



Sex differences in the blood biochemical parameters of the fresh water fish, *Notopterus notopterus* (Pallas, 1789)

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ABSTRACT

Before blood constituents data can be applied as a diagnostic tool, general patterns related to sex, season and collection of fish and location need to be well documented and understood. The sex of the fish may also influence the blood parameters. In turn, the values of the different blood biochemical parameters significantly influence the physiological factors and sex of the fish. Hence, there is a need to assess sex before reporting the biochemical indices of the fish species. The present study was undertaken to know the difference between male and female blood biochemical parameters of the fish, *Notopterus notopterus*. The blood biochemical measurements included glucose, protein, total cholesterol, HDL and LDL-cholesterol, triglycerides, creatinine, enzymes-SGPT, SGOT and alkaline phosphatase. Furthermore, the electrolytes of - sodium, potassium, calcium and phosphorous were compared with regard to sex. Moreover, the effect of sex on quantitative changes in fish blood was studied. The blood parameters between the sexes indicated that male fishes consistently had higher level of all biochemical parameters than did the female fishes, and this study may be a proposal as the means of sexing fish. This indicates that the male fish is biochemically and nutritionally richer than the female; in female fish this effect may come about because much of the metabolites and nutrients are continuously being exhausted in the development of gonads (ovary). The results of this study could be used for monitoring the health and the physiological condition of the fish, *Notopterus notopterus* in a particular aquatic body.

Keywords: Blood biochemical parameters, sex difference, fresh water fish, *Notopterus notopterus*

1. INTRODUCTION

Blood chemistry and hematological measurements can provide valuable tools for monitoring the health and condition of both wild and cultured fish. Physiological indices can offer critical feedback on rearing conditions and nutritional status and can aid in the diagnosis of disease. Some authors reported evident quantitative difference between the blood parameters of male and female fishes (Ezzat *et al.*, 1974; Van Vuren and Hatting, 1978). The values of the different biochemical and haematological parameters significantly influence the physiological factors and sex of the fish. In the assessment of the blood parameters it was revealed that the sex and period of acclimation may exert some degrees of influence on some of the haematological characteristics of *C. gariepinus* (Gabriel *et al.*, 2004) and hence, the need to reckon with these factors in the assessment and reporting of the biochemical indices of fish species. The present study has been undertaken to know the difference between the blood biochemical parameters of male and female fish, *Notopterus notopterus* (**Figure 1**). The blood biochemical measurements were compared with regard to sex.



Figure 1. *Notopterus notopterus* (Pallas, 1789)

2. MATERIAL AND METHODS

Live specimens of Fresh water fish, *Notopterus notopterus* were collected from Bheema River, around 45 km away from Gulbarga and transported in aerated containers to the laboratory. The fish were placed belly upwards and blood samples obtained from the caudal circulation with the aid of a disposable plastic syringe and a 21 gauge disposable hypodermic needle. After collecting blood sample, each fish was sacrificed and dissected to know the sex and accordingly the test tubes were marked as 'M' for male and 'F' for female, as the sex of this fish cannot be identified morphologically.

One portion of the sample serum was obtained by centrifuging and is used for determination of biochemical parameters. All determination was carried out for each sample.

The total serum proteins were measured by using the modified Biuret method, end point assay as described by Lawrence (1986), Glucose was determined by (GOD-POD) Glucose

oxidase – peroxidase, by end point and assay method. Urea and urea nitrogen were determined by modified Berthelot method, total cholesterol was determined by (CHOD-PAP) cholesterol oxidase - phenol aminophenazone method, HDL cholesterol was determined by (CHOD-PAP) cholesterol oxidase - phenol aminophenazone method, LDL cholesterol was determined by Friedewald's equation, HDL cholesterol Triglycerides (TG) was determined by (GPO-PAP) glycerol-3-phosphate oxidase - phenol aminophenazone end point assay method. Creatinine was determined by modified Jaffe's method, using kinetic test without deproteinisation according to the Jaffe's method. Serum SGOT and SGPT activity was assayed following modified International Federation for Clinical Chemistry (IFCC) method using commercial kit, Serum Alkaline phosphatase activity was determined by kinetic assay (IFCC) method using commercial kit. Sodium and potassium were determined by colorimetric method. Calcium was determined by Modified Arsenazo method. Phosphorus was determined by Molybdate UV method.

Statistical treatment of the data: Difference in biochemical parameters between male and female was studied by adopting varied statistical methods. Standard deviation and probability test, *i.e.*, 't' test were calculated. The student -'t' test was carried out to know the levels of significance using the standard formula. All the values of p below 5% level are designated as significant. Correlations of the tests were carried out by using statistical software SPSS 7.5

3. OBSERVATION

The studies have been carried out to know the blood biochemical variables in the fish, *Notopterus notopterus* and the measurements were compared with regard to the sex of fish. The blood biochemical studies, such as protein, glucose, urea, urea nitrogen, creatinine, cholesterol, HDL, LDL and triglycerides (TG) are presented with each mean and standard deviation in **Table 1**. Blood enzymes, such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP) and electrolytes, such as sodium (Na^+), potassium (K^+), calcium (Ca^{2+}) and phosphorus (P) are determined and presented in the Table 1.

The males are having higher levels of protein and glucose as compared to females. Urea is present in surprisingly small quantities in the blood and is in greater concentration in the corpuscles than in the plasma. The concentration of urea in the blood represents mainly a balance between urea formation from protein catabolism and urea excretion by the kidneys. Urea formation takes place mainly in liver. Males are having higher levels of urea nitrogen than females. Creatinine, an end product of creatine metabolism, is a normal and alkaline component of blood. Male fishes are having higher levels of creatinine than the females. The preponderance of creatine over creatinine in the plasma of fish corresponds to the relationship of the two compounds in the blood and emphasizes the relative importance of the creatine-creatinine metabolism of these animals at least in so far it as concerns with excretion. The importance of such study in connection with the nitrogenous substances has not been sufficiently emphasized. The known compounds making up the non-protein nitrogenous fraction of the blood are for the most part materials of interest chiefly as end- products ready for elimination from the body. Serum lipids (TG and CHOL) and lipoproteins, males are having higher levels of triglycerides, cholesterol, HDL and LDL than the females. In the fish *N. notopterus*, serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate

transaminase (SGOT) males are having high levels of enzyme as compared to females. Alkaline phosphatase (ALP) is present in practically all tissue of the body. It occurs at particularly high levels in kidney tubules, intestinal epithelium, and bone (osteoblasts). The precise metabolic function of ALP is not known but it appears that the enzyme is associated with calcification process in bones and with lipid transport in the intestine. Male fish has higher level than female fish.

Electrolyte (Na^+ , K^+ , P , and Ca^{2+}) levels indicate the operation of a variety of homeostatic mechanisms in the body. Sodium (chief cation of extracellular fluid) and potassium (chief cation of intracellular fluid) are the most important osmotically effective electrolytes. The serum sodium content in the fish *N. notopterus*, males are having higher values than females. Potassium is higher in intracellular fluid compared to the plasma and potassium ratio is vital for ion permeability barriers in the cell membrane. Males are having higher contents than females. Calcium serves a number of functions, it combines with phosphorus for the deposition of bone calcium and appears to have role in reproduction. The serum calcium in males are having higher values than females. Phosphorus plays important roles in growth and bone mineralization and also in lipid and carbohydrate metabolism. The serum potassium level in males are having higher values than females.

4. DISCUSSION

Measurement of biochemical parameters is a commonly used diagnostic tool in aquatic toxicology and bio-monitoring (Xiaoyun, *et al.*, 2009). In the present study the total protein, blood glucose, urea, urea nitrogen (BUN), creatinine, total cholesterol, triglycerides, HDL and LDL levels have been studied in the fish *N. notopterus*. The fish *N. notopterus* is very active as observed under laboratory condition. It comes to the surface for gulping air and for feeding frequently and needs more glucose for metabolism for its activity. The male fish has increased concentration of blood glucose than females, indicating that male is more active than female fishes. Hence, it is probable that based on active movement of the fish, the sex of the fish; *N. notopterus* may be identified as one of the factors for identification of sex in fishes. Similar values for glucose were found in the fish, *P. obscura*, (Kori-Siakpere *et al.*, 2005). During burst type of exercise in fish the working muscle derives energy almost exclusively, forming the anaerobic metabolism of glycogen. Consequently, there is a near depletion of the muscle glycogen store and an accumulation of lactate (Milligan and Wood, 1986). The path way for the synthesis of muscle glycogen in fish is not clear, classically, muscle glycogen restoration has been described by the Cori cycle: lactate produced into glucose which intern, is released to the blood and utilized by the muscle to support glycogen re-synthesis (New sholme and Leech, 1983). The higher levels of total protein have been expected as these fishes were carnivores and adult fishes need more protein for their gonads (Tavares –Dias, *et al.*, 2007). In the present study also higher levels of total protein have been found in the fish *N. notopterus* since the fishes were adult and it is also carnivore.

Serum cholesterol concentration in male fish is higher than in female fish. In the fish *Arapaima gigas* reported by Tavares Dias *et al.*, (2007), the cholesterol range was 150.6 – 263.7 mg/dl and Triglycerides 154.6 – 622.7 mg/dl. Once synthesized, cholesterol is released into the circulation for transport in combination with specific Apo lipoproteins. Lipoproteins transport free cholesterol in the circulation, where it readily equilibrates with cholesterol in

other lipoproteins and in membranes. Cholesterol ester is storage form of cholesterol found in the most of the tissues. It is transported as cargo in the hydrophobic core of lipoproteins. LDL is the mediator of cholesterol and cholesterol ester uptake into many tissues. Free cholesterol is removed from tissues by HDL and transported to the liver for conversion to bile acids in the process known as reverse cholesterol transport. The LDL and HDL levels were found to be higher in the male fish, *Scorpaena porcus* in comparison with those in female (Celik, 2004). In *N. notopterus*, the HDL and LDL values were higher. It has been shown that the lipoprotein differs among species (Ando and Mori, 1993).

Urea efflux has been assumed to be passive, since urea production occurs in the liver and this supply could provide an outwardly directed gradient for simple passive diffusion. Urea transport has been studied several times in the blood of the spiny dogfish *Squalus acanthias*. The results appear to be conflicting, suggesting both carrier-mediated and passive (Rabinowitz and Gunther, 1973) diffusion. In the present study the mean value of urea in the male fish *N. notopterus* is found higher than in females. The declining BUN may indicate liver disease or starvation, a low of 10 mg/dl is common in healthy freshwater fish and a low of approximately 5 mg/dl, in marine freshwater fishes (Stoskopf, 1993). In the present study the BUN level was found to be higher in male fish, *N. notopterus* than that in females, it was normal in values for a healthy fish. Creatinine is present in muscle, brain and blood in free form as well as in the form of creatine phosphate creatinine. In the blood of fish *N. notopterus* mean values were found higher for males than those for females. Creatinine is excreted by the kidney in fish, and Shell (1961) interpreted periods of increased creatinine concentration as indicating kidney impairment in small mouth bass (*Micropterus dolomieu*). The creatinine level in the fish *N. notopterus* is low, indicating normal functioning of the kidney in both in male and female fishes.

Enzymes such as Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP) are present at all times in the circulation of normal animal. Their substrates are also present in the circulation of these functional enzymes, which perform physiologic function in the blood. They are generally synthesized in the liver but are present in equivalent or higher concentrations in the tissue. In the fish *N. notopterus* both sexes exhibited normal values. These enzymes belong to the 'family' of non-plasma-specific enzymes that are localized within the tissue cells of the liver, heart, gills, kidneys, muscles and other organs; when present in blood serum or plasma, they may provide specific information on organ dysfunction. Elevation of SGPT activity appears to reflect liver disease and it is more specific for liver disease than SGOT because of the biological location of these two enzymes. However, the activity of either enzyme, particularly SGOT, may also be elevated in acute liver necrosis. Alkaline phosphatase (ALP) is one of the first of the clinically important enzymes identified. The liver produces more ALP than the other organs or the bones. Some physiological conditions, such as liver and skeletal disorders, result in large amounts of ALP appearing in the blood. The serum levels of SGPT, SGOT, ACP, CK, LDH and ALP were studied in immature males and females of *A. stellatus* and found that the activity of LDH, ALP, ALT and ACP were not significantly different between males and females. However, CK and AST were significantly different with the levels in the female being significantly higher than those of males and suggested that the difference mirrors the effect of sex on the activity of these enzymes (Shahsavani *et al.*, 2010). In the present study, mature *N. notopterus* fishes were studied for these enzymes in the serum and results obtained indicated that all the three enzymes are found to be high in male fishes as compared to females.

The blood electrolytes such as sodium (Na^+), potassium (K^+), chloride (Cl^-), calcium (Ca^{2+}), magnesium (Mg^{2+}) and phosphorous (P) are commonly used to determine the physiological characteristics, toxicity and health status of the fish. The electrolyte levels indicate the operation of a variety of homeostatic mechanism in the body (Clarke, 1998). The monovalent ions, namely sodium (Na^+) and potassium (K^+) play an important role in osmoregulation and homeostasis, in fish sodium enters the gill cells from the blood, co-transported with potassium. One of the divalent ions calcium (Ca^{2+}) serves a number of functions in fish. It combines with phosphorus for the deposition of bone. It is possible that bone serves as a reservoir of calcium for plasma and tissues. Calcium appears to be important in the reproduction and mitochondrial function. It is generally recognized that calcium has an important role in osmoregulation (Wurst and Stickney, 1989). Thus the levels of serum electrolytes offer important knowledge concerning the health status of fish (Wurst and Stickney, 1989; Evans, 1993). Percin *et al.*, (2010) reported the electrolytes in the serum samples of Blue fin tuna (*Thunnus thynnus*) of male and female fish and found lower in wild female than male and indicated that the female fish is more sensitive and weaker than males. In the present study the female fish *N. notopterus* has lower values of Na^+ , K^+ , Ca^{2+} and P than males indicating females are sensitive and weaker than males (Kavya *et al.*, 2016).

Table 1. Blood biochemical parameters of the fish *Notopterus notopterus* in relation to sex.

Blood Parameters/Sex	Male	Female
Glucose mg/dl	57.94 ±6.72	42.4 ±5.79
Protein gr/dl	6.69 ±1.67	5.82 ±1.55
Urea mg/dl.	32.58 ±17.85	28.34 ±16.73
Urea Nitrogen mg/dl	14.56 ±9.63	13.21 ±7.79
Creatinine mg/dl	3.17 ±1.04	2.37 ±0.83
Total Cholesterol mg/dl	174.04 ±23.34	131.33 ±20.78
Triglycerides mg/dl	361.65 ±24.17	248.03 ±34.61
High density Lipoprotein mmol/l	67.32 ±18.45	39.13 ±2.02
Low Density lipoprotein mmol/l	41.54 ±12.38	30.54 ±12.46
SGOT (Serum glutamate oxaloacetatetransaminase) U/L	15.66 ±0.67	13.65 ±0.92
SGPT (serum glutamate pyruvatetransaminase) in U/L	16.94 ±0.26	15.38 ±0.24
Alkaline phosphatase (ALP) IU/L	59.55 ±7.56	40.51 ±5.13

Sodium (Na ⁺) mmol/l	94.31 ±9.61	67.48 ±8.17
Potassium (K ⁺) mmol/l	15.75 ±1.09	12.99 ±1.32
Calcium (Ca ²⁺) mg/dl	9.51 ±0.37	7.82 ±1.46
Phosphorus (P) mg/dl	0.096 ±0.001	0.089 ±0.005

Each value expressed as Mean ±SD, N = 6

5. CONCLUSIONS

The results of this study could be used for monitoring the health and studying the physiological condition of *Notopterus notopterus*. In general, males showed higher blood biochemical values than females. 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 exorted in the development of gonads (ovary). The results obtained in the present study may provide a contribution to the knowledge of the characteristics of biochemical parameters of the fresh water corni-omnivorous fish, *N. notopterus*. This study may be a proposal as means of sexing in fish where there is no sexual dimorphism.

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