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ALTERATIONS IN HEMATOLOGICAL AND BIOMETRIC PARAMETERS OF *CLARIAS BATRACHUS* (LINN, 1758) FOLLOWING SHORT-TERM STARVATION

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Abstract: The present study is carried out to analyze the effect of short-term starvation on biological condition indices, hematological and biometric parameters of *Clarias batrachus*. Two groups of test fish, *Clarias batrachus* were subjected to different treatments: Group A (control) fishes were then fed three times a day until apparent satiety for 14 days; Group B (experimental) fishes were starved for 14 days. A significant rise in WBC (7.16%) with substantial reduction in hematocrit (11.97 %), haemoglobin (37.04 %), RBC (35.51%), glucose (19.15%), protein (23.65%) and cholesterol (43.12%) were assessed in the blood of starved fish for 14 days, while triglyceride level decreased (14.79%) in fish starved for 7 days, subsequently returning to values almost similar to control by the end of the trial *i.e.* 14 days. A significant decrease in liver protein (36.08%), total lipid (17.96%) and glycogen (75.0%) contents, while in muscles, the protein, total lipid, and glycogen contents also reduced up to 39.70%, 28.60% and 65.90% respectively, after 14 days in starved fish. These hematological and biometric parameters seem to have potential as a predictive tool for establishing the nutritional status or physiological condition during short-term starvation of *Clarias batrachus*, which may be useful to manage and monitor feeding practices during culture of this species.

Keywords: Biometric parameters, *Clarias batrachus,* Hematological parameters, Starvation.

INTRODUCTION

Several metabolic reactions and physiological aspects are directly or indirectly affected by feeding and starvation particularly longevity, survival and growth of fish. Fishes are cold blooded aquatic vertebrates having very unique physiology and digestive potential that absorb the nutrients from their food (Verma and Prakash, 2020). Fishes in wild are generally deprived of food, *i.e.*, they face the situation of less availability of food because of seasonal changes, alternation in temperature, migration, etc. Intentional feed restriction (starvation) influences the digestive process in them.

Long-term starvation distorts the morphological and anatomical intestinal structure and also alters the composition of the intestinal microbiota (for example *Bacteroides*, *Plesiomonas*, *Clostridium*, *Cetobacterium* and *Romboutsia* are the principal intestinal bacteria of *Nile tilapia*, *Oreochromis niloticus*), consequently affecting the intestinal digestion and assimilation with the inflammatory response (Sun *et al.*, 2020). Feeding, starvation and re-feeding regulate the proportion and diversity of these microbial communities besides the metabolic process and immune response of fish (Sakyi *et al.*, 2020).

The growth, gut microbiota and the non-specific immunity provide new perceptions for selection of the fish habitat and adapt to changes in their environment underpinning environmental (stress) adaptation (Xiaochun *et al*., 2020). The abiotic factors (dissolved oxygen, temperature, pH, salinity etc.,) and biotic factors (health state, age, sex of fish etc.) influence the physiological aspects that affect the digestion and subsequently absorption of nutrient formation due to chemical transformation during cultivation (Furné *et al*., 2005).

Starvation causes oxidative stress and metabolic changes that affect the growth and development of fish. Feed restriction or starvation triggers the synthesis of acute- phase protein that defend the fish from cell injury (cellular damage) and oxidative stress (Arjona *et al*., 2009).

The liver is the primary organ which detoxifies and degrades metabolic waste product and is continuously defied by exogenous and endogenous free radicals. Starvation mobilizes the nutrient and energy reserves stored in the liver and skeletal muscles and also increases the hepatic antioxidant enzymes (Rahmati *et al*., 2015). Starvation is very common in fish because of food restriction, weather conditions and specific stages of its reproductive cycles. Short term starvation or feed restriction may be a right approach for finding the solution to solve the problems and in maintaining the water quality; it might reduce detrimental effects of stress resultant to handling and mortality drop due to diseases (Davis and Gaylord, 2011). As the days of starvation increase, there is significant increase in glucose 6-phosphatase (G6Pase) and lactate dehydrogenase (LDH) while a substantial decrease in activities of enzyme glucose6-phosphatase dehydrogenase (G6PDH) and hexokinase. There is remarkable reduction in glycogen ($p < 0.05$) throughout starvation period and it recuperates upto the same level of control after refeeding thus affirming the role of glycogen store (Dar *et al*., 2018). It is pertinent to see and reveal the effect of starvation on fish as there is a unique feature of fish to withstand prolonged starvation leading to physiological and biochemical changes (Mustafa, 1983). In fact, starvation affects the physiological status and biochemical constituents of fish.

Haematological and biochemical parameters are the excellent health indicators of an animal and adaption to the environment because these parameters are sensitive enough to evaluate the effect of physical (*i.e.*, temperature, salinity) and chemical (*i.e.*, metallic or organic compounds) stressors. Any alteration in these parameters is the result of intrinsic changes such as age of animal or extrinsic factors such as pathogenic exposure to toxicant (Sakyi *et al*., 2019). So, the measurement of hematological and biochemical parameters may be reliable diagnostic tools to determine the effect of starvation on the health condition of fish. Accordingly, the present investigation intended to determine the most sensitive haematological and biochemical parameters of freshwater catfish, *Clarias batrachus* (Linn) when exposed to short-term starvation.

MATERIALS AND METHODS

Healthy live specimens of *Clarias batrachus* (35±5.01 g and 15±3.06 cm) were collected from local fish market, Balrampur (UP), India and transported to laboratory. The fishes were treated with 0.1 % $KMNO_4$ solution to get rid of dermal infection. As an oxidizer, it is able to chemically burn up organic matter such as bacteria, parasites, and fungi present in mucous of skin and gill. Finally healthy fishes were selected and kept in large plastic jar containing 50L of clean tap water and acclimatized for 15 days in the laboratory conditions, during which time they were fed with commercial floating fish feed. The water in the tank was changed regularly to remove faeces and food remnants. Water parameters were monitored as per APHA (2005). The temperature 23-25 ^oC, pH 7.3-7.8 and dissolved oxygen 7.0 -7.5 mg/l were maintained.

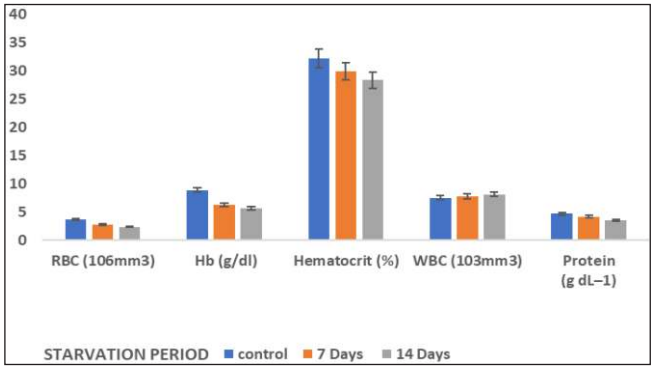
Two groups of test fish, *Clarias batrachus* were subjected to different treatments: Group A (control) fishes were then fed three times a day until apparent satiety, for 14 days; Group B (experimental) fishes were starved for 14 days.

Experiment I. Haematological and serum biochemical parameters: Fishes were anesthetized after 7 days and 14 days of both groups with 0.2 mL/L of clove oil in <3 min then blood samples were taken from each fish by puncturing the caudal vein using 1 mL tuberculin syringes. The collected blood was used to determine total erythrocyte (RBC) counts (by Neubauer haemocytometer), haemoglobin (by Sahli's acid haematin method), haematocrit percentage (by Wintrobe & Landsberg's method), total leucocytes (TLC) counts (by using blood smear method with Leishman's stain), Glucose (by Mendel *et al.*, 1954 method), protein (by Lowry *et al.*, 1951 method), triglycerides (by Neri and Frings, 1973 method) and cholesterol (by Sackett's, 1925 method, which is the modification of Bloor's, 1916 method).

Experiment II. Tissues Biochemical parameters: The fishes of both groups were sacrificed immediately at the end of 7th and 14th day and the desired tissues (liver and muscles) were dissected out and weighed on an electrical pan balance sensitive up to 0.030 mg, and their known weights processed separately for the estimation of protein and glycogen. The protein, glycogen and total lipids were estimated by Lowry *et al.* (1951), Carroll *et al.* (1965) and Barnes and Blackstock (1973) respectively. The experimental data were analyzed by student's 't' test to determine the significance of the changes from control.

RESULTS AND DISCUSSION

The effects of starvation on hematological parameters are shown in table 1 and graph 1. In the present study, hematocrit, hemoglobin and RBC count were significantly decreased to 7.18%, 29.54% and 24.59%, respectively after a week of starvation which further falls to 11.97%, 37.04% and 35.51%, respectively in two weeks starved fish as compared to control. The reduction in RBC counts, haemoglobin and haematcrit was found to cause anemia as noticed in stressed fishes was due to dysfunction/ suppression of haemopoitic organ (Verma and Prakash, 2019). The number of RBCs is an indicator of oxygen transfer efficiency from respiratory organs to tissues. Therefore, alterations in RBC count could be associated with changes in metabolic rate. Also, the RBC counts show the status of the fish immune system. Generally, the fish under starvation has a weaker immune system than fish with appropriate feeding. Thus, the starved fish is prone to pathogen attacks and usually its WBC level is higher than fish with adequate feeding as observed in the present study.



Graph 1: Alteration in haematological parameters and serum protein of *Clarias batrachus* during starvation.

Table 1: Alteration in haematological parameters of *Clarias batrachus* during starvation.

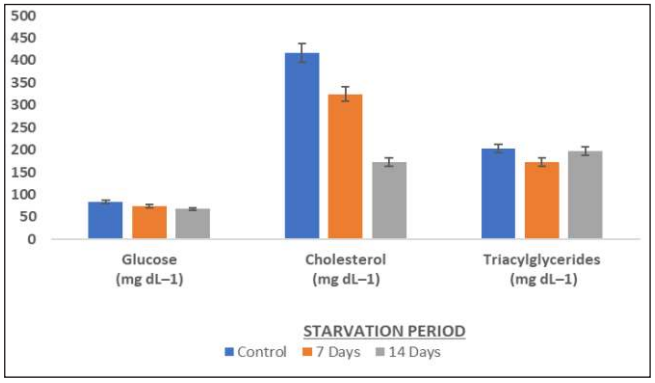
Sl. No.	Blood Parameters	Control	Starvation Period	
			7 days	14 days
1.	RBC (10^6mm^3)	3.66±0.37	2.76 ± 0.11* (-24.59%)	2.36 ±0.19** (35.51%)
2.	Hb (g/dl)	8.88±0.30	6.30±0.51* (-29.54%)	5.59±0.42** (37.04%)
3.	Hematocrit (%)	32.16±0.10	29.85±0.67* (-7.18%)	28.31±0.39** (-11.97%)
4.	WBC (10^3mm^3)	7.50±0.11	7.75±0.21 (+3.33%)	8.09±0.18* (+7.16%)

Significant: *P<0.05; **P<0.001

Table 2: Alteration in serum biochemical components of *Clarias batrachus* during starvation.

Sl. No.	Serum Parameters	Control	Starvation Period	
			7 days	14 days
1.	Protein (g dL^{-1})	4.65 ± 0.20	4.15±0.30 (-10.75%)	3.55±0.10* (-23.65%)
2.	Glucose (mg dL^{-1})	83.85± 3.0	73.43± 2.4 (-12.42%)	67.76±5.5* (-19.15%)
3.	Cholesterol (mg dL^{-1})	417.80±8.1	324.75±7.6* (-22.27%)	237.62 ± 9.1** (-43.12%)
4.	Triglycerides (mg dL^{-1})	202.69±7.44	172.71±8.29* (-14.79%)	197.11±9. 16 (-2.75%)

Significant: *P<0.05; **P<0.001



Graph 2: Showing changes in serum biochemical components *Clarias batrachus* during starvation.

The effects of starvation on biochemical parameters are presented in table 2 and graph 1 & 2. In the present study, serum glucose, protein, triglycerides and cholesterol levels were significantly decreased in starved fish as compared to control. This is likely the response to more consumption of energetic compounds of blood in response to acute starvation. During starvation, carbohydrates are used as primary reserves to produce energy and once these fall below the critical values; lipids are mobilized and metabolized to yield energy. With the catabolism of alcohol, the lipid is converted into glucose (Chatterjee, 1980).

Serum protein could serve as a good index of the severity of starvation in fish (Cho, 2009). The serum protein level in starved fish decreased up to 10.75% during the first starvation week, which further falls up to 23.65% during the second starvation week as compared to control (table 2).

The significant decreases in serum protein levels have also been observed in starved fishes by various researchers (Heming and Paleczny, 1987; Costas *et al.*, 2011; Kim *et al.*, 2014). In the present study, depletion of protein increased with increasing of duration of starvation due to its utilization in the conversion of glucose because during stress condition fish needs more energy to overcome the stress. Since fishes have less amount of carbohydrate so next alternative source of energy is protein to meet increased demand of energy during stress condition (Prakash and Verma, 2018).

In the present investigation, blood glucose level decreased up to 12.42% during the first week of starvation, which further falls up to 19.15 % during the second starvation week as compared to control (table 2). The significant decreases in blood glucose levels have also reported in starved fishes by other workers (Rahmati *et al.*, 2015; Hernández *et al.*, 2019).

In this study, serum cholesterol level is decreased up to 22.27% during the first week of starvation, which further falls up to 43.12 % during the second starvation week as compared to control (table 2). Chatterjee (1980) reported that the thyroid hormone and ACTH decrease the cholesterol content in the blood. Tang *et al*. (1983) reported that secretion of thyroid hormone and corticoids were disturbed in starving animals. Thus, in the present investigation, the intense and substantial reduction in the serum cholesterol level in starved fishes might be due to

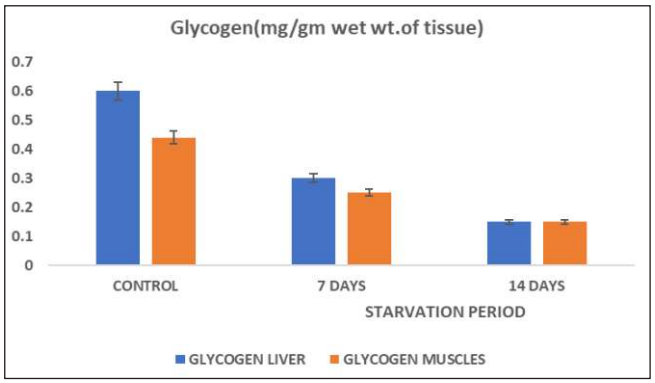
breakdown of cholesterol to produce energy and is the result of hyper secretion of thyroid and ACTH.

It is possible that *Clarias batrachus* exhausted 14.79 % of their triglyceride reserves in the blood plasma during the first seven days of starvation as a response to the depletion of glucose stores (table 2); but in the second week of starvation the lipid level restored up to 197.11 mg dL^{-1} following the starvation period, probably resulted from an increase in lipogenesis. A similar inclination in serum trigylglyceride level was observed in juvenile turbot, *Scophthalmus maximus* (Jia *et al.*, 2018) and in juveniles of *Sparus aur*ata after starvation (Peres *et al.*, 2012).

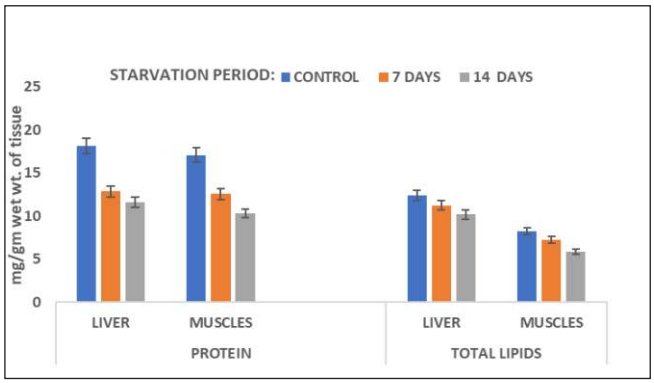
Table: 3 Alteration in tissues biochemical components of *Clarias batrachus* during starvation.

Biochemical Parameters	Tissue	Control	Starvation Period	
			7 days	14 days
Glycogen (mg/g wet wt. of tissue)	Liver	0.60±0.10	0.30 ± 0.03* (-50.0%%)	0.15 ± 0.07** (-75.0%)
	Muscle	0.44±0.11	0.25 ± 0.05* (-43.18%)	0.15 ± 0.03** (-65.90%)
Protein (mg/g wet wt. of tissue)	Liver	18.15±0.80	12.89 ± 0.36* (-28.98%)	11.60 ± 0.51** (-36.08%)
	Muscle	17.10±0.06	12.55 ± 0.51* (-26.60%)	10.31 ± 0.69** (-39.70%)
Total lipids (mg/g wet wt. of tissue)	Liver	12.41±0.21	11.25±0.21 (-9.34%)	10.18±0.17* (-17.96%)
	Muscle	8.25±0.32	7.25±0.25 (-12.12%)	5.89±0.38* (-28.60%)

Significant; *P<0.05; **P<0.001



Graph 3: Alteration in tissue glycogen of *Clarias batrachus* during starvation.



Graph 4: Alteration in tissue protein and lipid in *Clarias batrachus* during starvation.

In the present study, glycogen, protein and total lipids content in liver and muscles were decreased with increasing periods of starvation (table 3; graph 3 & 4). In fish, usually the liver glycogen and lipid are the first energy resources that are used for providing of energy during starvation periods (Black and Love, 1986). Of course, the nature of the energy resource (*i.e.*, carbohydrate, protein and lipid) is different depending on species, duration of starvation, environmental and nutritional conditions, reproductive stage and fish age (Rahmati *et al.*, 2015). The decline in liver and muscles glycogen content in starved fishes suggests enhance conversion of glycogen to glucose to fulfill the energy requirement under stress condition. The

possible reason of depletion of protein in liver and muscles of starved fishes suggests that during starvation period, fishes utilize the protein to convert into glucose. Since fishes have less amount of carbohydrate so next alternative source of energy is protein to meet increased demand of energy during stress condition (Prakash and Verma, 2018). The decreased total lipid content in muscles and liver of starved fishes may be due to increased utilization of stored lipid as a source of energy to conduct regular metabolic activities during starvation period.

CONCLUSION

In conclusion, the most sensitive physiological parameters during short-term starvation of *Clarias batrachus* were the blood hematocrit, hemoglobin, serum glucose, protein, cholesterol and liver glycogen, protein and lipid contents. Results demonstrated that these parameters could be useful as references to establish general health nutritional status of *Clarias batrachus*. The physiological strategy of this species for facing short-term starvation (up to 14 days) occurred as follows: firstly, serum glucose levels decreased, which is probably a reflection of tissues glycogen reserve depletion. Then endogenous protein was used as a primary energy source to meet metabolic energy requirements; and finally, lipid reserves of tissues was used to fulfill the energy requirement. Thus, from this study, it is evident that when freshwater catfish, *Clarias batrachus* was subjected to starvation stress, the fish successfully overcame the stressful period and survived all through the starvation, by mobilizing energy reserves such as glycogen, proteins and lipids, to combat the stress.

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