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Original Article

Assessment of fatty acid composition and amino acid profiles of three important fresh water fish species native to river Indus Pakistan

Avaliação da composição de ácidos graxos e perfis de aminoácidos de três importantes espécies de peixes de água doce nativas do rio Indo, Paquistão

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Abstract

This study was carried out to analyze the fatty acid and amino acid compositions of three economically important freshwater fish species, Mali (*Wallago attu*), Raho (*Labeo rohita*), and Mahseer (*Tor putitora*), indigenous to Indus River, Pakistan.. Amino acid profiling was done by high-performance liquid chromatography (HPLC) while gas chromatography (GC) was used for fatty acid analysis. Glutamic acid, aspartic acid, arginine, alanine, leucine, lysine and isoleucine were the most predominant amino acid while palmitic acid (C16:0), oleic acid (C18:1c), palmitoliec acid (C16:1c), linolenic acids (C18:2c) and docosahexaenoic acids (DHA C22:6) were the notable fatty acids present in these species. Our results indicated that all species have comparable nutritional composition and are good source of healthy human diet. Our findings will help the people to make informed choice while selecting fish for consumption and will pave the way for future researchers in planning new strategies to enhance the growth and production of commercial fish species. It will also be helpful for theFrom the current finding it was concluded that all fish species under discussion are rich in amino acids and fatty acids. These species contain essential amino acids and important fatty acid such as omega3 and omega 6, thus raising the nutritional quality of these species.

Keywords: fatty acids, amino acids, PUFAs, DHA, Indus River.

Resumo

Este estudo foi realizado para analisar as composições de ácidos graxos e aminoácidos de três espécies de peixes de água doce economicamente importantes, Mali (*Wallago attu*), Raho (*Labeo rohita*) e Mahseer (*Tor putitora*), nativas do rio Indo, Paquistão. O perfil de aminoácidos foi determinado por cromatografia líquida de alta eficiência (HPLC), enquanto a cromatografia gasosa (GC) foi usada para análise de ácidos graxos. Ácido glutâmico, ácido aspártico, arginina, alanina, leucina, lisina e isoleucina foram os aminoácidos mais predominantes, enquanto ácido palmítico (C16:0), ácido oleico (C18:1c), ácido palmitolieco (C16:1c), ácidos linolênicos (C18:2c) e ácidos docosahexaenoicos (DHA C22:6) foram os ácidos graxos notáveis presentes nessas espécies. Nossos resultados indicaram que todas as espécies têm composição nutricional comparável e são uma boa fonte de dieta saudável para seres humanos. Nossas descobertas ajudarão as pessoas a fazer escolhas informadas ao selecionar peixes para consumo e abrirão caminho para futuros pesquisadores no planejamento de novas estratégias para aumentar o crescimento e a produção de espécies comerciais de peixes. Além disso, essas informações são úteis para concluir que todas as espécies de peixes em discussão são ricas em aminoácidos e ácidos graxos. Essas espécies contêm aminoácidos essenciais e ácidos graxos importantes, como ômega 3 e ômega 6, aumentando assim a qualidade nutricional dessas espécies.

Palavras-chave: ácidos graxos, aminoácidos, PUFAs, DHA, Rio Indo.

1. Introduction

Fish and fish products holds a special dietary position as these are a valuable as well as cost effective source of good quality proteins, amino acids, fatty acids and minerals like vitamin A and D, iron, phosphorus, iodine, potassium and copper (Roos et al., 2007). Owing to easy digestibility (less connective tissue) and excellent flavor, fish is preferred by millions around the world as a source of animal protein (Mohanty et al., 2019; Vlieg and Body, 1988). These nutritional attributes makes fish an excellent replacement of red meat. Studies have shown that fish and

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its products are beneficial against a number of diseases including cardiovascular ailments (Mozaffarian and Rimm, 2006), disorders of nervous systems, degeneration of muscle tissues and depression (Peet and Stokes, 2005). Additionally, it is helpful for women during pregnancy, improves brain development of infants (Young and Conquer, 2005) and overall growth and development.

Fish has an immense importance in the nutrition, income generation, economic stability and earning of foreign exchange for country. Fisheries and aquaculture sectors, both are crucial for the economies worldwide, serving as an important source of income and employment for local communities. Fish industry is one of the prominent contributor to the economy of Pakistan (Rehman et al., 2019) and despite its relatively modest share of the GDP plays a significant role on securing foreign currency (Shamsuzzaman et al., 2020). Meanwhile fish business in Pakistan serves as a vital source of employment, benefitting nearly 400,000 individuals directly and supporting another 600,000 people indirectly (Laghari, 2018).

Freshwater fish has a significant dietary value for human since, it is a precious source of valuable nutrients (Fawole et al., 2007), both macro and micro thus, provides excellent nutritional value to fish meat (Kumar et al., 2020). Since majority of world population rely on fish as a source of nutrition it is important to know the chemical constitution of fish which is a reliable indicator of quality of fish, its nutritional value and other factors (Ravichandran et al., 2011). The composition of fish meat, as mentioned by Balami et al. (2019), is 65-80% moisture, 15-20% proteins, 5-20% fat and 0.5-2% ash.

Fish has a vast food potential, that is important to meet the dietary requirements of people in areas where other animal proteins are either less or hard to obtain (Hasselberg et al., 2020; Tran et al., 2023) thus improving the general health by treating nutrients deficiency. Fish consumption is beneficial for maintaining healthy weight because it is a rich source of protein, containing AAs (both essential and non-essential AAs) while being relatively low in calories (Pawar and Sonawane, 2013). Amino acids (AA) are elemental biomolecules serving as basic building units of proteins and form basis of all life processes. Also, these are precursors of vital biological substances like neurotransmitters and nucleotides. Additionally, they have crucial part in cell signaling and gene expression (Wu, 2010), transport of nutrients, metabolism, prevention and treatment of metabolic diseases (Mohanty et al., 2014a; Wang et al., 2013), and immune responses. AAs are also required for facilitating growth, reproduction, for synthesis of vitamins, tissue growth and all the repair processes within body (Amosu et al., 2023). Previous studies have shown that fish proteins have numerous health benefits for human (Khalili Tilami and Sampels, 2018).

In addition to proteins, freshwater fishes also contain nutritionally important lipids and fatty acids (FAs), crucial for human health. Fish and its by-products are rich in both omega-3 and omega-6 PUFAs, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Holub and Holub, 2004). Studies have proved that ω-3 PUFAs have important contribution in formation of bones and metabolic processes. These fatty acids are also helpful in lessening the probability of certain cancers and numerous illnesses like heart disease (Nøstbakken et al., 2021; Njinkoue et al., 2016; Von Schacky and Harris, 2007), rheumatoid arthritis (Berbert et al., 2005), Alzheimer's (Petot and Friedland, 2004), inflammatory conditions (Gogus and Smith, 2010) and autoimmune disorders (Simopoulos, 2002). In addition to this, both EPA and DHA function as precursors of prostanoids and eicosanoids, which in turn are important for cell membrane structure and various physiological processes of body (Duarte et al., 2021; Pino-Hernández et al., 2020). Several other FAs such as oleic and linoleic acids paly crucial parts in various biological processes. These fatty acids contribute in maintenance of cell membranes, support brain functions and facilitate transmission of nerve impulses (Nunes et al., 2012), hemoglobin production and cell division. However, human beings are unable to produce these fatty acids in body, thus must get these from other sources i.e. meat and fish (Duarte et al., 2021).

Since the information about nutritional and health benefits of fish consumption has widespread, its demand has increased (Tacon et al., 2020; Dantas Filho et al., 2021). Fish as a whole has great nutritional potential and is beneficial to overcome malnutrition especially in rapidly growing countries like Pakistan with limited sources of protein rich foods. Thus, there is an absolute need of knowing the nutritional content of fish eaten by majority of population in third world countries (Oladipo and Bankole, 2013). According to Azrita et al. (2021), AA and FA composition of fish must be known for better understanding of nutritional properties of fish (Béné et al., 2016) and for better selection of right type for balanced diet (Kingsley et al., 2022).

The River Indus, one of the Pakistan's longest river, is known for its rich ecological diversity including a variety of fish species (Abro et al., 2020) which are important both nutritionally and economically. Inhabitants of surrounding areas of Indus River use fish in abundance as their diet. Hence, the purpose of our investigation was to assess the composition of fatty acid and amino acid in Mahseer (Tor putitora), Rohu (Labeo rohita) and Mali (*Wallago attu*), commonly consumed by locals living in vicinity of Indus River, Pakistan.

Labeo rohita (raho), a herbivore specie, is one of the most abundant specie of Indus River, which mainly feeds on vegetations and phtoplankton. Raho has rapid growth and excellent taste which makes this specie economically important. *Wallago attu* (Mali), a predatory cat fish, is selected for this study because this specie is available throughout the year and has stable market price apart from having fast growing nature and flesh of exceptional nutritional quality. The third species selected for this study is *Tor putitora* (Mahseer) which is a freshwater carp and is an omnivore specie. Studies have indicated that Mahseer is a widely consumed fish owing to high quality of meat and is favored for its large size and weight which raises its market price and help locals to fetch good income (Ingram et al., 2005).

Although these fish species are readily available and have significant commercial values, yet it is required to comprehend the complex AA and FA profies of these species to optimize their nutritional values and improve aquaculture practices. This study thus, aims to investigate the AA and FA compositions of freshwater Raho, Mali, and Mahseer, native to Indus River, Pakistan.

2. Materials and Methods

2.1. Study area

The study was carried out in Khyber Pakhtunkhwa (KPK) province of Pakistan and samples were collected from Swabi, Nazampur, Khushal gath and Dera ismail khan fish sampling points which are all situated at the bank of River Indus. Indus River is the only source of freshwater in Pakistan and country's ecosystem and agriculture all depends on River Indus. It originates from Himalayas and extend upto the Arabian Sea and has rich biodiversity, covering mountains, plains and environments. Out of total drainage area of 1,165,000km^2, 712,000km^2 is located in Pakistan, making it important for ecological and economic landscape of country.

2.2. Sample collection

Adult Mahseer (Tor putitora), Rohu (Labeo rohita) and Mali (*Wallago attu*) were taken in present study. Total 10 specimens were collected for each species from their natural freshwater ecosystem. Figure 1 represents the fish sampling points in the study area. These samples were collected from Kurram River and Indus River, Pakistan. The selected fishes were immediately transferred to insulated ice box to limit the biological processes and were brought to Pakistan Council of Science and Industrial Research (PCSIR) Laboratories. Once reached the destination the fish were cleaned with water, scales and internal organs were removed and were dissected to get various parts i.e. head, abdomen and tail which were then gutted and homogenized and were stored in refrigerator at −20 °C until these were further analyzed.

2.3. Analysis of fatty acid

2.3.1. Lipid extraction

Lipid extraction was done by using method outlined by Bligh and Dyer (1959). Samples from head, abdomen and tail were homogenized individually in 30 volumes of mixture of chloroform, methanol and water (2:2:1) in homogenizer. After phase separation, the chloroform extracts were evaporated under nitrogen stream and residue obtained was quantified.

2.3.2. Fatty Acid methyl esters (FAME) preparation

In the next step trans-esterification of released lipids was performed to convert them into FAME by using 2% sulphuric acid in methanol and treatment with Boron trifluoride (BF_3). The methyl ester thus obtained were extracted using *n*-hexane (1ml).

2.3.3. FA analysis using gas chromatography

The extracted methyl esters were determined by capillary gas chromatography furnished with flame ionization detector and possessing capillary columns. FAs were then identified based on their retention time and peak areas compared to recognized standards.

2.4. Amino acid analysis

2.4.1. Sample collection and preparation

Method used by Ishida et al., was followed to determine the amino acid composition in tissues (Ishida et al., 1981). Initially the sample was dried and defatted using a mixture of chloroform and methanol. Then, by using 6N hydrochloric (HCL) proteins were hydrolyzed at 110° for 24 hours. Once hydrolyzation was completed, sample was neutralized by using 6N NaOH and was then derivatized samples were injected into a high-performance liquid chromatography (HPLC) system equipped with fluorescence detector and C18 RP column. The chromatographic conditions used were

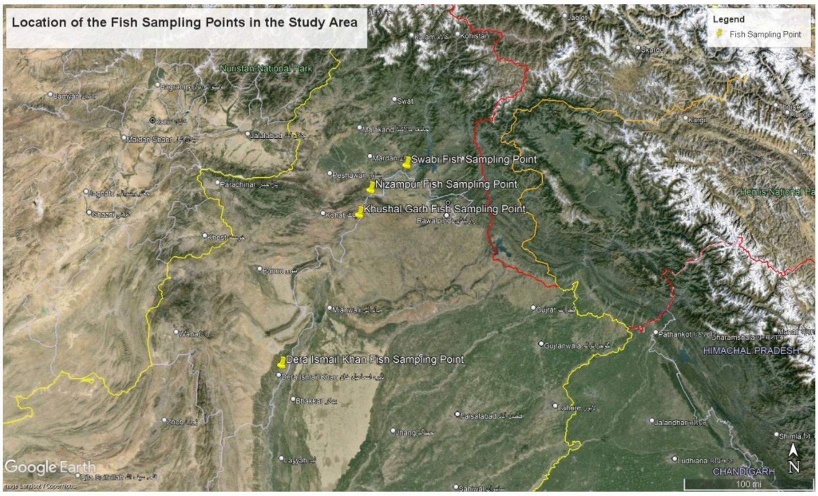

Figure 1. Map and location of fish sampling point in the study area.

in accordance with the Agilent method (Henderson et al., 2000) Retention time and peaks obtained were compared with the recognized standards to identify and quantify amino acids.

2.5. Statistical analysis

Each measurement was repeated thrice to avoid any inaccuracy. Results were expressed as mean ± standard deviation. Data analysis was done using Statistical Package for the Social Sciences (SPSS) version 22.0 and One-way analysis of variance (ANOVA) with Duncan multiple range test (DMRT). Significant means were determined at 5% confidence level ($P<0.05$).

For amino acid and fatty acid profile the Tukey-Kramer HSD test was used because this post hoc test works well for pairwise group differences. Standard deviation values were calculated during statistical analysis.

3. Results

3.1. Amino acid profile

Figure 2 represents a variation of amino acid contents in mali, raho and mahseer from Indus river. Glutamic acid was the most abundant amino acid in all fishes compared to other amino acids. Table 1 shows that raho had highest levels of glutamic acid i.e. 19.818 ± 0.71 while mali exhibited the lowest values (17.247 ± 1.60) showing a significant difference of $p<0.05$ between raho and mali.

In case of aspartic acid, mahseer had highest content of this amino acid (12.307 ± 0.58) followed by raho (11.117 ± 0.74) and mali (10.156 ± 3.38). A significant difference of $P<0.05$ can be seen in aspartic acid composition of mahseer and mali. A significantly high levels ($p<0.001$) of arginine and lysine was observed in mali as compared to raho and mahseer. Raho and mahseer had comparable levels of arginine and lysine. A similar trend was observed

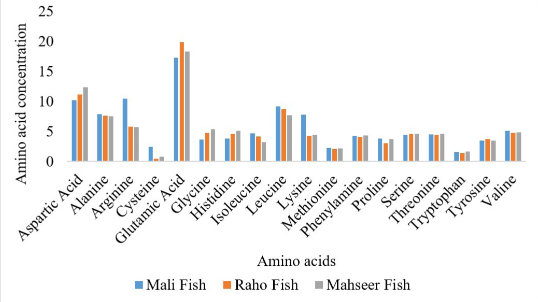

Figure 2. Comparison of amino acid concentration in Mali (*Wallago attu*), Rohu (*Labeo rohita*) and Mahseer (*Tor putitora*) from Indus River.

Table 1. Analysis of Amino Acid composition in Mali, Raho, and Mahseer Fish.

Amino Acid contents	Mali Fish	Raho Fish	Mahseer Fish
Aspartic Acid	$10.156^{b} \pm 3.38^{**}$	$11.117^{b} \pm 0.74^{**}$	$12.307^{b} \pm 0.58^{**}$
Alanine	$7.853^{c} \pm 1.10^{**}$	$7.576^{d} \pm 0.35^{**}$	$7.500^{c} \pm 0.22^{**}$
Arginine	$10.411^{b} \pm 2.47^{**}$	$5.753^{e} \pm 1.65^{**}$	$5.693^{d} \pm 0.24^{**}$
Cysteine	$2.414^{efg} \pm 0.33^{**}$	$0.466^{j} \pm 0.32^{**}$	$0.764^{k} \pm 0.20^{**}$
Glutamic Acid	$17.247^{a} \pm 1.60^{**}$	$19.818^{a} \pm 0.71^{**}$	$18.302^{a} \pm 0.42^{**}$
Glycine	$3.616^{def} \pm 0.41^{**}$	$4.718^{ef} \pm 0.24^{**}$	$5.324^{de} \pm 0.30^{**}$
Histidine	$3.813^{def} \pm 0.31^{**}$	$4.607^{f} \pm 1.01^{**}$	$5.094^{def} \pm 0.50^{**}$
Isoleucine	$4.619^{d} \pm 0.37^{**}$	$4.149^{f} \pm 0.59^{**}$	$3.156^{i} \pm 0.57^{**}$
Leucine	$9.172^{bc} \pm 0.68^{**}$	$8.747^{c} \pm 0.23^{**}$	$7.681^{c} \pm 0.34^{**}$
Lysine	$7.737^{c} \pm 1.43^{**}$	$4.263^{f} \pm 1.36^{**}$	$4.398^{g} \pm 0.99^{**}$
Methionine	$2.214^{fg} \pm 0.38^{**}$	$2.100^{hi} \pm 0.42^{**}$	$2.149^{j} \pm 0.53^{**}$
Phenylamine	$4.234^{de} \pm 0.35^{**}$	$4.049^{fg} \pm 0.79^{**}$	$4.319^{gh} \pm 0.31^{**}$
Proline	$3.753^{def} \pm 0.26^{**}$	$2.997^{gh} \pm 0.53^{**}$	$3.688^{hi} \pm 0.20^{**}$
Serine	$4.424^{d} \pm 0.27^{**}$	$4.574^{f} \pm 0.44^{**}$	$4.537^{fg} \pm 0.32^{**}$
Threonine	$4.514^{d} \pm 0.28^{**}$	$4.373^{f} \pm 0.78^{**}$	$4.558^{fg} \pm 0.30^{**}$
Tryptophan	$1.523^{g} \pm 0.29^{**}$	$1.370^{ij} \pm 0.27^{**}$	$1.666^{j} \pm 0.27^{**}$
Tyrosine	$3.479^{def} \pm 0.32^{**}$	$3.734^{fg} \pm 0.17^{**}$	$3.477^{i} \pm 0.27^{**}$
Valine	$5.076^{d} \pm 0.43^{**}$	$4.717^{ef} \pm 0.69^{**}$	$4.790^{efg} \pm 0.30^{**}$
HSD	**4.032**	**3.032**	**1.036**

$P<0.001$ = **, $P>0.05$ = ns. Superscript letters indicate statistically significant differences between groups; values with the same letter are not significantly different.

for cysteine where mali had highest content of this amino acid holding a value of 2.414±0.33 while raho and mahseer had a non-significant difference (0.466±0.32 and 0.764±0.20 respectively).

When compared the content of Leucine and isoleucine among all three species, the order was mali>raho>mahseer where mali had significantly higher levels of leucine and isoleucine (Figure 1). Mahseer, however had higher glycine and histidine content followed by raho and mali. A non-significant difference was seen for alanine, methionine, phenylamine etc.

3.2. Fatty acid analysis

Table 2 presents the comparison of some important fatty acids in different body regions of three freshwater fish species. The most prominent saturated fatty acid (SFA) among all three species was palmitic acid. Overall, Raho had significantly higher ($p<0.001$) percentage of palmitic acid 70.577% followed by mahseer 65.420% and mali 27.23%. Among other SFAs, myristic acid was present in higher percentage (5.750%) in mali compared to 3.270% in raho and 0.20% in mahseer. Similar results were seen for stearic acid, mali having highest percentage followed by raho and mahseer. Most of the SFAs, however, showed a non-significant difference ($p>0.05$) (Table S1).

Oleic acid was the predominant monounsaturated fatty acid (MUFA), palmitoleic acid being next in these species. Raho had 15.750% of this fatty acid significantly higher than 12.540% in mali and 8.230% in mahseer with probability of $p<0.05$.

Further, it was observed that, mali exhibited highest percentage (9.380%) of palmitoleic acid, followed by 7.720% in mahseer and 5.567% in raho. Among polyunsaturated fatty acids, Docosahexaenoic acid (DHA) was the predominant PUFA with percentages ranging from 27.530% to 27.940%. However, a non-significant difference was seen among all species. In contrast to this, a significantly high percentage of linoleic acid was noted in raho as compared to mali and mahseer respectively.

When compared, in raho, the head region displayed highest percentage of palmitic acid (70.577%) than 62.680% in abdomen and 61.050% in tail region. Alternatively, both myristic acid and stearic acid were in highest percentages in tail region followed by abdomen and head.

Among MUFAs, oleic acid was significantly higher in abdomen and tail region as compared to head with probability of $p<0.001$ while the head region had highest % of palmitoleic acid as compared to other body regions (Table 2).

Among polyunsaturated fatty acids, DHA and linoleic acid was present in higher amounts. DHA exhibited a non-significant difference among all body parts while a slightly higher value of linoleic acid was observed in head region as compared to other parts of raho.

The FA compositions of the lipid extracted from head, abdomen and tail regions of raho, mali, and mahseer are shown in Figures 3a, b, c, 4a, b, c and 5a, b, c repectively.

When SFAs were compared in mali, it was observed that palmitic acid was most abundant SFA present in mali followed by myristic acid and stearic acid. The tail and abdomen had statistically higher levels ($p<0.001$) of palmitic acid compared to head region. Additionally, myristic acid was highest in abdomen region while stearic acid was highest in tail region of mali.

Like raho, mali was also rich in oleic acid followed by palmitoleic acid. Analysis of samples from different body parts showed that highest percentages of oleic acid was present in the abdomen region while the head region had

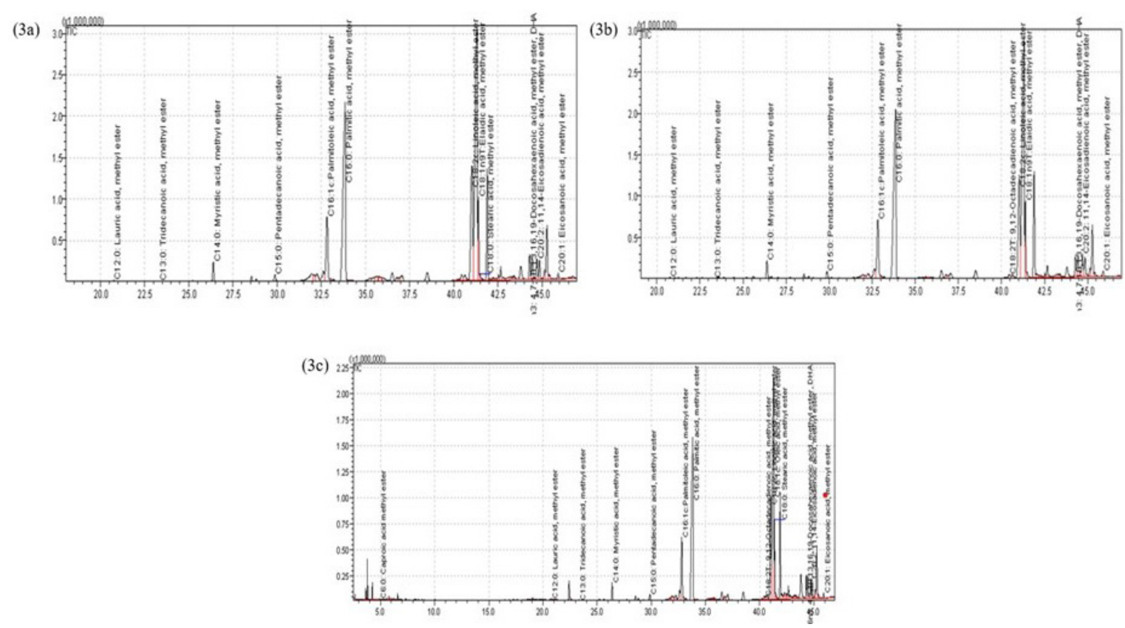

Figure 3. Chromatogram of the fatty acid composition of (3a): head region, (3b): abdomen region and (3c): tail region of *Labeo rohita*.

Table 2. Fatty acid profile in different body regions of mali, raho amd mahseer.

Fatty acid contents	Head Region			Abdomen Region			Tail Region		
	Mali Fish	Raho Fish	Mahseer Fish	Mali Fish	Raho Fish	Mahseer Fish	Mali Fish	Raho Fish	Mahseer Fish
C14:0 myristic	1.320g ± 0.01**	3.750e ± 0.01**	0.130mn ± 0.01**	5.750d ± 0.01**	3.270b ± 0.01**	0.200klm ± 0.10**	0.020n ± 0.01**	3.350de ± 0.01**	0.120mn ± 0.01**
C16:0 Palmitic acid	27.230b ± 0.01**	70.577a ± 0.02**	63.870a ± 0.01**	63.180a ± 0.01**	62.680a ± 0.01**	65.200a ± 0.10**	65.370a ± 0.01**	61.050a ± 0.01**	65.420a ± 0.01**
C16:1c Palmitoleic acid	1.760e ± 0.01**	5.567d ± 0.01**	7.720d ± 0.01**	5.320e ± 0.01**	3.170b ± 0.01**	7.520c ± 0.01**	9.380d ± 0.01**	3.440de ± 0.01**	7.480d ± 0.01**
C18:0 Stearic acid	0.057k ± 0.02**	1.447g ± 0.04**	0.170mn ± 0.01**	0.053r ± 0.02**	1.423b ± 0.04**	0.033n ± 0.02**	2.537g ± 0.03**	1.733e ± 0.03**	0.060no ± 0.02**
C18:1c Oleic acid	9.020d ± 0.01**	3.650e ± 0.53**	8.230c ± 0.01**	12.540c ± 0.01**	15.750b ± 0.01**	7.620c ± 0.01**	10.690c ± 0.39**	15.020c ± 0.01**	8.130c ± 0.01**
C18:2c Linoleic acid	1.550f ± 0.01**	11.993c ± 0.01**	4.730e ± 0.01**	5.260e ± 0.01**	10.220b ± 0.02**	4.600d ± 0.01**	4.220e ± 0.01**	10.630c ± 0.02**	4.450f ± 0.02**
C20:5n3 EPA	25.700c ± 0.20**	1.020h ± 0.01**	2.540g ± 0.01**	2.263g ± 0.02**	0.767b ± 0.02**	2.660e ± 0.01**	2.247h ± 0.04**	0.950e ± 0.02**	3.120g ± 0.01**
C22:6n3 DHA	27.830a ± 0.08**	27.920b ± 0.01**	27.620b ± 0.06**	27.940b ± 0.01**	27.880b ± 0.01**	27.560b ± 0.01**	27.970b ± 0.01**	27.840e ± 0.01**	27.530b ± 0.01**

P<0.001 = **, P>0.05 = ns. Superscript letters indicate statistically significant differences between groups; values with the same letter are not significantly different.

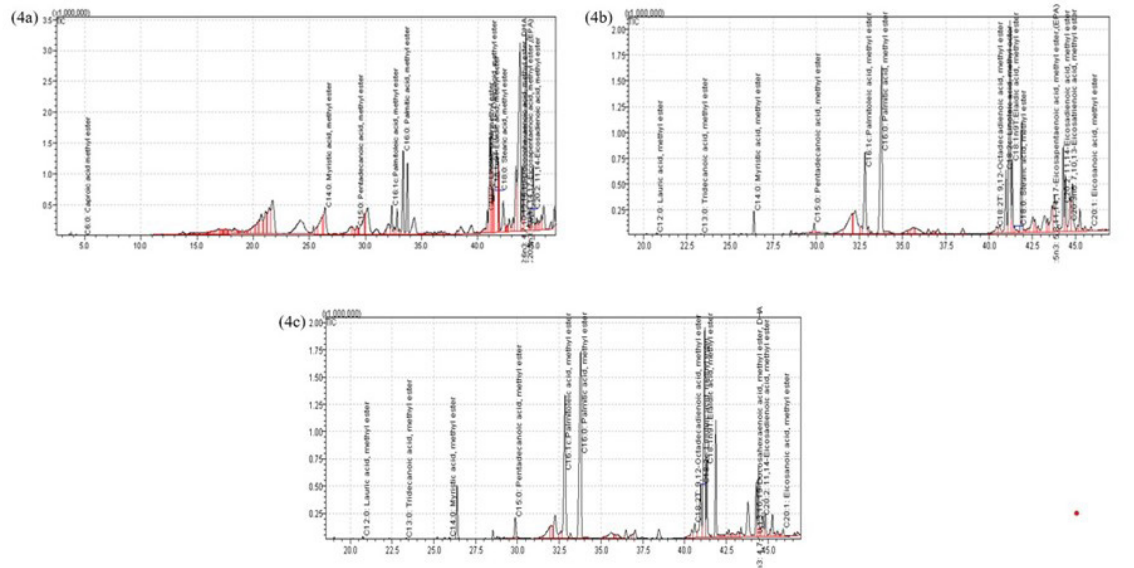

Figure 4. Chromatogram of the fatty acid composition of (4a): head region, (4b): abdomen region and (4c): tail region of *Wallago attu*.

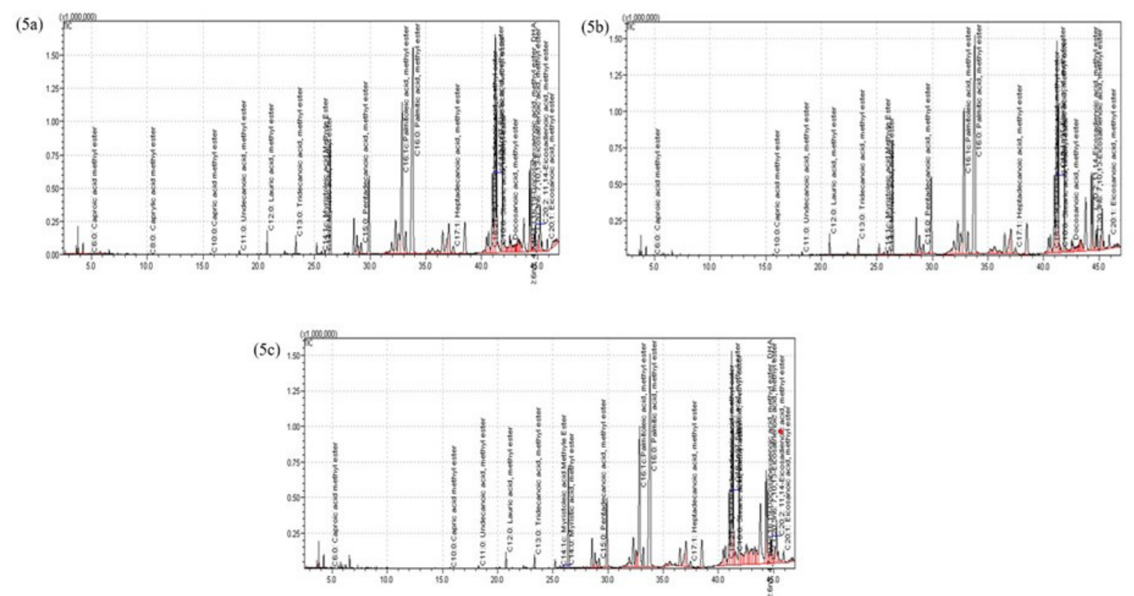

Figure 5. Chromatogram of the fatty acid composition of (5a): head region, (5b): abdomen region and (5c): tail region of *Tor putitora*.

lowest content of this FA. In case of palmitoleic acid, tail exhibited significantly higher levels ($p<0.05$) compared to other regions.

When PUFAs were compared, this specie also showed a non-significant difference in polyunsaturated fatty acids except for eicosapentaenoic acid (EPA) which was highest in the head region. A highly significant difference ($p<0.001$) was observed in EPA where head region had 25.70% EPA compared to 2.263% in abdomen region and 2.247% in tail region. The difference in content of linoleic acid was also significant with abdomen region showing the greatest percentage.

Similar to other two species the highest SFA in mahseer observed was also palmitic acid with tail exhibiting slightly higher values compared to other parts. Among MUFAs, the oleic acid followed by palmitoleic acid were highest like other 2 species. The percentage of Oleic acid was 8.230% in head region as compared to 8.130% in tail and 7.620%

in abdomen region. Also, palmitoleic acid was highest in head region in contrast to other body region although the difference was non-significant.

Like both previously discussed species, in mahseer, the most predominant PUFA was also DHA, however, no significant difference was observed in different regions. Also a non-significant difference was seen in case of linoleic acid and EPA.

4. Discussion

Fish is a protein rich food source, providing all the essential nutrients required for healthy human body (Polak-Juszczak and Podolska, 2021). The fish species selected for this study are the most preferred species in all areas of Pakistan being liked by both consumers and fishermen. All three species of this study hold high nutritional values and are good source of AAs and FAs. This study indicated that glutamic acid was the most predominant AAin these follwed by aspartic acid. Among EAA leucine was present in highest percentages followed by lysine and isoleucine. Similarly, all three species were rich in fatty acids and results showed that oleic acid, palmtoliec acid, palmitic acid, DHA, EPA and linoleic were present in abundance in these species.

Glutamic acid was the most prevalent AA these three fish species that aligned with the findings of Dezhabad, Dalirie and Toudar (2012) who reported that freshwater fish species are excellent source of glutamic acid which is an immune booster. It is also the most abundant amino acid in many small indigenous fish species (Nurullah et al., 2003). Glutamate and Glutamine, both, are important for regulation of gene expression, cell signaling, and immune responses (Wu, 2010). Similarly, highest content of glutamic acid in raho is in accordane with the findings of Mohanty et al. (2014a, b) who also find high content of glutamic acid in raho than mahseer.

After glutamic acid, aspartic acid was also present in abundance and the percentage was within range as observed in fresh water species investigated by other researchers (Azrita et al., 2024; Elaigwu, 2019). Similar studies with freshwater fish species have proven the abundance of glutamic acid and aspartic acid (Özden and Erkan, 2008). The highest content of aspartic aicd in Mahseer aligns with the findings of Chasanah et al. (2021) who also examined higher aspartic acid in mahseer than other freshwater species. Aspartic acid plays many important biological functions as it regulates hormone secretions of immense importance as well as is precursor of other AAs including leucine, isoleucine, methionine and threonine, isoleucine, and lysine.

All the species examined, also, appeared to have good amount of arginine, an amino acid necessary for wound healing (Williams and Barbul, 2012). Study carried by Azrita et al. (2024) showed almost a same range of arginine in Freshwater Bagridae Fish species as was observed in our studied species. In another study similar values of arginine was observed in 2 fresh water species i.e. catfish and tilapia (Osibona et al., 2009) further confirming our results.

Among essential amino acids EAAs, the highest amount was observed for leucine followed by lysine and isoleucine in all examined species. Studies have shown that leucine is helpful in healing of skin and muscle tissues as well as bone formation and holds therapeutic properties in stressful conditions such as sepsis and burn injuries (De Bandt and Cynober, 2006). Additionally, isoleucine promotes muscles (Charlton, 2006) and hemoglobin formation and lysine is precursor of glutamate which is an important neurotransmitter (Papes et al., 2001). Lysine is considered a limiting amino acid in foods of plant origin (Chapman et al., 2010) and its deficiency can cause mental and physical disorders. However this deficiency can be supplemented by adding these fish in diet.

Carnivore species are expected to have more EAA and NEAA compared to herbivore species which justifies our finding that mali exhibiting highest proportion of EAA and NEAA. However, this contradict with the previous findings showing high content of essential amino acid in herbivorous species (Mohammed and Alim, 2012).

Since all the studies fish species have both EAA and NEAA, these can be consumed along with plant diet for healthy body composition. According to Osibona et al. (2009) the AA composition of fish and human beings are similar, thus, fish consumption with cereals can fulfill body's protein requirement.

The fatty acid pattern of our study aligned closely with the pattern previously documented by Osman et al. (2007). Our research methodology successfully recovered all the major fatty acid, targeted in study (Table S1). Oliec acid appeared as most prevalent MUFA especially in rohu and since this fatty acid is not synthesize in fish body and must be sourced from outside, hence it reflects the diet fish takes (Ackman, 1989). The abundance of oleic acid and palmitoleic acid in these species aligned with previous studies that have proved that in freshwater species from Brazil, oleic acid and palmtoleic acid were most abundant MUFAs while most dominating SFAs were palmitic and stearic acid (Andrade et al., 1995), (Table 2). Among SFAs, palmitic acid was in prominent FA in species under discussion. Aggelousis and Lazos (1991) argued that freshwater species had palmitic acid, palmitoleic acid, oleic acid, EPA, and DHA as most prominent fatty acids similar to our findings suggesting high levels of plamitic acid.. Likewise, among the studied SFAs and MUFAs, the highest palmitic acid followed by oleic acid, myristic acid and stearic acid was similar with the results of Łuczyńska et al. (2008).

Both DHA and EPA have been known to have ameliorative effects against heart diseases (Nøstbakken et al., 2021) and are beneficial in reducing muscular inflammation and pain in human beings (Table 3).

Significant levels of DHA and EPA is indicative of the nutritional importance of these fishes. Similarly, linoleic acid is necessary for tissue development and general health. All species were rich in this fatty acid as well. Among all species raho was richest in SFAs, MUFAs and PUFAs which highlights the feeding habitat of this species whose prime diet is phytoplankons which in turn are source of essential FAs. However, overall, all the fishes investigated had comparable nutritional values. These Indus River fish species have high

Table 3. Sum of essential amino acids and non-essential amino acids in mali, raho and mahseer.

Amino Acid contents	Mali Fish	Raho Fish	Mahseer Fish
∑EAA	42.902	38.375	37.811
∑NEAA	63.353	60.753	61.592

Table 4. Sum of total SFAs, MUFAs, and PUFAs in mali, raho and mahseer.

Fatty acid contents	Head Region			Abdomen Region			Tail Region		
	Mali Fish	Raho Fish	Mahseer Fish	Mali Fish	Raho Fish	Mahseer Fish	Mali Fish	Raho Fish	Mahseer Fish
∑SFA	30.624	76.991	70.404	70.983	68.636	71.382	69.687	77.906	70.669
∑MUFA	13.403	12.817	21.257	21.46	47.107	20.593	24.61	21.377	20.47
∑PUFA	57.973	41.733	36.09	37.773	40.214	35.707	35.817	40.503	36.25

percentage of SFAs than MUFAs and PUFAs (Table 4). Earlier investigation also proved that freshwater fish species have lower PUFAs than SFAs as these fishes consume plant material and vegetation (Vlieg and Body, 1988).

This study showed that all the investigated species of Indus River had significant nutritional values and are advantageous to health.

5. Conclusion

Our results showed that all fish species under discussion are rich in amino acids and fatty acids. These species contain essential amino acids and important fatty acid such as omega3 and omega 6 thus raising the nutritional quality of these species. This research will help the people to make informed choice while selecting fish for consumption. Additionally, this study will provide useful insight into the nutritional composition of fish consumed by local community, will pave the way for future researches and will be helpful in planning new strategies to enhance the growth and production of commercial fish species thus setting the foundation for future researches in this field.

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Supplementary Material

Supplementary material accompanies this paper.

Table S1. (Supplimentary material) Fatty acid composition in the head, abdomen and tail region mali, raho and mahseer from Indus River.

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