JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2009. Vol. **3**(2), p. 715-718

Observation of Carbohydrate Levels in Liver and Intestine of *Nemipterus japonicus* in relation to *Klebsiella* spp infection

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(Received: 09 May 2009; accepted: 12 June 2009)

Nemipterus japonicus **of Visakhapatnam coast collected from the polluted harbour waters, infected by *Klebsiella* a Gram Negative bacteria showed an abnormal elevation in carbohydrate levels in liver and a decline of carbohydrate levels in intestine, when compared with the normal fish collected from the unpolluted waters of deep sea.**

Key words: Carbohydrate levels, Liver, Intestine, Infection, *Klebsiella* spp, *Nemipterus japonicus.*

The heavy metals being discharged into the aqueous environment are sublethal to the aquatic organisms on prolonged exposure. Fish populations are sensitive to environmental changes and show a drastic biochemical fluctuations in the tissues compared with populations of unpolluted aqueous environment. The carbohydrates are one of the biochemical constituents that are mostly affected by infection and environmental changes.

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Carbohydrates are basic substances of protoplasm and are involved in the storage and release of energy. They are 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. The present analysis is carbohydrates was done in comparison with normal and infected tissues of *N. japonicus*.

MATERIAL AND METHODS

The specimens were collected from the fishing harbour and brought to the laboratory. In the laboratory they were thoroughly cleaned with running tap water and the excess water was removed with blotting paper. The specimens were dissected immediately to avoid decomposition. Liver and Intestine were removed separately from

both uninfected and infected fish. The tissue was kept in hot air oven at 60° C for about a week to dry the material. The dry tissue was ground into a fine powder in a porcelain mortar. The powder was preserved in desiccators for later use. The samples thus obtained were used for the determination of carbohydrate levels in the tissue. All the chemicals used were of analar grade.

Carbohydrate of intestine and liver of normal and infected fish were estimated by the method of Dubois et al (1956). A weighed amount of finely ground powder of about 10mg was taken in a test tube. To this 5ml of trichloro acetic acid was added and homogenized thoroughly. The content was centrifuged at 2500rpm for about 15minutes. The supernatant solution was measured and used as a stock solution.

To 0.5ml of stock solution taken in triplicates, 0.5ml of distilled water was added to make 1ml of solution. 5ml of Anthrone Sulphuric acid AR was added directly to the surface of the solution and allow it to cool for 15 minutes. The solution was boiled in a water bath for 15min. A blank was prepared with distilled water. Samples were allowed to cool and readings were noted in Hitachi U-2001 Spectrophotometer against water blank. Total content of carbohydrates were calculated from a standard curve prepared earlier using D-glucose.

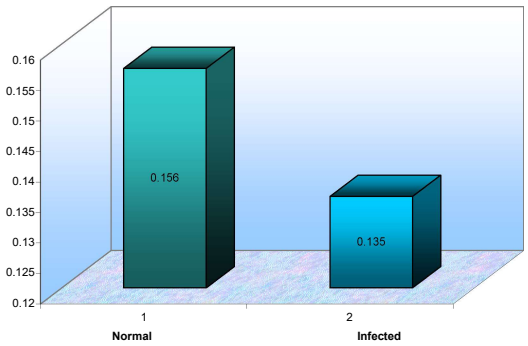

Fig. 1. Carbohydrate content in Intestine

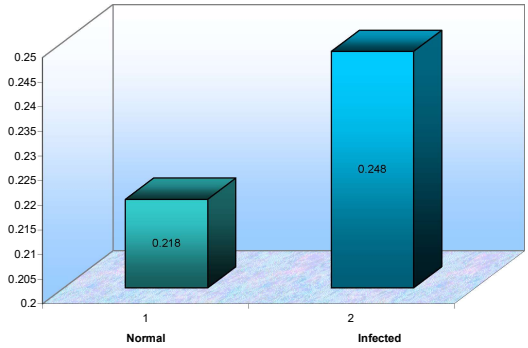

Fig. 2. Carbohydrate content in Liver

RESULTS

The results obtained in the present investigation are shown in Table. It is graphically represented in Histogram. There is an increase in carbohydrate level in the liver of infected fish than in the normal fish. Intestine carbohydrate levels had a slight fall in the infected fish when compared with normal.

The carbohydrate level in normal intestine is 0.156109 mg/gm, infected intestine is 0.135109 mg/gm, normal liver is 0.218109 mg/gm, and infected liver is 0.248109 mg/gm.

DISCUSSION

Investigations regarding the carbohydrate contents of fish and fish products were done earlier by many workers. The biochemical constituents of fish depend upon the growth, environment, diseases, stress, temperature etc. The present study on *Nemipterus japonicus* show an abnormal variation of carbohydrate contents in liver and intestine tissues of infected fish compared with the normal fish. This was supported by Southgate et al (1994) who made extensive study on the growth and biochemical composition of cultured seabass and reported a decline in carbohydrate contents. Cartee et al (2000) observed elevated levels of aminoacid in white muscle at different times of protein synthesis in Atlantic salmon. Venkaiah and Lakshmipathi (2000) observed and estimated that biochemical constituents on the skin are low of carbohydrates compared with protein and lipids in *Heteropneustes fossilis* and *Clarias batrachus*.

Table 1. Carbohydrate levels in normal fish tissues-mg/gm

Tissue	Sample-I	Sample-II	Sample-III	Average
Intestine	0.152	0.157	0.159	0.156
Liver	0.204	0.234	0.216	0.218

Table 2. Carbohydrate levels in infected fish tissues-mg/gm

Tissue	Sample-I	Sample-II	Sample-III	Average
Intestine	0.149	0.122	0.134	0.135
Liver	0.260	0.238	0.246	0.248

Ackerman et al (2000) showed the relationship between stress protein levels and hormonal stress response which resulted in increased glucose levels in hepatocytes of salmonids. Investigation made on carbohydrate levels in *C.nigrodigutatus, B.fitamentosus* and A.*occidentalis* reveal that carbohydrate contents are generally low in 3 sps {Abdullah (2001)}.

Bhuiyan *et al*. (2003) worked on carbohydrate fluctuations in *P. bilineatus* and *C. cynoglossus*. Mahboob *et al* (2003) worked on proximate composition of liver in wild and farmed *Labeo-rohita* resulted in high percentage of carbohydrate in wild spp. Nazrul Islam and Abdur Razzag Joadder (2005) estimated carbohydrate content in fresh water gobi, *Glossogobius giuris*. Nargis (2006) worked on muscle carbohydrate of Koi fish, *Anabas testudineus*. Jesu Arockia Raj et al (2008) worked on the different carbohydrate levels in fresh water catfish *Mystus montanus* and recommended optimum requirement of carbohydrate levels for better growth of fish.

The present study on *Nemipterus japonicus* revealed the high intensity and prevalence of infection at the polluted site of the Visakhapatnam port. As metal contaminants have a great effect on fish biochemical constituents abnormal fluctuations has been observed. This has been supported by Zinada (2000) who studied on the effect of niclosamide in *Liza ramada* detected in muscle an increased mulluscicide residues with increase in concentration of niclosamide, caused metabolic disturbances in fish. Solomatina et al (2001) stated that changes in indices of carbohydrate in carps is due to radionuclides accumulation in fish bodies. According to Sobha *et al* (2007) the levels of total proteins, in tissues of liver, exposed to cadmium chloride showed sub lethal concentrations of fall except glucose indicative of the organisms response to the toxicant stress. Martin deva prasanth and Arivoli (2008) studied the toxic effect of mercuric chloride on fresh water fish *Catla catla* which showed significant fluctuations in the values of carbohydrates in muscle, and intestine tissues.
The present investigation revealed that the bacterial infection of *Klebsiella* spp a Gram Negative capsulated form made of protein and lipid is one of the cause for the fluctuation of carbohydrate levels in infected tissues which in turn, effects the biochemical composition in fish tissues. This is supported by the following authors. According to Olsen et al (2002) acute stress led to significant alteration in enterocytes lining of gastro intestinal tract, while liver glycogen stores were depleted resulting in decrease of adherent bacteria in midgut and hindgut of stressed fish accompanied by bacterial contents of feaces due to sloughing of mucus, eliminating existing microflora and allowing to remain pathogenic bacteria in gut lumen to colonize the suface of the enterocytes in groups of Atlantic salmon.

In the present study carbohydrate levels were observed in both normal and infected fish. carbohydrate levels were elevated in infected fish liver where as declined in infected intestine tissues compared with the normal fish.

ACKNOWLEDGMENTS

One the author (Diana, T.C.) grateful to the authorities of Andhra University for allowing Full-Time Ph. D programme and provide the facilities during the course of study.

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