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Research Paper

ESTIMATION OF AMINO ACIDS PROFILE OF HILSA, *Tenualosa ilisha* IN UPPER AND LOWER REACHES OF BRAHMAPUTRA RIVER DURING MIGRATION

Makibur Rahman

Department of Zoology,
Pragjyotish College, Guwahati -781009, Assam,
India.

Abstract

The andromous migratory shad Hilsa, *Tenualosa ilisha* is a high nutritive fish rich in protein content. During migration the fish require a high energy to swim such a great distance from the estuary. The energy is metabolized from the proteins and lipids, where breakdown of protein ultimately affects the amino acids. A survey was done to estimate the protein and amino acids content of Hilsa from upper and lower reaches from the Brahmaputra river system. The fish lose a significant amount of protein after considering the value obtained from the fishes collected from the fishing at both reaches. The total protein content was found (19.5 ± 0.5) percent in lower reaches whereas (17.0 ± 0.5) percent in upper reaches. Among the essential amino acids, lysine (12.75 ±0.61^a) and, (11.53 ±0.84^a) Leucine (9.11±0.55^a) and (8.87 ± 0.84^a), Threonine (6.22±0.26^a), Phenylalanine (6.12 ±0.26^a) and (5.45 ±0.68^a) were predominant in both the reaches; whereas Tryptophan (0.77 ±0.26^a) and (0.71 ± 0.23^a) was found in trace amount. Among the non- essential amino acids Glutamic acid (17.58 ±0.68^a) and (16.12 ± 0.60^a), Aspartic acid (11.04 ±0.46^a) and (9.48 ±0.37^a), Alanine (7.68 ±0.47^a) and (6.45 ±0.39^a) were found to be predominant while Cysteine (0.81 ±0.34^a) and (0.78 ±0.62^a) was found in trace amount in both reaches in Brahmaputra river system. The fish burn about % of protein along with lowers both essential and non-essential amino acids. All the amino acids lose their concentration and are significantly different from their initial amount estimated from the examine samples.

Key words: Hilsa *Tenualosa ilisha, Biochemical compositions, Brahmaputra river, protein, amino acids*.

INTRODUCTION

The North East region shares its fish fauna predominantly with that of the Indo-Gangetic fauna and to a small extent with the Burmese and South China fish fauna

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[1](Yadav and Chandra, 1994). [2] Sen (1985) and [3]Mahanta *et al.,* (1998) recorded altogether 187 fish species from Assam and the neighbouring North Eastern states of India. Compilation of [4]Yadav and Chandra (1994) listed a total of 129 species. [5]Sinha (1994) in his comprehensive review gave a list of 230 fishes as available from North Eastern region. Recently, [6]Nath and Dey (1989) recorded a total of 131 species from the drainages of Arunachal Pradesh. [7]Sen (2000) has indicated that more number of species has been reported from North East India. The various reports show a wide variation in the total number of species reported. In the present situation 186 potential food, sports and aquarium fish species belonging to 27 families under 84 genera, have been presented along with state wise distribution, abundance along with potential fisheries. While the list of 267 fish species given by [7]Sen (2000) includes all indigenous and exotic species found in North East India.

Hilsa *(Tenualosa ilisha)* any of the members of the genus Hilsa of the family Clupeidae, order Clupeiformes. Locally known as *Ilish*, the fish has been designated as the national fish of Bangladesh. The body is strongly compressed and moderately deep with dorsal and ventral profile equally convex. The upper jaw has a distinct median notch. Regularly arranged medium-sized scales cover the metallic silver-coloured body. Body length may reach up to 60 cm, but commonly found specimens measure 35 to 40 cm. Females grow faster, and are usually larger than males. The Hilsa is known to be a fast swimmer, and attains maturity in one to two years [8](Talwar and Jhingran, 1991).

The biochemical constituents with reference to protein, lipids, vitamins and minerals present in fish make it a nutritious food. It has been found that fish provide about 16% global animal protein [9](Gopakumar, 1997). The two million km^2 India EEZ provide rich marine flora and fauna comprising a wide variety of fish (including both teleostean and cartilaginous fish), crustaceans, mollusks and a large amount of edible sea-weeds, which provide an immense source of quality food for human nutrition. Estuarine species including Hilsa provide a wide variety of different species of fish in the coastal as well as estuarine belt including India and Bangladesh. In Bangladesh Hilsa is a major capture estuarine fishery providing quality nutritional requirements, which provide a good source of protein. There is no doubt that multispecies nature of tropical marine and estuarine fisheries necessitates a detailed understanding of the biochemical composition of different species, for evolving suitable utilization of fishes for human

composition. Several workers ([10]Mazumdar *et al.,* 2004) have studied the biochemical composition of fishes of Indian origin. However systematically compiled information and nutritional analysis of fish require through studies. Further, fishes exhibit considerable seasonal changes in their composition. Diverse environmental factors, age, sex and reproductive strategies etc. all play significant roles in the total biochemical composition. Migratory fish of various categories such as Hilsa, Salmon or Anguila undergo a series of changes owing to the migration from one environment to another which may be one of a totally opposite nature. The changes of environmental conditions as encountered in Hilsa are largely responsible for the changes of its biochemical composition. The importance of fish oil is associated with a series of physiological factors of human nutrition. It has been found that Hilsa being as anadromous migratory species travels more than 500 km. on its way from the estuary of the Bay of Bengal to the Brahmaputra river system. Further, it has been seen that the fish does not consume any food items and this time, which results in loss of weight. The basic question related to the energy metabolism that the fish spends a high and significant amount lipids reserve or fatty acids. The fish require a high energy to swim such a great distance from the estuary. The energy is metabolized from the proteins and lipids, where breakdown of protein ultimately affects the amino acids. [11]Gopalkrishnan (1973) reported the biochemical composition with reference to % of moisture, protein, fat and ash along with the amino acids and fatty acids profile. The protein content varies from 16-21%, while fat 10-12%. A thorough investigation on the energy metabolism with the protein turnover during the migration is extremely essential which would provide the utilization and energy budget of the fish during migration.

STUDY AREA

Survey was done during the period from January 2019 to December 2019. For estimate the protein and amino acid composition of Hilsa, *Tenualosa ilisha* from the upper and lower reaches of Brahmaputra river, mainly four locations were considered. Dhubri (N26:10:05, E 90:00:00) and Goalpara (N26:10:05,E 90:50:15) from lower reaches and Tezpur (N26:60:00, E 92:40:35) and Silghat (N26:40:45, E 92:30:00) from upper reaches of Brahmaputra river system. Fig-1(a) and 1(b)

Fig-1(a) Map of Brahmaputra river

Fig-1(b) Brahmaputra river

MATERIALS AND METHODS :

Fresh matured *Hilsa* (*Tenualosa*) *ilisha*, were collected during the experiment specially in breeding season from a fishing boat after netting. The body tissue samples were collected from different body parts in glass vials containing chloroform/methanol (2:1, v/v) and 0.01% w/w butylated hydroxytoluene (BHT). The protein estimation is followed after AOAC, 1995; Kjeldhal method. For analysis of amino acids in different tissue (1g from each samples), a 10% homogenate (w/v) was prepared with a motor driven Potter-Elvehjem type glass homogenizer fitted with a Teflon pestle in ice-cold HPLC grade water. Protein was immediately precipitated out from the homogenate by adding ice-cold 2M PCA in 1:1 ratio, followed by centrifugation at 10,000xg for 10 min. The plasma was also treated with 2 M PCA in a 1:1 ratio to precipitate out the protein, and further processed as above. All these steps were performed at 4^0C. The supernatant was passed through I Millipore microfilter (0.45 µm pore size) before using for amino acid analysis with HPLC [12](Alaiz et al., 1992). The derivatized sample was then analyzed with a water HPLC with a pre-column derivation method. Amino acids was derivatized with phenyl isothiocyanide (PITC) with the standard protocol provided by the company and was separated out with a Pico Tag column (Waters, USA). The eluted amino acids was detected in a UV-detector (Model-486) at 254 nm coupled with Milinium data processor for quantification of the eluted peak areas. Two eluting mobile phases were used in a gradient for 72 min, to separate out all the amino acids. Eluent A (70 mM Na-acetate buffer, pH-6.5) and eluent B (60% acetonitrile). Before starting the run, the column oven temperature was set at 38 ^{0}C and the column was purged with 90% eluent A and 10% eluent B and equilibrated for stabilization of the baseline. The

run was started with 100% mobile phase A and the flow rate was set at 0.5 ml/min through out the run. In the first 13.50 min, the linear gradient progressed to 3% mobile phase B, followed by increase to 6% mobile phase B in 24 min, 9% increase of mobile phase B in 30 min, 34% linear increase of mobile phase B in 50 min and was held there till 620 min. The gradient was then increased to 100% mobile phase B from 62.01 min and continued till 72 min. After the gradient elution was complete, the column was washed thoroughly with HPLC grade water for 30 min and the column was again re-equilibrated with 100% eluent A before subsequent injections. The mixture of standard physiological amino acids (sigma) containing 38 amino acids and some amino compounds was also eluted under identical conditions as mentioned above for identification and quantification of amino acids.

RESULTS AND DISCUSSIONS

The amount of protein and the amino acids in Hilsa has been estimated from the collection of upper and lower reaches of Brahmaputra river system. It has been found that the fish lose a significant amount of protein after considering the value obtained from the fishes collected from the fishing at both reaches. The total protein % is as shown in Table -1. The value shows that the protein % is significantly less ($P<0.05\%$) less as has been found from the sample collected at upper reaches.

Table-1: Total protein (%) of Hilsa

Location/ No of fish	Protein %
Lower reaches	19.5 (± 0.5)
Upper reaches	17.0 (± 0.5)

Table -2. Amino acids (g/100g. protein) profile of Hilsa.

Amino acids	Lower reaches	Upper reaches	F-value
Essential			
Arginine	5.11 ±0.38[a]	4.30 ±0.49[a]	0.044
Histidine	4.46 ±0.36[a]	2.95 ±0.25[a]	3.217
Isoleucine	4.13 ±0.36[a]	1.95 ±0.54[a]	13.444
Leucine	9.11 ±0.55[a]	8.87 ± 0.84[a]	1.333

Lysine	12.75 ±0.61[a]	11.53 ±0.84[a]	0.158
Methionine	3.08 ±0.36[a]	1.91 ±0.40[a]	8.251
Phenylalanine	6.12 ±0.26[a]	5.45 ±0.68[a]	1.783
Threonine	6.22 ±0.26[a]	4.71 ±0.65[a]	2.016
Valine	5.57 ±0.36[a]	4.83 ± 0.53[a]	1.475
Tryptophan	0.77 ±0.26[a]	0.71 ± 0.23[a]	0.322
Non-essential			
Aspartic acid	11.04 ±0.46[a]	9.48 ±0.37[a]	3.034
Asparagine	6.27 ±0.28[a]	5.50 ±0.42[a]	3.466
Alanine	7.68 ±0.47[a]	6.45 ±0.39[a]	1.754
Cysteine	0.81 ±0.34[a]	0.78 ±0.62[a]	1.554
Glutamic acid	17.58 ±0.68[a]	16.12 ± 0.60[a]	2.067
Glycine	5.7 ±0.63[a]	4.2 ±0.53[a]	1.234
Proline	1.33 ± 1.22 [a]	0.66 ±0.33[a]	11.672
Serine	4.31 ±0.45[a]	3.27 ± 0.63[a]	2.377
Tryosine	2.9 ±0.39	2.05±0.37	0.344

Same suffix differ significantly at 5%

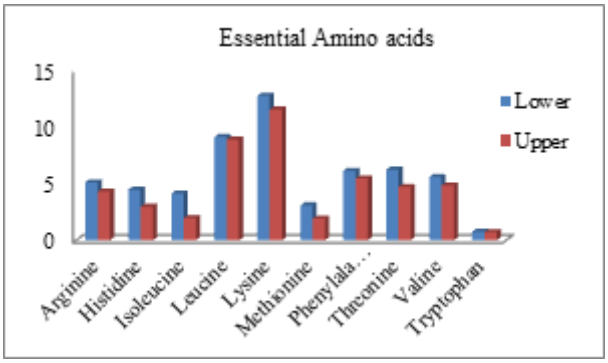

Fig-2: Essential amino acids of Hilsa

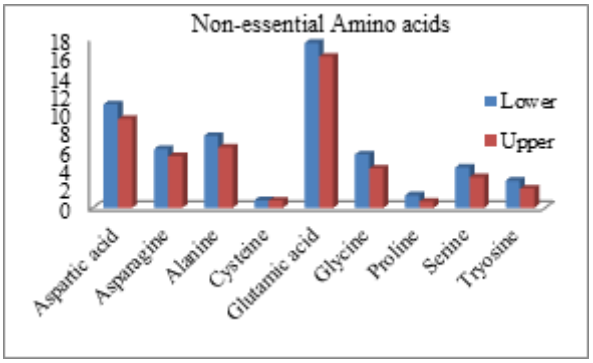

Fig-3: Non-essential amino acids of Hilsa

The amino acids content has been estimated (g/100g. protein). In Table-2 the values are shown with reference to essential and non-essential amino acids. Among the essential amino acids, lysine (12.75 ±0.61[a]) and, (11.53 ±0.84[a]) Leucine (9.11±0.55[a]) and (8.87 ± 0.84[a]), Threonine (6.22±0.26[a]), Phenylalanine (6.12 ±0.26[a]) and (5.45 ±0.68[a]) were predominant in both the reaches; whereas Tryptophan (0.77 ±0.26[a]) and (0.71 ± 0.23[a]) was found in trace amount. Among the non- essential amino acids Glutamic acid (17.58 ±0.68[a]) and (16.12 ± 0.60[a]), Aspartic acid (11.04 ±0.46[a]) and (9.48 ±0.37[a]), Alanine (7.68 ±0.47[a]) and (6.45 ±0.39[a]) were found to be predominant while Cysteine (0.81 ±0.34[a]) and (0.78 ±0.62[a]) was found in trace amount in both reaches in Brahmaputra river system. The essential amount from both the lower reaches as well as from the upper reaches is seen to be considerably less during migration. It can be concluded that the fish utilize the protein and its amino acid (including the essentials and non-essentials amino acid) during migration. Total protein percentage (%/100g.) of Hilsa collected at different reaches of Brahmaputra river it is seen that the protein decreases from lower to the upper reaches during migration. The same is observed in case of essential amino acids (Fig-2) and non-essential amino acids (Fig-3) of Hilsa collected at lower and upper reaches of the river. Protein is an indispensable nutrient required for the structure and function of all living organisms including fishes. Several investigations have determined the quantitative amino acid requirements for the growth and survival of different fish species [13](Wilson et al., 1980; [14]Santiago and Lovell, 1988; [15]Akiyama and Arai,1993). Fish muscle is known to contain an excellent amino acid composition ([16]Venugopal et al., [17]1996; Yanes et al.,1976) and is a unique source of physiological beneficial amino acids (arginine, histidine, lysine; [18]Marshall, 1994). In the present study, the amino acid composition shows difference in lower and upper reaches in Brahmaputra river system. The amino acid in Hilsa, *Tenualosa ilisha*

examined were particularly rich in lysine, leucine, threonine, Phenylalanine, Glutamic acid, Aspartic acid and Alanine

CONCLUSION :

In the present studies the protein parameters such as (i) protein (ii) essential and non-essential amino acids profile containing different categories of amino acids reveal interesting results. The fish burn about % of protein along with lowers both essential and non-essential amino acids. All the amino acids lose their concentration and are significantly different from their initial amount estimated from the examine samples. Total protein content of fish also decreases significantly. The overall total protein decrease from their initial amount. The present study revels the high nutritional significance of the fish.

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