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Alterations in Organic Reserves in *Channa punctatus* Infected with Ectoparasites

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Abstract: This investigation was carried out to investigate the effects of ectoparasites on the organic reserves of some tissues of fresh water snakeheaded fish, *Channa punctatus* collected from the polluted lotic fresh water body flowing through Balrampur city, India. The results indicated that the highest rate of parasitic infection in fish significantly reduce carbohydrate, protein and lipid contents in liver, muscles, gill, gut and kidney. Results of the present investigation also indicated that the Superoxide (SOD) and Peroxidase (POD) activities were significantly increased in liver, muscles, gills, gut and kidney tissues of the infected fish compared to uninfected fish. The differences in organic reserves between infected and uninfected fish, *Channa punctatus* indicated that the heavy infestation of parasites decreases the nutritional value such as moisture, ash, fat, protein and carbohydrate as well as energy production level. Infection with parasites in fish had the highest effect on liver, muscles, gills, gut and kidney.

Keywords: Ectoparasites, Organic Reserves, Antioxidant enzymes, Oxidative stress, *Channa punctatus*, Superoxide, Peroxidase

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Introduction

Parasites are the important group of pathogens, which occur at various stages of development in fishes. The parasites invade various tissues and organ of fish, including skin, gills, eye, kidney, liver, intestine, spleen, heart and brain. Parasites are metabolically dependent on their host mainly for their nutritional requirements (Marcogliese, 2004). Moreover, parasites may also affect its host in various ways such as utilization of host's food, destruction of host tissues, abnormal growth, mechanical inferences, biological effects, various kinds of tissue reactions as well as the effects of toxins, poisons or secretion of parasites itself. Like human and other animals, fish suffers from diseases and infected with parasites. Fish defenses against diseases are specific and non-specific, where non-specific defenses include skin and scales, as well as the mucus layer secreted by the epidermis that traps microorganisms and inhabits their growth.

Fish contain carbohydrates, vitamins A and D, iron, calcium and other mineral salts. The nutrients in fish are more than those found in eggs and poultry meat. Protein is an essential nutrient for both maintenance and growth of fish. The nutritive values of fishes can be greatly influenced by their chemical nature of their fats and other lipid components particularly cholesterol. Proximate composition has been reported to be a good pointer of physiology needed for routine analysis of fisheries. Biochemical composition of fish shows very wide variations from one species to another, within the same species in different portions of the body, from season to season, according to age, size, growth etc. (Murphy *et al*., 2021). Therefore, the alterations associated with biochemical parameters due to various parasites can act as an important tool for diagnosis of diseases that reveals the state of health of fish species and guidelines for the implementation of the treatment in the future. This information would be very much helpful in fish farming and fish industry.

The fishery industry of India is a potent food producing sector providing nutritional security, supporting livelihoods, providing employment and contributing to a major portion of agricultural exports. The inland fisheries sector in India has increased from 29% in 1950–1951 to 55% at present, supporting 25 million people and generating Rs. 15000 crores in revenue in 2020 (Prakash *et al*., 2021a). Despite the fact that India has seen a significant growth in fish production, freshwater productivity, particularly inland culture fishery, is declining as a result of numerous parasites that interfere with the metabolic processes, normal health conditions, and even the death of culturable fishes (Prakash, 2021a; Prakash *et al.*, 2021b).

Literature cautioned that diseases, especially the parasitic diseases caused great damage to fish production affecting their fry and fingerlings (Chakraborty *et al*., 2020). Since these pathogen affect the reproductive potential of host fishes, a parasitic disease reduces the fish production, profitability, and market as well as nutritive value, thus cause great economic loss (Prakash and Verma, 2017; Prakash and Singh, 2020).

Channa punctatus is one of the most common freshwater fish in India and has a great demand in the market because of its relatively low cost and high availability. These are the best sources of protein and high nutritive value (Nimbalkar and Deolalikar, 2015). The aim of present study was to investigate the impact of internal parasites on some biochemical parameters related to liver, muscles, gills, gut and kidney of selected infested and non-infested fish, *Channa punctatus*, which would form a baseline data for assessment of fish health.

Materials and Methods

Total 55 live fish, *Channa punctatus* of different sizes and sexes were collected randomly from the polluted lotic water bodies of Balrampur, U.P., India. Fish were transferred to the laboratory by using small containers containing water from Sawan nallaha with aeration. In order to check for parasites, fresh fish were dissected and examined microscopically for parasites. Out of 55 examined fish 38 fish were found infected with a total 176 parasites. Ectoparasitic protozoans (*Trichodon, Ichthyoptherius* and *Myxobolus*) were commonly found on the gills and skin whereas ectoparasitic crustaceans (*Ergasilus, Lernea, Lamproglena* and *Argulu*) were found on gill, skin and fin base of host fishes (Parmar and Prakash, 2022). The collected live fishes were washed and anaesthetized by 0.1 g/l of benzocaine then liver, kidney, gill, gut and muscles tissues were dissected out. The tissues of the infected and uninfected fish was collected, weighed and processed for biochemical estimations and enzymatic antioxidants using standard methods.

Table 1: Impact of Parasites on the organic reserves and antioxidant enzymes of infected and uninfected fish, *Channa punctatus*

Tissues	Fish status	Organic Reserves and Enzyme level in some tissues of fish				
		Glycogen (mg/g)	Protein (mg/g)	Lipid (mg/g)	SOD (Unit/mg)	POD (Unit/mg)
Liver	Uninfected	5.6±0.42	85.4±1.02	6.0±0.22	11.54±0.8	0.098±0.02
	Infected	4.2±0.31* (-25.0%)	71.6±1.01* (-16.16%)	3.9±0.32** (-33.33%)	15.43±0.7* (+33.79%)	0.125±0.04* (+27.55%)
Muscles	Uninfected	4.5±0.42	78.8±0.86	6.5±0.19	16.67±1.02	0.056±0.03
	Infected	3.8±0.39* (-15.55%)	67.4±0.64* (-14.47%)	4.6±0.23* (-29.23%)	21.31±1.11* (+27.83%)	0.065±0.21* (+13.85%)
Gill	Uninfected	4.7±0.32	70.5±1.12	4.2±0.14	17.52±0.43	0.084±0.05
	Infected	3.3±0.29* (-29.78%)	55.4±0.64** (-21.42%)	3.0±0.19* (-28.57%)	20.12±0.21* (+14.84%)	0.092±0.04* (+9.52%)
Gut	Uninfected	6.9±0.52	79.4±0.75	5.3±0.15	24.2±0.64	0.065±0.02
	Infected	4.4±0.19** (-36.23%)	53.6±1.11** (-32.49%)	3.6±0.22* (-32.07%)	31.4±0.51* (+29.75%)	0.071±0.04* (+9.23%)
Kidney	Uninfected	4.5±0.41	74.5±1.04	4.9±0.34	21.4±0.32	0.089±0.04
	Infected	3.1±0.34** (-45.16%)	55.4±1.02** (-25.63%)	3.7±0.32* (-24.48%)	25.2±0.12* (+17.76%)	0.097±0.03* (+8.99%)

*Significant at 5% level ($P<0.05$); ** Significant at 1% level ($P<0.01$)

The estimation of protein content in the host tissues was carried out by Lowry's method (1951), the glycogen estimation by the method of Carroll *et al.* (1956), lipid estimation as per method of Frings *et al*. (1972). Superoxide (SOD) was determined by method of Worthington (1988) and Peroxidase (POD) was determined by measuring its capacity to decrease the hydrogen peroxide concentration at 470 nm (Zia *et al*., 2011).The tissue of un-infected fish was taken as control. The experiments were conducted in triplicates. All the values are given as mean ± SE. The values of the biochemical data between the uninfected and infected groups of fish tissues were compared.

Results and Discussion

In the present investigation, the freshwater snakeheaded fish, *Channa punctatus* infected with parasites showed significant reduction in organic reserve i.e. glycogen, protein and lipid content in some tissues as compared to uninfected fish (Table 1).

Reduction in carbohydrate, lipid and protein in tissues of parasite infected fishes was reported by previous researchers (Mustafa, 2000; Hassan *et al*., 2010; Ashokan *et al*., 2013; Rajram *et al*., 2018; Grace *et al*., 2021; Murphy *et al*., 2021). In contrast significant increase of lipid level was observed in liver and gill of diseased fish than normal fish by Tanaka *et al*. (2002).

In the present investigation, the glycogen content in liver, muscles, gills, gut and kidney of infected fish were decreased by 25.0 %, 15.55 %, 29.78 %, 36.23 % and 45.16 %, respectively. The carbohydrate reduction observed in these tissues of infected fishes may be due to less intestinal absorption of glucose and due to the consumption of digested food by the parasites (Kaddumukara *et al*., 2006). In the present investigation, the protein content in liver, muscles, gills, gut and kidney of infected fish were decreased by 16.16 %, 14.47 %, 21.42 %, 32.49 % and 25.63 %, respectively. Depletion of protein in infected fish tissues may be due to the conversion of protein resources for the release of energy to withstand the wear and tear created by the parasites (Mustafa, 2000). In the present investigation, the lipid content in liver, muscles, gills, gut and kidney of infected fish were decreased by 33.33 %, 29.23 %, 28.57 %, 32.07 % and 24.48 %, respectively. Lipid serves as energy reserves to meet the metabolic demand for more energy to the fishes (Prakash and Verma, 2020). Reduction of lipid content may be due to the utilization of lipid by host and parasite.

Cholesterol which represents the stored energy is being rapidly utilized by the infected fish to withstand the stress brought by infestation.

In living organisms, oxidative stress is caused by a variety of endogenous and external sources. Organisms have adapted various mechanisms that protect against oxidative stress caused by a variety of biotic and abiotic agents, including parasitic infection and the induction of antioxidant enzymes SOD and POD (Livingstone, 2001). Results of the present investigation clearly indicated that the SOD and POD activities were significantly increased in liver, muscles, gills, gut and kidney tissues of the infected fish compared to uninfected fish. The SOD activity in liver, muscles, gills, gut and kidney of infected fish were increased by 33.79%, 27.83%, 14.84%, 29.75% and 17.76%, respectively. The POD activity in liver, muscles, gills, gut and kidney of infected fish were also increased by 27.55%, 13.85%, 9.52%, 9.23% and 8.99%, respectively. Increased levels of enzymatic activity indicated the oxidative stress. The results also indicated that the percentage of enzymatic activities of different antioxidant enzymes increases differently in different tissues. Invasion of any parasite can cause the stress and during stress condition fish would enhance the production of reactive oxygen species (ROS) in response to which antioxidant enzymes increases (Prakash, 2021b). The results of this study corroborate those of several other researchers who have found that parasite invasion alters antioxidant enzymatic activity in fish (Grace *et al*., 2021). However, the antioxidant system's reaction in parasitized fish varies depending on parasite species, examined tissues and enzyme specificity (Hursky and Pietrock, 2015). Many xenobiotics use general toxicity mechanism by causing oxidative stress in fish which is a pathological process related to increase production of ROS (Nazish *et al*., 2018). Samanta *et al.* (2018) and Srivastava and Reddy (2020) also confirmed increased activities of antioxidant enzymes in freshwater fishes collected from different rivers. Thus, increased activity of SOD and POD in the infected host's liver, muscles, gill, gut and kidney in the present study indicated enhanced oxidative stress caused by parasitic infection that disrupt the host's metabolic pathway (Buchmann and Lindenstrom, 2002). Thus, in the present investigation, increase in antioxidant enzymes in the tissues of *Channa punctatus* could also be ascribed to the parasite-induced metabolic activity modulation.

Due to the presence of these ectoparasites, the physiological and enzymatic activities of the infected host fishes are hindered and their developmental growth is retarded which cause economic loss to the fishery industry and piscine culture (Prakash and Singh, 2020).

Conclusion

The result of the present investigation revealed that the population of parasite burden and infestation rate in combination reduces the carbohydrate, protein and lipid content as well as energy production level in infected fishes. The infected fish's oxidative stress is modulated differently in different organs due to changed metabolic pathways. The parasitic infestation can affect the nutritional quality of fish thereby decreases the food values and marketability of fish. For human fishes are a good source of proteins and lipids. Therefore, health of fishes is also important for human beings.

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