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NUTRITIONAL COMPOSITION OF SEVEN COMMERCIALLY IMPORTANT FRESHWATER FISH SPECIES AND THE USE OF CLUSTER ANALYSIS AS A TOOL FOR THEIR CLASSIFICATION

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ABSTRACT

This study aimed to investigate the chemical composition and amino acid (AA) profile of seven freshwater fish species representing two mean live-weights inhabiting River Indus. For this purpose chemical composition was analysed by standard methods and amino acids were determined with an amino acid analyser (Biochrom-30 ion-exchange analyser). The fish species and live-weights differed significantly ($P<0.01$) for various nutrient compositions. Total fat and ash contents in all analysed fish species were higher in high weight (W2) than low weight (W1) category, while crude protein and total carbohydrates were higher in W1 than W2 category. These fish species contained most essential AA and particularly Lysine. Generally more amino acids were recorded in W1 than W2category. Cluster analysis showed that carnivorous fish species (*A. oar sarwari, C. marulius* and *W. attu*) were more similar to each other than herbivorous fish species (*L. rohita* and *C. mrigala*), while these differed from omnivorous fish species (*C. carpio* and *O. mossambicus*) with respect to amino acid contents. These fish offered good nutritional values and could be consumed to promote health and perhaps disease prevention in human beings.

Keywords: Amino acids, chemical composition, freshwater fish, nutritional value.

INTRODUCTION

The freshwater fish species provide food, subsistence and supplemental income to a wide range of people, especially those that live around various rivers (Mohammed and Alim, 2012). Fish and fishery products represent a valuable source of nutrients for diversified and healthy diets (Fawole *et al*., 2007). With a few exceptions of selected species, fish is usually low in saturated fats, carbohydrates and cholesterol. Fish provides not only high-value protein, but also a wide range of essential micronutrients, including various vitamins (A, B and D), minerals (e.g. calcium, iodine, zinc, iron and selenium) and omega-3 fatty acids (docosahexaenoic acid, DHA and eicosapentaenoic acid, EPA). Fish are known to be a source of protein rich in essential amino acids (lysine, methionine, cysteine, threonine and tryptophan) (FAO, 2011). It is recognised that essential amino acids play an important role in human nutrition and health promotion (Limin *et al*., 2006). There is an evidence of beneficial effects of fish consumption (FAO, 2011) in relation to coronary heart disease (Mozaffarian and Rimm, 2006), stroke, age-related muscular degeneration and mental health (Peet and Stokes, 2005). There is also convincing evidence of benefits in terms of growth and development, particularly for women during gestation and children during infancy for optimal brain development (Young and Conquer, 2005).

It is important to assess the meat quality of freshwater fish species that are less frequently analysed prior to their processing and storage. Such information can help to preserve the quality especially during post-harvest processing and storage of fish which otherwise could be affected by the level of moisture, protein and fat contents (Mohamed *et al*., 2010). Fish has an important role in food security and poverty alleviation in both rural and urban areas of Pakistan but little is known about the nutritional value of the Indus fishes of our selected study area. Better knowledge of their nutritional value, which is expected to be closely associated with fish species, could contribute to the understanding of variability in meat quality of different species of the Indus fish. Moreover, the measurement of some proximate profiles such as protein, lipids and moisture contents are often necessary to ensure that they meet the requirements of food regulations and commercial specifications (Waterman, 2000). Therefore, the present study compared the chemical and amino acid contents of the commercially most preferred seven species [*Cyprinus* (*C*) *carpio* (Common carp), *Labeo* (*L*) *rohita* (Rohu), *Cirrhinus* (*C*) *mrigala* (Mori), *Aorichthys* (*A*) *aorsarwari* (Singhari), *Channa* (*C*) *marulius* (Great snakehead or Giant Murrel or Sole), *Oreochromis* (*O*) *mossambicus* (Tilapia) and *Wallago* (*W*) *attu* (Malli)] from River Indus in Mianwali district. In spite of the commercial value and wide availability of these fish species, there is no available

report on their nutritive or caloric values and amino acid composition from the study area.

The selected fish represented typical habitats, food niches and market prices. For example, Tilapia (*O. mossambicus*) is a hardy, versatile and consumer popular white fish, whereas, *L. rohita*is the tastiest fish that can survive in different rivers and lakes. While *C. mrigala* are mostly herbivorous, can tolerate highly saline waters whereas, *C. carpio* is one of the most widely cultured fish species in the world. Moreover, *A. aorsarwari* is rich in vitamin A and other nutrients, *W. attu* is a fish of all seasons with a stable market price and *C. marulius* is not only tasty but also perceived to have wound healing characteristics. We also examined if variation in weights of selected fish could affect their muscle composition. This study will stimulate research on similar lines as the knowledge of chemical and amino acid compositions of different fish species is essential before selecting suitable methods of fish preservation, processing, product development and fish feed formulation. We anticipate that such information would facilitate the fish grading by using nutrient and amino acid compositions that will help to determine the market price of fish for health conscious consumers and the livelihood of fishing community of this study area and beyond.

MATERIALS AND METHODS

Study Area: This study was carried out in Mianwali District of Punjab Pakistan, which is situated at the bank of River Indus. This river is the prime water source and hence called the lifeline of Pakistan. The catchment area of River Indus covers one million km^2 involving several high mountains. There are approximately 200 species of fish in freshwaters of Pakistan, of which a large number is endemic (FAO, 2012).

Fish sampling: Fishing was performed with the help of professional local fishermen in March, 2014. Around 210 samples were collected by selecting thirty samples of each fish species (*C. carpio*, *L. rohita*, *C. mrigala*, *A. aorsarwari*, *C. marulius*, *O. mossambicus* and *W. attu*) of two different sizes [weight I (W1) = about 1kg; Weight 2 (W2) = about 2kg] by involving ten fish (five of each weight category) per net replicates on ice from different catchment sites of the study area. These catchment sites are known for the commercial supply of fish in the country. The weights of these fishes were selected on the basis of fish size normally caught, sold and bought in the study area. The fish samples were immediately transported in an ice chest at 4°C to the research laboratory of the Department of Zoology, Government College University Faisalabad Pakistan, where morphometric measurements by involving wet weight (WW), length, and width of each of these fish were carried out.

Fish dissection, preservation and sample preparation: After performing morphometric measurements, each fish was dissected. These fish were then skinned, gutted, filleted and transferred into marked sterilized polythene bags before their storage at -20°C until further analyses. Frozen fish samples were transported on dry ice in April 2014 to the research laboratory of the School of Agriculture Food and Rural Development, Newcastle University UK, where further analyses of fish samples were carried out. All fish samples of known weight were lyophilized by using a Lyo Lab G Freeze Dryer (Lyophilization Systems Inc., USA) at -50 °C for 72 h. These lyophilized samples were then ground and homogenized by using a homogenizer (Polytron, Kinematica GMBH). The homogenized samples were then used in triplicate for different chemical analyses.

Analysis of fish samples

Chemical composition: Moisture contents of the representative muscle samples of seven fish species of different weights were determined in triplicate by difference in weighed samples before and after their freeze drying in Lyo Lab G Freeze Dryer (Lyophilization Systems Inc., USA) at -50°C for 72 hours. Nitrogen (N) contents of lyophilized samples were determined by using the Dumas method on Leco (model FP-428, Leco Corporation St. Joseph. MI, USA). The N content was multiplied with 6.25 to estimate the crude protein (CP) of each sample. Ash was determined by burning the organic components from the known weights of the homogenized lyophilized fish muscles by using a furnace (AAF1100, Carbolyte, Parson Lane Hope valley, England) at 550 °C for 8 hours. Total crude fat from homogenized lyophilized muscles was determined by using non-polar organic solvent petroleum ether (Boiling point = 40–60 °C, Fisher Limited, UK) on a soxhlet apparatus. Total carbohydrates were determined by subtracting the sum of % values of crude fat (CF), CP and ash contents (A) from 100 (Onyeike *et al*., 2000) by using the following equation:

% Total carbohydrates = 100 – (CF + CP + A)

Total gross energy (caloric) value of each sample was determined by multiplying the percentage of CP, CF and total carbohydrate (C) contents with their respective energy values of 4, 9 and 4 kcal per 100 g of a fish sample to obtain the caloric values of these samples by using the following equation:

Caloric value = (4CP +9CF +4C) kcal/100 g weight

Amino acid composition: Amino acids composition of fish samples were determined by using an amino acid analyser (Biochrom-30 ion-exchange analyser, Biochrom Ltd, Cambridge, UK). The amino acids were expressed as percentages of total amino acids in each fish species for each mean live-weight and then the relevant ratios of essential amino acids were determined.

Sample preparation and description for amino acid analysis: Each lyophilized homogenized muscle sample was treated with norleucine as an internal standard and dried in a pyrolysed tube by placing it in a centrifugal evaporator. After this, gas phase hydrolysis of each sample was performed in a vial (Waters, WAT007363) at 115 ^{o}C for 22 hours. After adding hydrochloric acid (BDH, Aristar diluted 1:1 with water; 0.5ml) containing phenol (saturated solution in water; 7.5ul) followed by dodecanethiol (Sigma; 68 ul), the vial was evacuated, flushed with argon and evacuated several times before it was closed and placed in an oven at 115 ^{o}C for 22 h. The vial was cooled before it was opened and placed in desiccators under vacuum over sodium hydroxide for 40 minutes or longer if necessary. After removing traces of acid, sodium citrate loading buffer (pH 2.2) was added to dissolve the residue. The resulting solution was filtered under centrifugation through a 0.2 micron filter. An aliquot of the filtrate was injected into an amino acid analyser (Biochrom 30 instrument) and chromatography was performed on an ion exchange resin (sodium system) eluting with a series of buffers over the pH range of 3.2 to 6.45. Peak detection was achieved by mixing elute with ninhydrin at 135 ^{o}C and measuring the absorbance at 570 and 440 nm. Quantitation was performed by using Chromeleon software and calibration curves for each amino acid of interest.

Statistical Analysis: The data were statistically analysed by using the Minitab software 16 to compare the effects of fish species (S), Weight (W) and S x W interaction on different nutrient and amino acid components. These effects were declared significant if $P<0.05$, highly significant if $P<0.01$ and very highly significant if $P<0.001$. Tukey's test was used if there were more than two means to compare by using relevant standard errors of means (SEM). The amino acid composition of Indus fish protein was also compared with that of the standard protein being recommended by World Health Organization (FAO, 1991). Different fish species were also analysed by cluster analysis on the basis of total amino acid composition in the fish muscles by using Minitab16 software and the results were interpreted in the form of dendrograms (Single Linkage, Euclidean Distance).

RESULTS

Table 1 shows highly significant differences among species, weight categories and species x weight interactions ($P<0.001$) for most morphometric and chemical compositions. Total fat and ash contents in all analysed fish species were higher in W2 than the W1 category, while crude protein and total carbohydrates were higher in W1 than the W2 category. The average total fats in the analysed fishes varied from 11 to 21% where *L. rohita>C. marulius>A. aorsarwari> C. mrigala =O. mossambicus>C. carpio>W. attu*. Conversely, average total ash ranged from 3.2 to 10% and it was highest in *O. mossambicus>L. rohita>W. attu>C. carpio>C. mrigala>A. aorsarwari>C. marulius.* Total crude protein varied from 60-80% where *W. attu>C. marulius>A. aorsarwari>C. mrigala>C. carpio>L. rohita>O. mossambicus.* For total carbohydrates, the fish species showed that *O. mossambicus>C. carpio>L. rohita>A. aorsarwari>C. mrigala>C. marulius>W. attu*and total caloric value was greatest for *C. marulius> L. rohita>A. aorsarwari>C. mrigala>C. carpio>O. mossambicus*and *W. attu*that appeared to be high in crude protein contents but low in crude fat, total carbohydrates and energy value (Kcal/100g). *O. mossambicus* appeared to be high in ash and total carbohydrate contents but low in crude protein contents. *C. marulius* appeared to be high in energy value (Kcal/100g) and lowest in ash contents. All fish species of this study appeared to be good sources of nutrients (Table 1). Sixteen amino acids (mg/g) were identified in all analysed fish muscle samples except tryptophan which was lost due to acid hydrolysis (Table 2). All analysed amino acids showed highly significant differences for species, weight and species x weight interactions ($P<0.001$) except for serine where non-significant differences were observed for weight categories ($P>0.05$). Generally increased amount of amino acids was found in W1 category than W2. Out of 16 analysed amino acids 12 amino acids were higher in proportion in W1 category than W2. Among essential amino acids, Lysine, Leucine and Valine were higher in amounts while Tyrosine and Methionine were least in amount in these fish muscle samples (Table 3). The order of essential amino acids in *C. carpio, L. rohita, A. aorsarwari, C. marulius* and *W. Attu* was Lys>Leu>Val>Ile>Thr>Phe> Tyr>Met while in *C. mrigala* and *O. mossambicus* was Lys>Leu>Val>Ile>Phe>Thr>Tyr>Met. Among non-essential amino acids Glutamic acid, Aspartic acid and Arginine were higher while Proline, Serine and Histidine were lower in amount in all analysed muscle samples. A diverse order of non-essential amino acids was observed in all the analysed samples. In *C. carpio*and *C. mrigala*it varied from highest to lowest as Glu>Asp>Arg>Ala >Gly>Pro>Ser>His; in *L. rohita, A. aorsarwari, O. mossambicus* and *W. attu*t and he order of non-essential amino acids varied from highest to lowest as Glu>Asp >Arg>Ala>Gly>Ser>Pro>His; in *C. marulius* the order was Glu>Asp>Arg>Ala>Gly>Pro>Ser>His. More essential amino acids were found in *C. marulius* as compared to other Indus fishes. The ratio of essential to non-essential amino acids ranged from 0.81 to 0.86. The principal amino acids amongst the essential amino acids were lysine, leucine and valine and those amongst the non-essential amino acids were aspartic acid, glutamic acid and arginine in all analysed samples (Table 3). Table

4 presents the proportions of essential amino acids in a standard protein and in the muscles of different fishes of this study. The amounts (31.6 to 34.5 g/100 g protein) of essential amino acids in five Indus fishes (*L. rohita, C. mrigala, A. aorsarwari, C. marulius* and *W. attu*) were greater than that in the standard protein (30.9 g/ 100 g protein). However, the amount (30.1 and 29.9 g/ 100 g protein) of essential amino acids in *C. carpio* and *O. mossambicus* were slightly less than that in the standard protein (30.9 g/ 100 g protein). Here, *C. marulius* showed higher amount of all essential amino acids as compared to the standard protein (FAO, 1991). Lysine was found to be higher in all analysed Indus fishes as compared to the standard protein.

To classify the fishes according to different trophic levels, cluster analysis using single linkage, Euclidean distance method based on total amino acids was used. Cluster analysis divides the seven fish species into four clusters and three groups by similarity (Figure 1). The cluster analysis of total amino acids showed that group 1 is further subdivided into 1and 5 showing good similarity (74.44%) between *L. rohita* and *C. mrigala.* The group 2 is further divided into 2 and 3 followed by 6 showing that *A. oar sarwari* and *W. attu* showed good similarity (79%) followed by *C. marulius (*72.44%). Group 3 is further subdivided into 4 and 7 showing that *C. carpio* and *O. mossambicus* are similar (65%) but had no similarity with other species (Figure 1). Cluster analysis appeared to be an important tool in classifying the species according to their trophic level and feeding habit with regard to amino acid contents in to three groups i.e., group 1= herbivores = major carps, group 2 = carnivores and group 3= omnivores.

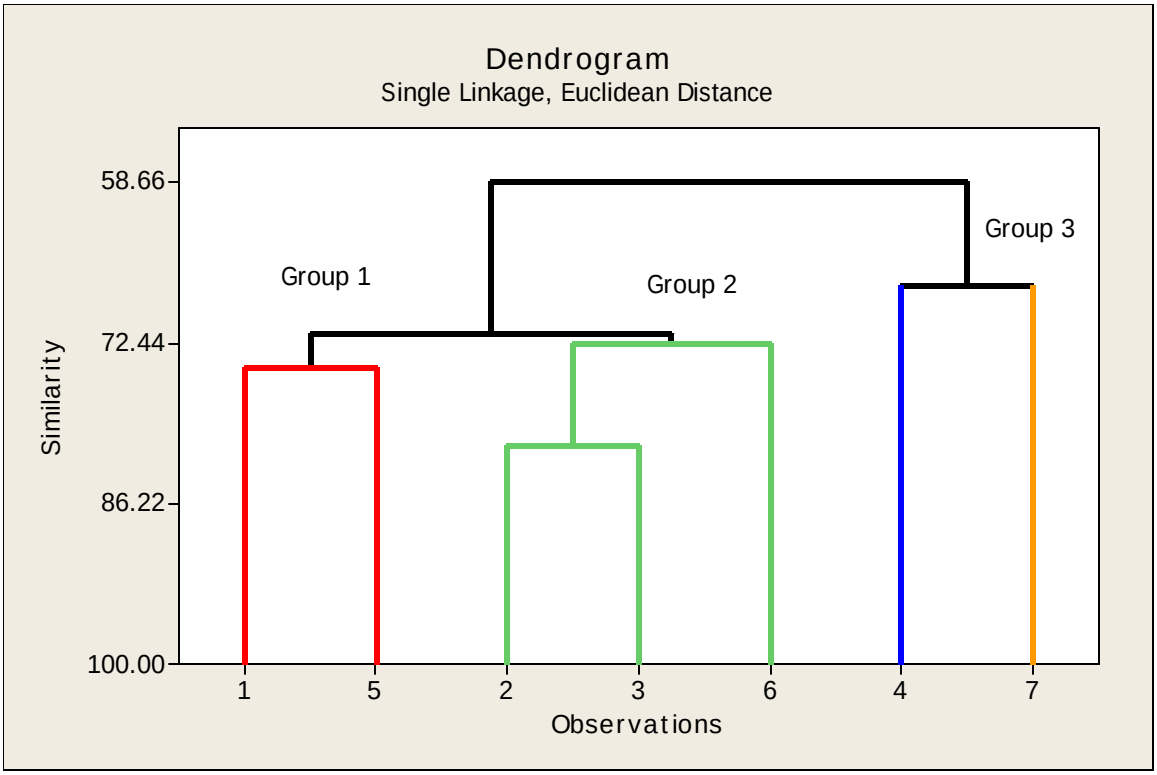

Figure 1 showing three trophic groups at 4 cluster levels of fish species based on their total amino acid contents where 1= *L.rohita,* 2= *A. aorsarwari,*3= *W.attu,* 4= *C.carpio* 5= *C.mrigala,* 6= *C.marulius* 7= *O.mossambicus*

Table1. Morphometric variables and chemical composition as % dry matter of muscles in different freshwater fish species from River Indus

Variables	*C. carpio*		*L. rohita*		*C. mrigala*		*A. aorsarwari*		*C. marulius*		*O. mossambicus*		*W. attu*		SEM and Significance		
	W1	**W2**	**W1**	**W2**	**W1**	**W2**	**W1**	**W2**	**W1**	**W2**	**W1**	**W2**	**W1**	**W2**	**Species (Sp)**	**Weight (W)**	**Sp x W**
Length (cm)	42.2	47.3	31	41	42.7	58.3	55	60.8	51	70.8	17.5	20.2	53.1	60.8	0.27***	0.14***	0.38***
Width (cm)	12.2	15.3	10.3	12.8	12.5	17.7	9.5	11.8	12.7	15.3	7.8	8.3	10.5	14.1	0.18***	0.09***	0.25***
Dry Matter	23.6	23.1	23.7	23.3	27	27	21.6	23	22	22.4	25.1	24.4	21.3	21.5	0.32	0.19	0.46
Fat	15.3	19.5	17.7	21.1	14.4	20.6	15.9	19.3	17	19.9	16	19	11.7	13.9	0.35***	0.19***	0.49***
Ash	5.4	5.4	5.6	6.6	4.5	5.3	3.7	4.8	3.2	4.5	7.5	10	5.3	5.9	0.17***	0.09***	0.24***
Crude Protein	75	74	73	71.3	78.6	73	77	75	78	74.2	68	67.7	80.5	79.6	0.40***	0.21***	0.57***
Carbohydrates	4.3	1	3.7	0.96	2.5	1.1	3.4	0.9	1.8	1.3	16.5	10.3	2.4	0.6	0.43***	0.23***	0.61***
Energy(Kcal/100g)	455.2	475.8	466	478.9	454	481.6	464.8	477.6	472.4	481.7	450	455	437	446	1.93***	1.03***	2.74***

SEM= Standard Error of Means;***=P<0.001;W1=1005±2.3/-g and W2= 2018±5.25

Table 2.Mean amino acid profile (mg/g) of different freshwater Indus fish species of two weight categories (W1= 1005±2.3 g and W2= 2018±5.25 g) together with the standard error of means (SEM) and significance.

AA	*C. carpio*		*L. rohita*		*C. mrigala*		*A. aorsarwari*		*C. marulius*		*O. mossambicus*		*W. attu*		SEM and Significance		
															Species (Sp)	Weight (w)	Sp x w
	W1	**W2**	**W1**	**W2**	**W1**	**W2**	**W1**	**W2**	**W1**	**W2**	**W1**	**W2**	**W1**	**W2**			
Ala	38.9	41.4	43.4	41	40.8	42.3	39.8	39.7	43	44.5	40.1	40.1	40.5	40	0.03^{***}	0.01^{***}	0.04^{***}
Arg	42.2	44.4	50.3	44.6	45.4	46.5	47.6	47.8	49.6	50.1	42.7	42.7	49.2	49.8	0.04^{***}	0.02^{***}	0.06^{***}
Asp	71.2	73	83.5	76.5	77.3	78	81.9	82.3	83	86.8	75.5	75.5	81	80.8	0.04^{***}	0.02^{***}	0.05^{***}
Glu	104.2	107.7	125.5	109.1	113.6	113.5	119.2	120	123.2	124.8	109.6	109.6	122.5	122.5	0.03^{***}	0.02^{***}	0.04^{***}
Gly	26.6	33.7	31.3	31.6	26.5	30	28.4	28.6	29.8	33.1	33.6	33.6	27.2	28.8	0.188^{***}	0.101^{***}	0.266^{***}
His	24.9	24.1	23.9	21.5	23.1	23.2	17.6	17.5	20.4	21.1	18.5	18.2	18	18.3	0.06^{***}	0.03^{***}	0.09^{***}
Ile	33	33.2	38	34.2	35.4	35.1	37	36.	37.9	39.7	33.5	33.5	38.5	38.2	0.03^{***}	0.02^{***}	0.04^{***}
Leu	59	57.2	66.7	60.3	61	60.8	64.4	64.2	65.4	67.3	57.9	57.9	65.3	65.3	0.19^{***}	0.102^{***}	0.27^{***}
Lys	66.4	68.9	78.9	70.7	72.6	71.9	77.1	77.4	76	78.5	67.7	67.7	77.7	77.6	0.003^{***}	0.002^{***}	0.005^{***}
Met	22.5	21.4	24.3	21.1	22	21.5	23.1	23.3	23.3	23.9	20.3	20.2	23.7	24.2	0.03^{***}	0.01^{***}	0.04^{***}
Phe	29.4	30.5	32.3	30.8	30.7	31.1	32.1	32.2	32.7	34.8	30.8	30.8	33.8	32.1	0.04^{***}	0.02^{***}	0.05^{***}
Pro	24.5	28	28	25.2	26.1	27.8	25.9	25.6	28.1	30.4	23.1	23.1	26.2	25.7	0.03^{***}	0.01^{***}	0.04^{***}
Ser	25.5	26	28.8	26.1	25.7	26.9	28.1	28	28.6	29.1	24.8	24.8	29.2	29.8	0.03^{***}	0.02^{NS}	0.05^{***}
Thr	30.2	30.3	34.1	30.3	30.1	31.1	34.5	34.4	34.2	35.9	30.5	30.5	33.9	34.4	0.03^{***}	0.02^{***}	0.04^{***}
Tyr	24.3	23.9	28.9	25.8	26	25.9	26.6	26.9	29	29.4	23.9	23.9	28.9	28.8	0.03^{***}	0.02^{***}	0.04^{***}
Val	35.6	35.6	41.2	37.9	38	38.2	37.5	37.5	40.1	41.9	34.7	34.7	39.6	39.7	0.03^{***}	0.02^{***}	0.04^{***}

*** = Significance at P<0.001; SEM= Standard Error of Means; NS= Non significant at P>0.05

Table 3.Mean (± SD) concentration (mg/g) of essential and non-essential amino acids in muscles of different freshwater fish species

Amino Acids	*C. carpio*	*L. rohita*	*C. mrigala*	*A. aorsarwari*	*C. marulius*	*O. mossambicus*	*W. attu*
Tyrosine	24.1 ± 0.21^{f}	27.3±1.68^{c}	26±0.08^{e}	26.8±0.14^{d}	29.2±0.21^{a}	23.9±0.01^{g}	28.9±0.05^{b}
Phenyl alanine	29.9±0.59^{g}	31.6±0.84^{d}	30.9±0.18^{e}	32.1±0.07^{c}	33.8±1.13^{a}	30.8±0.01^{f}	32.9±0.90^{b}
Isoleucine	33.1±0.11^{g}	36.1±2.10^{d}	35.3±0.18^{e}	36.7±0.35^{c}	38.8±1.01^{a}	33.5±0.01^{f}	38.3±0.17^{b}
Leucine	58.1±1.47^{e}	63.5±3.48^{c}	60.9±0.12^{d}	64.3±0.11^{c}	66.3±1.05^{a}	57.9±0.01^{e}	65.3±0.02^{b}
Lysine	67.7±1.34^{f}	74.8±4.50^{c}	72.3±0.36^{d}	77.3±0.16^{b}	77.3±1.41^{b}	67.7±0.01^{e}	77.6±0.28^{a}
Methionine	22±0.57^{e}	22.7±1.73^{d}	21.7±0.30^{f}	23.2±0.13^{c}	23.6±0.36^{b}	20.3±0.01^{g}	23.9±0.28^{a}
Threonine	30.2±0.05^{g}	32.2±2.04^{d}	30.6±0.56^{e}	34.5±0.06^{b}	35.1±0.91^{a}	30.5±0.01^{f}	34.2±0.31^{c}
Valine	35.6±0.03^{f}	39.6±1.84^{c}	38.1±0.13^{d}	37.5±0.02^{e}	41±0.98^{a}	34.7±0.01^{g}	39.7±0.02^{b}
∑ Essential Amino Acids	**300.7^{F}**	**327.8^{D}**	**315.8^{E}**	**332.4^{C}**	**345.1^{A}**	**299.3^{G}**	**340.8^{B}**
Aspartic acid	72.1±0.95^{g}	80±3.83^{d}	77.6±0.38^{e}	82.1±0.21^{b}	84.9±2.10^{a}	75.5±0.01^{f}	80.9±0.12^{c}
Serine	25.8±0.24^{f}	27.5±1.47^{d}	26.3±0.70^{e}	28±0.02^{c}	28.8±0.26^{b}	24.8±0.01^{g}	29.5±0.32^{a}
Glutamine	106±1.95^{g}	117±9.01^{d}	113.5±0.04^{e}	119.6±0.42^{c}	124±0.88^{a}	109.6±0.01^{f}	122.5±0.05^{b}
Glycine	30.1±4.03^{c}	31.5±0.19^{b}	28.2±1.90^{d}	28.5±0.10^{d}	31.4±1.83^{b}	33.6±0.01^{a}	28±0.84^{d}
Alanine	40.1±1.35^{e}	42.2±1.34^{b}	41.5±0.86^{c}	39.8±0.01^{g}	43.7±0.83^{a}	40.1±0.01^{f}	40.3±0.24^{d}
Histadine	24.5±0.43^{a}	22.7±1.29^{c}	23.2±0.05^{b}	17.6±0.02^{f}	20.7±0.40^{d}	18.3±0.41^{e}	18.1±0.17^{e}
Arginine	43.3±1.21^{f}	47.5±3.15^{d}	46±0.57^{e}	47.7±0.09^{c}	49.8±0.23^{a}	42.7±0.01^{g}	49.5±0.34^{b}
Proline	26.2±1.91^{d}	26.6±1.53^{c}	26.9±0.95^{b}	25.8±0.17^{f}	29.2±1.26^{a}	23.1±0.01^{g}	25.9±0.31^{e}
∑ Non-Essential Amino Acids	**368.1^{F}**	**395^{B}**	**383.2^{E}**	**389.1^{D}**	**412.5^{A}**	**367.7^{F}**	**394.7^{C}**
Essential AAs: Non-Essential AAs	**0.82**	**0.83**	**0.82**	**0.85**	**0.84**	**0.81**	**0.86**

Means with different letters in the same row differ significantly P<0.05

Table 4.Mean concentration (g/100g) of essential amino acids in muscles of different freshwater fish species of this study and a standard protein recommended by FAO (1991)

Amino Acids	FAO/WHOStandard	*C. carpio*	*L. rohita*	*C. mrigala*	*A. aorsarwari*	*C. marulius*	*O. mossambicus*	*W. attu*
Tyr+Phe	6.3	5.4^{f}	5.89^{c}	5.69^{d}	5.89^{c}	6.3^{a}	5.47^{e}	6.18^{b}
Ile	2.8	3.31^{g}	3.61^{d}	3.53^{e}	3.67^{c}	3.88^{a}	3.35^{f}	3.83^{b}
Leu	6.6	5.81^{e}	6.35^{c}	6.09^{d}	6.43^{c}	6.63^{a}	5.79^{e}	6.53^{b}
Lys	5.8	6.77^{f}	7.48^{c}	7.23^{d}	7.73^{b}	7.73^{b}	6.77^{e}	7.76^{a}
Met +Cys	2.5	2.2^{e}	22.7^{d}	2.17^{f}	2.32^{c}	2.36^{b}	2.03^{g}	2.39^{a}
Thr	3.4	3.02^{g}	3.22^{d}	3.06^{e}	3.45^{b}	3.51^{a}	3.05^{f}	3.42^{c}
Val	3.5	3.56^{f}	3.96^{c}	3.81^{d}	3.75^{e}	4.1^{a}	3.47^{g}	3.97^{b}
∑ Essential Amino Acids	**30.9**	**30.07^{F}**	**32.78^{D}**	**31.58^{E}**	**33.24^{C}**	**34.51^{A}**	**29.93^{G}**	**34.08^{B}**

Means containing different letters in the same row differed significantly at P<0.05

DISCUSSION

The seven Indus fish species being investigated in the present study are the most preferred market fish for both fishermen and consumers of rural and urban areas of Pakistan. In fact the selected seven fish species of this study represented different food niches comprising herbivorous, carnivorous and omnivorous. The nutritional quality of these selected fish species was assessed on the basis of essential amino acid profile of their muscle protein as compared to those of the standard protein of FAO (1991). The nutritional components showed variable values in all the analysed Indus fish species. It was reassuring to observe that all fish species of this study had high levels of crude protein and the essential amino acids, indicating their importance as a nutritional resource for a region where protein foods are in short supply and so fish as a quality protein food can fetch high market price (Jabeen and Chaudhry, 2011; Zuraini *et al*., 2006).

Amongst essential amino acids, lysine was present in higher amounts in all analysed Indus fishes as compared to the standard protein (FAO, 1991), which is severely restricted in cereals being the most important staple food in the world. A reduced supply of lysine in the diet may lead to mental and physical disorders as it is an essential precursor for the de novo synthesis of glutamate, which is the prime neurotransmitter in the mammalian central nervous system (Papes *et al*., 2001). It could be suggested that the presence of appreciable amounts of limiting essential amino acids like Lysine and Leucine in the Indus fish species can supplement the corresponding deficiency in plant proteins. Thus, these fish species can be utilised generously for a healthy body composition alongside cereals as a staple food.

As the amino acid composition of fish is similar to that in human beings, people can gain essential and non-essential amino acids by consuming fish alongside cereals to satisfy their body needs to maintain health (Osibona *et al*., 2009). In all the selected fish Lysine, Leucine and Arginine were more abundant than the other amino acids. While Leucine promotes the healing of bones, skin and muscle tissues; Isoleucine is necessary for haemoglobin formation and regulation of blood sugar and energy; and Arginine is important for wound healing (Williams and Barbul, 2012). Since the quantity of these amino acids in the selected fish were of sufficient level, the consumption of these fish could be beneficial in satisfying the body needs while treating metabolic disorders and wound healing of the neighbouring communities of the study area and beyond.

The highest amount of Glutamic acid being observed in all seven fish species was in line with the study of Dezhabad *et al*. (2012) and Kenari *et al.* (2009). All analysed freshwater fish species appeared to be an excellent source of Glutamic acid which could enhance the immune system. Both Glutamate and Glutamine play important roles in regulating gene expression, cell signalling, anti-oxidative responses and immunity (Wu, 2010).

With regard to their nutritional values, all selected fish contained all the essential amino acids that were needed by the human beings. It appeared that *C. marulius* was a richer source of total essential and non-essential amino acids as it had the highest respective amounts than the other species. While the proportions of essential to non-essential amino acid for the seven species of this study varied only slightly from 0.81 and 0.86, these were either comparable to those of Dezhabad *et al*. (2012) or greater than those of Kenari *et al*. (2009). Generally, the carnivorous fish are expected to contain more essential and non-essential amino acids than herbivorous fish. However, this is contrary to the findings of Mohammad and Alim (2012) who reported higher essential amino acid contents in herbivorous fish species. Such conflicting findings could be a result of changes in fish diets that are bound to change depending upon the region, season and quality of a freshwater system.

This study has revealed the importance of Indus fish as a good source of protein, essential amino acids, fat and energy. The results of this study are complementary to our previous work (Jabeen and Chaudhry, 2011) which reported good chemical composition and fatty acid profiles of only 3 fish species from the same area of River Indus. It is well known that a majority of consumers do eat fish because of its availability, price, flavour and palatability, while few do so because of its nutritional value. Therefore, the findings of this study would help the fishing community and consumers in their selection of fish species on the basis of their nutritional value along with their taste, size, freshness and external appearances (Mohamed *et al*., 2010). Such information about chemical composition can also influence the selection of a post-harvest processing and storage method that may be more suited to a particular fish species from a specific freshwater system.

The quality of food proteins can be judged against a standard protein, which is recognised as the most relevant for the assessment of the protein quality in the nutrition of all populations (Usydus *et al*., 2009). The amino acid composition of a WHO standard protein has been modified by the Joint Expert Committee of the FAO in 1991, with relevance to the present knowledge on the basis of the amounts of limiting amino acids. It was reassuring to find that the fish species of this study compared reasonably well with the FAO standard of a quality protein as reflected by their amino acid compositions. In fact the Indus fish species of this study were a good source of nutrition to supply excellent mixture of essential and non-essential amino acids.

Cluster analysis showed that carnivorous fish species (*A. oar sarwari, C. marulius* and *W. attu*) are

more similar to each other than herbivorous fish species (*L. rohita* and *C. mrigala*), while these are different from omnivorous fish species *C. carpio* and *O. mossambicus*) with respect to amino acid contents (Figures 1). It is interesting to note that this study revealed that generally fish of same feeding habit had similar amino acid contents and fish of low weight had more amino acids as compared to fish of high weight. So it will be convenient for the consumers who cannot buy large fish because of its cost can utilize the small fish which is cheaper and readily available in the local market to gain better nutritional value.

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