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BIOCHEMICAL STUDY OF FRESHWATER FISH *CLARIAS BATRACHUS* (L.) INFECTED WITH CESTODE PARASITE, *LYTOCESTUS* SP. FROM DISTRICT JALGAON, INDIA

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Abstract: This study involved analyzing bio-molecules including protein, glycogen and lipids in parasites as well as in both the infected and non-infected intestine of the host. The worms were sourced from the intestinal region of the freshwater fish *Clarias batrachus* and underwent a washing and other necessary processes. The dry weight was recorded after subjecting the samples to a temperature range of 50-60°C for duration of 24 hours. During the comparison between cestode parasites and the host intestine, it was observed that *Lytocestus* sp. displayed reduced concentration of protein and glycogen in comparison to both the infected and non-infected segments of the host's intestine. Moreover, the lipid concentration in the parasite exceeded that found in the host intestine, regardless of infection status. Author noticed that concentration of protein, glycogen, and lipids were notably high in the non-infected region compared to the infected section within the host intestine.

Keywords: *Clarias batrachus*, Fish, Glycogen, Intestine, Lipid, *Lytocestus*, Protein, Tissue.

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INTRODUCTION

Fishes are exclusively aquatic and poikilothermic vertebrates with streamlined body and anamniotic nature (Verma and Prakash, 2020; Ashok, 2020). Fishes are said to be gold in water and play an important role in national economy as it provides employment opportunity (Narayan *et al.*, 2021). They provide a high content of proteins to the daily growing population. 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. (Kumar and Prakash, 2021). The fishes also play a crucial role in the second tropic level of the aquatic ecosystem (Ashok, 2016); however, these are facing the problem of malnutrition and get infection from various kinds of parasites frequently (Peddinti *et al.*, 2021).

The tapeworms present in living beings cause considerable damage. The parasitic infections are

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common to vertebrates including fishes and human (Sushil *et al.,* 2011). The field of parasitic biochemistry is experiencing significant growth, particularly in tandem with the renewed interest in tropical diseases. In the past, parasitologists found it necessary to incorporate biochemical methodologies into their research practices to keep pace with ongoing developments in the field. Biochemistry came into its own as a distinct discipline when scientists integrated principles from biology with those of organic, inorganic, or physical chemistry. This interdisciplinary approach led to the exploration of various topics, including the mechanisms by which living organisms extract energy from food, the chemical foundations of heredity, and the fundamental alterations that occur in diseases. The scope of biochemistry encompasses diverse fields such as molecular biology, immunochemistry, neuro-chemistry, bioinorganic and biophysical chemistry.

Biochemistry is concerned with the study of the chemical processes that occur in living beings with the ultimate aim of understanding cell function in molecular term (Keith and John, 2006). Parasitology has developed into a multi-dimensional approach in helminth research. They serve as valuable models for the study of fundamental biological phenomena.

The proteins are absorbed by the parasites by diffusion and transfusion. Proteins have many different biological functions. They are everywhere in their distribution and there is really no satisfactory scheme of classifying them. The largest groups of proteins are the enzyme proteins provide rich environment for the nourishment of cestodes. The cestodes utilize different degrees of protein that producing energy. Literature reveals that the parasites able to adopt themselves to the parasitic mode of life, the protein usually constitutes between 20 to 40 % of the dry weight (John, 1981).

The glycogen content of various helminths fluctuates considerably and there is variation in habitat, though no similarity in nutrition of worms. Glucose is an important source of energy for cestodes, inhabiting the alimentary tract of vertebrates (John, 1981). Cestodes possess stored carbohydrate metabolism, with enormous amount of stored carbohydrate (Daugherty, 1956). Cestode parasites stores relatively large quantities of polysaccharides, which in most cases has been assumed to be glycogen (Reid, 1942).

Lipids are of great importance to the body of cestodes as the chief concentrated storage form of energy, besides their role in cellular structure and various other biochemical functions. The higher content of lipid is found in older proglottids (Von Brand, 1952). The present investigation deals with the biochemical studies of freshwater fish *Clarias batrachus* (L.) infected with cestode parasites, *Lytocestus* sp. from Jalgaon district (M. S.) India.

MATERIAL AND METHODS

Sample Collection

The worms were collected from the intestine of fresh water fish *Clarias batrachus* (L.) and then washed with distilled water. Collected worms were then dried on the blotting paper to remove excess water and transferred to watch glass and weight on sensitive balance. After 50-60 ^{0}C for 24 hours, the dry weight was taken.

Biochemical estimation

Several specimens of freshwater fish, *Clarias batrachus* (Linnaeus), was brought to the laboratory and carefully dissected. Some of these fishes were found to be infected with cestode parasites. Small sections of both infected and non-infected intestines were collected to perform the biochemical studies. Cestode parasites from the infected intestines were carefully collected and examined under a microscope. Identical worms were selected, with some fixed in 4% formalin for taxonomic identification. In the cestode parasites, protein content was estimated according to method given by Lowry *et al.* (1951); glycogen content by Kemp and Heijningen (1954) and lipid by Folch *et al.* (1957) method.

To estimate glycogen levels, the collected worms were first dried on blotting paper to eliminate excess water. Subsequently, the dried material was transferred to a pre-weighed watch glass and weighed using a sensitive balance. The material was then ground in a mortar and pestle. To this ground material, 1 ml of 30% KOH was added, and the mixture was transferred to a centrifuge tube. It was then placed in boiling water for 20

minutes, cooled to room temperature and 1.5 ml of 90% ethanol was added by gently stirring. The resultant solution was boiled in a hot water both. After that, the solution was centrifuged for 15 minutes at 2000 RPM and the supernatant was discarded, leaving the glycogen settled at the bottom. The residue was dissolved by adding 5 ml of distilled water. A 0.1 ml aliquot of the solution was taken, to which 0.9 ml of distilled water was added. Subsequently, 5 ml of anthrone reagent was added and the mixture was heated for 10 minutes. Readings were then taken using a colorimeter at 620 μm filter to quantify the glycogen concentration.

For protein estimation, the cestode worms were first dried on blotting paper to eliminate excess water, and the wet weight of the tissue was recorded. The material was then transferred to a pre-weighed watch glass and dried in an oven at 58 to 60°C for 24 hours. The resultant dry weight was used to prepare a powder, weighing 100 mg on a sensitive balance. This powder was homogenized in a mortar and pestle with 1 ml of 10% TCA solution and transferred to a centrifuge tube. After centrifugation for 15 minutes at 3000 RPM, the supernatant was discarded, and the residue was dissolved in 10 ml of NaOH. A 0.1ml aliquot of this solution was taken and mixed with 4 ml of Lowry's 'C' solution, followed by the addition of 0.4 ml of Folin phenol reagent. The resulting solution was left in the dark for half an hour until a blue color developed. The color was then read on a colorimeter with a 530 μm filter to calculate the protein content. Bovine serum albumin was used as a standard.

For lipid estimation, 100 ml of tissue was weighed and homogenized using a mortar and pestle with the addition of 10 ml of chloroform: methanol (2:1). The mixture was filtered using Whatman's filter paper and 1 ml of the filtrate was pipetted out and dried at 37°C or for 3-4 days at room temperature. To the dried filter, 1 ml of concentrated H_2SO_4 was added, followed by boiling in a water bath for 10 minutes and rapid cooling. A 0.2 ml aliquot of the solution was taken and mixed with 5 ml of Vanillin reagent, kept for 30 minutes at room temperature to develop a purple color. Readings were then taken with a colorimeter at 530 μm filter.

RESULTS AND DISCUSSION

In the present investigation, Cestode parasites i.e., *Lytocestus* sp. was carried out for biochemical estimation of primary metabolites such as protein, glycogen and lipid (Table 1; Graph 1). Author found the protein content of cestode studied (*Lytocestus* sp.) 0.44 ± 0.04 mg/100 mg dry wt. of tissue per ml solution. Author observed fresh water fish *Clarias batrachus* with infection 0.56 ± 0.02 mg/100 mg dry wt. of tissue per ml solution and without infection 0.66 ± 0.03 mg/100 mg dry wt. of tissue per ml solution. Protein content is lower in cestode parasites as compared to host (Sushil *et al.,* 2011; Amol *et al.,* 2014).

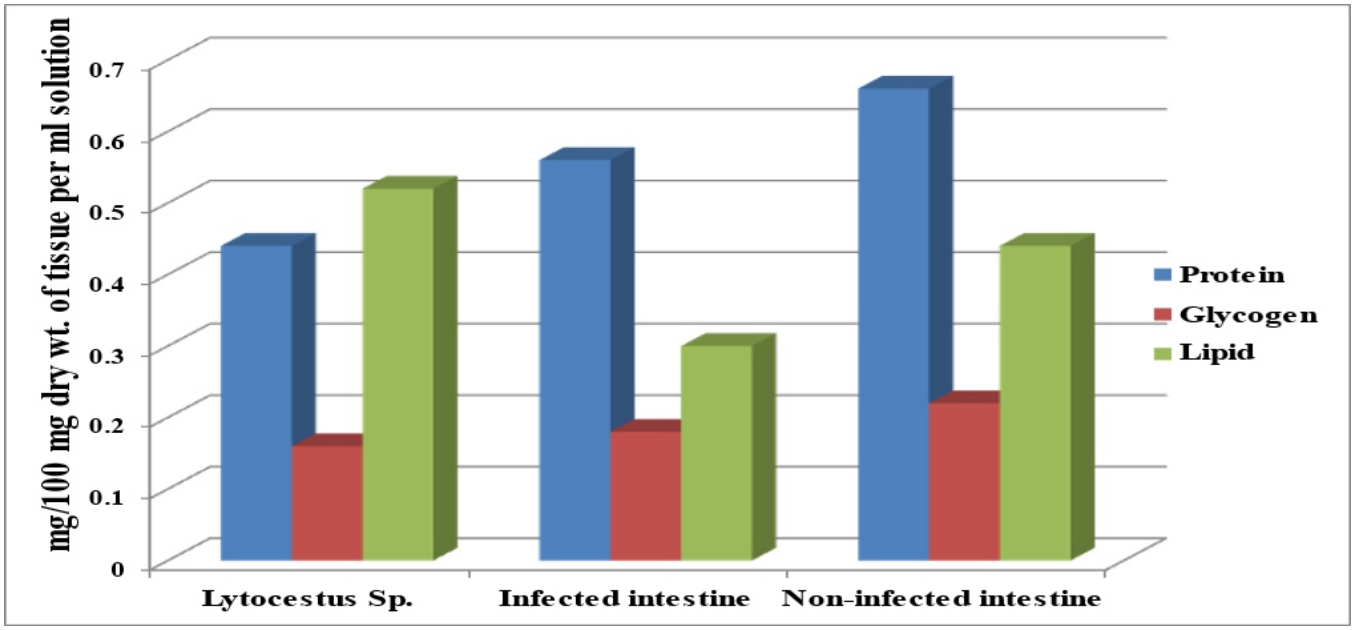
The glycogen content of parasite studied was found 0.16±0.004 mg/100 mg dry wt. of tissue per ml solution. Author observed fresh water fish *Clarias batrachus* with infection 0.18±0.006 mg/100 mg dry wt. of tissue per ml solution and without infection 0.22±0.019 mg/100 mg dry wt. of tissue per ml solution. Glycogen content is lower in cestode parasite as compared to infected and non-infected intestine of host (Pawar, 2020). Glycogen content is higher in cestode parasite as compared to infected and non-infected intestine of host (Sushil *et al.,* 2011).

Similarly, the lipid content of *Lytocestus* was obtained 0.52±0.02 mg/100 mg dry wt. of tissue per ml solution. Author reported fresh water fish *Clarias batrachus* with infection 0.30±0.020 mg/100 mg dry wt. of tissue per ml solution and without infection 0.44±0.15 mg/100 mg dry wt. of tissue per ml solution. Lipid content is higher in cestode parasites as compared to host intestine (Pawar, 2020). Lipid content is lower in cestode parasites as compared to host intestine (Amol *et al.,* 2014).

From the present experimental study, it has been observed that the lipid content is high in cestode parasites as compared to protein and glycogen. These parasites get most of nourishment from host and fulfilling its need causing hindrance in the proper development of tissue (Sushil *et al.,* 2011).

Table 1: Biochemical estimation of fresh water fish *Clarias batrachus* intestine and cestode parasite (*Lytocestus* sp.)

Parameters	*Lytocestus* sp.	Intestinal tissue of *Clarias batrachus*	
		Infected	Non-infected
Protein (mg/100mg dry wt. of tissue per ml soln.)	0.44 ± 0.04	0.56 ± 0.02	0.66 ± 0.03
Glycogen (mg/100mg dry wt. of tissue per ml soln.)	0.16±0.004	0.18±0.006	0.22±0.019
Lipid (mg/100mg dry wt. of tissue per ml soln.)	0.52±0.02	0.30±0.020	0.44±0.15



Graph 1: Biochemical estimation of fresh water fish *Clarias batrachus* intestine and cestode parasite (*Lytocestus* sp.).

CONCLUSION

The biochemical study of freshwater fish *Clarias batrachus* (L.) infected with the cestode parasite *Lytocestus* sp. from the Jalgaon district, India, yielded valuable insights into the physiological changes induced by parasitic infection. The findings provide a comprehensive understanding of the alterations in key biochemical parameters such as glycogen, protein and lipid concentration shedding light on the intricate host-parasite interactions in this specific ecological context.

The study further revealed that infected fish exhibited notable changes in glycogen, protein, and lipid levels compared to their non-infected counterparts. The lower concentration of glycogen and protein in the infected fish suggest a potential diversion of these resources by the cestode parasite, impacting the host's metabolic balance. Conversely, higher lipid concentration in infected fish may indicate a host response or a manipulation by the parasite to meet its energy requirements.

Additionally, this study contributes to the broader understanding of host-parasite dynamics in aquatic ecosystems, particularly in the Jalgaon district. Further research in this area could enhance the understanding of host adaptation and the potential implications for both host and parasite populations in aquatic environments.

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