The Asian Journal of Animal Science (December 2009 to May 2010) Vol. 4 Issue 2 : 143-145

RESEARCH PAPER

Changes in the protein content of liver and intestine in relation to *Klebsiella* infection in *Nemipterus japonicus* from the coast of Vishakhapatnam, India

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Accepted : July, 2009

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ABSTRACT

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

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

Proteins form one of the most important and complex groups of biological materials, 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 place in the body are also protein in nature. Proteins serve as structural components as biocatalysts, as hormones and as depositors for the genetic information characteristic of a species. These are colloidal in nature, non-diffusible and contain high molecular weights. Once the protein denatures, it looses all its biochemical unlike carbohydrates and fats. The proteins are stored in the body in only limited amount and is quickly available for use when needed.

In healthy fish, protein content tends to be relatively constant for a given species. In general, there is no significant seasonal variation in the protein content of fish, but during the periods of starvation and gonad development, non-fatty fish draw on their carcass protein.

In the present study, the changes in protein content of *Nemipterus japonicus* (Bloch) in relation to infestations of Gram negative bacteria of *Klebsiella* spp. has been carried out.

MATERIALS 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 60^0 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 protein levels in the tissue. All the chemicals used were of analar grade. The total proteins were estimated by the method of Lowry *et al.* (1951)

A weighed amount of material about 10mg was taken in a test tube. To this 5ml of 1% NaOH solution was added and homogenized thoroughly. The content was centrifuged at 2500 rpm, for 15 min. The supernatant solution was measured and this was used as stock solution.

To 0.1ml of stock solution, 0.9ml of distilled water was added to get 1ml of solution. To this solution, 5ml of Lowry C was added and kept for incubation for 15 min. Later, 0.5ml of Folin Phenol reagent was added and the colour intensities were measured at 720nm using Hitachi-U-2001-spectrophotometer. The protein content of sample was read from a standard calibration curve prepared earlier with Bovine Serum Albumin.

RESULTS AND DISCUSSION

The results of the present study are given in Table 1 and 2. The total proteins in intestine and liver are given for both normal and infected fish.

The total protein content in normal intestine was 0.436 mg/g, infected intestine was 0.654 mg/g, normal liver was 0.572 mg/g, infected liver was 0.354 mg/g. This suggests that the total protein in intestine of infected fish has been increased compared with protein content of the normal intestine. Likewise these levels decreased in liver of infected fish compared with the normal fish.(Table 1 and

Table 1 : Protein levels in normal fish tissues (mg/g)

Tissue	Sample I	Sample II	Sample-III	Average
Intestine	0.480	0.415	0.414	0.436
Liver	0.543	0.577	0.596	0.572

Table 2 : Protein levels in infected fish tissues (mg/g)

Tissue	Sample I	Sample II	Sample III	Average
Intestine	0.628	0.651	0.683	0.654
Liver	0.387	0.352	0.324	0.354

2).

Investigations regarding the protein contents of fish and fish products were estimated as early as 19th century. Saha and Guha (1939) have estimated the protein content of 24 different varieties of fresh water fish in Bengal.

Investigation made on carbohydrate, lipid and protein levels in *C.nigrodigutatus, B.fitamentosus* and A.*occidentalis* reveal that protein contents are generally high in 3 spp. Abdullah (2001), Martin *et al.* (2001) stated that the protein synthesis in liver protein degradation increased as the fish losts weight and decreased in the size of liver. Ellestad *et al.* (2002) observed a low level of intestinal phytase activity in the intestine brush border membrane of hybrid stripped bass (*Morone sexatilis x.m chrysops*). Mehboob *et al.* (2003) worked on proximate composition of liver of wild and farmed *Labeo rohita* which resulted in higher contents of protein in farmed fish, compared with wild *Labeo rohita.*

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 biochemistry, abnormal fluctuations has been observed. Zinada (2000) studied on the effect of niclosamide in *Liza ramada* and detected in muscle and increased mulluscicide residues with increase in concentration of niclosamid and changes in the liver enzymes, which caused metabolic disturbances in fish. Solomatina *et al.* (2001) stated that changes in indices of protein and energy metabolism in the liver in carps was 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.

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 causes for the elevation of protein levels in infected tissues which in turn, effects the biochemical composition of fish tissues. This is supported by Waagbo *et al.* (1998) who showed increased levels of protein, in liver and increase in level of valine in muscle tissues of infected Atlantic salmon (*Salmo salar* L.) suffering with cold water vibriosis, Hitra disease. Mustafa (1999) studied the relationship between infection and nutrient resulting in elevated levels of protein and lipid content of muscle, liver and ovaries in Powan, *Coregonus lavaratus*. Paige *et al.* (2001) found pathogen load increased maximum levels of protein in liver and kidney tissues of juvenile rainbow trout infected with *Vibriosis*. Dorocu *et al.* (2002) showed suggestive increased levels of protein giving rise to exophthalmia, local hemorrhage and lens cataract in *Acanthobrama marmid* infected with *Diplostomum* spp. Russel *et al.* (2008) observed increased distribution and density of immunoreactive ladderlectin and interlectin within inflammatory leucocytes in infected tissues and immuno related organs in infected rainbow trout. Gauther *et al.* (2008) observed several fold increase in serum levels of CRP like protein in carp infected with a pathogen, *Aeromonas hydrophila*.

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

Acknowledgement :

One the author (T.C.Diana) is 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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