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INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/11595
DOI URL: http://dx.doi.org/10.21474/IJAR01/11595

*RESEARCH ARTICLE*

# SERUM METABOLITES ANALYSIS ON ENDURANCE OF FRESH WATER FISH DUE TO REGULAR EXERCISE ROUTINE

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## *Manuscript Info*

***Manuscript History***
Received: 30 June 2020
Final Accepted: 31 July 2020
Published: August 2020

***Key words:-***
Serum, Blood, Metabolites, Carp

## *Abstract*

Blood serum metabolites of enzymes, lipids and proteins were chosen to compare between two groups of fresh water fish as control group and trained group. As a result of regular programmed workout regime some alterations in metabolites content were recorded. Comparative analysis of metabolite contents revealed minor fall down in HDL cholesterol whereas hepatic and bile activities were found normal as AST and ALT did not show reductive tendency. Ion metabolites did not show any major alteration except Na that may be due to age related changes during the training routine. On the basis of comparison, it may be concluded that regular but non-intense exercise will cause moderate changes in the basal metabolism of fresh water fishes.



## Introduction:-

Fishes inhabitants of fresh water undulate their body to perform complex body movements during swimming. Three different myotomal muscle fibers support the movement of fishes morphologically. Fishes perform cruise swimming with the help of 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 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 (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 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 (Bernard *et al.*, 1999; Rajput & Shah, 2020), 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 (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. At last, aerobic form of metabolism takes lead which indicates a comprehensive switchover to 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 exercise in marine fish (Bernal *et al.*, 2010) and this phenomenon takes place due to the absence of glycerokinase 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 lower level as rate of lipolysis is not affected by exercise of

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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 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.

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

## Material and Methods:-

Samples of *Labeo rohita* (mean weight- 56.71± 14.26 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 District 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 60 feet) 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 from caudal region at every $7^{th}$ 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).

## Results:-

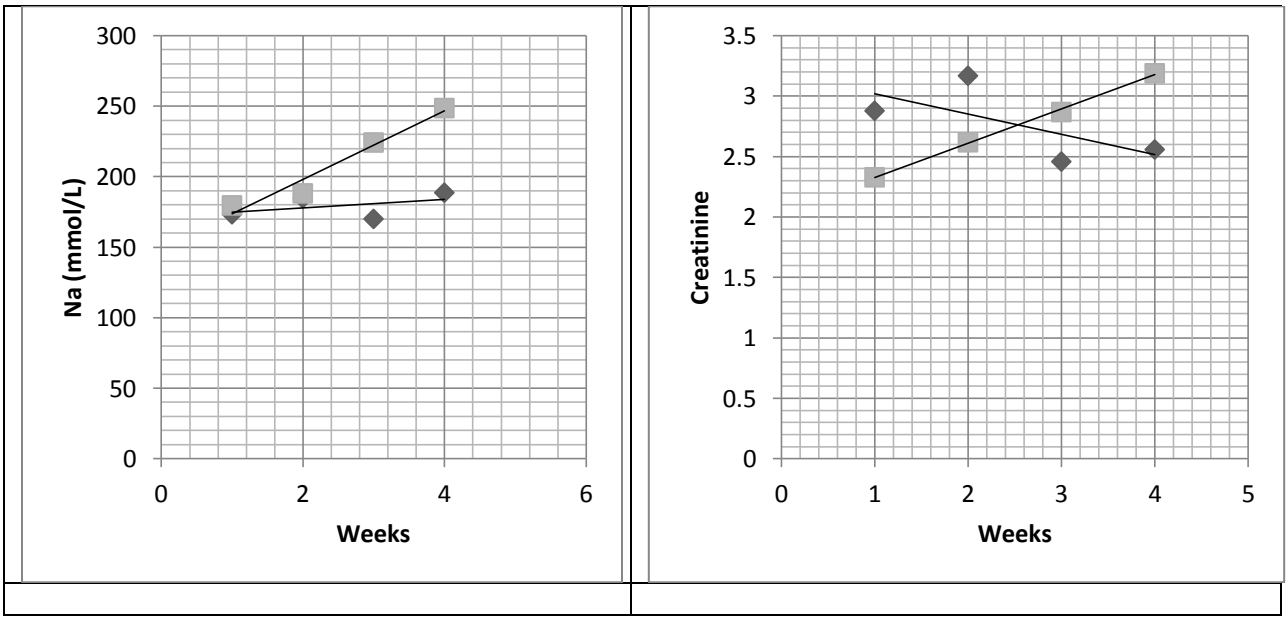
Serum Calcium concentration did not show remarkable changes and range between 4.32-4.82 mmol/L in control group and 4.26-4.63 mmol/L in trained group. Highest sodium concentration was recorded (248.8 mmol/L) in trained group and 188.8 mmol/L) in control at $21^{th}$ day. In serum Phosphate content ranges between 5.53-5.89 mmol/L in control and 5.38-6.83 mmol/L in trained group. Magnesium showed range of 2.39-3.13 mmol/L and 2.68-3.55 mmol/L, in control and trained group respectively (Table 1).

Alkaline phosphates in blood serum of samples range between 174.7-223.3 (IU/L) and 162.6-194.4 (IU/L) in control and trained group. LDH serum concentration recorded noticeable variation as range between 288.3-636.5(IU/L) in control and 255.1-788.6 (IU/L) in trained group. Serum content range of ALT was reported between 3.46-4.89 (IU/L) in control and 3.68-6.73 (IU/L) in trained group. Gamma-GT serum content ranged between 2.28-4.62 (IU/L) in control and 3.43-4.45(IU/L) in trained group. AST content showed the variation of 124.70-164.8 (IU/L) and 137.8-210.6(IU/L) respectively (Table 1).

**Table 1:-** Blood serum analysis of *Labeo rohita.*

| | **Control** | **After exercise** | **Control** | **After exercise** | **Control** | **After exercise** |
|---|---|---|---|---|---|---|
| **Minerals** | | | | | | |
| | **Na (mmol/L)** | | **Ca (mmol/L)** | | **P (mmol/L)** | |
| 1st day | 173.5±25.75 | 179.8±42.85 | 4.32±0.47 | 4.26±1.56 | 5.79±1.75 | 5.69±2.82 |
| $7^{th}$ day | 184.8±36.78 | 188.3±39.18 | 4.82±0.29 | 4.59±1.01 | 5.33±1.29 | 6.54±1.35 |
| $14^{th}$ ay | 170.2±44.53 | 224.4±28.36 | 4.68±0.86 | 4.49±1.58 | 5.89±1.74 | 6.83±2.14 |
| $21^{th}$ day | 188.8±37.76 | 248.8±33.12 | 4.69±0.43 | 4.63±1.43 | 5.52±1.52 | 5.38±1.68 |
| | **Mg (mmol/L)** | | | | | |
| 1st day | 2.39±0.56 | 2.91±0.56 | | | | |
| $7^{th}$ day | 2.80±0.78 | 2.86±0.76 | | | | |
| $14^{th}$ ay | 2.92±0.93 | 2.68±0.52 | | | | |
| $21^{th}$ day | 3.13±1.22 | 3.55±1.54 | | | | |
| **Enzymes** | | | | | | |
| | **Alkaline Phosphatase (IU/L)** | | **ALT (IU/L)** | | **AST (IU/L)** | |
| 1st day | 174.7±56.5 | 162.6±39.5 | 3.46±1.43 | 4.67±1.87 | 124.7±17.33 | 137.8±28.12 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 7th day | 196.5±43.5 | 167.1±55.8 | 4.81±1.58 | 3.82±1.43 | 164.8±21.56 | 151.1±35.67 |
| 14th ay | 198.8±49.9 | 186.2±49.3 | 4.89±1.83 | 3.68±1.83 | 129.5±14.45 | 166.2±42.61 |
| 21th day | 223.3±62.8 | 194.4±22.5 | 3.47±1.34 | 6.73±1.49 | 130.6±19.67 | 210.6±23.38 |
| | **Gamma-GT(IU/L)** | | **LDH (IU/L)** | | | |
| 1st day | 3.29±1.17 | 3.43±0.36 | 288.3±155.35 | 465.1±75.34 | | |
| 7th day | 2.28±1.19 | 4.45±0.78 | 373.4±78.2 | 648.6±56.65 | | |
| 14th ay | 4.61±1.28 | 3.63±0.49 | 358.6±83.46 | 255.1±32.45 | | |
| 21th day | 2.41±0.73 | 3.52±0.88 | 636.5±221.47 | 788.6±184.74 | | |
| **Nitrogenous metabolites** | | | | | | |
| | **Albumin (g/L)** | | **Creatinine (µmol/L)** | | **Total Protein (g/L)** | |
| 1st day | 33.6±21.8 | 38.1±9.5 | 2.88±0.86 | 2.33±1.48 | 42.3±5.78 | 45.3±10.99 |
| 7th day | 34.6±27.6 | 35.6±16.8 | 3.17±0.73 | 2.62±1.07 | 46.6±4.36 | 53.5±12.88 |
| 14th ay | 32.4±11.54 | 49.5±10.1 | 2.46±0.84 | 2.87±1.34 | 57.9±7.39 | 47.2±14.68 |
| 21th day | 35.4±21.3 | 43.7±17.2 | 2.56±0.44 | 3.19±1.28 | 51.1±3.48 | 54.2±13.84 |
| **Lipid metabolites** | | | | | | |
| | **Triacylglycerol (mmol/L)** | | **LDL Cholesterol (mmol/L)** | | **HDL cholesterol (mmol/L)** | |
| 1st day | 3.74±1.29 | 4.63±0.38 | 4.73±1.438 | 4.61±2.75 | 3.53±0.58 | 3.55±1.14 |
| 7th day | 4.21±1.11 | 4.27±0.46 | 4.74±1.56 | 5.71±2.87 | 3.73±0.84 | 3.78±1.49 |
| 14th ay | 4.74±1.31 | 4.98±0.29 | 5.12±1.53 | 5.53±1.51 | 3.35±0.73 | 3.95±1.48 |
| 21th day | 4.13±1.42 | 5.73±0.33 | 5.51±1.56 | 5.48±2.47 | 3.49±0.77 | 4.52±1.78 |



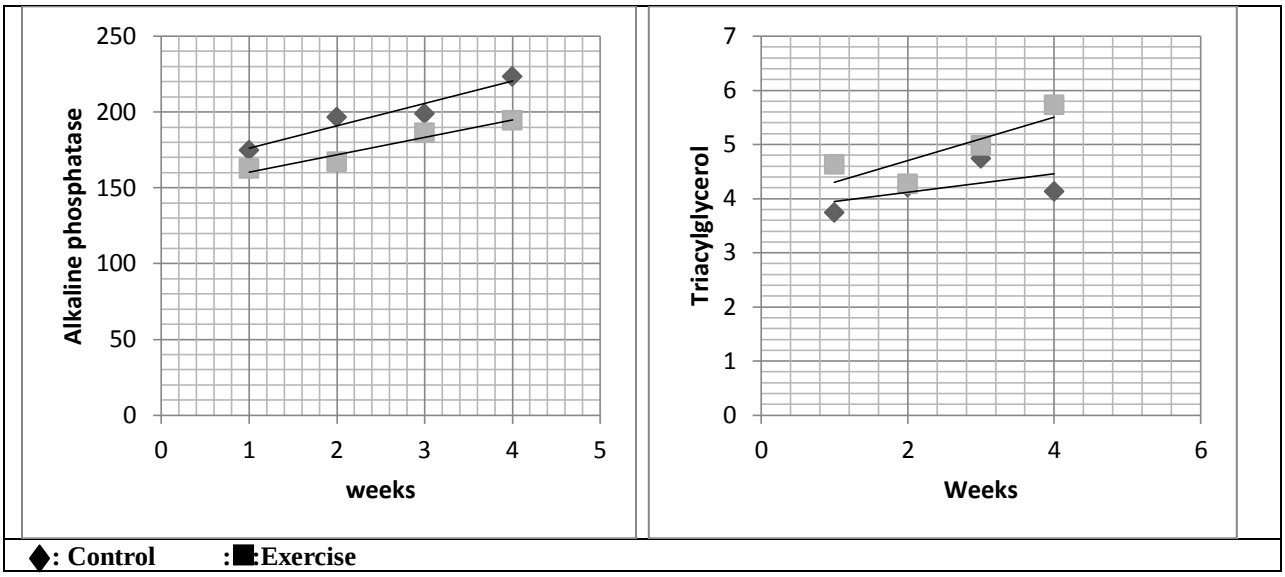



**Fig 1:-** Graphical representation of major serum parameters in fresh water fish.

Creatinine range was reported as 1.19 (µmol/L) at 24th day of routine and highest content was recorded 3.44(µmol/L) at 1st day of routine in control, whereas, in trained group it ranged between 1.41-3.76(µmol/L). Total protein range was recorded between 42.3-57.9 (g/L) in control group and 45.3-54.2 (g/L) in trained group. The range of albumin content was recorded as 32.4-35.4 (g/L) and 35.6-49.5 (g/L), in control and trained group, respectively (Table 1).

| | | | | | | | |
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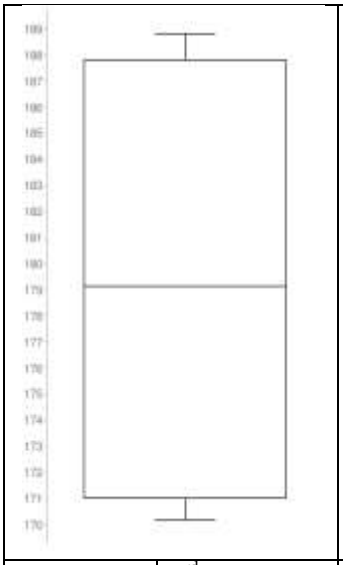
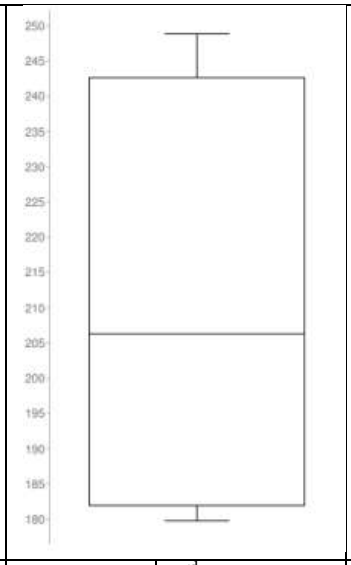
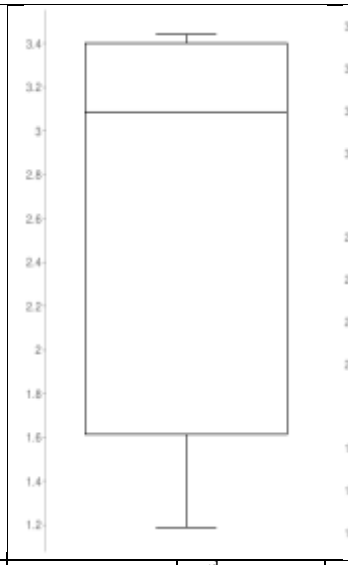
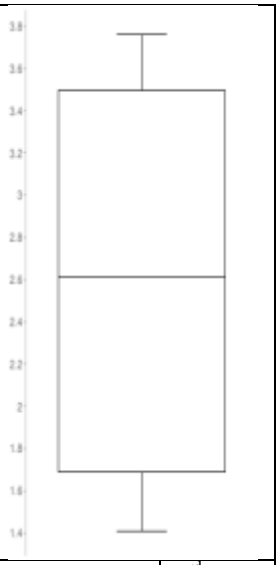
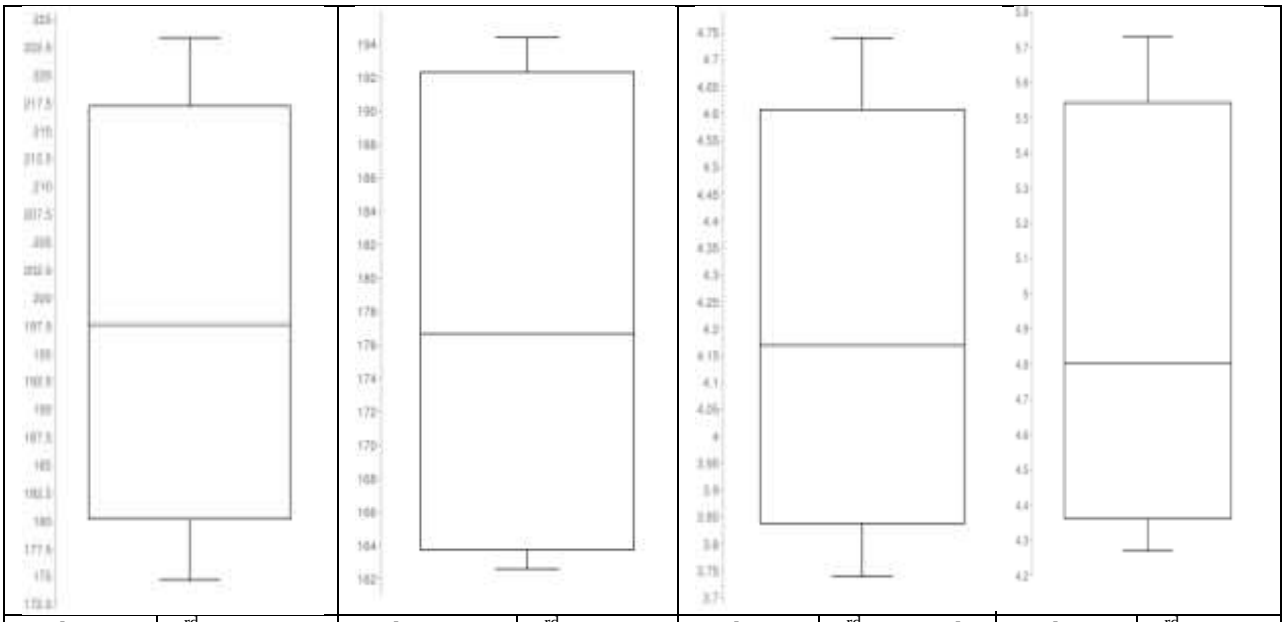
|  | |  | |  | |  | |
| Median: 179.15 1st quartile:171.02 | 3rd quartile:187.8 Interquartile: 16.77 | Median: 206.35 1st quartile:181.9 | 3rd quartile:242.7 Interquartile:60.77 | Median: 3.085 1st quartile:1.612 | 3rd quartile:3.40 Interquartile: 1.79 | Median: 2.615 1st quartile:1.687 | 3rd quartile:3.49 Interquartile: 1.81 |
| **Na (Control)** | | **Na (Exercise)** | | **Creatinine (Control)** | | **Creatinine (Exercise)** | |



| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Median: 197.65 1st quartile:180.15 | 3rd quartile:217.17 Interquartile: 37.02 | Median:176.65 1st quartile:163.72 | 3rd quartile:192.35 Interquartile :28.62 | Median: 4.17 1st quartile: 3.83 | 3rd quartile: 4.60 Interquartile: 0.77 | Median: 4.80 1st quartile: 4.36 | 3rd quartile: 5.54 Interquartile: 1.18 |
| **Alkaline Phosphatase (Control)** | | **Alkaline Phosphatase (Exercise)** | | **Triacylglycerol (Control)** | | **Triacylglycerol (Exercise)** | |

**Fig 2:-** Box plot analysis of major blood serum metabolites.

Serum HDL cholesterol (mmol/L) content was reported 3.35-4.74 (mmol/L) in control and 3.55-4.52 (mmol/L) in trained group. Triacylglycerol range was reported as 3.74-4.74 (mmol/L) and 4.27-5.73 (mmol/L) in control and trained group. Whereas, LDL cholesterol content was found between 4.73-5.51 (mmol/L) in control and in trained group ranged 4.61-5.79 (mmol/L) (Table 1) (Figure. 1& 2).

## Discussions:-

It is evident that workout or exercise may give remarkable changes on metabolic activities of all organisms. Generally, recorded effects of workout are dehydration, reduction of oxidizable fuel in cells and increased level of excretory products in plasma.

In the analysis of nitrogenous metabolites, the concentration of albumin was reported higher in trained group. As per the previous study albumin is believed as major serum fraction as well it also works as carrier substance for (NEFA) non-esterified fatty acid (Moyes *et al.*, 1992) therefore, increased 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 (Pagnota and Milligan, 1991) 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 (Davison, 1997), 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, where as intercellular fuels (glycogen) are minor source of energy (Kipreos *et al.*, 2010). 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 (Magnoni and Weber, 2007). 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 (Richard *et al.*, 2002). As contrary tendency of AST have been recorded by (Bernal *et al.*, 2010) 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 (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 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 (Hinterleitner *et al.*, 2011). 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 (Bernard *et al.*, 1999).

Kerksick and Willoughby (2005) mentioned about the fluctuations of serum ions concentrations due to regular swimming in the fresh water fishes. Any fluctuations in the serum ions concentration may be 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 (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 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.

## Conclusion:-

On the basis of this investigation, it may be concluded that sudden burst manner of swimming in natural habitat or routine regular training session may result in the form of minor decrease in HDL cholesterol, although bile and hepatic activities did not show remarkable alteration as ALT and AST parameters did not depict negative fluctuation. 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.

## Acknowledgments:-

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

**Conflict Of Interest**

The authors declare no conflicts of interest.

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