



Studies on the biochemical composition in the liver of *Labeo rohita* and *Anguilla bengalensis* in Mysore, Karnataka.

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ABSTRACT

Abstract: This study investigates the biochemical composition of the liver in two freshwater fish species, *Labeo rohita* and *Anguilla bengalensis*, to understand species-specific metabolic and nutritional characteristics. Liver tissues were analysed for protein, carbohydrate, lipid, and cholesterol contents using standard biochemical methods. The results indicated that *Labeo rohita* showed higher protein and carbohydrate levels, reflecting its active metabolism and feeding habits, while *Anguilla bengalensis* exhibited elevated lipid and cholesterol content, suggesting energy storage adaptations associated with its carnivorous and migratory behaviour. These findings provide baseline data on the biochemical profile of these species, contributing to insights into their physiological adaptations, nutritional assessment, and potential responses to environmental stressors.

1. Introduction

Fishes are animals that are of aquatic habitat, are cold blooded, breath using gills, have backbone, have a scaly skin and have different types of fins viz., dorsal fins, pelvic fins, pectoral fins, anal fins, and caudal fins. In fish limbs are modified into fins. Different species of fish require different aquatic habitats and food sources for survival like marine, fresh water, estuaries and brackish water. Different species in the same habitat occupy different niches as bottom feeder, column feeder and surface feeder based on their feeding habit. They are distributed among the different parts of the world. The main source of food for fishes includes planktons, invertebrates and small fishes. Fish has been recognized as an excellent food source for human beings for centuries and is preferred as a perfect diet not only due to its excellent taste and high digestibility but also because of having higher proportions of unsaturated fatty acids, essential amino acids and minerals for formation of functional and structural proteins.

Fish are a rich source of polyunsaturated fatty acids (PUFAs), namely the n-3 and n-6 PUFAs, which are beneficial to human health. Fish meat and oils are good sources of unsaturated omega-3 fatty acids, eicosapentacenoic acid Docosahexaenic acid (DHA), as well as its precursor, alpha linoleic acid. Compared to beef and chicken, fish meat contains higher levels of n-3 PUFAs, which is known as cardio-protective, anti-atherosclerotic, antithrombotic, and anti-arrhythmic and also play a role in reducing cholesterol level, regulate prostaglandin synthesis and hence induce wound healing and stabilizing the electrical activity of heart cells therefore, fish meat containing high rate of PUFAs. Fishery products are very important for food security, providing more than 15% of total animal protein supplies (FAO 2002). This sector is also crucial not only as a main source of animal protein to ensure food security, but also to improve employment and income for poverty elimination in developing countries including India. As the cold-blooded animal fish is easily influenced by various factors, environmental factors, and nutrition factors. The surrounding water temperature shows the prominent effect on body temperature, growth rate, feed consumption, and other metabolic function (Britz et al., 1997).

Condition factor compares the well-being of a fish which has been used as an index of growth and feeding intensity (Fagade and Adebise, 1979) and also influences the reproductive cycle in fish (Welcome 1979). The reproductive cycle of different species of fish has developed into their natural range and habitat.

1.1 Works on biochemistry of fish

The annual discards of fish industry is estimated to be approximately 20 million tons (or 25% of the total production) per year (Rustad, 2003). Unlike marine fish processing sector, fresh water fish or the inland fisheries sector in general is un-organized, especially in developing countries and hence poses a different level of waste disposal problems. The fish production from freshwater bodies through aquaculture alone stands currently at 25.75 million metric tons (FAO,

2006). Considering the fact that fish viscera are a rich source of protein and polyunsaturated lipids (Bhaskar et al.,2007). and constitute about 10–15% (depending on the species) of the fresh water fish biomass, it can be estimated that freshwater fish processing waste in the form of viscera. Major carps (Catla, Rohu, Mrigal and common carp) contribute mainly towards the freshwater fish production globally (FAO, 2006) and India alone generates >300,000 tons of visceral waste through freshwater fish processing (Mahendrakar, 2000).

The fact that these by-products are rich in protein and fat make them more perishable. These proteinaceous fish waste can be converted into hydrolysate (Aspmo et al.,2005) and thereby avoid any environmental, health and economic problems (Vidotti et al.,2003) that can arise. Traditional methods like fish silage have often been used for preparing autolytic hydrolysate like fish silage that makes use of the endogenous enzymes. However, such methods have the limitation of requiring longer time for the completion of the process; and, depending on the temperature the degree of hydrolysis varies. (Espe and Lied,1999). Alternatively, enzymatic proteolysis can be employed to recover biomass from fish visceral mass and results in a soluble product generally referred to as fish protein hydrolysate (Guerard et al.,2002). The soluble hydrolysate is subjected to dehydration to obtain a powdered form with a high protein content that is stable (Diniz and Martin,1997). Enzymatic hydrolysis, unlike autolytic hydrolysis, offers better control of the process apart from hastening it and resulting in a product of reproducible quality (Liaset et al.,2000). Further, hydrolysis of proteins by enzymes allow the proteins into more soluble forms as peptides and amino acids thereby making the hydrolyzed mass the most available amino acid source (Espeet al.,1989 and Vidotti et al., 2003). Such hydrolysates prepared from low quality materials like visceral waste has potential for application as ingredients in aquaculture feeds (Vidotti et al.,2003 and Nilsang et al.,2005) and as source of nitrogen in microbial growth media (Dufosse et al.,1997 and Guerard et al.,2001).

The major lipid storage sites in fish vary depending on species, they are primarily located in the subcutaneous tissue, belly flap, muscle tissue, liver, mesentric tissue, and the head (Ackman 1994). Saturated fatty acids in fish lipids are dominated by palmitic and myristic acids followed by stearic acid, whereas the major monounsaturated fatty acids (MUFA) are oleic and palmitoleic acids (Kolakowska et al.,2002). Fish oils have been considered as important sources of omega-3 fatty acids (Gbogouri et al.,2006), especially DHA and eicosapentaenoic acid (EPA) which reduce the risk of coronary heart diseases (Bhaskar et al.,2006). Both EPA and DHA lower the blood pressure and prevent the development of hypertension (Prisco et al.,1998, Mori et al.,1999, Frenoux et al.,2001) which is one of the critical factors resulting in cardiovascular pathologies like atherosclerosis or stroke (Tapiero et al.,2002). These fatty acids are the major constituents of phospholipids, which in turn are major molecules in most biological membranes.

Lipids are major sources of metabolic energy throughout the embryonic developmental stages in fish (Sargent, 1995). The amount of lipids as well as the lipid classes catabolized vary among species (Cetta and Capuzzo, 1982; Tocher et al., 1985; Fraser et al., 1988; Falk-Petersen et al., 1989; Finn, 1994), and other sources of energy, such as carbohydrates and proteins, are also utilized. Needham (1931) was the first to suggest that there was a succession in the use of endogenous sources of energy for respiration during embryonic development, where in carbohydrate proceeds protein and protein proceeds lipid. A number of studies have supported this statement (Terrier, 1968; Kimata, 1982; Finn, 1994).

Changes in fatty acid composition in muscle of three farmed carp fish species (Labeo rohita, Cirrhinus mrigala, Catla catla) study has shown that the all three analysed fish are quite nutritious in terms of fatty acid composition especially long-chain PUFA, high level of protein and low level of fat. As a consequence, when human health is taken into account, the carp species i.e. L. rohita, C. mrigala, C. catla species could be consider desirable source in the human diet. The low level of fat and higher levels of PUFA may originate from the feed. Thus, total lipids contents and fatty acids proportions of fish greatly depend upon the same diet consumption ie, the list of ingredients used in experimental diet of fish was rice bran, fish meal, poultry biproduct meal, wheat flour, soybean meal and Egg yolk. (Memon et al. 2010).

The biochemical composition of two marine eels (Thyrsoidea macrura and Congresox talabanoides) has been studied. The proximate analysis exposed that the protein content of T. macrura and C. talabanoides was 22.7%, 25.8%, respectively, the total lipid content was high, ranging from 5.8% and 7.9%, the crude ash ranged from 6.9% and 7.1%. The major amino acids were glutamic acid, histidine and glycine ranging from (0.28 and 4.7%). The other dominant fatty acids were detected T. macrura and C. talabanoides. The level of Arachidonic acid in Conger eel was 2.31% and Moray eel was about 2.01. The high levels of EPA and DHA content of Moray and Conger eel makes its fatty acid profile more favorable. (Peninal et al.,2012).

The *Sardinella longiceps* and *Plotosus lineatus* have the different range of protein, carbohydrate and lipid content. Total of 20 amino acids which exhibit high levels of phenylalanine followed by lysine and Iso leucine based on the quantum of availability in the *P. lineatus*. In the present investigation the fatty acid concentration is the major element in both investigated fish. In this result, Palmitic acid, Magaric acid and Stearic acid are the major component in both samples. Consumption of fish and other marine products has always been a major factor in the economy and nutrition of the coastal inhabitants. (Suvitha et al.,2015).

1.2. Effect of insecticides

The effect of starvation on the biochemical parameters and viscera in the white sturgeon, *Acipenser transmontanus* was studied by Hung et al., (1997). All parameters are significantly affected by duration of starvation. Proportionally viscera lost more weight than carcass and lipid of the tissues showed higher reduction than protein. Thus viscera was more preferred over muscle and lipid was preferred nutrient over protein for mobilization in the white sturgeon which is starved (Hung et al., 1997).

The effect of starvation on biochemical composition of *Anabas testudineus* was studied by Konnur and Iragoud, (2015). There was a gradual increase in water content from zero day to 30th day of starvation. Whereas protein, lipid, and carbohydrate content gradually decreased (Konnur and Iragoud, 2015).

2. Materials and methods

- **Collection of fishes:**
Labeo rohita and *Anguilla bengalensis* were collected from a government Licensed seller.
- **Sample preparation:**
The fishes were sacrificed and the liver tissue was removed free from Blood vessels and connective tissues.
- **Estimation of protein content:**
Estimation of total protein contents of the liver in the fishes, *L.rohita* and *A.bengalensis* was measured by the following methodology of Lowry et al. 1951.

3. Procedure

- 500 mg of the liver tissue was homogenised in 10ml of distilled water.
- The homogenate was centrifuged for 15 minutes and 1ml of supernatant was collected in the test tube.
- Standard protein solution was prepared by dissolving 0.015gm of Bovine, Serum Albumin in 20 ml distilled water. From the standard protein solution different concentrations were made in different test tube (0.2, 0.4, 0.6, 0.8, 1ml). The volume is made up to 1ml with distilled water.
- To all the test tubes, 5ml of alkaline copper reagent was added. Alkaline copper reagent was prepared by adding 100ml of solution A and 2ml of solution A was discarded and 1ml of each solution B and solution C were added. Solution A was prepared by dissolving 2g of Calcium carbonate in 100ml distilled water. Solution B was prepared by dissolving 0.2g of copper sulphate in 10ml distilled water. Solution B was prepared by Dissolving 0.2g of sodium Potassium tartrate in 10ml distilled water.
- After 20 minutes 0.5ml of Folin's reagent was added. Folin's reagent was prepared by adding 10ml of Folin's reagent and 10ml of distilled water.
- After 15 minutes the optical density (O.D) was read at 660nm wavelength using Spectrophotometer.
- The calculations were made based on the data obtained.

- The amount of protein in the liver tissue is calculated using the formula

$$\text{Total Protein content in Liver} = \frac{\text{O.D. of sample}}{\text{O.D. of standard}} \times \frac{\text{Concentration of standard}}{\text{Weight of the tissue}} \times 1000$$

4. Estimation of cholesterol content

Estimation of total Cholesterol content of the liver was measured in the fishes, *L. rohita* and *A. bengalensis* by the following methodology of Peters and Vanslyke (1946).

- **Procedure:**

- 1) 200mg of tissue is homogenised in 10ml of Alcohol-ether mixture.
- 2) The homogenate was centrifuged for 10 minutes and the supernatant was collected in a test tube and dried by boiling till it completely evaporates.
- 3) To the residue 5ml of chloroform is added.
- 4) Standard was prepared by dissolving 0.015gm of Cholesterol in 20ml chloroform. From the standard Cholesterol solution different concentrations were prepared (0.2, 0.4, 0.6, 0.8, 1 ml). The volume is made up to 5ml with chloroform.
- 5) To all the test tubes, 2ml of Acetic anhydride mixture was added. Acetic anhydride mixture was prepared by adding 1ml of concentrated Sulphuric acid to 20ml of Acetic anhydride.
- 6) The test tubes were kept in dark for 15-20 minutes.
- 7) The O.D. is read at 660 nm wavelength using spectrophotometer.
- 8) The concentration of Cholesterol was calculated using the formula

$$\text{Concentration of Cholesterol in liver} = \frac{\text{O.D. of the sample}}{\text{O.D. of the standard}} \times \frac{\text{Concentration of standard}}{\text{Weight of the tissue}} \times 1000$$

5. Results

Table.1
Mean concentration of
fishes *A. bengalensis* and

Mean concentration of Cholesterol	Eel	Rohu
	1.123±0.291	0.220±0.022

Cholesterol in the two
L. rohita

Note: All values are expressed as Mean ± SE. The data was analyzed by student "t" test values with same superscript better are not significantly (p<0.05), different where as those with different superscript * are significantly (p<0.05) different as judged by student "t" test.

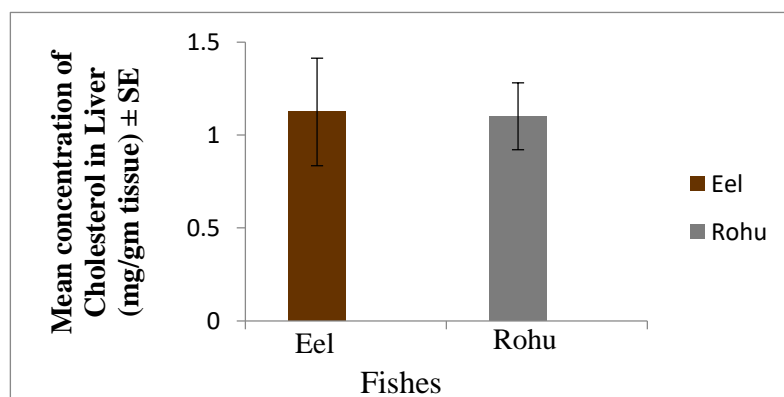


Fig. 1: Mean concentration of Cholesterol in the two fishes *A. bengalensis* and *L. rohita*

Note: All values are expressed as Mean ± SE. The data was analyzed by student "t" test values with same superscript better are not significantly (p<0.05), different where as those with different superscript * are significantly (p<0.05) different as judged by student "t" test.

Table.2
The mean concentration of protein in the fishes A. bengalensis and L. rohita.

Mean concentration of protein	Eel	Rohu
	0.793±0.116	1.298±0.199

of protein in the fishes A.

Note: All values are expressed as Mean ± SE. The data was analyzed by student "t" test values with same superscript are not significant (p<0.05), it differs such as the concentration of protein is little less in Eel compare to Rohu as judged by student "t" test.

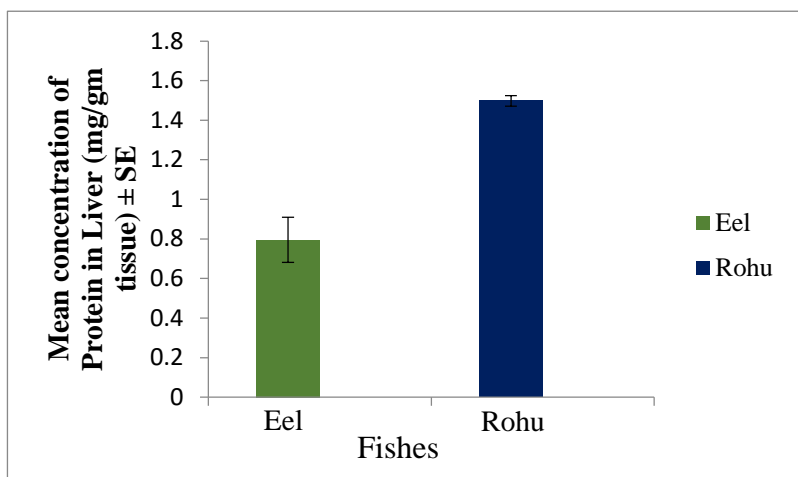


Fig.2: Mean concentration of Protein in the two fishes A. bengalensis and L. rohita

Note: All values are expressed as Mean ± SE. The data was analyzed by student "t" test values with same superscript are not significant (p<0.05), it differs such as the concentration of protein is little less in eel compare to rohu as judged by student "t" test.

6. Discussion

The two species of fresh water fishes were examined for protein and cholesterol content in liver sample, The values are shown in table 1,2, 3 and 4. Both were determined by taking optical density (O.D) in a spectroscopy using different methods as shown in materials and method. Cholesterol constitutes the vital organic substance playing an important role in energy metabolism.

According to (Shivaprasad Rao and Ramana Rao 1979), studied that considerable decrease in total lipids in tissue might be due to drastic decrease in glycogen content in the same tissue which is an immediate source of energy during toxic stress condition. (Ramana Rao and Ramamurthi 1980), studied that the increase in activity of enzymes lipase is for increasing lypolytic activity to meet the increased demand of energy during stress. Amudha et al. (2002) studied the Dairy effluent induced alterations in the protein, carbohydrate and lipid metabolism of a freshwater teleost fish. After glycogen, lipid content may be used for energy production to overcome toxic stress condition. Some workers support the results in which lipid content decreases. (Copuzzo and Lanacaster 1981), reported significant decrease in lipid of post larval lobster when exposed to pollutants. A decrease in total lipid content in tissue reported by (Ram and

Sathyanesan 1984) on *Channa punctatus* intoxicated with mercuric chloride. The loss of the lipid may be a consequence of inhibition of lipid synthesis and mobilization of stored lipids, (Mani and Saxena 1985).

During the stress organisms need sufficient energy which is supplied from reserve material (glycogen, lipid and protein), if the stress is mild then only stored glycogen is used as a source of energy, but when stress is strong then the energy stored in lipids and protein may be used. The heavy metals cause metabolic rearrangement in the living system. Due to their potential toxicity, biochemical changes occur in the organs of animals. (Ram and Sathyanesan, 1984). The toxicity stress which suppresses the activity of a number of enzymes responsible for lipid transformation ultimately causing disturbances in lipid metabolism and leads decreased value of cholesterol (Ganeshwade, 2012). The alteration in cholesterol content may be due to its utilization in corticosteroidogenesis and also impairment in the synthesis of cholesterol (Vasanthi et al., 2013). The depletion in cholesterol was increased as the period of exposure increased. The maximum depletion occurred in the liver followed by gonads.

In the present investigation the cholesterol content of *L.rohita* is little less compare to Eel because it is very active and utilize more energy for different physical and physiological processes. Decrease in cholesterol level in the liver is due to an increase in the lipid utilization to meet additional energy requirements under stress (Shelke Ahay D.,2013). Cholesterol content is altered in *L. rohita* after exposure to an insecticide polo (Savitha.Y Waghmare and G.P. wani 2014) so during stress condition cholesterol is less in liver tissue.

The protein content is less in eel compare to rohu because the physiological processes is slow because the activity of the fish is slow. Here rohu is very active and the physiological processes also very fast so it needs sufficient amount of diet containing protein for their growth and development.

Conclusion

The protein and cholesterol are the essential biochemical components required for the growth, development and leading normal physiological processes. Here we conclude that the results were obtained from the biochemical estimations between the liver of two fresh water fishes eel and rohu is significant. The protein content is more in rohu compare to eel because it is very active swimmer and the rate of physiological processes is high. The cholesterol content in is more in eel fish compare to rohu because it is a bottom dweller, getting sufficient amount of food, and it is less active so its physiological processes also slow.

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