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BIOCHEMICAL ANALYSIS OF *Labeo rohita* and *Threadfin bream* FISH

G. Vimala and M. Thangadurai*

G. Vimala
PG & Research Department of Zoology Rajah Serfoji Govt. College (Autonomous) Thanjavur - 613 005.

***Dr. M. Thangadurai**
Assistant professor, PG & Research Department of Zoology Rajah Serfoji Govt. College (Autonomous) Thanjavur - 613 005.

ABSTRACT

Fish is very important dietary animal protein source in human nutrition. Production of aquatic species through freshwater fisheries and aquaculture for protein supply is being encouraged in developed or developing countries but in under- developed countries, it is declining. The current examination on the nutritional profile of *Threadfin bream* and *Labeo rohita* bringing to our attention the richness of healthy nutrients present in the eatable portion such as muscle. The protein and amino acid content was higher in *Threadfin bream* as compared to *Labeo rohita*. The carbohydrate content was lower in *Labeo rohita* as compared to fresh water fish *Threadfin bream.* The lipid content was lower in *Threadfin bream* as compared to fresh water fish *Labeo rohita*. Among the two fishes, *Threadfin bream* has rich nutrients as compared with fresh water fish *Labeo rohita*. In *Threadfin bream* and *Labeo rohita* shows the presence of calcium, magnesium, potassium, sulphate, iron, sodium, phosphate, nitrate and chloride were presented.

Keywords: Biochemical analysis, *Labeo rohita, Threadfin bream*

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INTRODUCTION

India is the third largest producer of fish in the world next only to China and Peru and it ranks second in the production of Inland fishes. Fish production has increased from 0.75 million tons in 1950 to 6.90 million tons in 2006-2007, registering a compound growth rate of 4.53% per annum which has been the fastest growing one in respect of any item in the food sector. The fisheries sector contributes Rs. 19,555 cores to national income which is 1.4% of the GDP and 4.7% of the agricultural GDP. Out of total Indian exports, the share of export is 3.32%. The distribution however is that it is the 3rd largest contributor to the net foreign exchange earned by the country. This sector accounts for 13.95% of total exports of the Indian economy. Fishery sector, besides contributing towards nutritional security component of the food basket of India, is recognised for providing livelihood and employment to millions of people.

Nutrient content varies with fish species and depending on the health status of the fish. There are limited data on the nutritional composition of fish species which are commonly consumed by the poor in developing countries of Asia and sub-Saharan Africa. Therefore, the crust of this study was to evaluate the nutritional content in *Labeo rohita* and *Threadfin bream.*

MATERIALS AND METHODS

Collection of fish

Labeo rohita and *Threadfin bream* were collected from Keelavasal fish marker, Thanjavur. The fish was washed with saline and used for experimental work.

Preparation of homogenate

The *Labeo rohita* and *Threadfin bream* were sacrificed and flesh was dissected out, washed with ice-cold physiological saline. The 1g tissues was weighed and homogenized using a Teflon homogenizer. Tissue homogenate was prepared in 0.1 M Tris Hcl buffer (pH 7.4) and used for the estimation of various biochemical parameters.

Biochemical estimations

Estimation of Total protein:

Protein was estimated by the method of Lowry *et al* . (1951).

Estimation of total lipids:

Total lipids in tissues were estimated by the method of Folch *et al* (1957).

Determination of Carbohydrate by Anthrone Method

To estimate the amount of carbohydrate present in the given sample by using Anthrone method

Estimation of Amino acids (Ninhydrin method)

Amino acid in tissues were estimated by the method of Rosen (1957).

Qualitative analysis of Inorganic elements

Fish (500mg) was prepared and treated with HNO_3 and HCl (3:1 v/v) for 1 hour. After the filtration, the filtrate was used to perform the following tests (Khandelwal 2006).

Statistical analysis

The results were presented as mean ± SD. Data was statistically analyzed using student "t" test. P. values set as lower than 0.05 was considered as statistically significant.

Qualitative elements analysis in *Labeo rohita* and *Threadfin bream*

The following elements were found in *Labeo rohita* and *Threadfin bream*. In *Labeo rohita* shows the presence of calcium, phosphate, sodium, magnesium, potassium, sulphate, nitrate, iron and chloride were presented. In *Threadfin bream* shows the presence of calcium, phosphate, sodium, magnesium, potassium, sulphate, nitrate, iron and chloride were presented. (Table 2).

RESULTS

The present study was carried out to analyze the various biochemical parameters in *Labeo rohita* and *Threadfin bream*. The observations made on different fishes were compared as follows.

Table I - Shows the levels of Carbohydrate in *Labeo rohita* and *Threadfin bream*. Carbohydrate was decreased in *Threadfin bream* when compared to *Labeo rohita.* Protein, amino acid and lipids content in was decreased in *Threadfin bream* when compared to *Labeo rohita.*

Fish	Carbohydrate (mg/gm)	Protein (mg/gm)	Lipids (mg/gm)	Amino acids (mg/gm)
Labeo rohita	107.64±3.72	9.00±0.04	116.66±10.80	30.00±20
Threadfin bream	77.30±4.49	319.50±29.19	70±0.05	15.05±7.23

Values were expressed as mean ± SD.

* Significantly different from *Catla catla* (P< 0.05)

Table 2: Qualitative analysis of inorganic elements in *Labeo rohita* and *Threadfin bream*

S.No	Elements	*Labeo rohita*	*Threadfin bream*
1.	Calcium	+	+
2.	Magnesium	+	+
3.	Sodium	+	+
4.	Potassium	+	+
5.	Iron	+	+
6.	Sulphate	+	+
7.	Phosphate	++	+
8.	Chloride	+	+
9.	Nitrate	+	+

Note: (+) Presence

DISCUSSION

Biochemical studies are very important from the nutritional point of view. Protein is essential for the sustenance of life and accordingly exists in the largest quantity of all nutrients as a component of the human body (Sudhakar *et al* ., 2011). In various fish species, proteins are of important as structural compounds, biocatalysts and hormones for control of growth and differentiations (Amal and Naheb, 2012). Protein in fish is a main component constituent of tissue and organs. They are precursors of other nitrogen compounds (enzymes, hormones, slurry, neurotransmitters, cofactors, etc) and constitute an important energy source. The effect of dietary lipid levels on fish growth performance varies considerably within species, size, age, diet and composition, range of lipids level tested and rearing conditions (Arredondo *et al.*, 2012). Inadequate protein levels in the diets result in a reduction of growth and loss of weight. However, when an excess of protein is supplied in the diet, only part of it is used for protein synthesis (growth) and the remaining is transformed into energy (Arredondo *et al* ., 2012). Each body cell is composed mainly of protein. Protein makes up the membrane surrounding the cell and also occurs within the cell. During growth period, adolescence and pregnancy, the number of cell increases and more protein is required for cell growth. In all stages of life tissue protein is constantly being broken down and must be replaced by dietary protein. Protein plays a vital role in the formation of enzymes, antibodies and hormones and other substances that regulate the body process.

Protein and Amino acid

Muscle rich in proteins, forms mechanical tissue intended for mobility and do not participate in metabolism. Liver being the centre for various metabolisms is also rich inproteins (Agusa *et al* ., 2007). Fish and shellfish are important source of protein and income for people in Southeastern Asia They are also increasingly marketed for the health benefits to consumers (Schmidt *et al* ., 2006). The requirement of nitrogen and sulphur is regulated by dietary protein. The protein immunoglobins act as prime defense against bacterial and viral infections. Proteins by means of exerting osmotic pressure help in maintenance of electrolyte and water balance in human system. Several studies show that protein derived from fish, balances many body regulatory factors. It is well known that protein is the most important and expensive item that should be supplied in adequate amounts to support good growth with minimal cost (Wec *et al* ., 1982: Zehra *et al* ., 2011; Nurnadia *et al* ., 2011) determined the proximate composition and energetic values of selected marine fish and shellfish from West Coast of Penisular Malaysia. This study has included *Labeo rohita* and *Chenna striata*. The study revealed that *Catla catla* contained high protein content. Long-tailed butterfly ray contained the highest protein According to the work done by Anbuchezhian *et al* . (2012) in Catfish it is clear that antimicrobial proteins and peptides play key role in innate immunity and they had been observed from a wide variety of organisms in last few years. Hence, the fishes rich in protein will produce more innate immunity. *Labeo rohita* would be more useful in developing innate immunity.

Lipids

Cholesterol is undoubtedly the most publicized lipid in nature, because of the strong correlation between high levels of cholesterol in the blood and the incidence of disease of the cardiovascular system in humans. Usually, the cholesterol content will be more in fish liver oils but in the present investigation the consumable part of fish, muscle and brain were found to contain cholesterol. It is the essential constituent of cells. It aids in the permeability of the cells. It controls the red cells from being easily homolyzed. It functions as the defensive action and transports fat to liver in the form of cholesterol ester for oxidation. It assists the formation of bile acids and bile salts, 7-dehydrocholestrol and vitamin D3, corticosteroid hormone, androgens, estrogens and progesterone.

Cholesterol helps the granulation of cell division and acts as an antagonist to phospholipids. High Density Lipoprotein (HDL) transports cholesterol and its esters from peripheral tissues to the liver for its catabolism (scavenging action). Very Low Density Lipoprotein (VLDL) transports mainly endogenous triglycerides synthesized in hepatic cells from the liver to the extra-hepatic tissue including adipose tissue for storage. Low Density Lipoprotein (LDL) regulates cholesterol synthesis in extra-hepatic tissue. The triglycerides are the most abundant of all lipids. They constitute about 98% of total dietary lipids, the remaining 2% consists of phospholipids and cholesterol and its ester. They are major components of storage or

depot fats in animal cells but not normally found in membranes. Triglycerides can be stored in quantities, sufficient to supply the energy needs of the body for many months as in the case of obese person. They are not only stored for longer duration but also yield over twice as much energy as carbohydrates. Lipids and fatty acids play a significant role in membrane and have a direct impact on membrane mediated process such as osmoregulation, nutrient assimilation and transport. On the other hand, the nature and quantity of these lipids in fish vary according to species and habit. (Kumaran *et al* ., 2012). Previous studies correlate with our present investigation pertaining to lipid observations.

Carbohydrate

Components like carbohydrate play a vital role as energy precursors for fish under stress conditions (Umminger, 1970). Glucose is a carbohydrate that has a major role in the bioenergetics of animals, being transformed to chemical energy (ATP), which in turn can be expressed as mechanical energy (Lucas, 1996). Changes in carbohydrate metabolism measured as plasma glucose (energy substrate whose production is thought to metabolically assist the animal to cope with an increased energy demand caused by stress) used as general stress indicators in fish (Teles *et al*., 2007). Glucose (or glucose 6-phosphate) is released through the degradation of glycogen by glycogen phosphorylase (GP) (Roach *et al*., 1998), and energy is mainly supplied by the oxidation of glucose and lactate as a result of carbohydrate metabolism (Morgan *et al* ., 1997). The glucose concentration was proposed to be mediated by endocrine release such as cortisol (Hontela *et al* ., 1996). Silbergeld (1974) stated that assay of this important blood parameter can serve as an indicator of environmental stress. In the present study decreased the carbohydrate content in *Threadfin bream* as compared to *Labeo rohita.*

Elements in *Labeo rohita* and *Threadfin bream* fish

The elements are separate entities from the other essential nutrients like proteins, fats, carbohydrates, and vitamins. Animal husbandry had demonstrated the need for minerals in the diet (Hegsted *et al* ., 1976). In this century, biological assay methods clarified the significance and importance of mineral elements for human and animal nutrition and modern analytical techniques led to the detection of trace elements as essential nutrients and this is still an active area of current research. Micronutrient deficiencies are a major public health problem in many developing countries, with infants and pregnant women especially at risk (Batra and Seth, 2002). Infants deserve extra concern because they need adequate micronutrients to maintain normal growth and development (Rush, 2000). The micronutrient deficiencies which are of greatest public health significance are iron deficiency, causing varying degrees of impairment in cognitive performance, lowered work capacity, lowered immunity to infections, pregnancy complications e.g. babies with low birth weight, poor learning capacity and reduced psychomotor skills (Batra and Seth, 2002). In the present study all the elements present in *Labeo rohita* as compared to fresh water fish.

Minerals are inorganic substances, present in all body tissues and fluids and their presence is necessary for the maintenance of certain physicochemical processes which are essential to life. Minerals are chemical constituents used by the body in many ways. Although they yield no energy, they have important roles to play in many activities in the body (Eruvbetine, 2003). Every form of living matter requires these inorganic elements or minerals for their normal life processes (Ozcan, 2003). Minerals may be broadly classified as macro (major) or micro (trace) elements. The third category is the ultra trace elements. The macro-minerals include calcium, phosphorus, sodium and chloride, while the micro-elements include iron, copper, cobalt, potassium, magnesium, iodine, zinc, manganese, molybdenum, fluoride, chromium, selenium and sulfur (Eruvbetine, 2003). The macro-minerals are required in amounts greater than 100 mg/dl and the micro-minerals are required in amounts less than 100 mg/dl (Murray *et al* ., 2000). The ultra trace elements include boron, silicon, arsenic and nickel which have been found in animals and are believed to be essential for these animals. Evidence for requirements and essentialness of others like cadmium, lead, tin, lithium and vanadium is weak (Albion Research Notes, 1996).

Calcium (Ca)

Calcium functions as a constituent of bones and teeth, regulation of nerve and muscle function. In blood coagulation, calcium activates the conversion of prothrombin to thrombin and also takes part in milk clotting. It plays a vital role in enzyme activation. Calcium activates large number of enzymes such as adenosine triphosphatase (ATPase), succinic dehydrogenase, lipase etc. It is also required for membrane permeability, involved in muscle contraction, normal transmission of nerve impulses and in neuromuscular excitability. A reduced extracellular blood calcium increases the irritability of nerve tissue, and very low levels may cause spontaneous discharges of nerve impulses leading to tetany and convulsions (Hays and Swenson, 1985; Malhotra, 1998; Murray *et al* ., 2000).

Phosphorus (P)

Phosphorus is located in every cell of the body and is vitally concerned with many metabolic processes, including those involving the buffers in body fluids (Hays and Swenson, 1985). It functions as a constituent of bones, teeth, adenosine triphosphate (ATP), phosphorylated metabolic intermediates and

nucleic acids. It serves buffering action, that is, phosphate buffers, functions in the formation of high energy compounds, that is, adenosine triphosphate (ATP) and is involved in the synthesis of phospholipids and phosphoproteins. Decrease in serum phosphorus is found in rickets, hyperparathyroidism, De Toni-Fanconi Syndrome. Deficiency disease or symptoms in children causes rickets and in adults, it causes osteomalacia. Increase in serum phosphorus is found in chronic nephritis and hypoparathyroidism.

Sodium (Na)

Sodium is the principal cation in extracellular fluids. It regulates plasma volume and acid-base balance, involved in the maintenance of osmotic pressure of the body fluids, preserves normal irritability of muscles and cell permeability, activates nerve and muscle function and involved in Na+/K+-ATPase, maintenance of membrane potentials, transmission of nerve impulses and the absorptive processes of monosaccharides, amino acids, pyrimidines, and bile salts. The changes in osmotic pressure are largely dependent on sodium concentration (Hays and Swenson, 1985; Malhotra, 1998; Murray *et al* ., 2000). Sodium deficiency in young chicks cause growth retardation. Egg production and hatchability in laying chickens are depressed (Merck, 1986). Increased level of sodium in the serum is called hypernatraemia and this occurs in Cushion's disease, administration of adrenocorticotropic hormone (ACTH), administration of sex hormones, diabetes insipidous and after active sweating (Malhotra, 1998).

Potassium (K)

Potassium is the principal cation in intracellular fluid and functions in acid-base balance, regulation of osmotic pressure, conduction of nerve impulse, muscle contraction particularly the cardiac muscle, cell membrane function and Na+/K+-ATPase. Potassium is also required during glycogenesis. It also helps in the transfer of phosphate from ATP to pyruvic acid and probably has a role in many other basic cellular enzymatic reactions. Its metabolism is regulated by aldosterone. Hyperkalaemia is increased level in serum potassium and this occurs in Addison's disease, advanced chronic renal failure, shock and dehydration. Deficiency disease or symptoms occurs secondary to illness, functional and structural abnormalities including impaired neuromuscular functions of skeletal, smooth, and cardiac muscle, muscular weakness, paralysis, mental confusion (Hays and Swenson, 1985; Malhotra, 1998; Murray *et al* ., 2000). Others are cardiac arrythmias, impaired carbohydrate tolerance, altered electrocardiogram in calves. Potassium deficiency affects the collecting tubules of the kidney, resulting in the inability to concentrate urine, and also causes alterations of gastric secretions and intestinal motility (Streeten and Williams, 1952). The rapidly growing animals apparently have a higher requirement for potassium, and increasing the protein level increases the requirement. Plant products contain many times as much potassium as sodium. Sources include vegetables, fruits, nuts.

Chloride (Cl_2)

Chloride is the principal anion in extracellular fluid. It is involved in the regulation of extracellular osmotic pressure and makes up over 60% of the anions in this fluid compartment and is thus important in acid base balance. The concentration of chloride ion is subject to more variation than that of sodium, since other anions, especially bicarbonates, can exchange for the chloride. It is the chief anion of the gastric juice and is accompanied by the hydrogen ions in nearly equal amounts. The chloride of the gastric secretions is derived from blood chloride and is normally reabsorbed during the latter stages of digestion in the lower intestine (Hays and Swenson, 1985; Murray *et al* ., 2000).

Magnesium (Mg)

Magnesium is an active component of several enzyme systems in which thymine pyrophosphate is a cofactor. Oxidative phosphorylation is greatly reduced in the absence of magnesium. Mg is also an essential activator for the phosphate-transferring enzymes myokinase, diphophopyridinenucleotide kinase, and creatine kinase. It also activates pyruvic acid carboxylase, pyruvic acid oxidase, and the condensing enzyme for the reactions in the citric acid cycle. It is also a constituent of bones, teeth, enzyme cofactor, (kinases, etc) (Murray *et al* ., (2000). The health status of the digestive system and the kidneys significantly influence magnesium status. Magnesium is absorbed in the intestines and then transported through the blood to cells and tissues. Approximately one-third to one-half of dietary magnesium is absorbed into the body. Gastrointestinal disorders that impair absorption such as Crohn's disease can limit the body's ability to absorb magnesium. These disorders can deplete the body's stores of magnesium and in extreme cases may result in magnesium deficiency. When a magnesium-deficient diet is fed to young chicks, it leads to poor growth and feathering, decreased muscle tone, ataxia, progressive incoordination and convulsions followed by death (Merck, 1986).

Sulphate

Sulphate contributes to numerous physiological processes in mammalian physiology, particularly during development. Sulphotransferases mediate the sulphate conjugation (sulphonation) of numerous compounds, including steroids, glycosaminoglycans, proteins, neurotransmitters and xenobiotics, transforming their biological activities. Importantly, the ratio of sulphonated to unconjugated

molecules plays a significant physiological role in many of the molecular events that regulate mammalian growth and development. In humans, the fetus is unable to generate its own sulphate and therefore relies on sulphate being supplied from maternal circulation via the placenta (Dawson, 2011). To meet the gestational needs of the growing fetus, maternal blood sulphate concentrations double from mid-gestation. Maternal hyposulphataemia has been linked to fetal sulphate deficiency and late gestational fetal loss in mice. Disorders of sulphonation have also been linked to a number of developmental disorders in humans, including skeletal dysplasias and premature adrenarche. Whilst recognised as an important nutrient in mammalian physiology, sulphate is largely unappreciated in clinical settings (Daniel Markovich, 2001; Florin *et al* ., 1991).

CONCLUSION

Over all, the study concludes that locally obtainable *Threadfin bream* fish has rich nutrition which may be use growth and development and can be a substantial aid in redressing the problems of malnutrition, diabetic and cardiovascular patients in our country.

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