



## Biochemical effects on Protein and Free Amino acid metabolism in *Catla catla* and *Labeo rohita* due to *Pallisentis nagpurensis* infection.

Dr. P. Anil Kumar, PhD

Lecturer in Zoology, P.R. Government College(A), Kakinada, AP, India

**Abstract:** Fish is correctly regarded as a healthy component of the diet; it is an excellent source of protein and is low in saturated fats. However, there are risks associated with eating cultured fish owing to the infection by helminthic parasites. Parasitic fish diseases are posing a problem for breeders in India cultivating fish for food. Despite several attempts to control these parasites, infestation continues to grow and until effective measures of control are devised and implemented, a serious decline in fish population is expected. In carp culture, particularly Indian major carps, comprising of *Catla catla* and *Labeo rohita* contribute a large proportion of fish production from inland water bodies. Though parasitology is relatively young in biology, parasitism is deep rooted. The relation between the parasite and host is interesting and important. As the development of the parasite progresses, the host is weakened and deteriorated. Such deleterious effects of parasites on host are biologically and commercially important to us. That the parasites are biologically harmful is already documented (Holmes & Bethel, 1972; Holmes, 1979; Minchella & Scott, 1991, Bikshapathi, V.; Reddy, 2008, Applebaum S.L. & Rønnestad I. 2004 )

Basing on the available work and related scientific literature, the post-helminth parasitic effects due to infestation by *Pallisentis nagpurensis* in commercially important cultured fish, *Catla catla* and *Labeo rohita* are examined and the metabolic changes in protein and Free amino acids are studied. In the present study, an attempt has been made to understand the parasitic effects on physiological status of the fish by analyzing a few selected biochemical parameters, related to protein and free amino acid metabolism.

**Keywords:** *Catla catla*, *Labeo rohita*, *Pallisentis*, Protein metabolism, Free amino acid metabolism.

### I. Introduction

Proteins serve a physiological system in many ways with their ubiquitous nature. They build up new tissue and maintain the structure of every cell/ tissue including its content of protein-containing enzyme systems. They are hydrolyzed by the digestive enzymes and thus liberated amino acids are rapidly absorbed into the intestinal capillaries and thereby enter the general circulation by way of liver. The amino acids from the hydrolysis of dietary protein join the amino acids released from the continual disintegration of structural and functional protein from the tissues and become a part of the amino acid pool. From the common amino acid pool, amino acids are taken up by the cells, to be built into the cell structure as required.

### II. Materials and Methods

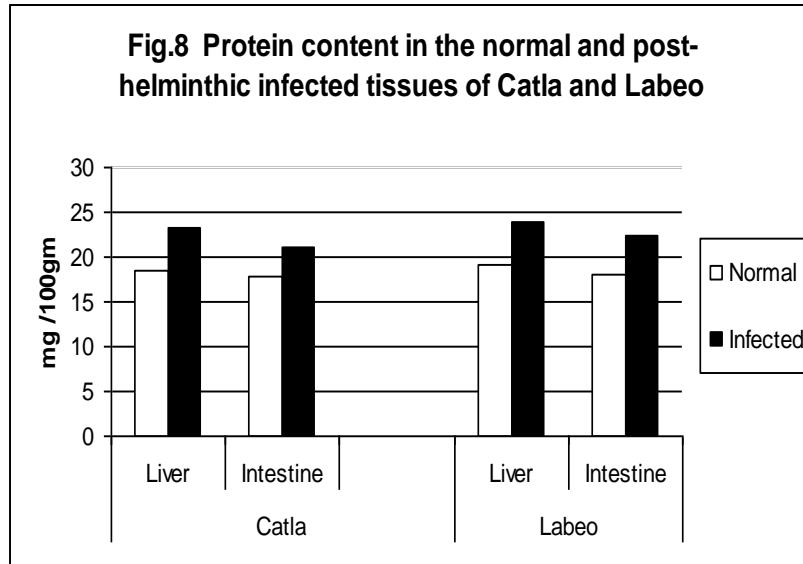
The fish, *Catla catla* and *Labeo rohita* were collected from the tanks surrounding Hyderabad. The fish were brought to the laboratory in aerated tanks and were acclimatized for 24 hours. Fish were sacrificed and dissected. The tissues were carefully examined for the presence of the parasites. The parasites were isolated and were preserved. The normal fish – without the parasites – were also sacrificed along with the infected fish. The tissues viz., liver and intestine were isolated into saline solution from both categories of fish for biochemical estimations. The identified and separated helminth parasites *Pallisentis nagpurensis* were stained for identification. They were taken on a glass slide and pressed under a cover glass. The glass slide was preserved in 4% formalin for 48 hrs. Before staining, the glass slides were removed from the preservative and washed thoroughly under running tap water. Alum carmine stain was used for staining the parasites. Total protein content was estimated by the method of Lowry et al., (1951). 2% tissue homogenates were prepared in 10% trichloro acetic acid. One ml of crude homogenate was centrifuged at 2500rpm for 15min. The sediment was dissolved in 5ml of 1N sodium hydroxide. To 0.1ml of the above solution, 4ml of alkaline copper reagent was added followed by 0.4ml Folin phenol reagent. Light blue colour that was developed was read at 600nm. Total free amino acids were estimated by the method of Moore and Stein (1954) as described by Colowick and Kaplan (1957). 2% tissue homogenates were prepared in 10% trichloroacetic acid. One ml of crude homogenate was centrifuged at 2500rpm for 15min. To 0.05ml of supernatant, 2ml of ninhydrin reagent was added and boiled. The cooled solution was made up to 10ml with distilled water. The bluish-pink colour that developed was read at 570nm.

### III. Results

#### Proteins

The total protein content in the normal and infected tissues of the two fish is shown in Table 8. The total protein content appeared to have increased during the infection. This increase is seen in the tissues studied viz., liver and intestine.

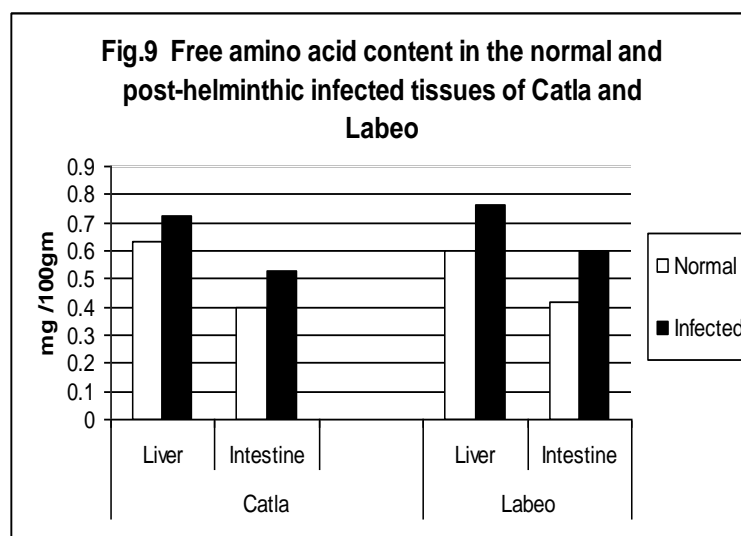
The total protein content was observed to be more in liver than in the intestine. The content was almost same in the tissues of both the fish. But the increase was seen to be more in the tissues of *Catla catla* than *Labeo rohita*. Proteins recorded and increase of 26% in the liver of *Catla* and 25.4% in *Labeo*. Intestine of *Catla* showed 17.9% increase while that of *Labeo* showed 23.1% increase. The actual content was shown in graph (Fig.8).



#### Free amino acids

The free amino acid content also showed an increase due to the effect of parasitic infection (Table.9). The increase was consistent in both the fish and both the tissues. Liver of *Catla* has recorded an increase of 14% while the intestine showed 32.9% increase. In *Labeo*, liver recorded an increase of 27% and intestine showed an increase of 48.8%.

Among the tissues studied, liver of *Catla* and *Labeo* showed more free amino acids than intestine. Similarly, the percent change was more in intestine in both the infected fish species. The results were graphically represented in Fig. 9.



### IV. Discussion

The study of protein content and its related components has shown that the parasitic infection indeed change the metabolism profile of the fresh water fish, *Catla catla* and *Labeo rohita*. The pathological changes induced on

the host metabolism altered the Protein metabolism. Some of the studies reported a drop in the protein levels during the helminth infections (Patwardhan, 1953, Lomukhin, 1971). But some other workers found the protein content did increase during the helminth infection in various organisms (Kameshwari, 1978, Bhonsle, 1980, Thabitha, 1982).

In the present study the proteins were observed to have increased upon the infection by acanthocephalan parasites. This increase could be attributed to the tissue repair mechanism operating in the host system in order to cope with the parasitic invasion. The repair mechanism can also be substantiated by the amount of free amino acid levels observed to be increased. Repair of connective tissue is a mandatory procedure in the infected hosts. And this process is supported and brought about by the increased shunt of proteins and free amino acid levels.

Amino acids were found to play an important role in meeting the energy demands of the animal by converting themselves into keto acids which run into citric acid cycle through transamination process. To enable this activity, the animal might have sought catabolic activities and degraded proteins to amino acids.

Thus, the parasites have altered the crucial physiological aspects of the host system and the host seems to try to face the infection by altering its physiology.

### References

- Aragão C., Conceição L.E.C., Dinis M.T. & Fyhn H.J. (2004b) Amino acid pools of rotifers and *Artemia* under different conditions: nutritional implications for fish larvae. *Aquaculture* 234, 429-445.
- Agosin, M. and Repetto, Y. 1963. Studies on the metabolism of *Echinococcus granulosus* scolices. *Comp. Biochem. Physiol.* 8: 245-261.
- Ansari, M.J. And Singh, K.S. 1974. Histochemical studies of liver in *Opistharchiasis*. *Ind. J. Animal Sci* 43(5): 438-446.
- Barrett, J. 1981. "Biochemistry of parasitic helminths" MacMillan publications, London.
- Bauer, O.N. Musselines, V.A. and Stelkov, YU.A., 1969. Diseases of Pond fishes *Kolos* 335.
- Bergmeyer, H.U. 1965. in methods of enzymatic analysis (H.U. Bergmeyer Ed) Academic Press, New York.
- Bergmeyer, H.U. 1965. In methods of enzymatic analysis (H.U. Bergmeyer Ed). Academic press, New York.
- Bikshapathi, V.; Reddy, K.S.; Kameswari, M., 2008: Post helminth infection changes in the protein metabolism of myotomal muscles in *Channa punctatus*. *Uttar Pradesh Journal Of Zoology*: 3, 289-295
- Blacklock, B. 1919. *Ancylostoma ceylanicum* in the cat in Durban. *Ann. Trop. Med. Parasit.* 13: 297.
- Bose, K.C. and Sinha, A.K. (1981), Histopathology of *Clarias batrachus* infected by *lytocestus indicus* (kughe) *SCI Cult.* 47: 186-187.
- Butter Worth, J. 1970. Studies on phosphomonoesterases of *Ascaris suum* (Goeze, 1782). Ph.D. Thesis. University of Wales.
- Carlton, P.L. 1969. Brain Acetyl choline and inhibition in reinforcement and behaviour (J.T.Tapp.Ed). *Acad. Press.* New York. 286-327.
- Caroll, N.V.; Lansley, R.W. and Rae, JH. 1956. Glycogen determination in liver and muscle of authrone reagent. *J.Biol. Chem.* 220 : 583-593.
- Chari, S.S. and Subramanian, G. 1972 Histopathological and histochemcial changes following invasion of mice by *Taxocara Canis* (warner 1782). *Ind. J. Anim. Sci.* 42(11), 957-960
- Cheng, T.C. (1974): *General Parasitology* (new York) Academic Press.
- Cherry and Grandall. 1932. In Hawk's physiological chemistry (edited by Oser, B.L., 1954) McGraw-Hill book company, New York.
- Claude Bernard, L. 1859. De la matiere glycogene chez les animaux depourvus de foie. *Comp. Rend. Soc. Biol. Paris.* 1: 53-44.
- Cobbold, T.S. 1883. On the destruction of fish and other aquatic animals by internal parasites. *Fish Exhibition. Lit. 6 Conference* 3,20.
- Cohen S., And Sadum, E.H., (Eds), 1976. *Immunology of parasitic infections*. Blackwell, Oxford.
- Coles, G.C. 1973a. The metabolism of *schistosoma* : A review. *Int. J.Biochem.* 4: 319-337.
- Cox, R.A. and Gokcen, M 1974. Circulating lipids in the golden hamster (*Mesocricetus auratus*). *Comp. Biochem. Physiol. B* 49B: 655-661.
- Crawford, N. 1958. An improved method for the determination of free and total cholesterol using the ferric chloride reaction. *Clinic. Chem. Acta.* 3: 357-367.
- Devlin, T.R. 1986. *Textbook of biochemistry with clinical correlations*, 2nd edn, Wiley, New York.
- Dhar, D.N. And Singh, K.S. 1963: Pathology of liver in *dicrocoeliasis*. *Ind. J. Vet. Sci.* 33(4), 142-151.
- Duncan, J.L. And Pirie, M. 1975. The Pathogenesis of single experimental infection with *strongylus valgaris* in fowls. *Research in Vet. Sci.* 10(1): 82-93.
- Endo, A 1981. Biological and pharmacological activity of inhibitors of 3-hydroxy -3 methyl glutaryl coenzyme A reductase. *Trends in Biological Sciences.* 6: 10-13.
- Eugster, A.K.; Albert, P.J. and Kalter, S.S. 1966. Multiple enzyme determinations I sera and livers of tumor bearing hamsters. *Proc. Soc. Exp. Biol. Med.* 123: 327-331.
- Faust, E.C., 1920: Pathological changes in the gastropod liver produced by fluke infection. *Johns Hospkins hops. Bull.* 31, 1976-1984.
- Fisher, Jr. F.M. 1965. Studies on the accumulation of hexose by cestodes. *J.parasitol.* 51(2). Sect 195-197.
- Friedmann And haugen- *J.Biol. Chem* 147, 415 (1943). Quoted in Hawk's physiological chemistry Ed. Oser. B.L. 1954. Mc. Graw hill book company New York.
- Furton, J.S. and Simmonds, S. 1960. *General biochemistry* 2nd edition New York. John Willey and sons.
- Ginger, C.D. I and Fairbairn, D. 1966a. Lipid metabolism in helminth parasites. I. the lipids of *Hy* *Govondyan, V.S.* 1975. Early Biochemical changes in the host as a response to infection. *Akad. Sdl'ske Nank.* V(1).
- Graff, D.J. and ead, C.P. 1967. Specific acetylcholinesterase in *Hymenolepis diminuta*. *J.parasit.* 53: 1030-1031.
- Graves, D.J. and Wang, J.H. 1972. *The enzymes* (Boyer, P.D.ed) 3rd ed. 7 : 435-482, Academic press, New York.
- Halton, D.W. 1967. Studies on phosphatase activity in trematoda. *J.Parasit* 53: 46-54.
- Huckabee, 1956. *J. Appl. physiol* 9, 163 (1956) *AM J.Med* 30, 833 1961. Quoted in Hawk's physiological chemistry ED Oser. B.L. 1954 Mc Gram Hill Book company, New York.
- Indian Council of medical research, 1983. *A manual of Laboratory Techniques*, NIN, Hyderabad, India.
- Jenkins, T 1973. Histochemical and fine structure observations of the allosteric reponse and comparison with other allosteric proteins. *The journal of Biological Chemistry.* 265 (50 : 2409-2419.
- kadav, M. and Agarwal, S.M. 1983. Amino acid picture (qualitative and quantitative) of host serum of uninfected and infected *Clarias batrachus* parasitized with caryophyllids. *Ind.,J.of Helmin* 33(1), 79-81.
- Kivy, E. 1947. The effect of roentgen irradiation on the testes of the golden hamster. *Proc. Soc. Lonaon. Ser. B.* 125: 548-564.

- Krebs, H.S. and Johnson, W.A. 1937. Enzymologia, 4. 148. In “ Energy in biological systems- molecular and cell biochemistry” 1 edn, chapman and Hall. India, CIT East, Madras.
- Levine, N.D. 1980. Parasitic nematodes of domestic animals and man minneapolis : Burgess.
- Lomukhin, V.P. 1971. Biochemical changes in the liver of cattle with chronice *Fasciola infenction*. Veterinaria 21, 127- 132.
- .Mohabey, 1979. Parasite effects on some aspects of Haematology and biochemisty of *Clarias batrachus* (linn) Found in nature heavily infected with caryaphyllaeid cestodes.
- Onwuliri, C.O.E., 1985. Energy metabolism in the developing larval stages of *Ancylostoma tubaeforme* and *Haemonchus contortus* : glycolytic and tricarboxylic acid cycle enzymes. Parasitology 90(1) : 169-177.
- Palmer, T. 1981. Understanding enzymes, Ellis Horwood Limited, England.
- Pearse, A.G.E. 1968. “Histochemistry, theoretical and applied, 3<sup>rd</sup> edition, Vol. I. Churchill, London.
- Peter, A. and Mayes. 1979. “Physiological Chemistry”, Lange medical publications, California.
- Pozdyakov, O.M. 1968. Electron histochemical investigation of Cholinesterase in the neuromuscular synapse of rats. Bull. Exp. Med. 66:924-927.
- Ramakrishnan, S. 1994. Biochemistry manual. T.R.Publications, Madras.
- Sanderson, B.E. 1972. Release of cholinesterase by adult *Nippostrongylus brasiliensis* in vitro. Zeitschrift fur parasitenkunde 40 : 17.
- Satpute, L.R. and Agarwal, S.M. 1974. A seasonal infection of *clarius batrachus* (L) by *Lytocestus indicys* Moghe, 1931 and parasite effects on its haematology and histopathology. Ind.J. Exp. Biol. 12 (6) 554-586.a
- Zambonino-Infante J.L., Gisbert E., Sarasquete C., Navarro L, Gutiérrez J. & Cahu C.L. (2008)
- Ontogeny and physiology of the digestive system of marine fish larvae. In: Feeding and digestive functions in fishes (ed. by J.E.P. Cyrino, D. Bureau & B.G. Kapoor). Science Publishers, Enfield, New Hampshire, USA.