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Comparative Analysis of Tissue Protein Isolated From *Labeorohita*, *Labeoboga* and *Catlacatla* and its Expression

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***Abstract*: Background: Fish are poikilothermic and live permanently immerse in water, they are directly affected by changes in ambient medium. The variable undergoing change may be variation related to protein content or other chemical constituent of the body.**

Aim: The aim of the study was to assess the protein content in fish from different location and also it's differently expression of protein.

Design: A commercially available carp (*L. rohita, L. boga* and *C. catla*) was collected from Hajo pond and Brahmaputra River at Guwahati in Assam district. The samples were then subjected for qualitative test and quantitative test by Lowry estimation and Bradford estimation. A comparative analysis of the tissue protein was done using SDS-PAGE to see differently expressed proteins in the fish samples.

Result: Lowry estimation of fish sample from Brahmaputra and Hajo pond showed 0.94mg/ml,0.936mg/ml for L. rohita, 0.94mg/ml,0.90mg/ml for C. catlaand 0.98mg/ml, 0.92mg/ml for L. bogarespectively. Bradford estimation of fish from Brahmaputra and Hajo pond show 0.99mg/ml, 0.97mg/ml for *L. rohita* 0.95mg/ml,0.90mg/ml for *C. catla* and 1mg/ml, 0.919 for *L. boga* respectively. The SDS-PAGE also showed many striking variation in number, density.

Conclusion: The result revealed that, Brahmaputra river fish show more protein content than Hajo pond fish. The SDS-PAGE also reveal that expression of protein band is depleted in Hajo pond fish compare to Brahmaputra River fish, the depletion of protein may be due to different diet and environmental condition that they adopt itself to the change metabolic system which might lead to degradation process like proteolysis and utilization of degraded products for increased metabolism.

***Keywords*: Lowry estimation, Bradford estimation, SDS-PAGE, proteolysis.**

1. Introduction

Fish and aquaculture products supply an important amount of animal protein as well as valuable nutrition to the diet. Fishes contains good quality, balanced and digestible protein. Commercially available carp (L. rohita , L. boga and C. catla) which are widely consumed by people are common Indian major carp found in lakes, streams and river. This carps are highly rich in protein and play a major nutritional role. However with increasing agricultural practices, development of various type of industries, increase in human population especially in urban centres brings undesirable changes in the environment, which could affect the biotic composition of the ecosystem. Fish growth is influence by number factors including food, space, salinity, season and physical activity (M. Ali et al., 2005). The variable undergoing change may be variation relate to protein content or other chemical constituent of the body (Weatherly and Gill, 1987). Proximate body composition analysis is a good indicator of the physiological condition of a fish but it is time consuming to measure (M.Aliet al., 2005). Carbohydrate and other non-protein compounds are present in negligible amount and are usually ignored for routine analysis (Cui and Wootton., 1988). Protein content, which is important component, tends to vary little in healthy fish (Weatherly and Gill, 1987). It is crucial to note that all research related to proteins increase our understanding of their levels, interaction, functions, modifications, regulations, and localization in cells.

Proteins are the polymers of amino acids. They are complex organic compound containing nitrogen, hydrogen, carbon and oxygen. Qualitatively they can be confirmed by several tests. Due to the presence of characteristic side chains in them, certain amino acids exhibits typical colour reactions or either coagulation or precipitation that forms the basis for their identification. Proteins respond to some colour reactions due to the presence of one or more radicals or groups of complex protein molecule. Coagulation of protein is caused by the denaturation of protein structure by heat or acids and the precipitation is cause by reagents like alcohols, alkaloids, salts etc. (Dr.A.C.Deb, concept of biochemistry). Also in this experiment, concentration of protein is estimated among the fishes collected from different location base on the principle of Lowry and Bradford. Lowry Principle lies in the reactivity of peptide nitrogen with alkaline copper sulphate solution and folins reagent giving blue colour according to the concentration of protein. The absorbance is read at 660 nm. (Gary L.Peterson, 1977). And Bradford estimation is based on protein binding with coomassive brilliant blue dye and reading the absorbance at 595 nm.The result give us how much protein is present in the sample or to measure the concentration or amount of protein in fish muscle samples. Quantitative evaluation of proteins can be

accomplished with protein profiling, which shows us a unique patterns expression (healthy fish and toxic exposed fish). Tissue proteins of aquatic animals under toxic stress are also known to play a pivotal role (Venkataramana, Sandhya 2006). In an electric field, protein move towards the electrode of opposite charge. The rate of which they move is governed by a complex relationship between the physical characteristic of both the electrophoresis system and the proteins. Factor affecting protein movement involve size, shape and charge of proteins (Garfin 1990). Proteome study of fish can help to identify protein and enzyme that are responsible for increasing meat yield, the commercially important fish vitamins and unsaturated fatty acids, as well as treatment of fish disease. Since protein are the main functional entities of the cell they should be expected to provide the most relevant information regarding cellular function (SerginScobioalaet al.,2005).

2. Methodology

A. Study setting

The present study was carried out in well-equipped Biochemistry and CIF laboratory of Assam down town University.

B. Preparation of extract

The collected fish sample was transported to the laboratory packed in polythene bag keeping at -4 degree and after which wash with deionise water. The tissue protein from L.rohita L. boga and C.catla was extracted by boiling the muscle tissue for 15 minutes in deionise water. 20 gram of boiled muscle tissue was crushed with mortar and pestle in 100 ml of phosphate buffer (ph-6.5). The crushed tissue muscle was centrifuge at 10000 rpm for 10 minutes. The supernatant was collected which will be used for further investigation (Blanchard JS 1984).

C. Qualitative test for protein

Preliminary protein test was done by using qualitative test to detect the presence of peptide linkage, aromatic amino acids of protein by:

- *Biuret test*. (concept of biochemistry, theory and practical by Dr.A.C.Deb 1999)
- *Ninhydrin test*.(concept of biochemistry., Dr. A. C. Deb)
- *Xanthoproteic reaction.* (Concept of biochemistry)
- *Sulphur reaction*. (concept of biochemistry., Dr. A. C. Deb)
- *Coagulation test* with con. HNO3. (concept of biochemistry., Dr. A. C. Deb)
- *Precipitation by neutral salts*. (concept of biochemistry., Dr. A. C. Deb)
- *Precipitation by alcohol*. (Concept of biochemistry, theory and practical by Dr. A. C. Deb 1999)

D. Qualitative test for carbohydrates and lipid

Preliminary carbohydrate test was done for the presence of monosaccharide, reducing and non-reducing sugars by:

- *Molisch test:* (AmalAlmari qualitative analysis of carbohydrate 1)
- *Benedict's test:(*AmalAlmari qualitative analysis of carbohydrate 1)
- *Iodine test: (*AmalAlmari qualitative analysis of carbohydrates 1)
- Preliminary lipid test was done for the presence of lipid by.
- *Emulsification test*:(Lieberman Burchard, qualitative test for protein 3)

E. Quantitative test

1) Quantitative test for protein

Standard quantitative test was done for the estimation of total protein content in the sample.

- *Lowry estimation of protein*. Principle lies in the reactivity of peptide nitrogen with alkaline copper sulphate solution and folins reagent giving blue colour according to the concentration of protein. The absorbance is read at 660 nm (Gary L. Peterson 1977)
- *Bradford estimation of protein:* The principle of this assay is that the binding of protein molecules to coomassie dye under acidic conditions result in a colour change from brown to blue. The absorbance is read at 595nm (Gary L. Peterson 1977).

2) SDS-page analysis of the extract

The tissue protein extracts obtained from the L. rohita, L. boga and C. catla was subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis to resolve the proteins and to analyse the protein(s) expression (Hames BD et.al., 1990).

3. Observation and result

1) Observation and result for qualitative test for protein.

The preliminary qualitative test was done to assess the presence of protein. The presence of protein was determine in Labeorohita, Labeoboga and Catlacatla collected from Brahmaputra river and Hajo pond as shown in the figures (Table 1).

2) Observation and result for qualitative test for carbohydrates.

The preliminary qualitative test was done to assess the presence of carbohydrate in the fish sample shown in table 2 Observation and result for qualitative test for lipids. The preliminary qualitative test was done to assess the presence of lipids in the fish sample shown in table 3.

3) Observation and results for quantitative test for protein concentration by lowry method

As shown in table 4, the two different location fish show variation of total protein (mg/ml) in body muscles of selected fishes *Labeorohita*, *Labeobogaand Catlacatla.* The total protein (mg/ml) content in the body muscle of *Labeorohit* arranged from 0.94 to 0.93 mg/ml collected from Brahmaputra River and Hajo pond respectively. Also for Catlacatlait ranged

Table 1
Qualitative test for protein

S. no	Test	Brahmaputra river			Hajo pond		
		L. rohita	*L. boga*	*C. catla*	*L. rohita*	*L. boga*	*C. catla*
1	Biuret test	+	+	+	+	+	+
2	Ninhydrin test	+	+	+	+	+	+
3	Xanthoproteic test	+	+	+	+	+	+
4	Sulphur reaction	+	+	+	+	+	+
5	Coagulation test	+	+	+	+	+	+
6	Precipitation by salts	+	+	+	+	+	+
7	Precipitation by alcohol	+	+	+	+	+	+

+: positive result

Table 2
Qualitative test for carbohydrate

Sr.no	Test	Brahmaputra river			Hajo pond		
		L. rohita	*L. boga*	*C. catla*	*L. rohita*	*L. boga*	*C. catla*
1	Molish	-	+	+	-	+	+
2	Benedict	-	-	-	-	-	-
3	Iodine	-	-	-	-	-	-

+: Positive result
-: Negative result

from 0.94 to 0.90, Labeobogafrom 0.98 to 0.92 from Brahmaputra River and Hajo pond respectively.

4) Observation and results for quantitative test for protein concentration by bradford method

As shown in table 5, the two different location fish show variation of total protein (mg/ml) in body muscles of selected fishes Labeorohita, Labeobogaand Catlacatla. The total protein (mg/ml) content in the body muscle of Labeorohitaranged from 0.99 to 0.97 mg/ml collected from Brahmaputra River and Hajo pond respectively. Also for Catlacatlait ranged from 0.95 to 0.90, Labeobogafrom 1 to 0.91 from Brahmaputra River and Hajo pond respectively. The total protein (mg/ml) content in the body muscle of all the three fishes collected from Brahmaputra River ranged from 0.94 to 0.98mg/ml from Lowry estimation and 0.95 to 1mg/ml from Bradford estimation. Also for Hajo pond fishes the value range from 0.92 to 0.93 from Lowry estimation and 0.90 to 0.97 from Bradford estimation. The result obtains from the above shows that the Brahmaputra River fishes show more protein concentration in it body as compare to the Hajo pond fishes.

5) Observation and result of SDS-PAGE.

After the completion of SDS-PAGE, the following expression of protein was observed as shown in Fig. 2. below.

B. Discussion

Proteins are the most important constituents of animal tissues, as they play an important role in spare energy. Tissue proteins of aquatic animals under unfavorable condition also play a pivotal role in the activation of compensatory mechanisms (VenkataramanaSandhya 2006).As per the result obtained on the total protein content in the selected fish species L. rohita, C. Catlaand L. boga from different location. There is significant difference in total protein content (mg/ml) in the body muscle of Hajo pond fish and Brahmaputra River fish as given in the above table 4 and 5. From the above result, the Hajo pond fish show less concentration of protein as compare to Brahmaputra river fish. The Hajo pond fish protein concentration is depleted due to physiological strategy played by animal to adopt itself to change metabolic system. The highest protein content was observed in Labeoboga collected from Brahmaputra River ranging from 0.98mg/ml to 1mg/ml. The lowest protein content was observed in Catla catla collected from Hajo pond ranging from 0.9mg/ml to 0.905mg/ml. The expression of protein by the SDS-PAGE also shows many striking variation between the two location fish species. The expressions of comparative protein analysis in fishes were investigated by various authors and correlated their results, which are summarized below. The quantity of protein is dependent on the rate of protein synthesis, or rate of its degradation. The quantity of protein may also be affected due to impaired incoperation of amino acids in the polypeptide chain (Singh et al., 1996).

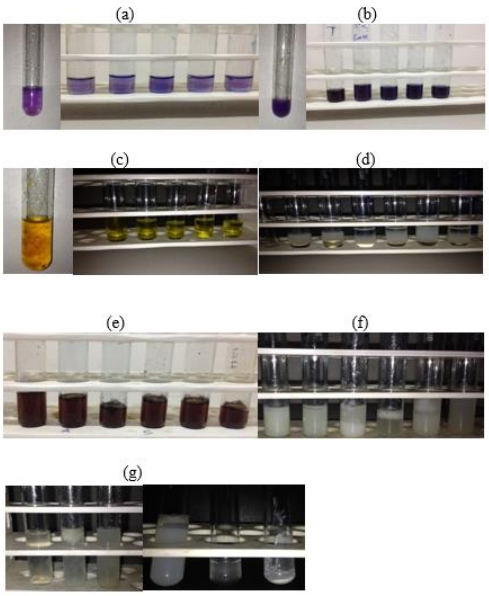

Fig. 1. Photographic representing presence of protein in the sample. (a) Biuret test (b) Ninhydrin test (c) Xanthoproteic test (d) Sulphur reaction test (e) Coagulation test (f) Precipitation by salts (g) Precipitation by alcohol

Table 3
Qualitative test for lipids

Sr. no	Test	Brahmaputra river			Hajo pond		
		L. rohita	*L. boga*	*C. catla*	*L. rohita*	*L. boga*	*C. catla*
1	Emulsification test	-	-	-	-	-	-

-: Negative result

Table 4
Protein concentration of selected fishes by Lowry method

Sr.no	Sample name	Con. mg/ml (Brahmaputra river)	Con. mg/ml (Hajo pond)
1	*L. rohita*	0.94	0.93
2	*C. catla*	0.94	0.90
3	*L. boga*	0.98	0.92

Table 5
Protein concentration of selected fishes by Bradford method

Sr.no	Sample name	Con. mg/ml (Brahmaputra river)	Con. mg/ml (Hajo pond)
1	*L. rohita*	0.99	0.97
2	*C. catla*	0.95	0.90
3	*L. boga*	1	0.91

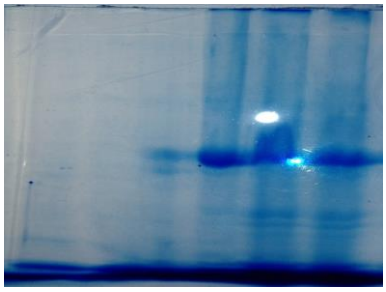

Fig. 2. SDS-Profile of the samples

1. *Labeo rohita* from Hajo pond
2. *Catla catla* from Hajo pond
3. *Labeo boga* from Hajo pond
4. *Labeo rohita* from Brahmaputra River
5. *Catla catla* from Brahmaputra River
6. *Labeo boga* from Brahmaputra River

The present study showed that the use of protein profiles may be helpful in parameters and particularly in fishes with unsuitable diet and environmental condition which give us clue to whether fishes are in favorable condition for their proper metabolism

4. Conclusion

Total protein content in body muscles of Brahmaputra River fishes was higher compared to the Hajo pond fishes. Among the three fishes collected, *Labeoboga* showed the highest protein content (mg/ml) from Brahmaputra River. The lowest protein content (mg/ml) was observed in *Catlacatla* collected from Hajo pond. Total protein content (mg/ml) in body muscle of three fishes collected from Brahmaputra River and Hajo pond show fluctuations in the results (Lowry estimation and Bradford estimation).however the significance obtained from this two method are in very little variation. Also both the estimation result show that the protein content is higher in Brahmaputra River fishes then that of Hajo pond fishes. From the SDS-PAGE the expression of protein varies. The Hajo pond show very little expression as shown above in the picture. However, the Brahmaputra river fish protein expression is higher and is in better amount. it show many different striking variation in number, density and the depletion of protein in Hajo pond protein may be due to different diet and environmental condition. In the present study it can be concluded that the fish samples collected from different region of Guwahati showed different pattern of protein expression as they are expose to different environment. The result revealed that, Brahmaputra river fish show more protein content than Hajo pond fish which indicate that they adopt it to change metabolic system which might lead to degradation process like proteolysis and utilization of degraded products for increased metabolism.

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