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PROXIMATE COMPOSITION, FATTY ACID PROFILE AND LIPID HEALTH INDICES OF AN IMPORTANT FRESHWATER FISH *LABEO ROHITA* OF LOKTAK LAKE, MANIPUR

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ABSTRACT

Proximate composition of the muscles of *Labeo rohita* was determined. The average moisture content of the fish was found to be 79.18±0.43% while that of protein, lipid and ash were 16.02±0.17%, 1.85±0.43% and 1.10±0.01% respectively. A total of 29 fatty acids were found to be present in the fish (7 Monounsaturated fatty acids, MUFA and 12 polyunsaturated fatty acids, PUFA). Among them, palmitic acid was found to be the most abundant monounsaturated fatty acid. *L. rohita* contains reasonable amount of essential PUFA such as docosahexaenoic, eicosapentaenoic and arachidonic acids. The n6/n3 ratio (1.34) was found to be < 3, which are believed to be healthy values. The indices of artherogenicity and thrombogenicity show favorable value for human health.

Keywords: Proximate composition, PUFA, lipid health indices

1. INTRODUCTION

Loktak Lake is the largest freshwater lake located in Manipur, northeast India. It is one of the important wetlands listed in Ramsar sites, harbouring many birds both local and migratory, fishes and also the endangered deer, *Rucervus eldii eldii*. The lake plays an important role in the economy of Manipur and is also a source of livelihood for the rural fishermen living in the surrounding areas and on the floating mats of weeds locally known as phumdi.

The proximate composition of fish includes mainly moisture, protein, lipid and ash and this composition generally indicates the nutritive value of a fish. Many studies on the proximate composition of fishes have been reported for a number of fishes, which is important in understanding the nutritional value of the fish [1-8]. Fishes are considered a good source of high quality dietary protein for a healthy human diet. Recent investigations on fish nutrition have been directed towards lipid composition and its importance. The nutritional importance of fish consumption is, in great extent, associated with the content of Omega-3 (n-3) fatty acids and Omega-6 (n-6) fatty acids [9]. Fish oils are an excellent source of long chain polyunsaturated fatty acids, namely Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). These fatty acids are known to lower the risk factors related to arteriosclerosis [10], support good health and promote brain development and eye function in infants [11-17]. n-3 polyunsaurated fatty acids play important role in preventing coronary heart disease [18-22], risk of cancer and inflammatory diseases [23-27]. Arachidonic acid and Eicosapentaenoic acid are the most important n-3 essential fatty acids for the production of eicosanoids [14, 28]. There have been reports of intensive studies on omega 3, 6, 9 and cholesterol content as well as fatty acid profile of some sea water and freshwater fish [29-32].

Labeo rohita, locally known as 'Rou' in Manipur, is one of the preferred and commonly available food fish in Manipur. Loktak lake is the main natural source of this economically important fish in Manipur. The proximate compositions of various freshwater fishes including Labeo rohita have been studied in different parts of India and outside India [3-8]. However, very few information is available on the proximate composition and fatty acid profile of *L. rohita* found in Loktak lake. It is important to understand the nutritional profile of this fish as it is one of the commonly available and extensively consumed fish of Manipur. It is widely consumed in almost all households in many cooked forms (frying, roasting, smoking and currying) and also at cultural and traditional feast in Manipur. Therefore, the aim of the present study was to determine the nutritional composition of *L. rohita* collected from Loktak lake of Manipur.

2. MATERIALS AND METHODS

2.1. Sample collection

Labeo rohita weighing 400-500gms with standard length 26.5-28cm were collected from Loktak lake with the help of local fishermen during the month of December 2018 and January 2019. The fishes were kept in cold ice-box and brought to the Fishery Research Laboratory, Department of Life Sciences, Manipur University, immediately for further analysis. On arrival at the laboratory, fishes were washed thoroughly with running tap water and were beheaded and eviscerated. The skinless and hand deboned muscles were ground in a domestic food processor to ensure homogeneity and representative samples taken for analysis.

2.2. Proximate composition analysis

Proximate composition analysis was done in triplicate for moisture, protein, lipid and ash contents. Moisture was determined by using the methods of AOAC [33]. Total lipid was extracted from the muscle tissues following the method described by Singh et al. [34]. Total nitrogen content was estimated by Micro-kjeldahl method [33]. The samples were subjected to digestion, distillation and titration. Crude protein was estimated by multiplying the total nitrogen content by the conversion factor 6.25. Ash content was determined by incinerating the sample in a muffle furnace at 550°C for 3hrs to obtain carbon free white ash [33].

2.3. Analysis of fatty acids

For determination of fatty acids, about 150-250 mg of the lipid extract was taken for the preparation of fatty acid methyl ester (FAME). FAME was prepared using boron-triflouride methanol following the method of Metcalfe [35]. The fatty acid methyl esters were analysed by gas chromatography (Shimadzu GCMS-QP2010 Plus) equipped with flame ionization detector and fitted with Rxi-5Sil MS capillary column (30m x 0.25mm x 0.25um), employing the following operating conditions: helium gas was used as carrier gas at a constant flow of 1.21ml min^{-1} and an injection volume of 2µl was employed (split ratio 10.0), injector temperature 260°C; ion source temperature 230 °C. After injection (2µl), the column temperature was held at 140°C for 5 minutes, then increased to 280°C at 4°C degree min^{-1} and held at this temperature for 10 minutes. The spectrum of unknown fatty acids was compared with spectrum stored in the National institute of standards and Technology (NIST) library. The eluted fatty acids were characterized on the basis of their molecular formula, structure, retention time and peak percentage area. The percentage compositions of individual fatty acids were calculated by using the method of internal normalization.

2.4. Lipid health indices

The fatty acid profiles obtained were used to calculate atherogenicity index (AI) and thrombogenicity (TI) index [36]. These indices relate the different effects of various fatty acids with the risk of cardiovascular disorders. AI indicates the relationship between the sum of the main saturated fatty acids and, the sum of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), the former being considered pro-atherogenic (favouring the adhesion of lipids to cells of the immunological and circulatory system), and the latter anti-atherogenic (inhibiting the aggregation of plaque and diminishing the levels of esterified fatty acid, cholesterol, and phospholipids, thereby preventing the appearance of micro- and macrocoronary diseases) [36, 37]. TI shows the tendency to form clots in the blood vessels [36, 38]. It is defined as the relationship between pro-thrombogenic (myristic, palmitic, and stearic) and anti-thrombogenic (MUFA, n-6 PUFA and n-3 PUFA) fatty acids [36, 37]. The following equations were applied:

$$\text{AI (Atherogenicity index)} = [C12{:}0 + (C14{:}0 \times 4) + C16{:}0] / (\text{Total unsaturated fatty acids})$$

$$\text{TI (Thrombogenicity index)} = \Sigma(C14{:}0 + C16{:}0 + C18{:}0) / [(0.5 \times \Sigma MUFA + 0.5 \times \Sigma(n\text{-}6) + 3 \times \Sigma(n\text{-}3) + (n\text{-}3/n\text{-}6)]$$

The nutritional evaluation of fat should be the utilization of indexes based on functional effects of fatty acids such as the ratio of hypocholesterolaemic:hypercholestero - laemic fatty acids (HH), computed according to the present knowledge of the effects of individual fatty acid on cholesterol metabolism [39-41] because index such as polyunsaturated fatty acid and saturated fatty acid ratio are based only on the chemical structure of fatty acid and may not be an adequate way to evaluate the nutritional value of fat as it considers that increase in cholesterol is induced by all saturated fatty acid and ignores the effect of monounsaturated fatty acid [41]. HH (hypocholesterolaemic/hypercholesterolaemic ratio] is calculated by using the following equation [42]

$$HH = (C18{:}1\,n9 + C18{:}2\,n6 + C20{:}4\,n6 + C18{:}3\,n3 + C20{:}5\,n3 + C22{:}5\,n3 + C22{:}6\,n3) / (C14{:}0 + C16{:}0)$$

3. RESULTS AND DISCUSSION

The proximate composition of a food provides the basic information about its nutritional properties.

Table 1. Proximate composition (%) of *Labeo rohita* of Loktak lake

Proximate composition	% composition
Moisture	79.18±0.43
Protein	16.02±0.17
Lipid	01.85±0.43
Ash	01.10±0.01

Table 1 shows the proximate composition of *Labeo rohita* found in Loktak lake. The average moisture content in *L. rohita* was found to be 79.18±0.43%. The moisture content was slightly higher compared to other studies in the same species [5, 43].

Table 2: Fatty acid composition (%) of *Labeo rohita* of Loktak lake

Sl.no.	Fatty acids	% Composition
1.	C12:0 Lauric	00.27
2.	C13:0 Tridecanoic	00.21
3.	C14:0 Myristic	02.36
4.	C15:0 Pentadecanoic	01.54
5.	C16:0 Palmitic	19.18
6.	C17:0 Heptadecanoic	02.98
7.	C18:0 Stearic	10.85
8.	C19:0 Nonadecanoic	01.28
9.	C20:0 Arachidic	00.29
10.	C22:0 Docosanoic	00.00
11.	C24:0 Lignoceric	00.13
12.	C16:1 n7 Palmitoleic	05.39
13.	C17:1 cis-10-heptadecanoic	00.67
14.	C18:1 n7 cis-11-octadecenoic	03.62
15.	C18:1 Oleic	13.08
16.	C20:1 cis-11 Eicosenoic	00.16
17.	C22:1 n9 13-docosenoic	00.16
18.	C18:4 n3 Stearidonic	00.00
19.	C20:3 n3 11,14,17-eicosatrienoic	02.14
20.	C20:4 n3 8,11,14,17-eicosatetraenoic	00.50
21.	C20:5 n3 Eicosapentaenoic (EPA)	01.81
22.	C22:5 n3 7,10,13,16,19-docosapentaenoic	03.66
23.	C22:5 n3 Heneicosapentaenoic	02.47
24.	C22:6 n3 Docosahexaenoic (DHA)	05.28
25.	C18:2 n6 Linoleic	09.81
26.	C20:2 n6 11,14-eicosadienoic	00.45
27.	C20:3 n6 8,11,14-eicosatrienoic	01.36
28.	C20:4 n6 Arachidonic	06.34
29.	C22:4 n6 Adrenic	00.97
30.	C22:5 n6 4,7,10,13,16-docosapentaenoic	02.33
31.	C20:3 n9 5, 8, 11-eicosatrienoic	00.55
	ΣSFA	39.09
	ΣMUFA	23.63
	Σn3	15.86
	Σn6	21.26
	Σn3+ Σn6	37.67

SFA=Saturated fatty acid; MUFA=Monounsaturated fatty acid

The moisture value is within the range, 70-85% as reported for freshwater fishes [44]. The protein content and lipid content were 16.02±0.17% and 1.85±0.43%, respectively. The ash content was found to be 1.10±0.01%. The lipid and ash content was found to be lower to those reported in the same species from West Bengal, Odisha, Andhra Pradesh, Tripura, Karnataka and Chhattisgarh [5] and, in Uttarakhand [4]. But the lipid and ash content in the present study were similar to those found in *Oreochromis mossambicus* [45]. The higher percentage of ash content in fish is attributed to the high content of inorganic matter in them [44, 46, 47]; therefore the lower ash content in the present study may be attributed to the lower content of inorganic matter in them. The variation of proximate composition of the present study to those reported by other researchers may be due to factors such as geographical location, the season, environmental conditions, age or sexual maturity, sex, size, feeding habit of the fish and storage condition of the fish.

A total of 29 fatty acids were found in *L. rohita* of Loktak lake in the present study (Table 2). The fatty acid composition of the fish consist of 39.09% saturated (SFA), 23.63% monounsaturated (MUFA) and 37.67% polyunsaturated (PUFA). Among them, the fatty acid occurring in high proportions were myristic acid (C14:0, 2.36%), pentadecanoic acid (C15:0, 1.54%), palmitic acid (C16:0, 19.18%), heptadecanoic acid (C17:0, 2.98%), stearic acid (C18:0, 10.85%), palmitoleic acid (C16:1n7, 5.39%), 11-Octadecenoic acid (C18:1n7, 3.62%), oleic acid (C18:1n9, 13.08%), eicosapentaenoic acid (EPA, C20:5n3, 1.81%), heneicosapentaenoic acid (C22:5n3, 2.47%), docosahexaenoic acid (DHA, C22:6n3, 5.28%), linoleic acid (C18:2n6, 9.81%), arachidonic acid (C20:4n6, 6.34%), docosapentaenoic acid (C22:5n6, 2.33%). In the present study, palmitic acid (C16:0) was the major saturated fatty acid present in highest amount followed by stearic acid (C18:0) whereas the monounsaturated fatty acid (MUFA) found in highest percentage was oleic (C18:1) and palmitoleic acid (C16:1). These findings were in agreement with the findings of [9, 48, 49]. The characteristic difference between marine fish and fresh water fish has been indicated to be the higher levels of C16:0 and C18:0 acid and lower levels of C20:0 and C22:0 fatty acid in fresh water fishes [3, 50, 51].

In the present study also, C22:0 acid was not detected in *L. rohita*. The fatty acid profile (Table 2) exhibits the dominance of saturated fatty acid and unsaturated fatty acids especially PUFA. These results are in agreement with previous studies on fatty acid of other species [33, 52]. Among the n-6 acids, the dominant fatty acids were linoleic acid (C18:2), arachidonic acid (C20:4) and docosapentaenoic acid (C22:5). Comparatively high amount of linoleic acid (9.81%) was found in *L. rohita*. Arachidonic acid (C20:4), a precursor of other eicosanoids was also found high in *L. rohita* (6.35%). The amount of docosapentaenoic acid found in our study was 2.33%. Essential n-3 and n-6 polyunsaturated fatty acid cannot be synthesized in the human body, they must be obtained through diet [53]. *L. rohita* in the present study contains considerable amount of n-3 and n-6 fatty acid and so it may serve as a good source of EPA and DHA required for human health.

The n6/n3 ratio is an important indicator of nutritive quality of a food. Low level of n6/n3 value, i.e. 1.34 was observed in our study (Table 3). Healthy values of this ratio for human diet are believed not to be higher than 3, while the current Western diet has n6/n3 from 10 to 25 [54]. The n-6/n-3 ratio of Zander (*Sander lucioperca*) from Swedish lakes was about 0.53 [55] and this ratio in filets of zander from Turkish lakes varied from 0.26 to 1.39 [56, 57]. These results are, on an average, close to that of *L. rohita* in the present work.

The artherogenicity index (AI) and thrombogenicity index (TI) value in the present study was found to be 0.47 and 0.45 respectively (Table 3). The result of this index is a number indicating the risk of formation i.e., atherosclerosis. The higher the AI is the higher risk it constitutes. It is assumed that AI below 1 is beneficial for human health [58]. An increasing TI indicates a risk of developing a blood clot [36, 38]. Favourable values of AI and TI indices were found in *L. rohita*. Consumption of this fish may be expected to be good for cardiovascular patients as well as for normal people. AI and TI are highest for most atherogenic and thrombogenic dietary components. Low indices are caused by a comparatively low content of SFA, especially palmitic and stearic acid, and a high content of n-3 PUFA. The values for the two indexes (AI and TI) showed that *L. rohita* of Loktak lake are beneficial to human health. The lower the atherogenicity (AI) and thrombogenicity (TI) index values, the healthier the food. This is because these indices report the relationship between fatty acid in food and their contribution to the prevention of coronary diseases [59]. However, the index related to ratio between hypocholesterolemic and hypercholesterolemic fatty acid (HH) was found to be 1.69. Higher HH values ranging from 2.03 to 2.46 were reported [42]. Moreover, the higher the ratio between hypocholesterolemic and hypercholesterolemic fatty acid, the more adequate that oil or fat is for the nutrition [41, 42, 60]. The values in this study are similar to those found in Tilapia (*Oreochromis niloticus*)[61, 62].

A number of factors influence the proximate composition and fatty acid profile of fish. The differences in fatty acid of fishes should not only be considered with respect to species habitat but also based on their natural diet, especially whether a species is herbivorous, omnivorous or carnivorous [63]. Apart from that, size, age, reproductive status of fish, environmental conditions, and especially water temperature, salinity, time of capture influence lipid content and fatty acid composition of fish muscle to a certain extent [64-66]. Therefore, this factor must be considered when analyzing differences among studies [67]. The result in our study shows high content of medically important n-3 fatty acid in *L. rohita* of Loktak lake.

Table 3: Lipid quality indices of *Labeo rohita* of Loktak lake

Ratio/Indices	Value
Σn6/ Σn3	1.34
Atherogenecity index (AI)	0.47
Thrombogenicity index (TI)	0.45
Hypocholesterolaemic/Hypercholesterolaemic (HH)	1.69

4. CONCLUSION

Labeo rohita of Loktak lake is a good source of protein and essential polyunsaturated fatty acid, EPA and DHA. The EPA and DHA were found in appreciable amounts and would be suitable for inclusion in highly unsaturated low fat diet of the population in Manipur. The n6/n3 ratio of was found to be in the favourable range for human health. The values of artherogenicity and thrombogenicity indices are low and would be beneficial to human health as low indices value indicates a healthier food. The present study has established some important nutritional composition of *Labeo rohita* from Loktak lake and its significance. The fish was found to be high in nutritional value and therefore its consumption may be recommended.

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