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*Full Length Research Paper*

# Influence of biometric characteristics and gender on serum IgM in the Benni, *Barbus sharpeyi* Gunther, 1874 (Osteichthyes: Cyprinidae)

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**IgM is the only known immunoglobulin in fishes with an important role in immune responses. This study was done to evaluate serum IgM level in *Barbus sharpeyi* and to study the influence of weight, length and sex on it. IgM value was determined in the serum of 42 male and female *B. sharpeyi* (Benni) fish using RIA method. Fish came from Dashte azadegan fish farm at the southwest of Iran. The mean value of IgM was 62.8 mg/dl (42 and 78.4 mg/dl in males and females, respectively). There was a significant difference between males and females IgM level ($p<0.05$). Serum IgM value was also compared in fishes with different weight and length and the results showed that serum IgM increases with increasing fish weight ($p<0.05$). The correlation coefficient between the serum IgM concentration and morphometric features of *B. sharpeyi* indicated that fish weight, length and sex have influence on serum IgM.**

**Key words:** Benni, immunoglobulin M, Iran.

## INTRODUCTION

*Barbus sharpeyi* which is locally known as Benni, is one of the most important fishes in the Tigris-Euphrates Basin. It is one of 300 *Barbus* species in the world and 15 known *Barbus* species in Iran. This species is enumerated as an important cultured species in south Iran and there is an increasing interest in this species for aquaculture purposes. It also comprises 23% of the total fish production in the neighboring country, Iraq (Abdoli, 1999). Studies of the haematology and blood biochemistry in different fishes are of comparative physiological interest (Oner et al., 2008; Parma De Croux, 1994). It contributes to a greater understanding of habitat, food selection and health status of the species. It is reported that fish blood is a suitable means of indicating the effects of stress, influence of environment and diseases in fish in a given area.

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Immunoglobulin M is the first immunoglobulin to appear in evolution and commonly the only immunoglobulin class described in different fish species. A better understanding of the structure and function of fish IgM has become all the more important in recent years due to the need of the fish farming industry for effective prevention and control of various fish diseases. IgM is pentameric in higher vertebrates and cartilaginous fish, tetrameric in teleosts (Pilstrom and Bengten, 1996) and hexameric form has been demonstrated in some amphibia (Hsu and Du Pasquier, 1984). There are also indications that another class of immunoglobulin may be found in some fish species. An unusual IgM type has been described in eggs of chum salmon (Fuda et al., 1992) and a second class of immunoglobulin has been described in some cartilaginous fishes (Tomonaga and Kabayashi, 1985).

Wilson et al. (1997) described a new chimeric Ig, heavy chain in channel catfish which showed similarities to mammalian IgD but also shared C termini and first constant domain of IgM heavy chain. As the culture of *B.*

**Table 1.** Biometric characteristics of studied fishes.

| Parameter | All samples | Males(18) | Females(24) |
|---|---|---|---|
| Weight (g) | 775.21±380.43 | 488.27±335.07 | 990.41±359.81 |
| Total length (cm) | 39.41±5.40 | 34.86±3.66 | 42.83±3.17 |
| Standard length (cm) | 33.73±4.82 | 28.61±2.85 | 36.83±3.49 |

**Table 2.** IgM level in fishes with different length and weight.

| Parameter | Total length | | | P-value | Standard length | | | P-value | Weight | | | P-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | <35 cm | 35-45 cm | > 45 cm | | <30 cm | 30-35 cm | 35-40 cm | | <500 g | 500-1000 g | >1000 g | |
| IgM (mg/dl) | 38.7±14.85 | 35.36±45.62 | 90.10±64.42 | 0.001 | 38.07±14.85 | 43.71±20.53 | 96.31±60.78 | 0.001 | 38.07±14.85 | 65.36±45.62 | 90.10±64.42 | 0.028 |

*sharpeyi* becomes more and popular recently in Iran, it is increasingly important to accurately evaluate different serum parameters of this species during the production cycle. There is no previous report on the serum IgM of *B. sharpeyi*. The aim of the present work is to determine the serum IgM level in *B. sharpeyi* fish and to compare IgM value with sex and biometric characteristics.

## MATERIALS AND METHODS

The fish came from Dashte Azadegan fish farm close to Ahvaz city in Khuzestan Province at the south west of Iran. A total number of 42 apparently healthy fish, all 2 years old, including 18 males and 24 females were studied during 2009. After total length and body weight were measured, fish were investigated for ecto and endoparasites and any other clinical sign of disease according to the instructions by Raissy et al. (2010, 2011). Biometric characteristics of randomly selected fishes ranged from 335 to 1815 g for weight (mean=775.2 g) and 26 to 45 cm for total length (mean=39.4). The blood samples were obtained with syringe, fitted with 21 gauge hypodermic needle. From 2 to 5 ml blood was collected from the caudal vein. The blood was allowed to clot at room temperature for 2 h and then overnight at 4°C then serum collected after centrifugation at 2000 rpm for 10 min. Serum samples were stored at -20°C. IgM level was detected by radioimmunoassay test according to Gharagouzlou (1997). RIA is a quantitative immunoassay technique used to detect the level of protein or antigen in a sample by measuring the diameter of the ring of precipitin formed by the complex of the protein and the antiserum. To perform a radioimmunoassay, a known quantity of an antigen is made radioactive, frequently by labeling it with gamma-radioactive isotopes of iodine attached to tyrosine. This radiolabeled antigen is then mixed with a known amount of antibody for that antigen, and as a result, the two chemically bind to one another. Then, a sample of serum from containing an unknown quantity of that same antigen is added. This causes the unlabeled (or "cold") antigen from the serum to compete with the radiolabeled antigen ("hot") for antibody binding sites. As the concentration of "cold" antigen is increased, more of it binds to the antibody, displacing the radiolabeled variant, and reducing the ratio of antibody-bound radiolabeled antigen to free radiolabeled antigen. The bound antigens are then separated from the unbound ones, and the radioactivity of the free antigen remaining in the supernatant is measured using a gamma counter. Using known standards, a binding curve can then be generated which allows the amount of antigen (IgM) in the serum to be derived (Yalow and Berson, 1960., Werner et al., 1974). Data were transferred to Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) for analysis. SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA), was used for ANOVA and Student's t-test analysis; differences were considered significant at values of $P<0.05$.

## RESULTS

Biometric characteristics including weight, total and standard length were measured and sex was identified after necropsy of each fish. Biometric characteristics of studied fishes are presented in Table 1. Using RIA, The mean level of IgM was 62.8 mg/dl in samples. A statistical difference was found between male and female serum IgM ($p<0.05$). Mean serum IgM value ranged from 31 to 53 mg/dl in males (mean = 42±18.29) and 43 to 82 mg/dl in females (mean = 78.41±56.59). In order to comparing mean IgM concentration in fishes with different length and weight fishes were divided into 3 groups based on their weight (<500 g, 500 to 1000 g and >1000 g) and 3 groups based on their length (<35 cm, 35 to 45 and >45 cm). Pearson's correlation indicates significant ($p<0.05$) interact-tion between total length, weight and IgM as IgM

value increases in large fish (Table 2).

## DISCUSSION

The immune system of fishes is conditioned by environment, but also by their poikilothermic condition. Most pathogenic bacteria are opportunistic microorganisms normally present in the aquatic microflora. The obligatory pathogens are scarce, but their virulence depends on environmental factors such as thermic, ionic and osmotic changes, iron and oxygen availability, pollutants, eutrophia, etc. In fish, immunocompetence depends more on fish weight rather than on age, mainly due to the need of a minimum number of immunocompetent cells. Thus, it is known that some fish larvae have the ability to process antigens, even when they are still dependent on yolk resources (Hansen and Olafsen, 1999., Zapata et al., 1997). As in all vertebrates, fish have cellular and humoral immune responses, and central organs whose the main function is involved in immune defense. Fish and higher vertebrates like mammals show some similarities and some differences regarding immune function. Most of the generative and secondary lymphoid organs present in mammals are also found in fish, except for the lymphatic nodules and the bone marrow. Instead, the anterior kidney, aglomerular, assumes hemopoietic functions, and unlike higher vertebrates is the principal immune organ responsible for phagocytosis, antigen processing and formation of IgM and immune memory through melanomacrophagic centers. The teleost Ig are limited to mainly a IgM tetramer of approximately 800 kD and no definitive evidence of Ig diversity has been demonstrated in fish.

Previous studies have reported great variation in fish blood chemistry (Stoskopf, 1993). To reduce intraspecific variability, biometric characteristics of fishes and site specific data (e.g., biochemical characteristics of water) could allow correlation of blood chemistry with physiological and environmental conditions. Several studies have demonstrated considerable individual variations in serum IgM levels amongst different fish species. This may be related to species of fish species, size, gender or age (Klesius, 1990; Magnadottir, 1990), the environmental conditions (Olesen and Vestergard-Jorgensen, 1986; Klesius, 1990; Magnadottir, 1990) or the disease status (Magnadottir et al., 1995).

IgM is pentameric in higher vertebrates and cartilagenous fish (Kobayashi et al., 1984), tetrameric in teleosts (Gharagouzlou., 1997) and hexameric form has been demonstrated in some amphibia (Hsu and Du Pasquier, 1984). Hexameric, as well as pentameric form has also been found in mice (Hughey et al., 1998). Monomeric form has similarly been described in some species and separate isotypes have, for example, been identified in salmon (Hordvik et al., 1992). The so-called J-chain, which is associated with the polymerisation of mammalian IgM, is commonly absent from fish IgM. However, its presence has been indicated in a few fish species like catfish and rainbow trout (Sanchez et al., 1989).

It must be stressed therefore, that the IgM concentration level in the present study only represent the mean of healthy individuals of similar size and kept under similar environmental conditions.

According to the results, the mean IgM value is significantly higher in females than males which show that females are able to produce a strong antibody response of high specifity. Many scientists (Akinrotimi et al., 2010) have reported significant differences between male and female haematologic parameters.

The correlation coefficient between the serum IgM concen-tration and morphometric feature of *B. sharpeyi* indicated that fish size have influence on serum IgM, a position that has been supported by other researchers. The results show that serum IgM level in fishes with > 1000 g weight (90.1 mg/dl) is more than two times higher than fishes with < 500 g weight (38 mg/dl). A recent study has also shown that the serum IgM level of Cod increases with increasing weight (Magnadottir et al., 1999) which is comparable to the values obtained in the present study.

## Conclusion

It is concluded that the lower level of IgM in smaller fishes in comparism with large fishes may correlate with their susceptibility to diseases as the bigger fishes have a more potent immune system against pathogens.

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