Original Article

Life Science

Seasonal Variation of Protein and Essential Amino Acid Contents in *Labeo gonius* from Lotic and Lentic Water Bodies

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ABSTRACT [ENGLISH/ANGLAIS]

Fish protein is regarded as quality protein being rich in essential amino acids with a high digestibility value. The biochemical composition of the fish differs with the change in habitat as well as season. Water quality has been the most important limitation to fish production. A detailed knowledge of the chemistry of the fishes in relation to varying environmental factors is essential to determine the requirements which affect the nutritional quality of fish along with its commercial production. The present paper reports on the differences in the quantitative compositions of major nutrients as proteins and essential amino acids of *Labeo gonius* in lotic and lentic habitats. The total protein and amino acid content were determined in muscle and liver tissues in four different seasons of the year (pre monsoon, monsoon, retreating monsoon and winter). In lotic habitat the highest amount of protein in muscle and liver tissues was observed in pre monsoon (138.22 ± 6.82 and 148.41±8.96) and lowest amount was observed in retreating monsoon (42.8±1.49 and 52.40±1.41). Similar trends were observed in lentic habitat but the present study depicts a significantly higher concentration ($p<0.01$) of these nutrient components in lotic habitat. Seasonal variation on the differential amino acid content was clearly observed in all the conditions. Comparative analysis of the nutrient components suggests a definite effect of habitat and seasonal variation in the nutritional quality of *Labeo gonius.*

Keywords: *Labeo gonius*, lotic, lentic, nutrient

RÉSUMÉ [FRANÇAIS/FRENCH]

Protéines de poisson est considéré comme étant des protéines de qualité riche enacides aminés essentiels avec une valeur haute digestibilité. La composition biochimiquedu poisson diffère avec le changement de l'habitat ainsi que la saison. Qualité de l'eau aété la limitation la plus importante à la production de poissons. Une connaissance détaillée de la chimie des poissons par rapport à divers facteurs environnementaux est essentielle pour déterminer les exigences qui affectent la qualité nutritionnelle despoissons ainsi que sa production commerciale. Le présent document rend sur lesdifférences dans les compositions quantitatives de nutriments majeurs comme les protéines et les acides aminés essentiels de Labeo gonius dans les habitats lotiques et lentiques. La protéine totale et teneur en acides aminés ont été déterminées dans le muscle et les tissus du foie en quatre différentes saisons de l'année (pré mousson, la mousson, la mousson et l'hiver en retraite). Dans l'habitat lotique montant le plus élevé de protéines dans les muscles et les tissus hépatiques a été observée dans le pré mousson (138,22 ± 6,82 et 148,41 ± 8,96) et le plus faible montant a été observée dans la retraitela mousson (42,8 ± 1,49 et 52,40 ± 1,41). Des tendances similaires ont été observés dans l'habitat lentique mais le present étude montre une concentration significativement plus élevée ($p <0,01$) de cescomposants nutritifs dans l'habitat lotique. La variation saisonnière de la teneur en acideaminé différentielle a été clairement observé dans toutes les conditions. L'analyse comparative des éléments nutritifs suggère un effet certain de l'habitat et la variation saisonnière de la qualité nutritionnelle de Labeo gonius.

Mots-clés: Labeo gonius, lotique, lentique, des éléments nutritifs

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Accepted/Accepté : February, 2012

Full Citation: Deka BK, Mahanta R, Goswam UC. Seasonal Variation of Protein and Essential Amino Acid Contents in *Labeo gonius* from Lotic and Lentic Water Bodies. World Journal of Life Sciences and Medical Research 2012;2(2):71-6.

INTRODUCTION

Fishes, one of the most preferred groups of animal foods for human beings often provide the much needed nutrient that cannot be provided by cereals only [1, 2, 3, 4, 5]. Fish protein provides between 30% to 80% of the total animal protein intake of human and is rich in amino acid

composition very well-suited to human dietary requirement. Besides being used as food, fish is also increasingly demanded for use as feed. High consumption of fish meat has a beneficial role on human health as it minimizes the appearance of cardiovascular diseases by decreasing the cholesterol and triglyceride level, moderates the inflammatory response as well as improves the carbohydrate metabolism.

However, information concerning the chemical composition of freshwater fishes in general is of great significance to nutritionists concerned with readily available fish sources of low fat and high protein food [6, 7, 8]. Food biotechnologists are also interested in developing the fishes into high protein food ensuring finest quality flavour, colour, odour, texture with maximum nutritive value. It is of utmost importance for the ecologists and environmentalists to determine the effects of changing biological and environmental conditions on the composition, survival and population changes within fish species.

The chemical composition of fish varies greatly from one species and one individual to another depending on age, sex, environment, season and geographical location [9, 10, 11]. Various workers reported their investigation on chemical composition of different fish species mostly marine as well as some freshwater fishes at different times. But reports of investigation on nutritional composition of local fish fauna and the impact of season and the habitat difference is still fragmentary. Therefore, in view of these facts, the present study was carried out on *Labeo gonius*, a commercial and most preferred species of local population to assess its nutritional composition with special reference to protein and the essential amino acids in muscle and liver in lotic and lentic habitat in four different seasons of the year

MATERIALS AND METHODS

Sample Collection

The fish sample used for this study includes *Labeo gonius.* The fishes were collected from the local fishing sites. The length and body weight ranges were 25 ± 3cm and 240 ± 20gm respectively. The muscle and liver tissues were collected from the fish in fresh condition and weighed.

Estimation of Protein

The measured amount of tissues were homogenized in water (@1mg/10 ml) with the aid of a homogenizer. The solution was stirred for another three hours in a magnetic stirrer plate in order to get more protein in the solution. Protein was estimated by following the method of Lowry [12].

A standard protein and distilled water were used as reference and blank with the following reagents for the estimation. 1.5 ml of alkaline solution (0.5 ml of 4% solution of sodium-potassium-tartarate was added to 50 ml of 2g% solution of Na_2CO_3 and shaked. Then 0.5 ml of 2g% $CuSO_4$ solution was added to the mixture and shaked well). Then 150µl folin reagent (freshly prepared by adding 0.5 ml of folin ciacaltau reagent in 1 ml of distilled water) was added. Optical densities were measured at 670 nm in a computerised photometer (BTS - 320).

Estimation of Amino Acids

Preparation of Tissue Sample for HPLC of Amino Acids

Muscle and liver homogenate were prepared in water from freshly collected sample. 100µl of tissue homogenate was taken in graduated eppendorf tube and 500µl of 50% HPLC grade propan -2- ol in water was added. It was mixed properly and then centrifuged at 5000 rpm for 15 minutes. The supernatant was collected in 1 ml volumetric flask. The process was repeated with the precipitate and the obtained supernatant was transferred to the volumetric flask. The content of the flask was then dried by using a vaccum drier. To the amino acids settled at the bottom of the flask, 200µl of 1 (N) $NaHCO_3$ buffer was added and then evaporated under vacuum. On completion of evaporation 100 µl of 0.3% Dns-Cl- solution and 10 µl of deionized water were added and the flask was incubated at 50°C for one hour in a thermostat control water bath. After incubation 100µl of 1% proline solution was added to absorb the excess Dns-Cl. After 5 minutes of equilibrium the volume was adjusted to 1 ml with addition of 1:1 methyl cyanide-water diluting solution was mixed using volumetric procedure. The sample was then ready for injection into the HPLC system and 100 µl of the prepared sample was injected for each analysis corresponding to the volume used for calibration and the dilution factor obtained during the sample processing was entered into the microprocessor to obtain the results.

The estimation of amino acids was analysed by using HPLC, Parkin Elmer System with a UV visible detector and carbon packed polar silica HPLC Column.

RESULTS AND DISCUSSION

The amount of total protein in muscle and liver tissues of *Labeo gonius* from lotic and lentic habitats in different

seasons are presented in table-1. The highest amount of protein in muscle tissue was observed in lotic habitat in premonsoon (138.22 ± 6.82) and winter season (139.22 ± 3.30) against the lowest amount in retreating monsoon (Table 1). Comparatively lower protein content was observed in all the seasons of lentic habitat. Similar trend was also observed in liver tissues both in lotic and lentic habitats.

Table 1: This table shows the significance of variation of total protein in muscle and liver tissues of *Labeo gonius* in different seasons under two habitats (Lotic and Lentic)

			Premonsoon	Monsoon	Retreating Monsoon	Winter
MUSCLE	Lotic	Mean	138.22*	121.91**	42.8**	139.22
		SEM±	6.82	6.15	1.49	3.30
	Lentic	Mean	120.80	109.30	39.50	127.60
		SEM±	1.87	1.45	0.49	4.38
LIVER	Lotic	Mean	148.41^{NS}	146.10^{NS}	52.40*	129.88
		SEM±	8.96	2.37	1.41	2.74
	Lentic	Mean	141.60	137.50	42.28	129.03
		SEM±	9.19	5.26	1.40	4.04

Values are mean ± SEM of twenty observations, significant at p<0.01, p<0.001**, NS-not significant*

Table 2: This table shows showing the significance of variation of total protein in muscle and liver tissues of *Labeo gonius* in different seasons under two habitats (Lotic and Lentic)

Significance of Variation	tissues	habitats	t values	p values
Between Premonsoon	Liver	Lotic	0.25	p>0.01
&		Lentic	0.39	p>0.01
Monsoon	Muscle	Lotic	1.78	p>0.01
		Lentic	4.85	p<0.001
Between Premonsoon	Liver	Lotic	10.59	p<0.001
&		Lentic	10.68	p<0.001
Retreating Monsoon	Muscle	Lotic	13.62	p<0.001
		Lentic	42.12	p<0.001
Between Premonsoon	Liver	Lotic	1.98	p>0.01
&		Lentic	1.25	p>0.01
Winter	Muscle	Lotic	0.13	p>0.01
		Lentic	1.43	p>0.01
Between Monsoon	Liver	Lotic	33.95	p<0.001
&		Lentic	17.5	p<0.001
Retreating Monsoon	Muscle	Lotic	12.49	p<0.001
		Lentic	45.92	p<0.001
Between Monsoon	Liver	Lotic	5.20	p<0.001
&		Lentic	1.28	p>0.01
Winter	Muscle	Lotic	2.48	p<0.001
		Lentic	3.97	p<0.001
Between Retreating	Liver	Lotic	25.16	p<0.001
Monsoon		Lentic	20.27	p<0.001
&	Muscle	Lotic	26.64	p<0.001
Winter		Lentic	19.98	p<0.001

Comparison between groups of 20 number of observations (df=38)

Out of the eight amino acids studied, Lysine was observed highest in muscle tissue in winter season under lotic habitat and in premonsoon of lentic habitat. In liver tissues, Lysine was observed highest in premonsoon of both lotic and lentic habitats followed by Leucine in premonsoon and winter. Amount of Valine was observed highest in muscle tissue in winter season of lotic and monsoon of lentic habitat which was followed by Threonine, Isoleucine, Phenylalanine and Methionine. In Liver tissue, Valine was found highest in premonsoon and

Table 3: This table shows essential Amino Acid profile in liver and muscle tissues of *Labeo gonius* in different seasons under Lotic and Lentic habitats

Amino Acids	Muscle (µg/g) Lotic				Muscle (µg/g) Lentic				Liver (µg/g) Lotic				Liver (µg/g) Lentic			
	Pre monsoon	Monsoon	Retreating Monsoon	Winter	Pre monsoon	Monsoon	Retreating monsoon	Winter	Pre monsoon	Monsoon	Retreating Monsoon	Winter	Pre monsoon	Monsoon	Retreating Monsoon	Winter
Leucine	0.432 ±0.02	0.39 ±0.02	0.146 ±0.01	0.439 ±0.02	0.39 ±0.01	0.364 ±0.01	0.122 ±0.005	0.39 ±0.01	0.488 ±0.02	0.464 ±0.02	0.171 ±0.004	0.442 ±0.03	0.488 ±0.02	0.440 ±0.04	0.122 ±0.01	0.415 ±0.03
Isoleucine	0.267 ±0.02	0.242 ±0.02	0.09 ±0.004	0.272 ±0.01	0.242 ±0.01	0.294 ±0.01	0.071 ±0.003	0.242 ±0.01	0.304 ±0.02	0.287 ±0.02	0.100 ±0.004	0.241 ±0.02	0.302 ±0.02	0.272 ±0.02	0.070 ±0.004	0.240 ±0.02
Valine	0.295 ±0.01	0.267 ±0.01	0.101 ±0.004	0.04 ±0.02	0.269 ±0.01	0.311 ±0.03	0.079 ±0.004	0.267 ±0.03	0.318 ±0.04	0.317 ±0.04	0.111 ±0.003	0.269 ±0.02	0.334 ±0.02	0.300 ±0.03	0.079 ±0.004	0.267 ±0.02
Threonine	0.272 ±0.02	0.246 ±0.02	0.092 ±0.003	0.279 ±0.02	0.274 ±0.02	0.308 ±0.02	0.077 ±0.005	0.245 ±0.01	0.342 ±0.02	0.293 ±0.01	0.108 ±0.004	0.291 ±0.02	0.308 ±0.03	0.276 ±0.02	0.086 ±0.004	0.260 ±0.02
Lysine	0.497 ±0.02	0.45 ±0.02	0.169 ±0.01	0.506 ±0.02	0.500 ±0.03	0.42 ±0.03	0.141 ±0.01	0.45 ±0.03	0.602 ±0.04	0.553 ±0.03	0.197 ±0.02	0.478 ±0.02	0.562 ±0.03	0.506 ±0.03	0.116 ±0.02	0.478 ±0.03
Histidine	0.187 ±0.03	0.168 ±0.01	0.085 ±0.004	0.256 ±0.02	0.186 ±0.004	0.148 ±0.004	0.053 ±0.005	0.168 ±0.01	0.284 ±0.02	0.201 ±0.02	0.074 ±0.004	0.18	0.210 ±0.03	0.189 ±0.01	0.053 ±0.004	0.179 ±0.01
Methionine	0.191 ±0.01	0.173 ±0.01	0.114 ±0.01	0.225 ±0.02	0.198 ±0.02	0.162 ±0.02	0.054 ±0.003	0.17 ±0.01	0.25 ±0.02	0.236 ±0.02	0.087 ±0.02	±0.211 ±0.02	0.214 ±0.03	0.223 ±0.01	0.062 ±0.004	0.182 ±0.01
Phenylalanine	0.248 ±0.02	0.222 ±0.03	0.123 ±0.01	0.281 ±0.01	0.25 ±0.004	0.218 ±0.004	0.07 0.005	0.222 ±0.01	0.312 ±0.02	0.296 ±0.02	0.100 ±0.004	0.248 ±0.002	0.280 ±0.001	0.250 ±0.02	0.078 ±0.004	0.265 ±0.002

monsoon under both lotic and lentic habitats. Lowest amount was observed in case of Histidine, but seasonal variation on the differential amino acid content was clearly observed in all the conditions.

Both muscle and liver tissues present minimum amount of essential amino acids in the retreating monsoon season both in lotic and lentic habitats; however, no significant ($p>0.01$) variation was observed between premonsoon and monsoon season, although slightly apparent variations were observed in premonsoon, monsoon and winter. Highest amount of non-polar amino acid with aliphatic side chains which altogether form about 40% of the total eight essential amino acids studied against 20% contributed by Lysine alone. While lowest amount of SH-containing side chains in all the situations was significant observation of the present study.

The present work has elucidated the importance of local freshwater commercial fish as good sources of protein having requisite amount of essential amino acids, though the consumers do eat fish- flesh because of its availability and palatability, but the present work has suggested significant richness of these nutritional components in liver tissue than that of flesh tissue. In addition, the nutrient composition of studied fish tissues (muscle and liver) was found to vary with seasons and geographical locality of harvest which agrees with the findings of the previous worker [3].

The nutrient composition of a particular species often appears to vary from one fishing ground to another and from season to season, but the basic causes of change in composition are usually variation in amount and quality of food that the fish eats and the amount of movement it makes. Abundance of food supply can markedly change the composition of a species. Moreover, fishes when overcrowded, insufficiency of food causes low intake and changes in composition accordingly. The findings of the present study with increase in nutrient composition (protein and essential amino acids) in lotic habitat than that of the lentic conditions are in conformity with the suggestions put forwarded by the previous workers [14, 8, 4].

Since the interest in commercial culture of fish has increased in present time to fill the gaps between supply and demand, the observation from the present work with differences in the habitat and seasonal variation may be useful in developing nutrient balanced, cost-effective production of studied fish by culture practices

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ACKNOWLEDGEMENT / SOURCE(S) OF SUPPORT

Nil

CONFLICT OF INTEREST

No conflict of interests was declared by authors