B-5410
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RESEARCH ARTICLE Indian Journal of Animal Research

Proximate and Amino Acid Composition of Freshwater Fish of Nagpur, Maharashtra, India

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10.18805/IJAR.B-5410

ABSTRACT

Background: The study aimed to evaluate proximate and amino acids profile of selected freshwater species from Nagpur, India. Nutritional composition of calbasu (*Labeo calbasu*), silver carp (*Hypophthalmichthys molitrix*), tilapia (*Oreochromis niloticus*) and pangasius (*Pangasianodon pangasius*) was investigated to make aware the consumer about the present nutrient composition of these fish.

Methods: Iced Calbasu, Silver carp, Tilapia and Pangasius fish were procured from Mayo fish market, Nagpur, Maharashtra, India and brought to the laboratory of Fish Processing Technology, College of Fishery Science, Nagpur. The marketable sized (average weight of 0.5-1.3 kg) fish were used for evaluation of proximate composition the methods of AOAC (2005). The amino acids profiling of fish samples were assessed by using High Performance Liquid Chromatography (HPLC, Shimadzu LC-6A).

Result: The variation ($p<0.05$) in nutrient composition *viz*. proximate and amino acids of these fish mainly due the effect of feed composition, feeding habits, geographical condition and availability of feed *etc*. The major components of proximate composition, moisture (75.75-77.53%), protein (14.52-18.01%), lipids (2.45-6.71%) and ash (1.10-2.09%) were recorded. The protein content was relatively stable in all species. The carbohydrate was found to be lower than 1.5%. The energy value of the fish species was between 94.81-123.51 Kcal. The pangasius had higher energy value due to high amount of fat content. During the study, the concentrations of essential amino acids (EAA) in all fish species ranged from 25.48 g/100 g to 29.41 g/100 g, while amounts of non-essential amino acids (NEAA) ranged between 27.94 g/100 g and 31.27 g/100 g. Lysine (5.14-6.31 g/100 g) and glutamic acid (6.26-8.72 g/100 g) were the most predominant amino acids found in EAA and NEAA respectively in all species. The higher level of essential amino acids was found in tilapia fish followed by silver carp, calbasu and pangasius. The results of the present study revealed that, all species have high nutritional value in terms of proximate and amino acids to offer positive health benefits upon consumption.

Key words: Amino acids, Freshwater fish, Nutritional quality, Proximate.

INTRODUCTION

Freshwater fish is recognized as a valuable source of nutrients for diversified and healthy diets (Fawole *et al*., 2007). Fish has been characterized by vital animal protein source accounting for 16% of the whole animal protein consumed by the world's population and it is a noteworthy source of vital nutrients for sustaining a healthy body (Ondo-Azi *et al*., 2013).

Fish are excellent sources of essential nutrients like protein, amino acids and fatty acids (Zula and Desta, 2021). Fish proteins have great biological significance due to the presence of enough amount of indispensable amino acids mostly methionine, lysine, histidine, threonine and valine (Tilami and Samples, 2018). Its major roles are in the creation of growth hormones, energy development, muscle development, absorption of calcium, immune function, good digestion, better sleep and sexual function of the body (Holecek, 2020). The dispensable amino acids such as glycine, glutamine, glutamate, proline and arginine show vital roles in maintaining gene expression (Kim *et al*., 2011). Apart from these, non-essential amino acids have biological functions, cell signaling, blood flow, nutrient transport and metabolism in animal cells (Wang *et al*., 2013). Works related to the chemical composition of fish are reported in the literature (Yalcin and Demet, 2014; Sarma *et al*., 2015).

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How to cite this article: Relekar, S.S., Koli, J.M., Gore, S.B., Mohite, A.S., Desai, A.S., Wasave, S.M., Telvekar, P.A. and Pagarkar, A.U. (2024). Proximate and Amino Acid Composition of Freshwater Fish of Nagpur, Maharashtra, India. Indian Journal of Animal Research. doi: 10.18805/IJAR.B-5410.

Submitted: 22-05-2024 **Accepted:** 20-09-2024 **Online:** 14-10-2024

Out of the total inland fish production (121.21 Lakh tonnes) of India in 2021-22, Maharashtra has contributed about 1.57 lakh tonnes, of which nearly 49.6% comes from Vidarbha region of Maharashtra, India (DoF, Maharashtra, 2022). Nagpur in the Vidarbha, is an important hub for varieties of freshwater fishes such as Indian major carps, tilapia, pangasius, murrel and minor carps, which are abundantly available in the fish market. Though fish are well known recognized to support the health of human beings, there is little known about the changes in the

centennial composition of these fish before consumption. Also, the quality of freshwater fish is essential to maintain during post-harvest processing and storage. Moreover, the previous work related to the present study is reported to a limited extent on proximate composition only.

Keeping in the above view, freshwater fishes such as calbasu, silver carp, tilapia and pangasius were selected to assess their nutritional quality for consumption. The nutritional information such as proximate composition and amino acids profile will reveal information to food scientists, nutritionists, dieticians, researchers, consumers and would be useful to small entrepreneurs for the preparation of health beneficial fortified or value-added fish products from freshwater fish.

MATERIALS AND METHODS

Materials

Calbasu (*Labeo calbasu*), Silver carp (*Hypophthalmichthys molitrix*), Tilapia (*Oreochromis niloticus*) and Pangasius (*Pangasianodon pangassius*) fish with an average weight of 0.8-1 kg, 0.9-1.2 kg, 0.5-0.6 kg and 1.1-1.3 kg respectively were procured from Mayo fish market, Nagpur, Maharashtra, India and brought to the laboratory of Fish Processing Technology, College of Fishery Science, Nagpur in iced condition (1:1 ratio of fish:ice). After arrival, fish were processed for dressing (de-headed, descaled, gutted) and washed with potable water. Fresh fish muscles were used for the estimation of proximate composition. Whilst, other fillets were minced, dried and converted into fine powder. The prepared fish powder was kept in appropriately labeled sterilized plastic bags and stored at -4°C for amino acid profiling. The study was carried out during the period from October 2023 to March, 2024 in the College of Fishery Science, Nagpur, India.

Proximate analysis

The proximate composition such as moisture, lipids, protein and ash content of fish samples was analyzed per the methods of AOAC (2005). The carbohydrate value was calculated as [100 - (sum of moisture, protein, lipids and ash %)]. While, Energy values were calculated as protein × 4 + lipids × 9 + carbohydrate × 4 (Jabeen and Chaudhry, 2011).

Amino acids profiling

The amino acid profiling of fish samples was assessed using HPLC (Shimadzu LC-6A). The fish samples were digested in 6 N HCL for 20 h, incubated in an oven (110°C) and neutralized with 4.8 mL of 10 M NaOH. The retention time and peak area of standards were used for quantifying and identifying individual amino acids (Herawati *et al*., 2018). The result was expressed in g per 100 g of dried fish powder.

Statistical analysis

The data obtained in triplicate were expressed as mean ± S.D. (Standard Deviation). All data were subjected to one-way analysis of variance (ANOVA). The means were compared using Tukey's multiple-range test. The statistical analysis of the data was performed by using Statistical Packages for Social Science (SPSS 8.0 for Windows developed by SPSS Inc., Chicago, IL). The *P* value ≤0.05 was measured as a significant difference.

RESULTS AND DISCUSSION

Proximate composition

The proximate composition of selected freshwater fish species analyzed in the present study is given in Table 1. The marketable-sized fish species were procured directly from the local fish market and therefore the origin of the species is unknown (wild or cultured). The nutrient composition of fish species varies with the growth rate and various factors such as available natural food, feed composition in cultured fish, trophic state of the water body, temperature as well as a lightening conditions influence the growth of fish species (Tilami *et al*., 2018). However, the present investigation aims to provide information on the nutrient composition of meat of fish for a better understanding of the health benefits they would offer to consumers.

From the Table 1, it can be seen that moisture, protein, lipid and ash content among the fish species studied varied significantly ($p<0.05$). All the fish species under study showed moisture content from 75.75% to 77.53%. Silver carp had a high amount of moisture and the lowest was found in pangasius. The moisture percentage usually ranges between 60-80% (Aberounmand, 2014). The

Table 1: Proximate composition (%) of four freshwater fish species from Nagpur, Maharashtra, India.

Species	Moisture (%)	Protein (%)	Lipids (%)	Ash (%)	Carbohydrate (%)	Energy value (Kcal)
Labeo calbasu	77.06±0.08^{b}	16.52±0.04^{b}	2.96±0.06^{c}	2.09±0.02^{c}	1.36±0.07^{b}	98.16±0.27^{b}
Hypophthalmichthys molitrix	77.53±0.03^{c}	16.86±0.02^{c}	2.45±0.01^{a}	1.83±0.01^{b}	1.33±0.02^{ab}	94.81±0.15^{a}
Oreochromis niloticus	77.12±0.03^{b}	18.01±0.05^{d}	2.56±0.04^{b}	1.10±0.02^{a}	1.19±0.08^{a}	99.92±0.17^{c}
Pangasianodon pangasius	75.75±0.09^{a}	14.52±0.09^{a}	6.71±0.04^{d}	1.75±0.15^{b}	1.24±0.41^{ab}	123.51±0.310^{d}

Data (n=3) are expressed as the mean±SD. According to Tukey's test, different superscripts in the same column signify statistical difference (P<0.05).

protein content of these fish species ranged from 14.52% to 18.01%. Lipid content varied from 2.45% in calbasu to 6.71% in pangasius. The values of moisture content recorded for tilapia in the present study was found lower than those reported for wild, pond, gher and cage cultured tilapia. Nevertheless, higher values of proteins and fat reported in the current study than those reported by Islam *et al*. (2021) for wild, pond, gher and cage cultured tilapia. The highest lipid content in pangasius reflected the muscle characteristics of fish. The reverse relationship was observed between moisture and lipids and was species-specific (Cardinal *et al*., 2011). The same was conspicuous in the case of pangasius. The result of the lipid content of pangasius is generally similar to those reported by Begum *et al.* (2019) for pangasius (6.99±0.36%) collected from the market of Bangladesh. Generally, pangasius in Nagpur fish market are sourced from aquaculture and their feed normally contains rice bran and cotton seed, which are rich in fat. On the contrary, Chakma *et al*. (2022) observed that farmed pangas contains higher protein and lower fat content than wild pangas. The usual range of ash content (1.10%-2.09%) was recorded in all species. The highest ash content was confirmed in *Labeo Calbasu*, may be due to the small intracellular bone in carp muscle. The carbohydrate content was found to be lower than 1.5%. The nearly similar result was observed by Kocatepe and Tarun (2012). The nutritional composition of muscle of fish species depends on the food and feeding habits of individual fish and varies from species to species (Islam and Joadder, 2005; Monalisa *et al*., 2013). To support the results of the present study, several authors globally reported slightly variable proximate composition of freshwater fish species of the world (Memon *et al*., 2010; Paul *et al*., 2018).

The energy value of the meat of fish under study varied between 94.81 Kcal to 123.51 Kcal. The highest energy value in pangasius (123.51 Kcal) was obviously due to the high lipid content of the muscle. The results similar to the current study were also reported for calorie content (89.3-90.2 Kcal) in freshwater carp species (Mukundan *et al*., 1986).

The analysis of the proximate composition of the present study revealed that the freshwater fish from Nagpur could be a good source of animal protein and would provide several health benefits such as helping in reducing oxidative stress of adipose tissue, reducing obesity, controlling type 2 diabetes and reducing inflammation (Tilami *et al*., 2018) through fish consumption.

Amino acid (AA) composition

In the present study, the concentrations of essential and non-essential amino acids were analyzed and listed in Table 2. Total 17 amino acids were recorded in the present study, which were higher than as reported by Begum *et al.*

Table 2: Amino acid (AA) composition (g/100 g) of four freshwater fish species in relation to the essential and non-essential amino acids along with reference value of FAO (2013).

Amino acids	*Labeo calbasu*	*Hypopthalmichthys molitrix*	*Oreochromis niloticus*	*Pangasianodon hypopthalmus*	FAO (2013)
Essential amino acids (EAA)					
Lysine	5.16±0.02^{a}	5.79±0.05^{c}	6.22±0.02^{d}	5.53±0.01^{b}	4.50
Isoleucine	3.15±0.02^{a}	3.19±0.02^{ab}	3.42±0.04^{c}	3.25±0.02^{b}	3.00
Leucine	4.85±0.02^{b}	5.15±0.01^{c}	5.44±0.04^{d}	4.77±0.02^{a}	5.90
Histidine	1.55±0.02^{b}	1.59±0.01^{b}	1.71±0.03^{c}	1.40±0.05^{a}	1.50
Methionine	2.22±0.02^{b}	2.62±0.02^{c}	2.86±0.02^{d}	1.90±0.02^{a}	2.20
Phenylalanine	2.51±0.02^{a}	2.55±0.02^{b}	2.85±0.02^{d}	2.64±0.01^{c}	3.80
Threonine	3.14±0.03^{b}	3.12±0.02^{b}	3.89±0.04^{c}	2.81±0.03^{a}	2.30
Valine	3.23±0.01^{b}	3.14±0.02^{a}	3.67±0.03^{c}	3.20±0.01^{b}	3.90
Total EAA	25.81	27.15	30.06	25.50	-
Non-essential amino acids (NEAA)					
Alanine	3.74±0.02^{b}	3.50±0.02^{a}	3.98±0.02^{c}	4.10±0.02^{d}	-
Arginine	3.67±.0.01^{c}	3.66±0.02^{c}	3.60±0.02^{b}	3.52±0.02^{a}	-
Aspartate	3.25±0.01^{a}	3.31±0.02^{b}	3.65±0.02^{d}	3.58±0.02^{c}	-
Cysteine	0.88±0.02^{b}	0.76±.0.02^{a}	0.98±0.02^{c}	0.73±0.03^{a}	2.20
Glutamate	8.70±0.01^{d}	8.36±0.03^{c}	8.26±0.02^{b}	7.88±0.02^{a}	-
Glycine	4.19±0.05^{b}	4.60±0.05^{d}	4.27±0.15^{c}	3.64±0.01^{a}	-
Proline	2.60±0.01^{c}	2.91±0.02^{d}	2.44±0.02^{b}	2.16±0.02^{a}	-
Serine	2.63±0.01^{c}	2.27±0.01^{a}	2.34±0.02^{b}	2.80±0.02^{d}	-
Tyrosine	2.59±0.01^{b}	2.05±0.03^{a}	2.09±0.01^{a}	2.85±0.01^{c}	3.80
Total NEAA	32.25	31.42	31.61	31.26	-

Data (n=3) are expressed as the mean±SD. According to Tukey's test, different superscripts in the same row signify statistical difference (P<0.05).

(2019) for cultured pangas and lower than those reported by Rieuwpassa *et al.* (2022) for farmed tilapia. Amino acids are the building blocks of proteins. Thus, proteins help in muscular body building, *etc*. The present study demonstrated that all species are good sources of essential and non-essential amino acids. The higher concentrations of all amino acids were analyzed in tilapia followed by silver carp, calbasu and pangasius. The total essential amino acids (EAA) content ranged from 25.50 g/ 100 g to 30.06 g/100 g, while non-essential amino acids (NEAA) were in the range of 31.26 g/100 g-32.25 g/100 g. Tryptophane was not detected in the study due to acid hydrolysis of the sample. The highest concentration of EAA was found in tilapia, whereas higher NEAA was observed in calbasu. The level of abundance of each amino acid varies with the species in the present study. This may be attributed particularly to variation in feed composition, nutrients availability, habitat, *etc*.

Lysine was the major essential amino acid found in tilapia followed by silver carp, pangasius and calbasu, which play major role in the creation of growth hormones and energy (Meybodi, 2019). Similar to the results of the present study, highest content of lysine was also observed in farmed pangas (Begum *et al*., 2019). However, lower content of lysine was noticed in the current study than reported by Rieuwpassa *et al*. (2022) for farmed tilapia. Other important EAAs in the present study were leucine, methionine, histidine, threonine, etc. also found in appreciable quantities. Leucine has healing and rejuvenation properties for skin, muscle and bone, whereas histidine assists in the formation of brain chemicals and is instrumental in the functioning of the immune system, digestion, sleep and sexual function of the body (Holecek, 2020). The lower content of histidine was observed during the study than those reported by Paul *et al*. (2018) for *L. calbasu* species collected from farm as well as market. In the present study, the phenylalanine and valine content ranged from 2.85 to 2.51 g/100 g and 3.67 to 3.14 g/100 g respectively. The species under study have shown a good source of EAA as values reported in the present study were found above the levels recommended by FAO (2013) for EAA except for valine and phenylalanine.

Among NEAA, glutamate was the primary NEAA found in large quantity in all the species under study and was followed by amino acid, glycine. The highest level of NEAA was found in calbasu (55.55%) and the lowest was observed in tilapia (51.26%). Total amino acids (TAA) observed for tilapia (95. 60 g/100 g) were found higher than the present study (61.61 g/100 g) (Abdulkarim *et al*., 2017) and out of TAA, EAA contained 41 g/100 g, which was also higher than observed for tilapia in the current study. The values of cysteine and tyrosine found in the present study were well below the level recommended by FAO (2013), which were 2.20 g/100 g and 3.80 g/100 g respectively.

Several researchers observed results similar to the current study for amino acid composition of fish. Lysine, leucine and glutamic acids were the most prominent amino acids found in tilapia fish procured from different culture systems (Islam *et al*., 2021), which are in line with the present study. Similarly, lysine and leucine among EAAS and glutamic acid in NEAAS were predominantly detected in all freshwater fish species of Indus River (Jabeen and Chaudhary, 2016). The present findings of amino acids composition were duly supported by Ashraf *et al*. (2011) in freshwater fishes. The lower content of all amino acids was observed for wild silver carp than farmed silver carp may be due to supply of artificial feed to the cultured species (Ashraf *et al*., 2011). The variation in the amino acid composition of species under study and those reported by other researchers may be because of different feed compositions, types of culture systems, environmental factors, physiology and morphology of fish species (Dogan and Ertan, 2017).

Therefore, the study inferred that the freshwater fish species may fulfill dietary requirements of EAAs of the human body and may help in alleviating nutritional disorders due to lack of amino acids to a certain extent.

CONCLUSION

According to the results of the current study, we conclude that freshwater fish species of Nagpur region of Maharashtra, India are a good source of essential and non-essential amino acids amino acids. The variation in nutrient composition was observed as fish are sourced from culture and wild environments as well. Natural and artificial diet, aquatic environment, habitat, physiology and morphology of fish influenced the nutrient composition of the fish. Nutrient information obtained in the present study provided insights into the nutritional value of these fish to consumers. The information would also be helpful to policymakers as well as health organizations to encourage fish consumption to achieve optimal health benefits to keep away nutritional disorders. The government should take steps to promote large-scale nutrition research and marketing of freshwater fish to sustain the fisheries sector as a whole.

ACKNOWLEDGEMENT

The authors are thankful to Vice-Chancellor, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist-Ratnagiri, Maharashtra, India. The first author is also grateful to authorities of Maharashtra Animal and Fishery Sciences University, Nagpur, Maharashtra, India for providing necessary facilities to conduct the research.

Conflict of interest

The authors declare no conflict of interest.

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