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Effects of feed on fatty acid composition in muscles and gonads of the Chinese mitten crab (*Eriocheir sinensis*)

by

Jianmin Zou[1,2], Chao Song[1,2,*], Shunlong Meng[1,2], Gengdong Hu[1,2], Liping Qiu[1,2], Limin Fan[1,2], Jiazhang Chen[1,2]

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[1]*Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Wuxi, China*

[2]*Laboratory of Quality & Safety Risk Assessment for Aquatic Products on Environmental Factors (Wuxi), Ministry of Agriculture and Rural Affairs, Wuxi, China*

* Corresponding author: songc@ffrc.cn

Abstract

In this study, the effects of different feeds on fatty acid composition in the Chinese mitten crab (*Eriocheir sinensis*) were investigated. The fatty acid composition in the Chinese mitten crab was significantly correlated with the type of feed source provided. Differences between the feed groups pertained mainly five fatty acids: oleic acid, linoleic acid, palmitic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The content of EPA and DHA was higher in the group of frozen trash fish than in the group of formulated feed. On the other hand, the content of oleic acid, linoleic acid and palmitic acid was higher in the formulated feed group than in the frozen trash fish group. There were significant differences in the nutritional value of the Chinese mitten crab reared under different feed sources, i.e. Chinese mitten crabs reared with the frozen trash fish feed were larger than those reared with the formulated feed, especially as regards the ω-3/ω-6 PUFA ratio and essential fatty acid levels.

Key words: Chinese mitten crab, fatty acids, formulated feed, frozen trash fish

1. Introduction

The Chinese mitten crab (*Eriocheir sinensis*) is widely distributed in China, especially in the Yangtze River basin (Chen et al. 2007; Wang et al. 2016). In 2019, the yield of the Chinese mitten crab was 778,682 tons (China Fisheries Statistics Yearbook 2020). The Yangtze River basin is currently the main breeding location, accounting for more than 80% of the total production (Liu et al. 2015). However, although the Chinese mitten crab culture is booming, it is also facing major changes.

The earthen pond culture is the main method of rearing Chinese mitten crabs, and frozen trash fish and other raw materials such as soybean meal and wheat are common natural feed sources (He et al. 2016). Trash fish include small-sized fish species that have lower economic value, are easily perishable, have poor edible qualities and are not suitable for direct human consumption (Hasan & Halwart 2009; Cao et al. 2015). Sources of traditional natural feed, such as frozen trash fish, become unstable. In addition, they tend to carry pathogens that can cause disease outbreaks, contaminate aquaculture water, reduce the quality of pond water, and severely compromise the healthy and sustainable development of Chinese mitten crab aquaculture (Ge et al. 2017). Frozen trash fish, as a feed source, result in a low feed conversion rate and thus can be considered as a waste of natural resources (Cao et al. 2015; Han et al. 2018; Li et al. 2009). Formulated feed, on the other hand, is convenient to use and cheap, in addition to being nutritionally balanced and quality controlled (He et al. 2016). With the rapid growth of aquaculture, fishery sources are unable to sustain the huge demand for feed (Ge et al. 2017). Meanwhile, formulated feed can completely replace the traditional feed source, including frozen trash fish and soybean meal (Zhuang et al. 2016). Replacing the natural feed with formulated feed has become an inevitable trend in the future development of the Chinese mitten crab aquaculture. The use of suitable formulated feed in the Chinese mitten crab aquaculture also attracts interest among farmers in China (Que et al. 2011). However, the effect of switching between these two feeds on the fatty acid composition and nutritional value of the Chinese mitten crab is unclear.

Due to different nutritional combinations that may affect the quality of crabs as a result changes in fatty acids, most culturists prefer to use trash fish feed. However, Que et al. (2012) proved that formulated feed could replace frozen trash fish feed and meet the nutritional requirements of Chinese mitten crabs, as there was no significant effect on the survival and growth rates or reproduction when the formulated feed was substituted for frozen trash fish during the aquaculture process (Que et al. 2012). Therefore, the formulated feed provides only basic growth requirements, and has the potential to reduce the nutritional and commercial value of Chinese mitten crabs. Additional research on three different types of feed (natural feed, consisting of trash fish and freshwater snails; traditional feed, consisting of juvenile crabs, trash fish, cooked beans, broad beans, corn, and wheat, and formulated feed) showed that they significantly altered volatile and non-volatile active components in female crabs (Zhuang et al. 2016). Further, they showed that formulated feed enhanced the sensory scores of Chinese mitten crabs when compared to those fed frozen trash fish feed (Zhuang et al. 2016). In addition, formulated feed can increase the viability of juvenile Chinese mitten crabs and promote gonadal development (Wu et al. 2011). Previous studies showed that different types of feed affected the water content, crude protein content, total lipid, and other biochemical components in the hepatopancreas, gonads and muscles of Chinese mitten crabs (Ge et al. 2017; Que et al. 2011, 2012). In general, substituting the frozen trash feed is feasible to some extent, but it is necessary to study the formula composition of formulated feed to meet the product quality requirement.

The objective of this study is to compare the effect of different aquaculture feeding methods on fatty acid composition and nutritional value of the Chinese mitten crab, and to determine the correlation between fatty acids in feeds and fatty acids in the Chinese mitten crab. The results of this work may provide suggestions for the formula composition of the formulated feed to replace frozen trash fish feed and promote environmentally friendly aquaculture.

2. Materials and methods

2.1. Chemicals

Thirty seven fatty acid standards were purchased from SUPELCO Inc. (US) (Supplementary material). Anhydrous ether, anhydrous ethanol, petroleum ether (boiling point range: 30–60°C), sodium hydroxide and sodium chloride were all analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd (China). Hexane and methanol were chromatographic grade and were purchased from Merck & Co Inc. (US); 14% boron trifluoride-methanol was purchased from Macklin Biochemical Co., Ltd (China).

2.2. Experimental design

All experiments were set up at the Yangchenghu Farm in the Jiangsu Province. Two Chinese mitten crab culture ponds were selected (Table 1) and different feed experiments were conducted, with artificially formulated feed in the first pond and frozen trash fish in the second pond. In the harvest season, nine individuals of the Chinese mitten crab were sampled from the first pond and eight individuals from the second pond. The Chinese mitten crabs arrived at the laboratory within 24 h of collection. Muscles (body, legs and claws) and gonads were collected from the Chinese mitten crabs, homogenized and stored at –20°C for three days.

2.3. Pretreatment and analysis

For pre-processing steps, approximately 0.5 g of gonad samples and 4 g of muscle samples were placed in a 250 ml stoppered flask. Then, 100 mg of pyrogallic acid, 2 ml of ethanol and 4 ml of ultrapure water were added and mixed. After that, 10 ml of concentrated hydrochloric acid was added and the flask was incubated at 70°C for 40 min. After cooling to room temperature, the extraction process was performed twice using the mixture of ether and petroleum at a ratio of 1:1 in a 40°C depressurized rotary evaporator. Then, 8 ml of 2% sodium hydroxide-methanol solution was added. After condensation and reflux at 75°C for 30 min, 7 ml of 14% boron trifluoride-methanol was added and the reflux step was continued for another 2 min. Approximately 50 ml of water was added for rinsing and the solution was cooled to room temperature. Next, 10 ml of hexane and 10 ml of saturated sodium chloride solution were successively added. The flask was mixed thoroughly and allowed to settle for 3 min before collecting the supernatant. Excess water was removed from the supernatant and samples were stored at –20°C.

For the preprocessing procedure of the formulated feed, a total of 5 g of the crushed sample was tightly wrapped using a filter paper and placed in a Soxhlet extractor for a 6 h extraction (with anhydrous ether and petroleum ether as extractants at a ratio of 1:1). The extracted solution was placed in a 40°C depressurized rotary evaporator. The subsequent steps followed the same procedure as described above, i.e. 8 ml of 2% sodium hydroxide-methanol solution was added. After condensation and reflux at 75°C for 30 min, 7 ml of 14% boron trifluoride-methanol was added and the reflux step was continued for another 2 min. Approximately 50 ml of water was added for rinsing and the solution was cooled to room temperature. Next, 10 ml of hexane and 10 ml of saturated sodium chloride solution were successively added. The flask was mixed thoroughly and allowed to settle for 3 min to collect the supernatant. Excess water was removed from the supernatant and samples were stored at –20°C.

An Agilent 6890N gas chromatograph and a flame ionization detector (FID, Agilent, US) were used for chromatographic analysis. Qualitative and quantitative analyses of samples were carried out based on retention time and the standard curve. Chromatographic conditions were as follows: the chromatographic column – CD-2560 (100 m × 0.25 mm × 0.20 μm); carrier gas – high-purity nitrogen gas (purity ≥ 99.999%); constant current mode; flow rate – 0.5 ml/min; makeup gas – nitrogen gas; flow rate – 28 ml min^{-1}; FID temperature – 250°C; air flow rate – 400 ml/min; hydrogen gas flow rate – 30 ml min^{-1}; inlet temperature – 240°C; split loading was used with a split ratio of 100:1 and a sample volume of 1 μl. The column temperature elevation program was as follows: initial temperature – 140°C for 5 min^{-1}, raised to 230°C at 4°C min^{-1} and maintained at this temperature for 30 min.

The chromatographic conditions were used to quantify 37 fatty acid methyl ester standards (Xu et al. 2020). Linear regression was carried out using the peak area (y) and the corresponding standard concentration (x) to obtain the linear equation. The detection limit was 0.49–1.00 mg/100 g and the mean recovery rate of 37 fatty acids was 49.53–123.90%.

2.4. Data analysis and processing

Experimental data were recorded and figures were created in Excel 2016 (Microsoft). All statistical analyses were conducted using SPSS (version 25.0) for Windows. Redundancy Analysis (RDA) was used to analyze differences in total fatty acids and the correlation between the feed and the Chinese mitten crab. Student's t-test and single-factor ANOVA analysis

Table 1

Description of aquaculture ponds for the Chinese mitten crab

Pond	Area (ha)	Feed	Feed coefficient	Rearing density (ind. ha^{-1})	Juvenile crab size (g)	Yield (kg ha^{-1})	Adult crab specifications (g)
Pond 1	1.2	Formulated feed	2.2	19 500	9.1	3988	175
Pond 2	0.2	Frozen trash fish	3.5	12 000	9.1	2578	190

were performed to test for differences in fatty acid composition between the two feed groups; $p < 0.05$ was considered significant.

3. Results

3.1. Description of fatty acids in the Chinese mitten crab

In this experiment, we analyzed fatty acids in muscles and gonads of Chinese mitten crabs that were fed different types of feed. A total of 34 types of fatty acids were detected in crab gonad samples, with only three fatty acids (C4:0, C11:0, and C15:1) not detected. The rate of detection in all samples was 100%, except for C6:0 with a detection rate of 52.94%. The mean total fatty acid content in all crab gonad samples was 11,922.26 mg 100 g^{-1} and the mean content of different fatty acids was 2.69–3036.25 mg 100 g^{-1} (Table 2).

The types and levels of fatty acids detected in crab muscles were much lower than those in crab gonads (Table 3). A total of 26 types of fatty acids were detected in crab muscle samples, with 11 fatty acids (such as C4:0, C11:0, C15:1, C18:2n6t, and C18:3n6) not detected. In addition, the detection rate of the remaining fatty acids was 41.18–100%. The mean total fatty acid content in all crab muscle samples was 432.73 mg 100 g^{-1} and the mean content of different fatty acids was 0.20–90.10 mg 100 g^{-1}.

The total fatty acid content in all samples was ranked accordingly (Fig. 1). The results showed that mainly seven fatty acids (C16:0, C16:1, C18:1n9c, C18:2n6c, C20:4n6, C20:5n3, and C22:6n3) were present in all crab gonad samples, accounting for 82.03% of the total content (Fig. 1A). The presence of mainly seven fatty acids (C16:0, C16:1, C18:1n9c, C18:2n6c, C20:4n6, C20:5n3, and C22:6n3) was determined in all crab muscle samples, accounting for 86.17% of the total content (Fig. 1B).

3.2. Differences in fatty acids caused by different types of feed

Redundancy analysis (RDA) was carried out on crab gonads and muscles (Fig. 2). The results showed that samples of the Chinese mitten crab from different feed groups clustered within two different regions for both crab gonad samples and crab muscle samples, and the separation effect was significant ($p < 0.05$). The levels of six major fatty acids (C20:5n3, C22:6n3, C14:0, C18:1n9c, C16:0, and C18:2n6c) were remarkably high in crab gonad samples, three of which (C20:5n3, C22:6n3, and C14:0) were significantly higher ($p < 0.05$) in the

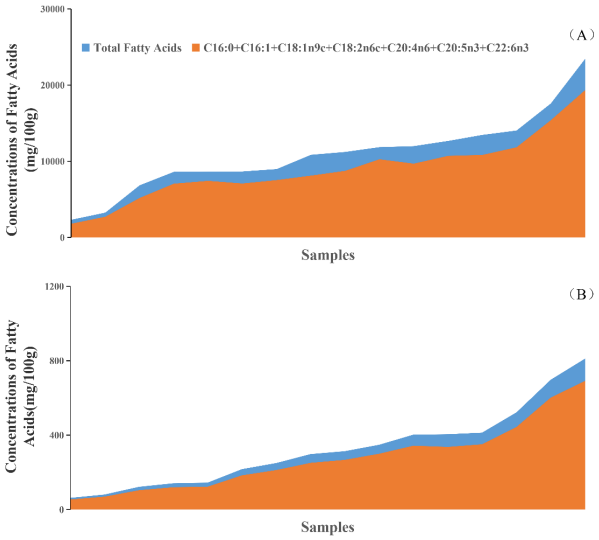

Figure 1

Area maps of six main fatty acids and total fatty acid content. (A) All gonad samples are ordered by total fatty acid content. (B) All muscle samples are ordered by total fatty acid content

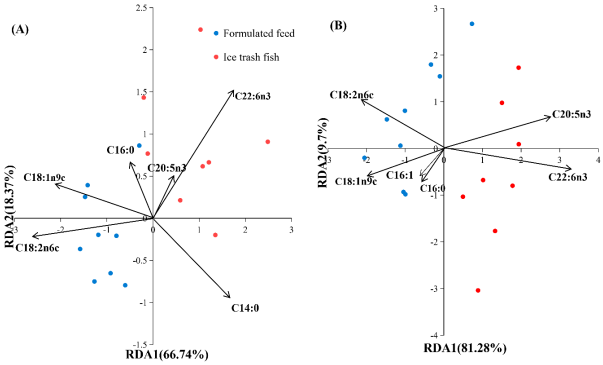

Figure 2

Redundancy analysis (RDA) graph of gonad and muscle samples. (A) Gonad samples from the Chinese mitten crab reared using two different types of feed are clustered in two different regions and showed significant separation ($p < 0.05$). (B) Muscle samples from the Chinese mitten crab reared using two different types of feed also are clustered in two different regions and showed significant separation ($p < 0.05$)

Table 2

Distribution of fatty acids in crab gonads

Abbreviation	Detection frequency (%)	Percentile (mg 100g^{-1})				
		25th	50th	75th	Maximum	Mean
C4:0	ND	-	-	-	-	-
C6:0	52.94	-	1.63	4.89	9.43	2.69
C8:0	100	5.59	6.06	6.57	24.81	7.13
C10:0	100	7.60	9.06	9.36	25.14	9.34
C11:0	ND	-	-	-	-	-
C12:0	100	18.91	29.81	31.87	73.16	29.17
C13:0	100	7.01	8.93	11.19	21.49	9.75
C14:0	100	178.49	338.62	537.07	755.70	350.50
C14:1	100	19.42	50.59	97.86	118.08	56.41
C15:0	100	54.70	92.82	144.12	181.37	96.38
C15:1	ND	-	-	-	-	-
C16:0	100	1669.37	2083.94	2519.33	5631.49	2341.52
C16:1	100	830.37	1047.71	1505.30	3402.25	1329.30
C17:0	100	48.88	80.70	118.06	173.58	87.65
C17:1	100	43.97	71.69	109.13	195.01	83.73
C18:0	100	267.84	336.02	395.19	841.47	363.02
C18:1n9t	100	37.27	56.52	82.12	231.50	73.50
C18:1n9c	100	2186.39	2571.46	3306.56	7773.32	3036.25
C18:2n6t	ND	-	-	-	-	-
C18:2n6c	100	1201.22	1656.27	2297.24	5048.97	1873.42
C20:0	100	31.13	35.94	54.50	84.40	41.83
C18:3n6	100	16.35	18.84	25.02	71.58	22.23
C20:1	100	12.50	16.75	20.80	102.28	20.30
C18:3n3	100	281.29	402.50	468.10	964.27	407.59
C21:0	100	9.23	10.24	15.66	38.93	13.75
C20:2	100	94.35	133.82	155.71	374.54	149.82
C22:0	100	15.03	23.47	34.29	66.24	25.97
C20:3n6	100	25.14	32.54	37.79	128.94	40.77
C22:1n9	100	10.21	19.63	31.64	43.70	21.85
C20:3n3	100	38.40	54.95	83.99	217.15	68.70
C20:4n6	100	96.68	150.36	174.32	407.14	167.52
C23:0	100	15.10	25.09	31.13	78.76	28.33
C22:2	100	2.73	3.34	4.38	9.74	4.24
C24:0	100	12.42	17.32	23.02	50.07	19.70
C20:5n3	100	206.15	338.46	476.44	691.65	344.24
C24:1	100	13.96	25.15	37.46	58.36	26.38
C22:6n3	100	482.72	891.35	1093.81	1324.45	769.28
Total fatty acids	/	8654.14	11227.59	13460.34	28208.63	11922.26

ND: Not Detected

Table 3

Distribution of fatty acids in crab muscles

Abbreviation	Detection frequency (%)	Percentile (mg 100g^{-1})				
		25th	50th	75th	Maximum	Mean
C4:0	88.24	0.70	0.87	0.96	4.65	1.18
C6:0	70.59	-	0.98	1.35	4.30	1.03
C8:0	58.82	-	0.49	0.80	4.22	0.72
C10:0	47.06	-	-	0.62	3.26	0.42
C11:0	ND	-	-	-	-	-
C12:0	ND	-	-	-	-	-
C13:0	ND	-	-	-	-	-
C14:0	100	1.03	1.74	3.74	34.66	4.48
C14:1	64.71	-	0.78	0.93	9.56	1.11
C15:0	100	0.46	0.72	1.29	6.83	1.17
C15:1	ND	-	-	-	-	-
C16:0	100	10.72	29.81	41.03	247.69	41.45
C16:1	100	7.10	13.92	25.73	133.61	21.57
C17:0	100	0.80	1.30	2.17	10.68	2.03
C17:1	94.12	0.79	1.31	2.04	9.72	1.99
C18:0	100	8.26	18.65	20.99	99.95	21.74
C18:1n9t	82.35	0.43	0.82	1.47	3.78	1.05
C18:1n9c	100	25.48	69.68	98.31	424.97	90.10
C18:2n6t	ND	-	-	-	-	-
C18:2n6c	100	15.61	46.25	55.20	177.76	50.97
C20:0	100	0.57	0.95	1.46	6.52	1.36
C18:3n6	ND	-	-	-	-	-
C20:1	100	1.08	1.84	2.64	23.57	3.53
C18:3n3	100	3.94	7.14	12.23	42.32	10.70
C21:0	52.94	-	0.19	0.31	0.82	0.20
C20:2	100	2.22	6.31	9.40	18.76	6.71
C22:0	100	0.71	1.27	1.94	6.21	1.68
C20:3n6	41.18	-	-	1.31	2.44	0.68
C22:1n9	ND	-	-	-	-	-
C20:3n3	100	0.65	1.03	1.63	3.58	1.22
C20:4n6	100	5.87	17.91	27.98	74.92	20.43
C23:0	ND	-	-	-	-	-
C22:2	ND	-	-	-	-	-
C24:0	ND	-	-	-	-	-
C20:5n3	100	24.24	43.82	56.79	304.72	61.02
C24:1	ND	-	-	-	-	-
C22:6n3	100	32.90	50.38	72.24	467.12	84.21
Total fatty acids	/	144.90	313.94	413.57	2122.05	432.73

ND: Not Detected

frozen trash fish group than in the formulated feed group. The levels of three other fatty acids (C18:1n9c, C16:0, and C18:2n6c) were higher ($p < 0.05$) in the formulated feed group than in the frozen trash fish group (Fig. 2A). In crab muscle samples, the levels of six major fatty acids (C18:1n9c, C18:2n6c, C16:0, C16:1, C20:5n3, and C22:6n3) were significantly ($p < 0.05$) higher than those of other fatty acids, two of which (C20:5n3 and C22:6n3) were present at significantly higher levels ($p < 0.05$) in the frozen trash fish group than in the formulated feed group and the levels of four fatty acids (C18:1n9c, C18:2n6c, C16:0, and C16:1) were higher ($p < 0.05$) in the formulated feed group than in the frozen trash fish group (Fig. 2B).

We also compared the mean content of different fatty acids in gonad and muscle samples from crabs in different feed groups. In gonad samples, the mean content of four major fatty acids (C18:1n9c, C16:0, C18:2n6c, and C20:4n6) was higher ($p < 0.05$) in the formulated feed group than in the frozen trash fish group, whereas the mean content of two fatty acids (C22:6n3 and C20:5n3) was significantly higher ($p < 0.05$) in the frozen trash fish group compared to the formulated feed group (Fig. 3A). In muscle samples, the mean content of four major fatty acids (C18:1n9c, C16:0, C22:6n3, and C20:5n3) was higher in the frozen trash fish group than in the formulated feed group, whereas the content of C18:2n6c was higher ($p < 0.05$) in the formulated feed group compared to the frozen trash fish group (Fig. 3B).

The differences in fatty acids between samples of crabs from different feed groups may be due to differences in the fatty acid composition of the two types of feed. To determine this possible correlation, we quantified the fatty acid composition of the two different types of feed (Fig. 4) to find significant differences ($p < 0.05$) in the fatty acid composition of crabs fed on the formulated feed and frozen trash fish (Fig. 5). The results showed a significant correlation (R^2 = 0.556, 0.684, 0.5004, 0.4149, respectively) between fatty acids in crab samples and feed samples regardless of the type of feed. In the artificially formulated feed, the total fatty acid content was 4015.23 mg 100 g^{-1} with six most abundant fatty acids (C20:5n3, C22:6n3, C18:0, C18:3n3, C16:0, and C18:2n6c) accounting for 86.59% of the total fatty acid content.

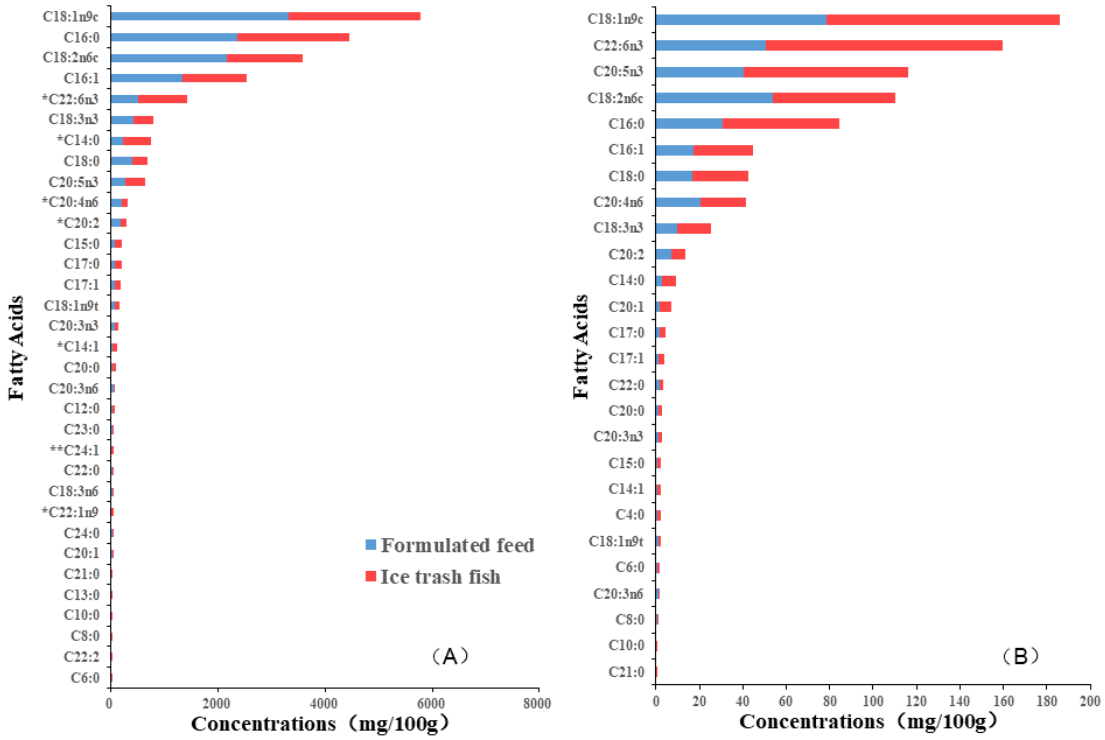

Figure 3

Comparison of average fatty acid content in samples from crabs receiving different types of feed. (A) Comparison of levels of different fatty acids in gonad samples from different feed groups. (B) Comparison of levels of various fatty acids from muscle samples from different feed groups

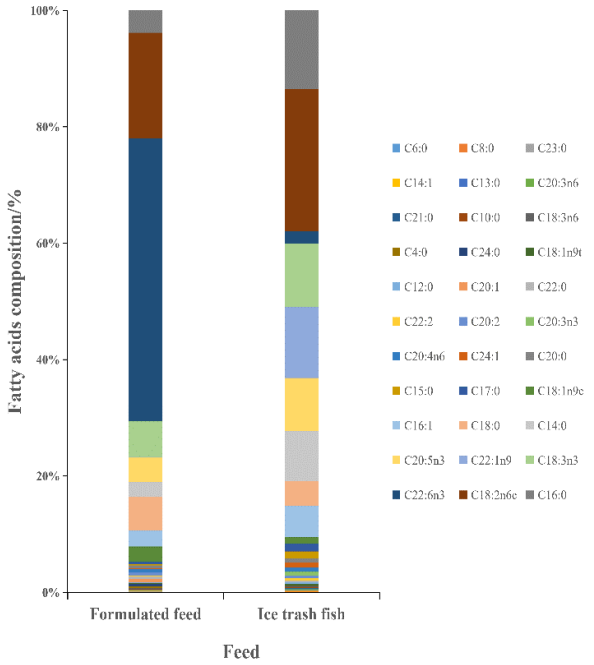

Figure 4

Fatty acid composition in formulated feed and frozen trash fish

In frozen trash fish, the total fatty acid content was 7255.68 mg 100 g^{-1} with eight major fatty acids (C18:0, C16:1, C14:0, C20:5n3, C18:3n3, C22:1n9, C22:6n3, and C16:0) accounting for 88.34% of the total fatty acid content.

During the phase of rapid gonadal development, oleic acid was found to be the most abundant fatty acid, and its ratio increased significantly throughout the development of gonads (Tang, et al. 2014; Teng et al. 2008). Oleic acid is not an essential fatty acid because it can be synthesized by the body. For this reason, we excluded oleic acid from our evaluation of the correlation between fatty acids in feed and crab samples.

3.3. Effects of differences in fatty acid content in Chinese mitten crabs from different feed groups on nutritional value

Fatty acid levels in the Chinese mitten crab were significantly higher than those described in other aquatic products. The mean fatty acid content in muscle samples from the Chinese mitten crab fed frozen trash fish and formulated feed were 529.04 mg 100 g^{-1} and 344.08 mg 100 g^{-1}, respectively, thus lower

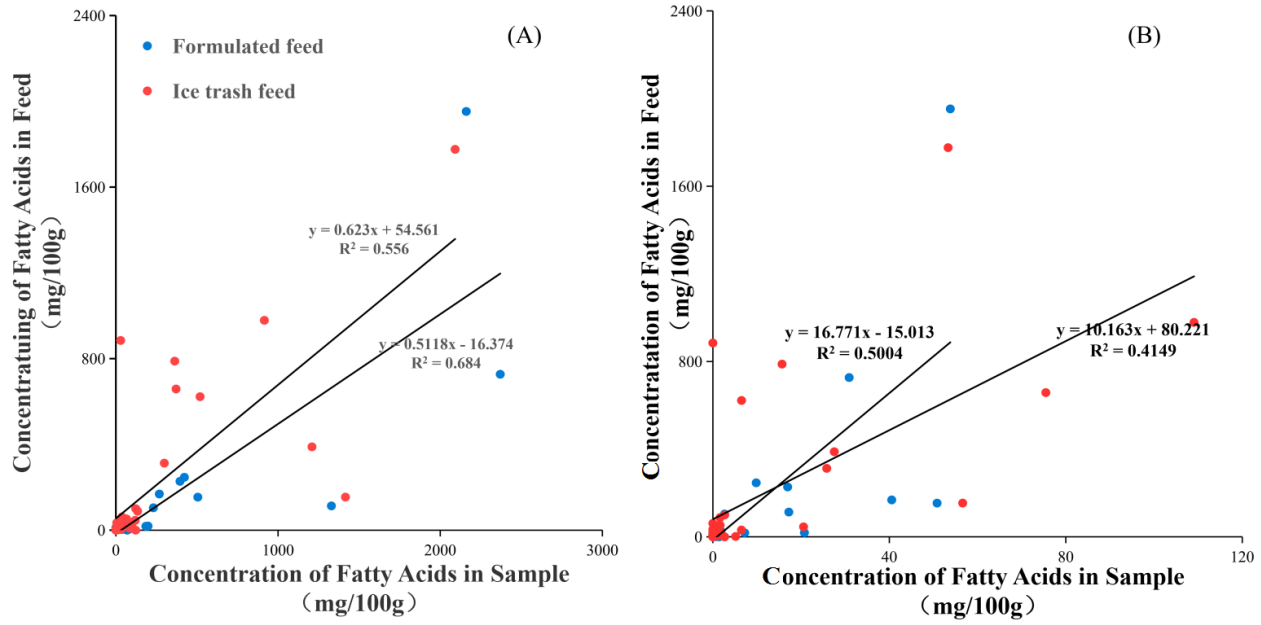

Figure 5

Correlations between fatty acids in feed and fatty acids in samples. (A) Analysis of correlation between levels of fatty acids in samples and their levels in feed by using levels of fatty acids in feed (y-axis coordinates) and their corresponding levels in crab gonad samples (x-axis coordinates). (B) Analysis of correlation between levels of fatty acids in samples and their levels in feed by using levels of fatty acids in feed (y-axis coordinates) and their levels in crab muscle samples (x-axis coordinates)

than in other aquatic products. The SFA:MUFA:PUFA ratios in gonad samples from crabs fed frozen trash fish and formulated feed were 1:1.19:0.98 and 1:1.47:1.11, respectively, whereas the SFA:MUFA:PUFA ratios in muscle samples from crabs fed frozen trash fish and formulated feed were 1:1.48:2.85 and 1:1.75:3.09, respectively (Fig. 6A). The mean fatty acid content in gonad samples from Chinese mitten crabs fed frozen trash fish and formulated feed were 10 818.11 mg 100 g^{-1} and 12 118.29 mg 100 g^{-1}, respectively (Fig. 6B). The ω-3PUFA+ω-6PUFA content in gonads of Chinese mitten crabs fed frozen trash fish and formulated feed were 3307.38 mg 100 g^{-1} and 3701.82 mg 100 g^{-1}, respectively, thus higher compared to other aquatic products. The ω-3PUFA+ω-6PUFA content in muscle samples of Chinese mitten crabs fed frozen trash fish and formulated feed were 279.31 mg 100 g^{-1} and 178.16 mg 100 g^{-1}, respectively. The ω-6 PUFA/ω-3 PUFA ratio in gonad samples from crabs fed frozen trash fish and formulated feed were 1.91:1 and 0.93:1, respectively, whereas the ω-6 PUFA/ω-3 PUFA ratio in muscle samples from crabs fed frozen trash fish and formulated feed were 0.74:1 and 0.39:1, respectively. We observed a significant difference in the mean

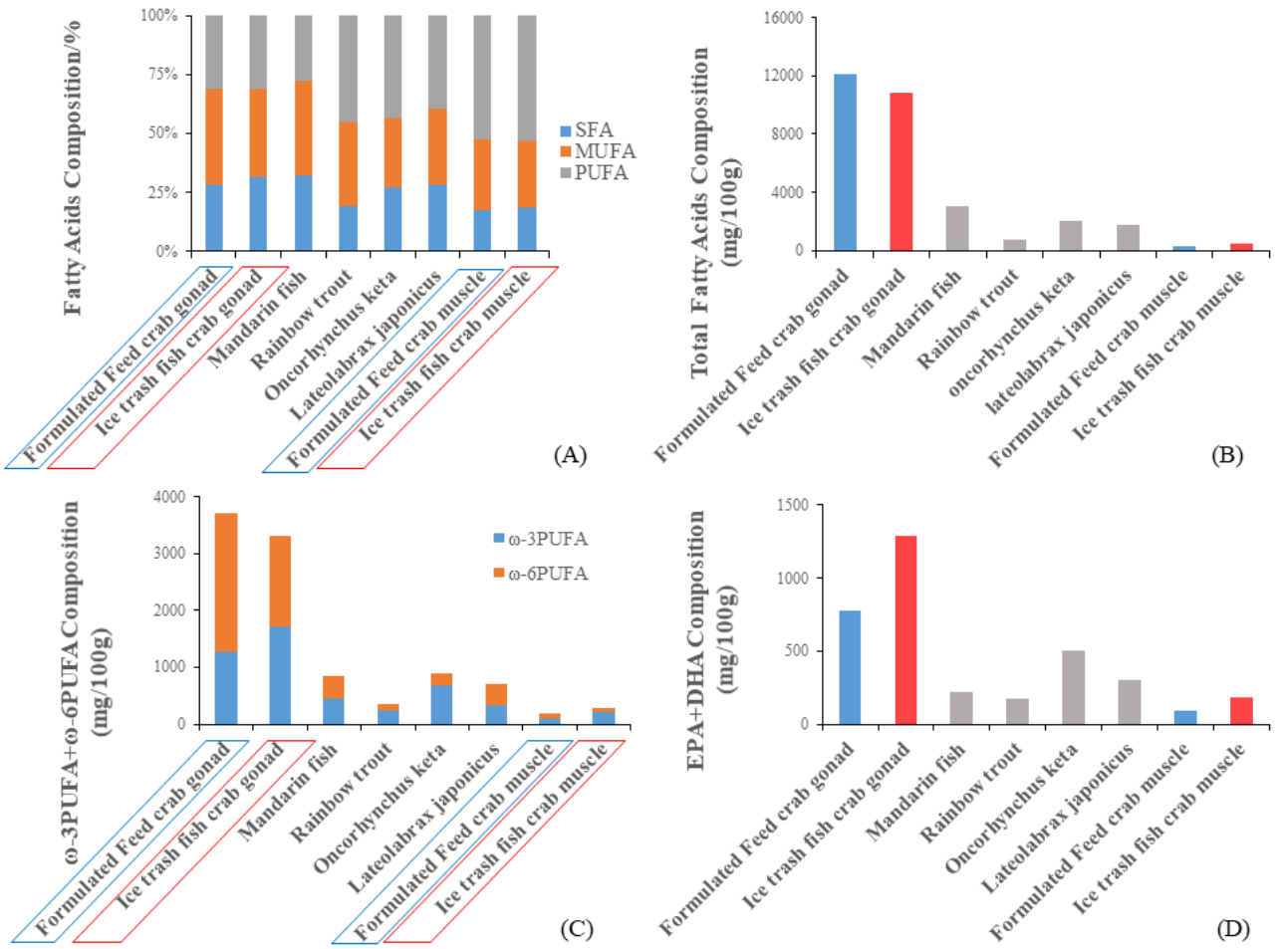

Figure 6

Nutritional value of fatty acids. (A) Comparison of SFA, MUFA, PUFA, and trans-fatty acid levels between gonad and muscle samples from two different feed groups and common food products. (B) Comparison of total fatty acid content between gonad and muscle samples from crabs receiving two different types of feed and different fish products. (C) Comparison of total ω-3PUFA and ω-6PUFA content between gonad and muscle samples from crabs receiving two different types of feed and different fish products. (D) Comparison of DHA+EPA content between gonad and muscle samples from crabs receiving two different types of feed and different fish products. Data on other fish products come from Zhang et al. (2014).

ω-3 PUFA content between the two feed groups. The gonads and muscles of crabs in the frozen trash fish group showed a higher content and a higher ratio of ω-3 PUFA than those in the group fed formulated feed (Fig. 6C). The DHA+EPA content in the gonads of crabs from the frozen trash fish group was the highest among aquatic products at 1286.66 mg 100 g^{-1} (Fig. 6D). The DHA+EPA content in the gonads of crabs fed formulated feed was significantly lower than that of crabs fed frozen trash fish, however, this level was higher than that observed in other aquatic products. The DHA+EPA content in muscle samples from crabs fed formulated feed and frozen trash fish was lower than that found in all other aquatic products, except rainbow trout.

4. Discussion

In this study, 34 types of fatty acids were found in crab gonads and 26 fatty acids in crab muscles, seven of which were primary fatty acids (C16:0, C18:1n9c, C18:2n6c, C20:4n6, C20:5n3, and C22:6n3). These findings are similar to those reported by Que et al. (2012). The content of PUFA, accounting for 53% of the total fatty acids, was higher compared to that of SFA and MUFA in crab muscles, which is generally consistent with the results reported by Shao (2012). Among the fatty acids, oleic acid showed the highest level in crab gonads, accounting for 24.68% of the total fatty acid content, while it accounted for 20.93% of the total fatty acid content in crab muscles. This level was lower compared to the results reported by Chen et al. (2007).

The fatty acid composition in crabs is affected by the type of fatty acids present in the feed source (Sui et al. 2007), which in turn affects the growth, reproduction and nutritional value of crabs (Wen et al. 2001; Que 2012). Changes in the type of feed inevitably lead to changes in fatty acids present in crab samples. The differences in the content of fatty acids in Chinese mitten crabs from different feed groups are mainly related to five fatty acids (C20:5n3, C22:6n3, C18:1n9c, C16:0, and C18:2n6c), including primarily C20:5n3, C22:6n3 and C18:2n6c, which can only be derived from feed administered to mitten crabs (Kan 2012). Therefore, differences in the content of essential fatty acids between formulated feed and frozen trash fish should be carefully considered when targeting efficient growth, reproduction, and survival of cultured Chinese mitten crabs. In this study, we quantified fatty acids in gonads and muscles of Chinese mitten crabs on different diets and analyzed the correlation between fatty acids in the feed and fatty acids in the gonads and muscles of the crabs. The results revealed that the fatty acid composition in Chinese mitten crabs is significantly correlated with the fatty acid composition of the provided feed, which is similar to the results reported by Wu et al. (2010) and Wang et al. (2017). The correlation between crab gonads and the type of feed was more significant than the correlation between crab muscles and the type of feed. This may be due to the fact that lipids in the feed are absorbed by the hepatopancreas before being selectively transported to the muscles (Li et al. 2011).

Chinese mitten crabs are rich in nutrients and are a good source of fatty acids, for example their gonads contain the highest levels of fatty acids and are regularly consumed by humans. The total content of fatty acids, ω-3PUFAs, and highly unsaturated fatty acids (HUFA) in gonads of these crabs is higher than that of snakehead or perch. Chinese mitten crabs are a rich source of ω-3PUFAs (Kong et al. 2012), which are extremely important in human nutrition and health (Elagizi et al. 2018; Lavie et al. 1995). A high level of ω-3PUFAs can improve the nutritional value of Chinese mitten crabs. Consumption of food containing ω-3PUFAs is beneficial for preventing cerebro-cardiovascular disease, inflammation, cancer, and kidney disease, as well as improving neurodevelopment (Rhee et al. 2017; Tocher 2015; Tur et al. 2012). In addition, muscles with high levels of ω-3PUFAs produce higher concentrations of volatile flavor products, including saturated and unsaturated aldehydes, alcohols, and ketones (Kong et al. 2012). High levels of ω-3PUFAs are necessary to enhance the flavor of Chinese mitten crabs. In the formulated feed group, the mean content of ω-3PUFAs accounted for 10.49% and 29.77% of the total fatty acids in the gonads and muscles of the crabs, respectively. In the frozen trash fish group, the content of ω-3PUFAs was 15.86% in crab gonads and 38.11% in crab muscles. Therefore, the proportion of ω-3 PUFAs in both gonads and muscles of the crabs from the frozen trash fish group is higher than in the formulated feed group. The ratio of ω-6/ω-3 PUFAs can reflect the nutritional value of a given type of food, making it an important criterion in assessing the nutritional value of Chinese mitten crabs (Çelik et al. 2004). A low ω-6/ω-3 PUFA ratio indicates a high nutritional value (Çelik et al. 2004; Jankowska et al. 2010). According to the Chinese DRIs (Chinese Nutrition Society, 2000), the proper ratio of ω-6/ω-3 PUFAs ranges from 4:1 to 6:1. However, previous research has demonstrated that the ratio of ω-6/ω-3 PUFAs in the diets of urban and rural adults, middle-aged and elderly people in China is 7.6:1, 7.5:1 and 8.0:1, respectively, which does not meet the recommended ratio (Meng et al. 2009 and Zhang et

al. 2009). In our study, the ratio of ω-6/ω-3 PUFAs in the gonads of crabs fed frozen trash fish was 1.91:1, whereas the ratio of PUFAs in the gonads of crabs fed formulated feed was 0.93:1. Given that Chinese mitten crabs are not considered a primary food source but a dietary supplement, there is a growing opportunity to change the dietary structure and increase their nutritional value by lowering the PUFA content.

The level of HUFA is considered an important indicator of the nutritional value of food (WHO/FAO 1994; Shao 2012). HUFAs, such as DHA, EPA, and ARA, are characterized by > two double bonds and > 20 carbon atoms. In terms of physiological functions, HUFAs play an important role in the process of reproduction and development. In particular, EPA and DHA play an important role in preventing cardiovascular disease, inhibiting cancer and inflammation, and promoting the development of the nervous system and vision (Harper & Jacobson 2005; Roynette et al. 2004; Zhu et al. 2007). DHA and ARA promote fetal and central nervous system development (Muskiet et al. 2006; Roynette et al. 2004).

The DHA+EPA content in the gonads and muscles of Chinese mitten crabs fed frozen trash fish was 1286.66 mg 100 g^{-1} and 184.54 mg 100 g^{-1}, respectively. Therefore, the levels of EPA, DHA, and other HUFAs in both the gonads and muscles of Chinese mitten crabs fed frozen trash fish were all significantly higher compared to those on formulated feed diet.

Chinese mitten crabs fed frozen trash fish are characterized by higher concentrations of EPA and other HUFAs due to different composition of feed. In this study, we confirmed a significant correlation between fatty acids in crab samples and feed composition. This is similar to the results presented by Xu et al. (2020) and Turchini et al. (2010), confirming that fatty acid profiles of farmed fish reflect their diet. At present, oil in the formulated feed is the most important fatty acid source for the Chinese mitten crab. However, with the increasing use of vegetable and rendered animal oils and fats, the addition of ω-3 PUFA-rich fish oil has decreased (Xu et al. 2020; Tocher et al. 2019). This reduces the nutritional value of Chinese mitten crabs fed formulated feed. By altering the fatty acid composition of cultured fish, the composition of aquatic feed directly affects the dietary fatty acid intake by humans (Xu et al. 2020).

In general, it is necessary to understand the relationship between the content of fatty acids in the feed and in the Chinese mitten crab, which is very important for improving the commercial value and nutritional benefits of the Chinese mitten crab. To meet the quality requirement, the formula of formulated feed needs to be improved. For example, alternative oils, consisting of genetically modified oilseeds and single cell oils containing ω-3 PUFA, which are currently being developed and are at different stages of commercial implementation are considered the most efficient feedstock (Osmond et al. 2019)..

5. Conclusion

The Chinese mitten crab is a high-quality aquatic product of China, ranking as an important product in China's aquaculture industry. The content of ω-3 PUFAs, such as EPA and DHA, is higher in crabs receiving frozen trash fish than in crabs from the formulated feed group. Fatty acids in crabs are significantly correlated with the feed composition. The oleic acid, linoleic acid, palmitic acid, EPA, and DHA are mainly affected by substituting trash fish feed for formulated feed, these fatty acids, to a certain extent, represent the nutritional value of Chinese mitten crabs. Compared with the frozen trash fish feed group, the level of the aforementioned fatty acids in the formulated feed group could reach a similar level by adding exogenous ω-3 PUFA to the formula. Thus, further research to improve the formula can significantly contribute to the promotion of environmentally friendly aquaculture.

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Supplementary material

Names and classification of 37 fatty acids

No	Names	Abbreviation	CAS Number	Category 1	Category 2
1	Methyl butyrate	C4:0	623-42-7	SFA	SCFA
2	Methyl hexanoate	C6:0	106-70-7	SFA	MCFA
3	Methyl octanoate	C8:0	111-11-5	SFA	MCFA
4	Methyl decanoate	C10:0	110-42-9	SFA	MCFA
5	Methyl undecanoate	C11:0	1731-86-8	SFA	MCFA
6	Methyl laurate	C12:0	111-82-0	SFA	MCFA
7	Methyl tridecanoate	C13:0	1731-88-0	SFA	LCFA
8	Methyl myristate	C14:0	124-10-7	SFA	LCFA
9	Methyl myristoleate	C14:1	56219-06-8	MUFA	LCFA
10	Methyl pentadecanoate	C15:0	7132-64-1	SFA	LCFA
11	Methyl cis-10-pentadecenoate	C15:1	90176-53-6	MUFA	LCFA
12	Methyl palmitate	C16:0	112-39-0	SFA	LCFA
13	Methyl palmitoleate	C16:1	1120-25-8	MUFA	LCFA
14	Methyl heptadecanoate	C17:0	1731-92-6	SFA	LCFA
15	cis-10-Heptadecanoic acid methyl ester	C17:1	75190-82-8	MUFA	LCFA
16	Methyl octadecanoate	C18:0	112-61-8	SFA	LCFA
17	trans-9-Elaidic acid methyl ester	C18:1n9t	1937-61-88	MUFA	LCFA
18	cis-9-Oleic acid methyl ester	C18:1n9c	112-62-9	MUFA	LCFA
19	Methyl linolelaidate	C18:2n6t	2566-97-4	PUFA	LCFA
20	Methyl linoleate	C18:2n6c	112-63-0	PUFA	LCFA
21	Methyl arachidate	C20:0	1120-28-1	SFA	LCFA
22	Methyl γ-linolenate	C18:3n6	16326-32-2	PUFA	LCFA
23	Methyl cis-11-eicosenoate	C20:1	2390/9/2	MUFA	LCFA
24	Methyl linolenate	C18:3n3	301-00-8	PUFA	LCFA
25	Methyl heneicosanoate	C21:0	6064-90-0	SFA	LCFA
26	cis-11,14-Eicosadienoic acid methyl ester	C20:2	2463/2/7	PUFA	LCFA
27	Methyl behenate	C22:0	929-77-1	SFA	LCFA
28	cis-8,11,14-Eicosatrienoic acid methyl ester	C20:3n6	21061-10-9	PUFA	LCFA
29	Methyl erucate	C22:1n9	1120-34-9	MUFA	LCFA
30	cis-11,14,17-Eicosatrienoic acid methyl ester	C20:3n3	55682-88-7	PUFA	LCFA
31	cis-5,8,11,14-Eicosatetraenoic acid methyl ester	C20:4n6	2433-97-8	PUFA	LCFA
32	Methyl tricosanoate	C23:0	2566-89-4	SFA	LCFA
33	cis-13,16-Docosadienoic acid methyl ester	C22:2	61012-47-6	PUFA	LCFA
34	Methyl lignocerate	C24:0	2442-49-1	SFA	LCFA
35	cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester	C20:5n3	2734-47-6	PUFA	LCFA
36	Methyl nervonate	C24:1	2733-88-2	MUFA	LCFA
37	cis-4,7,10,13,16,19-Docosahexaenoic acid methyl ester	C22:6n3	2566-90-7	PUFA	LCFA