Nutritional Evaluation of Some Freshwater Fishes

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The proximate composition, amino acid composition and the PER values of Rohu *(Labeo rohita)*, Mrigal *(Cirrhina mrigala)* and Calbasu *(Labeo calbasu)* are reported. The proximate composition of all the three fishes were similar. However the amino acid composition varied considerably. Protein quality index of the 3 fish proteins calculated from the amino acid composition are also reported. The nutritional quality of the 3 types of proteins to meet the growth requirements of infants, children and adult human beings is discussed. The possibility of substantial improvement in nutritional quality of the three fish proteins to suit the requirements of infants and children by marginal supplementation with valine/valine-rich food for rohu and valine and isoleucine/food rich in valine and isoleucine for mrigal and calbasu is also discussed. The results indicate that of the three fishes rohu is the best in protein quality followed by mrigal and calbasu. All of them have a better amino acid make up than casein to meet the amino acid requirement of adults.

Fresh water fish is a major source of animal protein, especially for the poor. As such there is great need for data on the nutrient content and nutritional quality of the fresh water fishes. Such data, apart from providing valuable information on the nutritional status of these poor people will also provide important tips for combating protein as well as calorie malnutrition among these people. It will also bring to light the presence/absence of any growth promoters/retarders if any present in them. The current knowledge of the nutritional quality of fresh water fishes is largely an extrapolation of the results of marine fishes. Excepting a few reports on the proximate composition of the freshwater fishes (Gopalan *et al.* 1980; Jafri 1973 and Jafri *et al.* 1964) there is no report of a scientific study on the nutritional quality of freshwater fishes.

The introduction of scientific acquaculture has enhanced the production as well as future production potential of freshwater fishes, substantially. Present day acquaculture heavily relies upon the growth rate namely, the amount of protein/edible mass produced per unit cost and the consumer acceptability. Much attention is not paid to the quality of the protein produced. For selecting suitable species for acquaculture the nutritional quality of the protein should have been the prime criterion, than growth rate alone. Considering all these points, three species of freshwater fishes used in acquaculture were selected for studying their nutrient composition and nutritional quality.

Materials and Methods

Ten kg of each of Rohu *(Labeo rohita)*, Mrigal *Cirrhina mrigala)* and Calbasu *(Labeo calbasu)* were collected from the local fish farm. The fishes weighed on an average 500 g. The muscle was minced, a small portion of which was used for determining moisture content. The rest of the mince was dried in a tunnel drier to 10% moisture level. The dried meat mince was sealed in polythene bags and stored at -18°C in a cold storage until further use.

The dried fish minces were analysed for proximate composition (AOAC 1975) and amino acid composition (Hirs, 1972; Liu, 1972). The dried fish mince was mixed with corn starch, ground nut oil, cellulose, salt mixture and vitamin mixture after giving due consideration for fat, carbohydrate and moisture content to give a 10% protein diet. The diets were designated control (casein) rohu, mrigal and calbasu. The compositions of the experimental and control diets are shown in Table 1. The vitamin mixture

Table 1. *Composition of the diets* (g/kg)

Ingredients	Diets I Control casein	II Rohu proteins	III Mrigal proteins	IV Calbasu proteins
Corn starch	720.0	706.8	708.0	711.7
Cellulose powder	45.0	45.0	45.0	45.0
Ground nut oil	90.0	88.8	89.4	86.9
Salt mixture	36.0	36.0	36.0	36.0
Vitamin mixture	9.0	9.0	9.0	9.0
Crude protein*	108.6	127.1	125.2	123.8
Total**	1,008.6	1,012.7	1,012.6	1,012.4

* The figures show the amount of the preparation to give 100 g protein. The rest being moisturte, fat and carbohydrate.
** The excess weight above 1000 g in case of diets I, II, III, IV is due to the moisture content of he respective proteins.

Table 2. *Proximate composition of the fishes on wet weight basis*

Name of fish	Moisture %	Protein %	Fat %	Carbohydrate %	Ash %	Calorie content K. Cal.
L. calbasu	76.0	20.5	0.6	1.6	1.75	93.8
C. mrigal	77.1	19.9	0.1	2.2	1.39	89.3
L. rohita	76.9	19.7	0.2	2.4	0.90	90.2

and salt mixture were prepared according to NAS/NRC (1963). The chemicals used for diet formulations were the best I.P. quality available in the market.

Forty male albino rats (Wister strian) of 21 ± 2 days age wer ecollected, they were separated into 4 groups of 10 each with due consideration for body weight and litter-mate distribution. The rats were caged individually and acclimatised for 5 days with the respective diets namely, group I control, group II rohu, group III mrigal and group IV calbasu. After acclimatisation the rats were weighed individually and fed with the respective diets for 4 weeks. Food and water were provided *adlibitum*. Each day the food supplied to and wasted by the animals were recorded. The growth and performance of the animals were monitored by weighing the animals once in a week and observing the animals for deficiency symptoms and physical debilities. As usual the weighing was done after depriving the animals from food and water for 3 h. At the end of the experiment the food intake and weight gains were worked out and PER values for each group were calculated according to NAS/NRC (1963). The animals were killed and samples of blood and liver were collected. The weight of liver and protein content of liver and serum were determined according to Hawk (1971).

Results and Discussion

The proximate composition of the 3 fishes are shown in Table 2. The moisture content of all the three fishes are almost equal, even though calbasu shows a water content of 76% which is about 1% less than that of the other two fishes. The crude protein content of the fishes was found almost equal in all these fishes. However the fat content of calbasu is 3 to 6 times that of rohu and mrigal. This high content of fat in calbasu reflects well in the moisture content of calbasu in accordance with the fat - moisture relationship (Idler & Bitners, 1959).

The amount of carbohydrate present in the fishes showed it to be a more significant consituent than fat, consequently carbohydrate earn the second place in nutrient content of the freshwater fishes. However the content of fat will vary with season and age of the samples. Carbohydrate content, varied between the 3 fishes with calbasu showing the lowest content of 1.6% against the 2.4% carbohydrate of rohu.

The ash content and hence the mineral content also varied considerably between the 3 fishes, with calbasu showing an ash content twice that of rohu. Mrigal with 1.4% of ash occupies an intermediate position.

The results of proximate composition reveal that all the 3 fishes are equally good in nutrient content. The fact that the fat and carbohydrate contents are very small compared to the protein content make the wide variations in fat and carbohydrate content of these fishes immaterial in computing the calorie content. This is reflected well in the calorie content of the 3 fishes which are around 90. The higher amounts of protein and fat in calbasu explains its high calorific value.

The values of proximate composition (Table 2) agree well with the results of Gopalan *et al.* (1980), even though the protein content of rohu and calbasu obtained by them are a little high. Most of the marine fishes show significant amounts of fat(Mukundan & James, 1978; Mukundan *et al.* 1981; Kutty Ayyappan *et al.* 1976) and some of the marine fishes show fat content in the range 8–16%(Gopakumar, 1965). This variation in lipid content between freshwater fish and marine fish is not known. Fat is an important source of bioenergy and that fish fat is an important source of Vitamin A and D and the essential fatty acids (Bailey, 1952; Gopakumar, 1965). Freshwater fishes are poor sources of fat soluble vitamins, essential fatty acids and the bioenergy compared to marine fishes.

Table 3 summerises the amino acid composition of the three fishes. Glutamic acid is the major constituent of the amino acids followed by alanine and lysine. In marine fishes also glutamic acid is the main amino acid constituent, but some marine fishes like grey mullet, Indian halibut and silver jew fish show still higher amounts of glutamic acid (Mukundan & James 1978; Mukundan *et al.* 1981). Incidently it can be seen that pomfret, mullet, crab and prawn contain glutamic acid in the range 15 to 20% (Mukundan *et al.*. 1981). It is seen that rohu also falls in this group with respect to glutamic acid content (Table 3). Glutamic acid, especially its monosodium salt is a known flavouring agent and hence its predominance in amino acid make up can be a reason for the prefered flavour of the table fishes.

Table 3. *Amino acid composition of the 3 fish proteins*

Amino acid	*L. calbasu* mg/g	*C. mrigal* mg/g	*L. rohita* mg/g
Aspartic acid	59.3	57.0	58.5
Theronine	34.9	59.8	40.1
Serine	75.6	66.0	60.9
Glutamic acid	138.2	135.8	180.2
Proline	8.7	11.7	30.8
Glycine	50.7	59.9	41.6
Alanine	120.2	110.3	103.1
Cystine + Cysteine	74.2	44.0	48.1
Valine	29.5	39.0	37.5
Methionine	67.2	28.0	26.6
Isoleucine	26.2	28.2	42.1
Leucine	87.3	85.1	103.5
Tyrosine	40.4	22.4	31.6
Phenylalanine	41.0	37.0	44.6
Histidine	15.5	44.0	36.3
Lysine	90.8	106.4	69.5
Argenine	30.4	52.0	30.3
Tryptophan	12.0	14.1	14.8
Total essential amino acids	388.9	397.6	378.7
Total sulphur amino acids	139.4	72.0	74.7
Total aromatic amino acids	93.4	73.5	91.0

All the three freshwater fishes are good source of lysine the nutritionally most important amino acid in accordance with the general observation that most fishes are rich sources of lysine (Mukundan & James, 1978). Incidently rohu contains the lowest amount of lysine, the highest value being observed in mrigal. However lysine content of these three fishes are enough to substitute its shortage in cereals like rice and wheat in which lysine is the first limiting amino acid (Jansen, 1981).

Table 3 also shows the total amount of sulphur amino acids in the 3 species of fishes. It is seen that the sulphur amino acid content of rohu and mrigal are the same but half that in calbasu.

The sulphur amino acid content of rohu and mrigal is similar to most of the marine fishes, which fall in the range of 4 to 6 (Mukundan & James, 1978; Mukundan *et al.* 1979 Mukundan *et al.* 1981). The high content of sulphur amino acid of calbasu is something peculiar.

Among the three fishes rohu is the richest in total essential amino acid content followed by mrigal and calbasu. However the total essential amino acid content of these freshwater fishes is marginally less than the total essential amino acid content of marine fishes, which is in the range of 45 to 50% (Mukundan *et al.* 1981). This variation is found to be due to the low levels of isoleucine, lysine and valine in freshwater fishes. Another interesting feature of amino acid composition of fresh water fishes is their lower content of proline and higher content of glycine. While proline in freshwater fishes is found to be less than 3.5% most of the marine fishes have a proline content between 4.5 and 7 (Mukundan & James, 1978). The lower content of proline will be reflecting on connective tissue content and the easy digestibility of freshwater fish. Another important feature of the amino acid composition is the glycine content which is 2 to 3% higher in the freshwater fishes than the average 2.5% reported for marine fishes (Mukundan and James, 1978). This high content of glycine can be one of the reason for the sweet taste of freshwater fishes.

Table 4 lists the amount of each essential amino acid required for balanced growth and maintenance of individuals of various age groups in mg/kg of body weight per day

Table 4. *Ideal protein requirement and its suggested amino acid composition for various age groups (FAO/WHO 1973)*

Protein/Amino acid	Requirement in mg/kg/day for		
	Infant	Pre-school child	Adult
Ideal protein[a]	1850	900	550.0
Isoleucine	65	36	9.0
Leucine	148	63	13.8
Lysine	96	50	12.1
Total sulphur amino acid	54	32	13.2
Total aromatic amino acid	116	54	13.8
Threonine	81	36	7.2
Tryptophan	15.7	9	3.6
Valine	87	45	9.9
Histidine	26	—	—

a = Arroyave (1975)

(FAO/WHO, 1973). A careful comparison of the data of Table 3 and Table 4 shows that all the three fish proteins can supply each essential amino acid in substantial quantities for infant, child and adult. However the full utility of the essential amino acids for infants and children are limited by the lower amounts of isoleucine and valine in the case of calbasu and mrigal. While in rohu only valine is limiting in this respect.

Table 5 shows the protein quality index calculated according to Arroyave (1975) based on FAO/WHO recommended pattern of essential amino acids requirement. From the table it can be seen that excepting for adults these fish proteins are not suitably balanced to meet the essential amino acid requirements.

For infants, the extent of insufficiency of valine with respect to the essential amino acid requirement are 39%, 20% and 20% respectively for calbasu, mrigal and rohu. Similar consideration of the requirements for children show that calbasu and mrigal are still limiting in isoleucine and valine and rohu in valine, and the deficiencies are respectively 41%, 25% and 25%. It can be

Table 5. *Protein quality index of the 3 fish protein and cow's milk protein*

Age group	*L. calbasu* protein	*C. mrigala* protein	*L. rohita* protein	Cow's milk protein*
Infant	61.0	79.7	79.7	100.0
Child	59.0	75.0	75.0	100.0
Adult	144.0	157.0	211.0	141.0

*Taken from Arroyave (1975)

seen that these fish proteins even though defficient in isoleucine and or valine the content of all other essential amino acids is sufficient enough to meet the recommended requirements. This suggests the chances of improving the quality of these proteins further by supplementing rohu proteins with valine and mrigal and calbasu proteins with valine and isoleucine.

A comparison of the requirements of adults on similar lines reveal that all the 3 experimental proteins are excessively rich to provide all essential amino acids to support balanced growth/maintenance as the protein quality indices are 144%, 157% and 211% respectively for calbasu, mrigal and rohu. The exceptionally rich quality of rohu protein in essential amino acids can be assumed from its very high protein quality index for adults. Further, marginal supplimentation of calbasu, and mrigal proteins with small but calculated amounts of valine and isoleucine and rohu proteins with valine alone can substantially increase their growth

Table 6. *Level of valine and isoleucine supplimentation required to increase protein quality index of the 3 fish proteins to the level of cow's milk protein (Infants)*

Protein source	Level of the essential amino acid supplementation		Resulting protein quality index
	Valine mg/1850 mg	Isoleucine mg/1850 mg	
L. calbasu	30.0	17.0	100
C. mrigala	15.0	13.0	100
L. rohita	15.0	—	100

promoting ability in infants and children. Tables 6 and 7 show the amount of valine and or isoleucine to be added to these proteins to raise the protein quality index to 100% or on par with casein to meet the essential amino acid requirement of infants and children. Even though the three fish proteins have a balanced distribution of all essential amino acids to meet the requirements of adults, it is seen that all of them have a surplus of all essential amino acids except valine and isoleucine.

Table 7. *Level of valine and isoleucine supplementation required to increase the protein quality index of the three fish proteins to the level of cow's milk protein (child)*

Protein source	Level of the essential amino acid supplementation		Resulting protein quality index
	Valine mg/900 mg	Isoleucioe mg/900 mg	
L. calbasu	19.0	18.0	100
C. mrigala	10.0	11.0	100
L. rohita	10.0	—	100

Protein synthesis for growth/maintenance involve the use of all amino acids in a definite proportion. Consequently all living organisms will be requiring the essential amino acids in a fixed proportion which will vary with species and age as shown by FAO/WHO (1973). The chance of a given protein to conform to this requirement is very rare. So far a protein consumed, the utilisation of its constituent amino acids for growth will be in accordance with the requirement of the individual. The amino acids consumed in excess of the requirement will be used only for deriving energy. In the case of the 3 fish proteins under study substantial amounts of all essential amino acids except valine and isoleucine will be lost. In the process the precious essential amino acids like lysine, sulphur amino acids and the aromatic amino acids will either get oxidised or will be converted to fatty acid for future energy requirements. On the other hand if calculated amounts of valine and isoleucine are supplemented the loss of precious essential amino acids can be prevented and at the same time the efficiency of the protein can be substantially increased.

Listed in Table 8 are the results of rat feeding studies of the native proteins of calbasu, mrigal and rohu along with the control protein casein. The results of protein quality index calculated for adults based on the essential amino acid composition (Table 5) agree well with the protein efficiency ratio (PER) values of Table 8. Rohu with highest protein quality index for adults gives highest PER followed by casein, mrigal and calbasu. The protein content of serum and liver shown in Table 8 is also reflective of the efficiency of the proteins as seen in PER and protein quality index values. From Tables 3 and 8 it is seen that the increased presence of some of the essential amino acids will not improve the PER as discussed in the case of protein quality index in preceeding paragraphs. The higher content of sulphur amino acids and aromatic amino acids and the lower PER of calbasu exemplify this, confirming the necessity for a definite pattern of essential amino acids in the diet. The excess amino acids over and above this pattern will be wasted as energy. However as described earlier if these proteins were supplemented with calculated quantities of the limiting amino acids valine and isoleucine the protein quality index and the PER can be increased making these fishes potent sources of quality protein.

Table 8. *Some parameters of growth of albino rats fed on casein and the 3 fish proteins*

Protein source of diet	PER	Protein content of Liver mg/g	Protein content of Serum mg/ml
Casein	2.5 ±	92.92	31.2
L. calbasu	2.4 ±	96.62	26.6
C. mrigala	2.68 ±	95.38	38.8
L. rohita	3.22 ±	91.85	42.8

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