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Biochemical and Nutritional Nvestigations of Bangladeshi Fresh Water Fish Puntius Gonionotus

Dutta, Prodip Kumar

University of Rajshahi

BIOCHEMICAL AND NUTRITIONAL NVESTIGATIONS OF BANGLADESHI FRESH WATER FISH *PUNTIUS GONIONOTUS*

Ph.D THESIS

A Dissertation

Submitted to the University of Rajshahi for the Fulfilment of the Requirements for Degree of Ph.D in Applied Chemistry and Chemical Engineering.

Submitted by
Prodip Kumar Dutta
Roll No. 12711
Registration No. 15975
Session: 2012-2013

Pharmaceutical Research Laboratory,
Department of Applied Chemistry and Chemical Engineering,
University of Rajshahi,
Rajshahi, Bangladesh.

Motiher Green, June 2016.

Dedicated

To

My Be-loved Sweet

Son and Daughter

Deshraj Dutta

and

Mohaswata Dutta (Dishana)

Dr. Md. AbulHossainMolla
Professor
Dept. of Applied Chemistry
and Chemical Engineering
University of Rajshahi
Rajshahi-6205, Bangladesh.

Phone: 0721-750498 (Res)
Mob: 01712-255636
Fax: 0721-750064
E-mail: ahmolla@ru.ac.bd

DECLARATION CERTIFICATE

We have much pleasure to certify that the thesis entitled **"BIOCHEMICAL AND NUTRITIONAL INVESTIGATIONS OF BANGLADESHI FRESH WATER FISH *PUNTIUS GONIONOTUS*"** submitted by Prodip Kumar Dutta to the University of Rajshahi, Rajshahi, Bangladesh, for the degree of "Doctor of Philosophy" is the original work of the author. He has fulfilled all the requirements according to the rules of this University. No part of this thesis has been submitted elsewhere for any degree, diploma or prize. To the best of our knowledge, the data presented in this thesis are genuine and original. We have gone through the final draft of the Thesis and recommend its submission for the award of the degree of "Doctor of Philosophy".

Dated, Rajshahi
/06/2016

Principal Supervisor

Professor Dr. Md. AbulHossainMolla

Co-supervisor:

Professor Dr. Md. ShameemAhsan
Department of Applied Chemistry
and Chemical Engineering
Rajshahi University, Rajshahi

University of Rajshahi
Rajshahi, Bangladesh

DECLARATION

I hereby declare that the research work presented in this dissertation is the result of my own investigation as a Ph. D Fellow Roll No. 12711, Registration No. 15975, Session 2012-2013 and when the works of others mentioned have properly been cited. This dissertation or no part of this work has not been submitted elsewhere for degree or prize.

June, 2016

(Prodip Kumar Dutta)
B.Sc (Hons), M.Sc

DECLARATION

I do hereby declare that the research work presented in this dissertation entitled"**BIOCHEMICAL AND NUTRITIONAL INVESTIGATIONS OF BANGLADESHI FRESH WATER FISH *PUNTIUS GONIONOTUS"***is my own achievement and is not a conjoint work with any one else. This is my original study and no part of this thesis has been submitted to any other University or Institution for any degree, diploma or prize. The thesis contains no materials written or published by any other person except when due reference in made in the text of the thesis. The thesis is submitted to the University of Rajshahi for partial fulfilment of "Doctor of Philosophy" Degree.

Dated, Rajshahi,
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(Prodip Kumar Dutta)
Ph.D. Fellow,
Roll No. 12711
Registration No. 15975
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The Author

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ABSTRACT

Bangladesh is one of the developing countries of the world. A large number of populations in Bangladesh have been suffering from malnutrition. To over come this problem, it is necessary to increase the production of protein rich food for her peoples. Most of the people of the developing countries are still dependent almost entirely on fish as a source of animal protein. In Bangladesh, fishes are common as well as cheap sources of protein, fat, mineral and some vitamins. It is said “Rice and Fish” is the staple food of the people of Bangladesh. Therefore, optimum utilization of fishes, rich in nutrients, is of paramount importance to minimize micronutrients gap and thereby it is possible to improve nutritional status in the country.

Puntiusgonionotus(*P. gonionotus*, locally known as China punti) fishes are being used as popular sources of fishes in some parts in Bangladesh. This fishes are found in Bangladesh mainly in the district of Pabna, Rajshahi, Bogra and Natore. Fishery of this fishes was carried out in three different ghers (polders). No remarkable change was found in pH, temperature and densities of water bodies used as fish habitat of these study areas.

Total dissolve solid (TDS), electrical conductivity (EC), salinity and free CO_2 were found maximum (22.3± 1.12 g/L; 33.85±0.82 ms/cm; 22±2.45 ppt, and 5.5±0.04 mg/L respectively) whereas hardness (mg $CaCO_3$/L); Ca^{2+} (mg/L); Mg (mg/L); dissolve oxygen ((mg/L) and total suspended solid (TSS) (g) were found minimum (630±2.54; 70±2.46; 110.75±4.75; 5.6±0.04 and 0.020±0.01 respectively) CO_2 and dissolved oxygen (D.O) are reliable for this fish culture but hardness’s were so high and may be concerned for the fish habitats. Alkalinity and chemical oxygen demand (COD) were found maximum (200±3.56 mg$CaCO_3$/L and 140±1.88 mg/L respectively). Different types of gears and crafts were found for fishing *P. gonionotus* fishes in these ghers and in the river during full moon. Fishermen handover their catches in different distannce and nearby market through a more or less distinguish channel. In between the fishermen and final consumers there are a few intermediates. The market price and landing of fish varied marginally from market to market. The price of this fish to the consumer varies from season to season. In the fishery,

maximum labour force is employed in the traditional sector. They have normally large family members. The fishermen of these areas are mostly poor and illiterate.

The proximate composition of flesh of this fish was determined using standard methods of analysis. The grand mean values were moisture 71.51±0.93%; protein 22.21±0.92%; lipid 5.24±0.64%; ash 1.02±0.05%; crude fibre4.655±0.32% and dry matter 28.50±0.65%.

The analytical properties of the crude lipid were evaluated. It was observed that lipid was reddish brown colour and density and refractive index of the lipid were 0.9516 g/cc and 1.4612–1.4635 respectively. Studies on the chemical characteristics of this fish lipid indicated that the iodine value (I.V), saponification value (S.V), saponification equivalent (S.Eq.), acid value (A.V), ester value (E.V), peroxide value (P.V), percentage of free fatty acids (as oleic) were found to be 108.72; 186.52; 296.52; 1.96 mg/KOH; 235.29; 1.78 mEq/Kg; 1.78 % respectively. The lipid is found to be edible as its acid value was estimated to be low (1.96 mg/KOH). The chemical indices indicate that the fish is a good source of protein. This fish contained several dietary minerals such as calcium (Ca), magnesium (Mg), sodium (Na), phosphorus (P), potassium (K), iron (Fe), copper (Cu) and zinc (zn).

The macro mineral elements calcium (Ca), sodium (Na), potassium (K), magnesium (Mg), and phosphorus (P) were found to be in the range of 276±4.54 mg; 76.01±2.16 mg; 294.4±2.01 mg; 48.1±1.83 mg and 210.0±1.80 mg per 100 g portion respectively. The micro minerals, iron (Fe), copper (Cu) and zinc (Zn), content in this fish were varied from 1.70±0.16 mg; 1.01±0.20 mg; 3.80±0.82 mg per 100 g portion respectively. The observations in dietary minerals suggested that the fish *P. gonionotus*could be provided a significant proportion of calcium (Ca), magnesium (Mg), sodium (Na) and also potassium (K).

The distribution pattern of fatty acids in fish lipid was separated and identified by Thin Layer Chromatography (TLC). Fish lipid under investigation contained myristic acid ($C_{14:0}$), palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$), lionoleic acid ($C_{18:2}$), lionolenic acid ($C_{18:3}$), arachidic acid ($C_{20:0}$) and behenic acid ($C_{22:0}$). These results revealed that the lipid is pharmacologically active owing to the fact that it is necessary for proper functioning of many metabolic processes. Fatty acids

composition and chain length of fatty acids in this fish lipid were determined by Gas-Liquid Chromatography (GLC). In GLC examination, the mixture of fatty acid methyl esters derived from fish lipid were separated on polar column having stationary phase diethyleneglycosuccinate (DEGS). The identities of the individual fatty acid were achieved by co-chromatography with standard reference compounds. It was established from the GLC examination, the mixture of fatty acid methyl esters derived from fish lipid that the mixture contained fatty acids consisting of carbon C_{14} to C_{22}. The percentage of each fatty acid methyl ester derived from fish lipid was provided by printed out record of the chromatogram of GLC. The percentages of composition of each fatty acid methyl ester in the fish lipid derived from the chromatogram were 6.33% myristic acid; 24.23% palmitic acid; 8.42% stearic acid; 30.29% oleic acid; 7.35% lionoleic acid; 5.46% lionolenic acid; 2.47% arachidic acid; 4.25% behenic acid and 11.13% unknown acid.

Analysis of this fish lipid for fatty acids indicates that the lipid contained 7.35% linoleic acid (ω-6 fatty acid) and 5.46% linolenic acid (ω -3 fatty acid). These fatty acids are an important part of the structural component of cell membranes and are necessary for the formation of eicosanoids which assist in blood pressure regulation, blood clot formation, maintenance of blood lipid levels and assist in the body immune response. The fish lipid contained about 43.1 oleic acid (monounsaturated fatty acids) can able to promote the high level of HDL cholesterol and hence decreasing the risk of heart diseases in human body. The concentrations of saturated fatty acids in this fish lipid were 42.43% of total lipids; monounsaturated fatty acids 43.1% and polyunsaturated fatty acids 14.43%. The fish lipid is suitable for edible purpose as it contained both ω-3 and ω-6 fatty acid especially linoleic and alpha-linolenic acids.

The effect of feeding Fish Protein Concentrate (FPC) at different protein levels with formulated cereal have been performed to identified the nutritional significance of fish *P. gonionotus* by determining the gain in body weights per one gram of food intake, Protein Efficiency Ratio (PER) and Calorie Efficiency Ratio (CER) values in twelve male young male albino rats for 32 consecutive days. For this purpose, five experimental diets were prepared at different protein levels (5% to 25%) with supplied formulated cereal where the control diet was prepared with supplied

formulated cereal only. Each mean gain in body weight per one gram of food intake, PER and CER were increased with increasing protein levels supplemented with formulated cereal and showed maximum value of 0.534g, 2.53 and 13.02 respectively at 15% protein level. When protein levels were further increased through FPC, fall in gain in body weights per one gram of food, PER and CER have been observed in diet-E (0.457g, 2.11 and 11.15 respectively) and diet-F (0.432g, 1.02 and 10.53 respectively).

Locomotive behaviour, central nervous system, excitation, weakness, reflexes, salivation, urination, diarrhoea, etc. of all albino rats are found normal during the experimental period. After consuming the diets containing FPC at different protein levels produces no observable changes in blood constituents like total RBC count ($10^6/\mu l$), hemoglobin (g/dl), erythrocyte sedimentation rate (ESR)(mm), total WBC Count ($10^3/\mu l$), differential count of WBC (neutrophil, lymphocytes, monocytes, eosoniphils) with that of control group and the blood cholesterol (mg/dl) levels of the treated rats were increased gradually than control group. Histopathological examination of the heart, liver, lung, kidney and spleen of all treated rats were found normal and no morphological change were found in their microscopic view with respect to the control group.

In Brine Shrimp Lethality bioassay, the chloroform-methanol extract of this fish lipid showed negative results indicating that the fish lipid was biologically inactive. From this experiment, it is revealed that the fish lipid has no toxicity against any living organisms.

CHAPTER 01

INTRODUCTION

Local Name : Thai shar phuti/China punti
Scientific Name : *Puntius gonionotus*

Introduction

1.1. General facts:

Most of the people of our country have been suffering from mental and physical disease for the deficiency of nutrition. The lack of balanced food ingredients is the main cause of their malnutrition. The lack of protein is very conspicuous in various kinds of food ingredients. There are two kinds of proteins such as plant protein and animal protein. Generally animal protein is better than plant protein in respect of qualities.

Fish gives us proteins. It can be used to make other products to make our visions better, but is much underappreciated for its significance in the life of all mankind. Fish is a good tasting and cheap source of daily nutrients. They can cure and prevent many illnesses. The total catch offish and other aquatic organisms in the world has reached the enormous figure of about 30 million metric tons or more[1]. Being a country of rivers, ponds and flood plains with a high potential of aquatic resources, fish plays a very important role in daily lives of many Bengalee people. In fact, fish is the most important part of a Bengaleefood menu. There is a very common expression in Bengali *"machebhateBengalee"* which can be translated as "fish and rice make a Bengalee". This expression is very true considering the amount of fish consumed and produced in the country. Fish is the only principal item of our food which we don't import from other countries[1]. Fish is the principal source of animal protein in a Bengalee diet. More than 63% of animal protein comes from fish alone [2]. Fish protein has been proven to be healthier and bad cholesterol free. It rather helps in cholesterol absorption in the body tissue. It also contains fatty acids. People are advised by dietarians to consume more fish than meat, as it decreaseshealth risks. The demand of fish as a source of animal protein is increasing day by day mainly for two inversely related factors

which increase the growth rate of population and decrease natural fish production. Traditionally, the people consume fishes of fresh water and nearshore brackish water origin[1].The quality of the fish depends greatly on the conditions of water in which they were bred. There are three basic elements to a fishery: the resources itself, its aquatic environment and the people who harvest the resources or change the condition of its aquatic surroundings [3]. Geographically, Bangladesh is located at the delta region [4] and blessed with freshwater and marine fisheries resources[1]. Bangladesh is endowed with vast and varied aquatic resources such as rivers, canals, floodplains, lakes, tanks, ponds, lagoons, estuaries and a long coastline displaying light diversities in their biotic and abiotic characteristic. Bangladesh is also composed of vast potential areas comprising 4.33 million hectares of flood plains, 4.04 million hectares of coastal shrimp polders and 1.66 million hectares (0.16 million sq. kilometers) of marinewater. These areas are regarded as being the Exclusive Economic Zone (EEZ), and suitable for capture fisheries [4]. According to the fisheries statistics of the 1 .4 million tons of fish produced annually, about 42% are obtained from inland fisheries. About 20% of the fish come from marine fisheries and 25% and 6% come from inland freshwater pond cultures and shrimp farming respectively[5]. Total production of *Puntiusgonionotus*fish in Bangladesh is 601 metric tons and is about 0.79% of total annual fishery.[6] Fishdoes not only meet the demands of our protein in food, it also plays a very important role in prosper of Bangladeshi economy. The total fish production accounts for 6% of Gross Domestic Production (GDP) and 12% of the total export earnings. About 12 million people depend on fisheries for jobs and foods. Of these people 1.2 million are dependent full-time of fish and fish related activities. With a total population of 1 17.5 million, the availability is 32 g offish per person per day which is 5.6 g of protein per person per day [5]. Bangladeshi fishery earning comes mostly from the marine, estuarine and inland water fishes. Several dozens of marine fish species are of considerable economic importance. There is a bright

scope of export of fisheries, lobsters, crabs, catfish, turtles etc to foreign countries. During the 2001-2002 fiscal year more than 16371.40 million Taka worth of foreign currencies have been earned by exporting fish and fishing products [6]. There is a possibility that the yield from these sources could be raised many times more than what it is now[2]. The sustainable utilization of genetic resources including fish plays a vital part in improving the standards of living in a populated country like as Bangladesh [7].

The importance of marine fishes in the economy of Bangladesh cannot be over looked. Fishery is one of the main economic activities in the coastal zone[8]. The coastal zone of Bangladesh has been officially defined as consisting of 19 districts and the Exclusive Economic Zone (EEZ). The land is intensively used for agriculture, settlements, forests, shrimp ghers. water bodies and fisheries. Most polders (ghers) in South- West region in Bangladesh depend on tidal saline water from adjacent rivers and canals and are thus dependent on the tides for water bodies [8]

The dark colouration contrasting against the almost white underside and a stocky build with extended belly identifies the species. It generally feeds along the bottom digging and turning over debris. It wiggles entire body while clumsily swimming[9]. The *P. gonionotus*fishes are desirable fish with good flesh, texture and taste, particularly when captured or taken from water of high salinity. This fish has a capacity for tolerating extreme condition of temperature, salinity and dissolved oxygen content. The fish is very popular and familiar in the region but still is not widely explored in a large scale for the local people of Bangladesh. In an improved traditional ghers (polder), this fish species may be cultured with shrimps and milkfish with increased economic benefit. This particular species, together with other commercially important brackish water fishes have been contributing a lot to our coastal fishery.

Fishes are the important source of dietary lipid, protein, minerals, vitamins and other nutrients needed for human body for proper growth to sustain normal life.

1.2: LIPID

Lipids are generic names assigned to a group of fatlike chemical compounds that are insoluble in water but soluble in organic solvents (diethyl ether, petroleum ether, chloroform, hot alcohol, benzene, carbon tetrachloride, acetone, etc.) which are related either actually or potentially to fatty acids esters, and which at the same time are utilizable by the animal organism[10]. Lipid compounds include monoglycerides, diglycerides, triglycerides, phisphatides, cerebrosides, sterols, terpenes, fatty alcohols and fatty acids[11]. The major structural units of naturally occurring forms of simple and complex lipids are the aliphatic long chain mono-carboxylic acid [12].

1.2.1. Classification of lipid:[10,11,13-18]

Lipids may be classified in different ways. Bloor [13] has suggested the most convenient classification of lipids on their chemical composition.

(1) Simlpe lipid: Simple lipids are those which on saponification yield two types of hydrolysis products (fatty acids, glycerol etc) per mole, e.g., triglycerols and cholesterol esters [14]. The hydrolysis of simple lipids may be expressed as

$$\text{Simple lipid} + H_2O \xrightarrow{\text{hydrolysis}} \text{Fatty acid} + \text{Alcohol}$$

(a) Fats and oils: fats and oils are the simple lipids which are the fatty acid esters of glycerol [15]. The term fats and oils are generally to refer to substances which have a similar chemical structure and which have the same metabolism in the animal body. Fats are those substances which are solids at room temperatures [16] due to preponderance of long chain saturated fatty acids[17] while

oils are liquid due to contain higher proportion of unsaturated fatty acid components [16]. Nearly all the commercially important fats and oils of animal and plant origins consist almost exclusively of this simple lipid class [14].

(b) Waxes: Waxes are fatty acid esters of long–chain alcohols [15]. Waxes, in general, can contain a wide range of different compounds including aliphatic diols, free alcohols, hydrocarbons (especially squalene), aldehydes, ketones, hydroxyl–ketones, β–diketones and sesquiterpenes [14]. Waxes consist of products of both animal and plant origin in which the esters are composed of palmitate, stearate or other higher fatty acid esters of cetyl (CH_3 (CH_2) $_{14}$ CH_2OH), octadecyl or steryl(CH_3 (CH_2) $_{16}$ CH_2OH) or higher straight–chain alcohols [10].

(c) Cholesterol and cholesterol esters: Cholesterol is by far the most common number of a group of steroids with a tetracyclic ring system; it has a double bond in one of the rings and one free hydroxyl group. Cholesterol is hydrolysed or trans esterified much more slowly than most other O–acyllipids. Steroid hormones and bile acids are structurally related compounds which differ in function from lipids as defined above [14].

(2) Compound lipids or conjugate lipid: This group is distinguished by the presence in the molecule of products other than fatty acids and alcohol. In some cases no alcohol is present and the fatty acids are combined in a peptide–linkage rather than as esters [10].

(a) Phospholipids or phosphatides: Phospholipids are the esters of fatty acids and phosphatidic acid [15]. In the phospholipids of animals and microorganisms, analogues containing vinyl ether and ether bonds are much more abundant than in the simple lipids [14]. These are the main constituent lipids of cellular membranes allowing the membrane surfaces to be hydrophobic or hydrophilic depending on the orientation of the lipid compounds into the intra or extracellular spaces [15].

(b) Glycolipids: Glycolipid consists of a lipid component that is attached to a carbohydrate component by a glycoside bond. These compound lipids undergo hydrolysis as follows:

Glycolipid $+H_2O \xrightarrow{\text{hydrolysis}}$ Fatty acid + A carbohydrate + Sphingosine (a nitrogen containing alcohol)

Glycolipids, unlike phospholipids, are soluble in acetone and this property can be used in isolating them by chromatographic means [14].

(c) Lipoprotein: These compounds found in mammalian plasma are composed of lipid material bound to protein.

(3) Derived proteins: This class includes derivatives of the first two classes of lipids obtained by hydrolysis which still retain the lipid characteristic of solubility in ether, etc., and insolubility in water [10].

(**a) Fatty acids:** Fatty acids are carboxylic acids with hydrocarbon chains of 4 to 36 carbons [18]. This group includes all fatty acids obtained from natural products which are soluble in fat solvents and which are insoluble in water [10]. Fatty acids are consisting of the elements of carbon (C), hydrogen (H) and oxygen (O) arranged as a carbon chain skeleton with a carboxylic acid group (–COOH) at one end [11]. The fatty acids starting with caprylic acid ($CH_3 (CH_2)_6$ COOH) and higher ones belong in this category [10].

(b) Alcohols: The alcohols of higher molecular weight which are obtained by hydrolysis of waxes and which are insoluble in water belong in this category. Glycerol is water-soluble and hence is not included [10].

(c) Hydrocarbons: This group includes products which contain no alcohol groups and which cannot be saponified.

1.2.2. Function of lipids:

Lipids are important constituents of the diet because of their high-energy value and also because of the fat-soluble vitamins (A, D, E and K) and the essential fatty acids found with the fat of the natural food stuffs. Phospholipids and sterols make up about half of the mass of biological membranes [18] and play an important role in cell structure and function. The phospholipids make up the integral structure of the unit membranes in the cells; thus, they are often called structural lipids [19]. Different cells have different quantities of phospholipid in their membranes. Gray matter in the brain is nearly 70% phospholipid, whereas brain white matter is less than half phospholipid because of high concentration of glycolipid (sugar fat) [20]. Lipids serve as transporters for fat-soluble vitamins A, D, and K into the cells [21] and can be utilized to partially spare (substitute for) protein. Lipids supply about twice the energy as proteins and carbohydrate. Although increasing dietary lipids can help reduce the high costs of diets by partially sparing protein in the feed, problems such as excessive fat deposition in the liver can decrease the health[22]. High fat diets are well known to be associated with certain kinds of cancers, including breast cancer, in particular [23]. Although butterfat stimulates breast cancer when compared with a fat-free diet, sunflower oil margarine has been shown to induce breast cancer much more strongly [24]. Fat is one last storage place of energy and are the principal stored forms of energy in many organisms [18]. Fat is stored in the adipose tissue, with about 85% of the total weight in actual fat [21].

Cholesterol forms the building blocks of several compounds (eg., bile, sex hormones, adrenal hormones and vitamin D) with important physiological functions and is a major structural component of cell membranes [25]. Cholesterol guards the cell membranes phospholipid from free radical damage and protects it against atherosclerosis, cancer and other free radical attack. The total circulating cholesterol remains relatively constant at between 170–200 mg/dl for the normal

adult. A low total cholesterol level (below 150 mg/dl) has been linked with an increased risk of cancer and stroke. Cholesterol-carrying lipoproteins play central roles in the development of artherosclerotic plaque and cardiovascular disease. The two main types of lipoproteins basically work in opposite directions. Low-density lipoproteins (LDL) carry cholesterol from the liver to the rest of the body. When there is too much LDL cholesterol in the blood, it can be deposited on the walls of the coronary arteries. Because of this, LDL cholesterol is often referred to as the "bad" cholesterol. High-density lipoproteins (HDL) carry cholesterol from the blood back to the liver which processes the cholesterol for elimination from the body. Because of this, HDL is often referred to as good cholesterol[26].

In body, all cells except erythrocytes and nervous system use fatty acids as source of energy. Ketone bodies, which are the derivatives of these acids, are used by brain in starvation [27]. Saturated fatty acids (stearic acid, palmitic acid and butyric acid etc) are found most in animal foods like meat, fish, coconut, palm and palm carnel oils. Specific foods which contain a significant amount of saturated fat include chicken (30%), vegetable shortening (31%), lard (40%), beef fat (50%), butter (62%), palm carnel oils (81%) and coconut oil (86%) [21]. Saturated fats raise total blood cholesterol levels more than dietary cholesterol because they tend to boost both good HDL and bad LDL [26]. Only the saturated fats of chain–length 12, 14 and 16 (lauric acid, myristic acid and palmitic acid) have been shown to elevate blood cholesterol. Of these, myristic acid elevates cholesterol the most [28]. Stearic acid has been shown to lower cholesterol by 21% even more than oleic acid ($C_{18:1}$) which lowers LDL by 15% [29].

The largest benefits in terms of reduced cardiovascular disease are found when saturated fatty acids are replaced with monounsaturated fatty acids (MUFAs). Studies have shown that for each 10 g increment intake of monounsaturated fat

in the diet, there is a significant reduction in the relative risk for invasive breast cancer. The anti–aging properties of monounsaturated fatty acids are:

(a) Lowering the oxidation of LDL cholesterol and thus slowing a crucial process of atherosclerotic plaque.

(b) Lowering the triglyceride levels, a form of circulating fat found in plaque and cholesterol [21].

Unsaturated fatty acids are important for membrane activity at the site of hormone receptors. Insulin resistance in adult-onset diabetes is associated with fewer membrane long-chain unsaturated fatty acids due to impaired desaturase and elongase enzyme function [30]. Polyunsaturated fatty acids will lower both LDL and HDL levels [21] and have often been recommended to reduce coronary heart disease [31].

The human bodycan manufacture most of the fats it needs including cholesterol, saturated fatty acids and unsaturated fatty acids [20].There is a subset of dietary fatty acids known as essential fatty acids (EFAs) which are essential for normal body function but that cannot be made endogenously and must be consumed. The two most important group of essential fatty acid is called ω–6 (N6 EFA) and ω–3 (N3 EFA), both come from consumption of PUFA and MUFA. The primary head of the ω–6 (N6 EFA) family is linoleic acid and the other sub–division to this similar category is alpha linolenic acid [21]. Linoleic and linolenic acid are essential fatty acids that are not produced in the body and must be obtained from dietary sources. These fatty acids are an important part of the structural component of cell membranes, and are necessary for the formation of eicosanoids which assist in blood pressure regulation, blood clot formation, maintenance of blood lipid levels, and assist in the body immune response [32]. An important product of linoleic acid is arachidonic acid ($C_{20:4}$). Arachidonic acid, in turns, gives rise to a whole group of 20 carbon,

biologically important substances known as eicosanoids, including prostaglandins, thromboxanes, lipoxins, and leukotrienes which affect immunity inflammation, and blood clotting. Members of ω–3 family of fatty acids manufactured from alpha linolenic acid are eicosapentaenoic acid (EPA) and docasahexaenoic acid (DHA). DHA and arachidonic acid are both crucial to the optimal development of the brain and eyes. Excessive amounts of ω–6 PUFAs and a very high ω–6/ ω–3 ratio have been linked with pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune disease. The ratio of ω–6 to ω–3 in modern diets is approximately 15:1, whereas ratios of 2:1 to 4:1 have been associated with reduced mortality from cardiovascular disease, suppressed inflammation in patients with rheumatoid arthritis, and decreased risk of breast cancer [11].

Fish, an important source of polyunsaturated fat known as ω–3 fatty acid, has received much attention for its potential to lower heart disease risk. There is strong evidence that fish and fish oil consumption reduces the risk of heart disease deaths and so–called "sudden deaths" [26]. The lower rates of cardiovascular disease is not directly related to total fat intake, but more with the kind of fat consumed. For example, Japanese who live in Japan have the lowest rate of heart attacks in the world. They consume an abundance of raw fish (Sashimi) that are high in natural saturated fatty acids (SFA) and N3 essential fatty acids (EFA). Fish are at the top of food chain based on phytoplankton (algae) that manufactures large amounts of EPA and DHA [21].

Polyunsaturated fatty acids (PUFAs) can be health hazard because carbon-carbon double bonds can lead to free radical formation and reactions with oxygen to form unstable lipid, peroxide compounds containing the same unstable oxygen–oxygen bond found in hydrogen peroxide. Lipid peroxidation and free radicals can cause cancer and may accelerate aging. High rates of lung cancer among women in China have been associated with lipid–peroxidized

oils in fumes from cooking polyunsaturated vegetable oils in a work [33]. During the hydrogenation process, the chemical structure of the natural fatty acid is changed from their original *cis*–configuration to unnatural *trans*-configuration and is called trans-fat. The trans-fat is the worse kind of fat. *Trans*–isomerization alters the three dimensional configuration of dietary fatty acid, causing damage to cell membranes and altering the function of phospholipid–dependent enzymes contained in these membranes. Trans fat increases the level of triglyceride more significantly; reduces the level of "good" HDL cholesterol that often related to the lower risk of cardiovascular diseases. A high intake of trans fat has, therefore, been linked to a variety of free radical and degenerative conditions such as cancer, arthritis and cardiovascular diseases.

A study published in the *New England Journal of Medicine* reported that trans fat linked to a 93% rise in the risk of cardiovascular disease. The research also revealed that a replacement of 2% transfat consumed with MUFA could reduce heart disease risk by 53% [21].

On exposure to the atmosphere, oils and fats gradually undergo certain changes. The oils and fats are become hydrolysed first slowly by the moisture and a free fatty acids are formed. The fatty acids so set free are then more readily attacked by the oxygen of the air and oxygenated products are formed, which impart to the oils and fats the rancid smell and taste [34].

1.3. Classification and function of proteins:

Proteins are a class of most important compounds that are found in living organisms [16]. Protein is composed of amino acids; nine of these (leucine, isoleucine, valine, lysine, tryptophan, threonine, methionine, phenylalanine, and histidine) are considered nutritionally essential or "indispensable" in the human diet, because they cannot be synthesized in the body [32]. Proteins occupy a central position in the architecture and functioning of living matter. They are

intimately connected with all phases of chemical and physical activity that constitute the life of the cell [35]. Different categories of proteins include enzymes, required as catalyst in most of the body's chemical reactions, peptide hormones (such as insulin, thyroid hormones, and the growth hormone somatotropin), structural proteins (in muscle and connective tissue), transport proteins (e.g., albumin, transferring, haemoglobin), and immunoproteins or antibodies [32]. Some proteins are associated with the genes, the heredity factors [36]. The proteins with catalytic activity (enzymes) are largely responsible for determining phenotype or properties of a cell in a particular environment. A protein also supplies nitrogen, for the internal synthesis of other amino acids required by the body [32]. Proteins are composed of carbon (50%), hydrogen (6.5%), oxygen (21.5%), nitrogen (16%) and very rarely sulphur also [22]. In certain proteins, phosphorus also occurs as well. Most animal proteins contain from 0.5 to 2.0 percent sulphur. Insulin is, however, a notable exception to this in processing about 3.4% sulphur. Proteins have been traditionally divided into two well–defined groups: animal protein and plant proteins. Animal proteins are the proteins derived from animal sources such as eggs, milk, meat and fish. They are usually called higher–quality proteins because they contain (and hence supply) adequate amounts of all the essential amino acids [35]. The proteins in fish muscle tissue can be divided into the following three groups:

1. Structural proteins (actin, myosin, tropormyosin and actomyosin), which constitute 70-80% of the total protein content (compared with 40% in mammals). These proteins are soluble in neutral salt solutions of fairly high ionic strength (0.5 M).

2. Sarcoplasmic proteins (myoalbumin, globulin and enzymes) which are soluble in neutral salt solutions of low ionic strength (< 0.15 M). This fraction constitutes 25–30% of the protein.

3. Connective tissue proteins (collagen), which constitute approximately 3% of the protein in teleostei and about 10% in elasmobranchii (compared with 17% in mammals). The structural proteins make up the contractile apparatus responsible for the muscle movement. The majority of the sarcoplasmic proteins are enzymes participating in the cell metabolism, such as the anaerobic energy conversion from glycogen to ATP. Changing the physical environment [19] easily changes the conformational structure of fish protein. Generally proteins are denatured by (physical agents) heat, surface action, ultra violet light, ultra sound, high pressure etc, and chemical agent acids, alkalis, heavy metals salts, urea, ethanol, guanidine, detergents [37]. The quality of protein is mainly determined by the specific amounts and relative proportions of its essential amino acids, their availability to the body, and to a lesser extent, the protein's digestibility. While the amount of protein required by individuals depends on their body weight and height, energy (calorie) intake, and physiological condition (e.g., infancy, pregnancy), it is the quality of the protein, which is most important in determining daily requirements. PER is another measure of protein quality, usually calculated by putting young animals on diets with various test proteins, and monitoring their growth. The PER is a ratio of the gain in weight divided by the weight of the protein consumed.

$$\text{PER} = \frac{\text{gain in body weight (gram)}}{\text{gram of protein consumed}}$$

1.4. Classification and fuction of minerals:

The minerals elements present in the animal body are supplied by the diet [38]. They are found in all body tissues and fluids [37]. They help in the production of energy and chemical reactions in the body. Unlike carbohydrates, fats and proteins, mineral elements don't furnish energy [37]. The mineral elements can be classified as principal elements (macro minerals) and trace elements (micro minerals). Seven essential elements like calcium (Ca), phosphorus (P),

magnesium (Mg), sodium (Na), potassium (K) sulphur and chlorine are known as principal mineral elements or macro mineral. The trace or micro minerals are present in living tissues in small amounts. Iron (Fe), zinc (Zn), iodine, copper (Cu) manganese (Mn) cobalt (Co), molybdenum (Mo), selenium (Se), chromium (Cr) and fluorine are possibly essential trace elements [38].

Both macro and minerals commonly obtained from fish, which could be regarded as public health concern. In other words, fish would be considered good source of these minerals. Fish with soft bones can also be important dietary sources; for example, walleye, bass and yellow perch provide 141, 103 and 102 mg of calcium, respectively, in a 100 g portion. Small fish eaten whole, such as sardines and smelts, as well as canned fish with bones, such as salmon also contribute appreciable amounts of dietary calcium [32]. Calcium along with phosphorus is essential for the formation and development of bones and teeth [38]. As estimated 99% of the body's calcium resides in the teeth and bones, where it is extracted and re-deposited as needed to keep blood levels of calcium constant. The one percent found in the blood, lymph and other body fluids is critical to the intracellular and extracellular environments of all living cells.

Fish and other animal foods contain heme iron, which only accounts for about 15% of dietary iron (85% is nonheme) but which is absorbed at over twice the rate of non-heme iron. Fish species differ in the amount of dietary iron they provide-channel catfish has 0.35 mg/100 g while the same size portion of bass contains 1.91 mg. In general fish contain less iron than beef, but comparable levels to pork and chicken. Men typically have approximately 3.8 g of iron on their bodies, one-third of which is stored as ferritin and hemosiderin in the liver, bone narrow and spleen, and two–thirds of which is functional iron, mostly in the form of haemoglobin and myoglobin, Women's bodies have about 2.3 g of iron, and only about one–eigth (0.3 g) is in storage. The body-increased demand for iron in pregnancy (1.0 g) is much greater than the average women's iron stores. Zinc is part of many enzymes, biomembranes, and is involved in RNA transcription, among other activities too numerous to

mention here. It has held tremendous public health significance in developing countries since 1960s, when zinc deficiency was linked to stunted growth and delayed sexual maturations. While widely distributed among plant and animals foods, zinc often has low bioavailability because of its interactions with copper, iron, and other food components such as phytates [32]. Sodium is the major component of the cations of the extra-cellular fluid and exists in the body in association with the anions chloride, bicarbonate, phosphate and lactate. It is largely associated with chloride and bicarbonate in regulation of acid base equilibrium. While potassium is largely present in the intracellular fluid and is also present in small amounts in the extra-cellular fluid because it influences cardiac muscle activity [38].

1.5.Introduction to the present work:

The fish *Puntius gonionotus* belongs to the family cyprinidae of the tribe osteichtheys. The fish is vernacularly known as Thai sharpunti, Rajpunti or chinapunti. This fresh water fish is an excellent food and a rich source of protein. It is indigenous species. Now a days this fish is being cultured in large scale in Bangladesh.

The fish *Puntius gonionotus* locally known as China puthi is found in everywhere in Bangladesh and are Consumed by the prople of the country for their delicious taste, availability, therapeutic value and relatively cheaper source of proteins. Local people believe that this fish is highly nutrious and they get better results after eating this fish. It is obvious that some sorts of nutritionally active compounds may be present in this fish and the people consume this fish as such without any knowledge about what types of compounds are present in this fish. The nutritionally active compounds from this fish are great importance from the medicinal point of view. Lipids, protein and minerals play an Important role for proper functioning of metabolic processes.

Although this fish occurs widely in every districts in Bangladesh, no up to date chemical or biological works on this fish has so far been reported. The present work is a step towards the direction of the efforts to get much information on this species about the physical and chemical conditions of water bodies as fish habitats, polders' status, and socio-economic condition of peoples. The study is carried out to determine the physical and chemical characteristics of this fish lipid and proximate analysis of fish flesh and qualitative and quantitative analysis of fatty acids of fish lipid. In view of recognized usefulness of this fish as a source of nutrion, this study is conducted to update information of micro and macro minerals of fish flesh. The cytotoxic effects of puntius gonionotus fish lipid have been determined to find any toxicological effect on living organisms. Finally the dietary effect of Fish Protein Concentrate on young albino rats at different protein levels and the haematological profile and histopathological examination of treated rats have been performed to find out any alternation in comparison with control rats.

A side view of *P. gonionotus* has been shown in the figure-1.

Figure-1: Photograph of *P. gonionotus.*

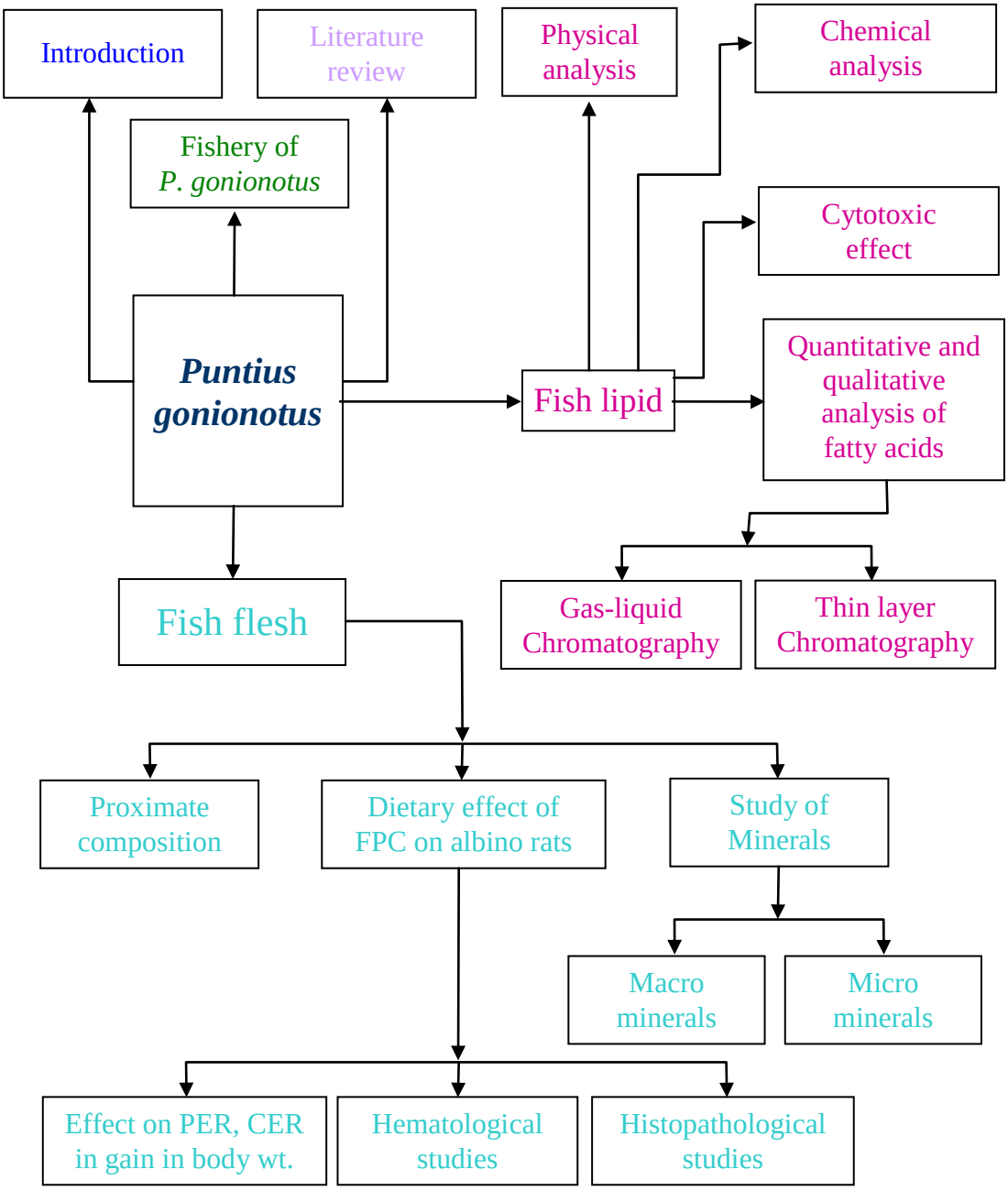

Fig. **2**: Conceptual framework of present study.

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CHAPTER 02

REVIEW OF LITERATURE

Local Name : Thai shar phuti/China punti
Scientific Name : *Puntius gonionotus*

Literature Review

A search of literature reveals that fisheries researches so far conducted in Bangladesh is mostly biological in character. No detailed research work has been performed on *P. gonionotus* fish in Bangladesh. A little information is available on the biology and culture practices of this fish species. Sporadic information that are available are mostly very specific and incomplete in nature. A literature survey on marine and fresh water fish reveals that the following research works have been carried out described below.

P.K. Ray[1] provided a graphic account of his experiments in successfully inducing *P. gonionotus* fish to breed injecting ovatide and also rearing the larvae triumphantly. In the ongoing situation of efforts at diversification away from shrimp farming, production of *P. gonionotus* seed at shrimp hatcheries for supply to farmers to culture this fish may prove to be a good protection.

M.J. Alam et al., [2] made an attempt to breed the estuarine catfish, *P. gonionotus* in captivity through hormone induction. After 6–7 hrs of a single dose injection with 2 ml ovaprim per kg body weight for both female and male, the catfish started spawning. Hatching of fertilized eggs completed within 18–22 hrs of spawning. The rate of hatching was 84–88%. At five days after hatching, the fry attained an average size of 5 mm in length and 1 mg in weight from a pair of female *P. gonionotus*; with an individual body weight of 140–150 gm, about 56,000–65,000 fry were produced.5 day old *P. gonionotus* fry produced by induced breeding were transferred to earthen nursery ponds and have been found to survive and grow well with an average size of 21–23 cm in length and 90–94 mg in weight at 19 day of rearing period.

Biodiversity Research Center, KUNHM, [3] suggested a geographical map of specimen collection localities and predicted distribution for "*P. gonionotus*".

Eli.,[4] made a report on distribution of *P. gonionotus* fish species in the world. This fish species are available in Bangladesh, India, Indonesia, Malaysia, Myanmar, Nepal, Pakistan, Sri Lanka, Thailand and Vietnam. Eli, showed the ecosystem, type and status on *P. gonionotus*. Ecosystem and types were found Chao Phraya River (basin), Chilka Lake (lake), Ganges (River basin), Godavari (River basin), Mekong (river basin) and Oriental (Zoogeographic realm). All status are native except oriental is endemic

R. Pethiyagoda, [5] reported the family, order, class, fish base name, size, environment, climate, distribution, biology, nature etc of *P. gonionotus*. This fish are primarily a brackishwater fish that enters and lives in fresh water and are available in tropical climatic region.

G.S.M. Asmat, [6] studied on the biodiversity of trichodinid ciliates from the state of West Bengal, India. The gills of the Indian estuarine fish, *P. gonionotus* (Hamilton, Bagridae) was found to be the host to a new species *Trichodina canningensis* sp. n. This ciliate is characterized by reduced blade: stout and robust central part; elongated ray having parallel borders and a large clear circle at the centre containing argentophilic particles. The body diameter ranged from 47.0 to 56.1 mum, whilst denticle number ranged 22 to 29. Approximately, 3.6% of the host fishes (6 out of 165) were infested with this ciliate, sometimes in concurrent infestation with *Trichodina mystusi* and other species of T*richodina* from November to January 1996. This trichodinid closely resembles *Hemitrichodina robusta* but differs with slightly developed blade having sharp tangent point and without any protrusion on the distal margin.

P.K. Sarker, et al., [7] investigated the fecundity and Ganado–somatic index (GSI) of *P. gonionotus* fish species. Fecundity was determined at different length, weight and ovary ranges of different individual fishes. At different

length ranges from 10–12 cm of fish showed different fecundity and significantly highest mean fecundity was found 21589 (19394-23481) from 20–22 cm length ranges of fish whereas significantly lowest mean fecundity was 11887 (11436–12712) from 10–12 cm length ranges of fish. Fecundity also determined from a wide weight ranges (15–110 g) of fishes. Study revealed that significantly highest mean fecundity was found 22545 (19741–23009) from 90–110g weight ranges of fishes whereas lowest mean fecundity was 10982 (10831–12481) from 15–20 g ranges of fish. Ovary weight ranges from 16–18 g showed significantly highest mean fecundity 21378 (19611–22825). This study stated that the fecundity of fish increased with the increase in size, weight and gonad weight as well as. The highest GSI values were found in July. Furthermore, GSI showed that *P. gonionotus* has a wide spawning season.

K. Anbarasu and M. R. Chandran, [8] studied on an immunostimulatory effect of vitamin C on the humoral and cell mediated immunity of the bagrid catfish, *M.gulio,* using different bacterins of *Aeromonas hydrophila*. Humoral as well as cell mediated immune responses were elucidated in the vitamin supplemented, vaccinated and unvaccinated groups

M.A. Karim, [9] recommended some standards for fishing water. Fish requires a minimum concentration of 4 ppm dissolved oxygen (D.O), pH value of above 8.5 and below 6.5 may directly lethal, at 25°C, electric conductivity is 800×10^{-6} micro-ohm/cm culture for inland surface water, maximum concentration of ammonia and its compound to be allowed 3.0 ppm, maximum concentration of CO_2 is 2.0 ppm to be allowed and 20–32°C temperature is suitable for fishing water.

Hilsa fish

NFEP [10] made a study on the socio-economic status of demonstration farmers, the investigation showed that the socio-economic condition of the farmers had

gradually improved in the intervening four years of 1993 to 1997. This study clearly showed that fish farming was just one activity of many which farming household's conduct to produce food and income. The demonstration farmers have adopted better fish farming practices and this resulted in sustained increases in fish production from 4.5 to 8.0 kg/decimal. Moreover, while fish production declined following withdrawal of project support, fish production of the same farmers was still 33% above the baseline figure at 6 kg/decimal.

M. A. Islam, [11] conducted a research on hydrological condition of fish habitants and reported that *Mugil Parsia* was influenced by physico–chemical factors like temperature, turbidity, pH, dissolve oxygen (DO), CO_2 and salinity. The relationship between fecundity with total length, standard length, total weight, ovary length and weight of this fish were estimated. The nutrient content and physico–chemical properties of different parts of *Mugil Persia* fish in different stages of fish oil varied remarkably.

A.F.M. Shofiquzzoha and S.U. Ahmed, [12] worked on improving soil, water and productivity of gher (polder) fishery. Yearly variations in water temperature, salinity and pH were studied.

M.A. Wahab et al., [13] made an observation on some of the environmental parameters of selected shrimp farms in Khulna, Bangladesh.

A. J. Arockiaraj et al., [14] stated that *Mystus monatanus* eggs contain a relatively small yolk (1.0-1.2 mm) and are strongly adhesive. The egg envelope is thick and transparent. Embryos hatched 23.0–24.0 h after activation and the incubation period was 23.0 h long. The circulatory system appeared early (at 17.75 h) and was characterized by a complex anastomosing vitelline system and by well-developed segmental vessels suggesting that *Mystus monatanus* embryos can exploit available oxygen sources veryefficiently. The post larvae observed to have begun aerial respiration on the seventh-ninth day after

hatching. After 20 days, the length of fry ranged 15.0–16.0 mm and resembled the adult in its external features. The length of fingerlings ranged from 25.0–30.0 mm after 30 days and their external features were just like those of an adult except that they were not sexually mature.

According to FAO report, [15] fish contains 72% moisture, 19% protein, 8% fat, 0.15% calcium, 0.25% posphorus and 0.01% vitamin A, B, C & D.

K. Azam, et al., [16] investigated fourteen selected dried fishes and evaluated the biochemical parameters. The samples had moisture content ranging from 18.23 to 24.46%. Protein varied between 40.69 to 68.09%. Ash and fat content were 5.08 to 16.02% and 2.97 to 26.13% respectively. The proximate composition of *arius caelatus* (Tangra) were found 19.22 %, 5.08 %, 66.52 % and 9.03% moisture, ash, protein and fat respectively in dry weight basis.

S. S. Jahan, et al., [17] conducted a study on chemical properties of fats of some varieties of fatty fish of Bangladesh. Three categories of fish as high-, medium- and low-fat fish muscle were analysed and the observed values revealed that the lower the fat content, the higher the moisture content and vice-versa. The moderately high iodine value of lean fish might be due to the presence of PUFA and the high iodine value of lean- and medium-fat fish was due to the presence of unsaturated fatty acids capable of reducing high serum lipids.

C. E. Thurston, [18] reported that Alaska pink salmon maintained inverse relationship between fat and moisture and positive correlation between protein and moisture.

Borgstrom.,[19] observed that the fat and protein contents in fish depended on some factors such as size, age species, sex, seasonal changes and season of capture.

M.S. Islam, et al., [20] analysed the fish protein concentrate of three varieties of marine fishes: Jew fish (powa), Mackerel fish (Maitta), Catfish (Kata machh) and from mixed fishes (small fishes). The percentage of protein content of Jew, Mackerel, Catfish and mixed fish were 70.58, 68.87,66.29, 57.50 and that of fat were 5.3, 2.42, 4.13, 2.42 respectively. The moisture content of the above four samples were Jew fish 5.02, Mackerel 4.5, Catfish 7.3 and mixed fish 3.9 (dry weight basis). The presence of all essential amino acids was detected in all the samples.

Stansby and Love, [21] evaluated the chemical composition of fish fillet and showed that the value of principal constituents of fish fillet such as protein, lipid, carbohydrate, ash and water were usually varied in the range from 16–21%, 0.2–25%, <0.5%, 1.2–1.5% and 66–81% respectively.

F.O. Abulude, et al., [22] determined the proximate composition, mineral and functional properties of *Penaeus motialis, Macrobrachium vollenhovenii, Palaenonspecies* and *Procambanis clorkii* obtained from coastal area of Ondo State, Nigeria. The grand mean value in g /100g DM was: moisture 47.94; fibre 13.27; protein 21.59; ash 10.13; fat 6.88 and carbohydrate 47. 26. All the four samples had good gelation property, high values of water absorption and least gelation capacities and oil emulsion stability, but lower values for oil absorption, foaming capacities and foaming stabilities.

A. Herzberg, et al., [23] determined the proximate composition of 10 species native to the Mediterranean and Red Sea. The proportions of protein, oil, ash and water in the fishes were examined on a year-round basis and showed that the concentration of fish protein, oil, ash and water varies considerably depending on the geographic fishing area.

G. Meyer, et al., [24] estimated nutritional requirements and essential amino acid (EEA) requirements through the determination of muscle tissue amino acid

composition for four groups of jundia (Rhamdia quelen), a fresh water fishes. Great similarity among the amino acid composition of all groups, either from the wild or from artificial spawning was observed. Essential amino acid (EAA) concentration in jundia muscle tissue did not depend on fish weight or dietary history.

C. Gopalan, et al., [25] determined the moisture content in *Mystus vittatus, Labeo rohita, Catla catla, Puntius* and they obtained the results at 70%, 72%, and 75% respectively. Kamaluddin et al., [26] reported almost the same result in case of fresh water tengra fish. They again quoted the values of fat and protein content in the species *Mystus vittatus, Mystus blekeeri,Amblypharyngodon mola,Catla catla* and *Puntius.* They observed that *Mystus vittatus* contained 6.4% fat and 19.2% protein. *Mystus bleekeri* contained 2.73% fat and 18.85% protein. *Amblypharyngodon mola,* contained 2.4% fat and 19.5% protein and *Puntius* contained 2.4% fat and 18.1% protein.

M. A. Hossian, et al., [27] evaluated nutritive values of twenty-three small indigenous fish species (SIS) in the size range of 3-18 cm. The moisture content of different species ranged between 71.0–81.94%. Small sized fishes showed higher moisture content. The muscle protein content among the species varied widely (16.16–22.28%). The muscle protein of fishes showed higher value than the whole carcass protein content. The carcass lipid content varied between 1.87–9.55% and showed an inverse relationship with the moisture content. The gross energy content ranged from 19.51–27.3 kj/g on dry matter basis. Calcium and phosphorus contents ranged between 0.85–3.20 and 1.01–3.29% respectively and ratio Ca/P varied between 0.44 and 2.00. From the nutritional point of view, these results showing that the SIS are good source of protein and minerals especially calcium and phosphors.

H.W. Ockerman and H. Yetim, [28] showed that egg white (1%) and intermittent tumbling (20 minutes work and 40 minutes rest) (12 hours at 6°C) positively influenced the physiochemical properties of fish muscle tissue when compared with non-treated tissues. Salt-soluble protein extraction allowed the production of a fish log out of fish pieces. With these treatments, little proteolysis and a satisfactory flavour were noted and maintained during extended refrigerated storage.

American Oil Chemists Society, [29] made a report on some chemical characteristics of Menhaden oil and found that the oil have saponification value between 180–200, iodine value less than 120, unsaponifiable matter not more than 1.5%, free fatty acids not more than 0.1%, peroxide value not more than 5 mEq/kg, lead not more than 0.1 part per million and mercury not more than 0.5 part per million.

Department of Health and Ageing Therapeutic Goods Administration, [30] reported some specifications of oil derived from raw, unprocessed or frozen whole body of finfish. Acid value was found not more than 2.0, iodine value 150–210, peroxide value not more than 10 $mEqO_2$/kg. Total eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) was found in minimum 34%, arsenic content was found not more than 1.0 mg/kg, cadmium, lead and mercury of finfish were observed not more than 0.5 mg/kg.

T. Macnair, [31] stated that cholesterol ensures the body's normal function and it forms part of the outer membrane that surrounded every cell. It's used to insulate nerve fibres and make hormones, which carry chemical signals around the body. Total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides were recommended less than 4.0 mmol/L, less than 2.0 mmol/ L, more than 1.15 mmol/ L and less than 1.5 mmol/L for human.

O.D. Adeniyi and A.A. Bawa, [32] extracted oil from Mackerel and analytical properties of the crude and refined oil were evaluated and found that the crude oil consists from: acid value 2.5 mg/KOH, peroxide value 2.19 mEq/kg, saponification value 201.6 mg KOH/g, iodine value 108.09 I_2/100g, specific gravity 0.911, refractive index 1.485 and reddish brown colour. The refined oil was also evaluated as follows: acid value 2.27 mg/KOH, peroxide value 1.00 mEq/kg, saponification value 147.84 mg KOH/g, iodine value 106.93 I_2/100 g and golden brown colour.

J. B. Field, et al., [33] analysed the blood constituents in two fresh water fish species, carp and trout. pH, RBC (10^3/c.mm),WBC(10^3/c.mm), Hb (%), glucose (%), total lipids (%), total cholesterol (%) and total plasma protein (gm) found in carp were 7.67, 841.5, 3.675 ,10.5, 111.2, 1.233, 0.662, 4.15 respectively and in trout pH, RBC (10^3/c.mm),WBC(10^3/c.mm), Hb (%), glucose (%) and total plasma protein (gm) were 7.33, 1013.9, 3.910, 8.5, 70.2 and 3.46 respectively.

R. G. Myers, [34] made an experiment on the blood of Humback whale and Sperm- whale and found that blood of Humback whale contain 42.6 mg cholesterol, 266 mg sugar, 5.1 mg uric acid, 14.5 mg NH_3N and 2,237 mg total N and the Sperm- whale has 65 mg cholesterol, 118 mg sugar, 2.1 mg uric acid, 2.4 mg NH_3N and 3,518 mg total nitrogen.

Dipl-lng, et al., [35] determined the cholesterol content of one hundred and eighteen crude fish oils and found that the value of cholesterol content in crude fish oils varied from 0.37 to 1.96% with a mean of 0.95%. Anchovy oils reached a maximum cholesterol levels in the winter months of July and August and a minimum in March and April. An inverse relationship was found to exist between the cholesterol level of fish oil and the oil yield of the fish.

M. D.O. Neill, et al., [36] analysed the blood serum for blood urea nitrogen (BUN), cholesterol, calcium, aspartate aminotransferase (GOT), alanine aminotransferase (GPT), hematocrit and glucose during the month of June and July. Cholesterol values were found 123.6±8.2 (n = 16) and 112.6±6.5 (n = 31) in July 1997 and June 1998 respectively.

S. O. salawu, et al., [37] determined the proximate and mineral compositions of different sizes of catfish (*clarias gariepinus*) collected from both in brackish water (lgbokoda) and fresh water (Ogbese) bodies subjected to different treatments (fried, boiled, with/ without salt solution and roasted). The highest protein concentrations of fish samples were recorded by boiling with water, while the ones roasted recorded the lowest value. The lowest iodine concentrations were observed Ogbese fish boiled with sodium chloride solution and lgbokoda fish boiled with either water or sodium chloride solution. On the whole, Igbokoda brackish water fish had higher iodine content then Ogbese fresh water fish with the highest concentration in fried and roasted fish samples. Higher mineral compositions were also found in the brackish water fish and also increased with size and were reduced by some treatments (boiling and frying) in the flesh of fish samples.

E. H. Robinson, et al., [38] conducted an experiment on nutrient profile of pond-raised channel catfish. 100 g fillet of this fish contain 16.3 g crude protein, 5.4 g crude fat, 77.38 g moisture and 1.1g ashes. The major fatty acids which accounted for about 75% of the total-lipids were 16:0,18:0,18:1, and 18:2. Concentration of saturated fatty acids was 23.8% of total lipids; monoeonic 43%; dienoic 15.3% and trieonic 4.1%. Pond-raised catfish are a good source of phosphorus, potassium and selenium.

Murrey and Burt, [39] examined some mineral constituents of fish muscle and found that fish muscle contained 30–134 mg sodium, 19–502 mg potassium,

19–881 mg calcium, 4.5–452 mg magnesium, 68–550 mg phosphorus per 100 g portion.

M. Ashraf, et al., [40] conducted an experiment on concentrations of AS, Cd, Cr,Cu, Fe, Mn, Ni,Pb, Zn and Hg in eleven common fish species including *Mystus Vittatus* and *Mystus seenghala*, along with relevant sediment and water, from three freshwater reservoir and the outfall area of the Indus River, Pakistan. In all fish species, arsenic (As) ranged from 0.005 to 0.110 μg/g, chromium (Cr) from 0.065 to 0.44 μg/g, cupper (Cu) from 0.002 to 0.180 μg/g, iron (Fe) from 0.725 to 3.445 μg/g except the fish *Rita rita*.

M. L. Garg, et al., [41] conducted an observational study with rats by feeding diets high in either saturated fat (beef tallow), alpha-linolenic acid (linseed oil) and eicosapentaenoic acids (EPAs) and docosahexaenoic acids (DHAs) acids (fish oil) with or without 2% cholesterol. Consumption of linseed oil and fish oil diets lowered arachidonic acid content of plasma, liver and heart phospholipids. Addition of 2% cholesterol to diets containing beef tallow or linseed oil increased plasma cholesterol concentrations but not when fish oil was fed. Dietary cholesterol supplementation elevated the cholesterol concentration in liver in the order: linseed oil greater than beef tallow greater than fish oil (8.6, 5.5, 2.6 fold, respectively).

R. Rossano, et al., [42] analysed the chemical composition and nutritional value of the fish species Mora moro (Risso, 1810). The fatty acid profile and the principal water–soluble proteins present in the white muscle of this fish have also been determined. The major fatty acids found in this fish were 22:6 n–6, 16:0, 18:1 n–9, and 20: 5 n–3.

O.D. Adeniyi, [43] extracted and refined oil from herring fish and quantitative and qualitative analysis were performed to provide the oil for industrial process. The experimental results revealed that the rate of extraction increases

with time until maximum extraction took place using an average size of 780 μm. Every 10.64 g of dried fish sample used has about 4.34 g of oil extracted for five hours. The extracted herring fish oil contains two essential fatty acids, eciosapentaeoic acid (EPA) and docosahexaenoic acid (DHA), which could be of great industrial importance.

D.S. Sachan, et al., [44] conducted a study to determine the comparative effects of dietary fish oil (FO), plam (PO), safflower oil (SO) and corn oil (CO) on carnitine status and ethanol metabolism in rats. The diets containing FO and PO retarded ethanol metabolism compared to the diets containing coconut oil (CO) and soybean oil (oil). The liver carnitine content was higher in the PO group after dietary and ethanol treatment.

A.J. De Koning, [45] established a high significant correlation between the refractive index of fish oil and their content of eciosapentaeoic acid (EPA), the sum of eciosapentaeoic acid (EPA) and (docosahexaenoic acid) DHA and the total polyunsaturated fatty acids.

M. Stuchlik and S. Zak, [46] stated typical fatty acid composition of the pharmacopoeial fish oils. Cod liver oils contain 2–6% $C_{14:0}$, 7–14% $C_{16:0}$, 4.5–11.5% $C_{16:1\ (n-7)}$, 1–4% $C_{18:0}$, 2–7% $C_{18:1\ (n-7)}$, 12–21% $C_{18:1\ (n-9)}$, 0.5–3% $C_{18:2\ (n-6)}$, <0.2% $C_{18:3\ (n-3)}$,, 0.5–4% $C_{18:4\ (n-3)}$, 1–5.5% $C_{20:1\ (n-11)}$, 5–17% $C_{20:1\ (n-9)}$, 7–16% $C_{:20:5\ (n-3)}$, 5–12% $C_{22:1\ (n-11)}$,, >1.5% $C_{22:1\ (n-9)}$,, 6–18% $C_{22:6\ (n-3)}$, and 15.5–38% total ω–3 Acids.

A. A. Aguilera, et al., [47] investigated the effect of dietary fish oil.rich in n-3 polyunsaturated fatty acids (PUFAs), on metabolic syndrome in a high-sucrose-fed rat model. Metabolic syndromes rats had a significant reduction in blood pressure, serum insulin, triacylglycerols, cholesterol, free fatty acids and total lipids, but no change was observed in serum tumor necrosis factor-α (TNF-α) concentration or fat accumulation after administration dietary fish oil (rich in n–3 acids).

W. E. Hardman, et al., [48] reported that ω–3 polyunsaturated fatty acids (the type of fat found in fish oil) have been used to kill or slow the growth of cancer cells in culture and in animal models and to increase the effectiveness of cancer chemotherapeutic drugs.

M. Mijkherjee, et al., [49] identified 39 fish species of fresh water, cold water, brackish water and marine water that are going to disappear from their natural habitat in the West Bengal. *P. gonionotus* (Brackish and Marine) is vulnerable fish: standardized techniques for artificial propagation can be used to help conserve threatened species, through captive breeding programs and also to generate new employment opportunities for rural people. Artificial reproduction techniques were applied of two endangered species: a fresh water fish Pabda, *Ompok pabo* and a brackish water fish, *P. gonionotus.*

P.K. Talwar and A.G. Jhirgran, [50] reported the common name; distinguish characters, colour, geographical distribution and fishery information of the fish species *P. gonionotus*.

R.J. Ranjit Daniels, [51] reported on morphological characters, related taxa, diversity, distribution, habitat preference, adult behaviur, life cycle and human significance of long-whiskered catfish *P. gonionotus.*

Institute of Nutrition and Food Science [52] recommended the daily requirement of calcium for different age group. For normal healthy condition 450 mg calcium is recommended for 20–39 age group. Calcium content in different foods (cereal food, vegetable, spices, fruits, fish, meat and oil) in Bangladesh was determined and 270 mg calcium was found in tengra fish.

E. Mahmud, [53] reported Bangladesh as a vast water resources with about 1.47 lakh hectare of ponds, 40.47 lakh hectare of flood plains, 0.054 lakh hectare of oxbow lakes and 1.41 lakh hectare of coastal farms. He also suggested that the

production from these water bodies could be increased through scientific management and proper policy making for poverty alleviation of the rural people.

M.S. Islam, et al., [54] conducted a survey and assessment of shrimp fry and other aquatic resources of Bangladesh. They observed the value of pH of different water bodies in Khulna region varies from 6.3 to 8.8.

M. A. H. Molla, [55] conducted a research on biochemical and nutritional studies on fish lipid and of Fish Protein Concentrate (FPC) along with the isolation, purification and characterization of protein from the fish, EEL, *Angullia bengalensis*. The fish oil afforded 6.62-12.06% oil, 20.83 –22.17% protein, 63.65–67.21% moisture, 24.29–26.17% dry solid matter and 1.23–1.68% ash in different seasons. The oil was observed to contain more unsaturation in the winter and less in the summer. Fatty acids composition of fish oil indicated 49.65% saturated acid, 40.43% monounsaturated acid and 3.47% diunsaturated acid, 2.82% triunsaturated acid and 3.38% polyunsaturated acid. These results suggested that the fish oil is pharmacologically active. The effect of fish oil at 5% fat level is highly significant and 9% FPC is recommended for fortification with other cereal to produce the maximum nutritive value.

According to G.N. Chattopadhyay, [56] productivity of a fishpond depends largely on the abundance of fish food organisms and also on occurrence of congenial environment in the pond. Generally 20–35^{0}C temperature is considered to be optimum for fish culture. Cold water fishes are adapted to grow well under very low temperature. Neutral to slightly alkaline pH ranges for both soil and water are considered to be congenial for aquatic production. Less than 5 ppm Dissolved oxygen is recommended for normal survival and growth of fish population and 10–20 ppm biological oxygen demaned (BOD) load of pond water to be optimum for aquaculture. Less than 5 ppm free

CO_2containing water supports good fish population and 0.05 ppm H_2S is generally considered to be critical.

M. F. Hasan, et al., [57] conducted a study on the effect of season on the Physicochemical Properties and Fatty Acid distribution Pattern of the Lipids of *Eutropiichthys vacha.* No wide variation in fatty acids distribution profile was observed by the influence of seasonal changes but the fatty acids present were found to be mainly palmitic, oleic and linoleic acids besides small quantities of myristic, stearic, linolenic and arachidic acids. The physical and chemical characteristic of this fish lipid varies a little bit from season to season.

A. H. Molla, [58] extracted lipid from fresh water fish *Rita rita* and determined the lipid content in summer season (12.86 %), rainy season (13.94%)and winter season (13.52%). Physical and chemical studies were carried out for the characterization of the lipid. The fatty acid composition of the lipid was determined qualitatively and quantitatively by Gas Liquid Chromatography. The analysis revealed that the fatty acid composition of the lipid lies between C_{14} to C_{22}. The fish lipid of *Rita rita* was found to contain myristic acid, palmitic acid linoleic acid oleic acid, stearic acid arachidic acid and behenic acid in different seasons mentioned above.

M. Castillo, et al., [59] conducted an experiment to showed that replacement of saturated fat in young chick diet with menhaden oil produced a significant reversion of the hypercholesterolemia previously induced by coconut oil feeding. Fish oil also produced a clear decrease of plasma triacylglycerol levels. Coconut oil increased the percentages of 12:0 and 14:0 fatty acids, while menhaden oil increased those of 20:5 n–3 and 22:6 n–3. Percentages of 20:4 n–6, 18:2 n–6 and 18:1 n–9 significantly decreased by fish oil addition to the diet. Total cholesterol, phospholipid and protein contents of high and low density lipoproteins increased by coconut oil feeding. When coconut oil was

replacement by menhaden oil, total cholesterol was significantly reduced in high, low and very low-density lipoproteins. Menhaden oil feeding decreased all chemical components of VLDL. The clear decrease found in arachidonic acid content of chick plasma and lipoproteins may contribute to the beneficial effects of fish oil consumption by lowering the production of its derived eicosanoids.

A. K. M.A. Bhuiyan, [60] reported that the fatty acid composition of Atlantic mackerel was studied by using GLC system. C_{20} and C_{22} polyunsaturated fatty acids that are known to have beneficial effects in human body cardiovascular system were found to be present in considerable quantities. The prevailing general idea that the composition pattern of a dietary lipid, especially of its unsaturated, is indicative of its usefulness as a dietary source of energy was further supported by the overall fatty acid composition.

H. S. Olcott, et al., [61] analysed fatty acids of total fish lipid and showed that the saturated fatty acids were mainly palmitic and stearic and the unsaturated fatty acid were mainly oleic and palmitoleic. The observed low content of polyenoic fatty acids may account in part for the high stability of the extracts to oxidation.

W.S. Harris et al., [62] made a comparative study of the effect of fish oil supplements low in saturated fat and cholesterol on plasma lipid and lipoprotein in hypertriglyceridemic patients. Compared with the placebo (safflower oil), fish oil lowered plasma triglycerides levels and raised LDL cholesterol levels, apolipoprotein B levels, and the ratio of LDL cholesterol to HDL cholesterol. No significant changes were seen in levels of HDL or HDL cholesterol sub fractions.

P. M. Newberne, et al., [63] studied on growth, reproduction and location of five generations of rats by feeding Fish Protein Concentrate (FPC), prepared from whole red hake (*Urophycis chuss*) as a sole source of protein. The growth,

reproduction and lactation of these groups were compared to control groups fed laboratory chow or a semi synthetic diet containing casein as the protein source. Selected biochemical parameters were also evaluated and tissues from all groups were assessed for histopathological alternations. Major differences noted were better reproduction and decreased food efficiency in the chow group, compared to either FPC or casein groups; toxicological and pathologic data were essentially the same for all groups

B.M. Mitruka and H. M. Ramsley, [64] made a report on haematological parameters (RBC, TWBC, lymphocyte, monocyte, Neutrophil, Eosonophil, and Basophil value) and normal serum cholesterol value of albino rats. All serum biochemical constituents studied were failed to show any sex dependent difference.

J. O. Adebayo, [65] investigated the effect of ethanolic extract of *Bougainvillea spectabilis* leaves on some haematological and serum lipid parameters in rats during a seven day administration of the doses of 50, 100 and 200 mg/kg body weight. The results show that the extract administered significantly reduced packed cell volume (PVC), haemoglobin concentration (Hb%) and red blood cell count (RBC) at the dose of 200 mg/kg body weight when compared with controls while other doses administered had no significant effect on these parameters. The extract significantly reduced the white blood cell (WBC) count and total cholesterol concentration at all doses when compared with control. The extract had no significant effect on MCH, MCV, platelet count and serum HDL-cholesterol concentration. The extract significantly increased serum triacylglycerol concentration at the dose of 50 mg/kg-body weight while other doses administered had no significant effect on serum triacylglycerol concentration.

O. E Mesembe, et al., [66] evaluated the effect of fresh and thermoxidized palm oil (TPO) diets on some haematological indices in albino rats for consecutive fourteen weeks. Each of the plam oil diets contained 15% (w/w) thermoxidized or fresh Palm oil (FPO). The packed cell volume (PVC), haemoglobin concentration (Hb%) and red blood cell count (RBC) of the TPO group were significantly than that of FPO group and control group. The white blood cell of the TPO group was significantly higher that of control and FPO group. There were no significant differences between the haematological indices of the fresh palm oil and control groups. These results suggest that chronic consumption of thermoxidized palm oil diet may result in anaemia and leucocytosis in the rat.

M. Asuduzzman, [67] reported that huge intake of fish oil rich in ω–3 reduces depression and also reduces the tendency for suicide.

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CHAPTER 03

Fishery of *Puntius gonionotus*

Local Name : Thai shar phuti/China punti
Scientific Name : *Puntius gonionotus*

Fishery of*Puntius gonionotus*

3.1.Introduction

Bangladesh is fortunate enough in vast fisheries resources due to favourable climatic condition and geographical location. The importance of fish and fishery in this country has been very great from time immemorial. Fisheries sector is playing a vital role in employment generation, animal protein supply, and foreign currency earning and also poverty alleviation. From to prehistoric time, fish is considered as most important item of human diet[1]. About 90 % offish are collected from inland waters [2]. Fish is the primary source of animal protein in human diet throughout the whole Indo-Pak-Bangladesh subcontinent South East Asia and African countries [3]. It is not only meeting the demand of protein in food, but also takes a most important part in our national economics.

*Puntius gonionotus (P. gonionotus)*is both a saline and brackish water long-whiskered catfish [4].

This fish is valuable in our diet because apart from supplying of good quality proteins,lipids. It also supplies several minerals which are beneficial to man and animal. In Bangladesh, this fish species is harvested into shrimp farms locally known ghers (ghers-the place used for shrimp cultivation by entering tidal saline water) in a selective areas. The Exclusive Economic Zone (EEZ). Innumerable network of rivers, beels, haor-boars and flood lands, and ponds provide opportunities for both capture and culture fisheries in this country. The land in selective areas are extensively used for agriculture, settlements, forest, shrimp ghers, water bodies and fisheries, industrial and infra-structure developments and tourism [6]. About 1900 sq. km. lands are used to cultivate the brackish water fishes which are located within the polders [7]. The present fishery statistics showed that 1.9 million tones of fish is produced annually, about 40% is obtained from inland fisheries, 25% from inland fresh pond water culture and 6%

from shrimp farming [8]. The *P. gonionotus*is the important section of riverine and brackish water fisheries in Indian sub-continent[9].

This fish like taxonomy, distribution, identification, habitat, behaviour, feeding habits, breeding .etc were also included in this investigation. Socio-economic conditions of the fishermen related with these ghers were also studied.

The species has became the abundance of the species has been reduced in the natural habitats in West Bengal [9]. In Bangladesh, total yield of this species in the shrimp ghers can be increased by applying modern farming techniques such as intensification of culture operation through regularizations of ghers size, increasing stocking density, employment of operating system, application of lime, fertilizer, feed etc. Little information is available on the biology and culture practices of this species [9]. The study may be helpful to understand the migratory nature, ecological adaptability, breeding nature, spawning areas of the fish *in* the ghers.

3.2. Taxonomy of *P. gonionotus*:

Classification [10]:

Phylum	:	Chordata
Subphylum	:	Vertebrata
Class	:	Osteichthyes
Sobclass	:	Actinopterrygii
Order	:	Cypriniformes
Family	:	Cyprinidae
Genus	:	Puntius
Species	:	*P. gonionotus*

3.3: Distinguishing Characters [4, 11]:

Head depressed, its upper surface rough and granulated; occipital process triangular, about 1.5 times longer than broad at this base, extending (in adults) to basal bone of dorsal fine; median longitudinal groove on head short, not very conspicuous, not reaching base of occipital processes. Eye-diameter 5 to 6 times in head. Mouth terminal; teeth villiform in bands on jaws; vomerine tooth patch narrow, continuous and crescentic. Barbeles four pairs; two pairs of barbels on upper lips, maxillary barbels extend posteriorly to end of pelvic fins. Dorsal spine strong, serrated on its inner edge; adipose fin small, inserted considerably behind rayed dorsal fin. Caudal fin forked and deeply divided; least height of caudal penuncle equals its height. Branchiostegal rays 9. Body elongate and compressed, its depth 3.8 to 4.1 times in standard length. *P. gonionotus* (Hamilton, 1822) specimen with distinguish characters is shown in figure **2.1**.

3.4. Geographical distribution [12, 13]: *P. gonionotus*species are found in South-East Asia, mainly in the countries of Bangladesh**,** India, Pakistan, Indonesia, Malaysia, Myanmar, Nepal, Sri Lanka, Thailand and Vietnam. Geographical distribution of*P. gonionotus* in the world [14] is shown in figure **2.3.1**.

In Bangladesh, this fish species is widely distributed in the district of Pabna, Rajshahi, Bogra and districts. The distribution of *P. gonionotus* in Bangladesh is shown in figure **2.3.2**.

3.5. Adult behaviour [4]: Like other species of catfishes, adults *P. gonionotus*do not attack or fight with other species when kept together. Young fishes are gregarious but adults are often territorial. Adults and young are both have an enormous appetite and feed on almost anything that they can gorge. Like other catfishes, this species is very active at night. When kept inside well-lit aquarium, they are shy and hiding.

3.6. Habitat preference[4, 6, 15]:

*P. gonionotus*has a greater preference for salt water, inhabiting estuaries tidal swamps and even invading the sea. In Bangladesh this species cultured as a brackish water fish with shrimp species in ghers. Brackish water environments are also fluctuating environments. The salinity is variable depending on the tide, the amount of fresh water entering from rivers or as rain and the rate of evaporation. *P. gonionotus* fish is tolerant of changes in salinity and in fact many positively benefit from similar periodic changes in aquaria. The soils of fish habitants are mainly non-calcareous and saline which have severe limitations to crop cultivation especially in dry season. The zone is characterized by inter connected tidal rivers and khals (Creeks).

3.7. Size and weights:

Maximum lengths of *P. gonionotus* as reported by several authors [11, 12,16, 17] is 22–46 cm and weight varies from 15–110 g in maturate state [17].

3.8. Food and feeding habits[18]:

Feeding is the essential component for culture of *P. gonionotus* in shrimp ghers. The natural food of fishes can be divided in to the following groups:

I) Main or basic food: Which is the natural food consumed by fish under favourable condition.
II) Occasional food: Fish when available eats this types food in small quantities.
III) Incidental food: Which rarely enters in the gut along with other item
IV) Emergency food: which is ingested in the absence of food or which the fish is able survives.

3.9. Breeding nature[9, 17]:

P. gonionotus dwells and breeds in the estuaries during the monsoon. An attempt has been made for artificial breeding and fry production of *P. gonionotus* both in Bangladesh and India. For artificial breeding, fully gravid female (the genital papilla was found to have a thick muscular ring round the opening) and male *P. gonionotus* (had a muscular, conical reddish-pink genital papilla) were collected and kept in rectangular cement tank providing suitable condition. After 3 days, a synthetic hormone named "ovaprim" at the rate 2.0 ml/kg body weight was injected and breeding hapas were provided with gentle aeration and overhead water shower.

3.10.Materials and Methods:

Fish and fisheries of *P. gonionotus* were investigated a selective area consisting of the district of Pabna, Rajshahi, Bgora and Natore. For this purposes, three shrimp's ghers and one river were selected. In our investigation, the study area–1, study area–2, study area–3 and study area–4, indicated these studied areas respectively. The studies were carried out during the month of June-2014. The data were collected through questionnaire survey from fishermen and different fish traders. The study areas are showing in Fig. 2.4.

Physical conditions of ghers, like gher dike, water depth of ghers, colour of water, temperature of water in ghers and river, sources of water, gher's preparations etc were observed. To know about the fish habitat water quality such as, TDS (total dissolve solid), TSS (total suspended solid), salinity, conductivity, dissolve oxygen (D.O), free carbon dioxide (CO_2), chemical oxygen demand (COD), hardness, Ca^{2+}, mg^{2+} were also measured. For these investigations, water sample was collected from the respective ghers and river. Some parameters like dissolve oxygen (DO), free CO_2, salinity, pH and temperature were analysed in *situ.* For the estimation of hardness, calcium and

magnesium water samples were preserved with 1 ml. HNO_3 and for COD determination the sample was preserved by 1 ml. of concentrated H_2SO_4. Different water quality parameters were measured in different methods which are shown on Table **1.1**.

At the beginning of gher preparation, bottom mud was removed, dike repairment was carried out. Aquatic weeds (harmful) became removed and predator control was done by using rotenoneon. Then lime was used to regulate the pH of water and soils of the ghers. For this purpose 15–100 kg/hactre (ha) of lime was spread over the gher bottom including dike walls. Besides these, fertilizers were used to enhance the neutral productivity by promoting the growth of phytoplankton and zooplankton those services as a feed of *P. gonionotus* and shrimp. For this purposes different types of fertilizer such as cow dung, urea, TSP etc in a required quantity were used.

The average value and standard deviation (± SD) of physical and chemical factors of water in different study areas were recorded and presented in table **1.3** and table **1.4** respectively.

Finally, the details information of all the studied gher were collected from the ghers owner's through a structural questionnaire.

Questionnaires for collection of data in present of gher farming in the selective zone of Bangladesh:

Name of the interviewee:

Age:

Village:

Thana:

District:

Questions and Informations:

1. Gher ownership — O Single; O Multiple; O Public
2. Whether the gher by the genuine owner? — O Yes; O No
3. Terms and condition of present use. — O Lease; O Share; O Others
4. Ghers area (excluding embankments):
5. Age of ghers.
6. Culture systems: — O Extensive; O Improve; O Traditional
7. Culture able species:
8. Condition of embankment: — O Complete; O Incomplete
9. Water colour: — O Clear; O Light green; O Green; O Turbid
10. Whether there are weeds or not: — O Presence of weeds; O Absence of weeds

11. Minimum and maximum water level in rainy and dry season:

12. Water turbidity: O Low

O High

O Moderate

13. Dose of manuring during gher preparation: O Cow dung

O Poultry faces

O Urea

O TSP

O MP

O No manuring

14. Dose of liming:

15. Frequency of monitoring system of water quality: O Daily

O Weekly

O Nothing

15. Water source:

16. Do you give supplementary feed? O Yes

O No

17. If yes what type of feed given: O Oil cake

O Rice bran

O Wheat bran

O Others

18. Time of interval in feeding?

19. When do you give food?

20. Harvesting system: O Single

O Multiple

(If multiple, how often)

21. Mention the harvesting system?
22. Mention the harvesting procedure?
23. Mention the gear used for harvesting?
24. Are you illiterate?
25. Location of fishing area:
26. When the larger sized fish obtained?
27. In which month the total catch is high?
28. Fish selling area?
29. Mode of transportation?
30. Socio- economic status:

Signature of responded Signature of investigator

3.11. Results and observations:

The size of study area–1, study area-–2 and study area–3 were 10 ha, 15 ha and 23 ha respectively. Dike of study area–1 and study area–3 were found properly constructed (Fig. **2.4.2**) and damaged dikes (study area–2) were found in other gher due to various reasons. During the study period, water depth was found highest in study area–2 and study area–3 (0.95 meter and 1.3 meter respectively) and minimum in study area–1 (0.75 meter). During the rainy season, the ghers in the studied areas contained 0.76–1.7 meter water depth and in the dry season the ghers in the studied areas contained no water. From this study, it was found that during gher preparation, most of the fishermen use lime; different types of fertilizer (inorganic and organic) were used to promote

the growth of natural feeds. The amount of lime and organic fertilizer used in gher depends on the pH and organic substances present in soil.

Water from Padma, Kalindi and Ichamoti rivers are the main source of water for all respective studied ghers. Water from these rivers is collected with the canals during the month of February–March in the time of full moon and new moon. During full moon, *P. gonionotus* fishes are migrated over shorter distance (ghers) through effective screen (Fig. **2.4.3**) with other estuarine fishes. A typical source of water used in gher is shown in (Fig. **2.4.4**). During the time of water collection, some other fish fry locally known as vetki, liza parsia, horina chingri, chaka chingri, tilapia etc are also easily moved towards with rivers water.

During the investigation, the colour of waters for both ghers and river was not clear. Watercolour of all study areas was found as lemon colour except river where the water was turbid.

Different types of feed such as oil cake (Khoile), rice bran (Vushi), wheat bran etc were used as feed for shrimp fry and also for *P. gonionotus.* Beneficial aquatic weeds and some zooplankton are also used as feed for these fishes. In studied areas, fishermen supplied supplementary food daily in the morning and evening in certain spot in the gher. A watching room used by the fishermen for overall inspection of ghers (Fig. **2.4.7**). Total dissolved solid in water body were found to be 17.6 ±2.25 g/L, 21.7±1.63 g/L, 19.1±1.98 g/L and 22.3±1.12 g/L for study area-1, study area-2, study area-3 and study area-4 respectively. Highest value of total suspended solid (TSS) (0.051±0.01g) was found in study area-1 and lowest (0.020±0.01) in study area-4. For study area-2 and study area-3 the amounts of TSS were 0.025±0.01g and 0.046±0.02 g respectively. The conductivities of water were found 24.7±0.95 ms/cm, 30.7±1.26 ms/cm, 27.1±1.50 ms/cm and 33.85±0.82 ms/cm for four respective investigated areas.

Density of water in all study areas were found identical and the values were 1.011±0.01 g/cc, 1.012±0.02 g/cc, 1.010±0.04 g/cc and 1.022±0.01 g/cc for respective ghers and river.

Alkalinity in study area 4 (Padma river) had minimum value (101±5.28 mg$CaCO_3$/L) than any other study areas. Maximum value of alkalinity (200±3.56 mg$CaCO_3$/L) was found in study area 3 and for the rest of two study areas (study area-1 and study area-2), the values were found 150±5.24 mg $CaCO_3$/L and 165±4.52 mg $CaCO_3$/L respectively. Highest amount of salinity (22±2.45 ppt) was recorded in study area 4 where as lowest salinity (14±1.98 ppt) was observed in study area 3. In the present study, it is found that dissolved oxygen (D.O) in different study areas were varied from 5.6±0.04 mg/L to 6.7±0.04 mg/L. The maximum value of dissolved oxygen was found in study area 3 and minimum was in study area 4 (Padma river). The values of free CO_2 in the water body of study areas were 4.7±0.05 mg/L, 4.1±0.01 mg/L, 3.9±0.03 mg/L and 5.5±0.04 mg/L for the study area-1, area-2, area-3 and area-4 respectively. Hardness (mg $CaCO_3$/L), Ca^{+2} (mg/L), and also Mg^{+2} (mg/L) were found highest (3420±8.78, 761.50±7.58, 2658.50±15.87 respectively) in study area–2 and the lowest was found (630±2.54, 70±2.46 and 110.75±4.75 respectively) in study area-4. The values of hardness (mg $CaCO_3$/L), Ca^{+2} (mg/L), and also Mg^{+2} (mg/L) for study area-1 and for study area-2 were 2540±8.64, 561.12±5.76 and 1978.88±7.65 respectively and 2620±5.66, 601.20±2.54 and 2018.80±12.87 respectively.

Fishes are available in ghers during full moon but opposite cases are observed in river. During full moon, naturally adult fishes are available in a specific place in ghers locally known as Goie (Fig.**2.4.8**). Fishermen partially harvest *P. gonionotus* by traditional method of fishing. For these purpose, different types of crafts and gears like throwing net (Fig. **2.4.9**), drang nets and other nets were also used. Fish trapped in a net is shown in figure **2.4.11**. After harvesting shrimps, finally, fishermen carried out complete harvest of *P. gonionotus* in the month of Februarywith others species (Parsia, vetki, tilapia etc locally known

as white fish) by draining out the gher's water. Different types of storage bin (Fig. **2.4.12**) were used for carrying fish.

The cost of ghers preparation including fertilization, liming, labour and harvesting was considered as the production cost. It also includes purchasing of shrimp fry in which *P. gonionotus* fry are enter in to the gher automatically. Total production costs per ha gher was found around 150,000.00 TK. Total production of fish per ha was 750 kg shrimp, 40 kg *P. gonionotus* species and 95 kg of others fishes. The price rate was about TK.375/kg for shrimp, TK. 110/kg for *P. gonionotus* and TK. 100/kg for other species and the total income was 295150.00 TK. So the net profit per ha gher was 145000.00 TK.

Marketing of the fish *P. gonionotus*received a limited attention because quantities marketed on a daily basis, in a given locality, are often in small quantities. The marketing system consisted with a market and marketing channel along a transportation system and some storage facilities. The marketing system lies between the producer and the final consumer [19]. Pabna, Rajshahi and Bogra, district are the regions where*P. gonionotus* species is sold. Most traders buy their consignment from specific fishermen and operate a push-bicycle. Sometimes, dealers come in lorries or small vans for their daily purchasing. It is rare, that a fishermen himself is engaged in trading. There has been a gradual increase in the price obtained by the fishermen, as well as in the selling price by the trader.

The price of the species varied marginally from area to area and market to market. The price also varied with the size and quality of fish. Bigger fishes selling for about 1.5 times higher price than the smaller fish. The price to the consumer varied from season to season. In rural markets, the consumer has to pay more during the paddy harvesting season when migrant labour is utilized to work on the rice fields because of the increased demand for fresh fish to feed this labour force.

3.12. Discussion:

Being a most important and cheap source of protein and providing certain useful nutrients, fishes have great significance in the life of mankind. Fishes, especially the brackish water catfish, found in coastal region in Bangladesh are the sole contributor of nutrients, protein and minerals.*P. gonionotus*, provide an easily accessible and affordable animal protein sources to the rural and urban sectors of the communities in the district of Pabna, Bogra, Rajshahi and Natore district. The fisheries sub-sector of Bangladesh is broadly divided into inland and marine fisheries. Marine fisheries sectors in Bangladesh are associated with coastal region. In the coastal region, marine fishes are cultured mainly in ghers especially for shrimp species.

Areas of ghers and water depth of ghers should be 0.5 ha to 1. to1.2 meter respectively and also the location of ghers should be in a region where tidal action occurs regularly [20].

The dike of a gher is an important physical structure particularly in Bangladesh, where flood is a recurring and divesting phenomenon. The top of the dike around gher should be about 1 meter in width to allow workers to walk round the gher for carrying feeding and harvesting [21]. The height of dike should be high than the water level expected in the gher. Every year all the farmers in these study areas try to improve their gher dike. Improvement of gher dike is a major problem of coastal region because dike preparation is too much costly. In the canals, effective screens at both inlet and outlet are important criteria for gher as they provide the main barriers against entry of predators and pests. Each gher should be an outer protective barrier made with tightly packed bamboo poles which can protect the inner screens from physical damage.

The selective areas are the prominent for fish culture but in dry season ghers were disconnected from the rivers and this period paddy culture is carried out.

Food used in fish farming should have high nutritional values that promote optimal fish growth. High quality diet contains protein, carbohydrates, fats, vitamins and minerals. These supplied diets are intended only to support the growth natural food (insects, algae, and small fish) normally available in ghers.

Feeding rate and frequencies are important in a function of fish size. The most important rule in fish nutrition is to avoidover feeding. Over feeding is a waste of expensive feed. It is also results in water pollution, lowering dissolved oxygen levels, increased BOD and increased bacterial loads. Usually fish should be fed only the amount of feed that they can be consume quickly (less than 25 minutes). Off-feed behaviour is the first signal of trouble such as disease or water quality deterioration in the fish growing system [21]. The presence of helpful aquatic weeds is responsible for high productivity of fish in ghers. Stocking is a useful measure for the management small or seasonal water bodies. The Fishermen stocked the ghers disregarding the fry size, soil condition, and presence of natural feed, water depth and water quality. Stocking rate varies from area to area. The proper stocking rate in improved culture is 60–120 of PL/dec [22]. But in the surveyed areas, farmers did not follow any scientific method for fry stocking. Though the owners of the small ghers are try to follow the proper ratio but on the aspect of big gher owner's it is not possible because of their money problem and also lack of knowledge. The daily feeding rate will be raised gradually from the initial (6.25 kg/10^4 sq. metre/day) to a much higher level at harvesting time [23].

In the surveyed areas most of the farmers give supplementary feed in their ghers but they not do perform this regularly. As a result their production was lower than other fishermen who used supplementary feed regularly. Liming plays a significant role in the production of*P. gonionotus.* Post stocking lime was used to practice by the farmers whenever any out break of disease or any

abnormal situation prevailed. Feed supply should not carry out before harvesting period for affordable, safe and high-quality fish.

Physico-chemical characteristics of fish habitat are important factors for sufficient growth and production of fishes. Colour of water is the physical parameter which indicates the presence or absence of natural feed in the ghers and river. Colour of water should be monitored with secchi-disc 25 to35 cm. Transparency indicates optimum natural food of the gher [24]. Water may be coloured by addition of artificial feed and other foreign materials. Natural colour exists in water primarily as negatively charged colloidal particles [25]. Due to rainfall during investigation; the colour of water in study area-4 was turbid. Surface water may be appeared highly coloured, because of coloured suspended matter when in reality there not.

No remarkable changes were found in pH, temperature and density of water in different ghers. For living fish, pH must be controlled within a favourable range (7.5 to 9.0) [26]. Water of investigated fish habitats is slightly alkaline. From this study, it is said that pH range in the study areas is most favourable for fish culture. The values of total dissolved solid (TDS) and total suspended solid (TSS) in normal drinking water are 1 g/L and 0.001 g/L respectively as recommended by environmental quality standard [27]. The observed TDS and TSS in different fish habitats were higher than normal drinking water. This may be due to the addition of artificial feed and other chemicals in fish habitats. Densities were almost similar in different habitats and these values were slightly higher than the density of drinking water (1g/cc).

Electrical conductivity (EC) values indicate the total concentration of ionised constituents of a water sample. Electrical conductivity (EC) is closely related to the amount of total dissolved solid (TDS) and is also used as an index of salt content of water [28]. In the observation areas, electrical conductivity was found

24.7±0.95 to 33.85±0.82 ms/cm. Maximum value of TDS and conductivity were observed in study area-4. Numbers of bases like hydroxide, carbonates, bicarbonates etc may contribute in alkalinity of water [28]. Maximum alkalinity was observed in study area–3. This value indicates the relatively higher amount of bases present in water body in study area–3 than other studied ghers. Apart from the important role in photosynthesis of primary fish food organisms, free CO_2 has interdependence with pH and bicarbonate- carbonate equilibrium. Water having more than 8.3 pH does not contain appreciable amount of free CO_2. Water supporting good fish habitats usually contains less than 5 ppm of free CO_2 [30]. The values of free CO_2 are normal in all studied fish habitats (3.94 mg/L to 5.05 mg/L).

The solubility of oxygen is less in salt containing water than in clear water [25]. More than 5 ppm D.O level is suitable for normal survival and growth of fish [28]. Aeration should be carried out if dissolve oxygen level exist lower than 3.5 in gher [26].The COD is a measure of the total amount of oxygen which is required to oxidize all the organic matter in a sample to CO_2, and H_2O [25]. Highest COD and DO value were found in study area–2 and study area–3 respectively than any other study area. Fishponds generally have COD values of 10-200 ppm [28].

Divalent cation such as Ca^{+2}, Mg^{+2}, Sr^{+2}, and Fe^{+2} are sometimes considered as contributing to the hardness of water [25]. The hardness of water in all investigated fish habitats was very high caused due to the presence of Ca^{+2} and Mg^{+2}.Since lime and other fertilizers were used during ghers preparation, so amount of Ca^{+2} and Mg^{+2} were relatively higher in different ghers than study area–4 (Mongla River). The recommended water quality ranges is salinity 5–25 ppt, dissolve oxygen (DO) 5–7 mg/L, pH 6.5–8.5, transparency 30–35 cm and temperature 24–28°C dec [22]. A previous studied was conducted by BFRI [30]to

determine some water quality parameters (water temperature, pH, salinity and also total hardness) in different rivers which is shown table **1.5**.

The socio-economic survey indicates that most of the fishermen in the study areas were living in huts and their living space are less than 0.5 ha. Fishermen in these study areas are partially depending on indigenous fishing during the fishing season and other periods they are involved in agricultural sector. Most of the fishermen do not have any land ownership. Most of the fishermen are illiterate, having moderately large family members. The fishermen are mostly poor. Their original sources of income depend on fishing in the respective ghers and adjacent areas. The earning from fishing is not sufficient for the fishermen to maintain and living standard. Due to the over all poverty of marine fishermen families, many children of school going age are also involved in fishing or in fishing related activities. It is observed that most of the fishermen households have a monthly income of TK. 5000.00–5500.00.

Table **1. 1**: Methods used to analyse water quality parameters of *P. gonionotus* habitat [28, 31].

Name of parameters	Methods/Instruments.
Temperature	Laboratory of Field Methods.
Colour	Visual Comparison Method.
TDS, conductivity	Instrumental Methods.
	TDS/Conductivity Meter. HANNA, HI 9635.
TSS	Evaporating Method.
Density	Density Method.
	Density Analyser, DSA-5000.
pH	Electrometric Method.
	pH Meter (HANNA)
Dissolve oxygen (D.O)	Winkleris Method.
Chemical oxygen demand (C.O.D)	Open reflux Method.
Free CO_2	Titrametric Method
Total hardness	EDTA Titrimetric Method
Ca^{+2}	EDTA Titrimetric Method
Mg^{+2}	Calculation Method
Salinity	Electrical Conductivity Method
	Refractometer
Alkalinity	Titration Method

Table **1.2:** Physical factors of water as fish habitat.

Study area	Colour water	Temperature (°C)	Conductivity (ms/cm)	Density g/cc	Total dissolved so1lid (TDS gm/L)	TSS (g)
1	Lemon	30 °C±0.07	24.7±0.95	1.011±0.01	17.6±2.25	0.051±0.01
2	Lemon	31°C±0.06	30.7±1.26	1.012±0.02	21.7±1.63	0.025±0.01
3	Lemon	30°C±0.04	27.1±1.50	1.010±0.04	19.1±1.98	0046±0.02
4	Light turbid	30°C±0.02	33.85±0.82	1.011±0.01	22.3±1.12	0.020±0.01

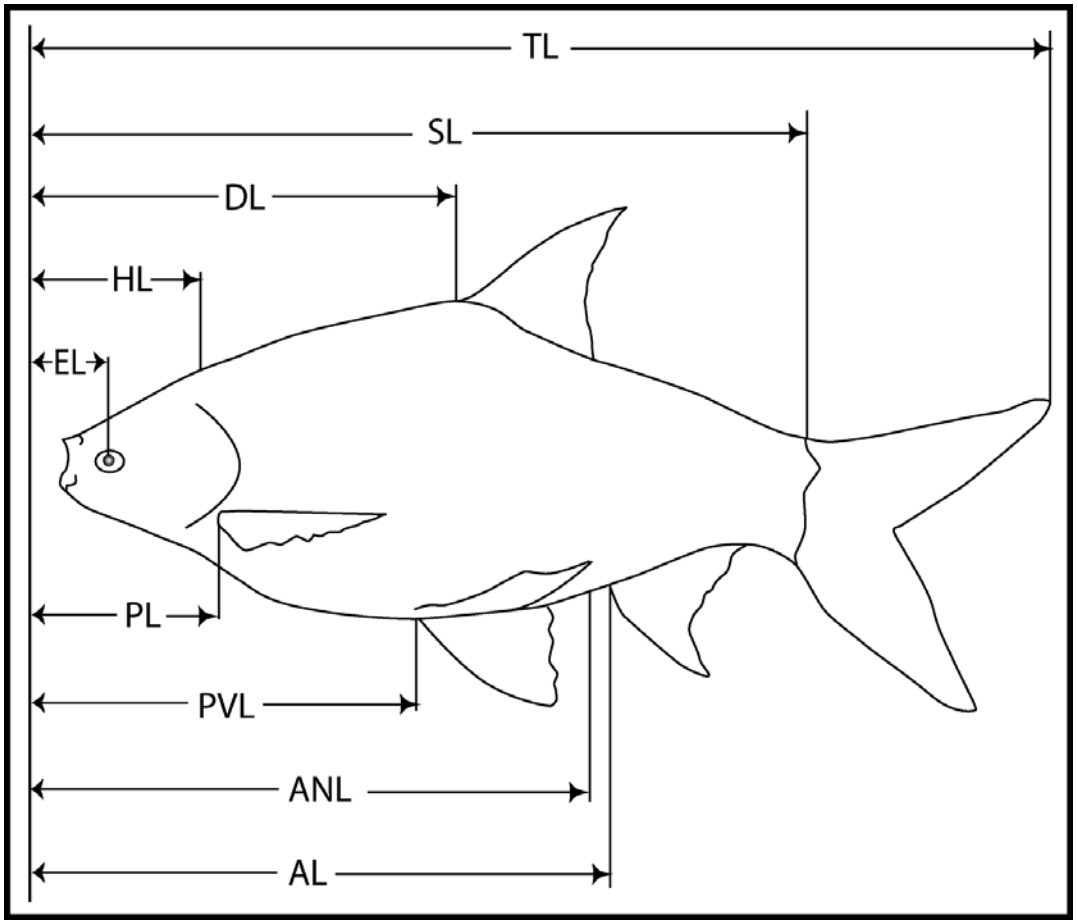
Table **1. 3:** Chemical factors of fish habitats.

Study area	PH	Alkalinity ($mgCaCO_3$/L)	Salinity (ppt)	D.O (mg/L)	Free CO_2 (mg/L)	C.O.D (mg /L)	Hardness ($mgCaCO_3$ /L)	Ca^{+2} (mg/L)	Mg^{+2} (mg/L)
1	8.1±0.02	150±5.24	15±1.54	6.3±0.03	4.7±0.05	20±1.45	2540±8.64	561.12±5.76	1978.88±7.65
2	7.5±0.32	165±4.52	18±2.76	6.0±0.08	4.1±0.01	140±1.88	3420±8.78	761.50±7.58	2658.50±15.87
3	8.0±0.56	200±3.56	14±1.98	6.7±0.04	3.9±0.03	40±2.65	2620±5.66	601.20±2.54	2018.80±12.87
4	7.9±0.01	101±5.28	22±2.45	5.6±0.04	5.5±0.04	39±3.45	630±2.54	70±2.46	110.75±4.75

Table **1.4**: Water temperature (°C), pH, salinity (ppt) and total hardness (mg/L) of different rivers during July-2000 [31].

Study area	Water temperature (°C)	pH	Salinity (ppt)	Total hardness (mg/L)
Padma	30.0	7.1	4.0	645
Ischamoti	28.0	6.7	12.0	2460
Kalimdi river	31.0	7.8	3.0	630

Source: BFRI, 2003.



TL = Total length; SL = Standard length; EL = Eye length; HL = Head length; DL = Dorsal length; PL = Pectoral length; PVL = Pelvic length; ANL = Anal length; AL = Anus length

Fig. **2.1**: *P. gonionotus*specimen with distinguishes characters.

Fig. **2.2.1**: Side view of *P. gonionotus* (Hamilton, 1822)

Fig. **2.3.1**: Geographical distribution of*P. gonionotus* in the world adapted from Worldatlas.com

Fig. **2.3.2**: (●) Distribution of *P. gonionotus* in Bangladesh adapted from DoF 2004.

Fig. **2.4.1**: Partial view of gher (polder).

Fig.**2.4.2**: Gher (polder) dike and traditional fishing craft (Bachari Nauka).

Fig. **2.4.3**: Collection of fry in gher (polder) from adjacent river.

Fig. **2.4.4**: River as a source of fish habitat.

Fig.**2.4.5:** Watching room in gher (polder).

Fig.**2.4.6**: Goie where adult fishes are available during full moon.

Fig. **2.4.7**: Throwing net used in gher (polder).

Fig. **2.4.8**: Trap (Kholson) used in gher (polder).

Fig.**2.4.9**: Basket used for carrying fish.

Summary and conclusion

Bangladesh is blessed with both fresh water and marine fisheries resources. A decline in marine fish production is noticed in recent years. Bangladesh Fisheries Development Corporation (BFDC) has remained fully dedicated to the development of fisheries in Bangladesh especially in the field marine fisheries. In Bangladesh *P. gonionotus*, a brackish water fish is found mainly in the district of Pabna, Rajshahi, Bogra and Natore. In these regions, *P. gonionotus* may be most important fish source available to rural and urban communities. In these regions, this fish species are found in ghers in shrimp farms. The study was carried out in three ghers and one river .The physical and chemical parameters were studied of these study area. Physical condition of all ghers were found both properly (for two ghers) and improperly (for one gher) constructed. In the month of June to September, the water level in ghers and river greatly increased by rainfall and flood water. If ghers dike are not properly constructed the ghers may be flooded and fish culture may be affected. During the study period, it is seen that two types of food (both natural and artificial foods) were used for shrimp's culture which is also used as feed for *P. gonionotus*. There are various types of aquatic weeds were found in the investigation period which are including Kata saula and pata saula. Chemical condition of these study areas that is water quality was observed different in different areas. Temperature, pH, and density were found similar and favourable for *P. gonionotus* where colour of water was found lemon for all ghers. For river turbid water was found.

TDS, conductivity, salinity and free CO_2 were found maximum where hardness (mg $CaCO_3$/L), Ca^{2+} (mg/L), Mg^{2+} (mg/L), dissolve oxygen ((mg/L) and TSS (g) were found minimum for Padma river (Study area-4) than the ghers. Free CO_2 and D.O were reliable for this fish culture but hardness's were so high and influenced the fish habitats.

In the ghers and river there are different types of crafts and gears found to engaged in fishing. Among these, the net (Bhesal Jal) was found to use extensively and next khepla jal and gill net. Besides these, there are several traps were mostly used for fishing. Marketing system of this fish was found in local market in urban and village's hats. Most of the fishermen have to sales their fish directly to the holders. Transportation media is comparatively well in all study areas. Adequate number of carrier boats should be introduced for its transportation and better utilization. The weight standard is mainly in kilogram (kg) and the prices of fish vary according to season.

In the fishery, maximum labour force is employed in the traditional manner. They have normally large family members. Most of the families were found engaged in fishing and most of them are illiterate and their incomes from fishing are not sufficient for their families. The fishermen of this of areas are mostly poor. Fishermen are much less familiar with the culture of *P. gonionotus*in shrimp's farms because of the lack of breeding and feeding techniques and non-availability fish fry from wild. It is expected that when *P. gonionotus*fish species are properly cultured in a commercial grade in these ghers the local people will provide a source of much needed animal protein for Bangladesh peoples and the excess of production can be exported and earned a lot foreign money. For these purposes it has been needed to develop a new breeding and modern cultured technology. In order to make this fish culture successful there is an urgent need for development low cost feeds, primarily using locally available ingredients. Processing techniques have to be developed to make them most attractive for consumption. Breeding and seed production of *P. gonionotus* in captive conditions would open a new era in the country for aquaculture and conservation of this commercially important brackish water species.

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CHAPTER 04

STUDIES ON PROXIMATE COMPOSITION OF FISH FLESH AND PHYSICO-CHEMICAL CHARACTERISTICS OF FISH LIPID IN *P. GONIONOTUS*

Local Name : Thai shar phuti/China punti
Scientific Name : *Puntius gonionotus*

Studies on proximate composition of fish flesh and physico-chemical characteristics of fish lipid in *Puntius gonionotus*

4.1. Introduction

Lipids are the generic names assigned to a group of fat-soluble compounds found in the tissues of plants and animals[1]. The lipids are important constituents of the diet because of their higher energy value and also because that the fat-soluble vitamins and the essential fatty acids are found within the fat of the normal foodstuffs. In the human body, fat serves as efficient source of energy which is stored in the adipose tissues. They also serve as an insulating material in the sub-cataneous tissues and around certain organs [2]. Specialized lipids serve as pigments (retinal), cofactors (vitamin-K), detergents (bile salts), transporters (dolichols), hormones (vitamin D derivatives sex hormones), extra cellular and intracellular messengers and anchors for membrane proteins [3].

Dietary fats facilitate absorption and transportation of fat-soluble vitamins [4, 5]. These are the concentrate source of energy which provides energy at the rate of 9 calories per gram, more than twice as available from an equal mass of carbohydrates and proteins [6]. High accumulation of fat in body develops heart disease, breast's cancer and obesity. Fishes are the good natural sources of dietary fat, protein, vitamins and minerals [7].

ω- 3 polyunsaturated fatty acids (the type of fat found in fish oil) have been used to kill or slow down the growth of cancer cells in the culture media and model animals and to increases the effectiveness of cancer chemotherapeutic drugs[8]. It has been considered for some time that many animals need polyunsaturated fatty acids (FUFAs) in their diet if proper growth is to be maintained and that reproduction, lactation, longevity of tissue structures as

well as regulation of the plasma, liver lipid and liver cholesterol levels are also depended on these fatty acids [9].

Fishes have the unique capability of metabolising these compounds readily and, as a result, can exist for long periods of time under conditions of food deprivation [1]. There is substantial evidence that diets rich in polyunsaturated fatty acids (FUFAs) such as those found in fish oil (eicosapentaenoic acid and docosahexaenoic acid) protect against colon carcinogenesis [10]. Polyunsaturated fatty acids (FUFAs) especially linoleic acid is necessary for the proper functioning of many metabolic processes. In view of these recognized usefulness of this fish with respect to lipid characteristics, the present investigation was undertaken. The present work reported the proximate composition of fish flesh; extraction of lipid from fish flesh and to carried out analytical tests of the crude lipid to ascertain some of its physical and chemical characteristics of the fish, *Puntius gonionotus* (*P. gonionotus*).

4.2. Methodology

4.2.1. Sample collection:

Standard medium sized alive *P. gonionotus* were collected from the local market of Shaheb Bazar. Keeping into ice, the samples of fish were brought into the laboratory for analysis within hours of collection. Finally, they were cleansed by discarding their scales, bones, stomach, viscera, fins etc. and only the body flesh of the fishes were preserved in frozen condition in a refrigerator.

4.2.2. Determination of proximate composition of fish flesh

4.2.2.1. Lipid: Lipid was extracted from *P. gonionotus* by Bligh –Dyer method [11]. For this purpose, 30 g of fish flesh was blended into a slushy mass with a blender machine (Blender Mill-2) and the lipid from the fish was extracted by using a mixture of chloroform and methanol (2: 1 v/v). For complete

extraction, this mixture was kept overnight at room temperature and then the resulting mixture was subjected to centrifugation. Clear lower layer of chloroform, containing lipid was carefully collected in a pre-weighed beaker and the solvent was removed from the lipid at low temperature (to prevent oxidation) with the help of a rotary film evaporator. When the lipid was free from organic solvent, the weight of lipid was determined. The lipid was then stored at 4°C in the presence of an inert gas (N_2 gas) for further experiment [12].

4.2.2.2. Moisture[9]: A quantity of 5.87 g fish flesh (previously dried at room temperature after collection) was weight in a porcelain crucible which was previously cleaned, heated to about 100°C and cooled. The crucible with the sample was heated in an electric oven (W.C. HERAEOUS GMBH, HABAU) at 100°C keeping constant weight. The loss of weight represented the moisture content and was calculated as a percentage of the weight of fish employed.

4.2.2.3. Ash[13]: A fixed amount of fish flesh (15 g) was weighed in a porcelain crucible (previously cleaned, heated to about 600°C and cooled) and was ignited in a muffle furnace at 600°C for about 6 hours. For ensuring completion of ashing, the crucible was heated again in the muffle furnace for half an hour; cooled and weighed. This was repeated till two consecutive weights were same and the fish flesh was almost white in colour. The ash was cooled, weighed and the percentage of total ash was calculated from the weight of the fish flesh taken.

4.2.2.4. Dry solid substance[14]: The amount of dry solid substance in wet fish was calculated by subtracting the amount of moisture from the amount of wet fish flesh.

4.2.2.5. Crude fibre[15, 16]:About 6.77g of moisture and fat free sample was weighed into a 500 ml beaker and 200 ml of boiled 0.225 N sulphuric acids was added to the sample. The mixture was boiled for 30 minutes keeping the volume constant by the addition of water at frequent intervals. At the end of

this period, the mixture was filtered through a muslin cloth and the residue was washed with hot water till free from acid. The materials were then transferred to the same beaker and 200 ml of boiled 0.313N NaOH was added. After boiling for 30 minutes (keeping the volume constant as before) the mixture was filtered through a muslin cloth. The residue was washed with hot water till it was free from alkali; followed by washing with few ml of alcohol and ether. It was then transferred in a preheated and weighed crucible, dried at 100 – 105°C and later weighed. The crucible was then heated in a muffle furnace at 600°C for 2 – 3 hours and then cooled in desiccators and weighed. The loss in weights represented the crude fibre content. The percentage of crude fibre was calculated by the following formula.

$$\% \text{ Crude fibre} = \frac{\text{Loss in weight noted}}{\text{weight of fish taken}} \times 100$$

4.2.2.6 Protein [17, 18]: The estimation of nitrogen was done by Kjeldahl method which depended upon the fact that organic nitrogen when digested with sulphuric acid in presence of catalyst (copper sulphate) was converted to ammonium sulphate. Ammonia liberated by making the solution alkaline was distilled into a known volume of a standard acid; collected ammonia was then measured by back titration. The protein content was obtained by estimating the nitrogen content of the nitrogenous material and multiplying the nitrogen value by 6.25 [13].

Procedure:

The wet defatted fish flesh (1g) was accurately weighed out into a long neck round-bottomed flask commonly known as Kjeldahl digestion flask. Then, 4–6 ml of concentrated sulphuric acid was added to it carefully. About 1 g of K_2SO_4 solution (catalyst) and some quartz of chips (to avoid bumping) were also poured into the flask. The flask was placed in Micro-Kjeldahl's digestion rack in a slightly inclined position, at an angle of about 60 degree. The flask

was heated with a low flame (310–410ºC) in a fume cupboard. When foaming had ceased, the flame was increased until the mixture was boiling gently. The heating was continued for 30 minutes after the solution became colourless or clear (usually 90–120 minutes) and then the solution was allowed for cooling. While it was cold, 100 ml of water was added carefully; a little at a time and with frequent shaking. The flask was then cooled under the tap water. The contained of the flask was transferred completely to the ammonia-distillation apparatus and excess of cold 50% NaOH solution with a few fragments of porous porcelain was also added.

The ammonia was distilled off into excess of standard acid. A blank determination exactly above was carried out omitting the nitrogen-containing sample. The blank reading was subtracted from the actual one. The liberated ammonia absorbed in an almost saturated solution of boric acid.

$$NH_3 + H_3BO_3 = NH_4^+ + H_2BO_3^-$$

The borate formed was determined by titration with standard 0.1N HCl using a mixed indicator bromocresol green.

$$H_2BO_3^- + H^+ = H_2BO_3$$

The percentage of nitrogen was calculated from the following formula:

$$\% N = \frac{(V_2 - V_1) \times 0.01401}{W} \times 100$$

Where,

V_2 = Volume in ml of standard HCl used in the actual titration.

V_1 = Volume in ml of standard HCl used in the actual titration.

N_A = Normality of the HCl and.

W = Weight of the sample in gram.

The total nitrogen content when multiplied by a factor 6.25, afforded the amount of total protein in the sample.

4.2.3. Physical characteristics of fish lipid

4.2.3.1. Refractive Index: Refractive index is often very useful in identifying a liquid substance. This is determined from the ratio of the speed of light through a vacuum to the speed of light through the substance [19]. When a ray of light passes from air into a denser medium, e.g. in a liquid, it is bent or refracted towards the normal. The sine of the angle of incidence and that of refraction is constant and characteristic of that liquid (Snell's law). The constant ratio is called the refractive index [20]. The refractive index may be determined by means of the Abbe refractometer, (Standard model 60/70) which gives the true refractive index, n_D [9].

Procedure [19]:

The double prism was opened by means of the screw head and a few drops of lipid were placed on the lower prism so that the entire width of the prism plate was covered. The upper prism part was then brought into contact with the lower prism so that the lipid forms an unbroken layer between the two. The cross wires of the telescope were focused by rotating the eye piece and the mirror to get good illumination. The alidade was moved on the side scale backsword and formed until the field of vision is divided into light and dark portions. The screw head of the compensator was rotated until a sharp colourless line was observed between the fields and then adjusted this line so that it falls on the point of intersection of the two (2) cross hairs. The reading of the index of refraction was taken off directly from the scale through eye piece.

4.2.3.2. Density [21]: The density of a liquid may be defined as the mass of unit volume of the liquid. For measurement of density of fish lipid, a high precision

vibrating-tube digital density meter (model DMA 5000, Anton Paar, Austira) was used. The method is based on the principle of time-lapse measurement for certain number of oscillations of a vibrating U-shaped sample tube filled with the sample liquid. At constant temperature, the natural vibration period of the U-tube is relatedto the density of lipid filling the tube. In the latest version of Anton Paar Digital Density Meter, DMA 5000, Austira, the natural vibration period is automatically converted into the density value and displayed directly on the LC display monitor of the density meter.

Using a polyethylene syringe the sample was continuously and slowly injected from the upper part of the U-tube until the excess fluid flowed out of the lower part. This was ensured that the inner surface of the cell was completely wet and there were no micro bubbles inside the U-tube. The syringe was kept as such in plugged. After the measurement, the sample was removed and air was passed by built-in pump, through the tube to remove excess lipid. The density meter was checked by measuring the density of standard water supplied with the density meter.

4.2.4. Chemical characteristics of fish lipid

4.2.4.1. Iodine value [9, 22]: One of the most important index upon which test of oil quality is done is the value of iodine. It is the most customary and generally useful means of assessing in the total unsaturation present and excepts when only two unsaturated species (e.g linoleic and oleic) are present, the same can not be used to determine the proportion of each unsaturated compound. It indicates the percentage of iodine (I_2) absorbed by an oil or fat when the latter is dissolved in chloroform or carbon tetrachloride and treated with an accurately measured amount of free iodine supplied in the form of iodine chloride. By this means a measure is obtained of the unsaturated fatty acids contained in oil or fat [23].

$$\text{Iodine value (I.V)} = \frac{(X - Y) \times I}{W} \times 100$$

Where,

X = Volume in ml of $Na_2S_2O_3$ solution required in the blank experiment.

Y = Volume in ml of $Na_2S_2O_3$ solution required in the sample.

S = Strength of $Na_2S_2O_3$.

W = Weight of the oil taken in grams.

I =Weight in grams of iodine equivalent to 1 ml of the $Na_2S_2O_3$.

Procedure: The fish lipid (0.5g) was dissolved in a glass-stoppered bottle containing 10 ml chloroform and then 25 ml of Hanus solution was added to the bottle. The contents of the bottle were allowed to stand in the dark place for 30 minutes with shaking occasionally. A quantity of 10 ml of 15 % KI solution was added to the above solution and the mixture was shaken well. Freshly boiled and cooled distilled water (100 ml) was added to the mixture and the liberated iodine was titrated with 0.1N $Na_2S_2O_3$ solution using starch solution as indicator. A blank experiment (without the sample) was performed exactly in the same procedure describe above.

4.2.4.2. Saponification value[15]: When a fat is boiled with an excess of alcoholic KOH, the triglycerides were hydrolysed and glycerol and soap were formed. The alkali consumed the hydrolysates is a measure of the saponification value. Saponification value is defined (S.V), as the number of milligrams of KOH required saponifying completely one gram of the oil or fat. It is also a measure of the mean molecular weight of the fatty acids originally bound as triglycerides.

Procedure: The fish lipid (1.25gm) was taken in a 250 ml flask resistant to the action of alkali and mixed with 25 ml of alcoholic KOH solution with some glass beds. The flask was connected to reflux condenser .The flask was then boiled gently but steadily until the sample was completely saponified as indicated by the absence of any lipid matter with the appearances of clear solution. A blank determination was also conducted alongside.

After cooling, about 1 ml of phenolphthalein solution was then added in both the saponified and the blank solution. Testing sample and blank solution was titrated against the standard (0.5N) HCl solution. The difference in the titration values gives the amount of alkali consumed in terms of standard acid.

If W is the weight of the lipid and V_b & Vw are the number of acid in ml required to neutralize the blank and testing solution respectively after refluxing, we have,

$$S.V = \frac{(Vb–Vw) \times 56.1 \times \text{strength of acid}}{W}$$

4.2.4.3. Sponification equivalent [9]: The saponification equivalent is the amount of fat saponified by 56.1 grams of KOH (i.e. one equivalent). The saponification equivalent is related to the saponification value (S.V) by the formula:

$$\text{Saponification equivalent} = \frac{56100}{S.V}$$

4.2.4.4. Ester value [22]: The ester value is a measure of the organic acids combined as esters and it has the same standard of reference as the acid and saponification values. It is obtained by deducting the acid value from the saponification value.

4.2.4.5. Unsaponifiable matter[15, 24]: The materials that present in oils and fats which after saponification of the oils or fats by caustic alkali and extraction by a suitable organic solvent specified remains non- volatile on drying at 80°C are

called unsaponifiable matter. Unsaponifiable matter thus defined includes hydrocarbons, higher alcohols and sterols such as cholesterol and polysterol.

Procedure:

The principle is based on the preliminary saponification of the lipid with caustic alkali and subsequent extraction of the soap formed by means of the solvent. About 0.49 g of lipid was weighed accurately and taken into a 250 ml round bottom flask and 50 ml of 1N ethanolic KOH solution was added to it. The flask was attached to the reflux condenser and the mixture was boiled for half an hour at 70–80°C on a steam bath with occasional shaking to effect saponification. The solution was then transferred to a 500 ml separating funnel and the flask was rinsed with 100 ml of distilled water and then with ether. The mixture was shaken well when it was still warmed and allowed to stand to separate the layers. The lower layer was drawn off in to the saponification flask and the ether extract was transferred to a second 500 ml separating funnel containing 40 ml water. In this way, two further extracts were carried out each with 100 ml ether and the extracts were combined.

The total ether solution was washed vigorously three times with 40 ml of water and then three times with similar quantities of KOH solution (0.5N). Finally, the solution was washed with water until the washed water was no longer alkaline towards phenolphthalein. The ether solution was concentrated on the water bath and transferred to a pre-weighed 250 ml conical flask and was dried to constant weight. Drying was assisted by adding 4 ml of acetone to the extract. The weight of residue was noted after cooling. The residue was then dissolved in 50 ml of neutral alcohol and titrated with 0.1N NaOH using phenolphthalein as indicator.

The following equation was used to calculate for estimation the percentage of unsaponifiable matter,

$$\frac{\text{Weight of residue – weight of fatty acids in the residue}}{\text{weight of sample taken}} \times 100$$

Where,

Fatty acid content in the ether extract as oleic acid in grams = ml of NaOH × N NaOH × 0.282.

4.2.4.6. Acetyl value[20]: The acetyl value is the number of milligrams of potassium hydroxide required to neutralize the acetic acid obtained when 1 gram of acetylated lipid or fat is saponified. The amount of acetic acid present in 1 gram acetylated material can be determined by diverse means. The bound acetic acid is liberated by complete saponification and after separating the water-soluble fatty acids, titrated in the aqueous phase with alkali. With the ml of 0.1N KOH used (a) and the weight of the acetylated sample in gram (W), it can be calculated as follows:

$$\text{Acetyl value} = \frac{a \times 5.61}{W}$$

Procedure:

The fish lipid (1.4850 g) was boiled with 20 g/ml of freshly distilled acetic anhydride for two hours under a reflusk condenser. The cooled product was poured in large beaker containing 500–600 ml of hot water and boiled gently for half an hour in presence of some boiling chips. The mixture was then cooled and allowed to stand for some times. The aqueous layer was settled and drawn off and the washing process repeated similarly until the layer was finally neutral to litmus paper. The acetylated oily layer was then filtered through a dry paper and dried in a steam oven for short time.

The dried acetylated product (0.8540g) was then saponified with 40 ml of alcoholic KOH solution (0.5N approximately). The alcohol was evaporated

after saponification and the soap was dissolved in water followed by the addition of 30 ml of sulphuric acid (0.5N approx.) and warmed gently. The free fatty acids which were collected on the top were separated and washed with boiling water until the washing were no longer acidic. The filtrate was titrated with 0.1 N standardized KOH solution using phenolphthalein as indicator.

4.2.4.7. Peroxide value[24]: The peroxide value is one of the most important chemical constant appraising the degree of deterioration of fats. The peroxide value gives the molar equivalent of oxygen contained in 1 Kg fat by using suitable procedures and strictly observed conditions. The most frequently used method depends on the reaction of alkali iodide in acid solution with oxygen bound as peroxide and on titration of the liberated iodine.

Procedure:

The fish lipid (0.75 g) was taken in a 250 ml glass-stoppered bottle and a mixture of 25 ml of glacial acetic acid and chloroform (2:1) was added to it. To the content of the bottle, 1ml of fresh saturated KI solution was added and the mixture was allowed to stand in dark with occasional shaking for half an hour. 30–75 ml of distilled water was added to the mixture and then liberated iodine was titrated with 0.0097 N $Na_2S_2O_3$, using starch solution as indicator. The titration was continued until the blue colour appeared. A blank experiment was performed exactly in the same procedure as describe above. Peroxide value was calculated by using the following formula:

$$\text{Peroxide value (P.V)} = \frac{(S-B) \times N \times 1000}{\text{weight of the lipid sample}}$$

Where,

S = Volume (ml) of $Na_2S_2O_3$ solution for sample.

B = Volume (ml) of $Na_2S_2O_3$ solution for blank.

N = Normality of $Na_2S_2O_3$ solution.

4.2.4.8. Acid value [24]: The acid value of an oil or fat is the number of milligrams of potassium hydroxide required to neutralize the free fatty acids in 1 gram of material [9]. The acid value is a measure of the extent to which the glycerides in the oil have been decomposed by lipase or other action. Its calculation is based on the molecular weight of a given fatty acid or a fatty acid mixture [22].

Procedure:

The fish lipid (1.44 g) was taken into a 250 ml conical flask and 50 ml of alcohol was added. The solution was boiled for two minutes and then few drops of phenolphthalein solution were added. The content of the flask was titrated with vigorous shaking against a solution approximately 0.1N alcoholic KOH solution (the exact strength of which was currently determined against standard acid) until pink colour (persisting for at least 15 seconds) was appeared.

$$A.V = \frac{V \times 56.1 \times \text{strength of KOH solution}}{W}$$

Where,

V = ml of KOH required to neutralize the lipid sample.

W = weight of fish lipid.

4.2.4.9. Free fatty acid [24]:As rancidity is usually accompanied by free fatty acid formation, the determination is often used as a general indication of the condition and edibility of oils. The percentage of free fatty acids implies knowledge of the mean molecular weight of the free fatty acids. The value of molecular weight may be deduced from the saponification equivalent in the case of glycerides containing little or no unsaponifiable matter. The percentage

of free fatty acid (as oleic) was determined from the acid value by the following formula:

$$\text{Free fatty acid} = \frac{\text{Acid value}}{2}$$

4.2.4.10. Cholesterol[25, 26, 27, 28]: 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 human. It is not only an important component of some cell membranes and of plasma lipoprotein but also the precursor of many other biologically important steroids such as bile acids and various steroid hormones. The main sources of cholesterol are fish liver oils and the brain and spinal cord of cattle.

The cholesterol was determined by CHOD-PAP-Method. The measurement was carried out after enzymatic hydrolysis and oxidation of cholesterol. The indicator quinonemine is formed from hydrogen peroxide and 4-aminophenazone in the presence of phenol and peroxide.

Reaction:

$$\text{Cholesterol} + H_2O \xrightarrow{CHE} \text{cholesterol} + \text{fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{CHO} \text{cholestene-3-one} + H_2O_2$$

$$2H_2O + \text{4- aminophenazone} + \text{phenol} \longrightarrow \text{quinonemine} + 4H_2O$$

Procedure:

To prepare standard solution 0.02 ml of supplied standard solution of cholesterol (200 mg/dl) was taken in a test tube and mixed with 2 ml of enzyme reagent. Blank solution contained only 2 ml of enzyme reagent. 0.02 ml of fish lipid was taken in a test tube containing 2 ml of enzyme reagent.

The sample solution was then diluted three times by adding 4 ml of distilled water. The test tubes were allowed to stand for ten minutes at 25°C for incubation. The absorbances of the solutions were then measured at 500 ηm against the reagent blank within 60 minutes.

$$C = \cdot \frac{200 \times \Delta A}{\Delta R} \text{ mg/dl}$$

Where,

ΔA = absorbance of sample and

ΔR = absorbance of standard sample

For estimation of total cholesterol in fish blood serum, blood samples were withdrawn by heart puncture into a syringe containing 0.1 M sodium oxalate solution. Total cholesterol was assayed by Liebermann-Burchard Reaction Methods.

4.3. Results and discussion:

Fishes are the good natural sources of dietary lipid, protein, vitamins and minerals [7].Fish lipids are ubiquitously distributed compounds that play fundamental roles in the architecture and functionality of all living cells and, therefore, are most commonly studied as components of foodstuffs and important energy source in the internal nutrition [29]. The fish flesh was analysed by carrying out some physical and chemical test on it and results were compared with proximate composition of other fish species which is shown in table **2.1.** Graphical comparison of proximate composition of this fish flesh and other fish species is shown in figure 3.

Physical and chemical constants were also determined for identification of the nature of fish lipid extracted from fish flesh of this fish. Physical and chemical

constants of the lipid of *P. gonionotus* are given in table **2.2** and table **2.3** respectively. A comparative study of physico-chemical characteristics of this fish lipid with other oils and fats is shown in table **2.4**.

The total percentage of lipid yield using solvent extraction by Bligh and Dryer method [11] was 5.24 ± 0.64%. Significant variation in the lipid content of individual catfish may be related to genetic variation, diet composition or feed intake [30]. When the lipid content exceeds the maximum that can be metabolised for energy purposes, the remainder will be deposited in the tissues, resulting in a fish with very high fat content [31]. The lipid content of this fish can be considered as low lipid class when compared with other fish *Anguilla Bengalensis*[32] (local name Bao Baim) (8.87%) and Herring (6.00) [33]. The lipid content of this fish was higher than *Ompok Pabda* (local name Madhu pabda) (3.56%) [34] and Channel Catfish (5.1%) [30] and also other *Mystus species*, *Mystus cavasius* and *Mystus vittatus* (2.26% and 2.76% respectively) [34].

Proteinis much more expensive feed ingredient than lipid. Usually most fish species will use some of the protein for energy purposes regardless of the lipid content [31]. The protein content of *P. gonionotus* in the present study was found to be 22.21± 0.92%. The protein content showed that the species is very rich as a source of protein as compared to the average value of other different fish species like *Anguilla Bengalensis*[32], *Ompok Pabda*[34], Channel Catfish [30] and Herring [33], containing 21.63%, 16.63%, 16.60% and 18.00% protein respectively and also other *Mystus* speices *(M. cavasius and M. vittatus* contain 16.16% and 17.59% protein respectively) [34]. Protein content of fish varies widely, depending on facts such as natural feeding habits and availability of feed, fasting during spawning, migration etc [35]. It could be concluded that 100 g serving of *P. gonionotus* samples would supply 22.21± 0.92% of protein to an adult when considering the recommended dietary allowances (RDA) 65 g/day [36].

Moisture content like fats is one of the most variable components of animal's tissues and differs among their species [7]; fat and moisture normally constitute around 80% of the fish flesh [31]. Moisture is essential for most of the physiological reactions in the plant and animal tissue and in absence of moisture, life does not exist [37]. The moisture content of this fish was found to be 71.51±0.93% and this value was less than *Ompok Pabda*[34], Channel Catfish [30] and Herring [33] containing 78.67%, 77.3%, and 78.00% respectively and also both for *Mystus cavasius* (78.9%) and *Mystus vittatus* (79.45%) [34]. The moisture content of this fish was higher than *Anguilla Bengalensis* (66.03%) [32]. The percentage of dry mater and crude fibre of this fish were estimated and found to be 28.50±0.65% and 4.655 ± 0.32% respectively.

The digestibility of animal feeding stuffs varies inversely with the fibre content [22]. Crude fibre content of *P. gonionotus* is less than prawn species (12.23% in dry basis) [38].

The ash figure can be regarded as a general measure of quality and often is useful criterion in identifying the authentically of the quality of a food. The high figure content has got a nutritional advantage in that it with assist to reducing constipation and other attendant problem in the human consumers [38]. The ash value of *P. gonionotus* was found to be 1.02±0.05% and this value was less than *Anguilla Bengalensis*[32], *Ompok Pabda*[34], Channel Catfish [30], Herring [33], containing 1.43%, 2.35%, 1.09% and 2.5% respectively and also both in *M.cavasius* and *M.vittatus* (4.77% and 4.30% respectively) [34]. The ash content is of significance in measuring of mineral contents of the sample suggests the presence of an inorganic adulterant [22]. The proximate composition of this fish are compared with the proximate composition of other fishes (table **2.1**) and found that protein content of this fish was remarkably high except *Anguilla Bengalensis*[32] and an appreciable variation was observed in lipid, moisture and ash.

Physical constants of lipid are also often capable of giving valuable information. A number of physical characters are also employed to determine the nature and, sometimes, for identification of fats and oils. These characters may also evaluate the suitability of given oil or fat for a given purpose. Refractive index, specific gravity etc are important physical constant for fat and oil.

The refractive index of fats and oils depends to some extent on their unsaturation [39]. In general, the refractive indices of natural fats and oils are related to their average degree of unsaturation in an approximately linear way. The refractive index is influenced by the hydration effect by which refractive index may be decreased. The refractive index of most fish oil is around 1.479 [40]. The refractive index of the investigated *P. gonionotus* fish lipid was found to be 1.4612 to 1.4635 which is very closed to that of other edible oils [6].

The density of the oils varies with their type and temperature and also treatment process. The range is from 0.91 to 0.93 g/cc between the temperatures of 15°C and 25° C. The density of this fish lipid was 0.9516 and was less dense than water whose density is 1.00 g/cc [41]. The specific gravity and apparent density of each kind of oil lie within a fairly narrow range if the determinations are made at a standard temperature and the figures are thus of diagnostic value in assessing the degree of purity of an oil [9].

Iodine value is defined as the number of grams of iodine (or iodine equivalent) absorbed by 100 g of fat or oil. Iodine value gives an estimate of the degree of unsaturation in fatty acids and so, of the relative amounts of unsaturated fatty acids in the triglycerides molecules of the fat. It may be suggested that the oil under investigation may be contained higher amounts of unsaturated fatty acids as its iodine value was calculated to be 108.72 I_2/100 g of the sample. This result was closed to that of Mackeral (*Scomber Scrombrus*) fish oil (108.09

I_2/100 g of the sample) [42]. Iodine value assists greatly in the identification of the components of a mixture. Thus, it points to the application of a further method to resolve the isolated fatty acids of an oil or fat into saturated fatty acids which do not absorb iodine and into unsaturated fatty acids which absorb iodine in various proportion [21]. The magnitude of the iodine value of the fats depends on various factors, primarily on the proportion of unsaturated fatty glycerides and also on the nature of fatty acids.

The unsaponifiable matter amounting to 0.5–2% represents a mixture of several lipid classes, viz., sterol, tocopherols, hydrocarbons, higher aliphatic and terpenoids alcohols[13]. The unsaponifiable matter in this fish lipid was found to be 1.85% which indicate that the fish lipid also contained sterols, tocopherols, hydrocarbons etc.

The saponification value is inversely proportional to the average molecular weight or chain length of the fatty acids present in the fats or oil. The shorter the average chain length in the fatty acids, the higher is the saponification number [22]. The saponification value of the fish lipid was found to be 186.52 mgKOH/g which was slightly higher than that of standard value for fish oil (165–195 mgKOH/g) [42].

Saponification equivalent is directly proportional to the average chain length of the fatty acids present. Fats and oils consisting largely of C_{18} fatty acids along with some palmitic acid; a little unsaponifiable matter and a low free acidity generally gave a saponification equivalent around 296.52; a higher value indicates the appreciable quantity of fatty acids [12]. The present result found for *P. gonionotus* was 296.52 indicated that the fish lipid contained mainly fatty acids of C_{18} molecular weight along with some longer chain fatty acids.

Many oils contain glycerides possessing one or more hydroxyl groups not in combination with glycerine, it is possible to obtain a measure of their presence

by replacing hydrogen of these groups by the acetyl radical. Any free alcohols such as glycerol, cholesterol or fatty alcohol will be included if they are present [9]. The acetyl value of the lipid of *P. gonionotus* was determined and was found to be 11.84 which was close to olive oil (10–11) [9]. The castor oils have a high acetyl number (146) because of high content of a hydroxyl acid. Increasing free fatty acid content of oil may lead to a slight increase in the acetyl value [9].

On exposure to the atmosphere, oils and fats gradually undergo certain changes and become a peculiar disagreeable smell and acrid taste which are defined by the term rancid [21]. The main causes of rancidity are oxidation of oil in which hydroperoxides compounds are formed. The peroxide value in oil is transitory intermediate which decompose into carbonyls and other compounds [43]. The peroxide value of the lipid of *P. gonionotus* was determined and was found to be 1.78 mEq/kg where as the standard value of fish oil is less than 10 mEq/kg [22]. Oils with high peroxide values tend to have strong bad odour and taste, so can oil with low quality. The specific limits of high quality oils are peroxide value of less than 3 when the oil leaves factory, less than 5 before it is encapsulated and less than 10 following encapsulation [43]. The peroxide value indicates that the fish lipid was in fresh condition.

The ester value of the fish lipid was found to be 235.29. The acid value and percentage of free fatty acid (as oleic) of the lipid of *P. gonionotus* were estimated and the amounts were found to be 1.96 mg/KOH and 1.24% respectively which were within the standard value of 5 mg/KOH and 0.5–1.5 respectively [22]. The low percentage of free fatty acid (bellow 1.15%) is an indication of suitability of the lipids for edible purpose. So, the present result suggests that the lipid of *P. gonionotus* might be useful for edible purpose.

Cholesterol is a fat (lipid) made by the body which is an important constituent of living cells and the coating of nerve tissues and is needed for digestion of

fat. Although nearly all body tissues can synthesize cholesterol, the liver and intestine [44, 45] synthesize most. The 2005 Dietary Guideline for Americans recommends that the intakes of cholesterol for human body should be less than 300 mg a day. Cholesterol is found in eggs, dairy products, meat, poultry and fish and shellfish. Fish generally have less cholesterol [46]. The cholesterol content of one hundred and eighteen fish oil varied from 0.37% to 1.96% with a mean of 0.95% [47]. The amount of cholesterol of the lipid of this fish was determined and was found to be 1.25% (1250 mg/dl). Form this result, it can be suggested that the lipid of *P. gonionotus* is more suitable for edible purposes with the existing cholesterol level. Total cholesterol in blood serum of this fish was also measured and was found to be 180 mg/dl. Blood cholesterol level of this fish is less than Carp (*Cyprinus carpio*) (0.662%) [48] but remarkably high than Humback whale, *Megaptera versabilis* Cope, and Sperm-Whale, *Physeter macrocephalus* Linneus contain 42.6 mg/dl and 65 mg/dl cholesterol respectively [49].

Table **2.1**:Proximate compositions of *P. gonionotus* and other fish species.

Name of species	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
P. gonionotus	71.51±0.93	22.21±0.92	5.24±0.64	1.02±0.05
Mystus vittatus (Tengra)	78.45	17.59	2.76	4.3
Mytus cavasius (Gulsha)	78.99	16.16	2.26	4.77
Ompok bimaculatus (Madhu Pabda)	78.67	16.63	3.56	2.35
Anguilla bengalensis (Bao Baim)	66.03	21.63	8.87	1.45
Herring (spring)	78.00	18.00	6.00	2.5
Channel Catfish	77.3	16.6	5.1	1.09

Table **2.2**: Physical constants of *P.gonionotus* fish lipid.

Physical constants	Value
Density (g/cc)	0.9516
Refractive index	1.4612-1.4635

Table **2.3**: Chemical constants of *P. gonionotus* fish lipid.

Chemical constants	**Values**
Iodine value (I_2/100 g)	108.72
Saponification value (mgKOH/g)	186.52
Saponification equivalent	296.52
Acid value (mg/KOH)	1.96
Ester value	235.29
Peroxide value (mEq/kg)	1.78
% of free fatty acids (as oleic acid)	1.24
Total cholesterol (mg/dl in lipid)	1.25

Table **2.4**: Comparative study of physico-chemical constants of *Puntius gonionotus* fish lipid with other oils and fats.

Name of species	I.V.	S.V.	S.E.V.	P.V.	A.V.
P. gonionotus	108.72	186.52	296.52	1.78	1.96
Herring oil [9]	105-128	185-192	289.17- 303.24	-	-
Menhaden oil [9]	150-170	188-194	-	Not more than 5	-
Salmon oil [9]	130-165	185-192	292.19- 303.24	-	-
Olive oil [9]	80-90	190-195		20 max.	0.2-3
Cod liver oil [9]	155-175	180.190	295.26- 311.67	-	-
Eutropiichthys vacha [50]	96.21	216.26	-	-	1.99
G. Centropus Sinensis [12]	98.37	189.43	296.14	10.58	1.87
Eel, Anguilla bengalensis (Bao Baim) [32]	96.68	189.05	296.75	7.46	3.55

I.V. = Iodine value (I_2/100 g)

S.E.V. = Saponification equivalent value

A.V. = Acid value (mg/g KOH)

S.V. = Saponification value (mg/g KOH)

P.V. = Peroxide value (mEq/kg)

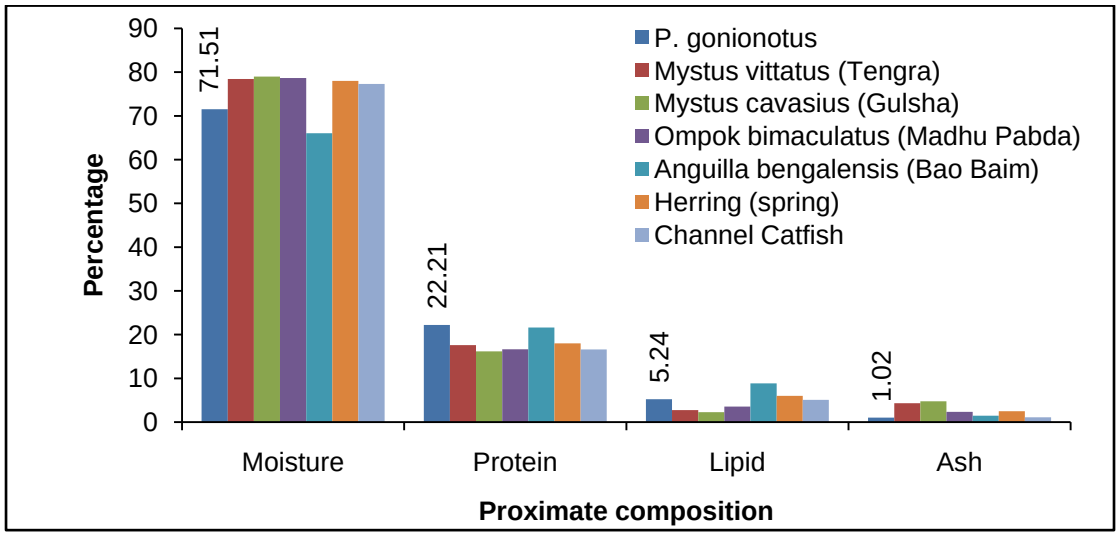

Fig. **3:** Proximate composition of *P. gonionotus* along with other fish species.

Summary and Conclusion:

Lipids are used for long-term energy requirements during periods of extensive exercise or during periods of inadequate food and energy intake. Edible oils extracted from fish sources are important in foods. The percentage of lipid yield from *P. gonionotus* using solvent extraction was 5.24±0.64%. This fish is lower in fat than most of other fishes. The lipid contained in this fish has become beneficial oils called omega-3 fatty acids. Based on the improved characteristics of the lipid, it could be suitable for applications in pharmaceutical and food industries. The proximate composition of fish flesh was determined and the values were found moisture 71.51±0.93%, protein 22.21±0.92%, ash 1.02±0.05%, crude fibre 4.655±0.32% and dry matter 28.50±0.65%. Most of these analytical results obtained were tolerable to the standard values. Some physical and chemical properties of this fish lipid were determined to know the lipid characteristics. The physical properties of this fish lipid such as density, refractive index were measured and found to be 0.9516 g/cm^3 and 1.4612–1.4635 respectively.

The iodine value, saponification value and unsaponifiable equivalent of this fish lipid were 108.72, 186.52 and 296.52 respectively. These values fall within the standard range may be attributed to the fatty acid composition.

The acid value and peroxide value of the fish lipid were found to be 1.96 mg/KOH and 1.78 mEq/kg respectively. Acid value depends upon the degree of rancidity which is used as an index of freshness. The appreciably lower value in peroxide could be attributed to the fact that the lipid was left for very few hours prior to the analysis of the lipid. Fish feed ingredients such as fishmeals should be protected against oxidation. The finished feed, if possible, should be stored in airtight containers at reduced temperature with minimum exposure to UV radiation and other factors accelerating the rate of lipid oxidation. Cholesterol, a pearly fat-likes substance, of this fish lipid was measured and found at 1.25% and this value lies within the cholesterol content of other fish lipids. Cholesterol in fish blood serum was found relatively high. There is every reason to believe that this lipid could be used as an edible one, since it has properties very similar to those of other edible fish oils.

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CHAPTER 05

STUDY OF MINERAL CONSTITUENTS OF FISH FLESH OF *P. GONIONOTUS*

Local Name : Thai shar phuti/China punti
Scientific Name : *Puntius gonionotus*

Study of mineral constituents of fish flesh of *Puntiusgonionotus*

5.1. Introduction

Minerals and vitamins are important micronutrients to sustain normal life. They help to provide a normal life and other chemical reactions in the body. Minerals are inorganic substances as contrast to such organic compounds as protein, fats, carbohydrates and vitamins. They are found in all body tissues and fluid. They occur in food as salts; for example sodium chloride. They may also be combined with organic compounds; for example, iron in haemoglobin and sulpher in almost all protein [1]. They can be divided in to two groups (macro-minerals and micro-minerals) based on the quantity required in the diet and the amount present in fish. Common macro-minerals are sodium (Na), chloride (Cl^-), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P) etc. These minerals regulate osmotic balance and aid in bone formation and integrity. Micro-minerals (trace elements), required in small amounts, are components in enzyme and hormone system. Common trace elements are copper (Cu), chromium (Cr), iodine (I), zinc (Zn) and selenium (Se) [2]. These mineral elements present in animal body are supplied by the diet. In tropical countries, addition of sodium chloride is of great importance because of the loss of NaCl in sweat [3]. On an average, a man excretes daily about 20 to 30gms of minerals salts and these outputs must be made by the intake through foodstuffs. In the case of growing body, provision must be made for additional amounts of many of the elements to ensure adequate growth of the tissues [4].

Fish is a nutrient-dense food which is high in protein and minerals (both micro and macro minerals). These are essential for healthy growth and development of human and animals. Normally, *P. species* are valuable and easily available source of food rich in proteins, lipids, vitamins and minerals not commonly available in other foods in Bangladesh. In a country with population like Bangladesh suffering from malnutrition and protein deficiency, consumption of

this fish may have positive effects in improving the health of the nation. *P. gonionotus* is preferred by the people of our country for their delicious tastes, therapeutic values and availability in coastal region in Bangladesh through out the year. Many minor and trace elements such as sodium, potassium, calcium, iron, iodine, zinc, magnesium and phosphorus may be present in this fish species. The need for thorough and long-term investigation on the nutritional value of this fish is urgently needed. Considering the importance, this study was under taken to assess the nutritional value of this fish available in coastal region in Bangladesh.

5.2. Materials and methods [5, 6, 7]

Atomic absorption spectrophotometer method was used for the determination of Na, K, Ca, Cu, and Zn contents in the species and Fe and P were determined by spectrophotometric method [5]. Atomic absorption spectroscopy is an absorption method where radiation is absorbed by non excited atoms in the vapour state [6]. The Atomic absorption spectrophotometer is normally arranged so that the radiation from the hollow cathode source is focused by a lens to a slightly reduced image at the centre of a long (10 cm) flame into which the solution to be measured is sprayed [7]. In this technique, the sample is first converted into an atomic vapour and then the absorption of atomic vapour is measured at a selected wave length which is characteristics of each individual's element. The amount of energy at the characteristics wave length absorbed in the flame is proportional to the concentration of the element in the sample over a limited concentration range.

A hollow cathode lamp for the desired metal was installed in the instrument and the wave length was roughly set according to atomic absorption concentration range with direct aspiration atomic absorption. The instrument was turned and the hollow cathode lamp was applied to the current suggested by the manufacturer. Then the instrument was allowed to warm up about 10─20 minutes until energy source was stabilized. Concentration of each

element was read out directly from the instrument. Na, K, Ca, Mg, Cu and Zn contentwere determined by atomic absorption spectrophotometer method (Direct air- acetylene flame) [7]. Metal free (deionised water) water was used for preparing all reagents and calibration standards and as dilution water.

5.2.1. Preparation of stock solution:

Sample preparation: The fish flesh of *P. gonionotus* was homogenized in a micro cutter and then mixed uniformly with precooled petroleum in a homogeniser and filtered through a clean muslin cloth. The process was repeated at least twice in order to obtained lipid free homogenate. Finally, filtrate was clarified further by centrifugation at 8 $\times 10^3$r.p.m for 10 minutes. Then this precipitate was air dried at room temperature [8].

Ash was prepared from this defatted fish sample by dry ashing method. For this purpose, defatted homogenate fish sample (about 5 g) was heated in pre-weighted crucible to a white ash 600°C. This was done for four hours in a muffle furnace. The Ash was dissolved in a minimum quantity of concentrated HNO_3 and warm water. The diluted sample was then filtered and adjusted to a known volume (100 ml) preferably, so that the final HNO_3 concentration is about 1% [7]. Small portion of this solution was taken for Na, K, Ca, Mg, Cu and Zn estimation. To determinate the amount of phosphorus, wet oxidation was performed by adding acid mixture (HNO_3: H_2SO_4: $HClO_4$ = 5: 1: 2) in to fish flesh (2.02 g). Then it was allowed to predigest at least for two hours and again digest until the content became colourless[9].

5.2.2.Preparation of standard solutions for estimation of Ca, Mg, Na, K, Cu and Zn[7]:

Calcium (Ca): Before weighting, $CaCO_3$ was dried at 180°C for 1 hour and then 0.247 g of dried $CaCO_3$ was dissolved cautiously in deionised water with a minimum amount of HNO_3 and then 10 ml of concentrated HNO_3 was added and diluted to 1000 ml with distilled water.

Magnesium: A quantity of 0.1658 g magnesium oxide was dissolved in a minimum amount of HNO_3 (1: 1). Then 10 ml of concentration HNO_3 was added to this solution and then diluted to 1000 ml with distilled water.

For determination of calcium and magnesium, 10 ml of lanthanum solution was mixed with per 100 ml of standard solutions.

Lanthanum solution: A quantity of 58.65 g of lanthanum oxide (La_2O_3) was dissolved in 250 ml concentrated HCl. In this case, acid was slowly added until the material was dissolved and then diluted to 1000 ml with distilled water.

Sodium (Na): A quantity of 0.2542 g of dried NaCl (dried at 140°C for 1 hour) was dissolved into distilled water and then 10 ml of concentrated HNO_3 was added and the solution was diluted with distilled water up to 1000 ml.

Copper (Cu): A quantity of 0.1 g copper metal was dissolved in 2 ml concentrated HNO_3 and then 10 ml of concentrated HNO_3 was added to this solution. The solution was then diluted to 1000 ml with distilled water.

Potassium (K): A quantity of 0.197 g KCl (dried at 110°C for 1 hour) was dissolved in distilled water and then filled up to marked with 1000 ml distilled water.

Zinc (Zn): A quantity of 0.1 g zinc–dust (Zn metal) was dissolved in 20 ml (1: 1) HCl and diluted to 1000 ml with distilled water.

5.2.3. Phosphorus estimation [5]:

Phosphorus content of this fish flesh wasdetermined spectrophotometrically by the phosphovanadomolybdate method.

Standard phosphorus solution: An amount of **0.**2197 g of analytical grade potassium dihydrogen phosphate (KH_2PO_4) was dissolved in distilled water and diluted to 1000 ml in a volumetric flask. From this solution, 2ml, 4 ml, 6 ml, 8 ml and 10 ml were taken in five different 50 ml volumetric flask and

marked with distilled water to prepare 2 ppm, 4 ppm, 6 ppm, 8 ppm and 10 ppm respectively standard phosphorus solution.

Procedure: Five ml of standard phophorus solution of different concentration were taken in to five different 50 ml volumetric flask. To each of the flask, 5 ml of 2.5 N HNO_3, 5 ml of molybdate and 5 ml of ammonium vanadate reagent were added to each flask and marked up to with distilled water. The mixture was then mixed thoroughly by shaking where by a yellow colour was obtained. The absorbance of these solutions was taken at 465 ηm using a reagent blank and data are tabulated in table **3.1**. The calibration curve was constructed by plotting absorbance against the respective concentration of the standard solution shown in figure **4.1**.

Five ml of sample solution which previously made was taken in a volumetric flask and 5 ml of ammonium vanadate solution, 5ml of ammonium molybdate solution were added and diluted with distilled water up to mark. The mixture was mixed thoroughly where by a yellow colour was obtained. The absorbance of the solution was measured at 465 ηm against a blank reagent.

The amount of phosphorus present in fish flesh was calculated from the standard curve and result is presented in table **3.2**.

5.2.4. Determination of calcium (Ca) [7]:

For estimation of calcium, a calcium hollow-cathode lamp was installed in the instrument and set the instrument at wave length 422.7 ηm and then warmed up for ten minutes until energy sources stabilized. Then air- acetylene flame was turned on at a rate for few minutes so that maximum sensitivity for calcium metal was obtained. Then the blank solution was run out and the instrument was adjusted to zero. Starting with the least concentrated solution, the standard calcium solutions were aspirated in turned in the flame and for each three readings was taken. These data checked the consistency of instrument set up. Finally, the sample solution was aspirated and the concentration was recorded

directly. Calcium content was found out by multiplying with dilution factor and the result is shown in table **3.2**.

Similarly, the concentration of sodium (Na), potassium (K), magnesium (Mg), copper (Cu) and zinc (Zn) were estimated with the help of atomic absorption spectrophotometer by Direct Air-Acetylene Flame Method in the same manner at 589.0 ηm, 766.5 ηm, 285.2 ηm, 324.7 ηm and 213.9 ηm respectively by using corresponding hollow-cathode lamp their results are shown in table **3.2**.

5.2.5. Determination of iron (Fe) [5]:

Iron content in *P. gonionotus* was determined spectrophotometrically by the thiocyanate method.

Preparation of standard solution: An amount of 0.864 gm ammonium iron (III) sulphate ($NH_4Fe\ (SO_4)_2\ 12\ H_2O$) was dissolved in water and then 10 ml concentrated HCl was added to this solution and diluted to 1000 ml with deionised water. 10 ml of this solution was taken in a 50 ml volumetric flask and marked up to point with distilled water. From this solution, 2.5 ml, 5 ml, 7.5 ml, 10 ml and 12.5 ml portions were taken in a five different 50 ml of volumetric flask and filled up to desired marked with deionised water to prepare 0.1 ppm, 0.2 ppm, 0.3 ppm, 0.4 ppm and 0.5 ppm iron solution respectively.

Preparation of stock solution: The amount of ash from 2 g fish fleshwas taken in a porcelain crucible and 15 ml of concentrated HCl was added to its. The resulting solution was evaporated nearly to dryness to expel excess of acid and then diluted slightly with water; the iron oxidized to the ferric state with bromine water and made up to 100 ml with distilled water to give the stock solution. Similarly, blank solution was prepared by the same quantities of above reagent except fish sample.

Procedure: Ten ml of stock sample solution was taken in 50 ml volumetric flask and 5 ml of 20% potassium thiocyanate and 2 ml of 4 N HCl was added

in this flask and mixed well. Deionised water was then added to the flask up to mark. The absorbance of the solution was measured at 480 ηm (blue green filter) against a blank reagent and the experimental data is shown in **table 3.3**. Iron content of the sample solution was determined from a standard curve (figure **4.2**) constructed by using standard iron solution of different concentration in the sample manner as before. The amount of iron found in this experiment is shown in table **3.2**.

5.3. Results and discussion

Minerals are important elements in human nutrition and these elements play an important role in bone formation, tooth formation, nervous response, muscle function, water balance and carbohydrate metabolism etc[4]. These elements are essential for life which acts as a cofactor of enzyme and as an organizer of the molecular structure of the cell (e.g. mitochondia) and its membrane [10]. The human body required a balance proportion of protein, vitamins, fats and minerals for proper growth and maintenance of health. Nutrients like calcium, potassium, magnesium, phosphorus, sodium, iron, copper and zinc are responsible for blood (coping with anaemia) and bone (reducing risk for osteopoesis) formation. Bone loss occurs naturally with age in both males and females; therefore, it is important that children and young and adults achieve their optimum bone density so that age related losses would not result in osteoporosis. Fish with soft bone can also be important as dietary sources. Small fish eaten whole, contribute appreciable amount of dietary calcium [11]. Imbalance of trace elements may be consequence of certain disorders [12].

The nutritional qualities of the fish *P. gonionotus* that are available in selected zone in Bangladesh were studied in the laboratory. An attempt was made to evaluate the nutrients contents in this fish. The macro and micro minerals constituents in this fish are shown in table **3.2.** The study revealed that the nutritional components of this fish were relatively rich in minerals like calcium, potassium, magnesium, phosphorus, and sodium, iron, copper and zinc.

Utilization of this fish in our diet may be considered as an important strategy to ensure the supply of minerals particularly to the communities who do not have enough purchasing capacity.

The fresh fish flesh contained 76.01±2.16 mg/100 g sodium which implies that this fish has an role in regulating plasma volume, acid base balance, nerve and muscle function and many other purpose within cell [13].Sodium content per 100 g fish is varied from 30–150 mg for different fish [14].

Calcium along with phosphorus is essential for the formation and development of bones and teeth. Calcium in normal ratio with potassium maintains the normal activity of muscle [3]. An estimated 99% of the body's calcium resides in the teeth and bones, where it is extracted and redeposited as needed to keep blood levels of calcium constant. The one percent found in the blood, lymph and other body fluid is critical to the intracellular and extra cellular environments of all living cells [11]. From table **3.2,** it is seen that *P. gonionotus*contained 266± 4.54 mg calcium. This is comparable to the amounts found in tengra (270 mg) [15] and was lower than the levels found in *M. cavasius and M. vittatus* (300 mg and 430 mg respectively) [16]. Small fish like pabda, bacha, kajali, and chala (local name) provide 310, 520, 175 and 590 mg of calcium respectively in a 100 g portion [14]. In general, the amount of calcium in different fish species varied from 5–200 mg [14]. From this result, it is seen that this fish species is a rich source of calcium.

Potassium plays an important role in the regulation of acid-base balance in the cell and also regulates the transmission of the nerve impulse and the contraction of the muscle [3]. Soft tissue contains many minerals in their structure including potassium [17]. The amount of potassium found in *P. gonionotus* fish potassium was 294 ± 2.01 mg. Potassium content in different fish species is varied from 250–500 mg [14]. Thus this fishmay be considered as a great supplement source of potassium.

Phosphorus is required for the formation of phospholipids, nucleic acid and phosphoproteins. It is also essential for utilization calcium in the body and in the assimilation of carbohydrate and fat [3]. From the calibration curve, phosphorus content in *P. gonionotus* was found at 210±1.80 mg and this value was lower than *M. cavasius*(430 mg)and *M. vittatus* (490 mg) [16].

Seventy percent of the total magnesium content of the body is combined with calcium and phosphorus in the complex soft bones. It is the principal content of the soft tissue [3]. Magnesium contained of this fish was found to be 45.1±1.83 mg. The magnesium content in different fish is between 10–60 mg [14].

The function of iron is mainly in the transport of oxygen to the tissues (haemoglobin). It is also involved in the process of cellular respiration [3]. A well balanced diet for growing children or for adults should contain sufficient amount of iron to meet the iron deficiency of the body and to allow for possible regional and seasonal variation in the iron contents of food [4]. *P. gonionotus* contains 1.70±0.16 mg iron which is less than tengra fish (2.1 mg) [15].

Copper is required for melanin formation and also for haemoglobin, phospholipids and collagen synthesis. Zinc is a part of many enzymes bio-membranes and is involved in RNA transcription, among other activities. Zinc deficiency is linked to stunted grown and delayed sexual maturation [11]. From this study, it is found that *P. gonionotus*contains 101±0.20 mg copper and 3.80±0.82 mg zinc. For adults, the daily requirements of sodium, calcium, potassium, magnesium, phosphorus, iron, copper and zinc are1100–3300 mg, 800 mg, 1875–5625 mg, 800 mg, 350 mg, 10 mg, 1.5–3.0 mg and 12 mg respectively [18].

The overall mineral contents of this fish was compared with the concentration of selected minerals (table **3.3**) in the flesh of pond–raised Channel Catfish of wet tissue [19] and found that the minerals content in this fish were higher than that of Channel Catfish except potassium contents. The value of Na, K, Ca, Mg

and P contents of this fish lies within the range of some minerals constituents of fish muscle [20](**table 3.4**). The comparative distribution of macro minerals and micro minerals in *P. gonionotus* fish flesh and pond–raised Channel Catfish are shown in figure **4.3** and figure **4.4** respectively.

Table **3.1:** Determination of phosphorus.

Experiment number	Concentration (ppm)	Absorbance	Absorbance of the sample
1	0	0.00	0.098
2	2	0.04	
3	4	0.08	
4	6	0.12	
5	8	0.161	
6	10	0.19	

Table **3.2:** Macro and Micro mineral constituents in *Puntiusgonionotus* along with pond-raised Channel Catfish.

Species	**Macro minerals (mg/100g)**					**Micro minerals (mg/100g)**		
	Ca	**K**	**P**	**Na**	**Mg**	**Fe**	**Zn**	**Cu**
P. gonionotus	276.00 ±4.54	294.00 ±2.01	210.00 ±1.80	76.01± 2.16	48.1± 1.83	1.70±0. 16	3.80±0. 82	1.01±0. 20
M. golio[15]	256.0	288.41	220.0	86.01	45.1	1.50	3.0	1.01
Rita rita[16]	908	276.42	151.63	63.9		1.69	0	0
Channel catfish[17]	9.1	353.60	179.90	40.40	22.4	0.5	0.59	0.03
Liza parsid[18]	653.42	0	3.47	0	0	5.67	0	0

Table **3.3**: Determination of iron.

Experiment number	Concentration (ppm)	Absorbance	Absorbance of the sample
1	0	0.00	0.008
2	0.1	0.011	
3	0.2	0.027	
4	0.3	0.039	
5	0.4	0.055	
6	0.5	0.069	

Table **3. 4**: Some mineral constituents of fish flesh.

Fish muscle	Macro minerals (mg/100g)				
	Ca	K	P	Na	Mg
	19–881	19–502	68–550	30–134	4.5–452

Source: Murray and Burt 1969.

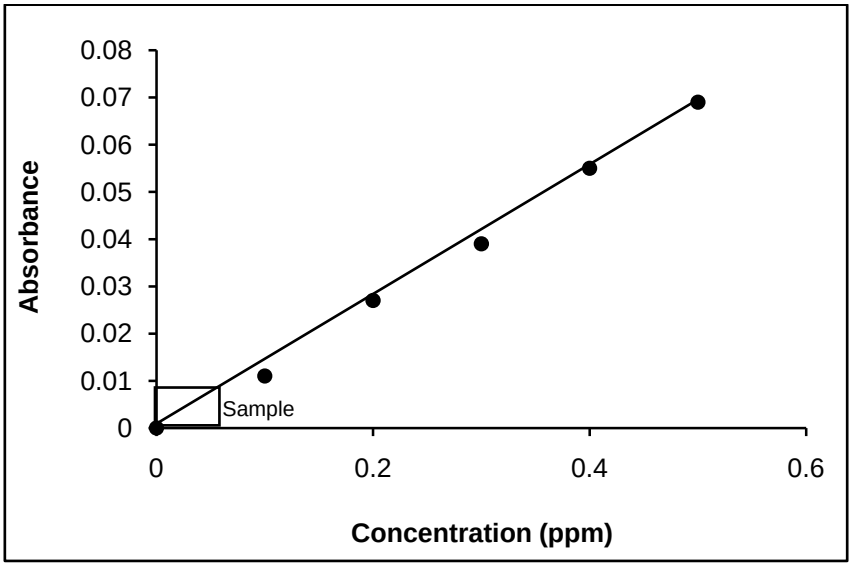

Figure **4.1**: Standard curve for phosphorus estimation.

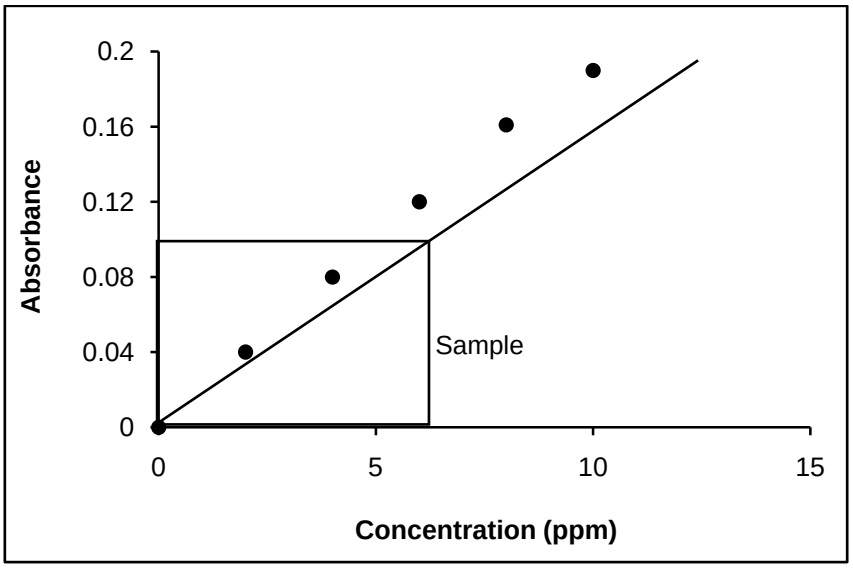

Fig. **4.2**: Standard curve for iron estimation.

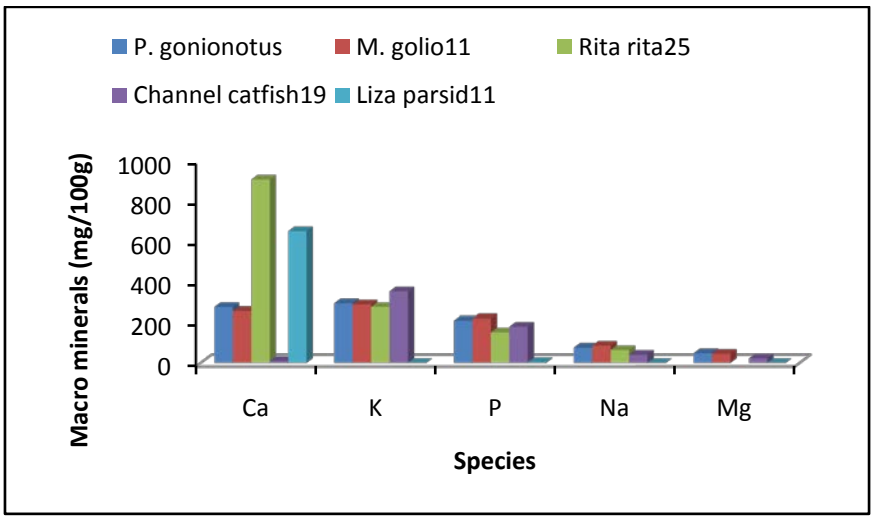

Fig. **4.3**: Graphical representation of macro minerals of *P. gonionotus*along with some other fish species.

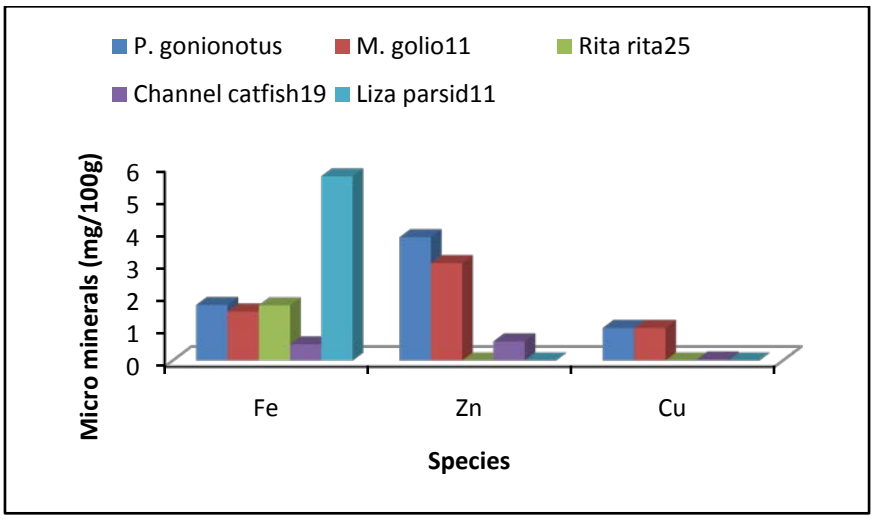

Fig. **4.4**: Graphical representation of micro minerals of *P. gonionotus*along with some other fish species.

Summary and Conclusion

Minerals elements, found in all animals and plants tissues in very small amounts, play a crucial role in virtually all biological and biochemical process and therefore, important in human nutrition. Majority of the diseases begin when biochemical imbalance occur in the cellular level. Micronutrients deficiency disease is a major public health problem in Bangladesh. Sodium (Na), Potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), iron (Fe), copper (Cu) and zinc (Zn) etc are known to play important role in physiochemical processes and their deficiency may cause certain diseases. These and other minerals are playing important role in metabolic processes of the body. There is a growing interest in the understanding of the role played by the micronutrients in biological system, as essential for human metabolism.

In Bangladesh, fishes are common as well as cheap sources of vitamins and minerals. In the present investigation, it was found that the fish *P. gonionotus* contained 276±4.54 mg calcium, 48.1±1.83 mg magnesium, 76.01±2.16 mg sodium, 294±2.00 mg potassium, 210± 1.80 mg phosphorus, 1.70±0.16 mg iron, 1.01±0.20 mg copper and 3.80±0.82 mg zinc. The study also revealed that the minerals sodium (Na), potassium (K), magnesium (Mg), calcium (Ca) and phosphorus (P) content of this fish are relatively rich than other small indigenous fishes. Therefore, optimum utilization of this fish rich in minerals is of paramount importance to minimize the mineral gap thereby improve nutritional status and also can play a vital role on preventing minerals deficiency and malnutrition problem in Bangladesh. Considering such importance the study was undertaken to assess the nutritional value of this fish available in coastal region in Bangladesh. The need for thorough and long-term investigation on the nutritional value of this fish *Puntiusgonionotus* is urgently needed.

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CHAPTER 06

QUALITATIVE AND QUANTITATIVE ANALYSIS OF FATTY ACIDS OF *P. GONIONOTUS* FISH LIPID

Local Name : Thai shar phuti/China punti
Scientific Name : *Puntius gonionotus*

PART-A

Separation and identification of fatty acids in fish lipid by Thin Layer Chromatography (TLC).

6.1. Introduction

Thin Layer Chromatography (TLC) is an excellent tool for micro-preparative separation of methyl esters obtains from lipid sample. In TLC, separation occurs on a layer of finely divided adsorbent (usually silica gel or alumina as a stationary phase), which is supported on a glass plate. A thin layer plate may be prepared by spreading an aqueous slurry of the finely ground adsorbent over the surface of a glass plate or a microscope slide [1]. The chemical mixtures applied to this film are separated into their respective components by means of a suitable solvent system. The solvent is drawn up to the adsorbent layer by capillary action. Due to their different rate of adsorption on the adsorbent, the components in a mixture migrate differently along the TLC plates. Separation of components also depends on the solubility of components into solvent. The migration rate to the solute in the direction of solvent flow is characterized by the term R_f which is the distance the solute migrated from the starting point divided by the distance travelled by the solvent [2]. i.e.,

$$R_f = \frac{\text{Distance travelled by the sample (cm)}}{\text{Distance travelled by the solvent (cm)}}$$

In hydrophobic solvent system, saturated fatty acids have the highest R_f values which decrease with the increasing degree of unsaturation and for a particular acid, the *trans* isomer usually travels ahead of its corresponding *cis*-isomer [3].

6.2. Materials and methods

6.2.1. Preparation of methyl esters from fish lipid [4, 5, 6]:

The fish lipid (1g) was taken in a round bottom flask (25ml) and 50ml of 2N alcoholic potassium hydroxide solution was added to it. The mixture was then refluxed for 45 minutes on a water bath until it became clear. The reaction mixture was allowed to cool and diluted with water. The unsaponifiable matters were removed with diethyl ether and the pH of the aqueous solution was adjusted to 1–2 by adding 2N hydrochloric acid drops wise. The acidified aqueous mixture was then extracted with 20 ml of diethyl ether in a separating funnel and the extraction was repeated for three times. The combined ether extract was washed twice with 50 ml portions of water in order to remove any adhering hydrochloric acid. Ether was then removed from the extract to give fatty acid mixture.

The fatty acid mixture was taken in a 250 ml round flask and 12 ml of methanolic boron trifluride (BF_3) solution was added. The flask was then attached with a condenser and boiled for 2 minutes. Finally, 2–5 ml of *n*-heptane was added to the flask through the condenser and then boiled for 1 minute for complete esterification. The heat was removed and then NaCl solution was added upto neck of the flask to float heptanes solution containing methyl esters. The methyl esters was collected and dissolved in diethyl ether in a separating funnel and washed with dilute sodium carbonate solution until the effervescence ceased. The esters were dried over anhydrous sodium sulphate and finally ether was removed to give dry methyl esters mixture.

6.2.2. Preparation of methyl esters of standard fatty acids:

Methyl eaters of standard fatty acids were also prepared by the same procedure as described above. In this experiment, standard fatty acids, capric acid, lauric

acid, myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid and behenic acid were used to prepare their corresponding methyl ester.

6.2.3. Preparation of TLC plates [7, 8]:

For the preparation of TLC plates, a number of glass plates of equal thickness (20 cm×20 cm×0.5 cm) were thoroughly washed with water to remove any dirty substances and were dried in a hot oven. The plates were then washed with acetone to remove greasy or fatty materials. The plates were placed in a plastic template tray specially made for TLC. The spreader was adjusted in such a way that the thin layer of silica gel in the plates had a thickness of 0.25 mm and was placed on one end of the glass plates. To make the slurry, silica gel GF254 (12 g/plate) and distilled water (2 ml/g of silica gel) were kept in a beaker. The beaker was shaken gently to obtain the slurry. When the gel mixed uniformly with water, it was poured quickly in the spreader. The lever of the spreader was turned to 180° and it was drawn immediately towards the other end of the glass plates so that the plates were coated uniformly. After air drying, the plates were activated by heating at 110°C in oven for 60 minutes [9]. The activated plates were kept in desiccators on a drying rack.

6. 2.4. Application of samples and development of the chromatograms:

The following solvent systems (v/v proportion) were used as a mobile phase [4, 7, 8]:

(a) Petroleum ether: diethyl ether (60:40)
(b) Petroleum ether: diethyl ether (80:20)
(c) Petroleum ether: diethyl ether: acetic acid (80:20:1)
(d) Petroleum ether: diethyl ether: acetic acid (85:15:1)
(e) Hexane: diethyl ether (80:20)

Standard substances like methyl caprate, methyl laurate, methyl myristate, methyl palmitate, methyl palmitoleate, methyl stearate, methyl oleate, methyl linoleate, methyl linoleniate, methyl arachidiate, and methyl behenate were run simultaneously with the mixtures of fatty acid methyl esters mixture derived from fish lipid in each of the five chromatograms. After development, each of the chromatogram was dried at 150°C for fifteen minutes. The dried plates were then placed in a sealed tank containing a few iodine crystals when the dark yellow-brown spots appeared within few minutes where esters of fatty acids had absorbed the iodine.

In the chromatogram, the substances in the mixture were found to move relative to some of the standard substances to constant ratios of relative R_f values. The spots were marked and R_f values of the spots were calculated. The R_f values of the standard substances and the fatty acids mixtures derived from fish oil have been given in table **4.1** and table **4.2** respectively.

6.3. Results and discussion

Thin layer chromatography has been used for isolation, purification and identification of organic compounds because of its high speed of separation and easy technique [1]. Thin layer chromatographic systems achieve their ability to separate mixtures of fatty acid methyl eaters by selectively retarding the passage of some fatty acid methyl esters through the stationary phase while permitting others to move more freely [2]. The fatty acids esters derived from fish lipid was resolved on chromatographic plates and eight spots of dark yellow-brown were appeared within few minutes. When the R_f values of these spots were compared with the R_f values of standard substances, it was found that these values were identical with some of standard substances. By comparing the R_f values of the eight spots obtained from the fish lipid (table **4.2**) with those of standard substances (table **4.1**), it is concluded that the fish

lipid contain myristic acid ($C_{14:0}$), palmitic acid ($C_{16:0}$), stearic acid($C_{18:0}$), oleic acid($C_{18:1}$), lionoleic acid($C_{18:2}$), lionolenic acid($C_{18:3}$), arachidic acid($C_{20:0}$) and behenic acid($C_{22:0}$).

Table **4.1**: Separation and identification of standard fatty acid methyl esters mixture by TLC.

Fatty acid methyl esters standard	R_f value obtained from the spot in developing solvent system				
	P: E (80:20)	P: E (60:40)	P: E: A (85:15:1)	P: E: A (80:20:1)	H: E (80:20)
Methyl laurate ($C_{12:0}$)	0.925	0.672	0.857	0.911	0.875
Methyl myristate ($C_{14:0}$)	0.892	0.765	0.812	0.623	0.729
Methyl palmitate($C_{16:0}$)	0.635	0.530	0.637	0.429	0.552
Methyl palmitoleate ($C_{16:1}$)	0.523	0.497	0.561	0.552	0.469
Methyl stearate($C_{18:0}$)	0.765	0.829	0.911	0.752	0.833
Methyl oleate ($C_{18:1}$)	0.466	0.323	0.492	0.411	0.385
Methyl linileate ($C_{18:2}$)	0.565	0.395	0.433	0.295	0.356
Methyl linolineate ($C_{18:3}$)	0.615	0.556	0.492	0.523	0.442
Methyl arachidate($C_{20:0}$)	0.632	0.721	0.554	0.332	0.497
Methyl arachidonate ($C_{20:4}$)	0.332	0.352	0.491	0.432	0.450
Methyl behanate ($C_{24:0}$)	0.910	0.812	0.937	0.650	0.720

P: E = petroleum ether (40-60°C): Ether

P: E: A = petroleum ether (40-60°C): Ether: Acetic acid

H: E = Hexane: Ether

Table **4.2**: Separation and identification of fatty acid methyl esters derived from fish lipid by TLC.

Spots in the mixture resolved	Fatty acid methyl esters identified	R_f value obtained from the spot in developing solvent system				
		P: E (80:20)	P: E (60:40)	P: E: A (85:15:1)	P: E: A (80:20:1)	H: E (80:20)
S_1	Methyl myristate ($C_{14:0}$)	0.894	0.762	0.814	0.624	0.727
S_2	Methyl palmitate ($C_{16:0}$)	0.632	0.540	0.634	0.425	0.557
S_3	Methyl stearate ($C_{18:0}$)	0.764	0.820	0.912	0.761	0.834
S_4	Methyl oleate ($C_{18:1}$)	0.465	0.328	0.496	0.418	0.381
S_5	Methyl linileate ($C_{18:2}$)	0.570	0.396	0.437	0.297	0.351
S_6	Methyl linolineate ($C_{18:3}$)	0.611	0.554	0.495	0.526	0.447
S_7	Methyl arachidate ($C_{20:0}$)	0.632	0.726	0.558	0.339	0.494
S_8	Methyl behanate ($C_{24:0}$)	0.912	0.818	0.932	0.658	0.724

P: E = Petroleum ether (40-60°C): Ether

P: E: A = Petroleum ether (40-60°C): Ether: Acetic acid

H: E = Hexane: Ether

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PART-B

Determination of fatty acids composition and chain length of fatty acids of fish lipid by Gas Liquid Chromatography (GLC)

6.4. Introduction

Lipids consist essentially of glycerides, compounds of glycerol and fatty acids; they are usually tri-glycerides in which one molecule of glycerol has combined with three molecules of fatty acids with the elimination of three molecules of water [1]. The fatty acids can be classified into several series. The first of these is referred to as the saturated fatty acid series or simply as the fatty acid series. The second group is characterized by the presence of one double bond and is called the oleic acid series (or monounsaturated fatty acids). A third group is the linoleic acid series which is characterized by two unsaturated linkage [2].

The fatty acids containing two or more unsaturated linkage are generally referred to as polyunsaturated fatty acids (FUFAs). Fatty acids are important building blocks of lipid and play a vital role in the body. They perform a variety of specific and essential functions. Fatty acids form an integral component of the plasma membrane of every cell in the body [3]. The human body can manufacture most of the fats it needs, including cholesterol, saturated fatty acids and unsaturated fatty acids. But there are two fatty acids which can not be manufactured in the body, and which must be obtained from dietary sources: linoleic acid (ω–6 fatty acid) and alpha linolenic acid (ω–3 fatty acid). These are the essential fatty acids [4]. Essential fatty acids (EFAs) are vital for a healthy immune system. ω–3, essential fatty acid is critical for eye, brain, and neurological function. Deficiencies of ω–3 essential fatty acids can cause impaired brain function and decrease I.Q. ω–6, essential fatty acid is also important. It plays a major role in the texture and appearance of the skin and blood vessel structure [3]. Administration of ω–3 polyunsaturated fatty acids was

shown to reduce proteinuria and ameliorate renal injury in murine and lupus nephritis, experimental focal segmental glomerulosclerosis and other type of renal diseases [5]. The body must receive a constant and balanced supply of essential fatty acids (EPAs) to ensure proper prostaglandin production. Long chain ω-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are found in cold-water fish such as sardines, mackerel, anchovies, and cod liver. Fish, on the other hand, is a direct source of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [6]. There is growing evidence that increased consumption of fish oil may be beneficial to health but nutritive value depends on the fish processing methods [7]. Fats can be converted into trans fats when it is heated with liquid oils in presence of hydrogen. Hydrogenated fats (trans fats) increase the level of triglyceride and LDL cholesterol [3]. They may be caused fire inflammation [8] and over activity of the immune system that has been implicated in the heart disease, stroke, diabetes and other chronic conditions [3]. Recent studies have found an association between consumption of fish oil and reduced risk of cardiovascular disease, as well as improvements in other health conditions, such as psoriasis and rheumatoid arthritis [9]. High density lipoproteins (HDL) carry cholesterol from the blood back to the liver which processes the cholesterol for elimination from the body. Because of these, HDL is often referred to as good cholesterol[8] Fatty acids as source of energy are important. Laurie acid, myristic acid and palmitic acid have been shown to elevate blood cholesterol. Of these, myristic acid elevates the cholesterol most[9]. Stearic acid has been shown to lower cholesterol by 21% even more than oleic acid which lowers LDL by 15%[10] The largest benefits in terms of reducing cardiovascular disease are found when saturated fatty acids are replaced with monounsaturated fatty acids (MUFAs). Monounsaturated fatty acids are involved in the significant reduction in the relative risk for invasive breast cancer. MUFAs are associated with the

lowering of the oxidation of LDL cholesterol and triglyceride level in the blood.[11]

Most current research on the benefits of consuming more fish is directed at the effects derived from ω–3 fatty acids in many fish species. Fish oil depresses the synthesis of hepatic fatty acids and triglycerides and the secretion of very low density lipoprotein cholesterol (VIDL). There is considerable evidence that fish and fish oils are beneficial to heart health, reduce the risk of cancer and benefit mental health. The "active" components of fish oils are eicosapentaenoic acid (EPA), polyunsaturated fatty acids with 20 carbon atoms in its backbone, and docosahexaenoic acid (DHA), polyunsaturated fatty acids with 22 carbon atoms. Both are members of the ω–3 group of essential fatty acids, EPA and DHA are found exclusively in marine animals; fatty fish such as herring, sardines, salmon and fresh tuna are the best sources [9]. The present investigation was done to find out the fatty acids composition and amount of individual fatty acids in the lipid obtained from *Puntius gonionotus* (*P. gonionotus*) fish.

6.5. Materials and methods

Gas-Liquid Chromatography (GLC) is useful for both qualitative and quantitative analysis of the fatty acid composition of a sample in a very short time [10] with a remarkable degree of accuracy and precision [1]. The advent of GLC revolutionized the analysis of fatty acids components of lipids and it is undoubtedly the technique that would be chosen in most circumstances for the purpose particularly for the separation of very similar compound within the classes [10]. GLC achieves separation by partioning between a mobile gas phase and stationary liquid held on a solid support [11]. The test substance is introduced into the carrier gas by means of a micro syringe of the Hamilton or similar type which discharges through a suitable sealing device directly into a vaporizing chamber which may be maintained at a suitable temperature [1]. Elution is then

carried out by forcing an inert gas (such as nitrogen or helium) through the column. The rate of movements of the various components along the column depends upon their tendency to dissolve in the stationary liquid phase. Components having a negligible solubility in the stationary phase move rapidly through the column, while those components whose distribution coefficient favours the solvent liquid phase, move with a low rate through the column. A record is made of the signal which is produced by the suitable detector [12].

Fatty acid methyl esters derived from the *P. gonionotus* fish lipid were chromatographed on the polar stationary phase. The experiment was carried out with a "PYE UNICAM" 4500 U model gas chromatograph equipped with a flame ionization detector. A glass coiled column (3 mm ID × 2.1 m) packed with 70-100 mesh chromosorb after impregnating it with 10% diethylene glycol succinate (DEGS) was used for the regular polar packed column GLC. The methyl esters of fatty acids obtained from fish lipid was introduced in to the column at a temperature 130°C and was blown down to the column by the nitrogen carrier gas. The stream of nitrogen used was at 30 ml/minute. Upon initial contact of the solute with the liquid stationary phase, equilibrium was rapidly established between the amount of solute which dissolved in the liquid phase and the amount of solute remaining in the vapour. The equilibrium would always be displaced towards the vapour phase because the temperature of the column was raised to a final temperature 230°C and the raising rate was 4°C per minute. The oven, injector and detector temperatures were 190°C, 200°C and 250°C respectively. The vapour fraction of the solute was moved down the column by the carrier gas and the equilibrium between the two phases was destroyed. The solute vapour, which had been moved down the column, encountered fresh solvent and a new equilibrium was established. The sample vapours were detected as they left the column and the compounds, which emerged first, had either the lowest solubility in the stationary phase or the highest volatility. The detector response was displayed on a recorder (PM

8251, Philips) and the chromatogram was produced as a printed out record of the area under each peak. The speed of the chromatogram was at 0.5 cm/ minute [13, 14, 15].

Standard fatty acid esters mixture was also chromatographed using same procedure as described above.

The fatty acids in the mixtures were identified by comparing its relative retention time [16] and $\log_{10}$ relative retention time with those of standard fatty acids. Relative retention time may be defined as the time elapsed between the injection of the sample and its appearance at the detector [12]. The relative retention time of a substance is its own retention time compared to that of a chosen standard of known chain length, e.g. methyl palmitate. The relative retention time was calculated for each of the standard and sample substances on polar column and the results have been computed in table **4.3**.

The area of each chromatographic peak was determined [17] by multiplying the height of the peak by the width of the peak at one-half of the height. The area under each peak down by the recorder was proportional to the amount of substance detected. The areas of all peaks were summed and the percentage of each fatty acid was calculated by the following formula:

$$\text{Percentage of fatty acid} = \frac{\text{area of each peak}}{\text{total area of all peak}} \times 100$$

6.6. Equipments

- Gas chromatogram: PYE UNICAM UP 4500 capillary chromatograph (Philips-England) equipped with flame ionization detector.
- Glass column: (1500 mm x 4 mm) Temperature maintained at 190- 220°C.
- Syringe: Maximum volume 10 ml, graduated 0.1 ml.

- Recorder: LKB 2220 Recording integrator (BROMMA-England).
- Packing materials: Columan packing was done with 10% diethylene glycol succinate (DEGS) on 100-120 mesh diatomic CAW.

6.7. Chemicals and reagents

- Standard Samples: Methyl esters of Caprylic, Nonanoic, Capric, Undecanoic, Laurie, Myristic, Palmitic, Stearic, Oliec, Arachidic, Behenic (Sigma Chemical Ltd.)
- Preparation of BF_3 solution: About 125 g BF_3/L methanol was prepared by the following method. MeOH (IL) was taken in a 2L flask and weighed. The flask was cooled on an ice bath and while cooling, BF_3 was bubbled into MeOH until 125 gm was absorbed from a cylinder through a glass tube. The work was carried out in a hood with special care so that BF_3 was flown through the glass tube with very slow flow rate.
- Methanolic sodium hydroxide solution (0.5N) was prepared by dissolving 2g NaOH (BDH, England) in 100 ml MeOH.
- Heptane (E. Merck, Germany.).
- Methyl red (E. Merck, Germany) solution: 0.1% in 60% alcohol.
- Nitrogen.

6.8. Preparation of fatty acid methyl esters mixture from the lipid

About 30.9 mg of lipid sample was taken into a 125 ml round bottomed flask and 50ml methanolic NaOH solution and boiling chips were added to the flask. The flask was saponified under reflux condenser for 45 minutes on a water bath until it became clear. About 31g of BF_3 solution was added from a bulb through a condenser and boiled for 2 minutes. Heptane (2ml) was added through condenser and boiled for 1 minute longer. The heat was removed and then saturated NaCl solution was added. The flask was rotated gently for several times. Additional saturated NaCl solution was added to float heptane

solution into neck of the flask. The dry methyl esters were recovered by transferring aqueous and heptane phases to 250 ml separator. The contents were extracted with successive 20 ml water until the solution was acid free to methyl red indicator. The esters were dried over anhydrous Na_2SO_4 and filtered and the solvent was evaporated under a stream of N_2. The methyl esters thus prepared were preserved for GLC experiment.

6.9.Procedure

Mixture of fatty acid methyl esters derived from the lipid was diluted to 7% using hexane solvent. The sample was then injected to the injecting port of the column. Temperature programming of the column was maintained 190-220°C at the rate of rising 4°C /min. Oven temperature, injector temperature and detector temperatures were 190, 195, and 205°C respectively. Nitrogen was used as a carrier gas at the flow rate of 32 ml/min. The identification of fatty acid methyl esters from GLC analyses was carried out on the basis of relative retention time and were quantified by measuring the peak area in comparison with standard chromatogram which was presented in table 5 gas chromatograms were given in figure 6.

6.10. Results and discussion:

Fish oils are unique in the variety of fatty acids of which they composed and their degree of unsaturation [18]. Fatty acid analysis of the fish lipid was carried out by GLC after esterification of the glycerides to their methyl esters on polar stationary phase (diethylene glycol succinate (DEGS) and the chromatogram as printed out has been shown in figure **5.1**. Standard fatty acid methyl esters mixtures of known structures were separated on the same column and the separation pattern has been shown in figure **5.2**. The order of emergence of fatty acid methyl esters from the polar stationary phase was observed to be

lauric ($C_{12:0}$), myristic ($C_{14:0}$), palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), linolenic ($C_{18:3}$), ahachidic ($C_{20:0}$), behenic ($C_{22:0}$) and lignoceric ($C_{24:0}$). From figure **5.1**, it is evident that the substances have been smoothly separated on polar column. The polar column stationary phase gives separations based on molecular mass (i.e., chain length) as well as number of double bonds. Relative retention time vary with the conditions of the column packing materials and with other operating parameters, such as temperature or flow-rate, but these variations are comparatively small and are in the same direction for all components [10]. The identities of the individual fatty acids were achieved by co-chromatography with standard reference compounds.

The peaks of figure **5.1** were tentatively identified by comparing their relative retention times with the relative retention times of standard fatty acids methyl ester mixtures [19] and the analytical data were summarized in table **4.4**. It is evident from chromatogram (figure **5.1**), that the fatty acid composition of fish lipid obtained from *P. gonionotus* were myristic acid ($C_{14:0}$), palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$), lionoleic acid ($C_{18:2}$), lionolenic acid ($C_{18:3}$), arachidic acid ($C_{20:0}$) and behenic acid ($C_{22:0}$).

From figure **5.1**, it is revealed that saturated fatty acid methyl esters chromatographed on polar stationary phase have the shortest retention times followed by corresponding monoenoic, dienoic, trienoic etc. The percentage of each fatty acid methyl esters derived from fish lipid was determined by calculating the area under each peak. The percentages of composition of each fatty acid methyl esters in the fish lipid from the polar stationary phase have been summarized in table **4.4**. From table **4.4**, it is evident that fish lipid contains 6.33% myristic acid, 24.23% palmitic acid, 8.42% stearic acid,

30.29% oleic acid, 7.35% lionoleic acid, 5.46% lionolenic acid, 2.47% arachidic acid, 4.25% behenic acid and 11.13% unknown acid.

Analysis of this fish lipid for fatty acids indicate that the lipid contain 7.35% linoleic acid (ω–6 fatty acid) and 5.46% linolenic acid (ω–3 fatty acid). In human nutrition, fatty acids such as lionoleic acid and lionolenic acid are regarded as essential since they can not be synthesized [20]. ω–3 polyunsaturated fatty acids have been used to kill or slow the growth of cancer cells in culture and animals models and to increase the effectiveness of cancer chemotherapeutic drugs [21].

The two distinct families of essential fatty acids compete for the same enzymes for forming double bonds (desaturase enzymes) and enzymes for longthering carbon chain (elongase enzymes).

The body can not convert a ω–3 to ω–6 fatty acid, or vice-versa [4]. Linoleic acid ($C_{18:2}$, ω–6) is converted to archidonic acid ($C_{20:4}$, ω–6) which is the immediate precursor to prostaglandins, hormone-like regulating substances which protect the body from deleterious effects (sticky platelets, high blood pressure, inflammation, water retention, lowered immune function) [9].

The fish lipid contained about 34.1% oleic acid (monounsaturated fatty acids) can able to promote the high level of HDL cholesterol and hence, decreasing the risk of heart diseases in human body [3]. From table **4.4,** it is evident that the percentage of saturated fatty acids in this fish lipid was 42.43%; monounsaturated fatty acids 43.1% and polyunsaturated fatty acids 14.43%. These values when compared with others oil derived from different sources (table **4.5**), it is found that, these values have different trend for different oils. Saturated fat diets seem to be protective against alcohol-induced liver injury.

Saturated fat is correlated with a lower incidence of alcoholic cirrhosis [22]beef fat [23] and coconut oil [24] but not lard [25] suppressed liver damage due to alcohol [6].

The fatty acids composition of marine fish oils is influenced considerably by seasonal, age and other factors [11]. The fatty acids profile of this fish when compared with other edible fish, meat, and seed oil (table **4.6**), it is found that this fish lipid is the reliable sources of saturated, monounsaturated and polyunsaturated fatty acids. The result indicated that unsaturated fatty acids in fish oils under present investigation are necessary for the proper functioning of many metabolic processes in human beings. There is strong evidence that fish and fish oil consumption reduces the risk of heart diseases and so-called "sudden deaths"[32].The present findings suggested that the fish lipid is suitable for edible purpose as it contained both ω –3 and ω–6 fatty acid especially linoleic and alpha-linolenic acids.

Table **4.3**: Relative retention time of standard fatty acid methyl esters mixture and fatty acid methyl esters derived from fish lipid on a polar stationary phase.

Name of sample	Peak no.	Chain length	Relative Retention time	Relative retention time with respect to methyl palmitate ($C_{16:0}$)	Log_{10} relative retention time	Name of fatty acids
Standard	1	$C_{12:0}$	4.42	0.55	-0.26	Lauric
	2	$C_{14:0}$	6.16	0.76	-0.12	Myristic
	3	$C_{16:0}$	8.06	1	0.00	Palmitic
	4	$C_{18:0}$	10.52	1.31	0.12	Stearic
	5	$C_{18:1}$	11.18	1.39	0.14	Oleic
	6	$C_{18:2}$	12.06	1.50	0.18	Linoleic
	7	$C_{18:3}$	13.15	1.63	0.21	Linolenic
	8	$C_{20:0}$	14.11	1.75	0.24	Arachidic
	9	$C_{22:0}$	18.51	2.30	0.36	Behenic
	10	$C_{24:0}$	25.68	3.19	0.50	Lignoceric
Fish lipid	1	$C_{14:0}$	6.15	0.76	-0.12	Myristic
	2	$C_{16:0}$	8.06	1	0.00	Palmitic
	3	$C_{18:0}$	10.52	1.31	0.12	Stearic
	4	$C_{18:1}$	11.18	1.39	0.14	Oleic
	5	$C_{18:2}$	12.05	1.50	0.18	Linoleic
	6	$C_{18:3}$	13.16	1.63	0.21	Linolenic
	7	$C_{20:0}$	14.12	1.75	0.24	Arachidic
	8	$C_{22:0}$	18.50	2.30	0.36	Behenic
	9	Unknown	21.95	2.72	0.43	Unidentified
	10	Unknown	25.68	3.19	0.50	Unidentified
	11	Unknown	26.62	3.30	0.53	Unidentified

Table **4.4**: Fatty acid composition of fish lipid with respect to standard methyl esters.

Name of sample	Peak no.	Chain length	Relative Retention time	Name of fatty acids	Area under each peak (sq. mm)	Weight %
Standard	1	$C_{12:0}$	4.42	Lauric	35.95	-
	2	$C_{14:0}$	6.16	Myristic	29.68	-
	3	$C_{16:0}$	8.06	Palmitic	107.17	-
	4	$C_{18:0}$	10.52	Stearic	96.49	-
	5	$C_{18:1}$	11.18	Oleic	71.22	-
	6	$C_{18:2}$	12.06	Linoleic	24.58	-
	7	$C_{18:3}$	13.15	Linolenic	12.39	-
	8	$C_{20:0}$	14.11	Arachidic	40.75	-
	9	$C_{22:0}$	18.51	Behenic	31.64	-
	10	$C_{24:0}$	25.68	Lignoceric	1548	-
Fish lipid	1	$C_{14:0}$	6.15	Myristic	35.17	6.33
	2	$C_{16:0}$	8.06	Palmitic	134.52	24.23
	3	$C_{18:0}$	10.52	Stearic	46.75	8.42
	4	$C_{18:1}$	11.18	Oleic	168.15	30.29
	5	$C_{18:2}$	12.05	Linoleic	40.84	7.35
	6	$C_{18:3}$	13.16	Linolenic	30.34	5.46
	7	$C_{20:0}$	14.12	Arachidic	13.73	2.47
	8	$C_{22:0}$	18.50	Behenic	23.59	4.25
	9	Unknown	21.95	Unidentified	30.41	5.47
	10	Unknown	25.68	Unidentified	12.94	2.33
	11	Unknown	26.62	Unidentified	18.53	3.33

Table **4.5**: Saturated and unsaturated fatty acid present in *P. gonionotus* along with some oils and fats [3, 29].

Name of oil derived from different sources	Saturated fatty acid	Mono unsaturated fatty acid	Poly unsaturated fatty acids			
P. gonionotus	**42.43%**	**43.1%**	**14.43%**			
Olive oil	13	72	08			
Soyabean oil	16	44	37			
Coconut oil	87	06	02			
Lard	39	44	11			
Butter	60	26	05			
Pond raised Channel Catfish	23.76± 0.12	43.75±4.48	Diens	Triens	n_3	n_6
			15.31±1.30	4.07± 0.75	3.74± 1.44	21.46± 3.04

Table **4.6**: Fatty acid compositions of *P. gonionotus* fish lipid and approximate fatty acid composition of some fats and oil (in %).

Sources of oil	Myristic ($C_{14:0}$)	Palmitic ($C_{16:0}$)	Palmitoleic ($C_{16:1}$)	Stearic ($C_{18:0}$)	Oleic ($C_{18:1}$)	Linoleic ($C_{18:2}$)	Linolenic ($C_{18:3}$)	Arachidic ($C_{20:0}$)	Arachidonic ($C_{20:4}$)	Behenic ($C_{22:0}$)
P. gonionotus	6.33	24.23	-	8.42	30.29	7.36	5.46	2.47	-	4.25
G. Centropus[26]	-	13.57	-	5.53	51.16	20.60	7.83	1.30	-	-
Rita rita[27]	1.63	19.95	-	9.15	38.31	-	-	-	-	27.94
Eutropiichthys vacha[28]	3.10	46.30	-	0.95	25.30	20.30	1.53	0.47	-	-
Pond raised Channel Catfish [29]	0.97± 0.06	16.26± 0.49	2.75± 0.28	6.36± 0.36	39.37± 4.34	13.56± 1.24	0.92± 0.19	-	2.55± 0.92	-
Mackerel [30]	4	19	5	5	27	1	-	-	-	-
Herring [30]	8	14	7	1	22	1	-	-	-	-
Tuna [30]	3	20	4	9	25	1	-	-	-	-
Cod liver oil[30]	4	11	8	2	22	1	-	-	-	-
Olive oil [30]	<0.5	7.5-20	0.3-3.5	0.5-3.5	56-83	3.5-20	<1.5	Trace	-	Trace
Chicken [31]	0.9	22.1	5.5	7.7	34.7	26.5	1.1	-	-	-
Egg chicken[31]	0.3	26.6	4.0	9.3	44.1	13.4	-	-	-	-
Beef fat [31]	3.4	30.5	4.3	14.7	45.8	3.2	0.4	-	-	-

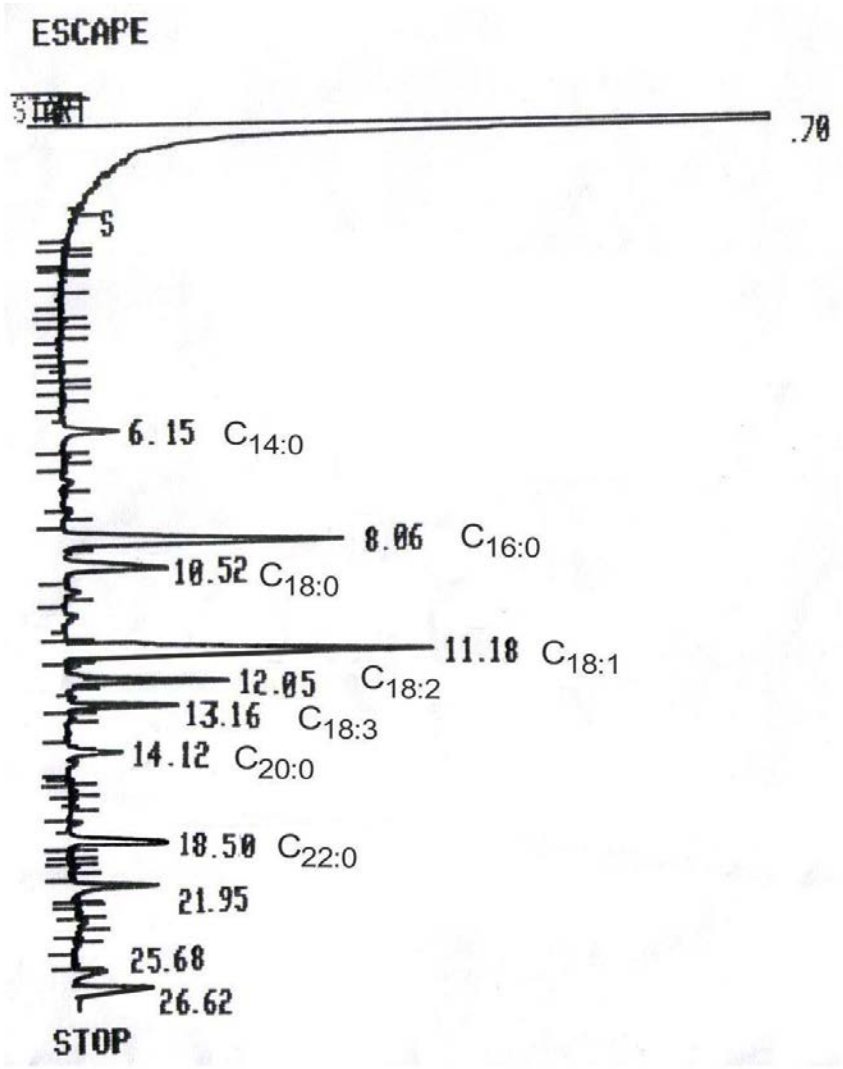

Fig. **5.1:** Gas-liquid chromatography separation of the fatty acid methyl ester mixture derived from fish lipid on a polar stationary phase (EGS).

RUN # 14

AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.70	5382300	SBB	0.045	98.979
6.15	3517	PB	0.116	0.064
8.06	13452	PB	0.127	0.247
10.52	4675	BB	0.138	0.085
11.18	16815	PB	0.131	0.309
12.05	4084	PB	0.081	0.075
13.16	3034	PB	0.071	0.055
14.12	1373	PB	0.121	0.025
18.50	2359	PB	0.076	0.043
21.95	3041	PB	0.106	0.055
25.68	1294	PP	0.123	0.023
26.62	1853	I PH	0.070	0.034

TOTAL AREA= 5437797
MUL FACTOR= 1.0000E+00

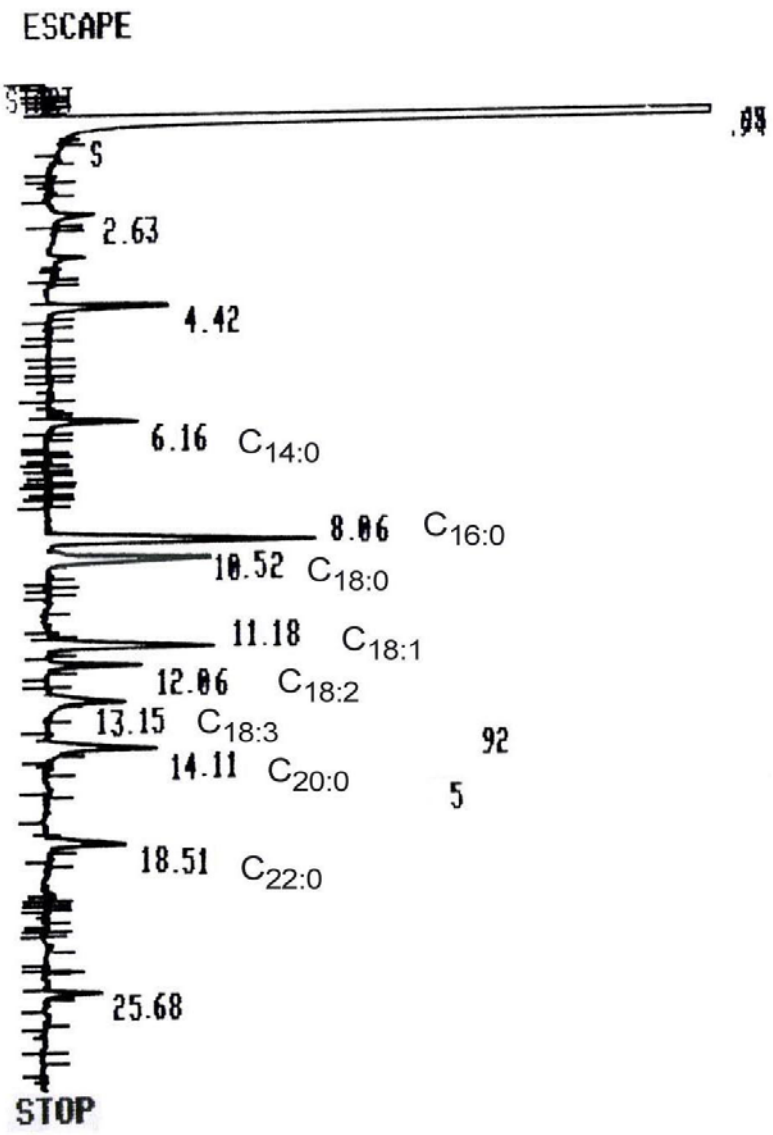

Fig **5.2:** Gas-liquid chromatography separation of standard fatty acid methyl esters on a polar stationary phase (EGS).

RUN # 9

AREA%

RT	AREA	TYPE	AR/HT	AREA%
0.65	7263700	SBB	0.037	97.250
0.74	156990	DTBB	0.027	2.100
2.63	1575	PB	0.103	0.021
4.42	3595	BB	0.083	0.048
6.16	2968	BB	0.092	0.040
8.06	10717	PB	0.111	0.143
10.52	9649	PV	0.098	0.129
11.18	7122	BB	0.119	0.095
12.06	2458	BB	0.073	0.033
13.15	1239	PP	0.085	0.016
14.11	4075	PB	0.107	0.055
18.51	3164	VB	0.117	0.042
25.68	1548	BB	0.078	0.020

TOTAL AREA= 7468849
MUL FACTOR= 1.0000E+00

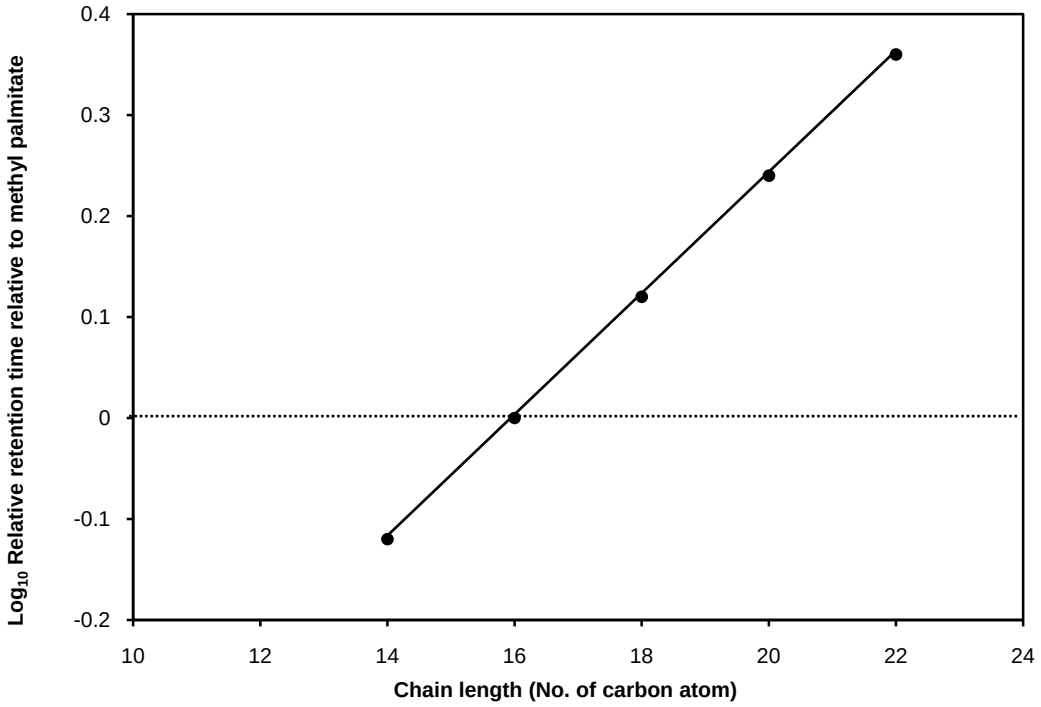

Fig.**5.3**: Relationship between $\log_{10}$ relative retention time relative to methyl palmitate and the number of carbon atoms of fatty acid methyl esters.

Summary and Conclusion

Fat is made up of fatty acid. Fatty acids are chains of carbon atoms which can either be saturated with hydrogen atoms or contain relatively fewer hydrogen atoms (the unsaturated and poly unsaturated fatty acids. Fats are an important component of membrane in our hearts, brains, immune cells and most of the other tissues of our body. Some fats are essential for good health, like MUFA and PUFA, while others fat such as *trans* fat are harmful to our bodies. A high intake of *trans* fat has been linked to a variety of free radical and degenerative conditions such as cancer, arthritis, and cardiovascular disease. The lower rates of cardiovascular disease is not directly related to total fat intake, but more with the kind of fat consumed.

TLC has been used for identification of fatty acids composition of *P. gonionotus* fish lipid. For this purpose, methyl esters obtained from this fish lipid fish was subjected TLC examination and their fatty acids composition were identified by comparing the R_f values of methyl esters of standard fatty acids. Using the different solvent systems, it was found from the chromatogram that the lipid gave eight spots under iodine vapour in a sealed tank. The spots were identified as myristic acid ($C_{14:0}$), palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), oleic acid ($C_{18:\ 1}$), lionoleic acid ($C_{18:\ 2}$), lionolenic acid ($C_{18:\ 3}$), arachidic acid ($C_{20:0}$) and behenic acid ($C_{22:0}$) in all solvent systems by comparison with the R_f values of standard methyl myristate, methyl palmitate, methyl stearate, methyl oleate, methyl linoleate, methyl linoleniate, methyl arachidiate, and methyl beheniate.

Analysis of fatty acid methyl esters derived from fish oil was also performed by GLC equipped with a flame ionization detector. A glass-coiled column packed with 70-100-mesh chromosorb after impregnating it with 10% diethylene glycol succinate (DEGS) was used. The operating conditions were

as follows: column temperature, 130-230°C; oven temperature, 190°C; injector temperature 200°C; detector temperature, 205°C. The carrier gas used was nitrogen, at flow rate of 30 ml/minute. The speed of the chromatogram was at 0.5 cm/ minute. Fatty acids were identified by comparison with the relative retention time and $\log_{10}$ relative retention time with those of standards. The polar column stationary phase gives separations based on molecular mass (i.e., chain length) as well as number of double bonds. The fatty acids profile of this fish oil were 6.33% myristic acid, 24.23% palmitic acid, 8.42% stearic acid, 30.29% oleic acid, 7.35% lionoleic acid, 5.46% lionolenic acid, 2.47% arachidic acid, 4.25% behenic acid and 11.13% unknown acid. A plot of the logarithm of retention times against the number of carbon atoms for saturated fatty acids gives a straight line. The most important characteristics feature of this fish lipid are content of both omega-3 and omega-6 type of polyunsaturated fatty acids (linolenic acid and linoleic acid). Fish lipid containing 5.46% linolenic acid (omega-3) and 7.35% (omega-6) that are combined in mixed triglycerols with ordinary fatty acids, are the main sources of omega-3 and omega-6 polyunsaturated fatty acids respectively. These PUFA containing lipid can be used as food additives or supplements. Monounsaturated fatty acid, oleic acid (43.1%) found in this fish lipid, can increase HDL cholesterol levels without increasing total cholesterol. Total saturated fatty acids found in this fish lipid were 42.43%, which is necessary normal functioning of the body..

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CHAPTER 07

CYTOTOXIC EFFECT OF *P. GONIONTOUS* FISH LIPID

Local Name : Thai shar phuti/China punti
Scientific Name : *Puntius gonionotus*

Cytotoxic effect of *Puntiusgonionotus* fish lipid

7.1. Introduction

Brine shrimp lethality bioassay is a recent development for the bioactive compounds. Fish lipid of *P. gonionotus* can be tested for its bioactivity by this method. Here, in vivo lethality in simple zoological organism (brine shrimp nauplii) is used as a convenient monitor for screening and fractionation in the discovery of new bioactive compounds. This bioassay indicates cytotoxicity as well as a wide range of pharmacological activities of the compounds[1-5].

The brine shrimp bioassay has several advantages such as:

(i) Rapid in process (24 hrs.).
(ii) Inexpensive and simple.
(iii) It easily utilizes a large number of organisms for statistical validation and requires no special equipment.
(iv) It requires a relatively small amount of sample. It does not require animal serum, as it is needed furthermore, for determination of cytotoxicity.

7.2. Materials

(i) Micropipette (10-100µl).
(ii) Small tank with perforated dividing dam to grow shrimp, cover and lamp to attack shrimp.
(iii) Magnifying glass.
(iv) Pipettes (5 ml, 1ml).
(v) *Artemiasalina* leach (brine shrimp eggs from store).
(vi) Vials (5 ml).
(vii) Sea salt.

7.3. Procedure

7.3.1. Preparation of seawater:

Seawater contains about 3.8% of sodium chloride. So, 38 g of sodium chloride (pure NaCl) was weighed accurately and dissolved in distilled water to make a volume of 1 (one) litre and then filtered off.

7.3.2. Hatching of shrimps:

Seawater was kept in the tank and shrimp eggs were added to one side of the perforated divided tank where constant oxygen supply was ensured at constant temperature (around 37°C). Two days were allowed to hatch and mature as nauplii (larvae).

7.3.3. Preparation of samples:

Exactly 10 mg fish lipid was accurately weighed and dissolved in10 ml chloroform to get a concentration of 1 µg/µl.

7.3.4. Application of the test sample and brine shrimp nauplii to the vials:

Cleaned 6 vials were taken for the sample of five different concentrations (one vial for each concentration) and one vial also taken for control test. Seawater (5 ml) was given to each of the vials. Then with the help of micropipette specific volumes of sample were transferred from stock solution to the vials to get final sample concentrations of 10 µg/ml, 20 µg/ml, 40 µg/ml and 80 µg/ml respectively. With the help of a pasteur pipette 10, 12, 12, 11, and 12 living shrimps were taken to sample and control vial respectively.

Counting of nauplii:

After 24 hrs, the vials were observed and the survived nauplii in each vial were counted and the results were noted.

Results and discussion:

Brine shrimp lethality bioassay indicates toxicity as well as a wide range of pharmacological activities (i.e. anticancer, antiviral, insectidal and pesticidaletc). The fish lipid of *P. gonionotus* showed negative results in brine shrimp lethality bioassay. The negative response obtained in this assay suggested that fish lipid have no antibacterial and antifungal activities.

Summary and Conclusion

In this bioassay, the chloroform-methanol extract fish lipid showed negative results indicating that the fish lipid was biologically inactive. From this experiment, it is revealed that the fish lipid has no toxicity against any living organisms.

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CHAPTER 08

DIETARY EFFECT OF FISH PROTEIN CONCENTRATE ON ALBINO RATS

Local Name : Thai shar phuti/China punti
Scientific Name : *Puntius gonionotus*

PART-A

Dietary effect of feeding[1] Fish Protein Concentrate supplemented with formulated cereal at different protein levels on young male albino rats for consecutive 32 days.

8.1. Introduction

For man and all animals protein is an essential constituent of diet and without this death is inevitable. The major percentage of Bangladesh peoples is suffering from malnutrion, physical and manual diseases. Now a day, the greatest challenge of Bangladesh is to combat prevalence malnutrion. To overcome this problem it is necessary to supply protein rich diets to the people. Fish is the principle source of protein for the people of Bangladesh. The biological value and protein efficiency ratio, indices of the amino acid profile and ability to support growth, are higher for fish than for beef, pork, chicken and milk proteins[1]. Traditionally the people consume fishes of fresh water and near shore brackish water origin [2]. *Puntiusgonionotus* (*P. gonionotus*) locally known as China punti (Bangladesh) is both saline and brackish water fish. The quantity of fish protein that is available from this fish is of excellent to quality which can be conventionally used to improve the protein quality in the diet of the people of Bangladesh. Besides this, our diet is known to be deficient in micronutrients, like iron, calcium magnesium, sodium, potassium and zinc. All this micronutrients can be derived from this fish as discussed in the foregoing. In this chapter the supplementary effect of feeding Fish Protein Concentrate (FPC) with formulated cereal under different protein levels was carried out to evaluating nutritional significance of the fish flesh of *P. gonionotus*. Nutritional significance of the FPC have been evaluated by determining gain in body weight per one gram of food intake, Protein Efficiency Ratio (PER) [3] and Calorie Efficiency Ratio (CER)[4] values in young albino rats after feeding fish

flesh at different protein levels supplemented with FPC formulated cereal. In this case, male young albino rats were selected to avoid any physiological differences that might be arises due to sex and young albino rats show maximum physiological responses [4].

8.2. Materials and methods

Twelve male albino rats (Wistar Kyoto Outbreed) [5] having body weight 20–30 g were collected from the animal house facility of the University Institute. After collection, they were kept in another animal house (used for research purposes) and fed them only supplied formulated cereal as their food for several days till their body weight obtained 50–60 g. Formulated cereal was collected from ICDDRB. This standard formulated cereal [6] is widely used as food for rats, mice and other related animals used for research purposes. Then the rats were grouped by randomised design into six groups where one was control group (diet–A) and rest were experimental groups (diet–B, diet–C, diet–D, diet–E and diet–F) of two rats each. They were housed individually in stainless steel cages with screen bottoms. The animal house was kept clean with optimal room temperature and exposed to a light and dark cycle of 12 hours duration each [7]. The experimental rats have been shown in figure 6.1.1 and the rat being fed with diet is shown in figure 6.1.2.

The quantitative composition of the ingredients and nutritive values of the formulated cereal are shown in table **5.1** and table **5.2** respectively. The major ingredients of formulated cereal were corn, wheat, wheat bran, gram, til oil cake, soya–44, salt, fish meal-60, rice polishing, and soybean oil. Five experimental diets (diet–B, diet–C, diet–D, diet–E and diet–F) were prepared by supplementing FPC (5%, 10%, 15%, 20% and 25% respectively) with formulated cereal at different protein levels as shown in table **5.3**. Rats of group diet–A (control group) were fed only supplied formulated cereal. The

percentage of proteins and percentage of lipid were plotted against different diets are presented in figure **6.2**.

The experimental period was 32 consecutive days. During this period of experiment, distilled water was provided all the time in watering bottles and changed daily [7]. Total food consumed, gain in body weight for each rat after 32 consecutive days and their initial body weight have been shown in table **5.4.1**. During experimental period, gain in body weight for each rat were observed at every four days interval. Data for these observations are shown in table **5.4.2** and plotted against corresponding days and shown in figure **6.3**. The nutritive values of each diet evaluated by PER and CER values were calculated by comparing the consumption of food and gain in body weight by each rat. Protein Efficiency Ratio (PER) is defined as the gain in body weight (g) per gram of protein intake during the period of experiment. Calorie Efficiency Ratio (CER) was calculated from gain in body weight (g) per 100 calories intake during the experiment. The effect of feeding FPC supplemented with formulated cereal at different protein levels on gain in body weight per gram of food, PER and CER have been computed in table **5.5**. Figure 6.4 shows the comparison of mean PER and mean CER in different diets.

8.3. Results and discussion

Table **5.1** shows the percentage of different ingredients present in formulated cereal and table **5.2** indicates that the supplied formulated cereal contain 21% protein, 5% fat, 5.15% fibre and 5.9% ash. From table **5.3**, it is evident that FPC levels in five experimental diets (diet–B to diet–F) were varying from 1.11 % to 5.55 % and fat content varying from 0.2625 % to 1.3125 %. Figure **6.2** shows that the percentage of total protein in different diets (21.00 to 21.30) and percentage of total lipid in different diets (5.00 to 5.0625) increased linearly with increasing FPC in six diets. From table **5.4.1**, it is seen that total food

consumed by each rat were almost similar but their body weight increased gradually with increasing FPC. From this result, it is revealed that gain in body weight in each rat depended mainly on supplied FPC not on formulated cereal. From table **5.4.2**, it is seen that gain in body weight of all rats (diet–A, diet–B, diet–C, diet–D, diet–E and diet–F) at four days interval increased with increasing days and reached maximum values of 63.02 g, 79.95 g, 100.25 g, 116.14 g, 112.93 g and 118.9 g respectively. Figure **6.3,** illustrates that body weight of all rats increased rapidly up to 20 days and then increased very slowly.

From table **5.5**, it is found that mean gain in body weight per gram of food intake, PER and CER were increased with increasing protein levels supplemented with formulated cereal and showed maximum value of 0.534g, 2.53 and 13.02 respectively at 15% protein level present in diet–D. This table illustrates that gain in body weight increased up to15% FPC were evidently proportional to the protein level. When protein levels were further increased through FPC, fall in gain in body weight per gram of food, PER and CER have been observed in diet–E (0.457g, 2.16 and 11.15 respectively) and diet–F (0.432g, 2.03 and 10.54 respectively). Rats in control group (diet–A) had a little effect on gain in body weight (0.272g) per gram of food intake, PER (1.30), CER (6.63) on young male albino rats in comparison with other five experimental groups. From figure **6.4**, it is found that diet–D had highest PER and CER followed by diet–E, diet–C, diet–F, diet–B, and diet–A. From the table **5.5,** it is also found that PER values were directly proportional to their respective CER values. From the values of PER, CER and also gain in body weight per gram of food in table **5.5**, it is inferred that the effect of 15% FPC in diet–D was remarkably high.

Table **5.1**: Quantitative compositions of formulated cereal.

Ingredients	% of ingredients	Ingredients	% of ingredients
1. Corn	12.76	11. Sorbatox	0.25
2. Wheat	30.00	12. Limestone	0.30
3. Wheat bran	20.00	13. Dichloride phosphate	0.25
4. Til oil cake	6.00	14. Choline chloride	0.10
5. Gram	8.00	15. Uitamine premix	0.30
6. Soya-44	3.00	16. Salt	0.50
7. Skimoned milk powder	1.00	17. Fish meal–60	10.00
8. Lysin	0.09	18. Soyabean oil	1.00
9. Feedzine	0.05	19. Rice polishing	6.00
10. Sal kill	0.04		

* The formulated cereal contains 410 calories/100 g.

Table **5.2**: Nutritive values of formulated cereal.

Proximate composition of ingredients	% in formulated cereal
Protein	21
Fat	5
Fibre	5.15
Ash	5.9

Table **5.3**: Composition of experimental diets.

Name of diets	Composition of diets (g)	Percentage of protein (g) in diets	Percentage of lipid (g) in diets	Total protein in diets (g)	Total lipid in diets (g)
Diet–A	100% formulated cereal only	21	5	21	5
Diet–B	5% fish flesh in 95% formulated cereal	Fish flesh = 1.11 Formulated cereal = 19.95	Fish flesh = 0.2625 Formulated cereal = 4.750	21.06	5.0125
Diet–C	10% fish flesh in 90% formulated cereal	Fish flesh = 2.22 Formulated cereal = 18.90	Fish flesh = 0.5250 Formulated cereal = 4.500	21.12	5.025
Diet–D	15% fish flesh in 85% formulated cereal	Fish flesh = 3.33 Formulated cereal = 17.85	Fish flesh = 0.7875 Formulated cereal = 4.250	21.18	5.0375
Diet–E	20% fish flesh in 80% formulated cereal	Fish flesh = 4.44 Formulated cereal = 16.80	Fish flesh = 1.050 Formulated cereal = 4.000	21.24	5.050
Diet–F	25% fish flesh in 75% formulated cereal	Fish flesh = 5.55 Formulated cereal = 15.75	Fish flesh = 1.3125 Formulated cereal = 3.750	21.30	5.0625

Table **5.4.1.** The effect of feeding FPC at different protein levels supplemented with formulated cereal on gain in body weight of young male albino rats having body weight 53–64 g.

Diets	Percentage of formulated cereal in diets	Percentage of fish flesh in the diet	Protein in formulated cereal per 100 g diet	Protein (g) in fish flesh per 100 g diet	Rat No.	Initial body weight (g) of each rat	Food consumed (g) by each rat	Gain in body weight (g) by each rat
A	100	Null	21	Null	a	54.62	230.82	62.44
					b	57.51	232.86	63.60
B	95	5	19.95	1.11	a	61.03	215.72	81.51
					b	55.65	226.51	78.38
C	90	10	18.90	2.22	a	53.86	219.35	98.27
					b	61.70	235.87	102.27
D	85	15	17.85	3.33	a	59.55	215.03	115.94
					b	56.27	220.14	116.34
E	75	20	16.80	4.44	a	63.17	224.60	119.48
					b	58.15	278.35	106.37
F	75	25	15.75	5.55	a	58.92	262.08	120.32
					b	59.80	290.51	117.48

Table **5.4.2**: Mean gain in body weight at every four days interval of male albino rats after feeding FPC at different levels supplemented with formulated cereal.

Days	Gain in body weight (g)					
4	Diet-A	Diet-B	Diet-C	Diet-D	Diet-E	Diet-F
8	7.54	8.97	13.27	25.88	40.32	45.00
12	17.57	23.80	35.63	55.02	70.46	82.44
16	32.8	42.32	55.32	80.54	90.54	100.00
20	45.63	59.36	76.67	100.02	103.14	111.55
24	56.32	70.25	89.65	110.11	107.89	114.11
28	61.52	75.24	95.64	112.23	112.64	115.86
32	62.83	77.11	98.55	114.18	112.81	117.22
4	63.02	79.95	100.27	116.14	112.93	118.90

Table **5.5**: The effect of feeding FPC at different protein levels supplemented with formulated cereal on gain in body weight per gram food intake, PER and CER.

Diets	Rat No.	Gainin body weight (g) per gram of food	Mean gain in body weight	Protein (g) intake by each rat	PER	Mean PER	Caloric intake by each rat	CER	Mean CER
A	a	0.270	0.272	48.47	1.295	1.30	946.36	6.60	6.63
	b	0.273		48.90	1.30		954.72	6.66	
B	a	0.378	0.362	45.43	1.79	1.72	884.45	9.21	8.82
	b	0.346		47.70	1.64		928.69	8.43	
C	a	0.448	0.441	46.32	2.12	2.09	899.33	10.92	10.75
	b	0.433		49.81	2.05		967.06	10.58	
D	a	0.539	0.534	45.54	2.55	2.53	881.62	13.15	13.02
	b	0.528		46.62	2.50		902.57	12.89	
E	a	0.531	0.457	47.70	2.43	2.11	920.86	12.97	11.15
	b	0.382		59.12	1.79		1141.24	9.32	
F	a	0.459	0.432	55.82	2.15	1.02	1074.53	11.20	10.53
	b	0.404		61.88	1.89		1191.09	9.86	

Table **5.6**: Gain in body weight per gram of food for each rat at different proteinlevels supplemented with formulated cereal.

Replications	Treatments						
	Diet–A	Diet–B	Diet–C	Diet–D	Diet–E	Diet–F	Grand Total
1	0.270	0.378	0.448	0.539	0.532	0.460	2.627
2	0.273	0.348	0.433	0.529	0.382	0.404	2.369
Total	0.543	0.726	0.881	1.068	0.914	0.864	4.996
Mean	0.272	0.363	0.441	0.534	0.457	0.432	2.499

Table **5.7:** Protein Efficiency Ratio (PER) values at different protein levels supplemented with formulated cereal of twelve young albino rats.

Replications	Treatments						
	Diet–A	Diet–B	Diet–C	Diet–D	Diet–E	Diet–F	Grand Total
1	1.29	1.79	2.12	2.55	2.51	2.15	12.41
2	1.30	1.65	2.05	2.50	1.80	1.90	11.20
Total	2.59	3.44	4.17	5.05	4.31	4.05	23.61
Mean	1.30	1.72	2.09	2.53	2.16	2.03	11.83

Table **5.8**: CER values at different protein levels supplemented with formulated cereal of twelve young albino rats.

Replications	Treatments						
	Diet–A	Diet–B	Diet–C	Diet–D	Diet–E	Diet–F	Grand Total
1	6.60	9.22	10.92	13.15	12.98	11.20	64.07
2	6.66	8.48	10.58	12.89	9.32	9.87	57.80
Total	13.26	17.07	21.50	26.04	22.30	21.07	121.24
Mean	6.63	8.85	10.75	13.02	11.15	10.54	60.94

Fig.**6.1.1**: Experimental rats.

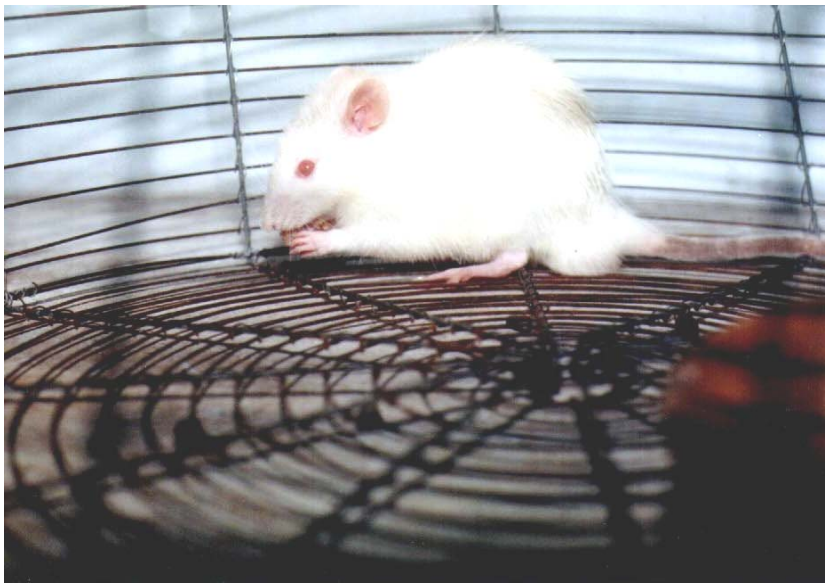

Fig. **6.1.2:** The rat being fed with diet.

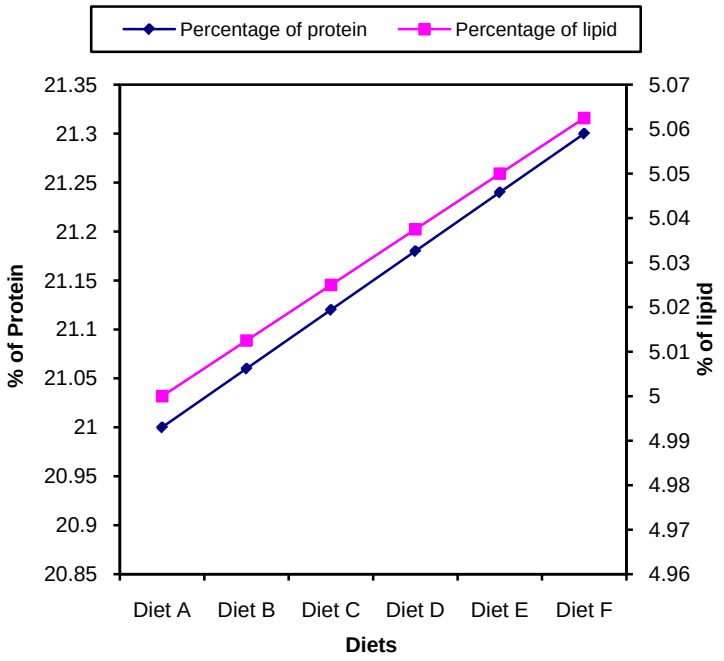

Fig. **6.2**: Comparison of percentage of protein and percentage of lipid in experimental diets.

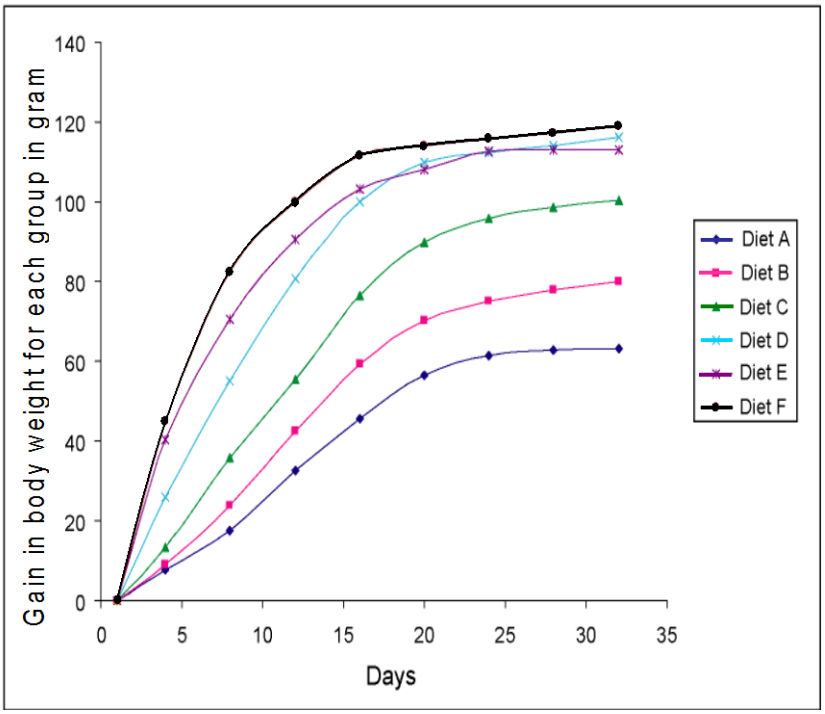

Fig. **6. 3**: The effect of feeding FPC supplemented with formulated cereal at different protein levels on average gain in body weight (g) for each group for every four days interval.

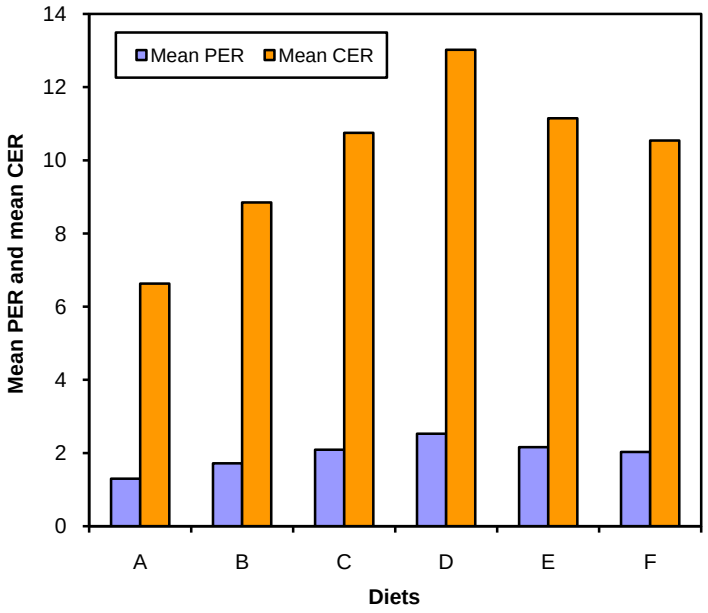

Fig.**6.4**: The effect of feeding the FPC at different protein levels supplemented with formulated cereal on mean PER and mean CER.

PART-B

Haematological and Histopathological Studies on young albino rats after feeding fish flesh (*Puntiusgonionotus*) in different protein levels supplemented with formulated cereal for consecutive 32 days both for the control group and experimental groups.[1]

8.5. Introduction

Haematology is the study of blood, blood forming tissues and relative substances concerned in blood production, conservation and destruction of blood and their disorders. Blood is made up of a fluid part called plasma and cells. The cells in the blood are of three types- red cells (erythrocytes), white cells (leucocytes) and platelets (thrombocytes). The white cells are divided into five series- esonophil, neutrophil, monocytes, basophil and lymphicytes[9, 10].Haematological parameters like haemoglobin, red blood cells (RBC), erythrocyte sedimentation rate (ESR), cholesterol, white blood cells (WBC) and total count of WBC of the both control group and experimental groups were studied after 32 consecutive days of feeding fish flesh at different protein levels with formulated cereal.

The present study was also aimed to identify any toxicological effect of the fish *Puntiusgonionotus* (*P. gonionotus*) in the major organs of liver, heart, spleen, lung and kidney of both control group and experimental groups of rats. These experiments were carried out by observing any changes in the cellular structures (degradation and regeneration) of the organs of the experimental rats after receiving fish flesh at different FPC levels for 32 consecutive days with respect to control group.

8.6. Materials and methods

At the end of 32 consecutive days, the albino rats were starved overnight and sacrificed under mild ether anaesthesia. Blood were collected from each rat of either group by cardiac puncture over EDTA for the measurements of various haematological parameters [7]. Haemoglobin (Hb) was measured according to the Acid Haematin Method of Shali; peripheral count of RBCs and total count of white blood cells (WBCs) and differential count of WBCs were measured according to Manual Method with the help of hematocytometer[9].

Erythrocyte sedimentation rate (ESR) was determined by Westergren Method. In this method, the blood was mixed with a suitable anticoagulant agent (3.1% trisodium citrate solution) and was made to stand vertically [9]. Blood serum cholesterol was determined by Spectrophotometrically according to Leibermann-Burchard Reaction Method [9]. The results were summarized in table **6.1**. Values of haematological parameters of the Sprague Dawley rats [11] were listed in reference table **6.2**. The comparison of RBC, WBC, Hb and cholesterol was shown in figure **6.5**. For histopathological investigation, the liver, kidney, heart, lung and spleen of all rats were isolated and weighed and recorded in table **6.3**. The table **6.4** shows the reference values for studied of SpagueDawley rats of different organs [5]. Percentage of liver fat (mean value) in each rat treated with different protein levels supplemented with formulated cereal is shown in table **6.6.1**. Comparison between percentage of liver protein and percentage of liver fat in each albino rat treated with different FPC was shown in figure **6.6**.

A small amount of these tissues were separately sliced in places, fixed in 10% formalin and processed for paraffin sections. After haematoxylin-eosin staining [14], the section of the control group and experimental groups were carefully examined under high power microscope and were recorded by photographs.

Histopathological observation results were computed in table **6.9**. Microscopic view of heart, kidney, liver, spleen and lung were shown in figure **6.7.1.1** – **6.7.1.3**, figure **6.7.2.1** – **6.7.2.3**, figure **6.7.3.1** – **6.7.3.3**, figure **6.7.4.1** – **6.7.4.3** and figure **6.7.5.1** – **6.7.5.3** respectively. The histopathological and haematological examinations were carried out at Rajshahi Medical College, Rajshahi, Bangladesh.

8.7. Results and discussion

Table **6.1** shows that the values of haematological parameters of all experimental rats were almost similar to that of control group and when compared with reference table [11]**6.2**, it was revealed that these values were in normal range. All collected organs appeared normal colour on gross necropsy evaluation. The weights of all organs in table **6.3** were within normal limits as compared with reference table **6.4** [5]. Figure **6.5**, shows the relationship among RBC, WBC, Hb and cholesterol with control group. Table **6.5** illustrates that fat deposit in liver was maximum (4.25%) when liver protein was minimum (16.975%). From table **6.5,** it is also revealed that percentage of protein in rat liver increased slowly up to diet–D and then decreased slowly though FPC increased. In table **6.8**, different superscripts in the same column were significantly different.

During the whole experimental period, locomotive behaviour, central nervous system, excitation, weakness, reflexes, salivation, urination, diarrhoeaetc of all albino rats were closely monitored and found normal. Reports of histopathological examination of the heart, liver, lung, kidney and spleen of all rats were normal as stated in table **6.9** and no morphological changes were found in their microscopic view.

Table **6.1:** Haematological profiles of albino rats after feeding FPC of P. gonionotus at different protein levels supplemented with formulated cereal after 32 consecutive days.

Haematological parameters	Rat No	Control rat with formulated cereal	Percentage of fish flesh of P. gonionotusin diet with formulated cereal				
			Diet–B	Diet–C	Diet–D	Diet– E	Diet– F
Total RBC count (10^6/μl)	a	7.4	7.8	9.3	9.7	8.3	8.2
	b	8.2	7.7	9.4	9.5	8.5	7.5
	Mean	7.8	7.75	8.85	9.6	8.4	7.85
Haemoglobin (g/dl)	a	14.4	14.87	16.44	15.62	13.16	15.75
	b	13.8	15.22	14.81	16.3	13.61	14.93
	Mean	14.1	15.04	15.62	16.07	13.38	15.34
Cholesterol (mg/dl)	a	136	140	146	148	155	161
	b	138	141	144	150	157	163
	Mean	137	140.5	145	149	156	162
Erythrocyte sedimentation rate (ESR) (mm)	a	05	04	01	02	07	03
	b	04	05	02	03	04	04
	Mean	4.5	4.5	1.5	2.5	5.5	3.5
Total WBC Count (10^3/μl)	a	10.20	12	12.5	14	11	13
	b	12.0	13	10.5	15.8	13	10
	Mean	11.1	12.5	11.5	14.9	12	11.5
Differential count a) Neutrophil (%)	a	07	16	09	15	8.5	13
	b	09	11	12	13	10.0	14
	Mean	8	13.5	10.5	14	9.25	13.5
b) Lymphocytes (%)	a	76	80	72	79	90	85
	b	78	77	86	81	83	90
	Mean	77	78.5	79	80	86.50	87.5
c) Monocytes (%)	a	02	04	02	03	02	02
	b	01	02	04	02	03	01
	Mean	1.5	3	03	2.5	2.5	1.5
d) Eosoniphils (%)	a	0.4	0.25	0.50	03	0.54	0.55
	b	0.10	0.50	0.40	02	01	0.60
	Mean	0.70	0.37	0.35	0.45	0.77	0.57

Table **6.2:** Reference values for studied haematological parameters of the Sprague Dawley rats[11].

RBC ($\times10^6$/μl)	TWBC ($\times10^3$/μl)	LYMPH (%)	NEUT (%)	MON (%)	EOS (%)
6.26–8.98	9.4–14.9	72–94	4.5–23.3	0.5–3.5	0.35–0.6

Table **6.3:** Weight of different organs of twelve albino rats after feeding FPC at different protein levels supplemented with formulated cereal.

Diets	Rat No.	Gain in body weight (g)	Weight of organs (g)				
			Liver	Kidney	Lung	Spleen	Heart
Diet–A	a	62.44	3.98	0.98	0.52	0.25	0.34
	b	63.60	4.35	0.63	0.47	0.26	0.32
Diet–B	a	81.51	4.66	0.81	0.51	0.32	0.52
	b	78.38	5.25	0.94	0.74	0.33	0.53
Diet–C	a	98.27	5.04	0.92	0.62	0.30	0.41
	b	102.27	5.47	0.94	0.83	0.34	0.42
Diet–D	a	115.94	3.96	0.95	0.57	0.31	0.47
	b	116.34	5.35	1.05	0.68	0.35	0.49
Diet–E	a	119.48	4.82	0.82	0.63	0.26	0.62
	b	106.33	6.15	1.08	0.76	0.36	0.64
Diet–F	a	120.32	4.96	0.76	0.72	0.29	0.44
	b	117.48	4.52	0.96	0.75	0.41	0.47

Table **6.4**: Histopathological examination of the section of Heart, Liver, Kidney, Lung and Spleen of all the rats of control group and experimental groups.

Observation	Liver	Kidney	Heart	Lung	Spleen
Control groups	No change occurred	Glomerulus's. No inflammatory cell was observed	Cardiac muscle, no morphological change occur, no congestion was found	Alveoli, no congestion occurred.	No abnormalities were detected.
Experimental groups	No abnormalities were detected.	No evidences of pathological changed.	As control group.	No change occurred.	Same as control group.

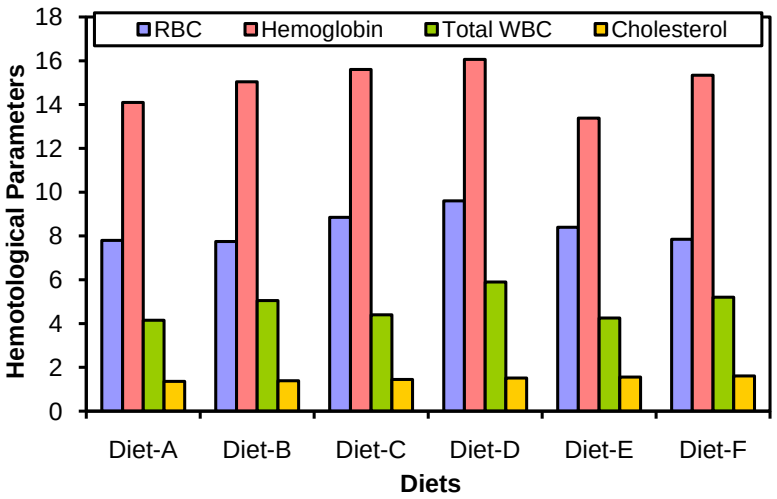

Fig.**6.5**: Graphical representation of haematological parameters (RBC, WBC, Hb, and cholesterol) both control and experimental groups

UNITS: RBC = $10^6/\mu l$ Haemoglobin = gm/dl

Cholesterol = mg/ml Total WBC Count = $10^3/\mu l$

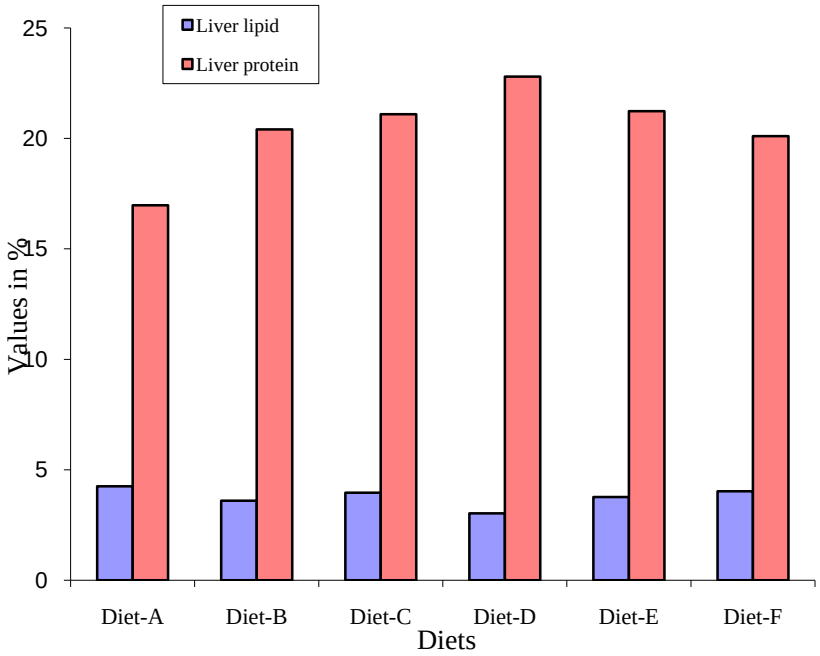

Fig.**6.6:** Comparison between percentage of liver protein and percentage of liver lipid in each albino rats treated with different FPC.

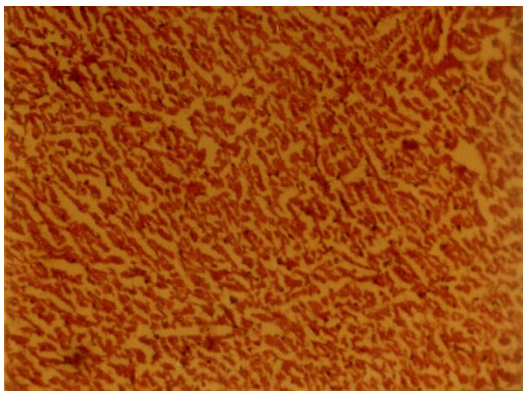

Fig. **6.7.1.1**: Microscopic view of heart tissues of control rat after 32 consecutive days (vehicle)× 400

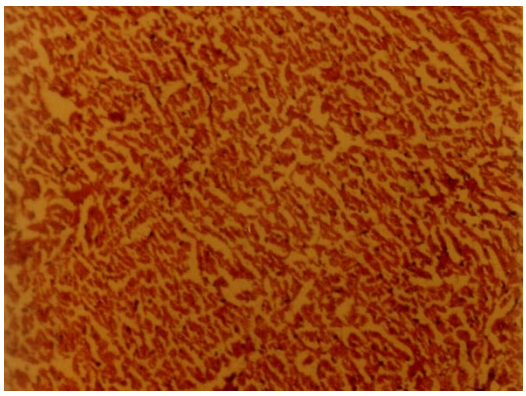

Fig**. 6.7.1.2**: Microscopic view of heart tissues treatment with 10 % FPC of after 32 consecutive days (vehicle)× 400

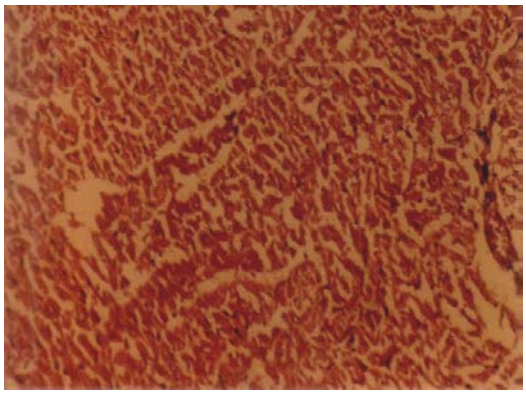

Fig.**6.7.1.3**: Microscopic view of heart tissues treatment with 20% FPC after 32 consecutive days (vehicle)× 400

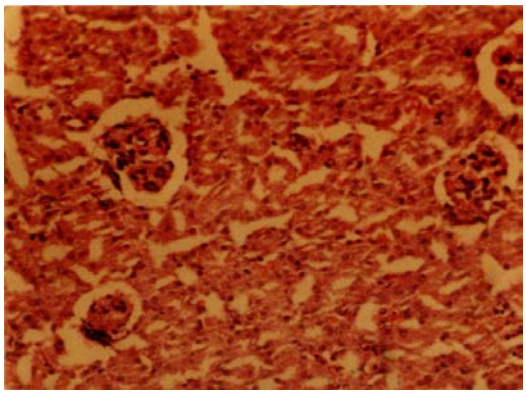

Fig.**6.7.2.1**: Microscopic view of kidney tissues of control rat after 32 consecutive days (vehicle)× 400

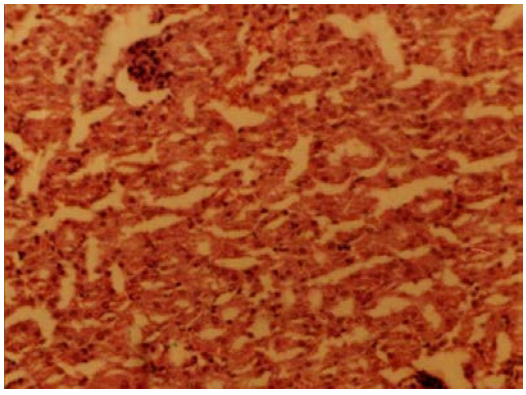

Fig**. 6.7.2.2**: Microscopic view of kidney tissues Treatment with10%FPC after 32 consecutive days (vehicle)× 400

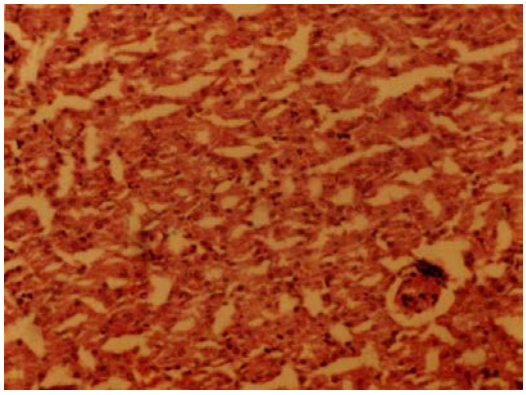

Fig. **6.7.2.3**: Microscopic view of kidney tissues Treatment with20% FPC after32 consecutive days (vehicle)×400

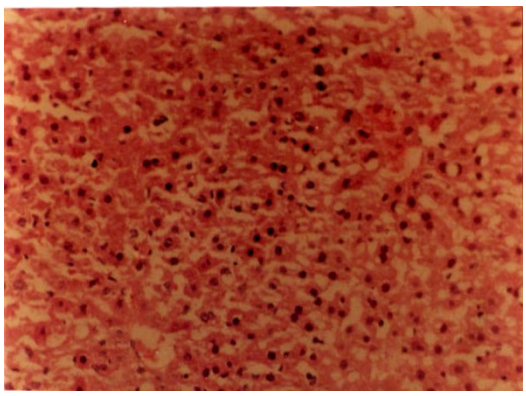

Fig**. 6.7.3.1**: Microscopic view of liver tissues of control rat after 32 consecutive days (vehicle)× 400

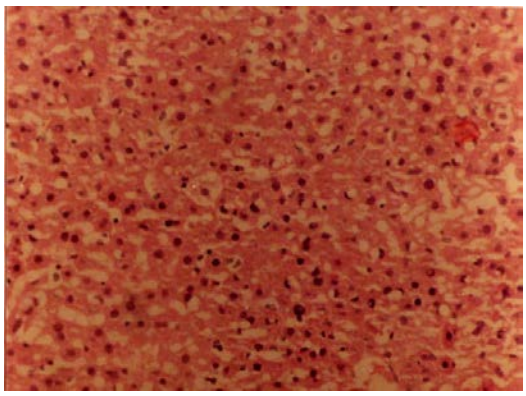

Fig**. 6.7.3.2**: Microscopic view of liver tissues treatment with 10 % FPC after 32 consecutive days (vehicle)× 400

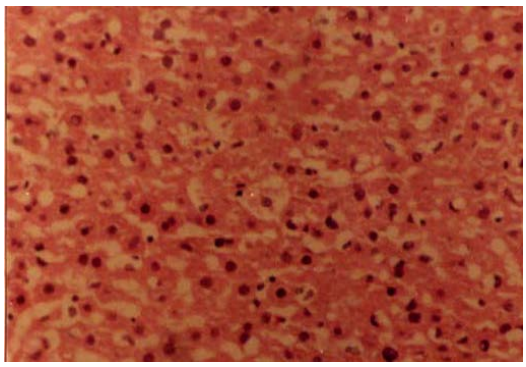

Fig. **6.7.3.3**: Microscopic view of liver tissues treatment with 10 % FPC after 32 consecutive days (vehicle)× 400

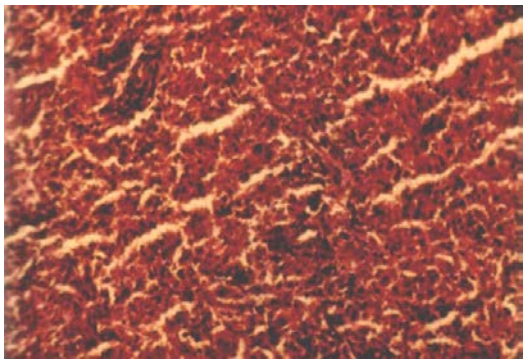

Fig. **6.7.4.1**: Microscopic view of spleen tissues of control rat after 32 consecutive days (vehicle)× 400

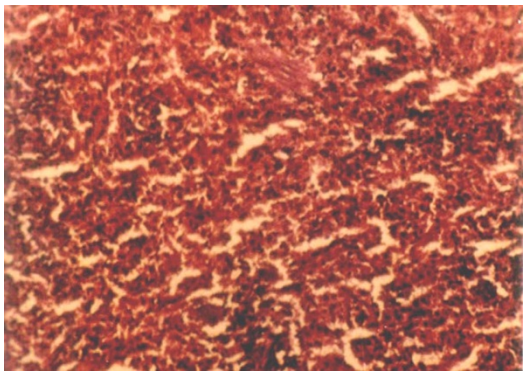

Fig.**6.7.4.2**: Microscopic view of spleen tissues treatment with 10 % FPC after 32 consecutive days (vehicle)× 400

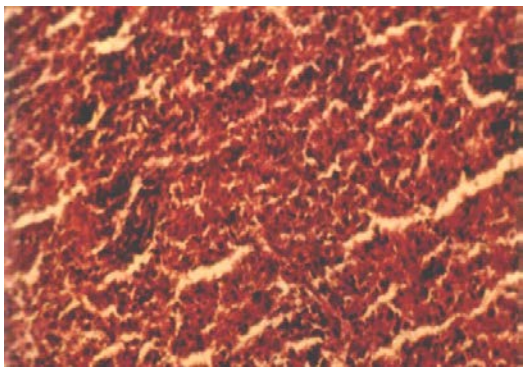

Fig **6.7.4.3**: Microscopic view of spleen tissues treatment with 20 %FPC after 32 consecutive days (vehicle)× 400

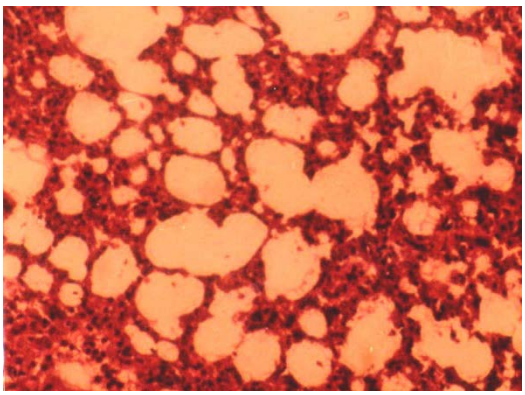

Fig. **6.7.5.1**: Microscopic view of lung tissues of control rat after 32 consecutive days (vehicle)× 400

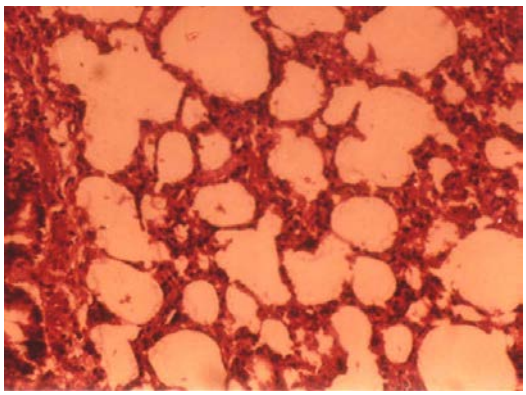

Fig**. 6.7.5.2**: Microscopic view of lung tissues treatment with 10 %FPC after 32 consecutive days (vehicle)× 400

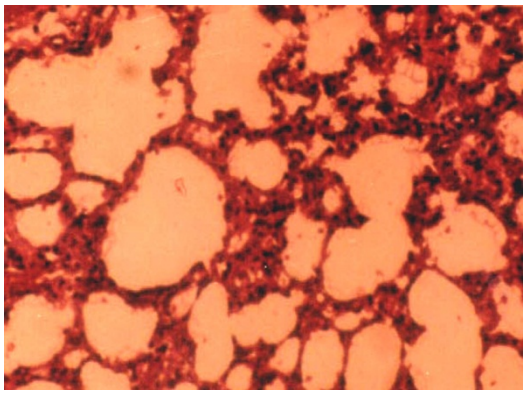

Fig. **6.7.5.3**: Microscopic view of lung tissues treatment with 20 %FPC after 32 consecutive days (vehicle)× 400

Summary and Conclusion

Being a most important and cheap source of protein, fishes have great significance in the life of mankind. It is the only principal item of food in Bangladesh that the country does not have to import from other countries. Fish is considered exceptionally valuable from the nutritional point of view primarily because of containing a high percentage of readily digestible animal protein.

The study on the effect of FPC at different protein levels with formulated cereal have been performed to identify the nutritional significance of fish *P. gonionotus* by determining the gain in body weight per gram of food intake, PER and CER values in twelve male young male albino rats for 32 consecutive days. During the experimental period, the rats were kept in a clean house with optimal temperature. Five experimental diets were prepared at different protein levels (5% to 25%) with supplied formulated cereal where the control diet was prepared with supplied formulated cereal only. During the experimental period gain in body weight for every four days interval was recorded and found that body weight of each experimental rats increased rapidly up to 20 days and then their body weight increased very slowly. After 32 consecutive days gain in body weight per gram of food, RER and also CER values have been calculated and recorded in table **5.5**.

Haematological studies revealed no obvious differences in any one of the haematological parameters between control groups and experimental groups. Microscopic examination of treated tissues in all group of rats had no abnormalities. Weights of major organs for treated rats were as like as normal rats. Liver fat and liver protein of experimental rats were estimated after 32 consecutive days. No toxicological effect and remarkable differences were found between control groups and experimental groups treated with different

FPC for 32 consecutive days.Protein, lipid, ash, and cholesterol contents of the flesh were tolerable to the standard values. Chemical characteristics of the lipid resembled that this values fall within the standard range which attributed to the fatty acid composition of the lipid. A low acid value and percentage of free fatty acid indicated that the lipid is less rancid. The macro and micro mineral constituents of the fish can be used to eliminate micro nutritional deficiency and malnutrition problem among the common people. The fish lipid contained 43.1% unsaturated fatty acids along with other fatty acids. This PUFA reduces the risk of heart attack.In view of these results discussed above, it may be concluded that intake of this species of fish can be used in required quantity (15%) for fortification of normal cereal to enhance their nutritive values.

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