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## Research Article

# Nutrients Quality and Chemical Composition of the Silver Carp (*Hypophthalmichthys molitrix*) from Lake Dianshan, Shanghai, China

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## Abstract

**Background and Objective:** Fish consumption offers important nutrients to all over the world and thus highly contributes to nutrition. Therefore, determining the biochemical composition of different fish species is of great importance to humans, this permits them to know which fish species are of high nutritive value and good for health. This study was therefore, undertaken to determine the biochemical composition, i.e., protein, carbohydrate, fat, amino acids and fatty acids from silver carp (*Hypophthalmichthys molitrix*). **Materials and Methods:** The study was carried out for a period of 3 months from Lake Dianshan in Shanghai, China. Forty-five muscle samples were taken and analyzed for their fatty acid, amino acid content and proximate compositions. For calculations used GraphPad prism 5 and MS Excel 2016. **Results:** The study revealed that the proximate composition of this species was 50% protein, 29% carbohydrate, 12% ash, 5% moisture and 4% fat. Nine indispensable essential amino acids beneficial for human health were present in this species, these amino acids namely in order of abundance were Threonine, Iso-Leucine, Tryptophan, Lysine, Phenylalanine, Valine, Histidine, Methionine and Leucine. The predominant amino acids recorded were proline, threonine, Iso-Leucine and arginine acids. For the saturated fatty acids, Palmitic acid had the highest proportion while Oleic acid was the main monounsaturated fatty acid. Alpha-linolenic, docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids were the dominant polyunsaturated fatty acids. The ratio of n3/n6 fatty acids was 1.15, this value shows that these species meat is optimal for nutritional purpose. **Conclusion:** Wild silver carp muscle Lake Dianshan had high-quality protein and also contains more essential amino acids and essential fatty acids predominantly EPA and DHA for promoting good health, prevention and healing of diseases in the human body principally DHA, which is associated with neural tissue, in particular the retina, playing an important role in neural development and vision functions. The findings of this study present a challenge to scientists to improve the nutrient quality and chemical contents of the cultured species to consumers.

**Key words:** Docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), fish consumption, fish muscle, Lake Dianshan, *Hypophthalmichthys molitrix*

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Fishes are valuable sources of high-grade protein and other organic products. Its consumption offers important nutrients to many communities around the world. Information related to the chemical composition of fishes is very much necessary to ensure that they meet requirements of man's diet. The contents of the proximate composition are traditionally used as indicators of the nutritional value of fish<sup>1</sup>.

Fish is a good source of essential fatty acids such as polyunsaturated fatty acids (PUFAs), which are known to regulate prostaglandin synthesis and hereafter induce wound healing<sup>2,3</sup>. The PUFAs such as n-3 and n-6, have been considered essential fatty acids and have been shown to have curative and preventive effects on cardiovascular diseases, neuro development in infants, cancers and fat glycemic control<sup>4,5</sup>. Numerous reports presented eicosapentaenoic acid (EPA or 20:5 $\omega$ 3) and docosahexaenoic acid (DHA or 22:6 $\omega$ 3) as the most frequent and present in tissues of many fish species and they also play a vital role in human nutrition<sup>6</sup>. Essential fatty acids are ( $\omega$ 3 and  $\omega$ 6 PUFAs), are known to have curative and preventive effects on human diseases such as cardiovascular diseases, neuro development in infants, cancers, fat glycemic control, rheumatoid arthritis and inflammation<sup>7</sup>. Many health experts suggest that two to three servings per week of seafood should be consumed in order to meet the recommended level of essential fatty acids for pregnant women, children and elderly people; it has also been suggested that consuming EPA and DHA may reduce the risk of mortality from cardiovascular disease in people who have already experienced a cardiac event<sup>8</sup>. The protein quality of food depends on their digestibility and content of essential amino acids like Leucine, Lysine and Phenylalanine<sup>9-11</sup>. Certain amino acids like aspartic acid, glycine and glutamic acid are also known to play a key role in the process of wound healing<sup>12</sup>. However, the composition of fish amino acids as one of the main protein component is influenced by various factors such as species, size, food resource, fishing season, water salinity and temperature<sup>11</sup>.

Cyprinids are multifariously located worldwide across lakes and rivers but are largely concentrated in lakes. Silver carps (*Hypophthalmichthys molitrix*) are one of the most common members of the *Cyprinidae* family. It's a freshwater species living in temperate conditions (6-28°C) and its natural distribution is in Asia. This species requires static or slow-flowing water, as found in impoundments or the backwaters of large rivers. The species is known for leaping out of the water when startled (e.g., by noises such as a boat motor). Silver carp can grow about 1 m in length and about

27 kg in weight<sup>13</sup>. The Dianshan lake is composed of about 40 fish species belonging to more than 15 families. Amongst these species, members belonging to the family *Cyprinidae* are always very common as well as dominant on the Lake. The 3 dominant and main economic fish species in Lake Dianshan are *Hypophthalmichthys molitrix*, *Carassius auratus*, *Cyprinus carpio* and all from this family. Reason, why this study is focused on *Hypophthalmichthys molitrix*, is to know its nutritional value to humans.

Presently, the proximate composition of fishery products has been widely investigated to analyze their nutritional quality<sup>14</sup>. Buchtova and Jezek<sup>15</sup> and Ashraf *et al.*<sup>16</sup> studied proximate composition, fatty and amino acids compositions of *Hypophthalmichthys molitrix* in the Czech Republic and Pakistani waters, respectively. Information given by these cited references isn't sufficient to compensate for the wild from the traditional waters in China and so there's a need for more studies on the nutritional quality of this fish species from a different source and geographical locations. This study is expected to ascertain the qualities of the Chinese native silver carp from the cultured species to which any lost nutrients in the culture can be further studied and improved for the benefit of consumers and perhaps for the utilization of other resources. The present study is therefore, undertaken to provide an insight into the proximate composition, fatty acid content and amino acid composition of the silver carp captured from Lake Dianshan, China as one of the main species (native in China) which has been broadly distributed and cultured worldwide.

## MATERIALS AND METHODS

**Description of study area:** Samples were collected from the study site for 3 months (October-December, 2016). The study site is located in China, a fresh water lake, in the suburb of Shanghai. Geographically, Dianshan Lake is found on latitude 31°11'N and longitude 120°96'E (Fig. 1). It is the largest freshwater lake in Shanghai with a total area of 63.7 km<sup>2</sup> at an average depth of 2.5 m and also with the deepest recorded depth to be 6.39 m. It is located in between Shanghai, Zhejiang and Kunshan of Jiangsu Province. This lake supports a lucrative fishery in Shanghai and fishermen involved in fishing bring in commercially important fish species.

**Sampling design and sample preparation:** A total of 20 fish species were captured during the 3 months with the help of gillnets and trawls. Gillnets (10 m long and 1.5 m wide) ranging from 2.0-10.0 cm mesh sizes were employed at each sampling point. Trawl nets (1.5 m height, 3.0 m long and

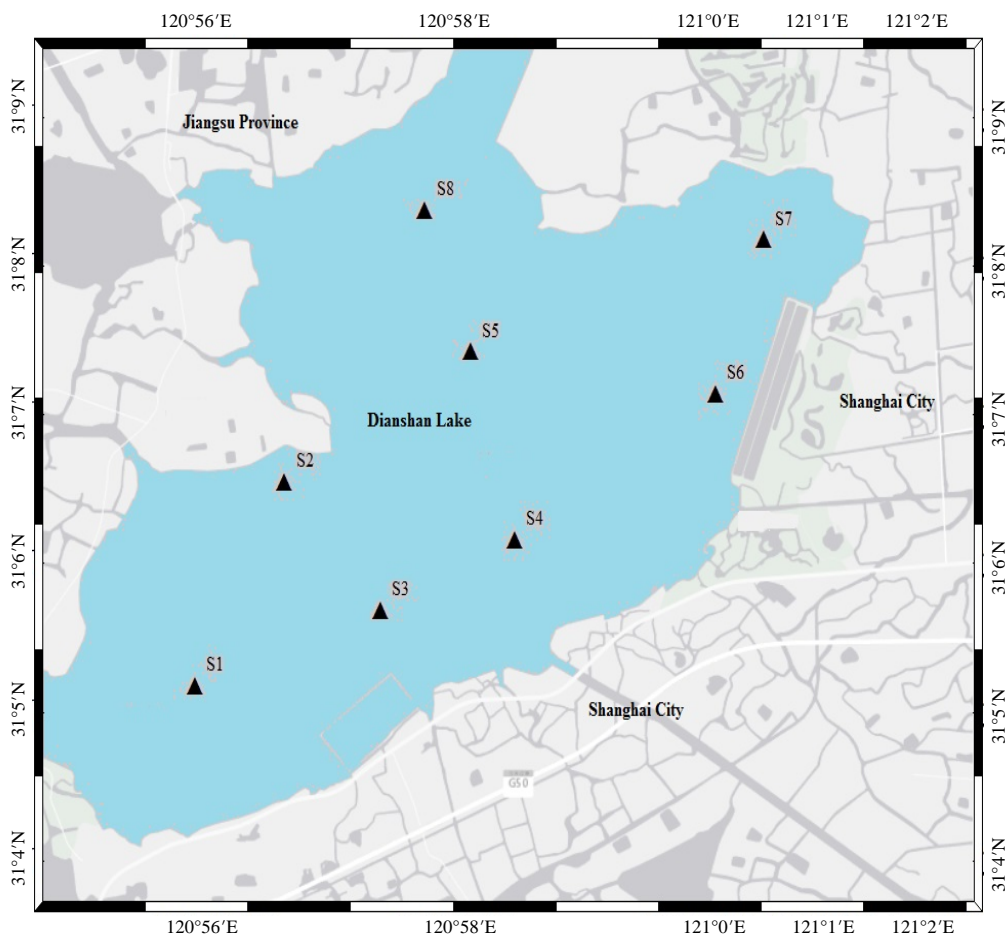


Fig. 1: Sampling site (Dianshan Lake)<sup>35</sup>

2.0 m wide), 2.0 cm mesh sizes, were also used. The sampling was done during morning hours and the randomly captured samples were selected, morphometrically identified and measured. Specimens collected were quickly sorted out then stored in coolers containing ice and later transported to the laboratory. Muscles of some selected fish were extracted after measuring the lengths and weights of each species. Separated muscle samples were homogenized and stored at -2°C temperature. These muscle tissues were dried in a lyophilizer (VIRTIS 6KBEL85) for 24 h to remove the water content in the samples. The samples were then ground in an agate pestle and mortar in order to obtain muscle powder. The muscle powder was then later used for proximate composition, fatty and amino acids analyses.

**Proximate composition:** To determine the moisture contents, fish muscle duplicate samples were kept in an oven, at 105°C for 24 h. Fat content was determined with the help of a Soxhlet apparatus (Germany) using the non-polar organic

solvent hexane analytical grade. Both moisture and fat contents analyses were carried out according to the method in AOAC<sup>17</sup>. For the protein determination, nitrogen (N) contents of the fish muscle samples were determined by the method in AOAC<sup>18</sup>. The N content was multiplied by 6.25 to estimate the protein of these samples. Ash content was determined by burning the organic components from the known weight of the homogenized dried fish muscle by using a furnace at 550°C<sup>18</sup>. On the other hand, total carbohydrates was estimated by using the procedure of DuBois *et al.*<sup>19</sup> with phenol-sulphuric acid.

**Amino acid composition analysis:** Amino acid compositions of the powdered muscle sample were determined with an amino acid analyzer (Lachrom D-7000 HPLC System) using a Shimadzu C-18 column with two solvent systems, (a) 0.1% TFA solution and (b) 0.1% TFA in 90% acetonitrile. The column was eluted at a flow rate of 1 mL min<sup>-1</sup> with 10-90% gradient solution B over 40 min of the total volume of 20 µL. The

RP-HPLC column elutes was monitored by their absorbance at 215 and 280 nm. The amino acids present in the sample were determined by comparing the relative to front value (Rf) band formation sample with that of 21 standard amino acids<sup>20</sup>. The Rf value is defined as the ratio of the distance moved by the solute (i.e., the dye or pigment under test) and the distance moved by the solvent (known as the solvent front) along the paper, where both distances are measured from the common origin or application baseline, that is the point where the sample is initially spotted on the paper.

**Fatty acid profile GC-MS analysis:** Fatty acid composition of the powdered muscle sample was analyzed by GC-MS using a Varian Saturn 2000R gas chromatograph (Hewlett Packard 5890 model) equipped with an OV-225 capillary column (30 m×0.25 mm), programmed from 50-225°C (40°C min<sup>-1</sup>), then kept constant for 30 min. The FAMES were identified by their typical electron impact MS spectra and retention times (Rt), shown by comparison with standards (Sigma) and quantified according to their relative peak areas<sup>21</sup>.

**Statistical analysis:** GraphPad prism 5 and MS Excel 2016 were used for different calculations.

## RESULTS AND DISCUSSION

**Proximate composition:** The results of the proximate composition of the experimental fish sample are: Protein, carbohydrate, ash, moisture and fat contents were 50, 29, 12, 5 and 4%, respectively.

**Amino acid composition:** Total amino acid content and composition of this species is shown in Table 1. The levels of different amino acids ranged from 56.7 mg (Asparagine) to 2093.6 mg (Proline). The major essential amino acids were Threonine (2045.6 mg) and Iso-Leucine (2015.7) while those for the non-essential amino acids were Proline (2093.6 mg), Arginine (1193.7 mg) and Cysteine (1093.3 mg).

**Fatty acid composition:** The fatty acid composition (mg/100 g) of silver carp is summarized in Table 2. A total of 9 fatty acids were analyzed for the fish samples. The

Table 1: Amino acid composition of the muscle of silver carp (mg/100 g)

Amino acid composition			
Essential amino acid	Total (mg/100 g)	Non-essential amino acid	Total (mg/100 g)
Threonine	2045.6	Proline	2093.6
Iso-Leucine	2015.7	Arginine	1193.7
Tryptophan	1192.1	Cysteine	1093.3
Lysine	1135.8	Tyrosine	547.8
Phenylalanine	1134.4	Aspartic acid	455.6
Valine	1093.6	Alanine	257.8
Histidine	893.7	Glycine	195.7
Methionine	456.2	Glutamic acid	102.5
Leucine	109.3	Asparagine	56.7
Total essential amino acid	10076.4	Total non-essential amino acid	5996.7
Total amino acid (essential+non-essential amino acids)		16073.1 mg	

Table 2: Fatty acid composition of the muscle of silver carp (mg/100 g)

Fatty acid composition			
Fatty acids	No. of carbon atoms	Fatty acid type	Total (mg/100 g)
Oleic acid	C18:1	MUFA	1306.8
Total monounsaturated fatty acids			1306.8
Margaric acid	C17:0	SFA	104.6
Palmitic acid	C16:0	SFA	1034.6
Stearic acid	C18:0	SFA	993.7
Total saturated fatty acids			2132.9 mg
Alpha Linolenic acid (n-3)	C18:3	PUFA	1035.7
Linoleic acid (n-6)	C18:2	PUFA	935.8
Stearidonic acid (SDA) (morotic acid)	C18:4	PUFA	223.7
Docosahexaenoic acid DHA (n-3)	C22:6	PUFA	24.7
EPA (eicosapentaenoic acid) (n-3)	C20:5	PUFA	12.3
Total polyunsaturated fatty acids			2232.2 mg

Monounsaturated fatty acids (MUFA), Saturated fatty acids (SFA), Polyunsaturated fatty acids (PUFA)

dominant individual fatty acids were Oleic acid C18:1 (1306.8 mg), Alpha Linolenic acid C18:3 (1035.7 mg) and Palmitic acid C16:0 (1034.6 mg). For the saturated fatty acids (SFA), Palmitic acid (C16:0) had the highest proportion while Oleic acid (C18:1) was the main monounsaturated fatty acid (MUFA). Alpha Linolenic acid (n-3) (C18:3) was the dominant polyunsaturated fatty acid (PUFA).

Biochemical studies from the nutritional point of view are very important. The biochemical constituents in animals are known to vary seasonally and also with the size of the animal, stage of maturity, temperature and availability of food. Protein is essential for the sustenance of life, it exists in large quantities in all nutrients to form the components of the human body. Information concerning the chemical composition of freshwater fishes, in general, is valuable to nutritionists concerned with readily available sources of low-fat, high protein foods such as most freshwater fishes<sup>22,23</sup>.

**Proximate composition:** It was indicated from this study that, silver carp contains a higher proportion of protein and a lower moisture content as showed in the results section. According to this species of low-fat content (4%), we can classify it as being a lean fish<sup>14</sup>. Previous studies on proximate analysis by Buchtova and Jezek<sup>15</sup> on this same species confirms the results obtained in this study however, reports from Ashraf *et al.*<sup>16</sup> are not in concordance with it as they reported higher moisture content and relatively lower protein content for the same fish species. This difference might be due to genetical factors as well as extrinsic factors such as feeding regimes and/or exercise significant change in some structure and flesh quality parameters of different fish species. Thus, there is no hard and fast rule applicable universally to all the fish species.

**Amino acid composition:** Fish muscle consists of an excellent amino acid composition and is a unique source of nutrients and easily digestible protein<sup>23</sup>. Besides, the constituent of amino acids of protein act as precursors of many coenzymes, hormones, nucleic acids and other molecules essential for life. Thus, an adequate supply of dietary protein is essential to maintain cellular integrity and function and for health and reproduction. There is growing recognition that besides their role as building blocks of proteins and polypeptides, some amino acids (functional amino acids) such as Arginine, Cysteine, Glutamine, Leucine, Proline and Tryptophan regulate key metabolic pathways that are necessary for maintenance, growth, reproduction and immunity<sup>24</sup>. The amount of non-essential amino acids in silver carp muscle was equal to the essential amino acids (Table 1) from the 18 different amino acids identified. The essential amino acid identified is within the spectrum of an index of the biological value of fish protein.

This shows that fish protein is well balanced with essential amino acids. According to FAO/WHO/UNU expert consultation on protein and amino acid requirements in human nutrition<sup>25</sup>, essential amino acids are required daily by humans for healthy living. This fish therefore, provides a good source of EAA for human consumption since the human system cannot synthesize it.

Ozden<sup>26</sup> and Iwasaki and Harada<sup>27</sup> reported aspartic acid, glutamic acid and Lysine as the main amino acids of fishes. However, this is very different from the results obtained in this study as the most abundant amino acid recorded for this fish species is proline (2093.6 mg/100 g), among the non-essential amino acids. The EAA Threonine acid (2045.6 mg) was also abundant in this species followed by Iso-Leucine (2015.7 mg). The lowest recorded amino acid is the NEAA Asparagine (56.7 mg). Glycine, Proline and Arginine play an important role in metabolic regulation, anti-oxidative reactions and neurological function. Thus, these nutrients have been used to prevent tissue injury, enhance anti-oxidative capacity, promote protein synthesis and wound healing and improve immunity to various inflammatory diseases<sup>28</sup>. In aqua culture, the concentrations of Lysine and Methionine in fish food are regarded as important indicators of the nutritional value of the diet<sup>29</sup>. They are known to have significant antioxidant properties as synergists or primary antioxidants and believed to be important metal chelators present in fish and most amino acids had significant antioxidative potential in Linoleic acid and methyl esters of the Linoleic acid system. It is therefore highly recommended that Amino acids necessary for growth and maintenance must be included in diets. Accordingly, silver carp fish muscle is wealthy in both essential and non-essential important amino acids and could be used as a valuable food source for human beings.

**Fatty acid composition:** Margaric (C17:0), palmitic (C16:0) and stearic acids (C18:0) were the saturated fatty acids (SFAs) detected, Oleic acid (C18:1) was the only monounsaturated fatty acid obtained, while polyunsaturated fatty acids were the most dominant fatty acids comprising of alpha-Linolenic (C18:3 n-3), Linoleic (C18:2 n-6), Myristic (C18:4), eicosapentaenoic acid (EPA) (C20:5 n-3) and docosahexaenoic acid (DHA) (C22:6 n-3). This result is in accordance with those presented by Ozden<sup>26</sup> and Testi *et al.*<sup>30</sup>, who found that PUFA was the highest in rainbow trout. Osibona<sup>31</sup> reported that similar studies on tropical and temperate freshwater fishes showed the dominance of these fatty acids (SFA, MUFA, PUFA) in their tissues. In this study, alpha-Linolenic was the primary n-3 PUFA, followed by DHA and then EPA. This result is confirmed by reports from Buchtova *et al.*<sup>15</sup> on the same fish species. Omega-6 and omega-3 fatty acids influence gene

expression which may be modulated by amino acids. Although, alpha-Linolenic acid seems to benefit the cardiovascular system and might reduce the risk of heart disease, further studies are yet to be done in order to confirm its effectiveness in cholesterol reduction. The EPA and DHA have the most potent anti-inflammatory effects that may be enhanced by amino acids. According to Piggott and Tucker<sup>32</sup>, the n-3/n-6 ratio is a better index in identifying nutritional value of fish oils of different species. In the present study, the n-3/n-6 ratio was found to range 1.15% in silver carp meat, while the ratio from 1:1 and higher is considered to be optimal for nutritional purpose<sup>33,34</sup>. The compositions of fatty acids in fish are also highly depending on feeding conditions, their habitats and physiological conditions such as maturity stage<sup>35</sup>.

### CONCLUSION

The results obtained from this study show that, *Hypophthalmichthys molitrix* harvested from Lake Dianshan contains essential polyunsaturated fatty acids such as EPA and DHA, which are good for human health, has high-quality protein, presents necessary fatty acids and essential amino acids which provide a good source of nutrients for human consumption.

### SIGNIFICANCE STATEMENTS

This study provides an insight of nutrient parameters of the fish species *Hypophthalmichthys molitrix* harvested from Lake Dianshan, Shanghai-China. This study will help scientists to uncover the critical areas of nutrients enrichment in the native Chinese silver carp species caught from Lake Dianshan that have not been analyzed before.

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