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Research Article

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A Comparative Study on Total Muscle Protein Content of *Anabas testudineus*, *Labeo gonius*, *Labeo rohita* and *Heteropneustes fossilis* and to analyze their Electrophoretic Banding Pattern using SDS-PAGE

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Abstract: India is undergoing nutrition transition and is facing the dual burden of malnutrition, problem of under-nutrition and malnutrition deficiencies. Fish is one such food that can solve the problem. Fresh fish flesh provides an excellent source of protein for human diet, which is relatively of digestibility, biological and growth promoting value for human consumption. The present study is aimed to determine the total muscle protein content of freshly killed fishes belonging to different species (*Anabas testudineus* [E1], *Labeo gonius* [E2], *Labeo rohita* [E3] and *Heteropneustes fossilis* [E4]) and also to analyze the electrophoretic banding pattern of muscle protein from the above species through SDS-PAGE gel electrophoresis. Electrophoresis can be used to separate and visualize proteins. In SDS-PAGE, proteins are separated based on size. When protein samples are applied to such gels, it is usually necessary to know the protein content or concentration of the sample. The total muscle protein estimation was done by Lowry's method using BSA protein as standard. The optical density was measured at 660 nm. The results showed that the total protein concentration of sample E1, E2, E3 and E4 was found to be 8.31±0.314 mg/ml, 7.65±0.333 mg/ml, 6.98±0.315 mg/ml and 8.98±0.483 mg/ml respectively. The SDS-PAGE electrophoresis done in case of different fish species showed variation in the banding patterns, in which E1 and E4 showed maximum number of bands as compared to E2 and E3. Fishes belonging to the group of catfishes and perches were found to contain more amount of protein as compared to Indian Major Carps and Minor Carps. Therefore, fishes play a vital role as it is an important and cheaper source of quality animal proteins.

Keywords: Muscle protein, Fish, Electrophoresis, Under-nutrition, Malnutrition, SDS-PAGE, Banding pattern

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1. INTRODUCTION

India is undergoing a nutrition transition and is facing the dual burden of malnutrition, i.e., the problem of under-nutrition and malnutrition deficiencies. Fish is one such food that can solve the problem unswervingly. The nutritive and medicinal value of fish has been recognized from time immemorial. Fresh fish flesh provides an excellent source of protein for human diet. This protein is relatively high protein content, digestibility, biological and growth promoting value for human consumption.[1] Fishes are known to be a very healthy food item. They are an excellent protein source that also provides essential fatty acids and micronutrients necessary for good health. Though fish is highly perishable, processing of fish into value added fish products can contribute greatly to extend the period of fish availability for all classes of people to be a source of animal protein and minerals such as calcium and phosphorus. It has been reported that societies with high fish intake have considerably lower rates of acute myocardial infarction , other ischemic heart diseases and atherosclerosis.[2,3] Fish proteins comprises of all the ten essential amino acids in required proportion, namely, lysine (high concentration), arginine, histidine, leucine, isoleucine, valine, threonine, methionine, phenylalanine and tryptophan, accounting for the high biological value of fish flesh.[1] Fishes are relatively cheaper protein sources of high biological value. Therefore, it's use may help bridge the protein gap because of its multifarious economic advantages and nutritional significance.[4] Fish is consumed either as a preparation from freshly caught fish or from those that have been preserved in some form. However, fish in fresh condition makes a difference. The nutritional value, the look, the flavor and the biochemical composition do not remain normal and undergo changes during preservation processes and storage. Also, same species of fish may show variation in biochemical composition of its flesh depending upon the fishing ground, fishing season, age and sex of the individual, fat and water content being most effected.[1] Fishes are quite different from the other animal food sources, because they provide calories with high quality proteins, which contain all essential amino acids in easily digestible form thereby being the most beneficial nutrition sources.[5] The principle biochemical contents of fish flesh are protein, fat and water. Protein constitutes about 20%, while fat and water vary widely. Proteins occur in fish as- Myosin, myogen, myo-albumin and globulin, these are all intracellular components of muscle fibre; collagen and connective tissue fibres; phosphoprotein and nucleoprotein.[6] The protein is present chiefly in the skeletal muscles- the fish flesh. Fishes can be classified as oily fish or fatty fish (fat content more than 8%), average fatty fish (fat content between 1- 8%) and lean fish (fat content less than 1%). These fats are chiefly the triglycerides esters of fatty acids with small amounts of free fatty acids, some vitamins, sterols, hydrocarbons, phospholipids etc.[7] In addition, a special type of fatty acid, omega-3 polyunsaturated fatty acid, recognized as an important drug to prevent a number of coronary heart diseases.[8] The present study was therefore undertaken to determine the total muscle protein content of freshly killed fishes belonging to four different species, i.e., *Anabas testudineus*, *Labeo gonius*, *Labeo rohita* and *Heteropneustes fossilis*. And also to analyze the electrophoretic banding pattern of muscle protein from the above mentioned species through SDS-PAGE.

2. MATERIALS AND METHODS

2.1 Collection and preparation of samples

Three different species of fish samples were collected: *Anabas testudineus* [E1], *Labeo gonius* [E2], *Labeo rohita* [E3] and *Heteropneustes fossilis* [E4]. All the samples were healthy at the time of collection. The fishes were bought live from the fish market and stored in a well maintained aquarium. In the experiment, all the samples were used in fresh condition.

2.2 Preparation of protein extract from muscle

The fishes were cut along the dorsal most part of their body, separating the skin from muscle, so that the muscles became well exposed. The bones were carefully removed, then to each 1g of sample (kept in separate test tubes), 0.1N saline was added using a micropipette. Since proteins are thermo-sensitive molecules, the test tubes containing the muscle extracts were kept in a beaker containing ice. The muscles were properly homogenized by using tissue homogenizer and centrifuged at 3000 rpm for 8-10 minutes. Supernatant was taken for experiment. The sediment was discarded. Supernatant kept in eppendorf tubes using micropipette and stored at -20°C for further analysis of the samples.

2.3 Estimation of protein concentration

The protein concentration of the samples were estimated by Lowry's Method by using standard protein (Bovine serum albumin).[9] This method is based on the reaction of copper ions produced by the oxidation of peptide bonds with Folin-Phenol reagent (a mixture of phosphotungstic acid and phosphomolybdic acid in the Folin-Phenol reagent). This method is more sensible than ultraviolet (280nm) absorbance reading, it is less disturbed by turbidity. The optical density of each sample was measured by using a spectrophotometer at a wavelength of 660 nm and recorded the data in tabular columns.

2.4 SDS- PAGE Gel Electrophoresis

Electrophoresis can be used to separate and visualize proteins. In sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE), proteins are separated based on size.[10] It uses sodium dodecyl sulfate (SDS) molecules to help identify and isolate protein molecules. The advantage of adding SDS to gel electrophoresis is that it denatures the proteins and gives them a negative charge. When doing gel electrophoresis for protein, it is important to denature the proteins first, thus removing their higher structures. When protein samples are applied to such gels it is usually necessary to know the protein content or the protein concentration of the sample. This makes it possible to apply a volume of samples of the gel so that samples have a comparable amount of total protein. An intact SDS-PAGE electrophoresis system should include a tank, lid with power cables, electrode assembly, cell buffer dam, casting stands, casting frames, combs (containing wells) and glass plates. The gel plates were assembled according to the manufacturer's instructions. The mixture for 12% resolving gel was poured inside glass mould and immediately overlaid with n-butanol. The casting frame was kept undisturbed for 30 minutes. n- butanol was discarded and the gel top washed with distilled water. The mixture for 5% stacking gel was then pipetted over the resolving gel up to top of the

plates. The comb was washed with the remaining solution and immediately inserted into the stacking gel. The casting frame was kept undisturbed for 30 minutes. Protein concentration of each sample was estimated after performing Lowry's method. Using the data of protein concentration, the volume of the required loading protein sample was calculated in such a way that each loading sample contained 40µg of protein. The loading protein samples were mixed with a sample buffer in properly labeled Eppendorf tubes (were kept on ice) using micropipette. The comb from the mould was removed carefully and the wells were properly washed with distilled water. Electrophoretic tank was filled with an electrophoresis buffer and the unit containing the prepared gel plates was placed at the right place. The loading samples were loaded in the wells using micropipette and the top of the chamber was covered. Then the apparatus was attached to the power supply unit and applied 70V for the stacking gel. Once the samples crossed the stacking gel, the voltage was adjusted to 150V. Power supply was turned off once the samples reached the bottom of the resolving gel. After that, the gel plates were carefully removed from the electrophoretic apparatus. The gel plates were removed and was placed in fixative solution and left for 30 minutes. After the fixation, the gel was placed in a petri dish containing staining solution for about 4 hours at room temperature. The petri dish was placed on an electronic rocker. Finally after the staining process, the gel was placed in destaining solution and left overnight.

3. STATISTICAL ANALYSIS

The data obtained were analyzed using Microsoft Excel 2010. The statistical data were presented as mean ± standard deviation (SD). Student's (paired) "t" test was used for analysis of comparison. Probability value (P) of less than 0.05 was considered statistically significant.

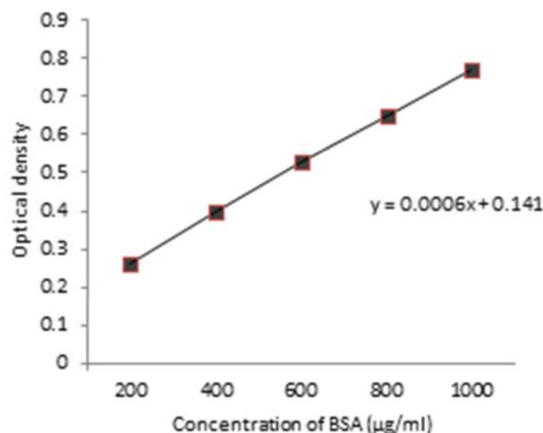
4. RESULTS

The measured optical density values of the standard BSA are given in the following table (Table 1).

Table 1. Optical density and concentration of BSA

Concentration of BSA (µg/ml)	Optical density
200	0.26
400	0.40
600	0.53
800	0.65
1000	0.77

The above mentioned concentrations and optical density were plotted on x-axis and y-axis respectively to get the calibration curve and hence the y = mx + c relation (Graph 1).



Graph 1. Relation between BSA concentration and optical density

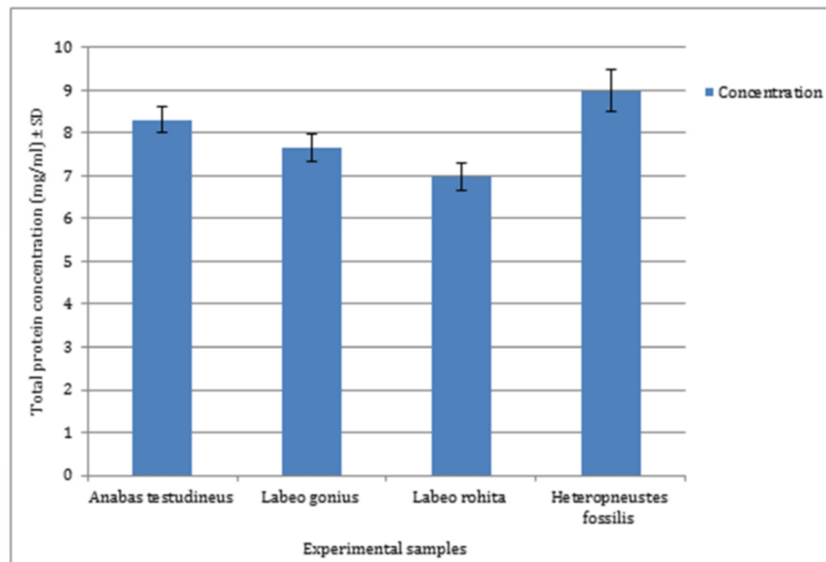
From the above graph (Graph 1), the relation between BSA protein concentration and respective optical density values were obtained by the relation, $y = 0.0006x + 0.141$.

Table 2. Optical density and protein concentration of the experimental samples

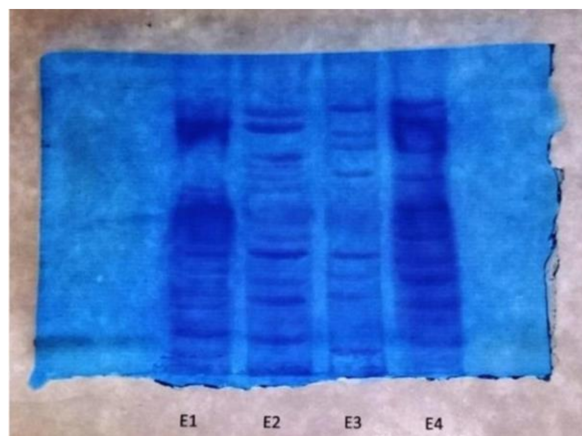
Experimental samples	Optical density ± SD	X value obtained from equation y=0.0006x+0.141	Concentration (µg/ml)	Concentration (mg/ml) ± SD
Anabas testudineus [E1]	0.64±0.018	831	8310	8.31±0.314
Labeo gonius [E2]	0.60±0.02	765	7650	7.65±0.333
Labeo rohita [E3]	0.56±0.018	698	6980	6.98±0.315
Heteropneustes fossilis [E4]	0.68±0.029	898	8980	8.98±0.483

Values are mean ± SD; (n=10), P<0.05

The protein extracts prepared from the experimental sample shows variation in terms of their total protein concentration. The total protein concentration of sample E1, E2, E3 and E4 was found to be 8.31±0.314 mg/ml, 7.65±0.333 mg/ml, 6.98±0.315 mg/ml and 8.98±0.483 mg/ml respectively. Thus, from the study it was found that the muscle protein concentration was highest in *Heteropneustes fossilis* [E4] and lowest in *Labeo rohita* [E3] out of the four experimental fishes (Table 2 and Graph 2).



Graph 2. Graphical representation of total protein concentration (mg/ml) in different experimental samples



***The SDS – PAGE electrophoresis done in the case of four different freshly killed fish species viz. Anabas testudineus* (E1), *Labeo gonius* (E2), *Labeo rohita* (E3) *and Heteropneustes fossilis* (E4) *shows the following electrophoretic banding patterns* (Fig. 1).**

Fig 1. SDS-PAGE gel showing muscle protein bands of four different fish species (E1= *Anabas testudineus*, E2=*Labeo gonius*, E3=*Labeo rohita* and E4=*Heteropneustes fossilis*)

5. DISCUSSION

In the field of fish nutrition, the preliminary evaluation of muscle protein can be used by SDS-PAGE electrophoresis. The SDS-PAGE electrophoresis is used to visualize and to quantify the level of protein through banding patterns. In Assam, among the fresh water fish species, *Anabas testudineus*, *Labeo gonius, Labeo rohita* and *Heteropneustes fossilis* are very delicious, have high market price, nutritious and popular to consumers. Therefore, these fishes are very important due to commercial purpose. Muscle proteins, the most abundant macromolecules found in biological systems are present in diverse forms in the muscles of different fish species. When these diverse groups of proteins are subjected to SDS-PAGE electrophoresis, it shows remarkable banding patterns. Hence, the electrophoretic banding patterns of muscle proteins of four different experimental fish species in the SDS-PAGE photograph show wide variation in each case.

From the gel photograph, it is seen that *Anabas testudineus* (E1) and *Heteropneustes fossilis* (E4) shows maximum number of bands as compared to *Labeo rohita* (E3) and *Labeo gonius* (E2). Bands in *Heteropneustes fossilis* are more distinct and thicker and also evenly spaced as compared to *Anabas testudineus*. As *Labeo rohita* (E3) and *Labeo gonius* (E2) belong to the same genus *Labeo*, their protein banding patterns are almost similar. In the case of these two species, bands are distinctly spaced and lighter as compared to E4 and E1. *H. fossilis* contains the highest amount of muscle protein. The protein extracts prepared from the experimental sample also showed variation in terms of their total protein concentration. The total protein concentration of sample E1, E2, E3 and E4 was found to be 8.31±0.314 mg/ml, 7.65±0.333 mg/ml, 6.98±0.315 mg/ml and 8.98±0.483 mg/ml respectively. Thus, from the study it was found that the muscle protein concentration was highest in *Heteropneustes fossilis* [E4] and lowest in *Labeo rohita* [E3] out of the four experimental

fishes. *A. testudineus* stands next to *H. fossilis* in its total muscle protein concentration. The two *Labeo* species have total protein concentration in lesser amounts as compared to the above two species. Our results corroborates with the findings of Singh *et al*. (2016) in case of *Anabas testudineus*.[11] Our results showed slight difference with the findings of Binukumari and Vasanthi (2013) and Mustafa (1983) in case of *Labeo rohita* and *Heteropneustes fossilis* respectively.[12,13] As fishes are an important source of quality animal proteins and it has been reported that fish protein has greater satiety effect than other sources of animal proteins.[14] Higher protein content means presence of more amino acids. Fish which contain high amounts of protein in them proves to be more nutritive thereby giving resistance to a number of diseases. The physiological role of dietary proteins is to provide substrates required for the synthesis of body proteins and other metabolically important nitrogen-containing compounds. Therefore, the content of the nutritionally indispensable amino acids in food proteins is usually the primary determinant of nutritional quality of protein.[15]

6. CONCLUSION

Fish protein is relatively of high digestibility, biological and growth promoting value for human consumption. The experiment proves that fishes belonging to the group of catfishes and perches contain more amount of protein as compared to Indian Major Carps and Minor Carps, revealing that carps have lower nutritional value as compared to the catfishes and perches. More protein contents directly related to the presence of more amino acids in the muscle tissues of fishes. Proteins containing amino acids like leucine, glutamic acid and histidine are present in plenty of amount in *Heteropneustes fossilis* and *Anabas testudineus* which has various therapeutic significance in the human body. Therefore, there is a need to generate and document nutritional information related to the presence of these amino acids in the muscles of numerous varieties of food fishes available.

7. ACKNOWLEDGEMENTS

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8. AUTHORS CONTRIBUTION STATEMENT

Dr. Devajit Basumatari derived the concept with proper planning and execution and carried out the research study. Ms. Dipika Doloi evaluated the results and contributed to the writing and revising of the manuscript.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

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