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Seasonal Changes in Free Amino Acid Contents in Lateral Muscles and Anterior Kidney of the Teleost Fish, *Nandus Nandus Cuv* .

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Summary : The free amino acids in the lateral muscles and anterior kidney are estimated both, qualitatively and quantitatively. The free amino acid pool of the lateral muscles exhibits almost definite constancy. The pool is observed to be disturbed due to occasional appearance of some amino acids. Roughly 5% amino acids in this tissue are extra additions during the course of the year. The maximum alterations are seen in the spawning season in the tissue studied. Tyrosine, tryptophan and ornithine appear only in the breeding season. The highest concentration of the free amino acids is anticipated in the spawning period. A continuous gradual increase in the quantity of the free amino acids is observed up to spawning period. In anterior kidney, besides other amino acids, arginine and ornithine are also detected. The results point to the fact that the amino acid pool in the fish tissues may be almost invariable yet quantity wise it is alternating according to breeding season. The amino acids in the intercellular fluid help to maintain the milieu and are taken up by the cells when required for some metabolic activity.

Key words : Free amino acids, Seasonal variations, Lateral muscles, Anterior kidney, Fish *Nandus nandus*

Introduction

In their aquatic environment fishes meet many stresses like temperature, light or photoperiod, salinity and pH etc. These factors affect their metabolic processes in multiple ways. Accordingly, the variations appear in many metabolites both, qualitatively and quantitatively. In a given season of the year environmental factors normally oscillate a little but differ much in the predeceasing season. The seasonal variations thus, alter the metabolite's concentrations. Amino acids are not exception to it. Tracing back to 1921 Sekine (Sekine, 1921) analyzed free amino acids in the muscles of male and female fish, followed by other scientists who analyzed free amino acids in the muscles of different fishes (Okuda and Matsuda, 1923; Delaunay and Serege, 1944; Argen, 1944). Many free amino acids in two

type of muscles and variations in these metabolites were studied (Drilhon, 1953). Work on free amino acids in the muscles of *Pleuronectes microcephalus* shows continuous variations in the amount of alanine, glutamic acid, glycine, leucine, lysine, B-alanine and taurine (Jones, 1959). When comparing free amino acid contents of fish muscles in fresh and preserved conditions, changes not only in amino acid pool but in their quantities were also found. Under several stress conditions, muscles and gill have been shown quantitative changes in the free amino acids (Eisq and Zaki, 1966). In higher vertebrates, free amino acid pool is reported to be constant even in histopathological conditions (Roberts and Frankel, 1949). In the present study, seasonal variations in the free amino acids are analyzed qualitatively and quantitatively in anterior kidney and lateral muscles of a teleost fish, *Nandus nandus* and the variations in the pool are correlated with the reproductive cycle. This is the only representative of the family Nandidae found in India.

Materials and Methods

The adult healthy specimens of *Nandus* were collected locally from Sagar lake and kept in aquarium for minimum ten days to acclimatize them in laboratory conditions. The fish were anaesthetized in 10% solution for 10 minutes. After dissections, which were done on every 14^{th} day of the month, the tissues were taken out, weighed on top pan balance and kept in the Young's ringer solution containing $CaCl_2$ – 0.011%, KCl – 0.014% and NaCl – 0.55%. The tissue homogenates were prepared in measured volume of 70% alcohol. The supernatant was subsequently mixed with chloroform and centrifuged. The aqueous part obtained after centrifugation was treated with 80% alcohol to ensure the elimination of peptide molecules. Synthetic semipermeable membrane was also used where it was found necessary. The extract, so procured was dried in vacuum at low temperature. A final solution of desired volume of the extract was applied with Hamilton's microsyringe on Whatman's chromatography paper no.-1. The papers were kept in an atmosphere saturated with the solvent system for 24 hours before use. The most suitable solvent system was n-Butanol, Acetic acid, Water in 4:1:1v/v proportion. Two-dimensional chromatograms were prepared by using saturated phenol solution as the second solvent. The separation of lysine and histidine was done by using pyrimidine : isoamyl alcohol : :

water : diphenylamine (w) in 10v : 10v : 7v : 3w. Ninhydrin solution (0.25% aqueous) was utilized as a developer (Awapara, 1948) . The extracts were prepared from five different specimens to observe any variation in amino acid contents. B.D.H. standard amino acids were used for identification. Rs values were calculated with respect to alanine. For quantitative analysis, standard curves of different amino acids were prepared with their various concentrations by using both, photodensitometry and photocolorimetry methods, after phtodensitometric evaluation resolved and identified amino acids eluted from the paper, colour density was measured.

Resulted and Discussion

The results are presented in Table –1 and 2. Cystine, lysine, aspartic acid, glycine, glutamic acid, threonine, alanine, tyrosine, histidine and valine are the normal constituents of the pool. Aspartic acid shows the maximum quantity, that is 38.4 mg and minimum is represented by 0.822 mg of tyrosine in 100 g of tissue. The normal constituents show at least one peak in the respective seasons, the highest generally being in the spawning season. The following amino acids are noticeable by their behaviour in the pool. Phenylalanine appears only in the month of July, amino butyric acid is absent in the spawning period while tryptophan is absent in the prespawning. Thus roughly 5% of the amino acids are extra additions during the course of the year. Major changes are mainly in the spawning season. Cystine, the sulfur containing amino acid remains constant during most of the part of the year but is estimated 16mg, the highest, in the month of October and since than its concentration declines up to January and remains stabilized during spawning season. Threonine, an essential amino acid conspicuously, is found to be undetectable in post-spawning period. In muscles, the concentration of methionine is not only remained very low in comparison to its fellow amino acid, the cystine but also observed the lowest amongst other amino acids. However, in *Nandus* the average glutamic acid storage is the least in the lateral muscles (than other tissues of the same fish, data is not given here. (Table-1).

The normal constituents of the pool of anterior kidney (Table-2) are cystine, ornithine, arginine, serine, glycine, glutamic acid, threonine, alanine, methionine and α-amino butyric acid. The maximum quantity reached by any particular amino acid is 41.69 mg of threonine and minimum

is represented by 1.162 mg of methionine in 100g of tissue. The normal constituents show at least one peak in the respective seasons Only two amino acids appear extra in the free amino acid pool, isoleucine in July and tyrosine in November. Roughly 5% of the amino acids in these organs are extra additions throughout the course of the year. Major changes are observed mainly in the spawning. Among the sulphur containing amino acids the indispensable methionine and cystine make their presence throughout the year. Their concentrations are roughly equal in anterior kidney while it is comparatively low in lateral muscles. Glycine is well for a number of metabolic reactions like the formation of porphyrins, purines, glutathione and many other metabolites. Alanine, serine and glycine are interconvertible and their presence in these tissues can be justified. The absence of serine in lateral muscles can be correlated with its conversion to alanine and glycine. Hence they can supplement each other so far as metabolic reactions are concerned. Threonine, one of the essential amino acids is strangely, not detected in most of the months of the post-spawning period and once in a month of the breeding period. Arginine is considered practically indispensable among higher vertebrates. But it is surprisingly absent in lateral muscles. Lysine, this essential amino acid is absent in anterior kidney. Only a limited portion of dietary lysine is known to be converted to glutamic acid. Its main use lies in the synthesis of protein. Its presence, at any time of the year may be considered as metabolically quite active, for example, the lateral muscles.(Table-1). An amino acid may be reported as missing due to its estimation in a particular season when it is absent.

Although the free amino acids have been studied from a number of view points (Awapara, 1948):, seasonal variations have ignored altogether. He stressed lack of constancy in the free amino acid pool of microbes and some invertebrates. The seasonal changes as regards the gonads have been histologically studied widely. The ovarian seasonal cycle corresponding to the breeding in the fish, *Nandus nandus* follows the underwritten pattern.

1. The prespawning period - January, February and March
2. Spawning period - May, June, July, August and September
3. Postspawning period - October, November and December.

Jones (1959) enumerated seasonal variations in the muscles and mentioned instability in the pool both, quantitatively and qualitatively. In the four species of fish, different amino acids were observed in their muscles (Okumura *et al*., 1959). In *Nandus* although the main ingredients of the pool are relatively characteristic for a tissue, the concentration is highly variable. Constancy of the pool is disturbed by the occasional appearances of extra amino acids during certain seasons of the year. Similarly, variations in free amino acid pool of red and white muscles of tuna and mahi-mahi led to the conclusion that the concentration of the free amino acids changes during prespawning period (Holden, 1962). The breeding cycle is also a major metabolically active period in living beings: the behavior of the free amino acids can be studied with benefit on that basis. Instead of the variations in the individual amino acids their total amount in a particular organ and in the various months is more indicative of the free amino acids also proportional to the amino nitrogen.

Qualitative and quantitative inconsistencies in the pool of free amino acids in the muscles of the various fishes were observed (Goncalvesferreira, 1952; Ranke, 1955 Shreenivasan. and Freda, 1961; Antonie *et al*, 2001). Such variations in the pool may be due to the influence of temperature, hormonal, biochemical and physiological changes. So far nutrition is concerned there is considerable controversy on its effects on the quantity and variability of the free amino acid pool and also, about the effect of living fish gain, feed efficiency and nutrients deposition of fish. Most of the free amino acids that are regular members of the pool increase in their concentration during December. It is one of the cold months when food availability becomes scanty. The absence of á-amino butyric acid in lateral muscles can be correlated with low temperature stress meet. And in anterior kidney its presence was interpreted with reference to the appearance of some new protein bands in gel electrophoresis of the particular organ (Bureau *et al*, 1999) (These results are not incorporated here). It is suggested that feeding level has no effect on efficiency of utilization of N and energy above maintenance (Rao and Rao, 2001). They have not found maximum protein deposition plateau in fish during spawning or than spawning period. Supplementation of diets with l-lysine or DL-methionine shows no effect on the performance of the fish and the quantity of the free amino acids is not found prominently elevated in the muscles. In *Glassogobius guiris (*Shad

et al., 2001) a fish, during its pre-spawning period, elevated lipids, carbohydrates and protein contents in muscles, kidney and ovary were estimate. But in this study higher amount of the total amino acids is observed in November, during the post-spawning period. Another interesting feature is that the anterior kidney, shows a two-month cycle– in which one month leads the other in the total amount of the free amino acids. Thus December, February, April and June have more quantity of free amino acids than January, March, May and July respectively. The stocks of amino acids are thus utilized actively and replenished as soon as possible. Quantitatively, the lateral muscles appear to follow a course similar to the anterior kidney as regards the free amino acids. There is alternate rise and fall in the quantity of the metabolites from one month to the other.

The fact presented above point out two important features. The alternate rise and fall in total free amino acid concentrations strongly suggestive of existence of basic monthly cycle. Perhaps, there are similar diurnal and nocturnal cycles. Since the estimations were carried out on the 14^{th} of every month, the basic cycle follows a period of about 30 days. The anterior kidney in teleost fishes has chromaffin cells and adrenocortical tissue scattered in the renal tissue, produce adrenaline and corticosteroids which control many metabolic activities. As such there appears to be a sort of balance and co-ordination between the metabolic reactions of the anterior kidney and the lateral muscle in the alternate month.

During the spawning period when the whole organism is triggered to the most important activity of life, the reproduction, most of the organs show an increase in the amount of metabolites though this is influenced by many factors like temperature, nutritional status and changing milieu.

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Table 1 showing monthly variations in the free amino acids and their concentrations in lateral muscles of *Nandus.*

Month	Cys	Lys	Asp	Gly	Glu	Thr	Ala	Tyr	Am.but Acid	Orni	Met	Val	Phe	Trp	His	Total
Jan	5.95	5.72	11.3	7.4	-	-	11.8	4.7	14.4	-	5.58	-	5.9	-	4.2	77.3
Feb	7.7	4.13	15.8	3.5	4.1	-	6.7	2.8	4.1	-	2.3	1.1	-	-	4.8	57.15
Mar	7.3	-	13.0	22.5	4.6	11.9	20.0	2.8	-	14.4	2.3	4.3	-	-	-	103.2
Apr	7.2	4.3	-	14.4	4.5	5.5	7.6	0.8	-	-	2.3	-	-	3.9	4.5	55.2
May	6.9	9.1	17.2	11.4	8.3	4.3	10.3	3.8	-	-	2.3	-	-	-	7.4	81.2
Jun	7.7	13.6	38.4	21.0	13.6	-	14.0	7.0	-	7.2	-	-	-	-	16.3	139.0
Jul	8.4	15.1	16.2	10.9	3.4	7.4	6.0	16.8	-	8.4	2.5	-	-	2.1	-	97.4
Aug	10.0	3.1	15.7	8.5	5.7	7.4	3.8	-	-	-	7.7	15.2	-	-	-	132.6
Oct	16.0	36.2	10.9	11.7	-	14.8	4.7	-	5.5	-	1.5	-	-	7.12	-	108.6
Nov	9.2	22.8	8.3	19.8	9.7	-	4.5	-	-	-	4.3	1.6	-	-	15.7	96.18
Dec	8.0	15.9	-	30.9	2.0	-	25.4	14.1	-	-	3.5	-	-	-	13.3	135.4

Table 2 monthly variations in the free amino acids and their concentrations in the anterior kidney of *Nandus*

Month	Cys	Orni	Arg	Ser	Gly	Glu	Thr	Ala	Met	Tyr	Iso	Am.but Acid	Total
Jan	21.6	21.6	31.6	13.4	22.9	11.1	7.8	9.6	13.9	-	-	14.4	168.5
Feb	24.0	19.2	30.34	16.5	14.4	10.3	24.1	7.6	1.1	-	-	3.6	151.0
Mar	24.0	2.4	10.1	4.0	7.6	3.9	12.0	11.8	5.8	-	-	3.6	85.4
Apr	28.8	12.0	16.8	23.1	34.7	13.4	8.9	34.4	2.9	-	-	24.0	198.3
May	25.3	14.4	14.6	24.7	21.3	14.0	13.8	24.1	7.1	-	-	16.8	176.4
Jun	22.8	4.7	13.5	37.0	19.8	15.9	41.6	15.2	10.3	-	-	13.3	194.6
Jul	21.4	-	10.7	25.6	3.2	3.8	2.2	2.2	6.9	-	3.0	-	79.3
Aug	24.0	14.4	25.9	21.8	26.5	24.4	3.1	2.8	12.4	-	-	-	155.6
Oct	16.8	14.4	8.1	19.2	33.4	17.6	4.4	1.3	2.4	-	-	-	117.9
Nov	23.6	33.6	40.7	0.8	19.9	16.1	14.6	15.2	4.1	8.4	-	21.6	209.0
Dec	28.8	26.4	25.5	4.8	8.1	15.9	22.2	15.3	9.7	-	-	24.0	181.0

➢ Values in mg/100gm of tissue.

References:

Antonie, C.I., Wei, R.C., Littell, B.P., Quinn, A.D.Hogle, and Marshall, M.R. (2001) : Free amino acids in dark and white muscles of of fish as determined by O-phthaldialdehyde precolumn derivatization. *J.Food Sci*., 66(1)1062-1068.

Argen, G. (1944) : Amino acid composition of the muscle protein from some species of Swedish fish. *Acta Physiol. Scand.*, 7, 134-146.

Awapara, J. (1948) : Application of paper chromatography to the estimation of free amino acids in tissues. *Arch. Biochem.*, 19, 172-173.

Bureau, D.P., Azevedo, P.A. and Cho, C.Y. (1999): Growth and nutrient deposition as a function of feeding level in salmonids. *International Workshop on Fish Nutrition and Growth*. Hubei, China, p.41-45.

Delaunay, H. and Serege, H. (1944) : Proteolytic and amino acid producing activity of the spleen. *Compt. Rend. Soc*. Biol., 88, 707-710.

Drilhon, A. (1953) : The free amino acids and the fluorescent compounds of fish autolyzates. *Bull. IInst. Oceanog.*, 1028, 4-7.

Eisq, E.A. and Zaki, M. (1966) : Chromatographic studies on free amino acid contents of fresh and preserved meat. Quantitative determination of amino acids using buffered paper at pH 2. A' in *Shams Sci. Bull*., 2, 219-224.

Goncalvesferreira, F.A. (1952) : Microbial determination of ten amino acids in four species of fish. *Cln. Hig. Ehiclorol.* (Lisbon), 18(2), 45-51.

Holden, J.T. (1962) : *Amino Acid Pools.* (New York, Elsevier Publishing Comp.)

Jones, N.R. (1959) : Free amino acids of fish .II. Fresh skeletal muscle from lemon sole. *J. Sc. Food Agr.*, 10, 282-6.

Okuda, Y. and Matsuda, S. (1923) : Hydrolysis of whole body of carps. : *J.Sci. Agr. Soc*. (Japan) 248, 337-41.

Okumura, N, Otsuki, S. and Aoyama, T. (1959) : Free amino acids and related compounds in fish, amphibia, reptiles, aves and mammals by ion exchange chromatography. *J.Biochem.(Tokyo)*, 207-212.

Ranke (1955) : Paper chromatographic study of the content of free amino acids in crustaceans and fishes. *Arch. Fischerei Wiss*., 6, 109-113.

Rao, P.S. and Rao, I.M. (2001) : Variations in biochemical composition of G.guiris(ham.) from Gosthani estuary in relation to sex, maturity, food and feeding intensity. *J.Curr. Sci*., 2(1) : 49-53.

Roberts, E. and Frankel, S. (1949) : Free amino acids in normal and neoplastic tissue of mice as studied by paper chromatography. *Cacer Res.*, 9, 645-648.

Sekine, H.(1921) : Muscle protein differences between the muscle proteins of male and female fishes. *J.Chem. Soc*. (Japan), 42, 548-58.

Shad, M.A., Ansari, T.M., Afzal, U., Kauser, S., Rafique, M. and Khan, M.I.(2001): Major constituents, free amino acids and metal level in renal canaliculi from Multan region. Online *J.Biol.Sc*.1 (11).

Shreenivasan, A. and Freda, C. (1961) : Free amino acids of some fresh water fish of Bhavanisagar reservoir. *J.Sci. Ind.Res.* (India), 195-213.