



Biochemical Compositions in Muscle and Liver of Normal and Infected Fish of *Johnius* off Visakhapatnam Coast

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Abstract: Proteins, lipids and carbohydrates in muscle and liver tissues were studied with respect to different seasons in *Johnius*. Marked changes were observed in the biochemical composition of the muscle and liver of normal and infected fish. High protein content was observed during pre-monsoon (81.7 ± 0.11 mg/g) in normal muscle. While high lipid concentration was seen in post-monsoon ($43.7 + 0.67$ mg/g) in normal liver. Whereas high concentration of carbohydrates was found in post-monsoon ($3.9 + 0.84$ mg/g) in infected muscle.

Keywords: Carbohydrates, lipids, proteins and *Johnius*.

1. INTRODUCTION

Fish is a vital source of human food particularly in terms of high quality proteins. For the people of Eastern India in general and North East India in particular fishes constitute a major component of diet. In the present study *Johnius* is taken since they occur throughout the year in considerable numbers at Visakhapatnam coast whereas other species occur rarely.

The genus *Johnius* belongs to the family Sciaenidae. The genus *Johnius* is represented by many species like *J. dussumieri*, *J. diacanthus*, *J. aneus*, *J. coibor*, *J. osseus*, *J. maculatus*, *J. sina*, *J. carutta*, *J. axillaris*, *J. soldado*, *J. argentatus* etc., But in and around Visakhapatnam coast only very few species are available like *J. aneus*, *J. maculatus* etc. *Johnius* is commercially known for their delicacy as food fish and have good quality of proteins and other nutrients.

2. MATERIAL AND METHODS

To find out the concentrations of proteins, lipids and carbohydrates in *Johnius* samples were collected from fishing harbour and local fish markets. In the laboratory they were thoroughly cleaned with running tap water and the excess water was removed with blotting paper. After recording the necessary morphometric and meristic characters of the fish collected from the study area the specimens were dissected immediately to avoid decomposition. Muscle samples were removed without skin and liver was removed separately from fish samples. The tissues were kept in hot air oven at 60°C for about a week to dry the material. After drying the tissue samples were pulverized and ground into a fine powder with the help of a porcelain mortar. The powder was preserved in desiccators for later use. Individually weighed powder samples were used for the quantitative estimation of proteins, lipids, carbohydrates, moisture, ash and fiber in muscle tissue. All the chemicals used were of analytical grade.

The proteins were homogenized in 10% Trichloroacetic acid (TCA) and centrifuged at 2000 rpm for 20 minutes at room temperature. The TCA supernatant was decanted into separate test tube. The sediment was in 1 N sodium hydroxide. Both sodium hydroxide dissolved sediment and TCA supernatant were estimated for proteins according to the method of Lowry et al, (1951) as modified by Schacterle and Pollack (1973). Content of the muscle tissue was estimated by following Lowry's method (1951), which involves two steps. In the 1st step, the carbonyl groups of protein molecules react with copper and potassium of the reagent resulting in the formation of a blue coloured copper potassium biuret complex. This complex together with tyrosine and phenol compounds present in the protein reduces the phospho-molybdate of the foline reagent to intensify the colour of the solution. The colour concentration (optical density) was measured by using an U.V. Spectrophotometer

(BioAurius CECIL CE7250 7000 series make) at the wave length of 620 nm to find out the protein content. To estimate the amount of protein, 10 mg of fine dried powder was taken to which 5 ml of 1N- sodium hydroxide was added and homogenized the content and centrifuged for about 15 min at 2500 rpm. 0.1 ml of solution was taken into a test tube and 0.9 ml of distilled water was added to get 1 ml of solution. Then 4 ml of Lowry's mixture was added (Lowry A + Lowry B; Lowry A is a mixture of 10 gm anhydrous Sodium carbonate and 2 gm of Sodium hydroxide in 500 ml of distilled water. Lowry B is also a mixture of 0.5 gm of Copper sulphate and 1 gm of Sodium potassium tartrate in 50 ml of distilled water each). Then shake the contents well and kept for incubation for about 15 min. To this 0.5 ml of Foline phenol reagent (phenol and distilled water at 1:1 ratio) was added and shake the contents once again for the development of colour. The colour concentration (optical density) of the samples were measured with an U.V. Spectrophotometer and noted down the readings of absorption. All the samples were taken in triplicates.

The dried tissue was homogenized washing of chloroform, methanol; 2:1 according to the procedure of Folch *et al.*, (1957). This was centrifuged at 2000rpm at room temperature and filtered. This was then dried in oven at 60°C for about until constant weight was obtained. After completion of drying the final weight was recorded. The difference between the initial and final weights gives the weight of the lipids which is expressed as percentage of the body weight.

Anthrone in sulphuric acid can be used for colorimetric determination of sugars, methylated sugars and polysaccharides (Dubois *et al.*, (1956). The assay is very simple, rapid, inexpensive and highly sensitive. The colour produced is very stable and the assay is largely unaffected the presence of proteins.

To estimate the amount of carbohydrate, 10 mg of a weighed amount of fine dried powder was taken in a test tube. To which 5 ml of 1N sodium hydroxide was added and homogenized the content and centrifuged for about 15 min at 2500 rpm. The supernatant was taken separately and kept for further analysis.

To estimate the amount of carbohydrates 0.5 ml of solution was taken into a test tube and to this 0.5 ml of distilled water was added to get 1 ml of solution. To this 5 ml of anthrone in sulphuric acid (50 mg of anthrone in 100 ml of concentrated sulphuric acid) was added. Then shake the contents well and kept for incubation for about 15 min. After completion of the process of incubation the test tubes were boiled in water bath for about 15 min. Colours were developed when the samples cooled to normal room temperature. The optical density of the samples were measured by using an U.V. spectrophotometer (BioAurius CECIL CE7250 7000 series make) and noted down the readings of absorption at the wave length of 600 nm. All the samples were taken in triplicates.

3. RESULTS

Analysis of biochemical composition was carried out in season wise samples of muscle and liver tissues of normal and infected fish of *Johniusaneus*.

4. PROTEINS

Protein was the most dominant biochemical constituent in the muscle of *Johniusaneus*. Proteins form one of the most important and complex group of biological material, as they form the chief nitrogenous constituents of the tissues of the body. A group of substances called enzymes which are agents responsible for all the chemical transformation taking places in the body are also protein in nature. Proteins serve as structural components as biocatalysts, as hormones and as depositors for the genetic information i.e., characteristic of species. These are colloidal in nature, non-diffusible and contain high molecular weights.

The maximum value of protein content in *Johniusaneus* muscle occurred as 81.2 (mg/g) during the year of 2011-2012 in post monsoon period and minimum values in pre- monsoon season. In the year 2012-2013 also having maximum value 81.7 (mg/g) in pre- monsoon and minimum value encountered as 79.9 (mg/g) in monsoon season. While the minimum values of protein content in infected *Johniusaneus* muscle occurred as 55.2 (mg/g) during the all seasons of 2011-2012 and 2012-2013.

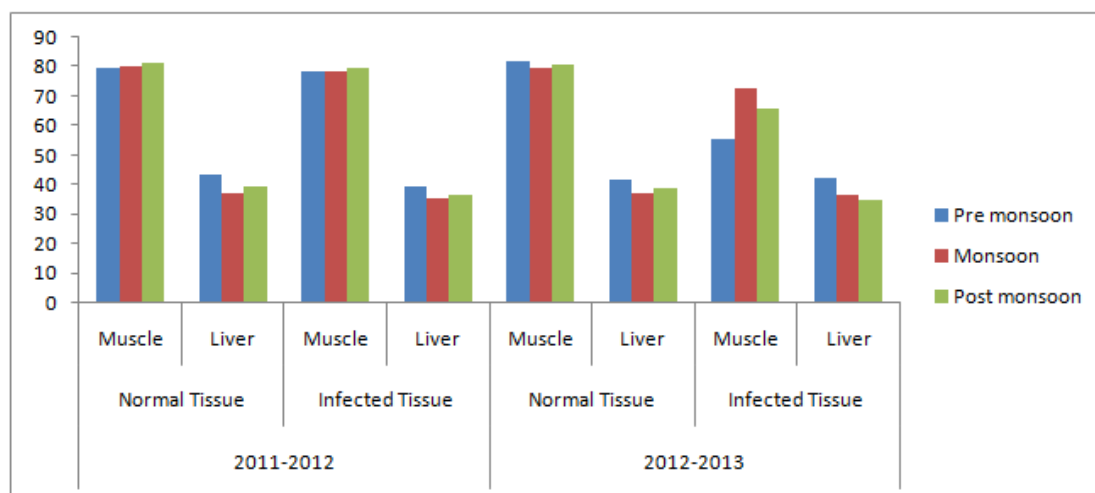
The maximum value of protein content in liver of *Johniusaneus* occurred, as 43(mg/g) during the pre-monsoon season and low value occurred in pre monsoon period during the year 2011-2012, in the year 2012-2013 high value 41.5 (mg/g) during pre-monsoon and minimum values encountered as 37 (mg/g) during monsoon. While the minimum values of protein content in infected *Johniusaneus* liver occurred as 35.2 (mg/g) during the season of 2011-2012 and 34.9 during 2012-2013 (Table: 11 & Graph: 11).

The maximum value of protein content in normal *Johniusaneus* muscle occurred as 81.7 (mg/g) in related all the seasons. While the minimum values of protein content noticed in the infected fishes. This suggests that the total protein in the muscle of normal fish was high compared with the infected muscle protein. Values obtained for total protein content of different fishes were found to be good indication of nutritional values.

Table11. Seasonal Distribution of Total Protein Levels in Normal and Infected Fish Tissues-mg/g

Seasons	2011-2012				2012-2013			
	Normal Tissue		Infected Tissue		Normal Tissue		Infected Tissue	
	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver
Pre monsoon	79.9±0.1	43.0±0.31	78.2±0.12	39.5±0.46	81.7±0.11	41.5±0.48	55.2±0.42	42.4±0.64
Monsoon	80.2±0.18	36.7±0.29	78.4±0.17	35.2±0.22	79.9±0.98	37±0.47	72.5±0.66	36.5±0.19
Post monsoon	81.2±0.9	39.2±0.14	79.9±0.19	36.4±0.45	80.9±0.79	38.6±0.54	65.8±0.12	34.9±0.03

All the data is based on average of three determinations.



Graph11. Seasonal Distribution of Total Protein Levels in Normal and Infected Fish Tissues-mg/g

5. LIPIDS

Lipids are organic substances insoluble in water, but soluble in organic solvents. They form important dietary constituents on account of their high calorific value and fat soluble vitamins and essential fatty acids contained in them. They are present in the cytoplasm as well as the cell wall and also in the specialized areas of the body as deposits of fat. Lipids are also the storage form of energy like glycogen.

The percentage of lipid content showed highest values in muscle tissue of infected fish. However, lowest lipid content was recorded for normal fish. Fat have a special importance to the animal body, which are primarily a source of energy in the diet. They offer higher calories percentage energy than that offer by protein and carbohydrate. Fats also pad to keep different body organs in place. Moreover, fats give the diet its particular flavors.

The maximum value of lipid content in the normal muscle of *Johniusaneus* occurred as 12.5 (mg/g) during the monsoon period and minimum values encountered as 10.5 in the pre monsoon season during the year of 2011-2012. While during the year 2012-2013, increased the lipid level as 12.8 (mg/g) in post monsoon period and decreased in the lipid values as 11.4 (mg/g) during the season of

pre- monsoon. When compared all the seasons during the study period of two years, the lipid levels decreased in pre monsoon.

In infected fishes, lipid levels in the muscle increased as 11.2 (mg/g) in the season of pre monsoon and decreased lipid value as 9.4 (mg/g) in post- monsoon season during the year 2011-2012, while lipid content increased as 16.5 (mg/g) in pre monsoon season and decreased to 10.4 (mg/g) in the season of monsoon during the year of 2012-2013.

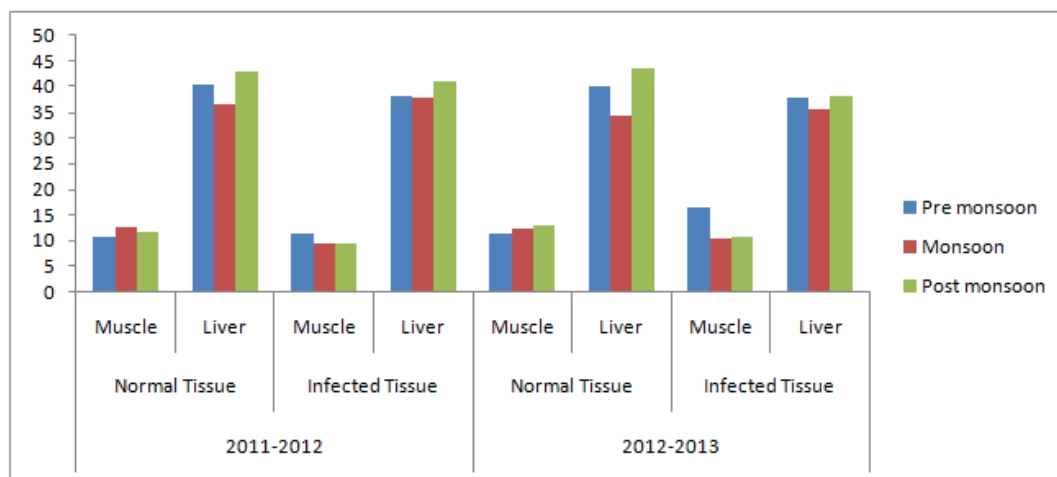
When compared all seasons of two years the lipid content in the infected muscle was elevated. Such distribution of lipids in the infected muscle suggests that are evidence for that fishes like other animals, store fat in their muscle for the supply of energy during starvation, reproductive phases and infestation period.

The maximum value of Lipid content in the liver of *Johniusaneus* occurred as 43.1 (mg/g) during the post-monsoon season and minimum value occurred as 36.9 (mg/g) during the monsoon season in the period of 2011-2012. While the lipid content level increased as 43.7 (mg/g) in the season of post-monsoon and decreased value as 34.5 (mg/g) during the monsoon season in the year of 2012-2013. While infected *Johniusaneus* liver maximum values of lipids were occurred as 40.9 (mg/g) during the post-monsoon season and decreased value 38.2 (mg/g) in the monsoon season in the period of 2011-2012 and minimum value 35.9 (mg/g) during post-monsoon, and maximum value 38.5(mg/g) in the year of 2012-2013.

Table12. Seasonal Distribution Of Total Lipids Levels In Normal And Infected Fish Tissues-mg/g

Seasons	2011-2012				2012-2013			
	Normal Tissue		Infected Tissue		Normal Tissue		Infected Tissue	
	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver
Pre monsoon	10.5±0.31	40.5±0.29	11.2±0.86	38.5±0.47	11.4±0.89	40.0±0.34	16.5±0.73	38.2±0.81
Monsoon	12.5±0.44	36.9±0.38	9.5±0.42	38.2±0.21	12.2±0.44	34.5±0.76	10.4±0.16	35.9±0.43
Post monsoon	11.5±0.21	43.1±0.24	9.4±0.83	40.9±0.37	12.8±0.57	43.7±0.67	10.8±0.92	38.5±0.76

All the data is based on average of three determinations.



Graph12. Seasonal Distribution Of Total Lipids Levels In Normal And Infected Fish Tissues-mg/g.

6. CARBOHYDRATES

Carbohydrates are basic substances of protoplasm and involved in the storage and release energy. They defined chemically as aldehyde or ketone derivatives of the higher polyhydric alcohols or as compounds which yield these derivatives on hydrolysis. Glucose, fructose, mannose, sucrose, galactose, maltose, lactose, trehalose, and glycogen are the important carbohydrates in the animal cells. Energy stored in carbohydrates is readily available for the cellular functions.

There is increase in carbohydrate level 3.8 (mg/g) in the muscle of normal fish during the monsoon season in the year 2011-2012, low level values are encountered as 2.2 (mg/g) during the post

monsoon season. While in 2012-2013 muscle carbohydrate value of normal fish shows high value 3.2 (mg/g) in the period of monsoon season and shows the low value 2.1 (mg/g) in the season of post monsoon.

The carbohydrate level in muscle of infected fish, in the season of monsoon occurred maximum value 3.5 (mg/g) and minimum value 3.1 (mg/g) during the post monsoon season in the year 2011-2012. While the maximum carbohydrate value as 3.9 (mg/g) occurred in monsoon season and minimum value as 1.5 (mg/g) encountered in the season of post monsoon during the year 2012-2013. There is increase in carbohydrate level in the infected muscle at all the samples, low level values are encountered in normal muscle tissues. This could be revealed that carbohydrates were utilized rapidly to meet the stress caused when fish was infected. This can be attributed to several factors and one of them is the decrease in the specific activity of some enzymes like phosphofructokinase, lactate dehydrogenase and citrate kinase that decrease the capacity of glycolysis metabolism in animals, followed by in muscle.

The liver of *Johniusaneus* carbohydrate values occurred, as maximum 14.5(mg/g) in the seasons of pre-monsoon and post monsoon whereas minimum values encountered as 13.5 (mg/g) in the monsoon season during the year of 2011-2012. While in the year 2012-2013, the carbohydrate level increased during the post- monsoon and decreased in the pre-monsoon period. While the minimum values of carbohydrate level in infected *Johniusaneus* liver 13.1 (mg/g) during the pre- monsoon season of 2012-2013 and maximum value 14.5 (mg/g) during 2012-2013.

7. DISCUSSION

Commonly fish is considered as a very good source of animal protein. The importance of fish in the diet is due to not merely to the percentage of protein it contains, but to the amino acid makeup of the protein and its availability to the system. Recent knowledge demonstrates that the biological value of food protein is dependent on the amino acid composition. Marine fish is supposed to be an integral part of a nutritious human diet. However, fish of various species do not provide the same nutrient profile to their consumers (Takama *et al.*, 1999)¹, and the nutritive value of a fish varies with season (Varljen *et al.*, 2003²; Imad Patrick Saoud *et al.*, 2008)³.

Proteins occur in the body in the form of amino acids and other metabolites, which serve as building blocks of the body. Hence, protein content of the cell is considered to be an important tool for evaluation the physiological standards Chezhian *et al.*, (2010)⁴. In the present study, the maximum value of total protein content reported in normal muscle of *Johniusaneus* in the pre monsoon during the study period 2011 to 2013. While the minimum values of total proteins noticed in the both pre monsoon and monsoon season during the study period. The maximum value of total protein content reported in normal liver of *Johniusaneus* in the pre monsoon during the study period of 2012 to 2013. While the minimum value of total protein content reported in the monsoon season during the study period of 2011 to 2012. In this study showed that protein was the most dominant biochemical constituent in the muscle of *Johniusaneus*. These findings correlated with many authors. Sivani (1994)⁵ and others reported that protein content was more in fishes during early summer and winter months corresponding to their maturity stages. Parulekar (1964)⁶ reported maximum protein content in the spawning specimens and the minimum associated with the spent and early maturation phases. Protein content can be correlated with the phases of maturity and spawning (Parulekar and Bal, 1969)⁷. Accordingly, protein content goes on increasing with the advancement in maturity. Similar elucidation in *Mugilcephalus* was suggested by Das (1978)⁸. Martinez *et al.*, (1999)⁹ stated that the increase in muscle aerobic capacity and protein contents. Bhuyan *et al.*, (2003)¹⁰ reported higher protein content observed.

Depletion in muscle-protein during spawning period has been reported in many fishes (Love 1970).¹¹ There was a decrease in total protein during starvation, this was principally conformed to the "soluble protein fraction" Johnston and Goldspink (1973). Medford and Mackay (1978)¹² showed that muscle protein and lipid of northern pike, *Esox lucius* were high before spawning and low after spawning. This could be attributed to the fact that these constituent might have been utilized for spawning and gonadal development. It may be due to the fact that the build-up of gonad is often accomplished at the expense of body-proteins. During the process of gluconeogenesis, protein is metabolized to yield glucose, which is utilized for energy synthesis in the form of ATP during stress conditions (Ghosh and

Chaterjee, 1985)¹³. Protein contents were greatly influenced by seasons. In general protein values are low during winter and high during summer or monsoon months (Shreni 1980)¹⁴. Plana *et al.*, (1996)¹⁵ reported protein content may be used because of this degeneration under stress conditions (starvation, emersion, abnormal temperature); a decrease in protein amounts was observed in starved infected individuals. The same results were obtained in *Ruditapes decussates* after a protracted starvation (Baghdiguan and Riva 1984). According to Satti Reddy (1992), the fall in the protein content is due to a fall in the rate of feeding, because of scarcity of food material due to turbidity and other ecological factors. Especially during monsoon months changes in the protein content during spawning season may occur due to changes in the endocrine system that monitors supply of nutrients to gonads from all parts of body including liver and muscles (Jyotsna *et al.*, 1995)¹⁶. The decline in protein might be because of a potential starvation due to the sharp decrease in the amount of plankton in January reported by Salihoğlu and Mutlu (2000)¹⁷ There was a significant seasonal effect on levels of protein or RNA, the lower values measured where those in the spring caught fish (Kemp *et al.*, 2008)¹⁸. Protein content decreased in horse mackerel in early spring (Gökhan Boran and HikemtKaraçam 2011)¹⁹.

The study thus supports the view that carbohydrate plays an insignificant role as energy reserve in aquatic animals (Love 1970), since it is in very low concentration in the muscle, liver and gonad, and that its role in the mobilization of energy during maturation and spawning. It is further supported by Vijay Kumaran (1979)²⁰ who stated that carbohydrates play a minor role in energy reserves of *Ambassisgymnoccephalus* and its depletion during the spawning season is insignificant. Carbohydrates formed a minor percentage of the total composition of the muscle. A critical appraisal of the observations indicates that the carbohydrates as energy reserves are comparatively insignificant in aquatic animals as mentioned by Love (1970). Very low values of carbohydrates recorded in the present study could be because of glycogen in many marine animals does not contribute much to the reserves in the body (Jayasree *et al.*, 1994)²¹. Ramaiyan *et al.*, (1976)²² reported similar findings in 11 species of clupeids. Phillips *et al.*, (1966) reported that carbohydrates are utilized for energy in trout, thus sparing protein for building of the body. Muscle carbohydrate content pattern in the present study showed variation from stage I to stage III. Muthukaruppan (1987)²³ also observed the same trend in *Liza parsia*. It is discerned that carbohydrate content decreased with the translocation of carbohydrates from depot site to where the energy prompt is required. Carbohydrates are considered to be the first degraded under the stress condition of animals (Chezhian *et al.*, 2010)²⁴. According to Dhavale and Masurekar, (1986), decreased level of carbohydrate constituents in tissues of toxicant exposed animals may be due to the prevalence of hypoxic condition in the tissues as a result of pollutant stress. Carbohydrate and mineral content of garfish were almost constant (Gökhan Boran and Hikemt Karaçam, 2011)²⁵. Whereas contrary with the highest amount of carbohydrate was found in *Latescalcarifer* (Ravichandran *et al.*, 2011)²⁶.

The carbohydrate levels in muscle of infected fish, in the season of monsoon maximum value occurred and declined value during the post monsoon season in the year 2011-2012. While the maximum carbohydrate value occurred in monsoon season and minimum encountered in the season of post monsoon during the year 2012-20113, which is in good agreement with previously reported results by Sivakami *et al.*, (1986)²⁷ observed gradual increase of muscle carbohydrate content with the maturation of gonads in the *Cyprinus carpio*. The increase of carbohydrate concentrations causes harmful physiological effects, reduces hormonal immune response and enhances dietary toxicity (Mona *et al.*, 2011)²⁸. We can conclude that carbohydrates are the major energy source in infected fishes.

Lipid content is an essential organic constituent of the tissues of all animals, and plays a key role in energy metabolism. Lipids are the best energy producers of the body next to carbohydrates (Chezhian *et al.*, 2010). Total lipids and cholesterol content found to be good indication of nutritional values (Sutharshiny and sivashanthini, 2011). In the present study, the increased value of lipid content in the normal muscle occurred during the monsoon and post monsoon whereas, the decline values encountered in the pre monsoon. A rise in the fat content of mackerel muscles before spawning followed by a fall after spawning was observed by Chidambaram *et al.*, (1952)²⁹. Fats also pad to keep different body organs in place. Moreover, fats give the diet its particular flavors. Seasonal differences in the availability of food and changes in the reproductive cycle have considerable effect on the tissue

biochemistry of the fish, particularly fat (Bumb, 1992). Fishes like other animals, store fat in their muscle for the supply of energy during starvation and reproductive phases. The greatest concentrations of fat may be found at the end of prolific feeding in summer and the least in winter (Love, 1980). There was a parallel decrease in the muscle lipid during same period. This could be attributed to less food intake. Richard (1997) suggested that during starvation the source of energy is lipid and carbohydrate in fishes. Higher lipid was observed in ripe and gravid fish where a low level of lipid was recorded in spent and young fish (Bhuyan *et al.*, 2003). The lipid content of the muscle showed two peak periods of accumulation- one during November and other during May–July Shreni (1980).

In the present study, proteins, carbohydrates and lipids were observed. Lipids elevated in infected muscle tissues. Whereas decline of protein has been observed in infected muscle tissue compared with normal fish. Some fluctuations have been observed in total carbohydrate content in infected fishes.

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