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In vitro biochemical analysis of fresh water fish *Mystus seengala* infected with cestode parasite

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Abstract
The biochemical composition of fishes collected from wakadi dam fresh water showed content of protein, glycogen and lipid in *Mystus seengala* tissue. The fishes showed successive decrease in protein and lipid contents from June to May whereas muscle glycogen content of fishes shows significant increase during June to May. Protein content in infected and non-infected fish 19.28 mg/gm of dry tissue in Mystus seengala and in non-infected protein content 21.78 mg/gm, 20.18, 22.09 and 18.12, & 20.21 respectively of tissue it was higher than infected one in fish tissue. Glycogen content in infected and non-infected fish *Mystus seengala* were recorded 24.70, 28.12, 25.16, 29.23 and22.89, 26.45 in between period of March to dec-2019 respectively comparatively low in infected fish than non-infected one. The lipid content in *Mystus seenghala* 11.23, 12.15, 10.14 mg/100mg dry wt. of tissue per ml sol^{n}) was higher than in the infected 12.90, 13.20 and 11.05 .g/100mg dry wt. of tissue per ml sol^{n}) and non-infected (0.42 ± 0.13 mg/100mg dry wt. of tissue per ml solution.

Keywords: Biochechemical analysis, infected, Cestode, *Mystus seengala* & wakadi dam etc

Introduction
Biochemical changes of fishes study are very important due to unwanted burden on fish infection by parasites may change their biochemical properties and these parasites considerable effect on human health those are taking infected with parasites in diet. Biochemistry is the role of many molecules in chemistry reaction and processing in live creature. Fishery Biochemical changes study is important due to the infection parasites or cestode is consuming essential protein and amino acid content in fish for their growth and reproduction. Also due to this fish nutritional value degrades and also mortality percentages increases. In present investigated fish selected on the basis their diet nature almost fishes depends on the living organism present in fresh water and consume them for his growth and development. Other think is that helminths infection found only in living dependent fishes.

Non vegetarian society mostly uses meat, egg, and fishes in his diet for fulfil his nutritional requirement of body. But fishes are cheap sources of protein and easily available in market but nutritional values of fishes degrades due to infection of helminth in water, helminth mostly infects to host fishes. Variety of fresh water fishes available in market but know about the selection of fish for his diet and also about the nutritional value of fishes and their health and how to identify infected fish by cestode parasites. So kindly in present investigation on selected fish for their infection by cestode and their effect on nutritional factors.

The research showed that fish contain high quality of protein. Because of that need method to calculate the protein levels. Immunological status of the host is very important of helminth infections, because gastrointestinal organs of the body and it serves as the first line or defense against orally administrated antigens (e.g. feed protein of carbohydrates) and intestinal pathogens. Gut associated lymphoid tissues make up about 25% by weight of the gut mucosa and sub mucosa and thus constitute the largest sites of lymphocytes (Mc Burney, 1973).

Protein, glycogen and lipid are important biomolecule of each and every organism. Caryophyllidean cestodes absorb all nutritive biomolecule from their host body. The present study was examine the relationship between Caryophyllidean cestodes infection and nutrient reserve in fresh water fish *Mystus seenghala* infected by cestode parasites collected from Wakadi Dam, Parbhani District, (MS) India.

Material and Method
Freshwater fishes taken for examination of cestode infection in intestine as well tissue and healthy of fishes. Then cestode parasite were preserved in 4% formalin, washed in saline and water, dehydrated in various alcoholic grades, stained with Harris haematoxylin and Borax carmine, cleared in xylene, mounted in D.P.X. Drawings were made with the aid of camera Lucida and identification by standard methods (Schmidt, 1934; Yamaguti, 1959; Hiware *et al*, 2003; Bhure, 2008) [1].

Estimation of Glycogen by (Kemp method)
The collected fish *Mystus seengala* were put on the thick blotting paper for absorb excess water from the body of cestodes and host. This material was transferred on a previously weighed watch glass. This weighted material was ground in to homogeneous paste in mortar pestle. To this paste 1ml of 30% KOH is added and taken in centrifuge tube and digested in hot water bath for 20 min. cooled and to same 1.5ml of 95% ethanol was added by stirring with glass rod. Gentle to boil in hot water bath, cooled and centrifuged for 15 min. at 2000 rpm supernatant was drained on filter and 5 ml distil water was added and reacted with 5 ml of test solution; 5 ml of glucose standard solution 5 ml of distil water. Separately in three test tube in each 10 ml of anthron reagent was added and mixed then heated for 10 min and immediately cooled and reading were taken with the help of colorimeter at 620mμ setting a blank 100.

Fresh water fishes *Mystus seenghala* and was brought to the laboratory, dissected carefully. Some of them were infected with cestode parasites; small pieces of infected and non-infected intestines were also collected to find the glycogen content in respective parasites and their hosts by using Kemp method.

Cestode parasites from the infected intestine were collected and observed under the microscope. Identical worms were sorted out; few of these were fixed in 4% formalin for taxonomic identification.

Estimation of Lipid by Folch *et al*. 1957

Mystus seengala dissect by sharp scissor and separate their intestine and intestine dissect by sharp scissor and separates their cestodes parasites in petri dishes contain water and also cut the muscle of fish in small pieces and small pieces kept in oven at 60^0C until the tissue become dry and taken 100 gm. of dry Tissue and tissue was homonized with ration of chloroform; methanol mixture (2:1) and 0.2 of Nacl (1%) centrifuged for 5 minutes at 3000 rpm. The lower phase comprising of chloroform methanol layer contained all the lipid was separated and evaporated at room temperature overnight. To this test tube 2 ml Concentrated H_2So_4 was added and boiled for 10 minutes in a water bath and cooled.

The sample volume 0.1 ml was taken in a clean test tube and made up to 1 ml with concentrated H_2So_4 acid to which 2.5 ml of phospho Vanillin reagent was added and incubated for 30 minutes. The color developed was read at 530 µm in a colorimeter against reagent blank. The amount of lipid was determined by referring to the standard graph prepared by using Cholesterol as standard lipid. The lipid concentration was expressed as mg/gm wet weight of the tissue.

Estimation of protein by lowr'y method

Small pieces of fish of were also collected from the infected intestine fish for the protein estimation. Protein content in muscle of tissue was carried out by using Lowry's method. The collected worms were kept on dried blotting paper to removed excess water. The material was transferred in to previously weighted watch glass and kept in oven at 60^0c until the muscle pieces become dry. Dry weight of tissue was taken. Tissue was weighted on mgs sensitive balance.100mg of tissue was taken and added 100ml TCA homogenate for 15min at 3000 RPM. Supernatant was drained and ppt was dissolved in 100ml 1N NaoH. Then o.1ml solution was taken and added 4ml Lowery's C solution and 0.4ml folin phenol reagent and cooled it for 30min (kept test tube in dark). Reading were taken with the help of colorimeter O.D at 530µm. setting blank 0.1ml (1N NaoH) and added 4ml Lowery's C and 0.4ml folin's phenol reagent. Standard blank solution was made by adding Bovins serum albumin in 100ml 1N NaoH same producer was applied for obtaining the protein from host intestine.

Mystus seengala

Collected cestodes from Mystus seengala fish

Fig 1

All results in three replicates

Result and Discussion

Protein

Fish is rich source of protein but nutrient value of selected fish decreases due to extra burden of cestode parasite depends on his protein source for his survive so, biochemical study of fish most important because fish is affordable source of protein consumed by society. In present biochemical analysis of fish *Mystus seengala* infected with cestode parasites. Table no. 1 showed that the protein content in infected and non-infected fish 19.28 mg/gm of dry tissue in Mystus seengala and in non-infected protein content 21.78 mg/gm, 20.18, 22.09 and 18.12,20.21 respectively of tissue it was higher than infected one in fish tissue.

Table 1: Protein in mg/g of dry weight of tissue in *Mystus seenghala*

Month	Protein in mg/g of dry weight of tissue in *Mystus seenghala*	
	Infected	Non-Infected
March-May2019	19.28	21.78
June-Sept 2019	20.18	22.09
Oct-Dec 2019	18.12	20.21

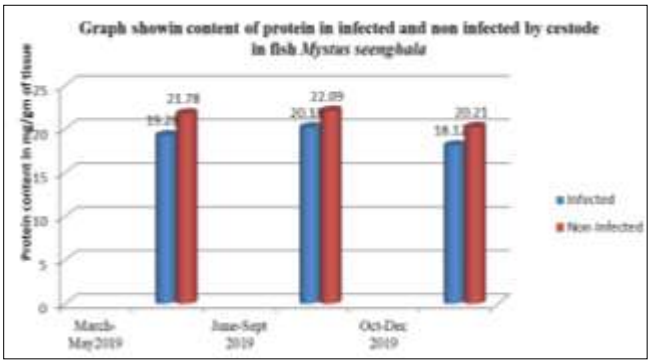

Fig 2: Graph showin content of protein in infected and non infected by cestode in fish *Mystus seenghala*

Glycogen

Fish is source Glycogen in low cost easily available in local market to society consumption need due its function in body need it maintain a physiological blood glucose

concentration, So, glycogen one of important factor in diet and this requirement full fill by fish but cestode parasite present in fish one the parasitic disease decreases nutritional value of fish and increase certain case of their mortality so, present work was carried on biochemical analysis of infected and non-infected fish glycogen content showing in table no.2. Results indicate that the glycogen content in infected and non-infected fish *Mystus seengala* were recorded 24.70, 28.12, 25.16,29.23 and22.89,26.45 in between period of March to dec-2019 respectively comparatively low in infected fish than non-infected one.

Table 2: Glycogen content in mg/100 ml of solution in of *Mystus seenghala*

Glycogen present in mg / 100 ml of solution in of *Mystus seenghala*		
	Infected	**Non-Infected**
March-May2019	24.70	28.12
June-Sept 2019	25.16	29.23
Oct-Dec 2019	22.89	26.45

Lipid

Table 3. shows the lipid content in *Mystus seenghala* 11.23, 12.15, 10.14 mg/100mg dry wt. of tissue per ml sol^{n}) was higher than in the infected 12.90, 13.20 and 11.05 .g/100mg dry wt. of tissue per ml sol^{n}) and non-infected (0.42 ± 0.13 mg/100mg dry wt. of tissue per ml sol^{n} .

Table 3: Lipids mg/gms in of *Mystus seenghala*

Month	**Lipids mg/gms in of *Mystus seenghala***	
	Infected	**Non-Infected**
March-May2019	11.23	12.90
June-Sept 2019	12.15	13.20
Oct-Dec 2019	10.14	11.05

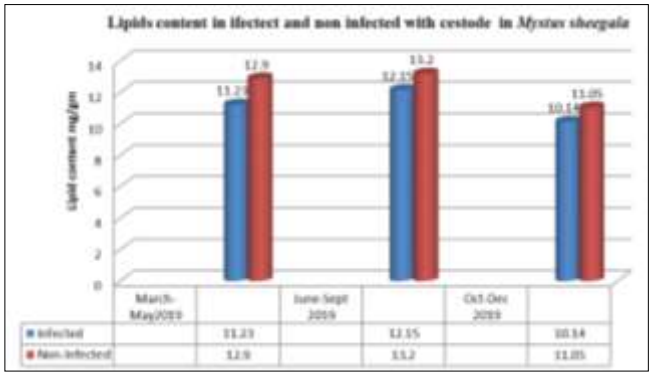

Fig 3: Lipids content in ifectect and non infected with cestode in *Mystus sheegala*

Discussion and Conclusion

The biochemical composition of fishes collected from wakadi dam fresh water shows *Mystus* species in their muscle protein, glycogen and lipid contents decreases in infected tissue as compared to normal and healthy fish. The fishes showed successive decrease in protein and lipid contents from June to May Whereas muscle glycogen content of fishes shows significant increase during June to May are shown in Table 1. Reay (1933), researcher carried out same work and they reported that deterioration of protein is linked with denaturation of fish protein are linked with frozen fish Das (2009) reported that Lipid content of Rohu (Labeo rohita), Grass carp (Ctenopharyngodon idella) and Tilapia (*Oreochromis mossambica*) were studied in fresh condition as well as freezing condition in different species showed different lipid level at different condition like Temperature, Freezing time, Location size. Padmawati and Prema Kumari (2006) also reported that changes in biochemical contents of muscles of fish species may also be attributed to alterations is due to increased glycogenesis in muscles and accelerated conversion of liver glycogen into muscle glycogen due to infection of cestode. Chamundeshwari Devi and Vijayaragahwan (2001) observed that changes in biochemical parameters in the fishes are linked to their habitat and nutritive values of each species. Protein, glycogen and lipid are important biomolecule of each and every organism. Caryophyllidean cestodes absorb all nutritive biomolecule from their host body. The present study was examining the relationship between cestodes infection and nutrient reserve in fresh water fish *Mystus seenghala* in relation to infection with cestodes parasite.

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