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Cholesterol, Lipid Content, and Fatty Acid Composition of Different Tissues of Farmed Cobia (*Rachycentron canadum*) from China

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Abstract Marine fishes are rich in n-3 polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are extremely important for human health. The objective of our work was to determine the content and composition of lipids and fatty acids in the different tissues of cobia from China and to evaluate their nutritional value. The results showed that cobia from China was rich in lipids; the neutral lipid content was above 82%; the content of cholesterol and phospholipid was low. Eighteen fatty acids were identified. Myristic (C14:0), palmitic (C16:0), and stearic acids (C18:0) were the main saturated acids; palmitoleic (C16:1n-7) and oleic acid (C18:1n-9) were the main monounsaturated fatty acids. EPA and DHA were the main PUFA; n-3 and n-6 PUFA were present as 12–18% and 2.6–3.2% of the total fatty acids, respectively. The n-6/n-3 ratio was in the range from 0.18 to 0.22, which was far lower than that (5:1) recommended by WHO/FAO. Therefore, cobia lipids from China have a high nutritional value.

Keywords Cobia · Lipid · Cholesterol · Phospholipid · Fatty acid

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Introduction

Cobia, *Rachycentron canadum*, is a large migratory coastal pelagic fish of the monotypic family *Rachycentridae* and is distributed worldwide in tropical and subtropical seas, except for the eastern Pacific [1]. The culture of cobia will presumably become an emerging aquaculture industry in the near future because of the fish's rapid growth and high quality flesh [2]. Cobia is now being cultured in many regions such as Hainan Province of China, Taiwan, Japan, and Vietnam, especially using sea-cage farming. Cobia is a famous deluxe food fish and highly preferred by Oriental consumers, especially people in Taiwan and Japan [3]. Recently, cobia has been cited as producing a high quality fillet suitable for sashimi and for restaurants [4].

Cobia has high nutritional and medicinal value due to its balanced composition of essential amino acids, its richness in polyunsaturated fatty acids, and its comprehensive supply of microelements [5]. In particular, the content of n-3 polyunsaturated fatty acids (n-3 PUFA), mainly eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), is high in the cobia lipids. The importance of n-3 PUFA, especially EPA and DHA, in human nutrition has been widely recognized [6]. Their potential for the prevention of certain cardiovascular conditions and other diseases has been reported [7–9]. n-3 PUFA has an indispensable role in the synthesis of prostaglandins, tromboxaines, leukotrienes, and eicosanoids [10, 11]. Therefore, the nutritional value of lipids is very important for human health.

For the nutritive evaluation of fish, the content of essential fatty acids is as important as the lipid content. Due to the great importance of n-3 PUFA, the quality of lipids is presently evaluated on the basis of the n-6

PUFA/n-3 PUFA ratio [10]. The WHO/FAO recommendation is that, in total daily diet, the n-6/n-3 ratio should be 5:1 [12, 13], but in fact the lower the ratio the better. According to the recommendations of the WHO, about 30% of human energy requirements should be satisfied from fats, whereby saturated fatty acids (SFA) should account for about 10% and PUFA up to 7% [12–14]. The recommended total intake of EPA and DHA is in the range of 0.3–0.4 g/day [15]. It is also thought that daily intake of 2 g/day of n-3 PUFA may completely satisfy the daily needs of the human organism [15]. On the basis of these data, it is possible to estimate whether the amount of consumed fish can satisfy the daily requirements of the human organism for EPA and DHA. Therefore, an understanding of fish intake is very important for evaluating whether the fish has sufficient nutritional value to satisfy daily EPA and DHA requirements [10].

At present, research on cobia mainly concentrates on breeding, culture, disease prevention, and feed. The study of cobia processing is, however, rarely reported. This work analyzes the lipid and fatty acid composition in the different tissues of cobia from China and evaluates their nutritional value to provide scientific data for food processing and pharmaceuticals.

Materials and Methods

The cobia (*Rachycentron canadum*) were cultured in marine cages located offshore of Hainan Province, China. When the cobia reached 5–6 kg, they were harvested and transported to a processing factory located in Zhanjiang City, Guangdong Province, China. Upon arrival, 10 specimens from 1,000 kg of cobia were collected, and each cobia was divided into five parts (fish back muscle, abdomen muscle, fish head, fish skin, viscera). Each part was ground and mixed by grinder. The samples (stored on ice during transportation) were taken to the laboratory, and they were immediately packed in plastic bags and stored at −20 °C until use. A 10-g sample of each part was used to extract total lipids every time. All reagents were of analytical grade.

Extraction of Total Lipids

The samples of back muscle, abdomen muscle, fish head, fish skin, and viscera from cobia were homogenized thoroughly with blenders and mixing mills. Lipid was then extracted in duplicate aliquots in chloroform:methanol (2:1; v:v) after Folch et al. [16] as modified by Iverson et al. [17]. Each part was analyzed in triplicate.

Separation of Neutral and Polar Lipids

Separation of neutral and polar lipids was accomplished on a preparative scale silica gel column (230–400 mesh; Sigma-Aldrich, St. Louis, MO, USA) [18]. A petroleum ether (30–60 °C) slurry of silica gel was poured to a depth of 1.3 cm into a column consisting of a disposable Pasteur pipette plugged with glass wool. The gel was washed with about 5 mL petroleum ether followed by 10 mL dichloromethane–methanol (19:1 by volume). A 2-mL aliquot of chloroform lipid extract containing approximately 10 mg total lipid was added to the column. Neutral lipids were eluted with 3 mL chloroform, followed by 14 mL dichloromethane–methanol solution. Polar lipids were then eluted with 24 mL methanol. Each part was analyzed in triplicate.

Analysis of Cholesterol

Analysis of cholesterol was carried out as described by Osman et al. [19] and Bohac et al. [20]. The procedure was as follows. Each part was analyzed in triplicate.

The stock reagent was prepared by dissolving 10 g of $FeCl_3 \cdot 6H_2O$ in glacial acetic acid using a 100-mL volumetric flask. Prior to use, 1.0 mL of the stock reagent was transferred into a 100-mL flask and concentrated H_2SO_4 was added to volume.

The stock solution (1 mg/mL) was prepared by dissolving 0.1 g of standard cholesterol (chromatography grade; Sigma Chemical, USA) in glacial acetic acid using a 100-mL volumetric flask. The stock solution was used to prepare working solutions containing 0.0, 0.05, 0.10, 0.15, and 0.20 mg cholesterol. Total volume of each working solution was added to 4 mL glacial acetic acid. Then, 2 mL of $FeCl_3$ coloring reagent was added, and the absorbance at 565 nm was determined in a UV-2450/2550 spectrophotometer (Shimadzu; Tokyo, Japan). Standard curve was determined by using the standard concentration of cholesterol for the abscissa and the absorbance of each working solution for the ordinate. The regression equation was obtained from the standard curve as $y = 0.004x - 0.0153$ ($R^2 = 0.99$).

Total lipid (0.5 g) was accurately weighed to 0.0001 g and saponified using 2% alcoholic KOH. The unsaponified fraction was extracted with 4 × 10 mL petroleum ether, and all the extracts were later combined. The extract was washed with 0.5 mol/L NaOH and repeatedly with distilled water until neutral. Traces of moisture were removed by the addition of a sufficient amount of anhydrous sodium sulphate. Finally, the extract was heated in a water bath to remove the petroleum ether.

The dried extract was resuspended in 4 mL glacial acetic acid, 2 mL of $FeCl_3$ coloring solution was added, and the resultant color was read at 565 nm. The cholesterol content was calculated using the following equation:

$$x(\%) = \frac{c}{m \times 1000} \times 100\%$$

where x (%) = the content of cholesterol in total lipid, c (mg) = concentration of cholesterol (from standard curve), m (g) = weight of sample.

Analysis of Phospholipid

The standard stock and working solutions were prepared according to the AOCS Official Method (method Ca 12-55) [21, 22]. Depending on the level of phosphorus in the sample, a standard curve was established from the series with 0.000, 0.001, 0.002, 0.003, 0.004, 0.005, and 0.006 mg phosphorus. Phosphorus standard solutions were placed individually in a 25-mL colorimetric tube. Deionized water was added to bring the volume to 10 mL. The following reagents were added in the following order: 1 mL sulfate solution, 1 mL ammonium molybdate solution, and 0.2 mL ascorbic acid solution. The reaction mixture was vortexed and then heated to 55 °C in a boiling water bath for l5 min with screw cap on loosely. After the reaction mixture had cooled to room temperature, the absorbance at 820 nm was determined in a UV-2450/2550 spectrophotometer (Shimadzu; Tokyo, Japan). Standard curve was determined using the standard concentration of phosphorus for the abscissa and the absorbance of each working solution for the ordinate. The regression equation was obtained from the standard curve as $y = 69.929x - 0.0012$ ($R^2 = 0.99$).

Total lipid (0.5 g) was accurately weighed to 0.0001 g into a 250-mL flat flask. Then, 10 mL concentrated sulfuric acid was added. The flat flask was placed in a block heater at 200 °C. After the flat flask was heated, it was removed and cooled. One drop of concentrated hydrogen peroxide was added to the flat flask, which was again placed in the block heater at 200 °C. The flat flask was removed and cooled. The flat flask that was not colorless was reheated with additional hydrogen peroxide to complete the wet ashing procedure. The wet ashing sample was then transferred to a 25-mL volumetric flask, and distilled water was added to 25 mL. The blank was prepared with the same treatments and reagents, except that the oil was omitted.

The sample and blank solution of accurate volume were placed in a 25-mL colorimetric tube and dealt with the same way as described for the standard phosphorus solution. Each part was analyzed in triplicate. Phospholipid fraction of the total lipids was calculated according to the following formula:

$$x(\%) = \frac{25 \times m \times 26.31}{V_1 \times M \times 10}$$

where x (%) = the content of phospholipids in total lipid, m (mg) = concentration of phosphorus (from standard curve), V_1 (mL) = the volume of sample solution, M (g) = weight of total lipid; 26.31 mg per phosphorus is equivalent to 26.31 mg phospholipids.

Analysis of Fatty Acids

Fatty acid profiles were determined by preparation of methyl esters as described by Metcalfe et al. [23]. The fatty acid methyl esters were identified by using a Shimadzu GC-MS-QP2010 system (Shimadzu; Tokyo, Japan). A free fatty acid phase (FFAP) capillary column (30 m × 0.25 mm × 0.25 m) was used to separate the fatty acid methyl esters. The capillary column with a stationary phase of polyethylene glycol was purchased from the Dalian Institute of Chemical Physics (Dalian City, Liaoning Province, China). For chromatography, temperature parameters were set as follows: injector at 240 °C, ion source 200 °C; for the oven program: initial temperature 90 °C for 3 min, 90–240 °C at 4 °C/min, 240 °C for 10 min. Carrier gas was helium, column pressure 100 kPa, split rate 1:30. A 1-μL sample was injected. The energy of the EI source of the mass spectrometer was 70 eV. Data were calculated using the normalized peak area percentages of total fatty acid content. Each part was determined in triplicate.

Statistical Analysis

Each sample was analyzed in triplicate, and all data were described as mean ± standard deviation. The significance was analyzed by one-way ANOVA using SAS (1997, SAS Institute, Cary, NC, USA). The calculated variances were used in the least significant difference (LSD) test.

Results and Discussion

Total Lipid Content in the Different Tissues of Cobia

There was a significant difference ($p < 0.01$) among the total lipid content in the different tissues of cobia from China (Fig. 1). The total lipid content was highest in viscera (16.34 ± 0.12%), followed by abdomen muscle (11.55 ± 0.20%), fish head (9.82 ± 0.35%), back muscle (6.23 ± 0.59%), and fish skin (2.89 ± 0.10%). The total lipid content in abdomen muscle was higher than back muscle, which is a general characteristic of fish and also the main reason that abdomen muscle tastes better than back muscle. The total lipid content in fish head was higher because of the orbital fat. These results showed that the lipids of cobia from China are mainly found in muscle, head, and viscera. Cobia muscle from China is mainly used for processing into sashimi, providing a high nutritional

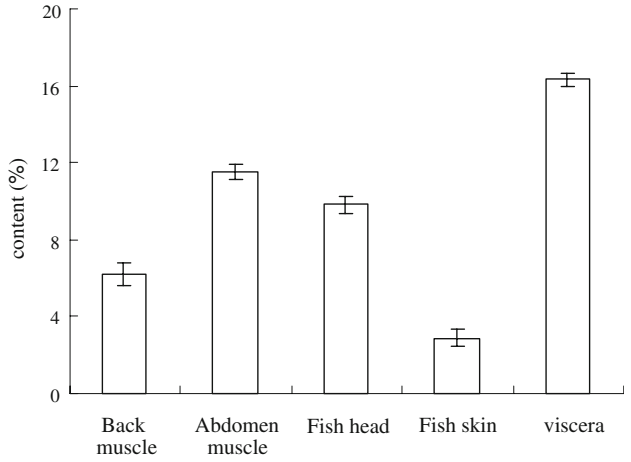

Fig. 1 Total lipid content in the different tissues of cobia

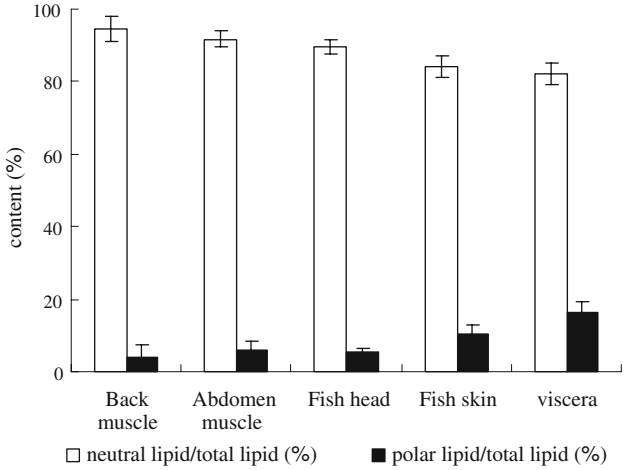

Fig. 2 Neutral and polar lipid contents in the different tissues of cobia

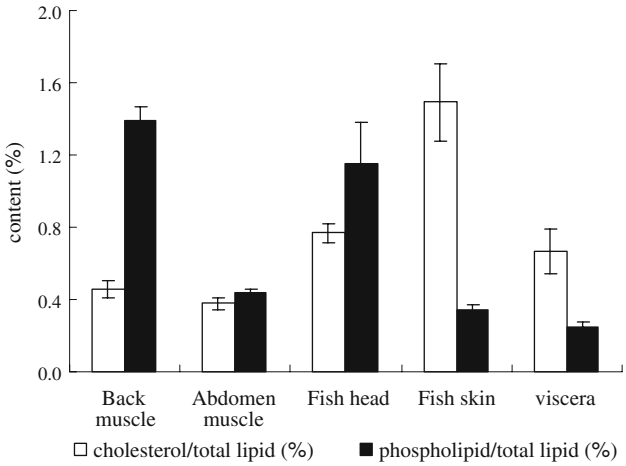

Fig. 3 Cholesterol and phospholipid contents in the different tissues of cobia

value for the product because of the richness of the fats. However, fish head, viscera, and fish skin are usually byproducts of processing sashimi. The lipid content of fish head and viscera is higher; therefore head and viscera are good raw materials for extraction of fish oil. Fish skin is usually used as a raw material for the production of collagen.

Neutral and Polar Lipid Content in the Different Tissues of Cobia

There was a significant difference ($p < 0.01$) between neutral and polar lipid content in all tissues of cobia from China (Fig. 2). There was also a significant difference ($p < 0.05$) across the different tissue types for both neutral and polar lipid content. Neutral lipids (glyceride) were the main component of cobia lipids, accounting for more than 82%. The content of neutral lipids was highest in muscle (above 91%), followed by fish head (89.68 ± 2.06%), fish skin (84.23 ± 3.14%), and viscera (82.09 ± 2.83%). The content of polar lipids in viscera (16.55 ± 2.64%) and skin (10.23 ± 2.51%) was relatively higher than in the other tissues. Neutral lipids—mainly in their function as storage lipids—provide energy for the fish activity, while polar lipids—as structure lipids—are an important part of the membrane structure.

Cholesterol and Phospholipid Contents in the Different Tissues of Cobia

The lower content of cholesterol and phospholipid was characteristic of the cobia from China, but the amount of cholesterol differed significantly ($p < 0.01$) in the different tissues (Fig. 3), ranging from 0.3 to 1.5% of total lipids, with the lowest content in the muscle (0.3–0.5%) and the highest content in the fish skin (1.49 ± 0.22%). The amount of phospholipid also differed significantly ($p < 0.01$) in the different tissues, ranging from 0.2 to 1.4% of total lipids, with the lowest content in the viscera (0.25 ± 0.03%) and the highest content in the back muscle (1.39 ± 0.08%). The content of phospholipids in fish head (1.15 ± 0.23%) was significantly higher than that of fish skin (0.34 ± 0.03%) and viscera (0.25 ± 0.03%).

According to Eilert [24] and Gertler et al. [25], rather than considering either cholesterol or phospholipid levels in serum, the cholesterol-to-phospholipid ratio as an index of atherogenicity should be considered more significant; Gertler et al. [26] suggested 0.78 as a normal cholesterol-to-phospholipid ratio [27, 28]. Their molar ratios differed along this series: back muscle (0.33)–fish head (0.67)–abdomen muscle (0.86)–viscera (2.66)–fish skin (4.39). The cholesterol-to-phospholipid ratios of back muscle and fish head are lower than the normal value, the ratio of

abdomen muscle is closer to the normal, and the ratios of viscera and fish skin are far higher than the normal value. Therefore, normal consumption of cobia from China does not present a danger of high cholesterol intake.

Fatty Acid Composition and Content in the Different Tissues of Cobia

Eighteen fatty acids were identified in the different tissues of cobia from China. Table 1 shows the content of these fatty acids in the different tissues. The data showed that the amount of fatty acids differed significantly among the different tissues, but the characteristic fatty acid composition was similar. Myristic (C14:0), palmitic (C16:0), and stearic acid (C18:0) were the predominant saturated acids, and there were also small but noteworthy amounts of tridecanoic acid (13:0), pentadecanoic acid (15:0), margaric acid (17:0), nonadecanoic acid (19:0), and arachidic acid (20:0). Palmitoleic (C16:1n-7) and oleic acid (C18:1n-9) were the major monounsaturated fatty acids, and there were also small but noteworthy amounts of gondoic acid (20:1n-9). EPA and DHA were the major PUFA, the total content of which was above 10%. The percentage of DHA exceeded that of the EPA in different tissues of cobia from China. There were also small but noteworthy amounts of linoleic acid (18:2n-6), α-linolenic acid (18:3n-3), arachidonic acid (20:4n-6), and docosapentaenoic acid (DPA, 22:5n-3).

Table 2 shows the total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), PUFA, n-6 PUFA, n-3 PUFA, and the n-6/n-3 ratios. The SFA were the most abundant fatty acids in the different tissues of cobia from China, accounting for 42–47% of the total fatty acids. The second-most abundant fatty acids were the MUFA, which

Table 1 Fatty acid composition and content in the different tissues of cobia (wt% of total fatty acids)

Fatty acids	Back muscle	Abdomen muscle	Fish head	Fish skin	Viscera
12:0	0.08 ± 0.01	0.07 ± 0.00	0.06 ± 0.00	0.07 ± 0.01	0.05 ± 0.02
13:0	0.03 ± 0.00	3.60 ± 0.03	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
14:0	4.10 ± 0.03	3.23 ± 0.01	3.49 ± 0.03	3.58 ± 0.02	3.58 ± 0.05
15:0	0.66 ± 0.04	0.55 ± 0.00	0.65 ± 0.01	0.60 ± 0.00	0.62 ± 0.02
16:0	27.61 ± 0.32	23.60 ± 0.41	26.48 ± 0.38	28.76 ± 0.52	27.25 ± 0.39
16:1 (n-7)	9.97 ± 0.11	8.15 ± 0.13	9.42 ± 0.20	9.61 ± 0.18	9.58 ± 0.21
17:0	0.94 ± 0.03	1.21 ± 0.05	1.04 ± 0.02	0.82 ± 0.02	0.92 ± 0.03
18:0	12.08 ± 0.15	9.87 ± 0.13	11.13 ± 0.14	10.70 ± 0.21	12.78 ± 0.35
18:1 (n-9)	27.09 ± 0.46	25.09 ± 0.45	24.32 ± 0.38	26.31 ± 0.24	27.68 ± 0.48
18:2 (n-6)	1.04 ± 0.04	1.11 ± 0.02	1.13 ± 0.04	0.80 ± 0.05	1.05 ± 0.03
19:0	0.34 ± 0.01	0.38 ± 0.02	0.40 ± 0.03	0.32 ± 0.01	0.39 ± 0.02
18:03 (n-3)	0.51 ± 0.03	0.53 ± 0.00	0.55 ± 0.05	0.41 ± 0.06	0.52 ± 0.03
20:0	0.51 ± 0.02	0.43 ± 0.01	0.57 ± 0.00	0.45 ± 0.02	0.58 ± 0.04
20:1 (n-9)	0.83 ± 0.05	1.49 ± 0.03	0.94 ± 0.01	0.92 ± 0.04	0.97 ± 0.03
20:4 (n-6)	1.62 ± 0.03	1.98 ± 0.05	1.95 ± 0.03	2.39 ± 0.02	1.67 ± 0.10
20:5 (EPA, n-3)	3.22 ± 0.04	4.37 ± 0.05	4.29 ± 0.07	3.87 ± 0.08	2.87 ± 0.11
22:5 (DPA, n-3)	1.62 ± 0.06	2.22 ± 0.03	2.27 ± 0.05	0.89 ± 0.01	1.81 ± 0.02
22:6 (DHA, n-3)	7.40 ± 0.17	10.16 ± 0.14	10.44 ± 0.18	9.33 ± 0.13	7.32 ± 0.15

The values are means ± SD in triplicate

Table 2 Fatty acid analysis in the different tissues of cobia (wt% of total fatty acids)

Fatty acids	Back muscle	Abdomen muscle	Fish head	Fish skin	Viscera
SFA	46.35 ± 0.25	42.94 ± 0.24	43.84 ± 0.28	45.32 ± 0.32	46.19 ± 0.40
MUFA	37.89 ± 0.32	34.73 ± 0.26	34.68 ± 0.38	36.84 ± 0.21	38.23 ± 0.18
PUFA	15.41 ± 0.15	20.37 ± 0.21	20.63 ± 0.18	17.69 ± 0.15	15.24 ± 0.17
n-3PUFA	12.75 ± 0.08	17.28 ± 0.10	17.55 ± 0.11	14.50 ± 0.09	12.52 ± 0.12
n-6PUFA	2.66 ± 0.12	3.09 ± 0.08	3.08 ± 0.14	3.19 ± 0.06	2.72 ± 0.08
n-6/n-3	0.21 ± 0.01	0.18 ± 0.02	0.18 ± 0.01	0.22 ± 0.02	0.22 ± 0.01

The values are means ± SD in triplicate

accounted for 34–39% of the total fatty acids. The PUFA accounted for 15–21% of the total fatty acids. The n-3 PUFA were present as 12–18% of the total fatty acids, most abundant of which was DHA (above 7%), while EPA and DPA were also present in important proportions. The n-6 PUFA were present as 2.6–3.2% of the total fatty acids and were mainly linoleic acid and arachidonic acid.

Total PUFA content, n-3 PUFA content, and n-6/n-3 ratios are essential for the evaluation of the biological and nutritional value of lipids. It is considered biologically more important that the n-3 PUFA content be enhanced relative to n-6 PUFA, which should result in a lower n-6/n-3 ratio [10]. As n-3 PUFA, especially those with 20 and 22 carbon atoms, shorten the process of biosynthesis of eicosanoids, their high lipid content can effectively prevent the appearance and development of cardiovascular and other diseases, as confirmed by clinical investigations [7–9]. Hence, concentrates of n-3 PUFA are already commercially available in capsule form [29].

Although the WHO/FAO recommendation is that in total daily diet the n-6/n-3 ratio should be 5:1 [12, 13], a low n-6/n-3 ratio is desirable. Lipids of cobia from China contain more n-3 PUFA (about 12.5-17.6%) and much less n-6 PUFA (about 2.6–3.1%), so that the n-6/n-3 ratio is in the range of about 0.18 to about 0.22 (nonsignificant, $p > 0.05$). Therefore, cobia lipids from China have a high biological and nutritional value, and their great importance as a significant dietary source of n-3 PUFA can be confirmed. In people consuming cobia, the risk of cardiovascular and other diseases should be lowered.

On the basis of the lipid content in the investigated muscle tissue of cobia and the composition of the fatty acids involved, the mean DHA and EPA content (back and abdomen muscle) can be calculated as 0.78 g/100 g and 0.34 g/100 g. In order to satisfy the daily needs for DHA and EPA of 0.4 and 0.3 g/day, respectively [10, 15], it can be calculated that daily consumption of cobia muscle from China should be in the range of 51–88 g. As for the intake of other essential fatty acids, in contrast to EPA and DHA, these fatty acids are also present in some other food products. Therefore, the consumption of cobia from China in normal amounts can satisfy the daily needs of DHA and EPA.

Conclusion

The study confirmed that the lipid content of cobia from China was high. Neutral lipids were the main component, and the content of cholesterol and phospholipid in total lipids was low. The PUFA accounted for 15–21% of the total fatty acids, and the ratio of n-6/n-3 PUFA was far lower than that recommended by WHO/FAO. Therefore, cobia from China have a high nutritional value in terms of lipids and can be a great dietary source of n-3 PUFA. In addition, the information presented in this study may be valuable for the food and pharmaceutical industries.

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References

1. Brown-Peterson NJ, Grier HJ, Overstreet RM (2002) Annual changes in germinal epithelium determine male reproductive classes of the cobia. J Fish Biol 60:178–202
2. Liu PC, Lin JY, Chuang WH, Lee KK (2004) Isolation and characterization of pathogenic *Vibrio harveyi* (*V. carchariae*) from the farmed marine cobia fish *Rachycentron canadum* L. with gastroenteritis syndrome. World J Microbiol Biotechnol 20:495–499
3. Miao S, Jen CC, Huang CT, Hu SH (2009) Ecological and economic analysis for cobia *Rachycentron canadum* commercial cage culture in Taiwan. Aquacult Int 17:125–141
4. Craig SR, Schwarz MH, McLean E (2006) Juvenile cobia (*Rachycentron canadum*) can utilize a wide range of protein and lipid levels without impacts on production characteristics. Aquaculture 261:384–391
5. Li LD, Chen BS, Feng J, Ke H, Cai JX, Fan K (2002) Analysis and evaluation in nutritive value of *Rachycentron canadum*. J Trop Oceanogr 21:76–82
6. Simopoulos AP (2004) Omega-6/omega-3 essential fatty acid ratio and chronic diseases. Food Rev Int 20:77–90
7. Connor WE (2000) Importance of n-3 fatty acids in health and disease. Am J Clin Nutr 71:171S–175S
8. Moyad MA (2005) An introduction to dietary/supplemental omega-3 fatty acids for general health and prevention: part II. Urol Oncol 23:36–48
9. Innis SM (2000) Essential fatty acids in infant nutrition: lessons and limitations from animal studies in relation to studies on infant fatty acid requirements. Am J Clin Nutr 71:238S–244S
10. Gordana V, Derd K, Ivan VV, Branislava J (1999) Composition of muscle tissue lipids of silver carp and bighead carp. J Am Oil Chem Soc 76:475–480
11. Jesui VV, Maximiliano DAN, Patricia DOC, Vanessa VDA, Claudio CDO, Nilson EDS (2007) Lipid content and fatty acid composition of 15 marine fish species from the southeast coast of Brazil. J Am Oil Chem Soc 84:543–547
12. FAO/WHO (1994) Lipids in early development. In: Fats and oils in human nutrition. 57:49–55
13. Newton IS (1996) Food enrichment with long-chain n-3 PUFA. INFORM 7:169–177
14. Czuczy P, Gaál Ö (1990) The importance of n-3 fatty acids in nutrition and their content in food products. Élelmezési Ipar XLVI 2:1–27
15. Peredi J (1995) Fats and health. Olaj Szappan Kozmetika 44:45–53
16. Folch J, Lees M, Sloan-Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226:497–509
17. Iverson SJ, Lang S, Cooper M (2001) Comparison of the Bligh and Dyer and Folch methods for total lipid determination in a broad range of marine tissue. Lipids 36:1283–1287

18. Wiegand MD, Johnston ATA, Martin J, Leggett WC (2004) Variation in neutral and polar lipid compositions of ova in ten reproductively isolated populations of walleye (*Sander vitreus*). Can J Fish Aquat Sci 61:110–121
19. Osman H, Yap KC (2006) Comparative sensitivities of cholesterol analysis using GC, HPLC and spectrophotometric methods. Malays J Anal Sci 10:205–210
20. Bohac CE, Rhee KS, Cross HR, Ono K (1988) Assessment of methodologies for colorimetric cholesterol assay of meats. J Food Sci 53:1642–1644
21. Firestone D (1989) Official methods and recommended practices of the American Oil Chemists' Society. AOCS Press, Champaign
22. Han TJ (1995) An improved phosphorus assay for oils without carcinogenic hydrazine sulfate. J Am Oil Chem Soc 72:881–885
23. Metcalfe LD, Schmitz AA, Pelka JR (1966) Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal Chem 38:514–515
24. Eilert ML (1949) The effect of estrogens upon the partition of the serum lipids in female patients. Am Heart J 38:472–476
25. Gertler MM, Garn SM (1950) Cholesterol esters and phospholipids in health and coronary artery disease. Science 112:14–16
26. Gertler MM, Garn SM, Bland EF (1950) Age, serum cholesterol and coronary artery disease. Circulation 2:517–522
27. Thomas HEJ, Kannel WB, Dawber TR, McNamara PM (1966) Cholesterol-phospholipid ratio in the prediction of coronary heart disease: the Framingham Study. New Engl J Med 274:701–705
28. Tanksale KG (1978) A study of cholesterol/phospholipid ratio and triene/tetraene ratio in coronary heart disease. J Postgrad Med 24:158–163
29. Haumann BF (1990) What's in those capsules? INFORM 1:117–120