04^{b}	0.24 ± 0.04^{a}	0.25 ± 0.04^{a}	0.24 ± 0.03^{a}	0.24 ± 0.03^{a}
20:2(n-6)	0.51 ± 0.06^{b}	0.63 ± 0.08^{a}	0.66 ± 0.06^{a}	0.64 ± 0.09^{a}	0.64 ± 0.07^{a}	0.64 ± 0.06^{a}
20:3(n-6)	1.21 ± 0.30^{c}	1.29 ± 0.29^{bc}	1.03 ± 0.23^{c}	1.65 ± 0.17^{a}	1.70 ± 0.21^{a}	1.57 ± 0.19^{ab}
20:4(n-6)	26.55 ± 1.68^{a}	26.97 ± 1.31^{a}	27.7 ± 1.33^{a}	23.15 ± 0.70^{b}	23.58 ± 1.38^{b}	24.10 ± 1.11^{b}
20:5(n-3)	0.22 ± 0.06^{a}	0.02 ± 0.01^{c}	0.01 ± 0.01^{c}	0.11 ± 0.06^{b}	0.10 ± 0.04^{b}	0.09 ± 0.04^{b}
22:0	0.30 ± 0.04^{b}	0.45 ± 0.05^{a}	0.43 ± 0.03^{a}	0.40 ± 0.04^{a}	0.40 ± 0.06^{a}	0.40 ± 0.04^{a}
22:1(n-9)	0.03 ± 0.01	0.03 ± 0.02	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.01	0.03 ± 0.01
22:4(n-6)	0.45 ± 0.04^{c}	0.95 ± 0.08^{a}	0.85 ± 0.08^{b}	0.32 ± 0.03^{d}	0.34 ± 0.02^{d}	0.33 ± 0.03^{d}
22:5(n-6)	0.86 ± 0.20^{c}	7.28 ± 0.93^{b}	8.64 ± 1.36^{a}	0.61 ± 0.17^{c}	0.73 ± 0.16^{c}	0.78 ± 0.34^{c}
22:5(n-3)	0.65 ± 0.12^{a}	0.12 ± 0.05^{b}	0.08 ± 0.02^{b}	0.16 ± 0.04^{b}	0.16 ± 0.04^{b}	0.16 ± 0.05^{b}
24:0	0.68 ± 0.06^{c}	1.03 ± 0.08^{a}	0.96 ± 0.07^{ab}	0.91 ± 0.07^{b}	0.90 ± 0.12^{b}	0.90 ± 0.10^{b}
22:6(n-3)	9.81 ± 0.87^{b}	2.11 ± 0.34^{c}	1.96 ± 0.22^{c}	13.25 ± 0.83^{a}	13.26 ± 0.87^{a}	12.83 ± 0.78^{a}
24:1(n-9)	0.26 ± 0.03^{a}	0.20 ± 0.02^{bc}	0.17 ± 0.03^{c}	0.22 ± 0.05^{b}	0.21 ± 0.03^{bc}	0.19 ± 0.02^{bc}
total SFA	41.04 ± 1.14^{ab}	42.04 ± 0.64^{a}	41.07 ± 0.87^{ab}	40.68 ± 0.47^{b}	40.42 ± 0.68^{b}	40.47 ± 0.54^{b}
total MUFA	7.28 ± 0.75^{b}	9.74 ± 0.93^{a}	9.98 ± 0.84^{a}	9.31 ± 0.79^{a}	9.98 ± 0.64^{a}	9.79 ± 0.92^{a}
total PUFA	51.69 ± 1.32^{a}	49.13 ± 0.91^{c}	49.83 ± 0.53^{bc}	50.76 ± 0.62^{ab}	50.33 ± 0.62^{b}	50.48 ± 0.73^{b}
total n-3	10.99 ± 1.01^{b}	2.27 ± 0.39^{c}	2.08 ± 0.23^{c}	13.54 ± 0.84^{a}	13.55 ± 0.90^{a}	13.10 ± 0.79^{a}
total n-6	40.70 ± 1.21^{b}	45.94 ± 0.77^{a}	46.87 ± 0.36^{a}	36.47 ± 0.67^{c}	36.05 ± 1.01^{c}	36.64 ± 0.85^{c}

Values are mean ± SD, n = 12. Values with different superscripts in the same row differ significantly ($p < 0.05$).

to the findings of the plasma FA profiles, feeding on an n-3 deficient diet decreased the level of n-3 PUFA in liver lipids of the deficiency and tripalmitin groups. The EPA and 22:5(n-3) (DPA) were not detected in the liver TAG fraction in the deficiency and tripalmitin groups. In the liver PL fraction, the normal feed group receiving soybean oil rich in ALA, the value of EPA was significantly higher than in the DHA groups, which was likely because of the conversion of ALA to EPA. The values of the 22:5(n-6) in both the TAG and the PL fractions were the highest in the tripalmitin group. The tripalmitin group and the deficiency group showed significantly higher levels of 22:5(n-6) than the values of the groups receiving normal feed or n-3 deficient feed supplemented with DHA. Also, the level of 22:4(n-6) was increased in the PL fraction of the deficiency and the tripalmitin groups compared to groups receiving n-3 PUFAs from soybean oil in the normal feed group or as DHA from structured TAGs in the DHA groups. This was consistent with the plasma FA results, which may indicate the increased elongation and desaturation of the LA in response to the n-3 deficient diet (Kulkarni et al., 2022).

3.4. Brain fatty acid composition

Table 7 shows the FA composition of the rat brain lipids. In contrast to the FA composition of the plasma and the liver, the percentage of DHA in brain fatty acids of the three DHA feeding groups showed no significant difference from the value in the normal feed group. In previous research, the DHA level in the brain did not change after dietary intake of DHA in mice fed with normal feed (Valentini et al., 2018) or after feeding fish oil to rats on normal feed (Gerbi et al., 1994). It was also reported that after 15 weeks of intervention with an n-3 deficiency diet, the expressions of elongase 2 and 5 and Δ5 and Δ6 desaturase genes were upregulated in rat liver, but not in the brain (Igarashi et al., 2007). The estimated rate of DHA synthesis in the brain is around 1 % of the DHA consumption rate in the brain (Rapoport et al., 2007). However, the rate of DHA synthesis in the liver is 10 times higher than the synthesis rate in the brain, forming a main source of DHA to maintain the brain's DHA level (Igarashi et al., 2007). Therefore, the soybean oil provided sufficient ALA for the normal feed group to synthesize DHA in the liver, which was transported to the brain after synthesis. In the case of a shortage of DHA, the body will allocate DHA from other organs to prioritize the supply of the brain. So the DHA content in the brain is buffered against short to mid-term changes in the dietary content of n-3 PUFAs. The DHA content in the brain is the most abundant among the plasma and three organs studied. Comparing the absolute content of DHA in the brain, the deficiency group showed the lowest value (431.5 ± 24.6 μg/100 mg fresh weight of the brain tissue), and the *sn*-2 group showed the highest (595.2 ± 26.6 μg/100 mg). The *sn*-2 DHA group showed a slightly higher content of DHA and total n-3 PUFA in the brain compared to the *sn*-1 and *sn*-3 groups, although the difference did not reach statistical significance. Bandarra et al. (2016) had a 12-week feeding trial on the hamster. The group feed with structured lipid with DHA located at the *sn*-2 position showed a higher level of DHA content in the brain, compared to the control, fish oil, and fish oil ethyl ester groups. The different results between the two studies may have been related to the different base diet used (n-3 adequate base feed used in the study of Bandarra et al., n-3 deficient base feed used in our study) as well as the shorter intervention time (4-week) used in our study compared to the 12-week intervention feeding in the study of Bandarra et al. Our findings are in line with the results of our previous research, which indicated higher bioavailability of DHA from the *sn*-2 position than in the *sn*-1/3 positions in a five-day feeding trial (Linderborg et al., 2019). The level of 16:1(n-7) in the *sn*-1 DHA and tripalmitin groups was significantly lower than the normal feed, the *sn*-2 and *sn*-3 DHA groups. No significant differences were found between the three DHA groups in the level of other FAs.

In agreement with the findings for the liver and plasma, the deficiency and the tripalmitin groups, both receiving an n-3 deficient diet, showed a significantly lower value of DHA than other groups in the brain. This indicates that 4 weeks of feeding with an n-3 deficiency diet was enough to induce a mild n-3 deficiency in the rats, a finding also supported by an earlier study in our group (Kulkarni et al., 2022). However, the EPA content of the deficiency group was close to the level of the normal feed group, which was significantly higher than the value of the tripalmitin group. Nevertheless, the EPA content only represents 0.03 % of the total FAs in the brain, and the level could have been maintained by increased conversion of ALA in the plasma and other

Table 7
The proportion of fatty acids (weight percentage of total fatty acids) in the rat brains of different intervention groups.

Fatty acids	Normal feed	Deficiency	Tripalmitin	*sn*-1 DHA	*sn*-2 DHA	*sn*-3 DHA
14:0	0.27 ± 0.02^{a}	0.26 ± 0.02^{ab}	0.25 ± 0.01^{b}	0.25 ± 0.01^{ab}	0.27 ± 0.03^{a}	0.27 ± 0.01^{a}
16:0	22.94 ± 0.63^{a}	22.9 ± 0.51^{a}	21.97 ± 0.85^{ab}	21.79 ± 0.68^{b}	22.66 ± 0.96^{ab}	22.7 ± 0.71^{ab}
16:1(n-7)	0.46 ± 0.03^{a}	0.41 ± 0.03^{bc}	0.40 ± 0.02^{c}	0.39 ± 0.02^{c}	0.44 ± 0.04^{ab}	0.44 ± 0.03^{ab}
18:0	23.67 ± 0.19^{a}	23.47 ± 0.28^{ab}	23.22 ± 0.49^{b}	23.48 ± 0.32^{ab}	23.62 ± 0.29^{ab}	23.67 ± 0.23^{a}
18:1(n-9)	15.31 ± 0.65^{b}	15.12 ± 0.55^{b}	16.07 ± 0.85^{ab}	16.63 ± 0.77^{a}	16.07 ± 1.13^{ab}	15.84 ± 0.80^{ab}
18:1(n-7)	3.04 ± 0.14	2.85 ± 0.15	3.07 ± 0.25	2.97 ± 0.28	2.91 ± 0.20	2.93 ± 0.16
18:2(n-6)	1.05 ± 0.12^{a}	0.72 ± 0.05^{c}	0.71 ± 0.06^{c}	0.79 ± 0.05^{bc}	0.88 ± 0.05^{b}	0.87 ± 0.08^{b}
18:3(n-3)	0.03 ± 0.01^{b}	0.05 ± 0.02^{a}	0.05 ± 0.00^{a}	0.05 ± 0.02^{a}	0.05 ± 0.02^{a}	0.06 ± 0.01^{a}
20:0	0.41 ± 0.05	0.41 ± 0.05	0.52 ± 0.13	0.51 ± 0.09	0.44 ± 0.09	0.42 ± 0.06
20:1(n-9)	0.72 ± 0.1^{ab}	0.70 ± 0.11^{b}	0.96 ± 0.30^{a}	0.96 ± 0.25^{a}	0.77 ± 0.16^{ab}	0.76 ± 0.12^{ab}
20:2(n-6)	0.21 ± 0.02	0.18 ± 0.02	0.20 ± 0.03	0.20 ± 0.02	0.18 ± 0.02	0.18 ± 0.02
20:3(n-6)	0.40 ± 0.02^{b}	0.34 ± 0.03^{c}	0.35 ± 0.04^{c}	0.45 ± 0.03^{a}	0.43 ± 0.04^{ab}	0.42 ± 0.03^{ab}
20:4(n-6)	11.67 ± 0.47^{ab}	12.11 ± 0.49^{a}	11.61 ± 0.66^{ab}	11.26 ± 0.60^{b}	11.37 ± 0.51^{b}	11.65 ± 0.36^{ab}
20:5(n-3)	0.04 ± 0.01^{a}	0.03 ± 0.01^{a}	0.02 ± 0.00^{b}	0.03 ± 0.01^{a}	0.03 ± 0.01^{ab}	0.03 ± 0.01^{ab}
22:0	0.44 ± 0.08	0.43 ± 0.08	0.54 ± 0.16	0.57 ± 0.11	0.45 ± 0.13	0.44 ± 0.09
22:1(n-9)	0.12 ± 0.02^{ab}	0.10 ± 0.02^{b}	0.14 ± 0.04^{ab}	0.15 ± 0.04^{a}	0.12 ± 0.04^{ab}	0.11 ± 0.02^{b}
22:4(n-6)	3.38 ± 0.25^{ab}	3.62 ± 0.15^{a}	3.61 ± 0.19^{a}	3.37 ± 0.57^{ab}	3.19 ± 0.13^{b}	3.28 ± 0.15^{ab}
22:5(n-6)	0.97 ± 0.06^{b}	4.44 ± 0.51^{a}	4.25 ± 0.43^{a}	0.68 ± 0.11^{b}	0.74 ± 0.16^{b}	0.79 ± 0.13^{b}
22:5(n-3)	0.13 ± 0.02^{a}	0.05 ± 0.01^{c}	0.05 ± 0.01^{c}	0.10 ± 0.01^{b}	0.11 ± 0.01^{b}	0.10 ± 0.02^{b}
24:0	0.58 ± 0.13^{ab}	0.61 ± 0.13^{ab}	0.77 ± 0.19^{a}	0.72 ± 0.12^{ab}	0.57 ± 0.15^{b}	0.53 ± 0.12^{b}
22:6(n-3)	13.24 ± 0.57^{a}	10.25 ± 0.52^{b}	9.97 ± 0.34^{b}	13.33 ± 0.39^{a}	13.73 ± 0.51^{a}	13.55 ± 0.74^{a}
24:1(n-9)	0.94 ± 0.25	0.94 ± 0.19	1.28 ± 0.39	1.31 ± 0.35	0.98 ± 0.29	0.95 ± 0.21
total SFA	48.31 ± 0.44^{a}	48.08 ± 0.50^{ab}	47.27 ± 0.81^{b}	47.33 ± 0.62^{b}	48.00 ± 0.80^{ab}	48.04 ± 0.51^{ab}
total MUFA	20.57 ± 1.08^{ab}	20.12 ± 0.91^{b}	21.91 ± 1.77^{ab}	22.41 ± 1.60^{a}	21.28 ± 1.74^{ab}	21.03 ± 1.20^{ab}
total PUFA	31.57 ± 0.58^{ab}	32.36 ± 0.38^{a}	31.54 ± 0.79^{ab}	30.90 ± 1.01^{b}	31.17 ± 0.90^{b}	31.37 ± 0.62^{b}
total n-3	13.44 ± 0.56^{a}	10.39 ± 0.52^{b}	10.09 ± 0.35^{b}	13.50 ± 0.39^{a}	13.91 ± 0.50^{a}	13.74 ± 0.73^{a}
total n-6	17.67 ± 0.58^{b}	21.41 ± 0.50^{a}	20.73 ± 0.96^{a}	16.76 ± 1.04^{b}	16.80 ± 0.66^{b}	17.19 ± 0.50^{b}

Values are mean ± SD, n = 12. Values with different superscripts in the same row differ significantly ($p < 0.05$).

tissues. The level of 18:2(n-6) of the normal feed group was significantly higher than in the other groups, which was in accordance with the results from the plasma and the liver reflecting the FA profile of the feed. However, the level of 18:1(n-9), 20:0, 22:0, and 24:0 did not show significant differences from the other groups. In contrast to the findings from the plasma and liver, the level of ALA in the brain showed a significantly lower level in the normal feed group compared with other groups except the *sn*-1 DHA group. However, both the proportion and the absolute content of ALA were very low in the brain and may not have a stronger association with the ALA content in the diet. It is generally known that dietary ALA is mostly used as a source of energy via β-oxidation, with only a minor proportion converted to long-chain PUFAs in the liver. Consistent with the results in the plasma and liver, the 22:5(n-6) contents in the brain of the deficiency and tripalmitin groups were significantly higher than the contents in the other groups. There was no significant difference between the total lipid content in the brain among the groups (data not shown). The most predominant FAs in the brain were 16:0, 18:0, and 18:1(n-9).

3.5. Visceral fat fatty acid composition

Table 8 shows the FA composition of the visceral fat. The lipid content in the visceral fat accounted for around 70 % of the fresh weight. There was no significant difference in the total lipids content between the groups. The most predominant FAs in the visceral fat were 16:0, 18:1 (n-9), and 18:2(n-6). It was interesting that the ALA content in the visceral fat was higher than in the liver, brain, and plasma, indicating that the dietary ALA was taken up effectively by the visceral fat without effective conversion to EPA and DHA. In the normal feed group, the content of 18:1(n-9) was significantly lower than in the other groups. However, the contents of LA, and ALA were significantly higher than the other groups, this is evidently because of the difference in fatty acid composition between the soybean oil and the peanut oil used in the normal feed and n-3 deficient feed (Table 3). The same was also reflected in the contents of long-chain SFAs such as 20:0, 22:0, and 24:0. In agreement with the results of the other organs, the deficiency, and the tripalmitin groups, both receiving an n-3 deficient diet, showed a significantly lower value of DHA in the visceral fat than in other groups. Even though the DHA was not prioritized for storage in the visceral fat, DHA accounted for 0.16 % of the total FA in the visceral fat in the normal feed group. The absolute content of DHA in the normal feed group was 113.5 μg/100 mg fresh weight of visceral fat (Fig. 2A). The DHA contents in the visceral fat of the DHA groups were increased by 3–4 folds compared to the normal feed group indicating that the dietary DHA content in this study was likely in surplus compared to the DHA level needed for normal physiology. The content of DHA and the total n-3 PUFAs in visceral fat of the *sn*-3 DHA group were significantly higher than the values in the *sn*-1 and *sn*-2 groups. Previously, Christensen & Høy studied the DHA distribution in new-born rats by an intervention with structured *sn*-2 DHA containing TAG and randomized oil with DHA equally distributed in the TAG molecule (M. M. Christensen & Høy, 1997). After 3 weeks of intervention feeding, higher DHA content in the visceral fat was found in the group fed with randomized fat. This indicates that the DHA located at *sn*-1/3 positions has a higher accumulation of visceral fat compared with *sn*-2 DHA. This may be related to the activity of lipoprotein lipase which is *sn*-1/3 specific, since the chylomicron TAG structure largely reflects the TAG structure of the dietary fat (M. S. Christensen et al., 1995). In our current study, among the three DHA-fed groups, the *sn*-2 DHA group had the lowest DHA level in the visceral fat, but the highest DHA content in plasma PL, liver PL, and brain lipids. Comparing the *sn*-1 and *sn*-3 DHA groups, *sn*-3 DHA was more effectively stored in the visceral fat, whereas *sn*-1 DHA resulted in higher DHA level in the plasma. These findings suggest differences in the bioavailability and metabolic fate of DHA from different *sn*-positions of dietary TAGs after digestion and absorption.

Based on the existing evidence, the regio- and stereospecific structures of the ingested TAGs are largely preserved in chylomicrons, but not in TAGs of chylomicron remnants or VLDL (Karupaiah & Sundram, 2007). After fat absorption, adipose tissue lipoprotein lipase (LpL) is activated leading to regiospecific cleavage of chylomicron TAGs, releasing *sn*-1/3 FAs, which are then stored as TAG in adipose tissue. Previous research findings indicated that LpL may have different efficiency in cleaving *sn*-1 and *sn*-3 FAs (Sato et al., 1998; Wang et al., 1982). Such stereospecific preference of LpL in hydrolysis of TAGs in

Table 8
The proportion of fatty acids (weight percentage of total fatty acids) in the rat visceral fat of different intervention groups.

Fatty acids	Normal feed	Deficiency	Tripalmitin	*sn*-1 DHA	*sn*-2 DHA	*sn*-3 DHA
14:0	1.57 ± 0.11	1.61 ± 0.14	1.63 ± 0.12	1.60 ± 0.07	1.52 ± 0.11	1.63 ± 0.12
16:0	25.44 ± 1.18	25.34 ± 1.29	26.22 ± 1.30	26.91 ± 1.06	26.92 ± 1.39	26.53 ± 1.47
16:1(n-7)	5.72 ± 0.54	5.60 ± 0.58	5.73 ± 0.83	5.60 ± 0.55	5.58 ± 0.66	5.34 ± 0.67
18:0	3.45 ± 0.18^{ab}	3.36 ± 0.21^{b}	3.40 ± 0.16^{ab}	3.63 ± 0.21^{a}	3.56 ± 0.21^{ab}	3.51 ± 0.19^{ab}
18:1(n-9)	29.86 ± 0.72^{c}	44.3 ± 1.23^{a}	43.66 ± 1.15^{ab}	42.57 ± 0.97^{b}	42.75 ± 1.23^{b}	42.34 ± 0.87^{b}
18:1(n-7)	2.62 ± 0.19	2.38 ± 0.20	2.19 ± 0.28	3.13 ± 3.49	2.02 ± 0.28	2.03 ± 0.17
18:2(n-6)	27.13 ± 2.05^{a}	15.56 ± 1.11^{b}	15.31 ± 1.33^{b}	14.42 ± 4.69^{b}	15.45 ± 1.30^{b}	16.16 ± 1.43^{b}
18:3(n-6)	0.09 ± 0.01^{a}	0.08 ± 0.01^{ab}	0.07 ± 0.01^{ab}	0.06 ± 0.02^{bc}	0.06 ± 0.03^{bc}	0.05 ± 0.02^{c}
18:3(n-3)	2.62 ± 0.16^{a}	0.14 ± 0.02^{b}	0.12 ± 0.02^{b}	0.12 ± 0.04^{b}	0.13 ± 0.01^{b}	0.15 ± 0.03^{b}
20:0	0.06 ± 0.01^{d}	0.15 ± 0.03^{c}	0.15 ± 0.03^{bc}	0.21 ± 0.06^{a}	0.20 ± 0.04^{ab}	0.20 ± 0.03^{a}
20:1(n-9)	0.19 ± 0.01^{b}	0.40 ± 0.02^{a}	0.39 ± 0.02^{a}	0.36 ± 0.09^{a}	0.39 ± 0.03^{a}	0.40 ± 0.03^{a}
20:2(n-6)	0.18 ± 0.04^{a}	0.11 ± 0.03^{b}	0.11 ± 0.02^{b}	0.08 ± 0.03^{b}	0.12 ± 0.08^{b}	0.08 ± 0.01^{b}
20:3(n-6)	0.09 ± 0.02^{a}	0.06 ± 0.01^{ab}	0.05 ± 0.01^{b}	0.08 ± 0.06^{ab}	0.06 ± 0.01^{ab}	0.07 ± 0.01^{ab}
20:4(n-6)	0.49 ± 0.12^{a}	0.39 ± 0.11^{ab}	0.41 ± 0.08^{ab}	0.33 ± 0.11^{b}	0.35 ± 0.06^{b}	0.41 ± 0.03^{ab}
22:0	0.04 ± 0.01^{c}	0.11 ± 0.01^{ab}	0.10 ± 0.01^{b}	0.12 ± 0.01^{ab}	0.13 ± 0.02^{a}	0.12 ± 0.02^{ab}
20:5(n-3)	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.00
22:2(n-6)	0.03 ± 0.00^{b}	0.03 ± 0.01^{b}	0.04 ± 0.00^{a}	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}
22:4(n-6)	0.11 ± 0.03^{a}	0.09 ± 0.03^{a}	0.10 ± 0.03^{a}	0.06 ± 0.01^{b}	0.06 ± 0.01^{b}	0.06 ± 0.01^{b}
22:5(n-6)	0.05 ± 0.01^{b}	0.12 ± 0.04^{a}	0.15 ± 0.04^{a}	0.03 ± 0.01^{b}	0.03 ± 0.02^{b}	0.03 ± 0.01^{b}
24:0	0.05 ± 0.01^{c}	0.08 ± 0.01^{bc}	0.08 ± 0.01^{bc}	0.07 ± 0.04^{bc}	0.10 ± 0.03^{ab}	0.13 ± 0.03^{a}
22:6(n-3)	0.16 ± 0.06^{c}	0.02 ± 0.00^{d}	0.02 ± 0.01^{d}	0.54 ± 0.17^{b}	0.49 ± 0.12^{b}	0.68 ± 0.08^{a}
24:1(n-9)	0.02 ± 0.01^{b}	0.04 ± 0.01^{a}	0.04 ± 0.02^{a}	0.04 ± 0.02^{a}	0.04 ± 0.02^{a}	0.04 ± 0.01^{a}
total SFA	30.62 ± 1.37	30.65 ± 1.46	31.58 ± 1.39	32.39 ± 1.22	32.43 ± 1.60	32.12 ± 1.67
total MUFA	38.41 ± 1.03^{c}	52.73 ± 0.95^{a}	52.02 ± 0.48^{ab}	51.70 ± 4.23^{ab}	50.77 ± 0.90^{ab}	50.15 ± 0.66^{b}
total PUFA	30.97 ± 2.09^{a}	16.62 ± 1.12^{b}	16.40 ± 1.27^{b}	15.76 ± 4.79^{b}	16.80 ± 1.31^{b}	17.73 ± 1.46^{b}
total n-3	2.8 ± 0.18^{a}	0.18 ± 0.02^{d}	0.16 ± 0.02^{d}	0.68 ± 0.18^{c}	0.63 ± 0.13^{c}	0.84 ± 0.09^{b}
total n-6	28.17 ± 1.97^{a}	16.34 ± 1.11^{b}	16.24 ± 1.27^{b}	15.08 ± 4.74^{b}	16.17 ± 1.31^{b}	16.89 ± 1.42^{b}

Values are mean ± SD, n = 12. Values with different superscripts in the same row differ significantly ($p < 0.05$).

chylomicron and adipose tissue may have been the mechanism causing the higher DHA level in visceral fat of the *sn*-3 DHA group as compared to *sn*-1 and *sn*-2 groups. During fat digestion, gastric lipase cleaves preferentially *sn*-3 FAs from dietary TAGs, whereas pancreatic lipase has higher efficiency for *sn*-1 FAs. The stereoselectivity of lipases may have resulted in different absorption and clearance between *sn*-1 and *sn*-3 DHA. Previous research has shown that in rats' plasma TAG levels remained high even at 10 h after fat ingestion, showing a significantly longer period of postprandial lipemia compared to humans (Panzoldo et al., 2011). The higher DHA level in plasma TAGs of the *sn*-1 group might have been related to delayed DHA absorption and/or slower clearance of TAGs compared to the *sn*-2 and *sn*-3 groups.

Feeding with DHA increased the DHA content but not the other n-3 PUFAs in the visceral fat. The contents of ALA (18:3n-3) in visceral fat of the DHA groups were significantly lower than the content in the normal feed group, whereas the content of EPA did not show a significant difference among the groups. Consistent with the results of the plasma, liver, and brain, the 22:5(n-6) levels in the visceral fat of the deficiency and the tripalmitin groups were significantly higher than in normal feed and the DHA groups, likely as a result of the compensatory response to maintain PUFA pool necessary for the fluidity of cell membranes as well as for the inflammatory and immune response.

3.6. Content of n-3 and n-6 PUFAs in different organs in different groups

The visceral fat showed the highest lipid content (0.69 ± 0.02 ~ 0.73 ± 0.03 g/g fresh weight) among all the organs, followed by the brain (0.041 ± 0.002 ~ 0.044 ± 0.002 g/g fresh weight) and liver PL (0.025 ± 0.001 ~ 0.027 ± 0.001 g/g fresh weight). The Fig. 2**.** shows the content of n-3 (Fig. 2A) and n-6 (Fig. 2B) PUFAs including 18:3(n-3), 20:5(n-3), 22:6(n-3), 18:2(n-6), 20:4(n-6), 22:4(n-6), 22:5(n-6), total n-3 PUFA, and total n-6 PUFA in different tissues and organs of different groups. The total n-6 PUFAs, mainly contributed by 18:2(n-6) and 20:4 (n-6), are the most abundant FAs in all organs. Consistent with the percentage of DHA in the total FAs of visceral fat, the absolute content of DHA in the visceral fat was significantly higher in the *sn*-3 group compared to the *sn*-1 and *sn*-2 groups, which indicates a higher accumulation of *sn*-3 DHA in the visceral fat. The content of 24:0 in the *sn*-1 group was significantly lower than the *sn*-2 and *sn*-3 groups in the visceral fat. In the brain lipids, the 16:0 and 16:1(n-7) showed lower values in the *sn*-1 group compared to the *sn*-2 group. There were no significant differences between the three DHA groups in the content of other FAs in the liver and plasma.

For TAG-rich organs, tissues, and fractions including the liver TAG, plasma TAG, and visceral fat, the normal feed group showed the highest content of total n-3 and n-6 PUFAs. However, in the PL-rich organs, tissues, and fractions including liver PL and plasma PL, the total n-3 PUFA content showed the highest value in the DHA groups. In the TAG-rich tissues and fractions, the 18:3(n-3) content was significantly higher in the normal feed group than in other groups. The content of 22:5(n-6) increased dramatically in all organs in the deficiency and the tripalmitin groups compared to the normal feed group and the DHA-fed groups, likely resulting from a compensation response to a diet low in n-3 PUFAs to keep the fluidity of the biofilm as discussed earlier. Likely for the same reason, the content of 22:4(n-6) also increased in the groups fed with n-3 deficient diet, showing significantly higher contents in plasma PL and liver PL in these groups than in the other groups. The total n-6 PUFA content in the deficiency and the tripalmitin groups show higher values than the normal feed group in the PL-rich organs and fractions, mainly due to the increase in the content of 22:5(n-6) existing mainly in the PL form (Fig. 2B).

4. Conclusion

Only a few studies have reported the synthesis of positional isomers of TAGs, and even fewer have reported findings of the bioavailability of DHA from regio- and enantiopure TAGs *in vivo*. In this study, we investigated the impact of a four-week intervention feeding with DHA from regio- and stereospecific positions of TAGs on the DHA content and the overall FA composition of organs and tissues in rats. The DHA groups showed significantly higher DHA content than the normal feed group in all the tissues except the brain. Intervention feeding with DHA located in the *sn*-1 position of TAGs resulted in a significantly higher content of DHA in the plasma TAG compared with feeding with *sn*-3 DHA, whereas

in visceral fat, the DHA content was highest in the *sn*-3 group among all the three DHA-fed groups. There was also a tendency towards a difference between the *sn*-1 DHA and *sn*-3 DHA groups regarding the DHA content in the plasma PL and liver, although the difference did not reach statistical significance. A future study with a longer intervention time could provide more evidence to confirm these findings. This study also demonstrated the effects of four-week n-3 deficient feeding on the FA composition of tissues and organs of rats in comparison with n-3 adequate feeding. Four-week feeding with an n-3 deficient diet as in the deficiency group and the tripalmitin group resulted in a clear decrease in DHA content and the total content of n-3 PUFAs accompanied by an increase in n-6 PUFAs, in comparison with the normal n-3 adequate feed group and the DHA groups. This is the first time that difference has been demonstrated in tissue accumulation of DHA from the *sn*-1 and *sn*-3 positions of dietary TAGs. The new findings contribute to improving the current knowledge on the bioavailability of dietary DHA from the regio- and enantiopure TAGs.

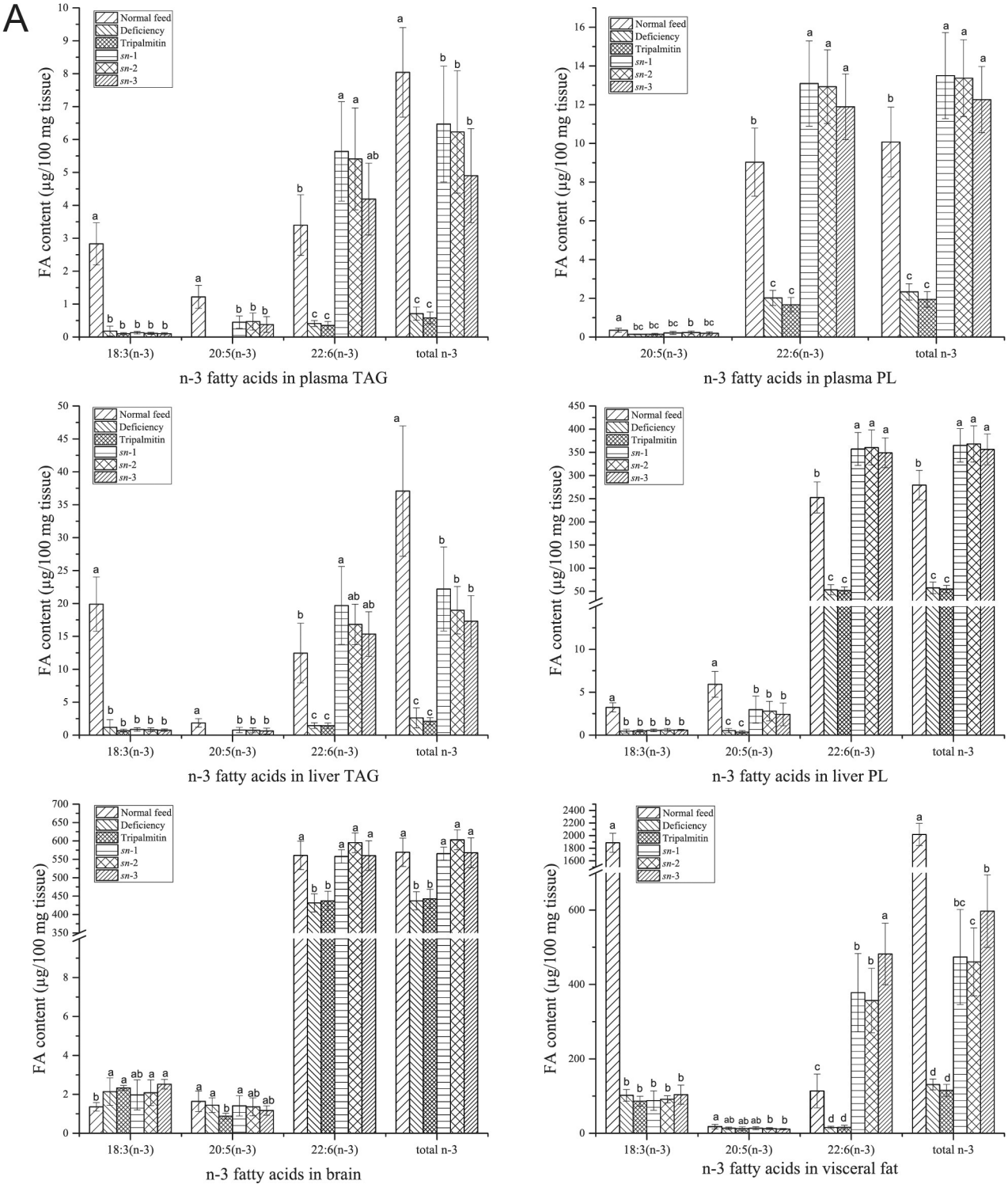

Fig. 2. Selected FA content in different organs. (A) Selected n-3 fatty acids in different organs; (B) Selected n-6 fatty acids in different organs. Values are expressed as means ± SD. Bars with different letters represent statistically significant differences ($p < 0.05$) between different intervention groups.

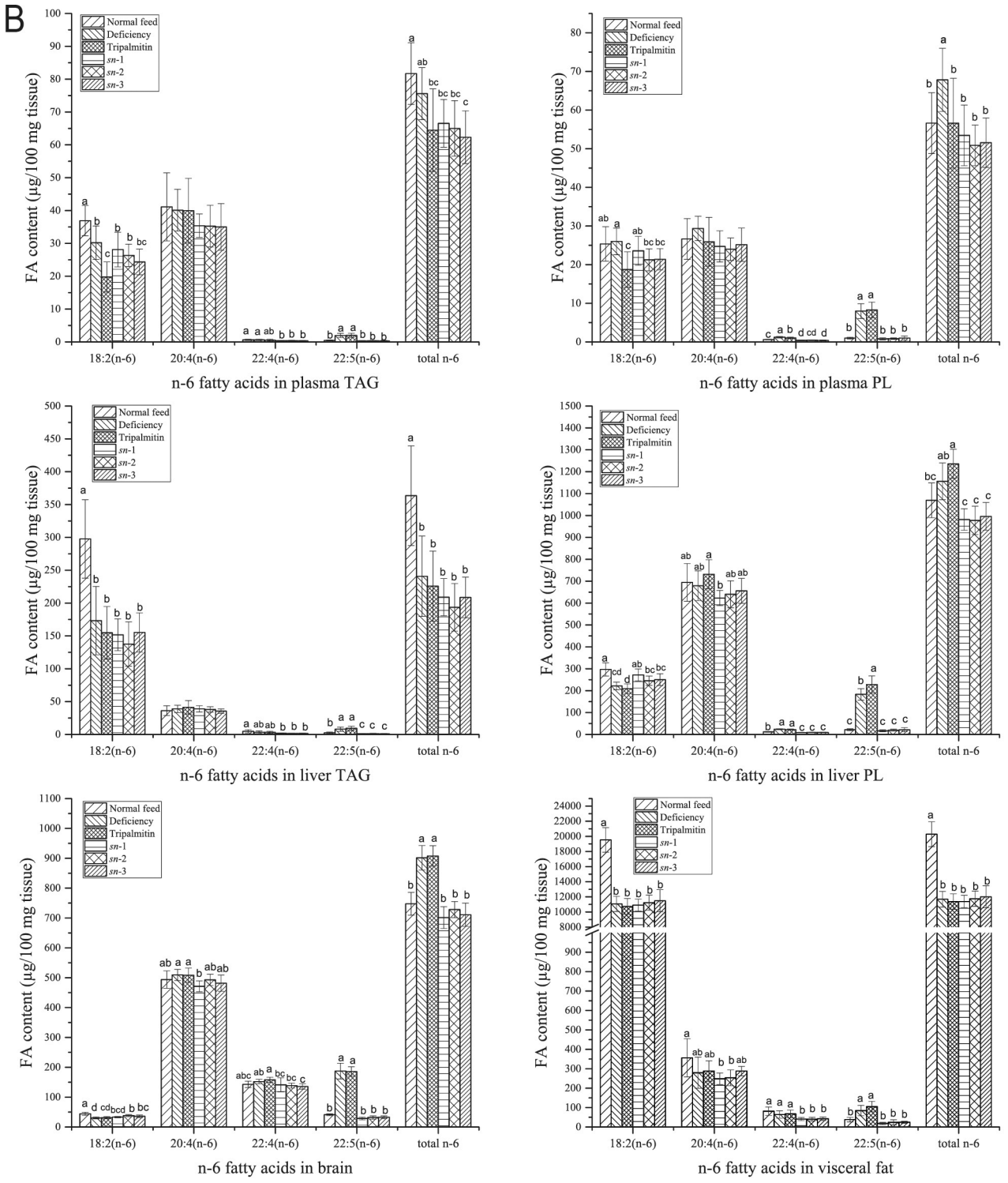

Fig. 2. (*continued*).

CRediT authorship contribution statement

Yuqing Zhang: Formal analysis, Methodology, Data curation, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition. **Marika Kalpio:** Supervision, Resources, Writing – review & editing. **Lingwei Tao:** Formal analysis. **Guðmundur G. Haraldsson:** Conceptualization, Methodology, Resources, Writing – review & editing. **Haraldur G. Guðmundsson:** Investigation, Methodology, Writing – review & editing. **Xiangrong Fang:** Formal analysis. **Kaisa M. Linderborg:** Resources, Writing – review & editing. **Yumei Zhang:** Conceptualization, Project administration, Resources, Methodology, Writing – review & editing. **Baoru Yang.:** Conceptualization, Methodology, Investigation, Project administration, Supervision, Resources, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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