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Investigation of different biochemical parameters in three economical important fish species captured from ponds of district Kashmore, Sindh, Pakistan

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Abstract

The aim of present study was to evaluate the biochemical composition in gills, liver and muscle of different fish species like *Catla catla, Cirrhinus mrigala* and *Labeo rohita* of different weight captured from ponds of Kashmore district. In this regard valuable biochemical factors were evaluated such as crude protein, % of fat and % of ash and also compared the outcomes with concentration found in samples of Indus River. Fish samples were caught with help of professional fishermen by using different size of fish nets. The samples of all three species *Catla catla, Cirrhinus mrigala* and *Labeo rohita* of four different weights were collected i.e. 250g, 500g, 750g and 1000g respectively were dissected with stainless steel cutters and required amount of specimens were extracted from fish samples. Further analytical processes were carried out in zoology laboratory for completion of possible objectives. The results revealed as protein contents, % of fat and % ash were found significantly high in *Catla catla* fish as compared to *Cirrhinus mrigala* and *Labeo rohita*. The concentration of biochemical parameters were found in greater quantity in gills of all fish species which were subjected in this research. Furthermore the comparative studies showed that the concentrations of all biochemical parameters were found high as compared to Samples of Indus River at Kashmore bank.

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Introduction

Fish is known to be one of the cheapest sources of animal protein and other essential nutrients required in human diets. The nature and quality of nutrients in most animals is dependent upon their food type. Also feeding habit of an individual fish species has great effect on its body nutrients composition (Ali Muhammad Yousafzai. 2010). The importance of fish in developing countries increased greatly after the which greatly decimated the cattle population, made the price of livestock virtually prohibitive, a trend which triggered a corresponding increase in the demand for fish which then is the main alternative animal protein source submitted that, fish is a highly protein food consumed by a larger percentage of populace because of its availability and palatability (Chattopadhyay B. 2002).

The food value of fish has been recognized all over the world. Proteins have a key role in human diet for proper growth and other vital activities. Fish is regarded as an excellent source of protein for human diet (Canli M and Atli G. 2003). As compared to other sources of animal protein, the fish provides highly digestible protein which has also much growth promoting value for humans (Indrajit Sen1, Ajay Shandil1. 2011).

The study of mineral elements present in living organisms is of biological importance; since many of such elements take part in some metabolic processes and are known to be indispensable to all living things (Jezierska. 2001). Present study was design to analysis the mineral contents in the commercially important fishes are *Labeo rohita* and *Cirrhinus mrigala* and *Catla catla* found in ponds of kashmore district.

So the knowledge of the proximate analyses of important carp fish species is desirable due to recent dietary and medical emphasis. Proximate body composition is analysis of moisture, crude fat, crude protein and ash and minerals contents of fish. Percentage of moisture content in fish muscles is a good indicator of its relative contents of energy, crude proteins and fat. Lower the percentage of moisture, greater the crude fat and protein contents higher the energy density of the fish meat although intake of trace and essential elements through food chain has significant role on human health. Similarly the real importance of fish in human diet is for normal growth where they reduce cholesterol levels and the incidence of heart disease, stroke, and preterm delivery.

Main objective of this study are to investigate the proximate composition of major biochemical constituents such as moisture, protein, fat and ash from different parts viz. Gill, liver and muscles of selected fish species available in ponds of Kashmore.

Materials and methods

Description of Study Area

District Kashmore is present in northern area of Sindh and its lies with Ghotki, Jacobabad, Shikarpur and Sukkur within Sindh. District is connected with the borders of Baluchistan on one side and another side with Punjab province. The latitude of Kashmore, Pakistan is 28° 25' 58.78 92" N and the longitude is 69° 35' 1.35 60" E.River Indus flowing therefore along with the Eastern side of Kashmore. Major source of irrigation is through the Guddu barrage and there is excess availability of water in this area due to this fish is to be considered as a cash product of this area.

Fig. 1. Map and site of sampling area.

Sample Collection and Preservation

120 total samples of economically important fishes were obtained through professional fishermen by using different size of fish nets from Chachar fish ponds. Fresh fish samples of three selected verities of fish species *Catla catla, Cirrhinus mrigala and Labeo rohita* of variable weights i-e 250g, 500g, 750g and 1000g were studied. Fresh fish samples were immediately stored in an ice box (at 4°C) and shifted

to the laboratory for further procedures, such as dissection, digestion and analysis. The different specimens of fish samples were obtained by dissection with sterilized stainless steel cutter equipments. Required grams of muscle, gills and liver specimen's samples from three varieties of fishes were accurately weighed by electronic balance.

Dissection and Digestion of fish's samples

Fresh fish samples were weighed with the help of electronic balance before dissection and extracted specimens were completely dried at 100°C until and unless constant weight was obtained.

Preparation of Biuret reagent

It is prepared with 10% dissolved sodium hydro-oxide and hydrated copper sulfate by adding potassium sodium tartrate and total volume made up to 1 liter. The reaction takes places with the change of peptide hydrogen atom in the alkaline conditions. Actually there is tri or tetra dentate with the peptide nitrogen which is responsible to produce the biuret color. This reagent was used to estimate the concentration of proteins due to occurrence of peptide bonds with the same frequency per amino acid in the peptide linkage. The reagent is mainly used in the determination of protein essay, a complex of colorimetric is formed and that is helpful for determination of protein contents by using UV spectroscopy at range of 540nm.

Determination of Crude Protein contents

All collected fish samples were dried and grinded using grinder machine in Lab. Accurately 01g of dried sample was weighed with electronic balance machine and mixed with 100 ml of de- ionized water. A series of standard were run with for obtaining a linear calibration curve. Blank reagents were made up by using 4ml of biuret reagent + 1ml of de-ionized water. The samples were filtered by using Whatman # 42 filter paper and 01 ml of sample was pipette out and mixed with 4ml of Biuret reagent. After 20 minutes the absorbance was measured at 540nm by using double beam UV-Visible spectrophotometer in Chemistry laboratory.

Method Determination of % Fat

Crude fat content of sample was determined by using solvent extraction technique with petroleum ether (B.P=40-60 0C) by using Soxtec. Briefly 1-5gm of dried fined powdered sample was placed in Whatman Thimble and defatted cotton is plugged on the top of the thimbles. These thimbles then put into the thimble holder and placed inside the machine i.e. attached with condenser. The aluminum made extraction cups were first dried and weighed. Then 60-70ml of petroleum ether was added and finally attached with thimbles already placed inside the machine. After full programming the extraction process was get start and then completed the whole extraction process, the equipment was display a message that extraction is now complete. Then the extraction cup containing fat content was removed from the extraction unit and placed in digital oven for about 60 minutes at 50-60 0C for the complete evaporation of petroleum ether, later on the aluminum cups containing samples will be placed in desiccators for complete coolness and finally the weight will be taken.

The total fat will be calculated by using following formulae:

Weight of fat (g)

$$(\%)\ \text{fat} = \frac{\text{Weight of fat (g)}}{\text{Weight of sample (g)}} \times (100)$$

Total conc: of extraction cup with fat- weight of empty extraction cup.

Method for Determination % of Ash

The empty crucibles were placed in oven 300°C overnight to ensure that impurities on the surface of crucible are burned off. The crucibles were kept in the desiccators (30 min) for cooling. The 01 g of each organ of fish varieties were weight with crucible and heated at 300°C overnight. After complete heating the samples were cooled down in the desiccators. The obtained weight was noted for both types of samples ash with crucible.

Instrumentation

UV-Visible spectroscopy

As for as National and International literature is concerned there were many methods which were reported and employed to estimate the concentration of protein contents which were mainly depends on

UV-visible spectroscopy. These techniques were employed depending on natural ability of proteins to absorb or physically modification of proteins to make them absorb in this region. Basically main principles behind each of these tests were always unique. Before this the calibration curves of absorbance vs protein conc: was made by using a series of protein solutions of known concentration.

Results and discussion

Crude Protein

Table 1. Average concentration of Crude protein in different species of fishes.

	C. catla			*C. mrigala*			*L. rohita*		
Specimens	Gills	Liver	Muscle	Gills	Liver	Muscle	Gills	Liver	Muscle
Average µg/g	4.2	1.3	2.4	2.5	1.2	1.8	2.1	1.3	2.2
STDV	0.08	0.02	0.04	0.04	0.02	0.04	0.02	0.02	0.02
% RSTD	2	1.4	1	1.3	1.2	1	1	1.2	1.2

Fat Concentration

Table 2. Average percentage of fat in different species of fishes.

	C. catla			*C. mrigala*			*L. rohita*		
Specimens	Gills	Liver	Muscle	Gills	Liver	Muscle	Gills	Liver	Muscle
Average (%)	0.21	0.24	0.41	0.21	0.18	0.23	0.24	0.19	0.23
STDV	0.002	0.002	0.003	0.002	0.002	0.002	0.003	0.002	0.003
% RSTD	0.04	0.04	0.05	0.03	0.04	0.04	0.05	0.04	0.04

Ash Determination

Table 3. Average percentage of ash in different species of fishes.

	C. catla			*C. mrigala*			*L. rohita*		
Specimens	Gills	Liver	Muscle	Gills	Liver	Muscle	Gills	Liver	Muscle
Average (%)	16	7.4	12	14.2	6.7	13	16	6.5	12
STDV	0.8	0.6	0.8	0.8	0.4	0.6	0.8	0.5	0.7
% RSTD	0.05	0.03	0.05	0.05	0.03	0.04	0.05	0.02	

Comparative Fig. Of Pond Fishes

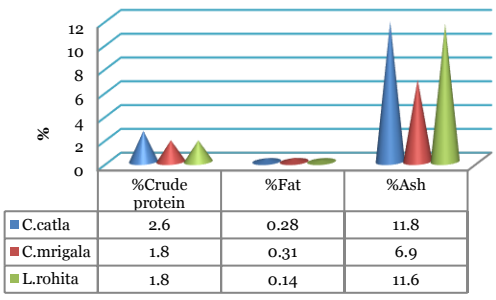

Fig. 2. Comparative fig. of different biochemical factors of three different fish species.

Comparative Fig. Of Indus River Fishes

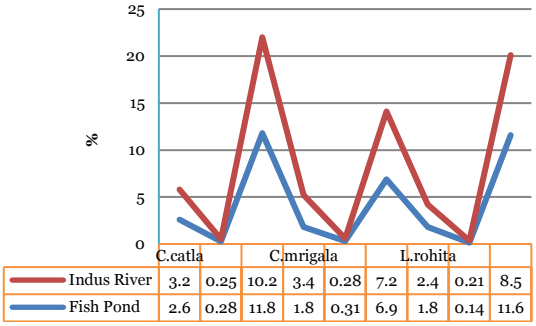

Fig. 3. Comparative values of biochemical parameters with Indus River fish species.

Crude Protein Contents

Table-01 Determines the average level of Crude protein concentration in gills, liver and muscle of three economically important fish species i.e. *Catla catla, Cirrhinus mrigala* and *Labeo rohita* of four different weighs 250g, 500g, 750g and 1000g respectively. The highest amount of protein contents (4.2µg/g) were found in gills of *Catla catla* fish species and in *C. mrigala* gills it was noted as (2.5µg/g).However the least amount was noted among all species as (1.3 µg/g) in gills of *L. rohita* fish samples respectively. The average concentrations of protein contents were found high (2.4µg/g) in muscle of *C. catla* as compare to other collected species muscles. However the liver of all three species contained lowest amount of crude protein as compared to other investigated parts of fish samples. In addition the proteins, peptides and amino acids from fish became more recently known for having positive health effects It's easy to understand the excitement. Protein is an important component of every cell in the body. Hair and nails are mostly made of protein. Your body uses protein to build and repair tissues. Protein makes enzymes, hormones, and other body chemicals. Protein is an important building block of bones, muscles, cartilage, skin, and blood.

Fat Contents

Table-02 Shows that the highest average percentage of fat (0.41%) in muscle of *Catla catla* fish species as compared to other organs of different fish species and lowest average percentage was noted in muscle (0.23%) of rest of species which were evaluated in our study. Similarly percentage of fat in liver of all

respective species was found in lowest concentration as compare to other biochemical parameters. Fish oil supplements have been promoted as easy way to protect the heart, ease inflammation, improve mental health, and lengthen life. Omega-3 fish oil contains both doco-sahexaenoic acid (DHA) and eico-sapentaenoic acid (EPA). Omega-3 fatty acids are essential nutrients that are important in preventing and managing heart disease.

Ash Contents

Table-03 Indicates that the maximum average percentage of ash was found (16%) in gills of both *Catla catla* and *Cirrhinus mrigla* fish species that were captured from fish ponds of kashmore district. Although the lowest average percentage was noted (6.7%) in liver of *Cirrhinus marigala* fish species. The gills are the good sites for metal uptake before entering other parts of the organisms. Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food .High fiber and ash which reduces digestibility may indicate there will be more fish waste in the water.

Comparison of different Bio chemical factors evaluated from fish pond fishes

Fig-02 Shows the average comparative concentration of different biochemical parameters among three economically important fish species. The highest concentration of ash was noted in *Catla catla* and maximum average concentration of fat was obtained in *Cirrhinus marigala*. Similarly the highest percentage of crude protein was noted in *Catla catla* fish species.

Comparison of different Bio chemical factors evaluated from Indus River fishes

Fig-03 Shows the comparative graph of three economically fish captured from Indus River Kashmore with fishes obtained from Ponds of kashmore district. The comparative graph shows that the highest level of all biochemical parameters which were studied found high in different organs of collected fish species captured from Indus River as compared to Fish pond fish species.

Conclusion

Bioaccumulation factor among different organs of all three fish varieties indicated that high accumulation of elements was seen in gills as compared to liver and muscle. Comparative study of Indus river fishes were exploited it indicates that almost all biochemical parameters were found as high concentrations in Indus River fish samples as compared to Pond fishes captured from kashmore district. The Protein contents, % of fat, and % ash were found significantly low in *C. catla* fish as compared to *C. mrigala* and *L. rohita*. It was further concluded that level of all parameters which were under studied significantly below than RDA as proposed by FAO and WHO. Therefore consumption of these fish varieties are to be considered as safe for people belongs to present study area.

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Conflicts

The authors have not declared any conflict of interests

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