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Comparative assessment of serum biochemical profile in riverine and cultured populations of *Channa marulius* (Hamilton, 1822)

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Serum biochemical assessment is an important tool to provide information about the internal organs, metabolic and nutritional parameters and electrolytes. Present study was conducted to determine the serum biochemistry of *Channa marulius* in hatchery and riverine populations. For this purpose blood samples were collected by caudal vein puncture. Inferences of the study showed non-significantly ($p \geq 0.05$) higher values of calcium 10.24 mmol/L as compared to lower 9.66 mmol/L whereas, significantly ($p \leq 0.05$) higher values of serum globulin 21.06 g/L, total protein 53.0 g/L, albumin 14.87 g/L, cholesterol 6.116 mmol/L, alanine transaminase 530 U/L and alkaline phosphatase 398 µ/L were observed in cultured populations as compared to lower 20.40 g/L, 44.84 g/L, 12.30 g/L, 5.084 mmol/L, 453 U/L and 255 µ/L in riverine populations. On the other side, non-significantly ($p \geq 0.05$) higher values of serum triglyceride 0.674 mmol/L as compared to lower 0.604 mmol/L, while significantly ($p \leq 0.05$) higher values of glucose 28.43 g/L, urea 2.541 mmol/L, bilirubin 28.20 µmol/L, chlorine 70.3 mmol/L, potassium 13.43 mmol/L, sodium 126.5 mmol/L and aspartate transaminase 1169 U/L in riverine populations as compared to lower 23.89 g/L, 2.17 mmol/L, 26.23 µmol/L, 61.9 mmol/L, 12.08 mmol/L, 111.2 mmol/L and 1029 U/L were recorded in cultured populations, respectively. Findings of the study will be helpful in the field of biochemistry, physiology and toxicology as well as to enhance the management and rearing potential of the *Chnanna marulius*.

[**Keywords**: Biochemistry, *Channa marulius*, Cultured, Serum, Wild]

Introduction

The serum biochemical surveys provide information about internal organs, proteins, metabolic and nutritional parameters and electrolytes[1]. Certain blood biochemical parameters are affected numerous environmental factors including supplying density and feeding management. External parameters such as infections, administration and stress mostly caused major changes in the blood configuration[2]. Thus, any modification in biochemical parameters could be serious in the form of numerous diseases[3]. Studies about serum biochemistry and their established baseline values for different fish species is commonly used tool for research purposes in North America and Europe. In developed countries biological and blood biochemical parameters of fish are used in fish vaccinology programs[4]. Fish serum biochemistry have proved valuable approach in integrated fisheries management to control the onset of diseases and allowed different strategies to mitigate the ultimate fish loss[5]. While, blood glucose levels along with albumin, serum protein, globulin and enzymatic activities in blood plasma are considered as the valuable indicators under stress conditions[6,7]. The internal conditions of organisms could be studied by the knowledge about their blood biochemical parameters[8,9]. Therefore, blood biochemical parameters are very essential for the exposure of physiological conditions and health status of fish[10].

Blood biochemistry is pivotal in the management of endangered fish species[11]. Number of endangered fish species can be monitored and well managed by the evaluation of their serum biochemical parameters[11]. Serum biochemical parameters are considered as important diagnostic tools in biomonitoring. These are used for the assessment of various diseases as well chronic and acute alterations in physiology that is correlated with nutrition and water quality[12,13]. Among the important diagnostic tool, blood enzyme values, cholesterol and triglycerides are more significant contributors to explore the inhabiting status of fish. Mostly, the impact of environmental stressors on fish health could be directly monitored by the assessment of serum

biochemical profile[14]. Fishes are important to observe the water quality because they respond quickly to direct and indirect changes in the aquatic environment[15].

The giant snakehead (*Channa marulius*), is a fast rising fish with high market value and consumer fondness[16]. In China, it is deliberated as a food fish and considered as important for wound healing. Tolerance to the variety of habitats and carnivorous nature make them a vital element of fish farming that demands to explore their biological and ecological requirements[17]. Various factors can seasonally affect alterations in the blood biochemistry of fish including diet, photoperiod, reproduction cycle, temperature, and pH[18]. However, it has been reported that the disease causing agents, environmental pollutants and scarcity of food could change the serum biochemistry of fish[19]. Blood biochemistry constraints can also be used to identify the health of fish. Basic features, such as feeding management and supplying density, also have a direct effect on certain serum biochemical parameters. Exogenous factors like infections, administration, and stress, always prompt major variations in blood configuration[2]. Like other aspects, there are several research gapes in the serum biochemistry of commercially important fishes. To fill much of this research gap the present study was conducted to assess the serum biochemical profile of *Channa marulius* in wild and cultured populations.

Materials and Methods

Samples of proposed fish (n = 60) *C. marulius* were collected from riverine (n = 30) and local fish hatcheries (n = 30). During the acclimation of few days the fish was shifted to glass aquaria of 50 litre water capacity containing tap water with normal pH, temperature (25 °C) and (5.65 mg/L) dissolved oxygen, respectively.

Blood collection and serum preparation

The blood samples were collected by caudal vein puncture using 2 ml syringes and transferred into serum tubes. Serum was prepared by centrifugation of fish blood at 3000 rpm, the serum was separated and preserved at -20 °C. All the vials were labelled and prior to use serum vials were brought to room temperature.

Estimation of serum total protein

Serum total protein was determined by taking 0.02 mL distilled water, standard and serum in separate test tubes by adding 1.0 mL biuret reagent in all test tubes, mixed it and incubated for 30 minutes at 20-25 °C, observed the absorbance at the wavelength of 546 nm.

$$\text{Total protein concentration} = \frac{\text{A sample}}{\text{A standard}} \times \text{Standard concentration}$$

Estimation of serum albumin

By taking 10 µL deionized water, standard and sample in separate test tubes, 2.0 mL reagent were added in all test tubes, mixed and incubated at 20 – 25 °C for 2 minutes then, the absorbance was noted at 600 nm wavelength.

$$\text{Albumin concentration} = \frac{\text{A sample}}{\text{A standard}} \times \text{standard concentration}$$

Estimation of serum cholesterol

About 5.0 mL reagent was taken in blank, standard and sample in separate test tubes, added 0.05 mL reagent in standard and sample test tubes, mixed it well and kept immediately in the boiling water bath exactly for 90 seconds. The tubes were cooled immediately at room temperature under running tap water and recorded the absorbance on photometer at 560 nm.

$$\text{Serum cholesterol mg/dL} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

Estimation of serum triglyceride

By taking 5.0 mL reagent in blank, standard and sample in separate test tubes, added 0.05 mL reagent in standard and sample test tubes and tubes, mixed it well and kept immediately in the boiling water bath for 90 seconds. The tubes were cooled immediately to room temperature under running tap water and the absorbance was observed at 560 nm.

$$\text{Serum triglyceride mg/dL} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

Estimation of serum Aspartate Transaminase and serum Alanine Transaminase

By taking 50 µL serum and standard in separate test tubes, added 0.25 mL substrate in all test tubes, incubated at 37 °C for 30 min, then added 0.25 mL dinitrophenyl hydrazine (DNPH) in all test tubes and

kept for 20 min at room temp. After that, added NaOH 2.5 mL in all test tubes, mixed and recorded the absorbance at 505 nm using spectrophotometer (Spectronic 21).

Estimation of serum alkaline phosphatase

About 1.0 mL buffer and substrate were taken in separate test tubes, incubated for 3 min at 37 °C, added 0.1 mL serum, standard and distilled water in all test tubes, and incubated for 15 min at 37 °C. Then 0.8 mL of 0.5 N NaOH was added in all test tubes followed by addition of 1.2 mL 0.5 N $NaHCO_3$ in all test tubes. Amino antipyrine and potassium ferricynide 1.0 mL were then added in all test tubes, mixed well and recorded the absorbance at 510 nm within 5 min.

$$\text{Alkaline phosphate concentration} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

Estimation of serum sodium

By taking 40 µL distilled water, standard and sample in separate test tubes, added buffers and enzyme 1000 µL in all test tubes, mixed and incubated for 5 min at 37 °C, then added diluent and substrate 400 µL in all test tubes, mixed and incubated at 37 °C for 1 min and absorbance recorded at 405 nm.

Estimation of serum potassium

2 mL of standard and macro samples and 1000 µL of standard and micro samples were taken in separate test tubes. Thereafter, 2000 µL and 1000 µL standard solution was added separately in micro and macro samples tests, respectively. Later on, 200 µL supernatant was added in macro sample test and 100 µL in micro sample test, respectively, and mixed carefully and allowed to stand for 5 minutes. The absorbance of standard solution and samples were measured against reagent blank.

$$\text{Potassium concentration} = \frac{\text{A sample}}{\text{A standard}} \text{x } 5$$

Estimation of serum calcium

The standard solution 2.5 mmol/L (10 mg/dL) was taken in a test tube, added buffer 2-Amino-2-methyl-propan-1-ol 3.5 mol/L, pH 10.7 and chromogen O-Cresolphthalein complexone, 8-Hydroxyquinoline Hydrochloric acid 0.16 mmol/L, 6.89 mol/L and 60 mmol/L. Then added EDTA 150 mmol/L.

$$\text{Calcium concentration} = \frac{\text{A sample}}{\text{A standard}} \times \text{concentration} \times 2.50 \text{ mmol/L}$$

Estimation of serum chloride

Synermed ISE testing reagents were used for the determination of the levels of these electrolytes in blood.

Statistical analysis

T-test analysis was performed using (MINITAB software) to compare the variations in hatchery and riverine groups. Differences in values of biochemical parameters between hatchery and riverine groups were expressed as Mean ± SD.

Results

Serum total protein

Proteins are very essential in the whole body of organisms and they reached to every cell by flowing through the blood. Fish is very rich with proteins. In the present study, serum protein contents were found lower 44.84 ± 6.50 g/L in riverine population as compared to the cultured group 53.0 ± 11.6 g/L (Table 1).

Serum albumin

Albumin is important to maintain the liquid, which leaks out from blood into the tissues. The measurement of albumin contents in blood and serum evaluates the presence of other specific molecules in blood. In the present study, higher contents of albumin 14.87 ± 1.35 g/L was found in cultured population as compared to the wild 12.30 ± 1.74 g/L ones (Table 1).

Serum cholesterol

Cholesterol plays very important role in maintenance and osmoregulation of blood. In this study, the cholesterol values were higher 6.116 ± 0.869 mmol/L in cultured populations as compared to the lower 5.084 ± 0.84 mmol/L in riverine populations (Table 1).

Serum triglyceride

Triglycerides are type of fats and essential source of energy in the body. Our study showed the higher values 0.674 ± 0.42 mmol/L of triglyceride in riverine group than that of lower 0.604 ± 0.202 mmol/L in cultured one (Table 1).

Table 1 — Comparative serum biochemistry profile of riverine and cultured *Channa marulius*

Parameters	Riverine populations Mean ± SD	Cultured populations Mean ± SD	P (*t*-test)
Serum Total Protein (g/L)	44.84±6.50	53.0±11.6	0.000**
Serum Albumin (g/L)	12.30±1.74	14.87±1.35	0.000**
Serum Cholesterol (mmol/L)	5.084±0.84	6.116±0.869	0.000**
Serum Triglyceride (mmol/L)	0.674±0.42	0.604±0.202	0.3077^{NS}
Serum Aspartate Transaminase (U/L)	1169±360	1029±186	0.000**
Serum Alanine Transaminase (U/L)	453±127	530±134	0.000**
Serum Alkaline Phosphatase (μ/L)	255.9±99.8	398±134	0.000**
Serum Sodium (mmol/L)	126.5±24.0	111.2±12.4	0.000**
Serum Potassium (mmol/L)	13.43±3.08	12.08±1.71	0.0115*
Serum Calcium (mmol/L)	9.66±2.58	10.24±3.89	0.4065^{NS}
Serum Chlorine (mmol/L)	70.3±20.1	61.9±14.9	0.0262*
Serum Glucose (g/L)	28.43±5.53	23.89±3.84	0.000**
Serum Bilirubin (μmol/L)	28.20±4.65	26.23±3.85	0.000**
Serum Urea (mmol/L)	2.541±0.54	2.117±0.413	0.000**
Serum Globulin (g/L)	20.40±1.65	21.06±1.39	0.000**

SD = Standard deviation ** = Highly significant ($p \leq 0.01$) * = Significant ($p \leq 0.05$)
NS = Non-Significant ($p \geq 0.05$)

Serum aspartate transaminase

Serum aspartate transaminase is an important enzyme, found in many parts of the body and especially produced in liver, heart, muscles and many other tissues of the body. In our study, higher 1169 ± 360 U/L serum aspartate transaminase levels were found in wild group as compared to lower 1029 ± 186 U/L in cultured populations (Table 1).

Serum alanine transaminase

An ALT test evaluates the quantity of this enzyme in the blood and ALT is usually examined to observe the liver functioning. In this study, the alanine transaminase contents in wild populations were lowered 453 ± 127 U/L when compared to the higher 530 ± 134 U/L in hatchery populations (Table 1).

Serum alkaline phosphatase

ALP is a very essential hydrolase enzyme. In the present study, the alkaline phosphatase values were found lowered 255.9 ± 99.8 μ/L in riverine populations when compared with higher 398 ± 134 μ/L in cultured ones (Table 1).

Serum sodium

Sodium is found in the body especially outside the cells, playing vital role in the transmission of nerve impulses and the osmoregulation of body. Our results showed higher sodium value 126.5 ± 24.0 mmol/L in riverine populations as compared to the lower 111.2 ± 12.4 mmol/L in cultured populations (Table 1).

Serum potassium

It is the main cation in the body and plays very important role in the functioning of several body organs specially heart, digestive tract, muscles and kidneys. In the present study, potassium contents in riverine populations was higher 13.43 ± 3.08 mmol/L when compared with lower 12.08 ± 1.71 mmol/L in farm raised populations (Table 1).

Serum calcium

Calcium contents are found in high amount in bones where, they are essential for their maintenance and strength. In the present study, calcium content in riverine populations was lower 9.66 ± 2.58 mmol/L when compared with the higher 10.24 ± 3.89 mmol/L in hatchery populations (Table 1).

Serum chlorine

Chlorine is found mainly outside the cells and plays significant role in the maintaining the equilibrium in body fluids and acid-base balance. Our study showed higher chlorine contents 70.3 ± 20.1 mmol/L in riverine populations as compared to lower 61.9 ± 14.9 mmol/L in hatchery populations (Table 1).

Serum glucose

Glucose is the main source of energy for body and is transported to all body cells, through blood stream from the liver or intestines. In this study, the glucose level in wild group was higher 28.43 ± 5.53 g/L when compared with lower 23.89 ± 3.84 g/L in cultured group of appraisal fish species (Table 1).

Serum bilirubin

Bilirubin is a yellow pigment produced by the catabolism of haemoglobin in red blood cells by liver.

In this study, the bilirubin level in wild group was higher 28.20 ± 4.65 µmol/L when compared with the lower 26.23 ± 3.85 µmol/L in cultured ones (Table 1).

Serum urea

Urea is the main source of nitrogen in aquatic organisms. In this study, the urea level in wild group was higher 2.541 ± 0.54 mmol/L when compared with the lower 2.117 ± 0.413 mmol/L in cultured group (Table 1).

Serum globulin

Globulins are major blood proteins with high molecular weight, produced by the immune system and liver. Our study showed the lower globulin levels 20.40 ± 1.65 g/L in wild populations when compared with the higher 21.06 ± 1.39 g/L in cultured populations of *C. marulius* (Table 1).

Discussion

Serum biochemical examination is imperative for the speculation of health status of fish species[10]. Serum biochemical assay also provides valuable information about different metabolic disorders and stress factors in farm raised fish populations. Various exogenous factors like diseases, management and stress are the causes of fluctuations in blood biochemistry[2]. In the present study, glucose level was examined in both riverine and cultured populations of *Channa marulis*. The glucose level was significantly ($p \leq 0.01$) higher in riverine group than that of cultured group. These results are supported by Pradhan *et al.*[20] who observed the higher values of glucose in riverine group *Cirrhinus mrigala.* Svoboda *et al*.[6] also supported same results by studying the biochemical profile of *Cyprinus carpio.* However, these results are contrary to Gul *et al.*[21] who reported the higher values of glucose in cultured group in serum biochemical profile of wild and cultured *Channa argus*. This series of experimental work indicated significantly higher ($p \leq 0.01$) albumin contents in cultured populations when compared with the riverine populations. These results are supported by Gul *et al.*[21] and De Pedro *et al.*[11], respectively. Significantly higher ($p \leq 0.01$) values of cholesterol were recorded in cultured group as compared to the riverine group. Coz-Rakovac *et al.*[22] also determined the lower levels of cholesterol in wild population than the cultured population. Gharaei *et al.*[23] also reported the same results by examining the serum biochemistry of *Huso huso.* Cholesterol contents are greater in cultured group due to differences in diet and other environmental factors in both habitats[22].

Triglycerides are important to explore the inhabiting status of fish and also played a vital role to provide energy to the body[14]. Our study, the triglyceride contents were non-significantly higher ($p \geq 0.05$) in wild population when compared with the cultured populations of *C. marulius*. These results showed similarity with the work done by Percin & Konyalioglu[24] they studied the serum biochemical profile and examined the higher values of triglycerides in the wild group. But these results are in contrary to the work done by Gul *et al.*[21] as there were higher values of triglycerides in the cultured population than the wild group. Here, our study indicated the significantly higher ($p \leq 0.01$) values of serum aspartate transaminase in the wild population when compared with the cultured population of species under examination. These findings are supported by Asadi *et al.*[11] who studied the same results by examining the serum biochemical profile of *Acipenser persicus* and the same results were also recorded by Owolabi.[25] during the examination of serum biochemical profile of *Synodontis membranacea*. In the present series of experiment, highly significant differences ($p \leq 0.01$) were recorded for alanine transaminase in riverine and hatchery populations of *Channa marulius.* These findings are according to the Gul *et al.*[21] who recorded the significant differences for wild and cultured *Channa argus*. Asadi *et al*.[11] also reported the same results in serum biochemical profile of *Acipenser persicus.*

Our study indicated significantly higher ($p \leq 0.01$) serum alkaline phosphatase contents in the cultured population as compared with the wild populations of *C. marulius*. These results are in accordance with Bayir *et al.*[26] which reported the higher values of alkaline phosphatase in the cultured population. The serum sodium contents were significantly higher ($p \leq 0.01$) in the wild population. These results are similar to Hollmén *et al.*[27] who studied the serum biochemical profile of *Somateria mollissima.* Faggio *et al.*[28] reported the same results with lower values of serum sodium levels in cultured *Mugil cephalus* whereas the serum potassium contents were higher with significant difference ($p \leq 0.05$) in riverine populations when compared with hatchery population of *Channa marulius.* These results are in accordance with Harms *et al.*[29] who studied the serum biochemical

indices of wild, *Sphyrna tiburo* and observed the higher values of potassium in wild population. A series of experimental work by Shahsavani *et al.*[30] also reported the same results by studying the serum biochemical parameters in starry sturgeon, *Acipenser stellatus*. Cataldi *et al.*[31] also supported these results by studying the serum biochemical profile of Adriatic sturgeon, *Acipenser naccarii.*

In this study, the serum calcium contents were found higher with non-significant differences ($p \geq 0.05$) in the cultured population when compared with the riverine group of *C. marulius*. These results were supported by Asadi *et al.*[11] with higher values of calcium in the cultured population as they determined the reference values of serum biochemical profile of *Huso huso.* Marco *et al*.[32] also reported the higher values of calcium contents in cultured fish population when they determined the effects of sampling on blood biochemistry of Adriatic sturgeon, *Acipenser naccarii.* In the present series of experiment, serum chlorine contents were higher in the wild population with significant difference at ($p \leq 0.05$) when compared with hatchery population of *Channa marulius*. These findings are supported by Bastami *et al.*[33] who observed the serum biochemical profile of common carp, *Cyprinus carpio* with similarly high values of chlorine in wild populations. Harms *et al*.[29] also reported the same results when studied the serum biochemical indices of wild Bonnet head Sharks, *Sphyrna tiburo.* In this study, the serum bilirubin contents were higher with significant difference ($p \leq 0.01$) in the wild population when compared with the hatchery populations this is similar to the findings of[21] in who reported significant difference in the values of bilirubin between the wild and cultured northern snakehead, *Channa argus*. While, serum urea level was significantly higher ($p \leq 0.01$) in wild, when compared with the farm raised populations of *Channa marulius.* These results are in contrary to Gul *et al.*[21] who reported the significantly higher levels of urea in cultured group.

The serum globulin was slightly higher with significant differences ($p \leq 0.01$) in cultured population as compared to the riverine population of *C. marulius.* These results are in accordance with the findings of Gul *et al.*[21] who reported a slight difference in the level of globulin between both wild and cultured *Channa argus*. The biochemical parameters including glucose, triglycerides, serum aspartate transaminase, sodium, potassium, chlorine, bilirubin and urea were determined and it was noticed that the levels of these parameters were higher in riverine population when compared with cultured populations of *Channa marulius.* On the other hand, the values of serum biochemical parameters including total protein, globulin, calcium, cholesterol, albumin, alanine transaminase and alkaline phosphatase were higher in cultured population than the wild population of *Channa marulius.* The study of the biochemical profile has a significant role in the determination of fish health and physiological status in both wild and cultured environments[10] whereas the difference between the serum biochemical profile is due to the difference in habitat of fish species[31].

Conclusion

In summary, variations in the serum biochemical profile were observed among the wild and cultured populations of *Channa marulius.* This difference is due to different habitats and different environmental factors. Thus, it is suggested that the alterations in serum biochemical parameters have very important role in the health status of fish as well as very essential for the better management of fish health conditions. However, further studies are required to verify this conclusion.

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Conflict of interest

The Authors declared no conflict of interest.

Author Contributions

Manuscript was prepared by SSUHK, MAK, and SBHS and other co-authors helped in editing and manuscript revision.

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