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Effect Analysis of Regular Exercise Routine on *Catla Catla* with the Help of Metabolites Level in Serum

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Abstract
The present study was focused on comparing the effect of non-intense exercise routines with normal swimming routine among fishes. To find out the comparison, serum metabolites of minerals, enzymes, nitrogenous substance, and lipids were selected between two groups of fresh water fish as trained and control group. Due to the regular non-intense exercise routine, some alterations in serum metabolites were reported. High-Density Lipoprotein (HDL) showed notable change due to the routine, whereas minor fluctuations were recorded in Low-Density Lipoprotein (HDL) content due to the absence of strength exercise routine. Hepatic and bile activities were reported normal as a minor fluctuation of AST and ALT recorded. A comparative study of lipid metabolite concentration showed noticeable changes in triacylglycerols. In mineral metabolites absence of remarkable fluctuation recorded may be due to age-related changes during the training routine. The comparative study may conclude that non-strength regular workout routine in fresh water fishes may moderate some moderate metabolic alterations.
Keywords: Metabolites, Minerals, Lipid, Serum

Introduction

Fishes inhabitants of fresh water undulate their bodies to perform complex body movements during swimming. Three different myotomal muscle fibers support the movement of fishes morphologically. Fishes perform cruise swimming with red oxidative muscles, where as a major proportion of white muscle fibers control short movements and burst movements. The metabolic process sustained demanding activities included altering basal metabolism, which is found strictly bound to a muscle fiber in the form of oxidation of protein and fat found in skeletal muscle increases remarkably. Simultaneously, in carbohydrates, a reduction in content has been recorded (Richard et al., 2002). 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 (Magnoni and Weber, 2007). In several fresh water species, hepatic glycogen was reduced while glucose and lactate concentrations have been recorded in the elevated form. Previous investigations revealed that in-situ glycogenic elimination of lactate is majorly reported in fresh water fishes (Bernard et al., 1999. Rajput & Shah 2020). A similar process is also recorded in slow swimming fishes, especially common carps. Normally determined, the anaerobic fermentative pathway in muscle utilizes ATP restored from the Phosphagenic hydrolysis Kerksick and Willoughby (2005). 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. Finally, an aerobic form of metabolism takes the lead, which indicates a comprehensive switchover to the metabolism of lipid Hinterleitner et al., (2011). The metabolic process of fishes is different from warm-blooded vertebrates, as recorded by Davison (1997) that NEFA oxidation does not activate through an exercise in marine fish (Bernal et al., 2010), and this phenomenon takes place due to the absence of glycerol kinase process. However, it is not recorded in fresh water fishes Dobsikova et al., (2009). Magoni et al. (2008) reported in rainbow trout that NEFA is utilized at a lower level as the rate of lipolysis is not affected by the 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 72 hrs of muscle exercise. In fish metabolic activities, regular long term training needs proper investigation. However, such type of metabolic adaptation is remarkably different from the effect of short duration movement.

This study was designed to find out the possible effect of regular planned exercise on changes that occur in blood serum. To find out any changes, blood serum metabolites of enzymes, lipids, and proteins in *Catla Catla* were analyzed.

Material and Methods

Samples of *Catla Catla* (mean weight - 56.71± 14.26 gm) were arranged with 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 an acclimatization period of 2 weeks. The exercise tank was divided into two unequal parts with the help of bamboos where in small portion (dimensions 5 X 5 feet) control group was kept, on the other hand in bigger portion (30 X 50 feet) trained group of samples was kept. During the acclimatization and experimentation phase, fishes were fed with wheat flour balls. To control the influx water velocity, a valve system was available to releases water in a tank at the time of exercise. A Group of trained samples was exercised every day for 45 minutes by opening the water valves. In contrast, the control group of samples remained in the same tank confined in the respective enclosure.

Figure 1: Fish Holding Pond

Blood samples were collected (sample I) from a caudal region with a syringe at the beginning of experimentation; later samples (sample II, III, and IV) were collected every 8th day. Collected blood samples were kept in tubes and placed in an ice box at the experimentation site. Samples were centrifuged at 12000 (rpm/min), and collected serum was stored at -700C. Chemical analysis of samples was done with the help of an automated analyzer (ERBA-Cam 7). Statistical analysis was performed with the help of the SPSS statistical software package (ver. 15.0).

Results

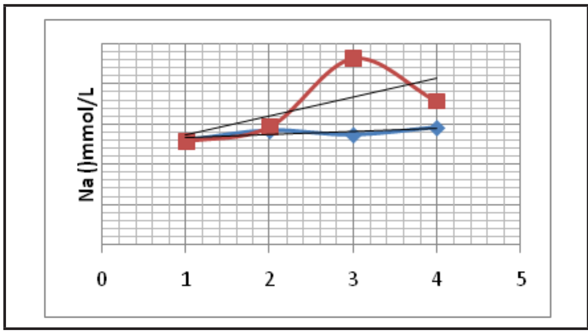
The concentration of serum Ca ranged between 2.14-2.78 mmol/L in the control group and 2.69-3.88 mmol/L in the trained group. The Highest Na concentration was recorded (145.7 mmol/L) in the control group and in the trained 178.3 mmol/L). Maximum serum Mg content recorded 1.91 mmol/L and 2.86 mmol/L in the control and trained group, respectively. (Table 1).

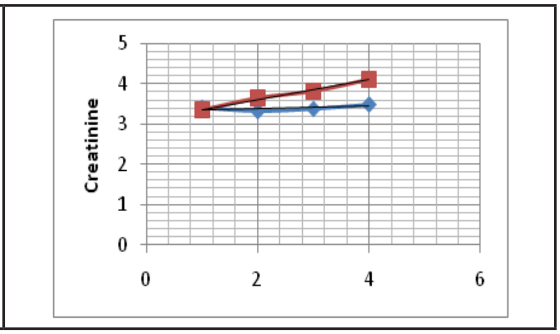
Table 1: Blood Serum Analysis of Catla Catla

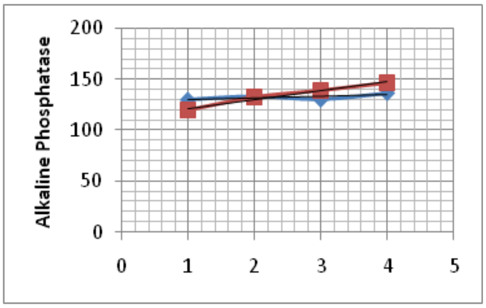
	Control Group	Exercised group	Control Group	Exercised group	Control Group	Exercised group
Minerals metabolites content						
	Na (mmol/L)		Ca (mmol/L)		Mg (mmol/L)	
1st day	130.4±15.65	128.6±22.35	2.54±0.67	2.69±0.54	1.41±0.79	1.62±0.86
7th day	142.1±16.28	146.5±19.15	2.78±0.79	2.89±0.17	1.83±0.62	1.88±0.56

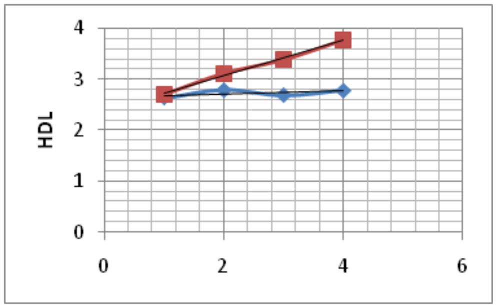
14th day	136.9±14.73	231.6±51.26	2.14±0.96	3.12±0.38	1.91±0.99	2.39±0.22
21th day	145.7±27.86	178.3±28.22	2.68±1.13	3.88±0.23	1.56±1.15	2.86±0.37
Enzyme metabolites content						
	Alkaline Phosphatase (IU/L)		ALT (IU/L)		AST (IU/L)	
1st day	129.8±26.4	119.9±28.4	2.31±1.46	2.39±1.82	93.8±27.33	101.7±18.12
7th day	133.2±33.3	132.7±35.8	2.56±1.54	2.78±1.47	102.5±29.56	119.4±15.87
14th day	130.1±29.6	139.2±37.3	2.48±1.77	2.84±1.81	123.3±44.45	134.6±12.81
21th day	136.4±42.4	146.5±28.5	2.36±1.31	3.13±1.51	95.2±39.69	128.8±13.28
	Gamma-GT(IU/L)		LDH (IU/L)			
1st day	1.49±0.18	1.32±0.76	214.5±85.55	233.6±79.94		
7th day	1.58±0.21	1.53±0.70	205.2±98.34	241.4±86.85		
14th day	1.53±0.38	1.68±0.69	219.4±63.76	263.2±81.85		
21th day	1.42±0.69	1.88±0.78	210.8±41.87	288.7±88.34		
Nitrogenous metabolites						
	Albumin (g/L)		Creatinine (µmol/L)		Total serum Protein (g/L)	
1st day	23.4±21.8	25.7±9.5	3.41±0.86	3.36±1.48	56.2±5.78	43.5±10.99
7th day	26.9±27.6	32.3±16.8	3.32±0.73	3.66±1.07	53.1±4.36	48.4±12.88
14th day	21.7±11.54	46.1±10.1	3.38±0.84	4.12±1.34	49.7±7.39	45.6±14.68
21th day	25.3±21.3	43.5±17.2	3.49±0.44	3.83±1.28	52.8±3.48	44.7±13.84
Lipid metabolites						
	Triacylglycerol (mmol/L)		LDL (mmol/L)		HDL (mmol/L)	
1st day	3.84±0.49	3.79±0.68	3.63±1.49	3.62±1.65	2.64±0.34	2.70±0.44
7th day	3.72±0.51	4.21±0.36	3.71±1.72	3.68±1.88	2.79±0.58	3.12±0.89
14th day	3.88±0.39	4.38±0.79	3.59±1.81	3.75±1.73	2.69±0.82	3.39±0.48
21th day	3.85±0.62	4.56±0.63	3.61±1.93	3.81±1.67	2.78±0.97	3.78±0.82

Alkaline phosphates in blood serum samples range between 129.8-136.4 (IU/L) and 119.9-146.5 (IU/L) in the control and trained group. LDH serum concentration recorded variation between 205.2-219.4(IU/L) in control and 233.6-288.7 (IU/L) in the trained group. The serum content range of ALT was reported between 2.31-2.56 (IU/L) in the control and 2.39-2.84 (IU/L) in the trained group. Gamma-GT serum content ranged between 1.42-1.58 (IU/L) in control and 1.32-1.88(IU/L) in the trained group. AST content showed a variation of 93.8-123.3 (IU/L) and 101.7-134.6(IU/L), respectively (Table 1).





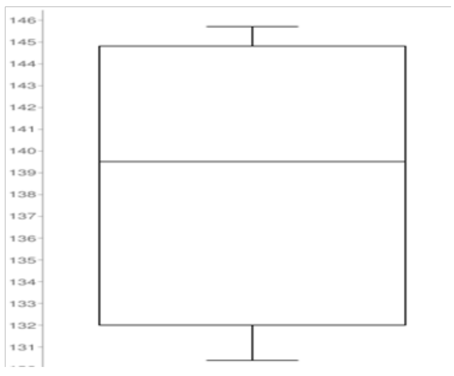




: Control : Exercise

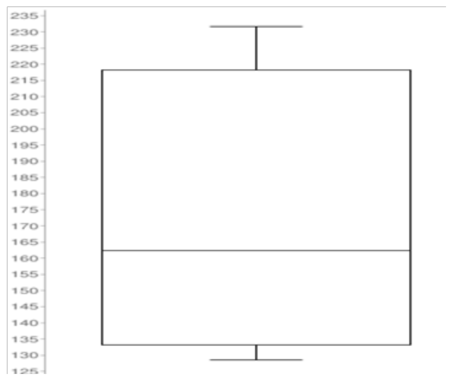
Figure 1: Graphical Representation of Major Serum Parameters in Fresh Water Fish

Creatinine range was reported as 3.32-3.49 (μmol/L) in control group and 3.66-4.12 (μmol/L) in trained group. Total serum protein range was recorded between 49.7-56.2 (g/L) in control group and 43.5-55.6 (g/L) in trained group. The range of albumin content was recorded as 21.7-26.9 (g/L) and 25.7-46.1 (g/L), in control and trained group, respectively (Table 1).



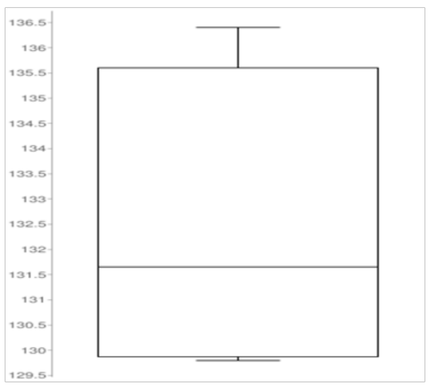
Median: 139.53
1st quartile:132.02
3rd quartile:144.8
Interquartile:12.77

Na (Control)



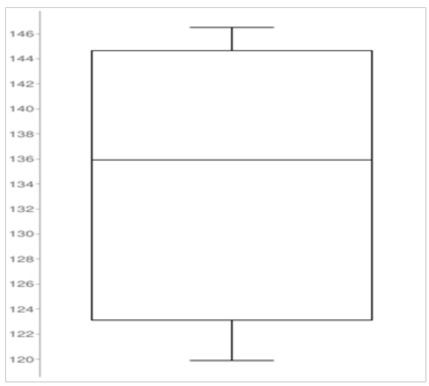
Median:162.42
1st quartile:133.0
3rd quartile:218.2
Interquartile:85.2

Na (Exercise)



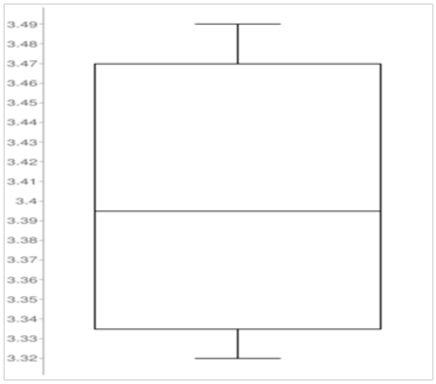
Median: 131.65
1st quartile:129.87
3rd quartile:135.6
Interquartile:5.72

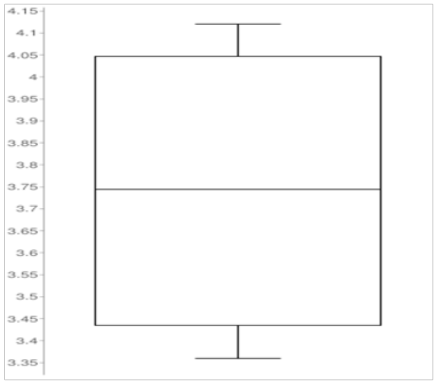
Alkaline Phosphatase (Control)



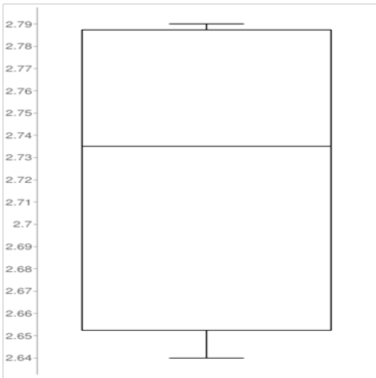
Median:135.95
1st quartile:123.1
3rd quartile:144.67
Interquartile:21.57

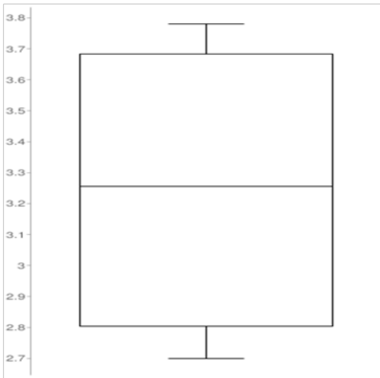
Alkaline Phosphatase (Exercise)





Median: 3.39 1st quartile:3.33	3rd quartile:3.47 Interquartile: 0.135	Median: 3.74 1st quartile:3.435	3rd quartile:4.04 Interquartile: 0.612
Creatinine (Control)		**Creatinine (Exercise)**	





Median: 2.73 1st quartile: 2.65	3rd quartile: 2.78 Interquartile:0.013	Median: 3.25 1st quartile: 2.80	3rd quartile: 3.68 Interquartile: 0.87
HDL (Control)		**HDL (Exercise)**	

Figure 1: Box Plot Analysis of Major Blood Serum Metabolites

Serum HDL cholesterol (mmol/L) content was reported 2.64-2.79 (mmol/L) in control and 2.70-3.78 (mmol/L) in trained group. Triacylglycerol range was reported as 3.72-3.88 (mmol/L) and 3.79-4.56 (mmol/L) in the control and trained group. Whereas LDL content was found between 3.61-3.71 (mmol/L) in the control and trained group ranged 3.62-3.81 (mmol/L) (Table 1).

Discussions

Previous investigations have produced remarkable evidence that a workout regime or routine exercise may result in noticeable changes in the metabolism of animals. According to Davison (1997), exercise routine may cause increased plasma content of nitrogenous substances, reduction of glucose and glycogen, and dehydration.

In the analysis of nitrogenous metabolites, the concentration of albumin was reported higher in the trained group. As per the previous study, albumin is believed a major serum fraction as well. It also works as a carrier substance for non-esterified fatty acids (Moyes et al., 1992); therefore, increased concentration of albumin indicates the storage of lipid hydrolysis and serum transport. Elevated albumin content may have been considered an indicator of increased fluid level in plasma Pagnota and Milligan (1991) to maintain osmotic pressure. In the case of creatinine and total protein, no remarkable effect was noted in response to exercise. Fish kidney filters, most of the nitrogenous waste in the form of creatinine Davison (1997), as creatinine content was remained unaffected throughout the study, indicates that protein metabolism remained unaffected from training.

In fresh water fishes, intramuscular lipids are majorly responsible for providing energy for intense although short thrusts, whereas intercellular fuels (glycogen) are a minor source of energy Kipreos et al., (2010). Increased tri-acyl-glycerol content is a symptom that the organism has been involved in routine workouts. Stable tri-acyl-glycerol is highly supportive of furnishing the need for swimming at a rapid and slow pace in fresh water fishes. Similar findings were recorded in the study, as tri-acyl-glycerol in serum was reported remarkably higher in the trained group when compared with control. On the other hand, HDL content is most sensitive towards the exercise routine, and HDL showed a remarkable elevation in the trained group Magnoni and Weber (2007). LDL shows the lower fluctuation in swimming or cardio workout, where as in the involvement of strength workout LDL content show high elevation. In the present study, LDL content showed minor elevation compared with HDL that may be due to the swimming type of workout.

In the enzymatic profile, AST and ALT are recorded as hepatic enzymes; in the present study, fluctuations were recorded during the exercise routine. This increasing tendency may be considered as evidence of hepato-cellular loss Richard et al., (2002) as the contrary tendency of AST has been recorded by Bernal et al. (2010) in fresh water carps, where fishes showed a 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 is considered as a glycogenic substrate in the tissues of fresh water fishes (Moyes et al., 1992). 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 a longer duration, event after the exercise routine. This might be considered the reason for the significant elevation of LDH. Enzyme alkaline phosphates are considered the marker of the proper function of live and bile activities (Hinterleitner et al., 2011). In the present investigation, no remarkable fluctuation was reported in the control and a trained group. Hepatic and bile activities remained unaffected of exercise routine as supported by gamma-GT concentration also did not show any significant increase (Bernard et al., 1999).

Earlier recorded by Kerksick and Willoughby (2005) about the fluctuations of serum ions concentrations due to regular swimming in the fresh water fishes. Any fluctuations in the serum ions concentration may occur due to water acidity or hardness fluctuation, or any inflammation. Na concentration showed noticeable change during the exercise regime in the trained group. Supporting Na's results was reported by Dobsikova et al. (2009) may be due to the relation of Na with the dry matter of the fresh water fish. Previously established investigations mentioned the relation of serum minerals concentration with stress, and this study showed supportive findings and reported a similar pattern. Based on these ion findings, we can state that most of the ions show minor fluctuations. Still, on the other hand, the amount of fluctuations is minor may be because gradual training did not affect the sarcolemma.

The majority of results concluded by this investigation were supported by previous studies.

Conclusion

The present study conducted on fresh water fish helps generate the conclusion that rapid splashing pattern of swimming during regular exercise or in natural habitat may result in the form of elevation in High-Density Lipoprotein (HDL). Aspartate Transaminase (AST) and Alanine Transaminase (ALT) showed minor positive fluctuations due to the response against exercise, but this minor alteration did not indicate tissue damage. Mineral profile recorded minor alteration may be due to age-related changes during the training routine. Based on this study, it may be stated that regular but non-intense exercise will cause moderate changes in the basal metabolism of fresh water fishes.

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Conflict of Interest

The authors declare no conflicts of interest.

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