International Journal of Fisheries and Aquatic Research
ISSN: 2456-7248; Impact Factor: RJIF 5.44
Received: 09-08-2020; Accepted: 25-08-2020; Published: 10-09-2020
www.fishjournals.com
Volume 5; Issue 3; 2020; Page No. 66-69

Comparative serum metabolite and ions analysis on two groups of Clarias batrachus

Vishal Rajput[1*], Garvita[2], Sakshi[3], Richa Gaur[4]
[1-3] Department of Biochemistry and Biotechnology, SBS University, India
[4] Department of Pharmaceutical Sciences, SBS University, India

Abstract
Present study was focused to investigate the major metabolic changes occur, due to a regular programmed workout regime in the fresh water fish by analyzing blood serum metabolites of enzymes, lipids and proteins in *Clarias batrachus.* On the basis of results it may be stated that natural burst pattern or regular exercise regime caused minor decrease in HDL cholesterol whereas hepatic and bile activities were found normal as AST and ALT did not show reductive tendency. Ion balance showed almost no alteration except Na that may be due to age related changes during the training routine. As a concluding remark, it may be assumed that regular but non-intense exercise will cause moderate changes in the basal metabolism of fresh water fishes.

Keywords: metabolites, fresh water fish, carp, metabolism

1. Introduction

In fresh water ecosystem thousands of fish species found and they swim through undulations of the body. Locomotion of fishes is supported by three morphologically myotomal muscle fibers. Approximately 10% of total muscles are found as red oxidative muscles and these muscles are responsible for constant cruise swimming in fresh water fishes, on other hand almost 50% of body muscles are white muscles fibers, white muscles fibers maintain lower myoglobin content and supports short movements as well as sudden movements. The metabolic process sustained demanding activities included the alteration of basal metabolism, which is found strictly bound to muscle fiber in the form of oxidation of protein and fat found in skeletal muscle increases remarkably, while in the case of carbohydrates reduction in content has been recorded [10]. Elevated breakdown of lipids and proteins results in the higher contents of their metabolites if aquatic organisms (mainly fresh water fishes), further lactate and blood glucose contents have been reported elevated due to the higher white muscles content [8]. In several fresh water species hepatic glycogen reduced while glucose and lactate concentrations have been recorded in elevated form. Previous investigations revealed that in-situ glycogenic elimination of lactate is majorly reported in fresh water fishes [4], similar process is also recorded in slow swimming fishes, especially common carps. Apart from this, normally determined, anaerobic fermentative pathway in muscle utilizes ATP restored from the Phosphagenic hydrolysis [12]. As reported earlier, the extended commencement of exercise results in the complete oxidation of carbohydrates, and later to that of fats and amino acids to fuel ATP replenishment within the muscle cell. At last, aerobic form of metabolism takes lead which indicates a comprehensive switchover to metabolism of lipid [5]. The metabolic process of fishes is different from warm-blooded vertebrates, as recorded by [2] that NEFA oxidation does not activate through exercise in marine fish [1] and this phenomenon takes place due to the absence of glycerokinase process, however it is not recorded in fresh water fishes [6]. In rainbow [7] trout NEFA is utilized at lower level as rate of lipolysis is not affected by exercise of muscles. Recent investigations reported that trout do not utilize triacylglycerol to exceed the stored plasma capacity to provide energy for muscles used in locomotion even after 72hrs of muscles exercise. In fish metabolic activities regular long term training need proper investigation, however, such type of metabolic adaptation is remarkably different from the effect short duration movement.

Present study was focused to, investigate the major metabolic changes occur, due to a regular programmed workout regime in the fresh water fish by analyzing blood serum metabolites of enzymes, lipids and proteins in *Clarias batrachus.*

2. Material and Methods

Samples of *Clarias batrachus* (mean weight- 68.78± 16.84 gm) were arranged with the help of local support. Samples were distributed in two groups of 20 each (20 Control and 20 Trained). All samples were kept in a cemented water tank (dimensions 40 X 60 feet, located in Distt. Saharanpur, U.P. India) for acclimatization period of 2 weeks. Exercise tank was divided in two unequal parts with the help of bamboos where, in small portion (dimensions 5 X 5) control group was kept, on other hand in bigger portion (30 X 50) trained group of samples were kept. During the acclimatization and experimentation phase fishes were fed with wheat flour balls. For control the influx water velocity, a valve system was available to releases water in tank at the time of exercise. Group of trained samples were exercised every day for 45 minutes by opening the water valves, whereas control group of samples remained in same tank confined in respective enclosure.

Blood samples were collected (sample I) from caudal region with syringe at the beginning of experimentation; later samples (sample II, III and IV) were collected at every 8th day. Collected blood samples were kept in tubes and placed in ice box at experimentation site. Samples were centrifuged at 12000 (rpm/min) and collected serum was stored at -70^{0}C. Chemical analysis of samples was done with the help of automated analyzer (ERBA-Cam 7). Statistical analysis was performed with the help of SPSS statistical software package (ver. 15.0).

3. Results & Discussions

Minerals: Highest Na concentration was recorded (236.8 mmol/L) at 24^{th} day of sampling in both groups. Serum Ca concentration did not show remarkable changes and range between 3.52-3.98 mmol/L in control group and 3.36-3.53 mmol/L in trained group. Similar pattern was recorded in Mg showed range of 1.69-2.03 mmol/L and 1.63-2.33 mmol/L, in control and trained group respectively (Table 1).

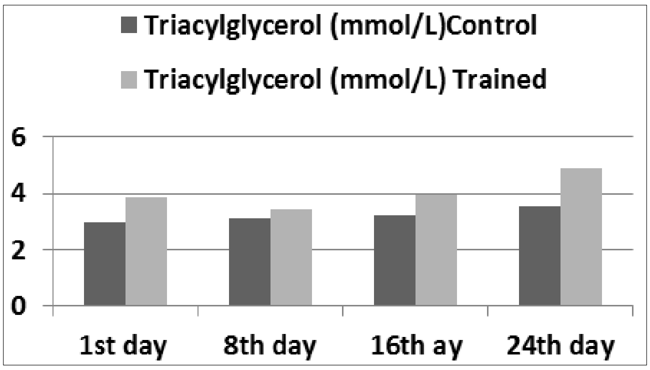
Enzyme profile: Serum concentration of alkaline phosphates ranges between 162.7-212.3 (IU/L) in control and trained group. Concentration range of ALT was reported as 2.17-3.93 (IU/L) in control and 2.65-5.43 (IU/L) in trained group. AST content showed the variation of 103.21-151.2 (IU/L) and 116.5-194.4(IU/L) respectively. Gamma-GT serum content ranged between 1.11-3.51 (IU/L) in control and 2.31-3.31(IU/L) in trained group. LDH serum concentration recorded noticeable variation as range between 287.8-714.3(IU/L) in control and 359.7-883.1 (IU/L) in trained group (Table 1).

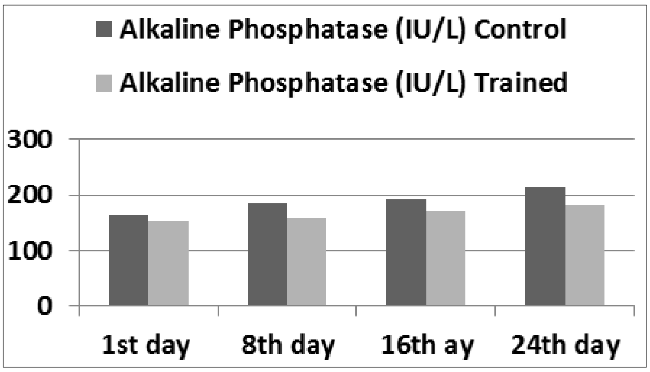
Nitrogenous profile: The range of albumin content was recorded as 23.4-30.7 (g/L) and 25.3-35.4 (g/L), in control and trained group, respectively. Creatinine range was reported as 0.79 (µmol/L) at 24^{th} day of routine and highest 2.19(µmol/L) at 1^{st} day of routine. Total protein (g/L) range was recorded between 31.8-46.4 (g/L) in control group and 33.2-42.7 (g/L) in trained group (Table 1).

Table 1: Blood serum analysis of *Clarias batrachus*

Minerals	**Na (mmol/L)**		**Ca (mmol/L)**		**Mg (mmol/L)**	
	Control	Trained	Control	Trained	Control	Trained
1st day	162.5±21.45	168.8±42.85	3.52±1.87	3.36±2.06	1.69±0.84	1.81±0.46
8^{th} day	173.8±26.48	179.3±39.18	3.72±2.04	3.49±2.15	1.70±0.95	1.77±0.58
16^{th} ay	169.2±31.83	202.4±28.36	3.98±1.56	3.41±2.28	1.82±0.63	1.63±0.84
24^{th} day	177.8±29.46	236.8±33.12	3.79±1.23	3.53±2.03	2.03±0.75	2.33±0.74
Enzymes	Alkaline Phosphatase (IU/L)		ALT (IU/L)		AST (IU/L)	
1st day	162.7±47.5	153.6±39.5	2.36±1.82	3.37±2.67	103.7±21.33	116.5±15.32
8^{th} day	183.5±38.5	159.1±55.8	3.71±1.37	2.65±1.23	151.2±28.56	138.1±28.97
16^{th} ay	192.8±53.9	172.2±49.3	3.93±2.65	2.88±2.67	100.5±16.45	144.2±32.81
24^{th} day	212.3±46.8	182.4±22.5	2.17±2.62	5.43±2.89	117.9±21.87	194.4±20.38
	Gamma-GT(IU/L)		LDH (IU/L)			
1st day	2.19±0.87	2.31±0.36	287.8±88.45	562.1±178.34		
8^{th} day	1.21±0.37	3.31±0.78	361.4±112.5	746.6±213.65		
16^{th} ay	3.51±0.68	2.88±0.49	294.6±94.76	359.7±88.45		
24^{th} day	1.11±0.83	2.33±0.88	714.3±198.47	883.1±256.74		
Nitrogenous metabolites	Albumin (g/L)		Creatinine (µmol/L)		Total Protein (g/L)	
1st day	23.8±11.9	27.1±9.3	2.19±1.41	1.51±0.78	31.8±5.78	33.3±6.79
8^{th} day	24.5±14.6	25.3±16.3	1.93±1.11	1.42±1.28	32.6±4.36	40.5±8.38
16^{th} ay	30.7±9.34	28.9±10.3	2.04±1.54	2.55±1.54	46.4±7.39	33.2±5.28
24^{th} day	23.4±17.3	35.4±17.3	0.79±0.54	0.33±0.38	40.1±3.48	42.7±11.34
Lipid metabolites	Triacylglycerol (mmol/L)		LDL Cholesterol (mmol/L)		HDL cholesterol (mmol/L)	
1st day	2.96±0.34	3.83±0.36	3.62±1.28	3.81±2.76	2.43±0.58	2.33±0.49
8^{th} day	3.11±0.29	3.41±0.44	3.84±1.86	4.11±2.84	2.63±0.84	2.88±1.23
16^{th} ay	3.24±0.17	3.96±0.26	4.08±1.72	4.23±1.54	2.85±0.73	2.68±0.58
24^{th} day	3.53±0.21	4.92±0.39	4.21±2.35	4.39±2.48	3.44±0.77	3.31±1.04

Lipid Profile: Triacylglycerol range was reported as 2.96-3.53 (mmol/L) and 3.41-4.92 (mmol/L) in control and trained group. Serum HDL cholesterol (mmol/L) content was reported 2.43-3.44 (mmol/L) in control and 2.33-3.31 (mmol/L) in trained group. Whereas, serum cholesterol content was found between 3.62-4.21 (mmol/L) in control and in trained group ranged 3.81-4.39 (mmol/L) (Table 1).





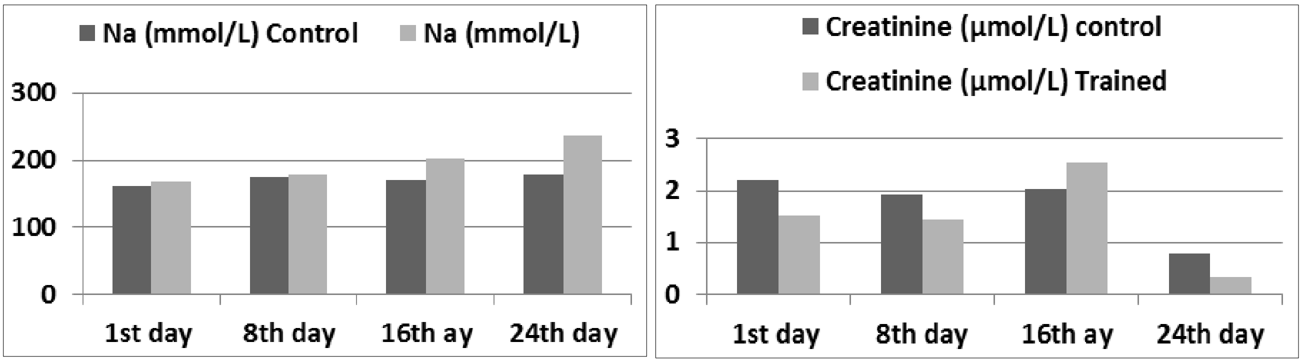


Fig 1: Graphical representation of serum parameters in fresh water fish

Exercise may result remarkable effects on metabolic activities of organism. Normally, noticeable effects of exercise may be dehydration, sudden fall in concentration of oxidizable fuel and increased accumulation of excretory products in blood.
During nitrogenous metabolites analysis, albumin concentration was recorded higher in trained group. As albumin is considered as major serum fraction as well it also works as carrier substance for (NEFA) non-esterified fatty acid [9] therefore, higher concentration of albumin indicates the storage of lipid hydrolysis as well as serum transport. Elevated albumin content may have been considered as an indicator of increased fluid level in plasma [3] to maintain osmotic pressure. In the case of creatinine and total protein, no remarkable effect was noted in response towards exercise. Fish kidney filters most of the nitrogenous waste in the form of creatinine [2], as creatinine content was remained unaffected throughout the study indicates that protein metabolism remained unaffected from training.
In major carps, intramuscular lipids are majorly responsible to provide energy for intense although short thrusts, whereas intercellular fuels (glycogen) are minor source of energy [11]. Stable tri-acyl-glycerol is highly supportive to furnish the need of swimming at rapid as well as slow pace in fresh water fishes. Similar findings were recorded in the study, as tri-acyl-glycerol in serum was reported higher in trained group, when compared with control. On other hand, total and HDL cholesterol were compared and both contents did not show similar pattern, in-fact unremarkable elevation was reported after exercise routine [8]. In present study, HDL cholesterol maintained almost equal concentration except 24th day of sampling.
AST and ALT are considered as hepatic enzymes, showed elevated serum contents during exercise routine. This increasing tendency may be considered as evidence of hepato-cellular loss [10]. As contrary tendency of AST have been recorded by [4] in fresh water carps, where fishes showed reductive tendency of serum AST due to the stress caused by transportation. In this investigation AST did not show the above mentioned response against exercise. LDH in considered as glycogenic substrate in the tissues of fresh water fishes [9]. In this case, LDH showed drastic fluctuations during the study. During the study, researchers observed that, fishes remained restless and kept performing burst-type of swimming for longer duration, event after the exercise routine. This might be considered the reason of significant elevation of LDH. Enzyme alkaline phosphates is considered as the marker of the proper function of live and bile activities [5]. In present investigation, no remarkable fluctuation was reported in control as well as trained group. Hepatic and bile activities remained unaffected of exercise routine as supported by gamma-GT concentration also did not show any significant increase [1].
Fluctuations of serum [12] ions concentrations due to regular swimming in the fresh water fishes. Any fluctuations in the serum ions concentration may occurred due to water acidity or hardness fluctuation or any inflammation. Na concentration showed noticeable change during exercise regime in trained group. Similar tendency of Na was reported [6] may be due to the relation of Na with the dry matter of the fresh water fish. Previously established investigations mentioned about the absence of any relation of serum Na, P, Cl and Ca concentration with stress and present study also reported similar pattern except Na molecule. On the basis of these ion findings, we can state that most of the ions did not show remarkable fluctuation may be because of gradual training did not cause any damage to sarcolemma.
Majority of results concluded by this investigation were supported by previous studies, mean while some parameters were found in contrast.

3. Conclusions
To summarize the whole study, it may be stated that natural burst pattern or regular exercise regime caused minor decrease in HDL cholesterol whereas hepatic and bile activities were found normal as AST and ALT did not show reductive tendency. Ion balance showed almost no alteration except Na that may be due to age related changes during the training routine. As a concluding remark, it may be assumed that regular but non-intense exercise will cause moderate changes in the basal metabolism of fresh water fishes.

4. Acknowledgment
The fish ponds used in this study were provided by Sahara Social Society, Dehradun. Chemicals were provided by SBS University, India. Sample analysis and instrumentation were provided by N H Pathology and Diagnostics, Haldwani, Uttarakhand, India.

5. Conflict Of Interest
The authors declare no conflicts of interest.

6. References
1. Bernal D, Donley JM, McGillivray DG, Aalbers SA, Syme DA. Function of the medial red muscle during sustained swimming in common thresher sharks: contrast and convergence with thunniform swimmers. Comp Biochem Physiol A Mol Integr Physiol, 2010; 155:454-463.
2. Davison W. The effects of exercise training on teleost

fish, a review of recent literature. Comparative Biochemistry and Physiology, 1997; 117:67-75.
3. Pagnotta A, Milligan CL. The role of blood glucose in the restoration of muscle glycogen during recovery from exhaustive exercise in rainbow trout (Oncorhynchus mykiss) and winter flounder (Pseudopleuronectes americanus). J Exp Biol, 1991; 161:489-508.
4. Bernard SF, Reidy SP, Zwingelstein G, Weber JM. Glycerol and fatty acid kinetics in rainbow trout: effects of endurance swimming. J Exp Biology, 1999; 202:279-288.
5. Hinterleitner S, Huber M, Lackner R, Wieser W. Systemic and Enzymatic Responses to Endurance Training in Two Cyprinid Species with Different Life Styles (Teleostei: Cyprinidae). Canadian Journal of Fisheries and Aquatic Sciences, 2011; 49:110-115.
6. Dobsiková R, Svobodova Z, Blahova J, Modra H, Velisek J. The effect of transport on biochemical and haematological indices of common carp (*Cyprinus carpio L*). Czech Journal of Animal Science, 2009; 54:510-518.
7. Magnoni L, Vaillancourt E, Weber JM. High resting triacylglycerol turnover of rainbow trout exceeds the energy requirements of endurance swimming. Animal J. Physiol Regul Integr Comp Physiology, 2008; 295:309-15.
8. Magnoni L, Weber JM. Endurance swimming activates trout lipoprotein lipase: serum lipids as a fuel for muscle. Journal of Experimental Biology, 2007; 210:4016-4023.
9. Moyes CD, Schulte PM, Hochachka PW. Recovery metabolism of trout white muscle: role of mitochondria. Animal J. Physiology, 1992; 262:295-304.
10. Richards JG, Mercado AJ, Clayton CA Heigenhauser GJF, Wood CM. Substrate utilization during graded aerobic exercise in rainbow trout. J Exp Biology, 2002; 205:2067-2077.
11. Kipreos G, Tripolitsioti A, Stergioulas A. The effects of anaerobic training in serum lipids and arachidonic acid metabolites. Biology of Exercise. 2010; 6(2):5-12.
12. Kerksick C, Willoughby D. The Antioxidant Role of Glutathione and N-Acetyl- Cysteine Supplements and Exercise-Induced Oxidative Stress. J International Soc. Sports Nutrition, 2005; 2:38-44.