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A STUDY ON DISTRIBUTION OF PROTEIN, LIPIDS AND CARBOHYDRATES IN MUSCLE AND LIVER OF MARINE ASSOCIATED *UPENEUS VITTATUS*

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ABSTRACT: Fish makes a vital contribution to the survival and health of a significant portion of the world's population. *Upeneus vittatus*, a marine species found in the coast of Bay of Bengal, Visakhapatnam was selected for the present study. An attempt is made to know the distribution of Protein, lipids and carbohydrates in muscle and liver of infected and normal *Upeneus vittatus*. It is observed that a significant difference between infected and normal fish in relation with Lipid content. Lipid levels were observed to be more in the fish with infection.

INTRODUCTION: Food is an important item next to air and water for the maintenance of the process of life on the earth. Strength to fight against diseases depends on the intake and purity of the vital constituents of food, such as proteins, carbohydrates, lipids, vitamins and minerals. The strength of a nation is by and large dependent on the health of its people. The proverb" health is wealth" and "sound mind in sound body" reiterate the importance of nutrients rich food. Therefore nutrition is of great importance for living organisms and the main factor, which influence the human health.[1] Fish makes a vital contribution to the survival and health of a significant portion of the world's population.

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It is especially important in the developing world. In some of Asia's poorest countries (Bangladesh, Cambodia) people derive as much as 75% of their daily protein from fish. In West Africa fish accounts for 30% of animal protein intake, and this number would be larger if the poor could afford to buy more.[2]

It also referred to as "rich food for poor people," fish provides essential nourishment, especially quality proteins and fats (macronutrients), vitamins and minerals (micronutrients). Second, for those involved in fisheries, aquaculture and fish trade, fish is a source of income, which can be used to purchase other additional food items. [2]

Malnutrition is a major problem of the developing countries and India has no exception to it. Many scientists, not only in India, but also worldwide are trying to find out the ways and means to solve this problem. Micronutrient deficiencies of Vitamin A, iron and iodine are also of public health concern in developing countries. Their consequences include

nutritional blindness, poor learning capabilities, poor growth and increased morbidity and mortality rates. Development and agricultural programmes including fisheries and aquaculture which mainstream nutrition issues can go a long way in alleviating the problem of malnutrition in this part of the world as well as in other countries.

The importance of nutritional studies was realized more than three decades ago when culture practices of certain fresh water and brackish water fishes seriously attempted in an impounded water bodies to raise protein rich crops.

Protein is essential for growth and maintenance of the vital functions of all organisms. It helps to maintain and replace the tissues in body, and it is found in almost every living cell and fluid. Muscles, organs and hormones were made up of protein. Protein deficiency leads to various clinical and sub clinical syndromes, such as impaired health, lowered resistance to infection and susceptibility to diseases.

Fish is excellent source of protein of high biological values, and man from the immemorial consumed it. Food may be broadly classified as energy and growth foods like carbohydrates, proteins and lipids, and non energy food like minerals, Vitamins, water, and oxygen. The nutritional value of the diet is measured by the presence of necessary element and catalyst (vitamins and mineral) and auxiliary food (oxygen and water). And a proper balanced should maintain between the energy and growth foods. The study of the growth food of the fishes of various groups is considered useful to evaluate the nutritive value.[3]

Investigations regarding the protein content of the fish and fish products have been initiated as early as 19th century. After 19th century several worldwide investigations were made on by biochemical composition of the fish. Note worthy contributions are those of Greene (1919) on the biochemical changes in the muscle tissue of king Salmon during spawning migration [4]. Bruce (1924) reported on the changes in the chemical composition of Herrings in relation to age and maturity.[5]

In the developed countries nutrition related health problems arise due to over consumption of fat, saturated fatty acids.[6] Various human health organization in the world have emphasized the importance of fish as a vital food, to meet the nutritional requirement for achieving the ideal body weight.

Fish is the rich source of nutrients (protein, vitamins and mineral.) with relatively low cholesterol content. The most important constituent of the fish muscle is protein, which generally varies from 17 to 25% in fresh condition. Fish is generally less expensive, in comparison with other protein foods. Because of its high protein quality and palatability. It is used in the diets of other fishes. Fish protein is highly digestible and contains a good number of essential amino acids, their by having high biological value. The cost per kilogram of edible flesh from most of the fish is less expensive than that of meat of domestic animals. The fat content of the fish varies greatly between different species and those with low amount of fat are particular suited for weight control diets.

The fat content of fish varies depending on the species as well as the season but, in general, fish have less fat than red meats. The fat content ranges from 0.2% to 25%. However, fats from fatty fish species contain the polyunsaturated fatty acids (PUFAs) namely EPA (Eicosapentaenoic acid) and DHA (Docosahexaenoic acid, omega 3 fatty acids), which are essential for proper growth of children and are not associated with the occurrence of cardiovascular diseases such as coronary heart disease.

In pregnant women, the presence of PUFAs in their diets has been associated with proper brain development among unborn babies. In other studies, omega 3 fatty acids have also been associated with reduced risk of preterm delivery and low birth weight.[6] The fat also contributes to energy supplies and assists in the proper absorption of fat-soluble Vitamins namely A, D, E, and K.

The minerals present in fish include iron, calcium, zinc, iodine (from marine fish), phosphorus, selenium and fluorine. These minerals are highly ‘bioavailable’ meaning that they are easily

absorbed by the body. Iron is important in the synthesis of hemoglobin in red blood cells, which is important for transporting oxygen to all parts of the body. Iron deficiency is associated with anemia, impaired brain function and in infants is associated with poor learning ability and poor behavior. Due to its role in the immune system, its deficiency may also be associated with increased risk of infection.

Paton (1898) in a series of valuable studies on the life history of the Scottish salmon followed the changes in fat content through the period of development of the ovaries. He also studied the composition of the protein fraction of the muscles.[7] Balland (1898) determined the composition of a number of fish with reference to their water content, fat, nitrogen, extractives, and ash [8]. Although several studies deal with the proximate composition of biochemical components of many commercially important fishes.[9]

Fish makes a vital contribution to the survival and health of a significant portion of the world's population. *Upeneus vittatus*, a marine species found in the coast of Bay of Bengal, Visakhapatnam was selected for the present study.[10] An attempt is made to know the distribution of Protein, lipids and carbohydrates in muscle and liver of infected and normal *Upeneus vittatus.*

MATERIAL AND METHODS:

To find out the concentrations of the proteins, carbohydrates and lipids in goatfishes, samples were collected at regular monthly intervals for a period of one year (March 2007 to February 2008) at Vishakapatnam fishing harbor. After recording the necessary morphometric and meristic characteristics of the fish collected from the study areas the muscle and liver samples were removed without skin and bone pieces and dried in hot air oven for about 48 hours at temperature of 58^{o}C.

After drying the samples were pulverized and ground into fine powder with the help of mortar individually weighed powder samples were used for quantitative estimation of proteins, carbohydrates and lipids.[11]

Proteins:

The protein content of the muscles tissue was estimated by following Lowry's method (1951), which involves two steps. In the 1st step, the carbamyl groups of protein molecules react with copper and potassium of the reagent resulting in the formation of a blue colored copper potassium biuret complex.[11] This complex together with tyrosine and phenol compounds present in the protein reduces the phospho-molybdate of the foline reagents to intensify the color of the solution. The colour concentration (optical density) was measured by using an U.V. Spectrophotometer (Bio Auarius CECIL CE7250 7000 series make) at the wavelength of 620nm to find the protein content.

To estimate the amount of protein 10mg of fine dried powder was taken to which to 5ml of IN sodium hydroxide was added and homogenized the content and centrifuged for about 15 mints at 2500rpm. The supernatant was taken separately and kept for further analysis. 0.1ml of solution was taken into a Test tube and 0.9ml of distilled water was added to get 1ml of solution. Then 4ml of Lowry's mixture (Lowry A + Lowry B. Lowry A is a mixture of 10gm anhydrous Sodium carbonate and 2gm of sodium hydroxide in 500ml of distilled water. Lowry B is also a mixture of 0.5gm of copper sulphate and 1gm of sodium potassium tartrate in 50ml of distilled water each).

Then the contents are shaken well and kept for incubation for about 15 mints. To this 0.5ml 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 was measured with an U.V Spectrophotometer and the readings of absorption were noted. All the samples were taken in triplicates. [11]

Carbohydrate:

Anthrone in Sulphuric acid can be used for colorimetric determination of sugar; methylated sugars and polysaccharides. The assay is very simple, rapid, inexpensive and highly sensitive. The color produced is very stable and the assay is largely unaffected the presence of proteins.

To estimate the amount of carbohydrate 0.5ml of solution was taken into test tube and to this 0.5ml of distilled water was added to get 1ml of solution.
To this 5ml of anthrone in Sulphuric acid (50mg of anthrone in 100ml of concentric Sulphuric acid) was added. Then the contents are shaken well and kept for incubation for about 15mints.After completion of the process of incubation the test tubers were boiled in water bath for 15mints.Colours were developed when the sample cooled to room temperature .The optical density of the samples were measured by using an U.V Spectrophotometer (Bio Aquarius CECIL CE7250 7000 series make) and the readings of absorption were noted at the wave length of 600nm. All the samples were taken in triplicates.[11]

LIPIDS:
The total lipids were extracted from the dry tissue. 50mg of the dry tissue was weighed and homogenized in 5ml Chloroform Methanol mixture (Chloroform and Methanol mixed at ratio of 2:1). The contents were centrifuged for 15 mints at 2500rpm by using laboratory centrifuge (REMI R8C Model). The supernatant was taken in an aluminium boat (pre weighed), dried in hot air oven at 60^0 C and the final weight was recorded. The difference between the initial and final gives total lipid content of the tissue. The concentrations were given as percentage dry weight of the tissue.[11]

RESULTS AND DISCUSSION: The results of biochemical composition of muscle tissue and liver of both normal and infected fish *Upeneus vittatus* have been presented .All the biochemical components of i.e proteins, carbohydrates and lipids were measured by following standard methods of universally accepted. Heavy infection occurred during the months of April and May in which more number of Nematodes were collected. Whereas during the month January to May the infection and prevalence of parasites increases. During the months of August to October no infection occurs. Month wise results of the biochemical composition of the muscle and liver were furnishes in tables (**Tables 1, 2, 3, 4, 5** and **6**).

Proteins:
The maximum value of protein content in muscle occurred as 677.50(mg/g) during the month of August 2007, while the minimum value observed as 516.10 (mg/g) during the month June 2007. The mean values of normal and infected fish were 626.11 (mg/g) and 564.64 (mg/g) respectively (**Table1** and **Fig.1**)

TABLE 1: PROTEIN CONTENT (mg/g) IN MUSCLE OF *UPENEUS VITTATUS*

S.No	Month	Protein (mg/g)
1.	March	621.34 (IN)
2.	April	614.18 (IN)
3.	May	653.48 (IN)
4.	June	516.10 (IN)
5.	July	499.95 (IN)
6.	August	677.50(N)
7.	September	628.25 (N)
8.	October	599.10 (N)
9.	November	526.70 (IN)
10.	December	520.75 (IN)
11.	January	590.46 (N)
12.	February	635.25 (N)

N – Normal individual, IN –Infected individual, Mean values: Normal – 626.11, Infected - 564.64.

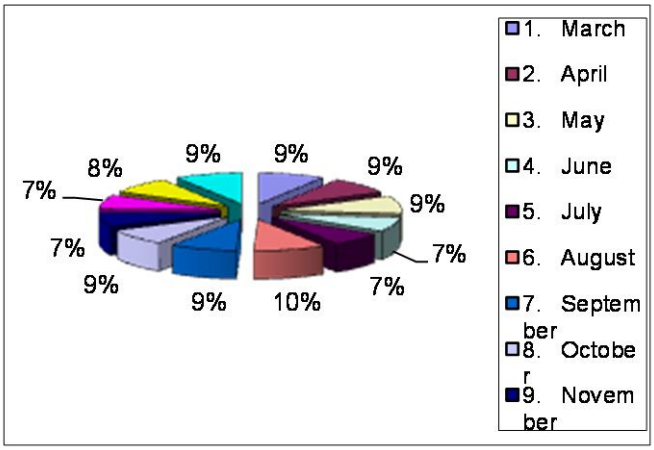

FIG.1: PROTEIN CONTENT (%) IN MUSCLE OF *UPENEUS VITTATUS* DURING MARCH 2007 –FEBRUARY 2008

The maximum value of protein content in liver occurred 398.10 (mg/g) during the month of October 2007, while the minimum value observed as 320.16(mg/g) during the month March 2007. The mean values of normal and infected fish were 377.21(mg/g) and 339.6 (mg/g) respectively (**Table 2** and **Fig. 2**).

Carbohydrate:
The carbohydrate content in the muscle maximum value occurred as 90.15 (mg/g) during the month of September 2007, while the minimum value observed as 39.10 (mg/g) during the month

March 2007. The mean values of normal and infected fish were 84.12 (mg/g) and 47.95 (mg/g) respectively. (**Table 3** and **Fig. 3**)

TABLE 2: PROTEIN CONTENT (mg/g) IN LIVER OF *UPENEUS VITTATUS*

S.No	Month	Protein (mg/g)
1	March	320.16(IN)
2	April	346.28 (IN)
3	May	339.46 (IN)
4	June	351.25 (IN)
5	July	343.56 (IN)
6	August	395.10 (N)
7	September	388.35 (N)
8	October	398.10 (N)
9	November	330.46(IN)
10	December	346.55(IN)
11	January	355.10 (N)
12	February	349.40 (N)

N – Normal individual, IN –Infected individual, Mean values: Normal - 377.21, Infected- 339.6

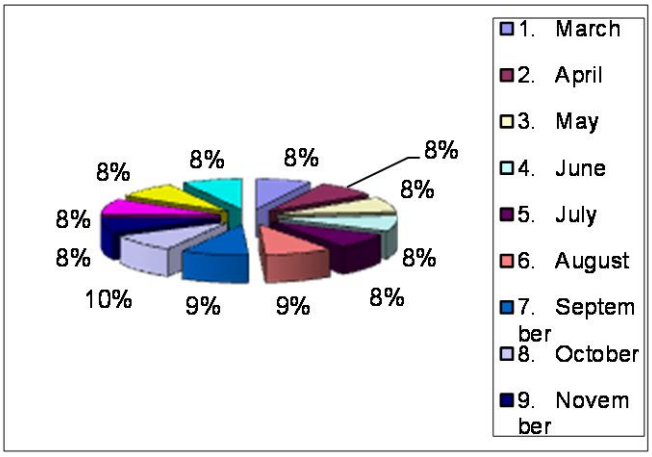

FIG.2: PROTEIN CONTENT (%) IN LIVER OF *UPENEUS VITTATUS* DURING MARCH 2007 –FEBRUARY 2008

TABLE 3: CARBOHYDRATE CONTENT (mg/g) IN MUSCLE OF *UPENEUS VITTATUS*

S.No	Month	Carbohydrate (mg/g)
1	March	39.10 (IN)
2	April	47.70 (IN)
3	May	55.35 (IN)
4	June	49.50 (IN)
5	July	56.40 (IN)
6	August	85.40 (N)
7	September	90.15 (N)
8	October	81.40 (N)
9	November	42.50(IN)
10	December	45.15 (IN)
11	January	80.44 (N)
12	February	83.21 (N)

N –Normal individual, IN –Infected individual, Mean values: Normal -84.12, Infected - 47.95

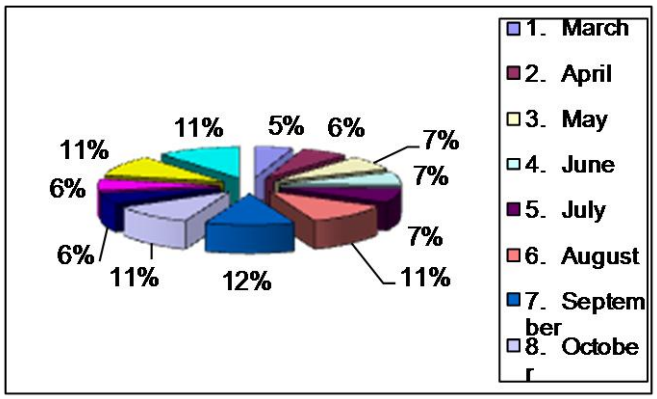

FIG.3: CARBOHYDRATE CONTENT (%)IN MUSCLE OF *UPENEUS VITTATUS* DURING MARCH 2007 –FEBRUARY 2008

The carbohydrate content in the liver maximum value occurred as 79.45(mg/g) during the month of August 2007, while the minimum value observed as 35.30(mg/g) during the month July 2007. The mean values of normal and infected fish were 73.3(mg/g) and 36.99(mg/g) respectively. (**Table 4** and **Fig.4**)

TABLE 4: CARBOHYDRATE CONTENT (mg/g) IN LIVER OF *UPENEUS VITTATUS*

S.No	Month	Carbohydrate (mg/g)
1	March	37.24 (IN)
2	April	35.42 (IN)
3	May	39.50 (IN)
4	June	36.40 (IN)
5	July	35.30 (IN)
6	August	79.45 (N)
7	September	76.10 (N)
8	October	71.50 (N)
9	November	38.60 (IN)
10	December	36.50 (N)
11	January	69.50 (N)
12	February	70.30 (N)

N – Normal individual, IN –Infected individual, Mean values: Normal -73.37, Infected - 36.99

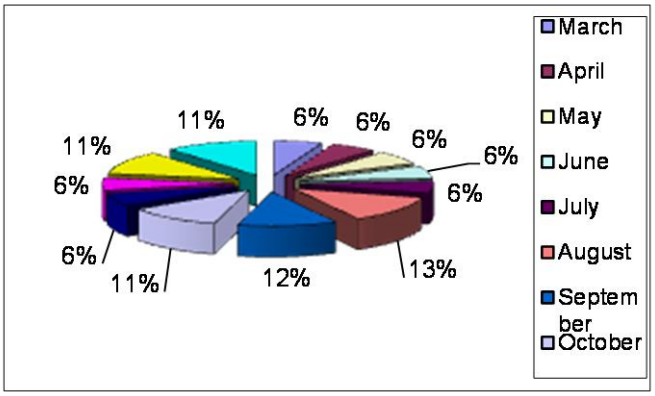

FIG.4: CARBOHYDRATE CONTENT (%) IN LIVER OF *UPENEUS VITTATUS* DURING MARCH 2007 –FEBRUARY 2008

Lipids:

The maximum value of lipids content in muscle occurred as 60.25 (mg/g) during the month of June 2007 while the minimum value observed as 41.30 (mg/g) during the month January 2008. The mean values of normal and infected fish were 45.05(mg/g) and 57.17(mg/g) respectively (**Table 5** and **Fig. 5**)

TABLE 5: LIPID CONTENT (mg/g) IN MUSCLE OF *UPENEUS VITTATUS*

S.No	Month	Lipid (mg/g)
1	March	56.10 (IN)
2	April	55.50(IN)
3	May	58.40 (IN)
4	June	60.25 (IN)
5	July	57.35 (IN)
6	August	45.10 (N)
7	September	48.30(N)
8	October	46.45 (N)
9	November	55.40 (IN)
10	December	56.25 (IN)
11	January	41.30 (N)
12	February	44.25 (N)

N – Normal individual, IN –Infected individual, Mean values: Normal - 45.05, Infected – 57.17

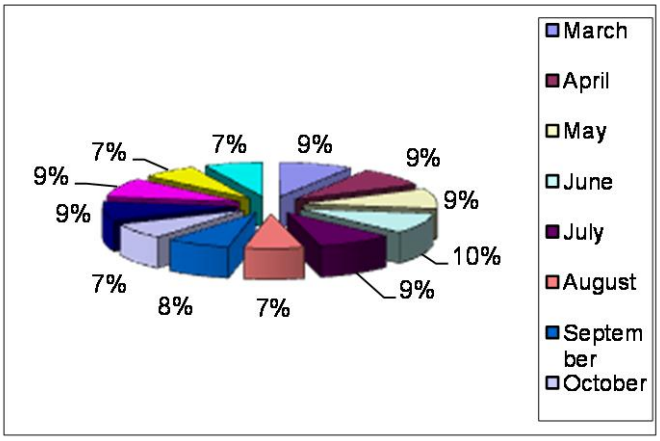

FIG.5: LIPID CONTENT (%) IN MUSCLE OF *UPENEUS VITTATUS* DURING MARCH 2007 –FEBRUARY 2008

The maximum value of lipids content in liver occurred as 61.60(mg/g) during the month of December 2007, while the minimum value observed as 43.35(mg/g) during the month January 2008. The mean values of normal and infected fish were 47.02(mg/g) and 58.9(mg/g) respectively (**Table 6** and **Fig.6**)

All the above observations are further verified by subjecting the detail on biochemical composition to the students't' test of significance.

TABLE 6: LIPID CONTENT (mg/g) IN LIVER OF *UPENEUS VITTATUS*

S.No	Month	Lipid (mg/g)
1	March	57.20 (IN)
2	April	58.50 (IN)
3	May	57.30 (IN)
4	June	59.60 (IN)
5	July	60.10 (IN)
6	August	47.40 (N)
7	September	48.60 (N)
8	October	49.30 (N)
9	November	59.25 (IN)
10	December	61.60 (IN)
11	January	43.35 (N)
12	February	46.45 (N)

N – Normal individual, IN –Infected individual, Mean values: Normal - 47.02, Infected - 58.9

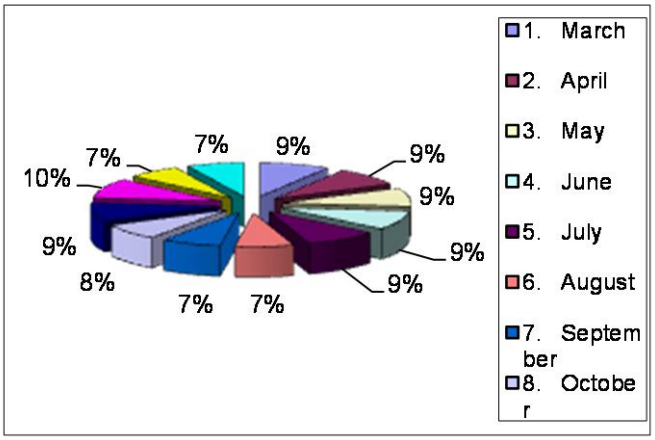

FIG.6: LIPID CONTENT (%) IN LIVER OF *UPENEUS VITTATUS* DURING MARCH 2007 –FEBRUARY 2008

It was already reported the changes in the composition of biochemical constituents of biota vary not only with environmental changes, but also with the seasons. Such changes were also be attributed to various physiological and other factors like maturation, spawning, feeding etc., Love (1957), Jacquot (1961) Saha and Guha (1940) reported that the chemical composition is dependent on age , sex, habitat and seasons. In tropical countries comparatively little is known about the chemical composition of the marine fishes.

Bruce (1924) and others reported that the protein content was more in fishes during early summer and winter months corresponding to their maturity stages. During maturity stages gonads get studded with proteins as well as fat and therefore the muscle generally contains relatively meager amount of protein and fat. [5]

A critical appraisal of the observation indicates that the carbohydrates as energy reserves are comparatively insignificant in aquatic animals as mention by Love (1970). In the present study the percentage of carbohydrate was very low when compared with protein and lipids. Bruce (1924) reported that percentage of protein was more in early summer and winter, which corresponds to the maturing stage of the fishes.[5]

During the maturity, the gonads get studded with proteins and fats, and hence the muscle contains comparatively less proteins and fat. Similar observations were made in the present study. Reported that lipids are present in the body in the form of non calorific metabolic proteins. According to Hickling (1947) lipid content is directly related to the intensity of the feeding.

Lipid content was observed to be more in the fish with infection and with nematode parasites, with favorable conditions might have produced abundant food material that is needed for the fish to grow well and to be rich in different biochemical constituents. Chemical composition of fish is affected by environment conditions and there are considerable differences in the chemical composition of reared and wild populations. The lipid content in the present study shows similar results and correlates with earlier studies. (**Plate 7**) A probe into the biochemical composition of the fish reveals the percentage decrease in the proteins, carbohydrates and lipids from the tables (**Tables 1, 2** and **3).**

The present observations are in strong concurrence with the similar studies by various authors. Sivakami *et al*. (1994) reported that there was a marked decrease in protein; carbohydrate and lipids content in fresh water fishes *Mystus vittatus* exposed chromium. They observed that the protein content decreased a quiet markedly in the exposed fish. The same was true for the carbohydrate content also, whereas the decrease was not so significant in the lipid content.[12] Reddy and Rao (1985) also observed a decrease in the carbohydrate in prawns exposed to Phosphamiolon.[13]

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. Higher fat and protein were observed in ripe and gravid fish where a low level of fat and protein was recorded in spent and young fish.[14]

In present study, It is observed that a significant difference between infected and normal fish in relation with Lipid content. Lipid levels were observed to be more in the fish with infection. Variation in biochemical composition in present study seems to be governed by spawning cycle and feeding activity.

CONCLUSION: In the present study an attempt is made to know the distribution of Protein, lipids and carbohydrates in muscle and liver of infected and normal *Upeneus vittatus.* We found that a significant difference between infected and normal fish in relation with Lipid content. Lipid levels were observed to be more in the fish with infection.

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