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ORIGINAL ARTICLE **OPEN ACCESS**

Effect of Cortisol on Carbohydrate Metabolism During Osmotic Adaptation of *Heteropneustes fossilis*

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ABSTRACT
The current study explored the relationship between osmoregulation and energy metabolism and their hormonal control in a stenohaline catfish, Heteropneustes fossilis. Improved plasma osmotic pressure after cortisol treatment at a higher salinity suggested its critical role in osmoregulation. Sustained muscle and blood water contents after treatment further highlighted the involvement of cortisol in osmotic adjustments. Significant increases in plasma glucose levels within 24-hours both in tap water and 30% sea water after cortisol treatment may be due to glycogenolysis as was evident from significantly decreased levels of glycogen in liver both in tap water and 30% sea water after treatment. However, no significant changes were observed in muscle glycogen after cortisol treatment, indicating that liver was the immediate source of energy during osmoionic regulation. These results indicated that the exogenous administration of cortisol at a dose of 10 µg/g body weight may improve the hypoosmoregulatory ability of the catfish and had a discernible effect on carbohydrate metabolism as was evident from the significant changes in the profiles of plasma glucose and liver glycogen.
Keywords: *Cortisol, Glycogen, Metabolism, Osmoionic, Osmoregulation, Teleost.*

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INTRODUCTION

Teleost fishes experience high osmotic stressors while adapting to different ambient salinities. To manage this condition teleosts require additional energy resources [1-6]. The increased energy demand during acclimatization of fish to varying salinities may occur due to the high transport rate of ions in various osmoregulatory organs, such as gills, kidney, and intestine[3,7]. To account for high energy demand during osmoionic regulation, teleosts utilize various substrates of energy metabolism, namely proteins, fats, and carbohydrates. Therefore, it is important to study the interrelationship between osmoregulation and energy metabolism.

Teleosts, when dealing with environmental stressors such as varying levels of salinities, utilize more energy reserves than when in isotonic conditions (state of equilibrium) [3, 8, 9]. This added energy expenditure can compromise the metabolism, growth, and immune response of fish. The redistribution of energy resources amid salinity crisis can lead to increased oxygen consumption and hyperstimulation of the ion transport mechanism [8, 10, 11]. The cascading effect due to energy and carbohydrate metabolism is compensated primarily by the oxidation of blood glucose. When experiencing salinity variations, how much oxygen is consumed during energy metabolism (adenosine triphosphate or ATP generation) and how much energy is produced by substrate metabolism can provide insights into the glucose oxidation pathway.

All biological systems strive for homeostasis or equilibrium. Fishes, especially teleosts, prefer osmotic (salt and water) balance across membranes as part of the osmoregulatory process [12]. When the osmotic balance is disrupted due to salinity variations, more energy is consumed by the fish to reach equilibrium. As a result, the active transport mechanism is activated which consumes ATP to move the solute (salt) against the concentration gradient, while depleting the precious energy reserves (ATP). These constant disruptions can be detrimental to the health, growth, and immune response of fishes.

Studies on the changes in the profile(s) of carbohydrate metabolism during osmoregulatory adjustments are important because teleosts have high ATP production rates, especially when experiencing salinity disruption, which allows for the ATPs to be employed readily. Carbohydrate metabolism has been studied in osmoregulatory organs (such as gills and kidneys) [6, 13-16] as well as non-osmoregulatory organs (such as, liver and muscle)[2, 4, 5, 17, 18]. Moreover, modification of carbohydrate metabolism during osmoionic regulation is considered to be a hormonally-mediated process [2, 6, 13, 19].

Of the vast number of hormones, cortisol, released by adrenal cortex, has been found to play a major role during saltwater adaptation in teleosts [6, 20-22]. Although considered a stress hormone, cortisol has been shown to play a significant role in osmoregulation and immune response [20, 23]. In teleosts, cortisol plays a dual role: it acts as a mineralocorticoid and a glucocorticoid. As a mineralocorticoid, cortisol allows the fish to cope with the fluctuation(s) in ambient salinity [6, 22-24]. On the other hand, as a glucocorticoid, it rearranges various sources of energy [25, 26], which modify dominant intermediary pathways of metabolism [27]. Besides, there are also reports to show that exogenous administration of cortisol affects carbohydrate metabolism [2, 6].

The catfish, *Heteropneustes fossilis*, is an economically important freshwater stenohaline fish that constitutes an important component of pisciculture and capture fishery. It is widely distributed in freshwater ponds and riverine backwaters of the Indian subcontinent. Various features of osmoregulation including the hormonal aspects have been elucidated in this fish species, and like other teleosts, cortisol has been found to play an important metabolic role in osmoionic regulation [22, 28-32]. Our previous study on *Heteropneustes fossilis* suggested that ambient salinities have a discernible effect on carbohydrate metabolism as was evident from the changes in the profiles of plasma glucose, liver glycogen, and muscle glycogen contents[4]. However, no evidence is available on the effect of cortisol on carbohydrates during osmotic adjustment of the catfish. Therefore, the aim of the present study was to study the effect of exogenous administration of cortisol on carbohydrate metabolism by monitoring the changes in the activity profiles of the readily oxidizable carbohydrates, glucose and carbohydrate reserves, mainly glycogen, during osmoionic regulation of *Heteropneustes fossilis.* Additionally, plasma osmolality, blood water content, and muscle water content were also analysed as they represent important indices of osmoregulation.

MATERIAL AND METHODS

Collection and Care of Fish

Adult specimen of the catfish, *Heteropneustes fossilis* weighing 40-50 g were obtained from the local fish market of Aligarh, Uttar Pradesh, India. They were acclimated to laboratory conditions (temperature 25+2ºC, photoperiod 12 L : 12 D) for 15 days in glass aquaria (60x25x30 cm), containing stored dechlorinated tap water (TW). During this period, the catfishes were fed daily *ad libitum* with Hindlever laboratory animal feed (Hindustan Lever Limited, Mumbai, India), and the water in the aquaria was changed daily by siphoning off and replenishing simultaneously with TW, adjusted to laboratory conditions.

Artificial Sea Water

Artificial sea water (SW) was prepared in dechlorinated TW according to Goswami et al.[28] and 30% SW was prepared by diluting full-strength artificial SW with dechlorinated TW.

Blood Collection and Plasma Separation

Blood was drawn from the caudal artery into heparinized glass syringes fitted with 24-gauge disposable needles. Immediately after collection, the blood was centrifuged for 10 minutes at 3000 rpm (Remi Ltd., India, Model No. R8C) and plasma was separated and stored at -20^{o}C until analyzed.

Plasma Osmolality

Plasma osmolality was measured in 10 µl sample with vapour pressure osmometer (Wescor 5500, Utah, USA) and expressed as mOsmol/kg.

Plasma Glucose

Plasma glucose was assayed by the o-Toluidine method [33].

Glycogen

Glycogen, both in muscle and liver, was estimated by the Anthrone method [34].

Blood and Muscle Water Contents

A freshly drawn blood drop (approximately, 50µl) and a small piece (approximately 0.1g) of muscle tissue from just beneath the dorsal fin of the catfish were collected and placed on the pre-weighed glass coverslips and immediately weighed using a weighing balance from Wensar (Chennai, India). The samples were kept at 110^{0}C for 24 hours to dry. The dried samples were weighed again and the difference between the wet and dry weight was utilized for determining the percentage water content in the tissues.

Statistical Analysis

Data for all parameters were expressed as mean $\pm$ standard error. Statistical comparisons between experimental and control groups were made by the Student's *t*-test [35].

Experimental Protocol

Fish were divided into 3 groups. Groups 1 and 2 were injected with cortisol acetate (FA) (hydrocortisone acetate, Wyeth Laboratories Limited, India, Batch No. 5C 1011) intramuscularly at a dose of 10 µg/g body

weight daily for 5 days and group 3 with DDW to serve as a control. This particular dose of cortisol was selected because of its effectiveness in rectifying deranged osmoregulatory parameters in hypophysectomised catfish [36]. After 5 days of treatment, Group 1 fish were transferred to 30% SW, while those of groups 2 and 3 were transferred to TW. Fish from each group were sampled at 24 hour, 3 days, and 6 days, post-transfer. Blood, from the caudal artery, was obtained for the estimation of plasma osmolality and plasma glucose. A piece of muscle was excised from just beneath the dorsal fin and the liver was dissected out to estimate the glycogen content. A blood drop and muscle tissue were collected on the pre-weighed glass coverslips for water content measurements. The results are presented in Figures 1-6.

RESULTS

Plasma Osmolality

The FA-treatment for 5 days at a dose of 10µg/g body weight/day did not exhibit any significant changes in plasma osmolality in the group maintained in TW. However, in the FA-injected fish transferred to 30% SW, a significant increase was observed within 24 hours of transfer ($p<0.005$), which increased further on day 3 ($p<0.001$) of the transfer, and the parity with DDW-injected control was obtained on day 6 of transfer to 30% SW (Figure 1).

Plasma Glucose

The FA-treatment for 5 days at a dose of 10µg/g body weight/day resulted in a significant increase in plasma glucose levels within 24 hours ($p<0.001$), both in TW and 30% SW maintained groups, beyond which the levels became equivalent to DDW-injected control group (Figure 2).

Liver Glycogen

Liver glycogen levels exhibited a significant decline after FA-treatment in both TW and 30% SW maintained groups as compared to DDW-injected control group throughout the duration of the experiment (Figure 3).

Muscle Glycogen

The FA-treatment for 5 days did not show significant change in the glycogen levels of the muscle in the fish transferred to TW and 30% SW throughout the duration of the experiment (Figure 4).

Blood Water Content

No significant change was observed in the % of the blood water content after FA-treatment in TW and 30% SW compared to DDW-injected control throughout the duration of the experiment (Figure 5).

Muscle Water Content

No significant change was observed in the % of the muscle water content after FA-treatment in TW and 30% SW compared to DDW-injected control throughout the duration of the experiment (Figure 6).

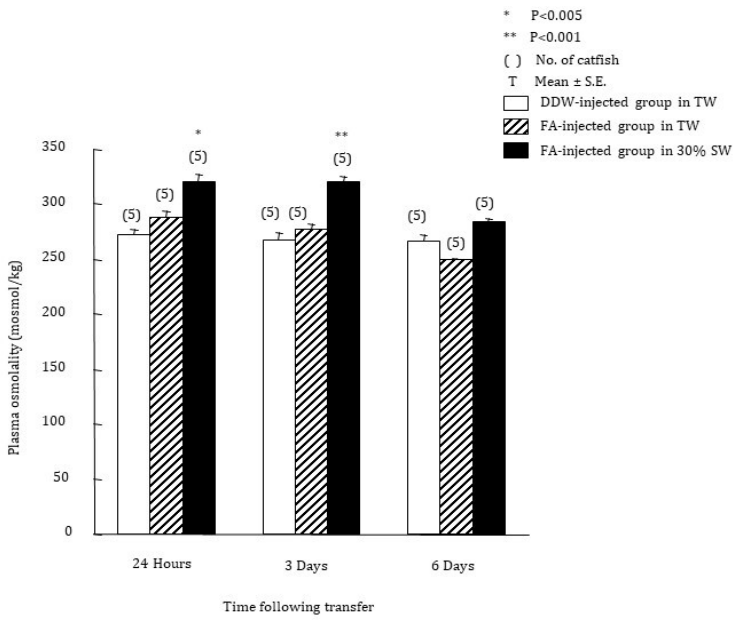

Figure 1: Plasma osmolality of *Heteropneustes fossilis* after cortisol acetate (FA) treatment in tap water (TW) and 30% sea water (SW)

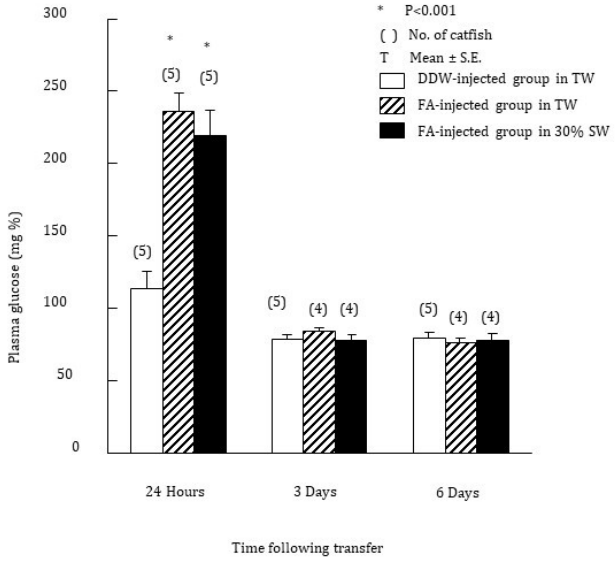

Figure 2: Plasma glucose of *Heteropneustes fossilis* after cortisol acetate (FA) treatment in tap water (TW) and 30% sea water (SW)

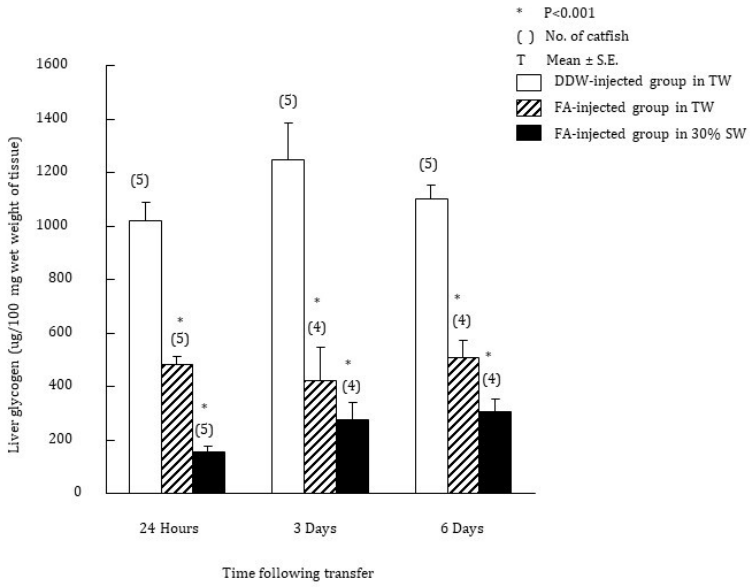

Figure 3: Liver glycogen of *Heteropneustes fossilis* after cortisol acetate (FA) treatment in tap water (TW) and 30% sea water (SW)

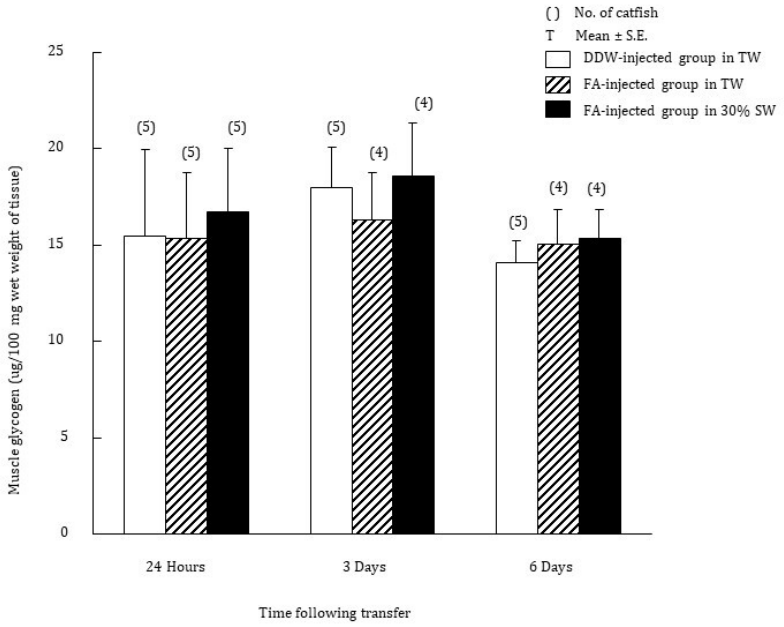

Figure 4: Muscle glycogen of *Heteropneustes fossilis* after cortisol acetate (FA) treatment in tap water (TW) and 30% sea water (SW)

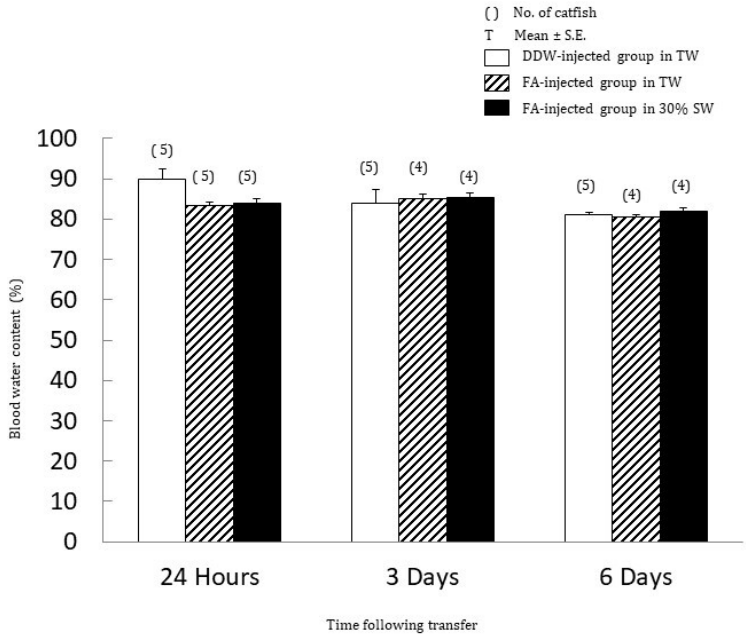

Figure 5: Blood water content of *Heteropneustes fossilis* after cortisol acetate (FA) treatment in tap water (TW) and 30% sea water (SW)

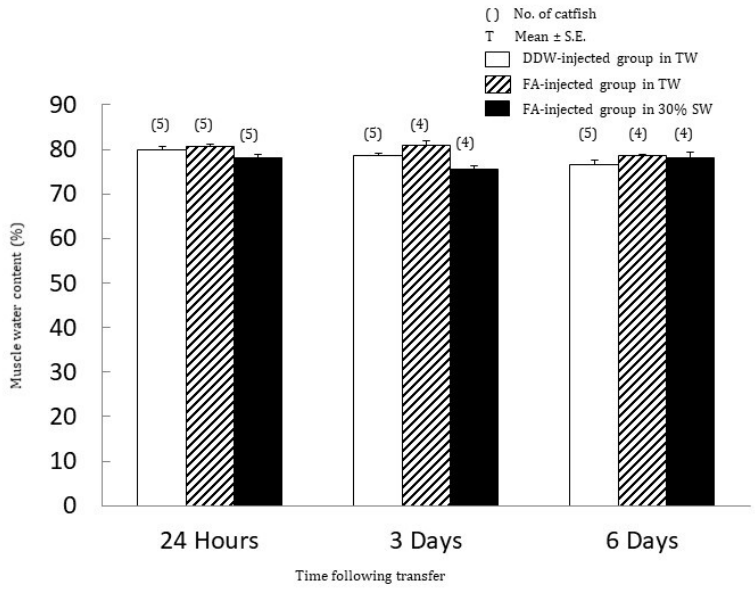

Figure 6: Muscle water content of *Heteropneustes fossilis* after cortisol acetate (FA) treatment in tap water (TW) and 30% sea water (SW)

DISCUSSION

Teleosts experience higher osmoregulatory stressors while experiencing variable salinities in ambient environments leading to physiological disruptions. There is abundant evidence to show that cortisol influences water and electrolyte exchange in fish organs, such as gills, gut, kidney, urinary bladder, and muscle, modifying concomitantly the concentration of electrolytes and osmolality of blood and urine[22,24,37]. In general, cortisol is thought to promote electrolyte excretion in fishes living in hypertonic media and that of its conservation in hypotonic environment [6, 38, 39]. In teleosts, the exogenous administration of cortisol impacted ion and water exchange in osmoregulatory organs (gills, gut, kidney, and urinary bladder) and also modified the concentration of electrolytes and osmolality of blood and urine [6, 22, 19].

The present study on the catfish did not show a significant change in plasma osmolality after cortisol treatment in TW. On the other hand, plasma osmolality was significantly increased within 24 hours when the catfish, pre-treated with FA, were transferred to 30% SW. The results of the present investigation on the catfish showed unchanged plasma osmotic pressure following FA treatment in TW, which resembled those on yearling coho salmon, *Oncorhynchus kisutch* in FW, where no effect of FA treatment was observed on plasma osmolality, Na^+, K^+, Ca^{2+}, or Mg^{2+} concentrations [40]. Cortisol treatment also did not affect the plasma Na^+ and Cl^- ions in rainbow trout, *Salmo gairdneri* and sea trout parr, *Salmo* trutta [41, 42]. Other scientists also have shown that plasma osmolality and steady-state levels of plasma ions in FW were not altered by cortisol [19, 43]. Regarding the results of plasma osmolality of FA-treated fish following transfer to 30% SW, the catfish exhibited significantly increased levels of plasma osmolality within 24 hours and attained parity with the control group by day 6, which could not be attained up to 15 days when the catfish were directly transferred to 30% and 35% SW [4, 22]. These observations indicate that the treatment of the catfish with FA may potentiate osmoionic regulatory adjustments in higher salinity [19, 22, 43]. These findings further confirmed our previous results on the catfish in which an improved plasma osmoregulatory ability of fish in 30% SW was observed after cortisol treatment [22].

Muscle and blood water content and haematocrit values are important indices for assessing the osmotic disruptions in cellular compartments [14]. No significant changes were observed in blood and muscle water contents both in TW and 30% SW after FA-treatment in *Heteropneustes fossilis* which further substantiated the hypoosmoregulatory role of cortisol [44] and indicated that the muscle and blood cells have the ability to regulate water in tissues[14]. However, decreased muscle water levels were observed in rainbow trout, *Salmo gairdneri* after cortisol treatment indicating some modification in osmoionic balance [41]. Significantly decreased levels of muscle water have also been reported in *Carassius auratus* after transfer to a higher salinity, suggesting muscle dehydration and the inability of the cells to function with increasing osmotic concentration [45].

Most of the reports on the effect of cortisol administration in fish deal with its action on carbohydrate metabolism [2, 6, 19, 46-52]. The present study on the catfish demonstrated a significant increase in plasma glucose concentrations both in TW and 30% SW after treatment of the fish with FA for 5 days. The hyperglycemia as observed in the catfish, *Heteropneustes fossilis* after FA-treatment, was in accordance with the findings of the earlier studies [19, 44, 53] but they were contrary to other studies that showed decreased [37, 54] or constant plasma glucose levels[2] after cortisol treatment. The hyperglycemic effect of FA-treatment in the catfish may be due to its repressive action on oxidation of glucose and its utilization in peripheral tissues [52]. The increase in plasma glucose after FA-treatment may be explained further by assuming that FA-treatment may supply glucose by the process of glycogenolysis or gluconeogenesis as cortisol has both the anabolic [2, 48] and the catabolic [46, 55] effects during stressful conditions. Several studies have also revealed that cortisol may have no effect in some species of fishes [51, 56, 57].

The current findings on the catfish, *Heteropneustes fossilis* demonstrated the catabolic action of cortisol more conspicuously, since liver glycogen was significantly decreased after FA-treatment both in TW and 30% SW throughout the duration of the experiment. The stimulation of glycogenolysis caused by FA-treatment is thought to be the probable cause for the lower hepatic glycogen levels in the FA-treated groups of catfish [46]. A decrease in liver glycogen levels was also reported in other species of fishes under stressful conditions after cortisol-treatment [46, 55]. Other reports have shown sustained [2, 49] or increased levels of liver glycogen [47, 50] after cortisol treatment.

This study on the catfish, *Heteropneustes fossilis* demonstrated no significant change in muscle glycogen levels after FA-treatment both in TW and 30% SW, which may be due to its negligible contribution in total energy expense [58]. Sustained muscle glycogen levels were also reported in gilthead seabream, *Sparus aurata* after treatment with cortisol and synthetic glucocorticoid, dexamethasone [2, 47]. Other reports have shown decreased[2] or increased [6, 51] levels of muscle glycogen after cortisol treatment both in FW and SW.

CONCLUSION

Cortisol is an osmoregulatory hormone which also plays an important role in glucose metabolism and helps the catfish, *Heteropneustes fossilis* to acclimatize to higher salinities. It regulates the osmotic adjustments such as ion regulation (salt and water secretion and uptake) following the transfer of the catfish to higher salinities. When administered exogenously at a dose of 10 μg/g body weight, cortisol may improve the hypoosmoregulatory ability of the catfish. In the catfish, cortisol was found to regulate the metabolic response to stressors by modulating the respiratory pathways (glycogenolysis). The limitations of the study were the limited sample size and the consideration of just one catfish species. As part of the future research directions, while building upon the findings of the present study, it would be important to consider, (a) including a larger sample size for accurate average values while identifying outliers, and (b) conducting research in more than one teleost species for comparative analysis of statistical data.

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Conflict of Interest

The author declares no conflict of interest.

Author's Contribution

The author conceived the ideas, designed the experiments, executed the experiments, collected samples, performed biochemical and tissue analyses, data analysis and interpretation, and wrote and edited the manuscript.

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No funding was available for conducting this research study and no personal assistance was sought.

Ethics Statement

Not applicable.

Informed Consent

Not applicable.

Data Availability

All data presented in this manuscript are original and not obtained from any other database or repository.

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