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Effects of Feed Deprivation on the Biochemical Responses of *Pangasianodon hypophthalmus*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The effects of feed deprivation, including feed restriction and starvation, were investigated on the biochemical responses of *Pangasianodon hypophthalmus* over a 28-day period. Juvenile *P. hypophthalmus* were divided into three groups: control (daily feeding), feed limited (fed every third day) and deprived (no feeding). Blood samples were collected weekly. Results showed a significant decrease ($P<0.05$) in total protein and calcium levels in fish subjected to feed restriction and starvation. Although glucose levels decreased, the difference was not statistically significant

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(P>0.05). Cortisol levels in feed-deprived fish showed significant variations (P<0.05) with an erratic trend. Notably, lactate dehydrogenase (LDH) and creatinine levels exhibited significant increases (P<0.05). Throughout the starvation period, alanine transaminase (ALT) and aspartate transaminase (AST) levels rose, yet compared to the control, their concentrations did not significantly differ (P>0.05). The study concludes that total protein, LDH, and calcium, among other biochemical markers, were particularly sensitive to hunger-induced stress in *P. hypophthalmus*. This kind of study already conducted on some freshwater catfishes (Mustafa S., Tripathi G.) and catfiahes.

Keywords: Stress biomarkers; Pangasianodon hypophthalmus; feed-deprivation stress; biochemical parameters; fish starvation; fish serum test.

1. INTRODUCTION

Pangasianodon hypophthalmus, also known as the iridescent shark or striped catfish, has become one of the fastest-growing species in the aquaculture industry worldwide, including in India. This growth is attributed to its rapid growth rate, improved management practices, and availability of essential nutrients, leading to widespread cultivation by farmers over the past decade [1].

Stress is an inevitable aspect of aquaculture, resulting in various physiological changes and increased susceptibility to diseases when fish are exposed to physical, chemical, and biological stressors such as feed deprivation, overcrowding, handling, and changes in water quality [2,3]. Feed restriction and starvation can induce diverse blood and serum responses, with the duration of restriction influencing the reactions. Feed deprivation typically triggers the mobilization of energy stores (glycogen, lipids, or proteins) to maintain fish homeostasis [4]. Prolonged starvation periods may lead to hormonal and biochemical changes. Therefore, understanding the effects of hunger on fish physiology and meeting their nutritional requirements are crucial for successful fish farming [5]. Blood analysis, encompassing both chemical and biological parameters, is a valuable tool in diagnosing and monitoring various conditions. Changes in enzyme profiles serve as important indicators (biomarkers) of toxicity, allowing the evaluation of the biochemical and physiological health of vital organs and tissues in fish (Gabriel *et al.,* 2007) [3].

2. MATERIALS AND METHODS

2.1 Experimental Design

The juveniles of *P. hypothalamus* (22.45 ± 1.66 cm and 77.37 ± 17.29 g) procured from Naihati fish market, S-24 Parganas, West Bengal were acclimated to laboratory conditions. All the juveniles were divided into three groups, each in triplicates , viz., control (fed daily with 28.0 % crude protein basal diet (commercial pelleted feed (CP 9951)), @ 3% body weight), feed restricted group (fed every third day with 28.0 % crude protein basal diet @ 3% body weight) and starved group (no feeding). The test fishes were stocked @ 15 numbers / tank total 135 fishes covered with fine mesh net and provided with aeration facility. The experiment was carried out for 28 days. At the start of the experiment, total length, body weight and blood sample (0th day) were measured. Following that, weekly sampling was carried out on the 7th, 14th, 21st, and 28th days.

2.2 Blood Collection

The experimental fish were starved (in case of fed fish) for 24 h before the blood collection to minimize the physiological stress during each sampling occasion. The fish were randomly sampled from the experimental FRP (Fiberglass Reinforced Plastic) tanks and transferred to plastic buckets containing water from the same FRP tank with same temperature. Fish were then partially anesthetized by exposing them to clove oil @ 50 µL/L of water of over one-minute period to minimize the activity of fish. Blood samples were collected from each anesthetized fishs'caudal vein individually to track biochemical responses. Unheparinized blood samples were allowed to coagulate at 4 °C for 10 minutes before being centrifuged at 2500 rpm for 15 min and kept at 20 °C for examination of serum biochemical parameters.

2.3 Biochemical Parameters

For all biochemical tests Commercial diagnostic kits were used. An enzyme-linked immunoassay kit (Accu Bind Elisa Microwells, Cortisol test

system) was used to assess the levels of cortisol in the blood. A kit based on the GOD-POD method was used to determine serum glucose (Span Diagnostics Ltd., India). The total serum protein was measured using the Biuret technique and a total protein test kit (Dia Sys Diagnosticspvt. Ltd., India). The ALT and AST were determined by using ALT and AST test kits (Span Diagnostics Ltd., India) following modified UV (IFCC) and kinetic assay methods, respectively. The serum LDH was determined by using LDH (P-L) test kit (DiaSys Diagnostics systems GmbH Germany) following modified IFCC method. The creatinine was determined by using creatinine test kit (DiaSys Diagnostics systems GmbH Germany) following modified kinetic test without deproteinization according to the Jaffe's reaction. The calcium was determined by using calcium test kits (Span Diagnostics Ltd., India) following Modified and end point assay methods, respectively.

2.4 Statistical Analysis

The results obtained for each examined parameter were expressed as mean ± standard deviation per each experimental group. Two-way analysis of variance (ANOVA) was done to assess significant differences in days and the experimental groups at the 0.05 Probability level.

3. RESULTS

In the control group (fed daily), the maximum level of glucose was observed at 138.50±11.59 mg/dl on the 7th day, while the minimum level recorded was 93.8±1.67 mg/dl on the 21st day. Regarding cortisol levels, the maximum level (41.66±2.04 µg/dl) was noted on the 21st day of stage 1, and the minimum level (30.2±2.04 µg/dl) was observed on the 0th day (beginning of the experiment) of stage 1 (Table 1). In the feed-restricted group (fed every 3rd day), the maximum levels of glucose and cortisol were recorded on the 21st and 28th days of stage 1, measuring 139.45±15.90 mg/dl and 45.59±0.27 µg/dl, respectively (Table 1). Conversely, the minimum levels of glucose and cortisol were 97.5±3.03 mg/dl and 42.32±0.27 µg/dl, respectively. For the starved (no feeding) group, the initial glucose level recorded was 118.01±11.61 mg/dl on the 0th day of stage 1, which decreased to 73.3±0.55 mg/dl by the 28th day. This indicates a gradual decline in glucose levels among starved fish, although no significant difference was found. Initially, the cortisol level in starved fish was minimum (30.20±2.04), but it reached its highest value (44.95±0.41) on the 7th day. Notably, cortisol levels exhibited significant variance among the experimental groups and days.

Lactate dehydrogenase (LDH) exhibited significant fluctuations across different groups. In the control group (fish fed daily), the maximum LDH level (435.00±19.79 IU/L) was observed on the 28th day, while the minimum level (323.5±34.64 IU/L) occurred on the 0th day (beginning of the experiment). Conversely, total serum protein varied between 2.76±0.07 g/dl (28th day of stage 1) and 2.53±0.15 g/dl (7th day of stage 1) in the control group. For the feed-restricted group (fed every 3rd day), the maximum and minimum total protein levels were found on the 0th day and 7th day of stage 1, measuring 2.56±0.02 g/dl and 2.13±0.18 g/dl, respectively (Table 1). In contrast, the lowest LDH level of 333.50±19.09 IU/L observed on the 0th day rose to the highest level of 881.5±64.34 IU/L on the 28th day (Table 1). In starved fishes, the total serum protein gradually decreased from an initial level of 2.56±0.02 g/dl to 1.46±0.14 g/dl observed on the 28th day. However, the lowest LDH level (333.50±19.09 IU/L) observed on the 0th day increased to the highest level (1101.25±56.77 IU/L) on the 28th day, showing significant variability between experimental days.

In the control group (fed daily), the highest and lowest values of alanine aminotransferase (ALT) fluctuated between 6.66±1.15 and 4.50±0.70 IU/L on the 7th and 21st days, respectively (Table 1). However, the mean aspartate aminotransferase (AST) levels did not vary significantly in the control group, with the highest mean value of AST being 109.00±14.14 IU/L on the 28th day. For the feed-restricted fish (fed every 3rd day), the maximum (8.66±0.57 IU/L) and minimum (5.66±0.57 IU/L) ALT levels were observed on the 14th and 7th days of stage 1, respectively (Table 1). The maximum AST level of 133.66±21.73 IU/L was recorded on the 7th day, which decreased to the minimum level of 88±3.46 IU/L on the 21st day (Table 1). In the starved fishes (no feeding) group, an overall increasing trend was observed in ALT levels as the duration of starvation advanced. The highest ALT level was observed on the 21st day of stage 1 (9.0±1.41 IU/L), while the lowest ALT level was observed on the 0th day of stage 1 (6.5±0.70 IU/L). There were no significant variances found between the groups and days. Similarly, the highest AST level (148±1.41 IU/L) was observed on the 28th day of stage 1, showing an increasing trend, although not significantly variable.

Table 1. Biochemical parameters (mean ± standard deviation) of *Pangasianodon hypophthalmus* subjected to feed deprivation stage

Biochemical parameters	0th day			7th day			14th day			21st day			28th day				
	Ctrl	FS	Stv	Ctrl	FS	Stv	Ctrl	FS	Stv	Ctrl	FS	Stv	Ctrl	FS	Stv	S	PV
Serum glucose	118.01±11.61	118.01±11.61	118.01±11.61	138.50±11.59	132.80±5.76	128.55±10.25	102.76±10.54	118.65±6.57	99.00±8.34	93.80±6.17	139.45±15.90	88.95±13.64	127.35±4.17	97.50±3.03	73.30±0.55	Groups	0.089
																Days	0.152
Serum cortisol(µg/dl)	30.20±2.04	30.20±2.04	30.20±2.04	30.78±5.48	42.32±0.27	44.95±0.41	35.76±5.19	44.37±0.93	44.10±0.86	41.66±2.04	45.59±0.27	43.60±1.76	34.93±2.28	42.47±0.54	39.40±4.71	Groups	0.005*
																Days	0.020*
Total serum protein(g/dl)	2.56±0.02	2.56±0.02	2.56±0.02	2.53±0.15	2.13±0.18	1.73±0.47	2.68±0.04	2.49±0.05	2.03±0.02	2.72±0.05	2.43±0.05	2.11±0.07	2.76±0.07	2.20±0.26	1.46±0.09	Groups	0.370
																Days	0.010*
ALT(IU/L)	6.50±0.70	6.50±0.70	6.50±0.70	6.66±1.15	5.66±0.57	7.33±2.30	6.33±1.52	8.66±0.57	8.66±3.78	4.50±0.70	6.25±1.25	9.00±1.41	5.00±1.00	6.66±1.15	8.75±0.95	Groups	0.754
																Days	0.146
AST (IU/L)	99.00±2.82	99.00±2.82	99.00±2.82	109.50±0.70	133.66±21.73	123.33±10.40	109.00±8.88	111.66±9.07	111.00±14.14	102.66±17.92	118.00±3.46	123.00±1.73	109.00±14.14	126.00±7.07	148.00±1.41	Groups	0.080
																Days	0.187
LDH(IU/L)	333.50±19.09	333.50±19.09	333.50±19.09	408.00±74.95	484.00±56.56	489.00±91.92	402.0±132.72	778.50±242.53	920.66±158.65	323.50±34.64	735.00±125.72	1014.33±146.7	435.00±19.79	881.50±64.34	1101.25±56.77	Groups	0.061
																Days	0.022*
serum creatinine (mg/dl)	0.375±0.035	0.375±0.035	0.375±0.035	0.456±0.015	0.456±0.049	0.513±0.025	0.370±0.020	0.495±0.045	0.506±0.032	0.386±0.011	0.430±0.014	0.473±0.023	0.283±0.005	0.373±0.014	0.410±0.014	Groups	0.009*
																Days	0.014*
serum calcium (mg/dl)	7.565±0.007	7.565±0.007	7.565±0.007	7.500±0.036	7.110±0.028	6.445±0.261	7.523±0.223	7.260±0.141	6.515±0.259	7.810±0.197	7.315±0.063	7.016±0.280	7.876±0.092	7.380±0.112	6.112±0.440	Groups	0.192
																Days	0.005*

*Note: * significant at 5 % level*
Ctrl: Control
FS: Feed Restriction
Stv: Starvation
S: Source
PV: P – value

In the control group (fed daily), the mean creatinine level was highest (0.456±0.015 mg/dl) on the 7th day and lowest (0.283±0.005 mg/dl) on the 28th day. Conversely, calcium levels ranged from a minimum of 7.500±0.036 mg/dl on the 7th day to a maximum of 7.876±0.092 mg/dl on the 28th day (Table 1). Fish fed every third day had maximum and minimum creatinine levels on the 14th and 28th days, with values of 0.495±0.045 mg/dl and 0.373±0.014 mg/dl, respectively (Table 1). Calcium levels in the same group ranged from a high of 7.380±0.112 mg/dl on the 28th day to a low of 7.110±0.028 mg/dl on the 7th day (Table 1). In the starved group (no feeding), the highest creatinine level was recorded on the 7th day (0.513±0.025 mg/dl), while the lowest creatinine level was observed on the 0th day (0.375±0.035 mg/dl). The initial calcium level in the starved group was 7.565±0.007 mg/dl on the 0th day, decreasing to a final level of 6.112±0.440 mg/dl on the 28th day. These values were significant at the 5% level both between the groups and days. Additionally, the calcium values showed significance at the 5% level between the experimental days.

4. DISCUSSION

4.1 Biochemical Parameters

4.1.1 Glucose

The blood glucose level in healthy and normal *Pangasianodon hypophthalmus* typically falls within the range of 90-139 mg/dl [6,7]. In our study, the average blood glucose level of *Pangasianodon hypophthalmus* in the control group ranged from 97.5±3.03 mg/dl to 143.007.21 mg/dl, consistent with previous findings [7]. The feed-restricted fish exhibited a minimum glucose level of 97.5±3.03 mg/dl, while the glucose level decreased to 70.5±3.39 mg/dl in starving fish (Table 1). This indicates a gradual decline in glucose levels among feed-restricted and starving fish. However, differences across days or groups were negligible (P>0.05).

The mobilization of energy stores such as lipids, stimulation of hepatic gluconeogenesis, and a decrease in the rate of glucose consumption are all linked to energy homeostasis in fish during food shortage [8]. However, the lower plasma glucose level in fasting fish could be explained by cortisol-mediated glycogenolysis or gluconeogenesis [9]. Various reactions in blood glucose concentration have been observed depending on the duration of fasting as well as species-specific variations in metabolism and its control (Caruso *et al.,* 2010).

Food restriction has been observed to induce a gradual decline in blood sugar levels in various fish species, with stabilization occurring after prolonged fasting periods. Studies on different species, including catfish (*Rhamdia hilarii*), brook trout (*Salvelinus fontinalis*), chinook salmon (*Oncorhynchus tshawytscha*), and rainbow trout (*Oncorhynchus mykiss*), have reported reductions in plasma glucose concentrations in response to fasting durations ranging from 20 to 42 days [10,11,12,13]. Additionally, in *Heteropneustes fossilis*, a significant reduction in glucose and glycogen levels in liver and muscle tissues was observed as hunger increased, indicating increased utilization of carbohydrate reserves during starvation [14]. Friedrich and Stepanowska [15] reported a substantial drop in blood glucose content in *Cyprinus carpio* during the initial 6 weeks of fasting, followed by stabilization at a lower level for the next 8 weeks, possibly indicating adaptation to a nutritionally challenging condition or mobilization of glycogen stores.

4.1.2 Cortisol

Soltanion et al. [16] reported cortisol levels in healthy *Pangasianodon hypophthalmus* to be in the range of 25-27 mg/ml. In the present study, both feed-restricted and starving fish exhibited a rise in cortisol levels during the first 7-14 days before experiencing a subsequent decline. Throughout the trial, cortisol levels displayed an erratic trend. Significant differences (P<0.05) were observed between the control group and both the feed-restricted and starving fish groups. However, there were no significant differences (P>0.05) observed between the feed-restricted and starving fish groups, both in terms of days and groups. These findings suggest that cortisol plays a functional role in energy mobilization in fish under conditions of feed limitation (feeding every third day) and famine (no feed). It is well-documented that fasting or malnutrition can lead to increased cortisol levels in animals [17]. However, in fish, there is conflicting evidence regarding the effect of hunger on cortisol levels, with some studies reporting decreases, increases, or no change in cortisol levels [18].

In fasting red porgy, serum cortisol levels significantly increased compared to fed individuals, a trend observed in other species like

Leiarius marmoratus and *Solea senegalensis Kaup* [19,20]. However, Barton *et al.* (1988) observed lower cortisol concentrations in starved fish (*Oncorhynchus tshawytscha* and *Ictalurus punctatus*) compared to fed fish. Additionally, Park et al. [21] reported that plasma cortisol concentrations in fed fish (*Paralichthys olivaceus*) were high at 4 weeks but remained unchanged in the deprived group throughout the 12-week trial. They suggested that cortisol and its metabolites increase in the early stages of hunger stress, facilitating rapid adaptability to potentially life-threatening situations. However, this response diminishes with prolonged starvation [22]. Increased corticosteroid levels in fish appear to serve as a mechanism for mobilizing energy reserves during periods of food scarcity [23].

4.1.3 Total protein

Serum total protein serves as a valuable biomarker for assessing nutritional status and metabolic activity in fish. Reports indicate that *Pangasianodon hypophthalmus* typically exhibits a total protein range of 4.07-5.30 g/dl [7]. In the present study, control fish exhibited low serum protein levels, ranging from 2.53±0.15 to 2.76±0.07 g/dl. However, during periods of feed restriction and starvation, total serum protein concentrations declined significantly to 2.13±0.18 g/dl and 1.46±0.14 g/dl, respectively (Table 1). A significant difference ($P<0.05$) was observed between control and starving fish, indicating a reduction in protein levels. This decline in protein levels suggests that the fish's basal metabolic rate necessitates the utilization of tissue protein to meet caloric needs [14]. Similar patterns have been observed in the liver and muscle tissues of Heteropneustes fossilis [14], as well as in other species such as freshwater catfish *Clarias batrachus* and *Notopterus notopterus*, and *Cyprinus carpio* [24,15]. Love [25] demonstrated that during prolonged starvation, fish utilize protein as an energy source through gluconeogenesis.

4.1.4 Alanine aminotransferase (ALT) and aspartate aminotransferase (AST)

Non-plasma specific enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are distributed in tissue cells across various organs including the liver, heart, gills, kidneys, and muscles [3]. These enzymes play a crucial role in linking carbohydrate and protein metabolism [26]. Yaghobi et al. [7] established 9±0.57 U/L as a suitable reference for *Pangasianodon hypophthalmus* ALT activity. In the control group (daily feeding), ALT levels fluctuated between 4.50±0.70 IU/L and 6.66±1.15 IU/L from the first day to the 21st day. Meanwhile, the mean AST levels remained relatively stable in the control group, with the highest and lowest mean values recorded as 109.00±14.14 IU/L and 99.00±2.82 IU/L, respectively, on the 28th day and 0th day of this stage (Table 1). In the case of starved fish, there was a consistent upward trend observed as the starvation period progressed. In the feed-restricted group (fed every third day), the highest ALT level (8.66±0.57 IU/L) and the minimum ALT level (5.66±0.57 IU/L) were measured on the 14th and 7th days of stage 1, respectively (Table 1). Similarly, the maximum AST level (133.66±21.73 IU/L) was recorded on the 7th day in the feed-restricted group, and it decreased to the minimum level (88±3.46 IU/L) on the 21st day (Table1). However, no significant differences were found in ALT and AST concentrations across groups ($P>0.05$).

Park et al. [21] observed a significant increase in the levels of ALT and AST in *Paralichthys olivaceus* subjected to starvation stress, indicating physiological strain on the liver and spleen due to fasting. This elevation in serum ALT and AST activities after fasting suggests potential hepatocellular damage or cellular degradation in the liver, spleen, or muscles [27]. The increased permeability of cells during fasting allows these enzymes to leak into the bloodstream, further contributing to the rise in serum enzyme activity [28]. Similar trends were observed by Olojo et al. [29] in *Clarias geriepinus*, where lead exposure led to enhanced AST and ALT activity, corroborating the findings of the present study.

4.1.5 Lactate dehydrogenase (LDH)

LDH, an enzyme ubiquitous in all tissues, primarily participates in carbohydrate metabolism (Sorensen et al., 1984). The levels of lactate dehydrogenase (LDH) varied significantly across all groups in this study. The highest LDH levels were observed in fish subjected to different feeding regimes: 435.50±19.79 IU/L in the control group (daily feeding), 881.5±64.34 IU/L in the feed-restricted group (fed every third day), and 1115.50±37.47 IU/L in the starving group (no feed). A significant difference ($P<0.05$) in serum LDH was noted between the control and starving fish, displaying an irregular pattern. The

significant variations in LDH activity suggest potential injury to organs such as the liver or kidneys, which produce this enzyme [30,31].

The findings of this study are consistent with those of Ramesh et al. [32], who observed a substantial increase in LDH activity in *Labeo rohita* induced by sodium selenite. Similarly, Chatterjee et al. [33] noted a significant rise in LDH activity in *Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala* stocked at high density. Exposure of early fingerlings of *Labeo rohita* and *Cyprinus carpio* to suboptimal temperatures resulted in a significant increase in LDH activity [33]. Das et al. [34] found a gradual increase in LDH activity in the gill, liver, kidney, and brain of *Cirrhinus mrigala* fingerlings exposed to elevated ammonia levels. Tkachenko et al. [35] reported a significant increase in LDH levels in *Oncorhynchus mykiss* due to exposure to chloramines-T.

4.1.6 Creatinine

In this study, the unstressed *Pangasianodon hypophthalmus* exhibited normal creatinine levels ranging from 0.283±0.005 to 0.456±0.015 mg/dl. While creatinine levels showed a slight increase in fish subjected to feed restriction and starvation, there was no clear trend or discernible difference. However, significant differences ($P<0.05$) were observed between the creatinine levels in control and starving fish. The increase in creatinine levels could potentially be attributed to glomerular insufficiency, increased catabolism of muscle tissue, or impairment in carbohydrate metabolism [36]. These findings align with the results reported by Osman and Haradawy [37], who observed an increase in creatinine levels in the muscle tissue of *Clarias geriepinus*, suggesting a steady rate of catabolism. Conversely, Kulkarni and Barad [38] found a significant decrease in creatinine levels in starved *Notopterus notopterus* fish. These discrepancies might be attributed to various factors including species-specific metabolic responses and environmental conditions.

4.1.7 Serum calcium

In the study, the normal calcium level in catfish was reported as 2.28 mmol/l (Clarias lazera) by Fathalla *et al.* (2004). However, in the current investigation, calcium levels exhibited significant differences ($P<0.05$) between the control and starved fish, as well as between the feed-restricted fish and starved fish. The decrease in calcium ion concentration could be attributed to tubular necrosis. Thangavel et al. [39] observed a reduction in serum calcium levels in *Sarotherodon mossambicus* when exposed to dimecron, while Prasad et al. [6] noted a gradual decrease in serum calcium levels in *Heteropneustes fossilis* after exposure to Euphorbia royleana latex. Similarly, Das et al. [34] reported hypocalcemia in *Heteropneustes fossilis* following dimethoate exposure. Studies on Clarias lazera subjected to fasting, high temperatures, and dim light conditions also revealed similar trends (Fathalla et al., 2004). Furthermore, Congleton and Wagner [40] observed a decline in plasma calcium concentrations in fasted Chinook salmon, *Oncorhynchus tshawytscha*. Hoseini and Ghelichpour [41] monitored serum calcium levels over the fasting period due to its association with serum proteins. Interestingly, in the current study, blood protein levels decreased more rapidly than serum calcium levels in fasting goldfish. It was observed that calcium levels only dropped significantly after prolonged periods of fasting. This suggests that the response of calcium levels to fasting may vary depending on the duration of fasting and other environmental factors [42-44].

5. CONCLUSION

The study revealed that starvation caused notable physiological changes in the *Pangasianodon hypophthalmus*. While various parameters responded to the altered physiological conditions, not all were equally effective as indicators of starvation stress. Total protein, lactate dehydrogenase, and calcium levels demonstrated heightened sensitivity to starvation stress. Overall, the results suggest that *Pangasianodon hypophthalmus* can endure food deprivation for as long as two months, underscoring its capacity for prolonged fasting adaptation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Singh AK, Lakra WS. Culture of *Pangasianodon hypophthalmus* into India: Impacts and present scenario. Pakistan Journal of Biological Sciences. 2012;15(1): 19.

2. Möck, A., & Peters, G. J. J. O. F. B. (1990). Lysozyme activity in rainbow trout, Oncorhynchus mykiss (Walbaum), stressed by handling, transport and water pollution. Journal of Fish Biology. *37*(6): 873-885.
3. Das A, Nagesh TS, Das SK, Abraham TJ. Stress responses of Indian major carps cultured in the East Kolkata Wetland, West Bengal, India. Aquatic Research. 2021; 4(4):351-362.
4. Gillis TE, Ballantyne JS. The effects of starvation on plasma free amino acid and glucose concentrations in lake sturgeon. Journal of Fish Biology. 1996;49(6):1306-1316.
5. Chatzifotis S, Papadaki M, Despoti S, Roufidou C, Antonopoulou E. Effect of starvation and re-feeding on reproductive indices, body weight, plasma metabolites and oxidative enzymes of sea bass (*Dicentrarchus labrax*). Aquaculture. 2011; 316(1-4):53-59.
6. Prasad G, Priyanka GL. Effect of fruit rind extract of Garcinia gummi-gutta on haematology and plasma biochemistry of catfish *Pangasianodon hypophthalmus*. 2011;6(3):240-251.
7. Yaghobi M, Dorafshan S, Akhlaghi M, Paykan Heyrati F, Mahmoudi N. Immune responses and intestinal morphology of striped catfish, *Pangasianodon hypophthalmus* (S auvage, 1878), fed dietary nucleotides. Journal of Applied Ichthyology. 2015;31(1):83-87.
8. Navarro I, Gutiérrez J. Fasting and starvation. In Biochemistry and molecular biology of fishes. Elsevier. 1995;4:393-434.
9. Mommsen TP, Vijayan MM, Moon TW. Cortisol in teleosts: Dynamics, mechanisms of action, and metabolic regulation. Reviews in Fish Biology and Fisheries. 1999;9:211-268.
10. Heming TA, Paleczny EJ. Compositional changes in skin mucus and blood serum during starvation of trout. Aquaculture. 1987;66(3-4):265-273.
11. Machado CR, Garofaloj MAR, Roselino JES, Kettelhut IDC, Migliorini RH. Effects of starvation, refeeding, and insulin on energy-linked metabolic processes in catfish (*Rhamdia hilarii*) adapted to a carbohydrate-rich diet. General and Comparative Endocrinology. 1988;71(3): 429-437.
12. Barton BA, Schreck CB, Fowler LG. Fasting and diet content affect stress-induced changes in plasma glucose and cortisol in juvenile Chinook salmon. The Progressive Fish-Culturist. 1988;50(1): 16-22.
13. Farbridge KJ, Leatherland JF. Plasma growth hormone levels in fed and fasted rainbow trout (*Oncorhynchus mykiss*) are decreased following handling stress. Fish Physiology and Biochemistry. 1992;10:67-73.
14. Borah S, Yadav RNS. Biochemical and haematological responses to starvation in an air breathing fresh water teleost *Heteropneustes fossilis* (Bloch). Indian J. Fish. 1996;43(3):307-311.
15. Friedrich M, Stepanowska K. Effect of starvation on nutritive value of carp (*Cyprinus carpio* L.) and selected biochemical components of its blood. Acta Ichthyologica et Piscatoria. 2001;31(2):29-36.
16. Soltanian S, Adloo MN, Hafeziyeh M, Ghadimi N. Effect of β-glucan on cold-stress resistance of striped catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878); 2014.
17. Ortiz RM, Wade CE, Ortiz CL. Effects of prolonged fasting on plasma cortisol and TH in postweaned northern elephant seal pups. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2001;280(3):R790-R795.
18. Pottinger TG, Rand-Weaver M, Sumpter JP. Overwinter fasting and re-feeding in rainbow trout: plasma growth hormone and cortisol levels in relation to energy mobilisation. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 2003;136(3):403-417.
19. Costas B, Aragão C, Ruiz-Jarabo I, Vargas-Chacoff L, Arjona FJ, Dinis MT, Conceição LE. Feed deprivation in Senegalese sole (*Solea senegalensis Kaup*, 1858) juveniles: Effects on blood plasma metabolites and free amino acid levels. Fish Physiology and Biochemistry. 2011;37:495-504.
20. Caruso G, Denaro MG, Caruso R, Genovese L, Mancari F, Maricchiolo G. Short fasting and refeeding in red porgy (*Pagrus pagrus*, Linnaeus 1758): Response of some haematological, biochemical and non specific immune parameters. Marine Environmental Research. 2012;81:18-25.
21. Park IS, Hur JW, Choi JW. Hematological responses, survival, and respiratory

exchange in the olive flounder, *Paralichthys olivaceus*, during starvation. Asian-Australasian Journal of Animal Sciences. 2012;25(9):1276.

22. Jung SH, Sim DS, Park MS, Jo Q, Kim Y. Effects of formalin on haematological and blood chemistry in olive flounder, *Paralichthys olivaceus* (Temminck et Schlegel). Aquaculture Research. 2003; 34(14):1269-1275.
23. Sheridan MA, Mommsen TP. Effects of nutritional state on in vivo lipid and carbohydrate metabolism of coho salmon, *Oncorhynchus kisutch*. General and Comparative Endocrinology. 1991;81(3): 473-483.
24. Tripathi G, Verma P. Starvation-induced impairment of metabolism in a freshwater catfish. Journal of Natural Sciences. 2003; 58:446–451
25. Love RM. The chemical biology of fishes. Vol.1. Acedamic press, New York. 1970;542.
26. Harper HA, Rodwell VW, Mayer PA. Review of physiological chemistry, seventeen ed. Lange Medical Publication, California. 1978;19-80.
27. Mohsen A, Momdouh AA, Maaly MA. Use of live Baker's yeast, *Saccharomyces cerevisiae*, in practical diet to enhance the growth performance of Galilee Tilapia, *Sarotherodon galilaeus* L., and its resistance to environmental copper toxicity. Journal of the World Aquaculture Society. 2010;41:214-223.
28. Yamawaki K, Hashimoto W, Fujii K, Koyama J, Ikeda Y, Ozaki H. Hemochemical changes in carp *Cyprinuscarpio* exposed to low cadmium concentration. Nippon Suisan Gakkaishi Bull. 1986;52:459- 466.
29. Olojo EAA, Abass AA, Olurin KB, Mbaka G. The potential use of certain protein metabolism parameters as biomarkers of heavy metal (lead) stress in the African cat fish, Clarias Gariepinus. Agricultural Journal. 2012;7(5):316-322.
30. Young SJ, Dowman AA, Cowell DC. The detection of pentachlorophenol by its inhibitory effectiveness on lactate dehydrogenase of rabbit muscle and Bovine Heart. Pesticide Biochemistry and Physiology. 1999;64(1):1-8.
31. De Coen WM, Janssen CR, Segner H. The use of biomarkers in Daphnia magna toxicity testing V. *In vivo* alterations in the carbohydrate metabolism of Daphnia magna exposed to sublethal concentrations of mercury and lindane. Ecotoxicology and Environmental Safety. 2001;48(3):223-234.
32. Ramesh M, Sankaran M, Veera-Gowtham V, Poopal RK. Hematological, biochemical and enzymological responses in an Indian major carp *Labeo rohita* induced by sublethal concentration of waterborne selenite exposure. Chemico-Biological Interactions. 2014;207:67-73.
33. Chatterjee N, Pal AK, Das T, Mohammed MS, Sarma K, Venkateshwarlu G, Mukherjee SC. Secondary stress responses in Indian major carps Labeo rohita (Hamilton), *Catla catla* (Hamilton) and *Cirrhinus mrigala* (Hamilton) fry to increasing packing densities. Aquaculture Research. 2006;37(5):472-476.
34. Das PC, Ayyappan S, Jena JK, Das BK. Acute toxicity of ammonia and its sub-lethal effects on selected haematological and enzymatic parameters of mrigal, *Cirrhinus mrigala* (Hamilton). Aquaculture Research. 2004;35(2):134-143.
35. Tkachenko H, Kurhaluk N, Grudniewska J. Effects of chloramine-T exposure on oxidative stress biomarkers and liver biochemistry of rainbow trout, Oncorhynchus mykiss (Walbaum), brown trout, *Salmo trutta* (L.), and grayling, *Thymallus thymallus (L.).* Fisheries and Aquatic Life. 2013;21(1):41-51.
36. Hadi A, Shokr A, Alwan S. Effects of aluminum on the biochemical parameters of fresh waterfish *Tilapia zillii*. J. Sci. Appl. 2009;3(1):33-41.
37. Osman AG, Koutb M, Sayed AEDH. Use of hematological parameters to assess the efficiency of quince (Cydonia oblonga Miller) leaf extract in alleviation of the effect of ultraviolet–A radiation on African catfish *Clarias gariepinus* (Burchell, 1822). Journal of Photochemistry and Photobiology B: Biology. 2010;99(1):1-8.
38. Kulkarni R, Barad V. Haematological and blood biochemical changes in the fresh water fish, *Notopterus notopterus* (Pallas) exposed to acidic medium. International Letters of Natural Sciences. 2015;45.
39. Thangavel P, Sumathiral K, Karthikeyan S, Ramaswamy M. Endocrine response of the freshwater teleost, *Sarotherodon mossambicus* (Peters) to dimecron exposure. Chemosphere. 2005;61(8): 1083-1092.

40. Congleton JL, Wagner T. Blood-chemistry indicators of nutritional status in juvenile salmonids. Journal of Fish Biology. 2006; 69(2):473-490.
41. Hoseini SM, Ghelichpour M. Effects of pre-sampling fasting on serum characteristics of common carp (*Cyprinus carpio* L.). International Journal of Aquatic Biology. 2013;1(1):6-13.
42. Das SK, Nagesh TS, Das A, Abraham TJ, Vishwanath TS. Stress Mitigating and Growth-Enhancing Effect of Dietary Vitamin E in Indian Major Carps Cultured in East Kolkata Wetlands, India. In Proceedings of the Zoological Society. New Delhi: Springer India. 2022;75(2):208-220.
43. Gabriel UU, George AOI, Plasma enzymes in Clariasgariepinusexposed to chronic levels of roundup (glyphosate). Environ Ecol. 2005;23(2):271–276.
44. Mustafa S. Changes in biochemical composition in starving catfish *heteropneustes fossilis*. Japanese Journal of Ichthyology. 1983;29(4):416-420.

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