Estimation of Monthly Variations of Lymphatic Proteins of Tilapia Reared in Pond Water

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The development of functional T- and B-cells from the thymocytes is a crucial step in development of functional immune system and the organs like spleen, kidney and thymus are vital sites to study the lymphatic proteins. Different lymphatic proteins isolated from teleosts but still a little bit known about these proteins. Estimation of proteins from tilapia was done first time in Pakistan. Isolation of lymphatic proteins was performed after tissue rupturing and all the cellular debris was removed. The determined average comparative quantitative estimation of lymphatic proteins concentrations in 6 months tenure in male tilapia's organs was as testes (8.705 mg/mL) > kidney (7.577 mg/mL) > gills (7.556 mg/mL) > spleen (7.455 mg/mL) > alimentary canal (7.243 mg/mL) and in female tilapia as ovaries (9.098 mg/mL) > kidney (7.742 mg/mL) > spleen (7.548 mg/mL) > gills (7.532 mg/mL) > alimentary canal (6.920 mg/mL). High concentration of protein was investigated in reproductive organs (testes, 9.41 mg/mL and ovaries, 9.82 mg/mL) in both sexes.

Keywords: Tilapia, Lymph proteins, Organs, PAGE.

INTRODUCTION

Fish has vital importance as human food source. High quality proteins are obtained from fish, almost 16 % animal proteins are provided by fish consumed by total world's population. Freshwater fishes are also major source of nutritionally important lipids and fatty acids. Long chain polyunsaturated fatty acids (PUFAs) are also obtained from fish. Polyunsaturated fatty acids play an essential role in natural growth, evolution and reproduction of all vertebrates [1]. Aquaculture can produce long-lasting profits for global food security and economic growth, if it is practiced with responsibility [2].

Tilapias are known as the second major group of wild-captured fish, with a commercial production of 2.5 million tons in 2007. Tilapia represented more or less 84 % of total worldwide production of tilapia in 2008 [3]. The expected worldwide production of Nile tilapia will be 2.5 million tons in 2020 [4]. Tilapia provides proteins, phosphorus, potassium, vitamin B-12, niacin and selenium. Its meat has low concentrations of fats, saturated fats, sodium, carbohydrates, calories and omega-3 fatty acids [5,6].

Fish live as a free organism since evolution of life. It depends on its natural (innate) immune system as compared to higher

vertebrates which depend on their acquired immune system [7,8]. Basic defence mechanism in fish is non-specific immunity. This nonspecific immunity also significantly affects the acquired immune response. A mechanism of receptor proteins affects homeostasis and acquired immune response. Specific molecular patterns are the distinctive feature of pathogenic microorganisms, viral RNA, bacterial DNA, peptidoglycan, polysaccharides, lipopolysaccharide (LPS) and other molecules. These molecular patterns are recognizable receptor proteins. Immune response is suppressed by various internal and external factors management and density, while efficiency of immune response can be increased by several food additives and immunostimulants [9].

Fish do not have bone marrow, even though T cells are generated in thymus [10]. Lymph nodes, peyer patches or germinal centers are absent in fish which describes that antigen production, may be different. Secondary humoral responses in fish are not as elevated as in mammals, although minor immunity maturation [11] and no immunoglobulin class switching is reconized [12].

Lymphoid organs of ample size in teleosts include spleen, thymus, anterior and middle kidneys [13]. The lymphoid bark tissue is covered with a capsule like organ called thymus. It

can be imagined as collection of macrophages which enhances the propagation of T cells in encapsulated form [14]. T cells are produced in thymus [15]. This organ takes part in many body functions including antigen processing, phagocytosis immunoglobulin M (Ig M) formation and immune memory with the help of melanomacrophage centers [16]. In addition, head kidney releases many endocrine hormones including corticosteroids and plays key role in immune-endocrine interactions. Kidney is the major place of haematopoiesis until the fish reaches its adult hood. It performs the similar functions in teleost fish as performed by bone marrow in vertebrates [13]. Spleen is involved in the processing of antigens, clearance of macromolecules and making of antibodies. It is situated in the gill chamber at dorsolateral part near to opercular cavity. It makes T-lymphocytes and antibodies which produce B cells [17]. The role of liver is not so important in immunity of fish, however it is revealed that it generates a few acute phase reactants used in immune system [18]. Gills play their role in the immune defense using mucosa which is associated with lymphoid tissues [19]. There is the occurrence of lymphoid cell aggregations (T cells). At the lower part of gill filaments, there are aggregations of lymphoid cells (T-cells) which supervise the immune system against gill infections [20]. Gut mucosa contains various types of immune cells like lymphocytes, eosinophilic granulocytes, plasma cells and macrophages [21]. Intestinal microbiota and antigens from the host fish use GIT mucosal surface as natural crossing point [22]. Non-specific immunity or innate response is supposed to be very important part of fish immune system in fighting pathogens because adaptive immune system has certain limitations, fish are poikilothermic in nature, the process of propagation, development and memory of lymphocytes is slow and confined range of antibodies [23]. Epithelial (mucosal) barrier, humoral components and cellular parameters are collectively responsible for generating the innate response in fish [24].

The aim of present study was to isolate lymphatic proteins from *Tilapia* especially from different organs along with a comparative study in both of the two sexes. The study conducted first time in Pakistan and showed the relationship between the immune system of selected fish of two different sites as well as between the organs of the same species. Moreover few research papers of this topic has been published. Therefore the main objective of present study was to compare lymphatic system of *Tilapia* and to analyze the quantitative and qualitative proteins profile of selected organs of *Tilapia*.

EXPERIMENTAL

Collection of different organs from *Tilapia* fish: Different organs (gills, kidney, spleen, Alimentary Canal and gonads) of *Tilapia* were collected through dissection of fish. The collected organs of fish were saved in PBS (pH 7.0) and 2 % EDTA at 4 °C.

Extraction of proteins from fish organs: Total fluid proteins from the lymphatic tissues of fish were isolated through 'one pot' procedure. The steps of this procedure are described as under.

- Different organs of dissected fish were washed with chilled PBS (pH 7).

- The organs of the fish were dried through lint free cloth and cut into small pieces of approximately 10-12 mg size.

- All the samples were placed in 1.5 mL micro centrifuge tubes.

- The extraction solution was added in the samples in the ratio of 1 g sample: 6.25 mL buffer.

- These samples were put in boiling water bath for 10-15 min at 90 °C and finally placed on ice for 5 min.

- The samples were centrifuged at 11000 rpm for 15 min and supernatants were collected in ependroff tubes.

- Bradford method was used for the protein estimation of these samples for protein quantification of different lymphatic organs.

For comparison, total proteins were collected from different organs like reproductive organs, gills, spleens and kidneys. Description of each organ is briefed below.

The tissue fluids of disrupted tissue of different organs were extracted through centrifugation and stored at 4 °C before characterization of proteins. Cellular materials were removed by centrifugation and the fluid containing proteins were separated. Anti-protease was added in order to inhibit the proteolytic activity of some lymphatic proteins. Then allocates were stored at -20 °C. Samples were defrosted for the identification of the proteins. Samples were refiltered before further investigation.

Bovine serum albumin (BSA) at a concentration of 1 mg/mL in deionized water was used as a stock solution. It was stored at a -20 °C. The standard solution was prepared by diluting the stock BSA solution in the range of 0.2 mg/mL, 0.4 mg/mL, 0.6 mg/mL, 0.8 mg/mL and 1.0 mg/mL. 20 µL of each dilution was added in 1 mL Bradford in each test tubes. Blank was prepared by adding 20 µL of deionized water in 1 mL Bradford and adjusted zero with it. Test tubes were kept for 10 min at room temperature and the absorbance was taken at 595 nm on spectrophotometer (Specto-20).

Estimation of proteins: The total lymphatic protein of different organs of *Tilapia* was estimated at 595 nm wavelength by Bradford method [25]. Bradford reagent is the main component of this assay, it is comprised of 100 mg Serva blue G dissolved in 100 mL of 85 % phosphoric acid and 50 mL of 95 % ethanol and made up to 1 L. 20 µL sample of protein solution to be measured was added to 50 µL of 1 M NaOH. To this 1.0 mL of Bradford reagent was added and the assay solution was vortexed immediately. Absorbance was read at 595 nm in a cuvette. The protein concentration was determined from a standard curve of bovine serum albumin (BSA) generated between the range of 0-1.0 mg/mL. All assay samples and standards were carried out in triplicate and the resultant means were determined. The standard curve of BSA concentrations against the absorbance at 595 nm produces a linear plot upon which linear regression was carried out. Linear regression was used on the sample readings so that unknown protein sample concentrations could be calculated.

The recent study was conducted for quantitative and qualitative analysis of total lymphatic proteins profile in different organs of fresh water fish *Oreochromis nilotica* (Nile *Tilapia*). The fish samples were collected from experimental fresh water pond of Fish Hatchery Farm Satyana Road

Faisalabad. The collection of fish samples was not possible from Chokera waste water treated ponds because of the water quality was not supportive for the survival of the fish. Physio-chemical parameters of water medium were checked on regular interval basis of both sites revealed a significant difference. The results of quantitative and qualitative protein estimation in different organs of fish were significant in both sexes.

RESULTS AND DISCUSSION

The study was conducted for the qualitative and quantitative comparison of lymphatic proteins of the tilapia reared in fresh water pond. The lymphatic system plays a pivotal role in the development of the adaptive immune system, an important circulation of nutrients, antibodies and electrolytes, etc.

Protein estimation: Tilapia samples were taken from experimental fresh water pond of Fish Hatchery Farms, Satayana Road, Faisalabad. The total lymphatic proteins were estimated through Bradford method and quantity of lymphatic proteins of different organs was observed in both sexes except liver as investigation indicated that the role of liver is not so important in immunity of fish, however it is revealed that it generates a few acute phase reactants used in immune system [18].

January: In the month of January 20 fish samples were collected from fish hatchery. Out of them 15 were male and remaining 5 were females. The concentration of total proteins (mg/mL) was found greater in reproductive organs in both sexes while in low concentration was investigated in alimentary canal. Mean concentrations of lymphatic proteins in kidney were 7.57 and 7.60 mg/mL in male and female respectively. Mean concentrations of lymphatic proteins in gills were 7.37 and 7.58 mg/mL in male and female respectively. Mean values of protein estimation in spleen were 7.43 and 7.54 mg/mL respectively.

February: In the month of February 20 fish samples were collected from fish hatchery. Out of them 13 were male and remaining 7 were females. The concentration of total proteins (mg/mL) was found greater in reproductive organs in both sexes while in low concentration was investigated in alimentary canal. Mean concentrations of lymphatic proteins in kidney were 7.55 and 8.11 mg/mL in male and female respectively. Mean concentrations of lymphatic proteins in gills were 7.45 and 7.63 mg/mL in male and female respectively. Mean values of protein estimation in spleen were 7.42 and 7.79 mg/mL respectively.

March: In the month of March 20 fish samples were collected from fish hatchery. Out of them 10 were male and remaining 10 were females. The concentration of total proteins (mg/mL) was found greater in reproductive organs in both sexes while in low concentration was investigated in alimentary canal. Mean concentrations of lymphatic proteins in kidney were 7.62 and 7.49 mg/mL in male and female respectively. Mean concentrations of lymphatic proteins in gills were 8.17 and 7.71 mg/mL in male and female respectively. Mean values of protein estimation in spleen were 7.59 and 7.41 mg/mL respectively.

April: In the month of April 20 fish samples were collected from fish hatchery. Out of them 11 were male and remaining

9 were females. The concentration of total proteins (mg/mL) was found greater in reproductive organs in both sexes while in low concentration was investigated in alimentary canal. Mean concentrations of lymphatic proteins in kidney were 7.64 and 7.70 mg/mL in male and female respectively. Mean concentrations of lymphatic proteins in gills were 7.50 and 7.39 mg/mL in male and female respectively. Mean values of protein estimation in spleen were 7.47 and 7.42 mg/mL respectively.

May: In the month of May 20 fish samples were collected from fish hatchery. Out of them 16 were male and remaining 4 were females. The concentration of total proteins (mg/mL) was found greater in reproductive organs in both sexes while in low concentration was investigated in spleen. Mean concentrations of lymphatic proteins in kidney were 7.50 and 7.50 mg/mL in male and female respectively. Mean concentrations of lymphatic proteins in gills were 7.44 and 7.35 mg/mL in male and female respectively. Mean values of protein estimation in alimentary canal were 8.49 and 8.33 mg/mL respectively.

June: In the month of June 20 fish samples were collected from fish hatchery. Out of them 12 were male and remaining 8 were females. The concentration of total proteins (mg/mL) was found greater in reproductive organs in both sexes while in low concentration was investigated in alimentary canal. Mean concentrations of lymphatic proteins in kidney were 7.59 and 8.05 mg/mL in male and female respectively. Mean concentrations of lymphatic proteins in gills were 7.40 and 7.53 mg/mL in male and female respectively. Mean values of protein estimation in spleen were 7.49 and 7.78 mg/mL respectively.

The present research tenure was of 6 months. Protein concentrations of different organs of tilapia *Oreochromis nilotica* was measured separately in each month by Bradford method. In the month of January the order of protein concentrations in different organs of tilapia was testes (8.42 mg/mL) > kidney (7.57 mg/mL) > spleen (7.43 mg/mL) > gills (7.37 mg/mL) > alimentary canal (6.59 mg/mL) for male tilapia (Table-1). In female tilapia order of protein concentrations in different organs in the month of January was ovaries (8.70 mg/mL) > kidney (7.60 mg/mL) > gills (7.58 mg/mL) > spleen (7.54 mg/mL) > alimentary canal (6.64 mg/mL) (Table-1). In the month of February results of protein concentrations in different organs of male and female tilapia were testes (8.52 mg/mL) > kidney (7.55 mg/mL) > gills (7.45 mg/mL) > spleen (7.42 mg/mL) > alimentary canal (6.51 mg/mL) and ovaries (8.9 mg/mL) > kidney (8.11 mg/mL) > spleen (7.79 mg/mL) > gills (7.63 mg/mL) > alimentary canal (6.69 mg/mL) (Table-1) respectively. In the month of March the order in male tilapia was testes (9.24 mg/mL) > gills (8.17 mg/mL) > kidney (7.62 mg/mL) > spleen (7.59 mg/mL) > alimentary canal (7.24 mg/mL). Female tilapia followed the same order in the month of March (Table-1). Protein estimation results of April revealed the order of protein concentration in different organs as testes (8.32 mg/mL) > kidney (7.64 mg/mL) > gills (7.50 mg/mL) > spleen (7.47 mg/mL) > alimentary canal (7.24 mg/mL) in male fish and in female fish this order was as ovaries (9.36 mg/mL) > kidney (7.70 mg/mL) > spleen (7.47 mg/mL) > gills

TABLE-1
ESTIMATION OF PROTEIN IN DIFFERENT ORGANS OF MALE & FEMALE FISH IN THE MONTHS (JANUARY-JUNE)

Organs	Protein estimation (mg/mL)					
	January		February		March	
	Male	Female	Male	Female	Male	Female
Gills	7.37 ± 0.19	7.58 ± 0.26	7.45 ± 0.26	7.63 ± 0.18	8.17 ± 0.43	7.71 ± 0.41
Kidney	7.57 ± 0.21	7.60 ± 0.25	7.55 ± 0.23	8.11 ± 0.35	7.62 ± 0.18	7.49 ± 0.24
Spleen	7.43 ± 0.24	7.54 ± 0.30	7.42 ± 0.11	7.79 ± 0.53	7.59 ± 0.23	7.41 ± 0.37
Alimentary canal	6.59 ± 0.34	6.64 ± 0.18	6.51 ± 0.23	6.69 ± 0.27	7.24 ± 0.50	6.64 ± 0.41
Testes/Ovaries	8.42 ± 0.17	8.70 ± 0.16	8.52 ± 0.22	8.90 ± 0.47	9.24 ± 0.35	9.37 ± 0.23
Organs	April		May		June	
	Male	Female	Male	Female	Male	Female
	Male	Female	Male	Female	Male	Female
Gills	7.50 ± 0.25	7.39 ± 0.14	7.44 ± 0.21	7.35 ± 0.31	7.40 ± 0.17	7.53 ± 0.34
Kidney	7.64 ± 0.22	7.70 ± 0.21	7.50 ± 0.14	7.50 ± 0.08	7.59 ± 0.22	8.05 ± 0.48
Spleen	7.47 ± 0.15	7.42 ± 0.14	7.33 ± 0.13	7.35 ± 0.06	7.49 ± 0.21	7.78 ± 0.38
Alimentary canal	7.24 ± 0.44	6.47 ± 0.21	8.49 ± 0.24	8.33 ± 0.17	7.40 ± 0.39	6.76 ± 0.78
Testes/Ovaries	8.32 ± 0.13	9.36 ± 0.21	9.41 ± 0.18	9.68 ± 0.35	8.34 ± 0.22	8.58 ± 0.30

(7.39 mg/mL) > alimentary canal (6.47 mg/mL) (Table-1) which was also investigated that efficiency of immune response can be increased by several food additives and immunostimulants [9]. In the month of May the protein concentration results showed the order testes (9.41 mg/mL) > alimentary canal (8.49 mg/mL) > kidney (7.50 mg/mL) > gills (7.44 mg/mL) > spleen (7.33 mg/mL) in male tilapia and in female tilapia this order was ovaries (9.68 mg/mL) > alimentary canal (8.33 mg/mL) > kidney (7.50 mg/mL) > gills (7.35 mg/mL) = spleen (7.35 mg/mL) (Table-1). In the month of June protein concentrations of different organs of male tilapia were as Testes (8.34 mg/mL) > kidney (7.59 mg/mL) > spleen (7.49 mg/mL) > alimentary canal (7.40 mg/mL) = Gills (7.40 mg/mL) and in female tilapia as ovaries (8.58 mg/mL) > kidney (8.05 mg/mL) > spleen (7.78 mg/mL) > gills (7.53 mg/mL) > alimentary canal (6.76 mg/mL) (Table-1).

Overall mean results of protein concentrations in 6 months tenure in male tilapia's organs were as testes > kidney > gills > spleen > alimentary canal and in female tilapia as ovaries > kidney > spleen > gills > alimentary canal while in case of mucoid lineage in different organs of *Clarius Butrachus* indicated that the kidney and the alimentary canal analysis showing the reverse results [26] and described protein concentrations of different organs was liver > skin > intestine > kidney, while gills play their role in the immune defense using mucosa which is associated with lymphoid tissues [19].

Salahudeen *et al.* [27] discussed the effect of urea on protein pattern of different organs of *Oreochromis mossambicus*. Before that they checked the protein concentrations in different organs of tilapia like gills, muscles, liver and intestine. According to the liver has the highest protein concentrations and intestine has the lowest protein concentrations. Present investigation indicated that in gonads of both sexes high concentration of proteins are found (Fig. 1). Stress response of biomolecules in fish *Channa punctatus* was studied by Javed and Usmani [28]. In their study they checked the protein profiles of serum, liver and muscles. For their study they quantified the proteins in these organs of fish by Bradford [25].

Moutou *et al.* [29] evaluated the activity of different oxidoreductases enzymes in kidneys and posterior intestine tissues of *Cyprinus carpio*. According to their results could

Fig. 1. Comparison of total protein concentrations in different organs of male and female tilapia

not related antioxidants. Rani *et al.* [30] also observed quantitative variations of proteins in the muscle tissues of fish *Clarias batrachus* by Bradford method. So it is clear that many of the scientists used the method of Bradford to find out activity of proteins in different organs of fish. In present research work we also determined the total proteins of the different organs of male and female *Oreochromis niloticus* by Bradford method. There was no significant difference in the total protein contents of male and female Tilapia (Figs. 2 and 3). As a general protein concentrations are highest in the gonads and lowest in the alimentary canal/intestine (Fig. 1).

Fig. 2. Protein estimation in different organs of male tilapia in different months

Fig. 3. Protein estimation in different organs of female tilapia in different months

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