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Immunoglobulin M (IgM) and Lipid-Profile (Cholesterol, Triglyceride, HDL and LDL) level in *Anabas testudineus*

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Abstract
Fish health is impaired by stress, disease, feeding activity and various environmental and physiological conditions. To improve fish health or nutritional quality, it is necessary to know their immunity and biochemical composition. The present study was thus to estimate the Immunoglobulin M (IgM) and Lipid-Profile (Cholesterol, Triglyceride, HDL and LDL) level in *Anabas testudineus* either affected by sex, gonad, and parasitic infection. Total 50 live fishes were collected during the period of November, 2018 to October, 2019 from Luxmibazar, Dhaka, Bangladesh. IgM (0.3097±0.051), Cholesterol (184.64±8.2) and HDL (66.64±9.08) were higher in male fishes than females but Triglyceride (291.16±84.45) was higher in female fishes. This may happen due to male fish is biochemically and nutritionally richer than female. IgM (0.237±0.037) and HDL (57.41±5.81) were higher in fishes without gonad than with gonad female, but Cholesterol (169±16.31), Triglyceride (388.71±218.87) and LDL (81.29±15.82) were higher in with gonad female fishes. Cholesterol (p=0.03) level showed a significant relationship between with gonad and without gonad female fishes because during gonadal enlargement Cholesterol level increase. IgM was higher in fishes without gonad because the incorporation of IgM into the eggs. The results of this study could be used for monitoring the fish health and disease resistance ability.

Keywords: IgM, cholesterol, triglyceride, HDL, LDL

1. Introduction
Fish is consumed by human for centuries and is preferred as a perfect diet not only due to its taste, but also having higher proportions of essential amino acids, vitamins and minerals. Bangladesh is one of the world's leading fish producing country. Fisheries sector is contributing significantly in food security through providing safe and quality animal protein and also plays an important role in the Bangladesh economy as it provides employment opportunity and a source of income.

Fish health affected by different factors such as temperature, water quality, stress and also various pathogens. Parasites are an important group of pathogens, which occurs in various developmental stages in fish. To get the perfect nutrition it is necessary that fishes must be healthy and free from parasitic infection and other diseases. The ability of fish to cope with infecting pathogens relies on a delicate balance between the environment and the components of the innate and acquired immune systems. The basic function of the immune system is to protect an organism against infection in order to minimize the fitness costs of being infected. Fish immune responses may be influenced by nutritional status and feed deprivation. Today fish immunology has developed into a flourishing and an independent scientific field with tight links with general immunology and aquaculture. A concise history of fish immunology was published [1, 2].

Immunoglobulins or antibodies which play a vital role in adaptive immune responses was first reported in 1890 as an agent in the serum that could neutralize diphtheria toxin [3]. Immunoglobulin M (IgM) is a major serum protein that plays role in blood, which an indicator of the physiological condition of an animal. IgM in normal serum is often found to bind to specific antigens; is produced automatically in any vertebrate body. If any type of infection or disease shown in vertebrate body IgM response first, thus the immune system plays important role in cellular and humoral components to defend the body against foreign substances. A potential candidate for IgM was discovered 50 years ago in spiny dogfish shark the *Squalu acanthias* serum [4] IgM contributes to both innate and adaptive immunity in fish.

IgM also mediates cellular cytotoxicity, agglutination for phagocytosis and removing pathogen [5]. Lipids and their components play a vital role in the ecological and biochemical adaptations, monitoring and testing of aquatic organisms. Cholesterol is a substance (a steroid) that is essential for life. It forms the membranes of cells in all organs and tissues in the body. It is used to make hormones that are essential for development, growth, and reproduction. It forms bile acids that are needed to absorb nutrients from food. The test for cholesterol measures total cholesterol that is carried in the blood by lipoproteins. Each particle contains a combination of protein, cholesterol, triglyceride, and phospholipid molecules and the particles are classified by their density into high density lipoproteins (HDL), low-density lipoproteins (LDL) and very low- density lipoproteins (VLDL). HDL-C particles, sometimes called "good" cholesterol, carry excess cholesterol away for disposal and LDL-C particles, or "bad" cholesterol, deposit cholesterol in tissues and organs. Triglycerides are a type of fat that found in fish blood. Fish oils are lowered by triglyceride but LDL level is increased and creating various problems in fish body. Age-sex related, and seasonal changes of plasma lipoprotein concentrations in trout was studied by [6] and find that season and the reproductive cycle are the two main factors affecting basal plasma lipid and lipoprotein levels in trout. LDL and HDL levels were higher in the male fish, *Scorpaena porcus* in comparison with female [7]. The objectives of the study were to measure the Immunoglobulin M (IgM) and Lipid-Profile level (Cholesterol, Triglyceride, HDL and LDL) either affected by sex, length, weight, gonad and parasitic infection in *A. testudineus*.

2. Materials and Methods

A total of 50 individuals of *Anabas testudineus* was collected from Luxmibazar, Dhaka, Bangladesh during the period of November, 2018 to October, 2019. Collected fish samples were transferred to the Fisheries Laboratory, Department of Zoology, Jagannath University, Dhaka, Bangladesh for further analysis. The collected fish samples were categorized into sex, gonad, parasitic infections etc. We estimated only 16 fishes for IgM and 30 fishes for Lipid- Profile level. It was not possible to estimate all fishes IgM and Lipid -Profile level because our blood sample volume was not enough to examine this level.

2.1 Blood collection Method: Blood samples (2-5 ml) were rapidly collected from the lateral line of live fish using heparinized syringes. Blood samples were put into an improvacuter tube. Centrifuging blood samples in 2000 × g for 10 min where the plasma was detached and stored at –70 °C. Plasma samples were immediately sent to the Department of microbiology, Bangabandhu Sheikh Mujib Medical University for measuring the Immunoglobulin M (IgM) and lipid profile level.

2.2 Measurement of IgM: Plasma biochemical parameters were analyzed using an auto analyzer (BN ProSpec system instruction manual and software of the instrument). Serum and plasma samples were automatically diluted 1:20 in the low concentration assay protocols with N dilute. The dilute samples were measured within four hours.

2.3 Determination of Lipid-Profile level (Cholesterol, Triglycerides, HDL, LDL): Cholesterol, Triglycerides were estimated using automatic serum chemistry auto analyser [24, 9].

HDL-C level was estimated by the phosphotungstic acid endpoint method. Serum TG level was estimated using the DES Trinder endpoint method with a lipid clearing agent. VLDL-C and LDL-C were calculated with Friedewald's formula [10].

LDL-C was calculated from the formula: TC− (HDL-C+VLDL-C) and VLDL-C was calculated from the formula: VLDL-C=TG/5, where TG<400 mg/dl. Using these two formulae, LDL-C levels were calculated as follows:

LDL-C=TC−HDLC−TG/5 [11].

The following were cutoff points for different lipoproteins, where elevated levels were determined as risk factors, according to the criteria of the American National Cholesterol Education Program:

HDL-C <35 mg/ml, LDL-C >130 mg/dl, LDL-C/HDL-C >2.0, and TC/HDL-C= 4.5 [11].

3. Results and Discussion

Total 16 fishes were used to estimate IgM and 30 fishes for Lipid-Profile level. Out of 16 host fishes 9 were females and 7 were males. Females and males IgM level were 0.1944±0.024 and 0.3097±0.051 respectively (Table 1).

Table 1: Immunoglobulin M (IgM) level based on sex.

Sex	Total number of fish	IgM	P value
Male	7	0.3097±0.051	1.53 ns
Female	9	0.1944±0.024	

All values are given in mg/dl and as mean ± SEM; s: Significant; $p< 0.05$, ns: Non-significant; p>0.05, SEM: Standard error of mean.

The present study showed that the IgM level was slightly higher in male than female fishes. According to literature [12], there was no relationship between fish sexes and IgM level and if the temperature changed seasonally, IgM may show significant difference between male and female fishes. Gender did not contribute significant differences in plasma IgM level and the levels remain constant in male-female rainbow trout [13, 14]. Out of 9 females, 5 were with gonad and 4 were without gonad. With gonad and without gonad IgM level were 0.1602±0.0256 and 0.237±0.037 respectively (Fig. 1)

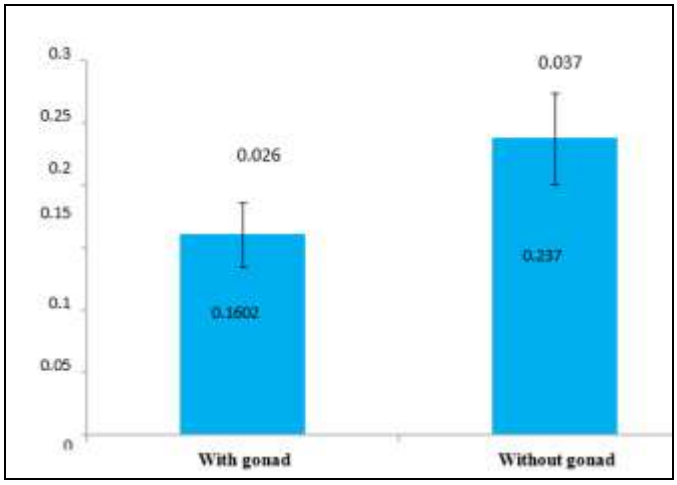

Fig 1: IgM level of with gonad and without gonad female fish.

The present study showed that IgM was also slightly higher in

without gonad than with gonad fishes. Previous literature also supports this view that the IgM level drop in female fish during the spawning period due to incorporation of IgM into the eggs. However, this incorporation is likely to occur at the level of nanograms of IgM per gram of egg, thus not significantly contributing to changes in circulating IgM [15, 16].
In case of 19 females and 11 males fishes Cholesterol levels were 168.58±7.03 and 184.64±8.2; Triglyceride were 291.16±84.45 and 283.18±58.87; High density lipoprotein (HDL) were 54.47±3.91 and 66.64±9.08; Low density lipoprotein (LDL) were 71.95±8.91 and 70.87±10.93 respectively (Fig. 2). The present study showed that Cholesterol and HDL were higher in males than females, but Triglyceride was higher in females. And LDL showed no difference between males and females. Present findings supported the view of [17] that the serum cholesterol concentration in *Arapaima gigas* male fish was higher than female fish. Male fishes showed higher blood biochemical values than females [18]. This indicates that the male fish is biochemically and nutritionally richer than the female. It is possible that in female fish, much of metabolites and nutrients are continuously being exhorted in the development of gonads (ovary). Total serum lipid concentrations in females were often significantly higher than in males [19] which relate our study. They also reported that serum Triglyceride level increase during feeding activity, seasonal change etc.

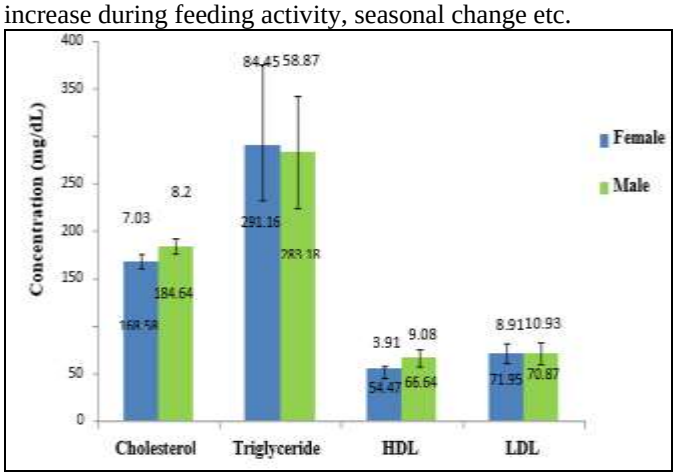

Fig 2: Lipid – Profile level based on sex

Among the 19 females host fish 7 were with gonad and 12 were without gonad. With gonad and without gonad female fishes Cholesterol level were 169±16.31 and 168±6.63; Triglyceride was 388.71±218.87 and 234.25±49.33; HDL was 49.42±3.42 and 57.41±5.81; LDL was 81.29±15.82 and 66.5±10.89 respectively (Table 2).

Table 2: Lipid Profile level based on gonad

Gonad	Total number of fish	Cholesterol	Triglyceride	HDL	LDL
With gonad	7	169±16.31	388.71±218.87	49.42±3.42	81.29±15.82
Without gonad	12	168±6.63	234.25±49.33	57.41±5.81	66.5±10.89
P value		*0.03 s	0.06 ns	0.09 ns	0.55 ns

All values are given in mg/dl and as mean ± SEM; s: *significant, $p<0.05$; ns: non-significant, $p>0.05$; SEM: standard error of mean.

Cholesterol, Triglyceride and LDL were higher in case of with gonad fishes than without gonad. But HDL was lower in with gonad fishes. *Cholesterol (p=0.03) level showed a significant relationship between with gonad and without gonad female fishes. Because during spawning season fishes showed higher feeding activity. Cholesterol level increased in with gonad female fishes. Reduction in plasma triglyceride and cholesterol levels during starvation was reported by [20]. Triglyceride and Cholesterol were increased with the gonadal enlargement but low levels of Triglyceride and Cholesterol at the time of spawning have been confirmed by [21, 22] and also was estimated that deposited energy was used for reproducing activity. Out of 30 fishes 28 were uninfected (both male and female), 2 were infected (female). In uninfected (male and female) and infected (female) Cholesterol level were 174.32±5.85, 176.5±7.5; Triglyceride were 295.96±60.80, 183±9; HDL were 59.35±4.49, 53±2; LDL were 70.48±7.26, 86.5±3.5 respectively (Table 3).

Table 3: Lipid Profile level based on infection

Factor	Total number of host	Cholesterol	Triglyceride	HDL	LDL
Uninfected (male and female)	28	174.32±5.85	295.96±60.80	59.35±4.49	70.48±7.26
Infected female	2	176.5±7.5	183±9	53±2	86.5±3.5
P value		0.2 ns	1.6 ns	0.9 ns	1.5 ns

All values are given in mg/dl and as mean ± SEM; s: Significant; $p< 0.05$, ns: Non-significant; p>0.05, SEM: Standard error of mean.

Here our study showed that Cholesterol and LDL were slightly higher in infected (female) than uninfected fish. Triglyceride and HDL were higher in uninfected (male and female) than infected (female). We could not properly compare between uninfected and infected fishes because our fish sample was insufficient. If we found a sufficient number of the infected fishes, then we compared the result appropriately.
Though we did not find any data of parasitic disease of Lipid - Profile but we found data on viral disease. If any kind of disease occurred in fish body; the plasma cholesterol level was significantly lowered [23]. These studies demonstrate that a reduction in the plasma total cholesterol level is linked to impaired disease resistance in fish.

4. Conclusion

The results of this study could be used for monitoring the immunological and the physiological condition of *A. testudineus*. Water quality, seasonal variation, feeding activity, habit and habitat etc. are the factors which influence or change the IgM and Lipid-Profile level. If we could estimate IgM and Lipid-Profile level by using a feed or observing seasonal variation, our result would have shown the more appropriate disease resistant ability of fish.

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