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Research Article **Zoology**

BIOCHEMICAL STUDIES ON NORMAL AND INFECTED *Sardina pilchardus* FISH

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ABSTRACT

Food borne infections and illnesses have become a major international health problem with consequent reduction in economic growth. It is also identified as a major cause of illness and death worldwide. In the developing world, foodborne infection leads to the death of many children, as well as resulting in diarrheal disease which can have long-term effects on children's growth as well as on their physical and noesis development and it also heavily affects the healthcare systems. the present study to investigate the carbohydrate, protein, lipids and amino acids in normal and infected *Sardina pilchardus* fish. The protein content was lower in infected fish as compared to normal fish. The Amino acid content was lower in infected fish as compared to normal fish. The carbohydrate content was lower in infected fish as compared to normal fish. The lipid content was lower in infected fish as compared to normal fish. The study concludes that locally obtainable infected fish shows nutritionally deficient and suggested that this type of fish is cannot be consider for eatable.

Keywords: *Sardina pilchardus*, protein, Amino acid, Carbohydrate and Lipid

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INTRODUCTION

Fishes are classified as any of the cold blooded aquatic vertebrates of the super class Pisces typically showing gills, fins and a streamline body. In addition, 'fish' also refers to the flesh of such animals used as food. There are about 22,000 species of fish that began evolving around 480 million years ago (Pal and Mahendra, 2015). Fish is an important part of a healthy diet due to its high quality protein, other essential nutrients and omega 3fatty acids, and its low fat content as compared to other meats (Rhea, 2009; Pal, 2010).Fish and seafood products constitute an important food commodity in the international trade due to its ever increasing consumption demand. Fish contributes about 60% of the world supply of protein, and 60% of the developing world derives more than 30% of their animal protein from fish (Emikpe *et al*., 2011). Fish allows for protein improved nutrition in that it has a high biological value in term of high protein retention in the body, low cholesterol level and presence of essential amino acids (Emikpe *et al*., 2011)

The microbiological flora in the intestines of sea foods such as finfish, shellfish, and cephalopods is quite different being psychotrophic in nature, and to some extent

believed to be a reflection of the general contamination in the aquatic environment (AdebayoTayo *et al.*, 2012a). Several studies have demonstrated a number of bacterial species encountered in different fish, which are potentially pathogenic under certain conditions as reported for Pseudomonas angulluseptica and Streptococcus spp. (Emikpe *et al.*, 2011). It is estimated that there are more than 80 million cases per annum of seafood borne illnesses on antibiotic resistance in the USA, and that the cost of these illnesses is in many billions of dollars per year (AdebayoTayo *et al.*, 2012a). The economic losses due to spoilage are rarely quantified but a report by the US National Research Council Committee (FND/NRC) estimated that onefourth of the world food supply is lost through microbial activity alone (EEC, 1992). Thus, the need for control of quality of the sea foods to avoid high microbial contamination, which may lead to antibiotics resistance, is well documented, and since the rate of seafood borne illnesses is increasing, there is an urgent means of assuring quality of sea food (AdebayoTayo *et al.*, 2012a).

Food borne infections and illnesses have become a major international health problem with consequent reduction in economic growth. It is also identified as a major cause of illness and death worldwide. Recognizing this, the World Health Organization (WHO) developed its Global Strategy for Food Safety. In the developing world, foodborne infection leads to the death of many children, as well as resulting in diarrheal disease which can have long-term effects on children's growth as well as on their physical and noesis development and it also heavily affects the healthcare systems. In the present study to investigate the carbohydrate, protein, lipids and amino acids in normal and infected *Sardina pilchardus* fish.

MATERIALS AND METHODS

Collection of Experimental Fishes

Normal and infected *Sardina pilchardus* fish (Fig 1 and Fig 2) were procured from Fish market, Kelavasal, Thanjavur Tamil Nadu, India.

Fig 1: Normal fish (*Sardina pilchardus*)

Fig 2: Infected fish (*Sardina pilchardus*)

Preparation of Homogenate

The Normal and infected *Sardina pilchardus* fish were sacrificed and flesh was dissected out, washed with ice-cold physiological saline. The required amount was weighed and homogenized using a Teflon homogenizer. Tissue homogenate was prepared in 0.1 M Tris Hcl buffer (pH 7.4) and used for the estimation of various biochemical parameters.

Biochemical estimations

Protein was estimated by the method of Lowry *et al.* (1951). Total lipids in tissues were estimated by the method of Folch et al (1957). To estimate the amount of carbohydrate present in the given sample by using Anthrone method. Amino acid in tissues were estimated by the method of Rosen (1957).

Qualitative analysis of Inorganic elements

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

Statistical analysis

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

RESULTS AND DISCUSSION

Biochemical analysis

The present study was carried out to analyze the various biochemical parameters in normal and infected fish. The observations made on different subjects of fishes were compared as follows.

Table I - Shows the levels of Carbohydrate in normal and infected fish. Carbohydrate was decreased in infected fish when compared to normal fish.

Table I: The levels of carbohydrate in normal fish and infected fish

FISH	CARBOHYDRATE (mg/gm)
Normal fish	196.40 ± 2.35
Infected fish	139.7±18.75*

Values were expressed as mean ± SD.
* Significantly different from normal fish (P< 0.05)

Table II - Shows the levels of protein in normal and infected fish. Protein content was increased in infected fish when compared to normal fish.

Table II: The levels of Protein in normal fish and infected fish

FISH	PROTEIN (mg/gm)
Normal fish	325.6 ± 28.72
Infected fish	214.66±21.01*

Values were expressed as mean ± SD.
* significantly different from normal fish (P< 0.05)

Table III - Shows the levels of amino acid in normal and infected fish. amino acid content was increased in infected fish when compared to normal fish.

Table III: The levels of amino acid in normal fish and infected fish

FISH	AMINO ACID (mg/gm)
Normal fish	145.66 ±20.20
Infected fish	106±11.23*

Values were expressed as mean ± SD.
* significantly different from normal fish (P< 0.05)

Table IV - Shows the levels of lipids in normal and infected fish. Lipids content was decreased in infected fish when compared to normal fish.

Table IV: The levels of lipids in normal fish and infected fish

FISH	LIPIDS (mg/gm)
Normal fish	41.01 ± 0.036
Infected fish	21.32 ±0.025*

Values were expressed as mean ± SD.
* Significantly different from normal fish (P< 0.05)

Analysis of inorganic elements in normal and infected Fish

The present study was carried out to analyze the important inorganic elements in normal and infected fish. The observations made on different subjects of fishes were compared as follows. The inorganic elements calcium, magnesium, sodium, potassium, sulphate and phosphate present in both fishes while iron only absent in normal fish but iron and nitrate were absent in infected fish.

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

Table V: Qualitative analysis of inorganic elements in normal and infected Fish

S.No	Inorganic elements	Normal Fish	Infected Fish
1	Calcium	+	+
2	Magnesium	+	+
3	Sodium	+	+
4	Potassium	+	+
5	Iron	-	-
6	Sulphate	+	+
7	Phosphate	+	+
8	Chloride	+	+
9	Nitrate	+	-

(+) Presence (-) Absence

DISCUSSION

Biochemical biomarkers have been used in order to prevent irreversible damage in whole organisms, communities and ecosystems (Lopez-Barea and Pueyo, 1998). Measurement of biochemical parameters is a commonly used diagnostic tool in aquatic toxicology and biomonitoring. The impact of a number of contaminants on aquatic ecosystems can be assessed by the measurement of their external levels in the surrounding water or sediments, or by determining some biochemical parameters in fish and other organisms that respond specially to the degree and type of contamination (Petrivalsky et al., 1997; Machala et al., 2001). Oner et al. (2009) reported that biochemical parameters assessed in fish may be a useful tool by providing quantitative measurement of metals impact as well as valuable information of ecological relevance on the effects of metals (Oner et al., 2009). Moreover, biochemical biomarkers are frequently used for detecting or diagnosing sublethal effects in fish exposed to toxic substances (Toguyeni et al., 1997). Sublethal effects are biochemical in origin as the most toxicants exert their effects at basic level of the organism by reacting with enzymes or metabolites and other functional components of the cell. Such effects might lead to irreversible and detrimental disturbances of integrated functions such as behavior, growth, reproduction and survival (Waldichuk, 1979).

Analysis of chemical substances in tissues and body fluids, toxic metabolites, enzymes activities and other biochemical variables have frequently been used in documenting the toxin interaction with biological systems. Plasma and serum reflect the physiologic state of an animal because they are the products of intermediate metabolism (Artacho et al., 2007). The levels of glucose, serum protein, albumin, globulin and activity of enzyme in blood plasma are considered to be specific indicators of sympathetic activation under stress conditions (Lermen et al., 2004; Velisek et al., 2009). Levels of blood or plasma ions and enzymes with important metabolic functions generally indicate the health status of fish (Hrubec and Smith,1999).

Analysis of chemical substances in tissues and body fluids, toxic metabolites, enzymes activities and other biochemical variables have frequently been used in documenting the toxin interaction with biological systems. Components like carbohydrate, protein and lipid play a vital role as energy precursors for fish under stress conditions (Umminger, 1970). Glucose is a carbohydrate that has a major role in the bioenergetics of animals, being transformed to chemical energy (ATP), which in turn can be expressed as mechanical energy (Lucas, 1996). Changes in carbohydrate metabolism measured as plasma glucose (energy substrate whose production is thought to metabolically assist the animal to cope with an increased energy demand caused by stress) used as general stress indicators in fish (Teles et al., 2007). Glucose (or glucose 6-phosphate) is released through the degradation of glycogen by glycogen phosphorylase (GP) and energy is mainly supplied by the oxidation of glucose and lactate as a result of carbohydrate metabolism (Morgan et al., 1997). The glucose concentration was proposed to be mediated by endocrine release such as cortisol (Hontela et al., 1996). Silbergeld (1974) stated that assay of this important parameter can serve as an indicator of environmental stress.

In a stress situation, glucose production provides energy substrates to tissues, in order to cope with the increased energy demand. Regardless of the wide use of glucose as an indicator of stress, some authors (Mommsen et al., 1999) emphasized that care has to be taken when using plasma glucose as the only indicator. It has been reported that glucose content is a less precise indicator of stress than cortisol (Pottinger, 1998). The storage or mobilization of metabolic substrates such as glucose, glycogen, lactate, lipid, and protein are disrupted by exposure to several trace metals, including cadmium (Rosen et al., 2003), manganese (Barnhoorn et al., 1999), nickel (Sreedevi *et al.*, 1992), and metal mixtures in a polluted habitat (Levesque et al., 2002). Many investigators have reported blood glucose levels under various toxicant exposure conditions; cadmium in Oncorhynchus mykiss, Salmo salar Ctenopharyngodon idellus Cyprinus carpio, copper in Oncorhynchus mykiss endosulfan in Salmo salar (Petri et al., 2006) and cyfluthrin in Cyprinus carpio (Sepici-Dincel et al., 2009).

Proteins are important organic substance required by organisms in tissue building. They are intimately related with almost all physiological processes, which maintain a simple biochemical system in 'living condition' (Joshi and Kulkarni, 2011).

Proteins are mainly involved in the architecture of the cell. Proteins occupy a unique position in the metabolism of cell because of the proteinaceous nature of all the enzymes which mediate at various metabolic pathways. During stress conditions fish need more energy to detoxify the toxicant and to overcome stress. Since fish have fewer amounts of carbohydrates so next alternative source of energy is protein and lipids to meet the increased energy demand (Singh et al., 2010).

Elements in fresh water fish and sea water fish

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

The current examination on the proximate profile of normal and infected fish, bringing to our attention the richness of healthy nutrients present in the eatable portion such as muscle. The protein content was lower in infected fish as compared to normal fish. The Amino acid content was lower in infected fish as compared to normal fish. The carbohydrate content was lower in infected fish as compared to normal fish. The lipid content was lower in infected fish as compared to normal fish. The study concludes that locally obtainable infected fish shows nutritionally deficient and suggested that this type of fish is cannot be consider for eatable.

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