Life Science Informatics Publications
Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences
Journal Home page http://www.rjlbpcs.com/
RJLBPCS
ISSN 2454-6348

Original Research Article **DOI: 10.26479/2020.0604.01**

EUS AFFECTED FISHES EXHIBITING DECREASE IN NUTRITIVE VALUE

Reena Laharia

Department of Zoology, Brijlal Biyani Science College, Amravati-444602, Maharashtra, India.

ABSTRACT: In developing countries like India fish is a dependable source of animal protein. Large-scale mortality occurs among the fresh water fishes often due to environmental stress followed by pathogenic attacks and parasitic afflictions causing a tremendous loss to the nation. This condition is called as Epizootic Ulcerative syndrome (EUS). It causes severe ulceration and heavy mortalities of fish in the wild and culture ponds. In the present study the healthy fishes were administered with the ulcerated tissue from the EUS affected fish and were studied for their muscle protein, Cholesterol and Glycogen as well as Serum protein. It was found that all the EUS affected fishes exhibited decreased nutritive value with significantly lowered muscle proteins, cholesterol and glycogen. Serum total protein level in EUS affected fishes was declined significantly with decreased A : G ratio. Thus it can be concluded that d EUS induces stress, making fishes weak and vulnerable to diseases.

Keywords: Protein, EUS, Cholesterol and Glycogen.

Article History: Received: May 25, 2020; Revised: June 15, 2020; Accepted: July 01, 2020.

Corresponding Author: Dr. R. R. Laharia* Ph.D.
Department of Zoology, Brijlal Biyani Science College, Amravati-444602, Maharashtra, India.
Email Address: dr.reenalaharia@gmail.com.

1. INTRODUCTION

In India, traditional aquaculture has in recent years turned into a science based economic and commercial activity involving heavy inputs and therefore diseases of all kinds are now known to occur on an increasingly larger scale. However, fish mortality is not the only criteria to evaluate the effect of fish disease. Even the morbidity, which leads to weight losses and poor growth in surviving fish, contributes substantial loss to the farmers. Since last decade there is an increased realization of

the necessity to avoid infectious agents to maintain healthy stocks to enhance productivity [1]. Like all animals, fish suffers from environmental, nutritional and infectious diseases. Organisms such as parasites, bacteria and viruses are pathogens capable of causing disease in cultured and wild populations of fish. Poor nutrition and water quality can also cause disease under stressful conditions. Epizootic Ulcerative Syndrome (EUS) causes severe ulceration and heavy mortalities of fish in the wild and culture ponds. This disease effected juveniles as well as adults of many species of both fresh water and brackish water fish and the extent of damage is very severe in some species . The quality of edible fish is severely reduced because the primary choice of fish lies in its protein and other constituents, which reduce considerably under EUS affected fishes. [2]; [3]; [4]; [5]; [6]; [7]; [8] have published their reports on the host biochemistry, comparison of diseased fishes and healthy fishes.

2. MATERIALS AND METHODS

The present study was carried out by collecting the suspected diseased fishes from nearby EUS prone water bodies (Wadali and Malkhed lakes) with the help of fishermen and were brought to the laboratory and maintained in glass aquaria for a week, to study the type of infection. General survey of fish market was also carried out simultaneously every week in the light of infection.The healthy fishes weighing about 25 gm and of approximately equal length were brought to the laboratory and kept in aquarium containing 75 litre of water and were acclimatized for 15 days. They were regularly fed on pellet feed and 25% water of the aquarium was also regularly changed at an interval of 7 days to maintain the water quality. 10 fishes were kept in one aquarium each.

Experimental set up: -

The experiment was carried out on six sets of 10 fishes in each group as below:

Group I: -Consisting of control (Healthy) fish (10, *Channa punctatus*).

Group II:-Consisting of Naturally EUS affected fish (10,*Channaspunctatus*).

Group III :- Consisting of artificially infected fishes injected with 0.2 ml homogenate of ulcerated skin tissue from diseased *Channa punctatus* (10, *Channa punctatus*).

Group IV :- Consisting of control (Healthy) fish (10, *Clarias batrachus*).

Group V :- Consisting of Naturally EUS affected fish (10, *Clarias batrachus*).

Group VI :- Consisting of artificially infected fishes injected with 0.2 ml homogenate of ulcerated skin tissue from diseased *Clarias batrachus*(10, *Clarias batrachus)*.

Suspension preparation:

The tissue suspension of ulcerated skin tissue was prepared in sterile fish saline (0.3%). A tissue (500 mg) homogenate was prepared by taking affected or ulcerated skin in fish saline (5ml).

Collection of Blood sample:

Blood was obtained by amputating the tail. Blood was collected in eppendorf tubes, allowed to clot at room temperature for 60 mins and then spun down at 2500 rpm and the serum was removed.

Serum of 6 fishes from the same group was pooled and then estimations were carried out. The serum was stored at -20°C until it was assayed for enzymes and also for RID and SDS-Phage.

Biochemical study:

After removal of blood from the control and experimental diseased fishes they were dissected with a sterilized scissor to remove the tissues like, kidney, liver, skin and gills. The tissues (liver and muscle) were weighed to the nearest of ± 0.1mg with electronic balance and were used for biochemical estimations like, glycogen, total protein and total cholesterol. Homogenates were prepared in different medium as per the reqiurement of the techniques involved. Cooling centrifuge was used to centrifuge the homogenates for varied time length as per the technique.

The Biochemical studies were carried out as per the methods given below.

Table: 1, Biochemical methods used :

Sr.no.	Biochemical Parameters	Tissue/Serum	Method
1.	Total Protein	Serum	Biuret [9].
2.	Total Protein	Tissue	Lowry *et al.,* [10].
3.	Glycogen	Tissue	Montgomery [11].
4.	Cholesterol	Tissue	Lieberman Burchard [12]

Electrophoresis (SDS-PAGE):

Serum proteins (albumin and immunoglobulin) from control, naturally EUS affected and artificially EUS infected fishes were separated by SDS-PAGE [13] and were run on the densitometer for finding out the fractions of immunological proteins.

3. RESULTS AND DISCUSSION

Effect on Nutritive values:

In control as well as EUS infected fishes nutritive values with respect to muscle proteins, cholesterol and glycogen were examined and the results are shown in Tables, 2 and 3 and Figs: 1 to 6. In naturally EUS affected *Channa punctatus* muscle proteins were found to be depleted by 20.48%, cholesterol by 49.73% and glycogen by 48.41%. However in artificially EUS infected fishes after 21 days of challenge infection slightly more depletion in all the three biochemicals was observed. The muscle protein depleted by 24.28%, cholesterol by 54.20% and glycogen by 58.36%. These depleted values are found to be significant at $p< 0.05$. Similarly in EUS infected *Clarias batrachus* also the nutritive values with respect to muscle proteins, cholesterol and glycogen were depleted, however the depletion was slightly less when compared with the depletion in EUS infected*Channa punctatus* (Tables, 2 and 3; Figs: 4 to 6).

Table 2: Alterations in muscle protein,cholesterol and glycogen contents (Nutritive value) of control as well as EUS infected fish, *Channa punctatus*.

Muscle biochemicals	Control	Naturally affected fish	Artificially infected fishes		
			7 days	14 days	21 days
Protein (mg/100g)	462.95 ±18.15	368.15 * ±10.16 (-20.48)	428.39 NS ±20.11 (-7.47)	400.15 NS ±35.88 (-13.17)	350.58* ±30.19 (-24.28)
Cholesterol (mg/100g)	22.16 ±2.22	11.14* ±0.98 (-49.73)	20.80 NS ±1.85 (-6.14)	15.38* ±1.33 (-30.60)	10.15* ±2.88 (-54.20)
Glycogen (mg/100g)	43.90 ±3.20	22.65* ±2.15 (-48.41)	39.89 NS ±3.16 (-9.14)	21.42* ±1.88 (-51.21)	18.28* ±0.95 (-58.36)

The values are mean of 6 replicates ± SE.

Figures in Parenthesis indicate percent change over control.

* P< 0.05, NS – Not significant.

Table 3: Alterations in muscle protein,cholesterol and glycogen contents (Nutritive value) of control as well as EUS infected fish, *Clarias batrachus*.

Muscle biochemicals	Control	Naturally affected fish	Artificially infected fishes		
			7 days	14 days	21 days
Protein (mg/100g)	522.15 ±18.08	428.92* ±28.11 (-17.86)	500.44NS ±32.08 (-4.16)	468.00 NS ±30.16 (-10.38)	432.72* ±22.22 (-17.13)
Cholesterol (mg/100g)	18.46 ±2.19	12.15* ±0.92 (-34.19)	16.15 NS ±1.11 (-12.52)	15.18 NS ±1.08 (-17.77)	13.55* ±0.98 (-26.60)
Glycogen (mg/100g)	52.62 ±3.66	30.18* ±1.15 (-42.65)	40.16 NS ±1.98 (-23.68)	36.88* ±2.15 (-29.92)	28.15* ±0.75 (-46.51)

The values are mean of 6 replicates ± SE.

Figures in Parenthesis indicate percent change over control.

* P< 0.05, NS – Not significant.

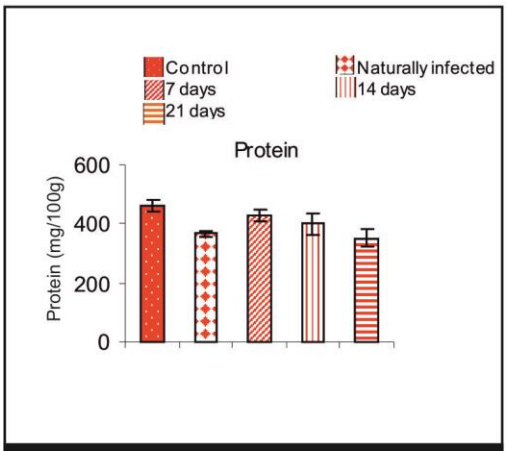

Fig: 1, Alterations in muscle protein in control, naturally and artificially EUS infected *Channa punctatus.*

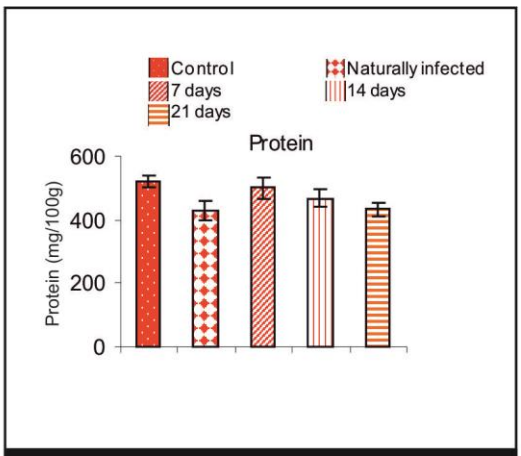

Fig: 4, Alterations in muscle protein in control , naturally and artificially EUS infected *Clarias batrachus.*

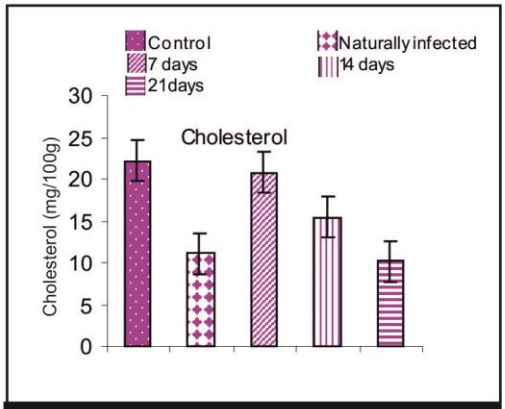

Fig:2, Alterations in muscle cholesterol in control, naturally and artificially EUS infected *Channa punctatus.*

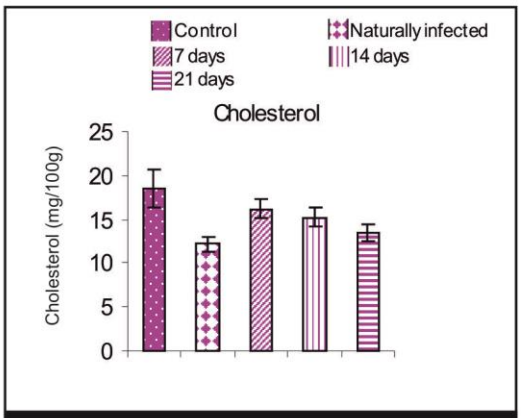

Fig:5, Alterations in muscle cholesterol in control , naturally and artificially EUS infected *Clarias batrachus.*

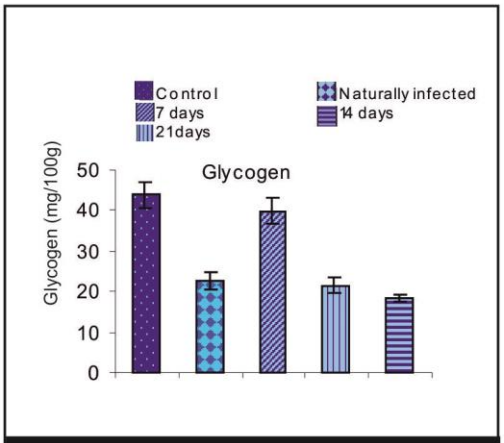

Fig:3, Alterations in muscle glycogen in control, naturally and artificially EUS infected *Channa punctatus.*

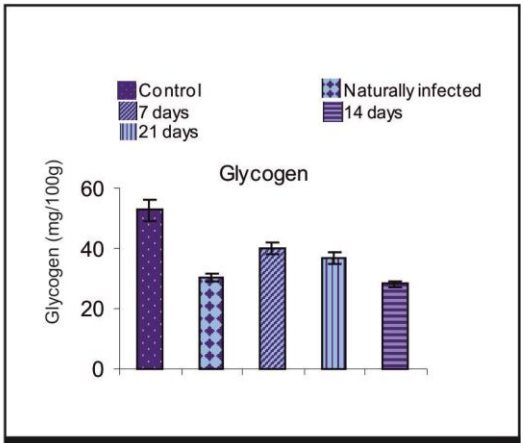

Fig: 6, Alterations in muscle glycogen in control , naturally and artificially EUS infected *Clarias batrachus.*

Alterations in Serum proteins of EUS infected fishes:-

In naturally EUS affected *Channa punctatus* as well as after 21 days of challenge infection (Artificially EUS infected) the serum protein level was declined significantly ($p<0.05$) when compared with serum protein level in healthy *Channa punctatus*. In *Clarias batrachus* similar observations are made. However, the serum proteins were found to be decreased more (34.14 to 38.75%) when compared with the results in *Channa punctatus* (Table,4 and 5 and Figs:7 and 8).

α_1, α_2, β as well as γ serum globulins were found to be elevated in all the naturally as well as artificially infected *Channa punctatus* and *Clarias batrachus*. This has resulted into decreased A:G ratio (Table, 4 and 5 and Figs 9 to 14). A:G ratio in naturally EUS affected *Channa punctatus* was 1.14 and that in artificially EUS infected fishes was found to be 0.72. These results are highly significant ($p<0.01$). Densitometric graphs were taken and are shown as Fig: 15 to 17.In *Clarias batrachus* the A:G ratio was 3.23, 0.94 and 1.49 in healthy, naturally EUS affected and artificially EUS infected fishes respectively. Densitometric graphs were taken and are shown as Fig: 18 to Fig: 20.

Table 4: Serum proteins in control and EUS infected *Channa punctatus*.

Serum proteins	Control	Naturally affected	Artificially infected (21 days)
Total Protein (mg/100g)	5.28 ±0.15	4.08* ±0.08 (-22.73)	3.98* ±0.22 (-24.63)
Albumin	74.64%	53.43%	42.09%
α_1	8.98%	15.96%	9.31%
α_2	5.55%	8.26%	14.37%
B	5.54%	12.67%	17.78%
γ	5.26%	11.66%	16.43%
Globulin	25.33%	46.55%	57.89%
A:G	2.94	1.14** (-61.23)	0.72** (-75.52)

The values are mean of 6 replicates ± SE.

Figures in Parenthesis indicate percent change over control.

* $P< 0.05$, ** $P<0.01$

Table 5: Serum proteins in control and EUS infected *Clarias batrachus*.

Serum proteins	Control	Naturally affected	Artificially infected (21 days)
Total Protein (mg/100g)	4.98 ±0.09	3.28** ±0.11 (-34.14)	3.05** ±0.08 (-38.75)
Albumin	76.37%	50.97%	59.84%
α_1	11.48%	19.97%	10.18%
α_2	4.10%	9.15%	14.30%
B	4.36%	8.36%	10.55%
γ	3.67%	1.53%	5.10%
Globulin	23.61%	48.01%	40.13%
A:G	3.23	0.94** (-70.9)	1.49** (-53.87)

The values are mean of 6 replicates ± SE.

Figures in Parenthesis indicate percent change over control.

** P< 0.01

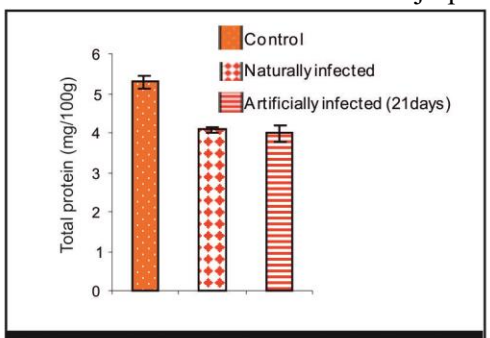

Fig:7, Serum total proteins in control,naturally and artificially EUS infected *Channa punctatus.*

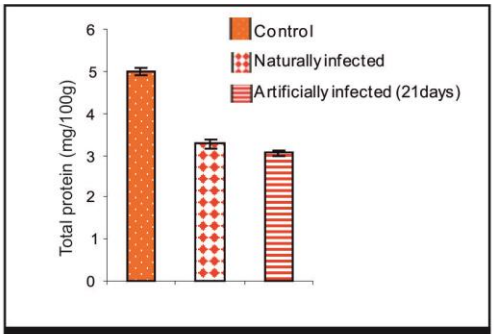

Fig: 8, Serum total proteins in control,naturally and artificially EUS infected *Clarias batrachus.*

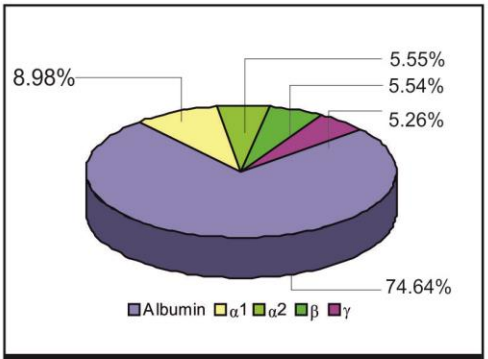

Fig: 9, Serum proteins in control *Channa punctatus.*

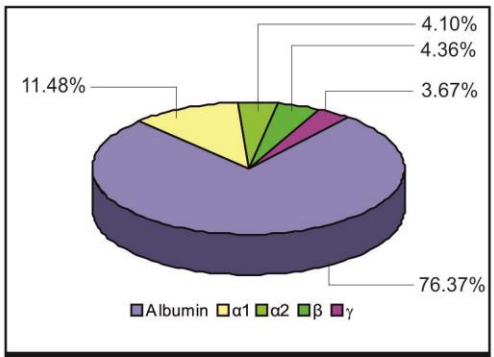

Fig:12, Serum proteins in control *Clarias batrachus.*

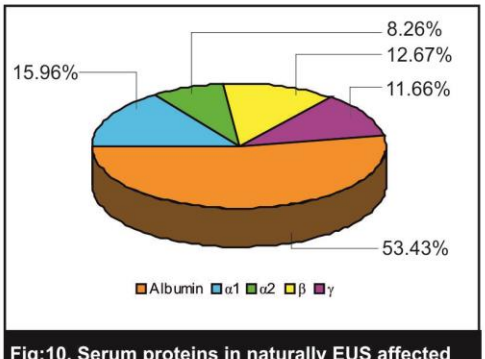

Fig:10, Serum proteins in naturally EUS affected *Channa punctatus.*

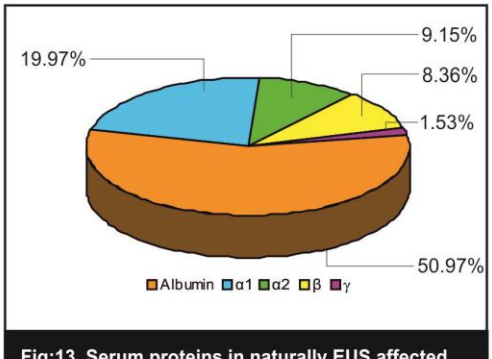

Fig:13, Serum proteins in naturally EUS affected *Clarias batrachus.*

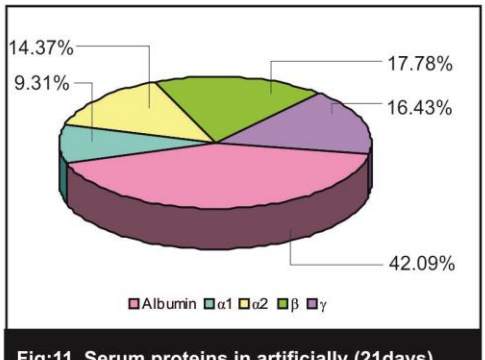

Fig:11, Serum proteins in artificially (21days) EUS infected *Channa punctatus.*

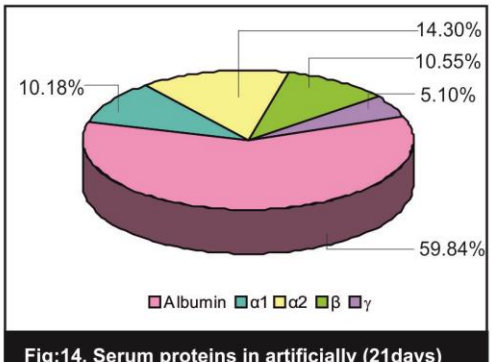

Fig:14, Serum proteins in artificially (21days) EUS infected *Clarias batrachus.*

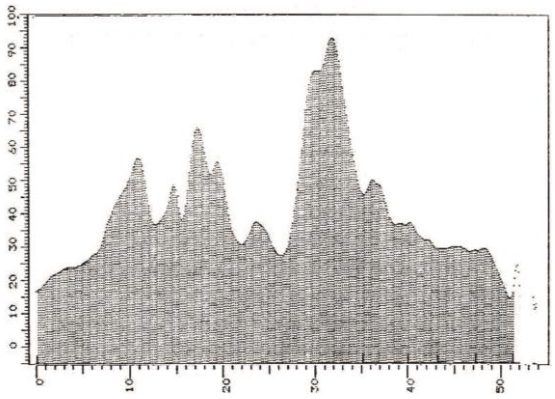

Fig: 15 ,Electrophoretic and densitometric record of serum proteins in control *Channa punctatus.*

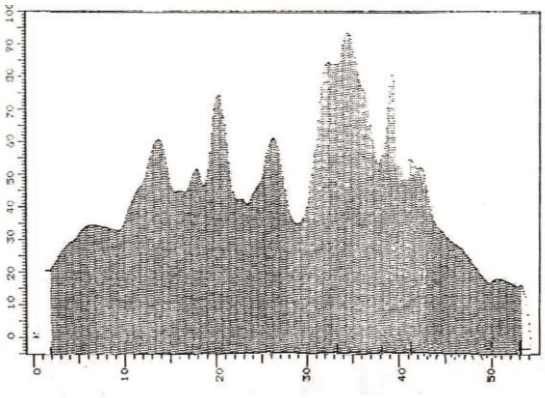

Fig: 16 Electrophoretic and densitometric record of serum proteins in EUS infected *Channa punctatus.*

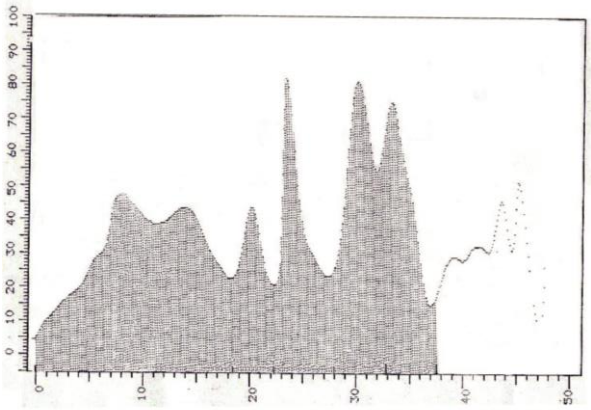

Fig: 17, Electrophoretic and densitometric record of serum proteins in EUS infected *Channa punctatus* 21days.

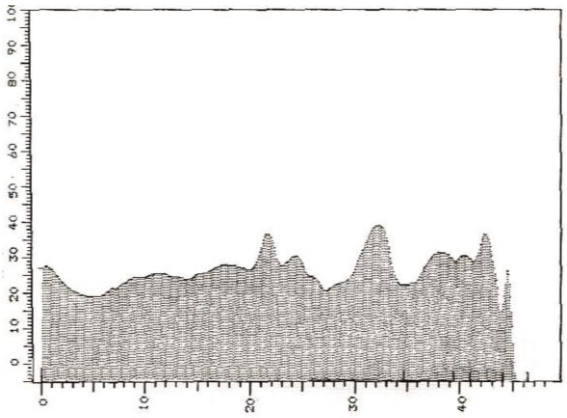

Fig: 18, Electrophoretic and densitometric record of serum proteins in control *Clarias batrachus.*

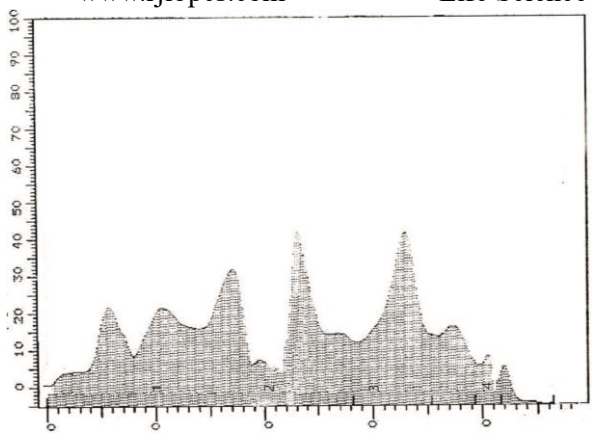

Fig:19, Electrophoretic and densitometric record of serum proteins in EUS infected *Clarias batrachus*.

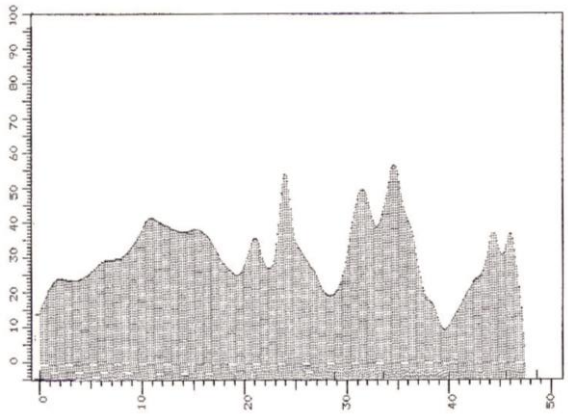

Fig: 20 , Electrophoretic and densitometric record of serum proteins in EUS infected *Clarias batrachus* 21 days

Electrophoretic studies:-

Serum proteins in muscles of healthy and EUS affected *Channa punctatus* and *Clarias batrachus* were analysed electrophoretically. The results are shown in Table: 6, and Fig: 21. Serum of healthy *Channa punctatus* exhibited 8 proteins bands with $R_f$values 0.31, 0.36, 0.39, 0.48, 0.54,0.59,0.67, and 0.72.However , the serum of naturally EUS affected fishes showed 8 bands of proteins with $R_f$values 0.31, 0.36, 0.48, 0.54, 0.59, 0.61, 0.67 and 0.72. Thus one new band of $R_f$value 0.61 was seen with absence of band with $R_f$value 0.39. Similarly serum of healthy and naturally EUS affected *Clarias batrachus* was analysed for different proteins (Table: 6 and Fig: 21). Serum of healthy *Clarias batrachus* exhibitted 7 bands on the gel with $R_f$values 0.48, 0.54, 0.70, 0.82, 0.85,0.90 and 0.93. Serum of naturally EUS Affected fish showed presence of 6 different proteins and $R_f$values 0.36, 0.48, 0.64, 0.74, 0.85 and 0.96.Standard Protein mixture with known molecular weights were also run simultaneously with R_f values, 0.31, 0.36, 0.48 and 0.54.

Table 6: Electrophoretic seperation of proteins from healthy and EUS affected *Channa punctatus* and *Clarias batrachus.*

R_f values of Protein Markers	Molecular weights of Protein Markers (KD)	R_f values of serum proteins from control *Channa punctatus*	R_f values of serum proteins from Infected *Channa punctatus*	R_f values of serum proteins from *control Clarias batrachus*	R_f values of serum proteins from Infected *Clarias batrachus*
0.31	66	0.31	0.31	0.48	0.36
0.36	43	0.36	0.36	0.54	0.48
0.48	29	0.39	0.48	0.70	0.64
0.54	14	0.48	0.54	0.85	0.74
		0.54	0.59	0.82	0.85
		0.59	0.61	0.90	0.96
		0.67	0.67	0.93	
		0.72	0.72		

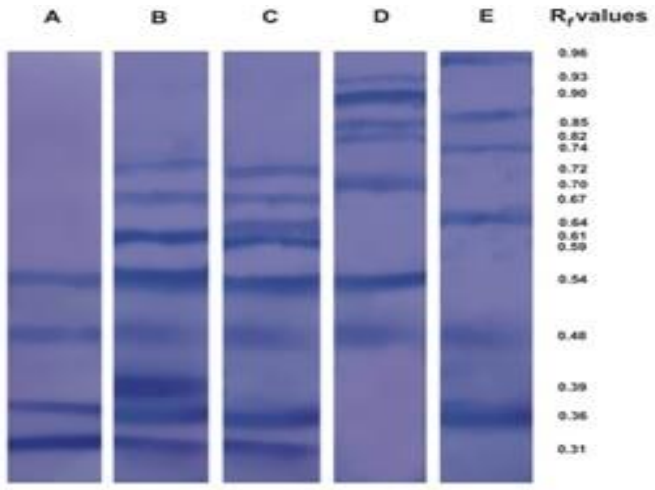

Fig : 21_ , Separation of serum proteins from naturally and artificially infected *Channa punctatus* and *Clarias batrachus*

A : Proteins with Known Molecular Weights

B: Separation of serum proteins of healthy *Channa punctatus.*

C : Separation of serum proteins of naturally EUS affected *Channa punctatus*

D : Separation of serum proteins of *healthy Clarias batrachus*

E : Separation of serum proteins of Naturally EUS affected *Clarias batrachus.*

The decreased glycogen and cholesterol contents observed in EUS affected fishes may be because of their early utilization during the stress of infection. In addition, the decrease in blood total protein (Table, 4 and 5) may account for the degeneration of muscular tissue. Blood total protein in fish is known to be very useful as a criterian for diagnosis of liver and muscle tissue function [14]; [15]. Omprakasham [16] reported that the total proteins and amino acids become gradually depleted in the muscle of *Rohu* fingerlings infected with *Psedomonas fluorescens*. Leibmann and Reidmuller [17] and Reidmuller [18] reported decreased quantities of serum proteins as well as albumin-globulin ratio in infected fishes. It was suggested that toxin produced by the pathogenic bacteria interfered with protein metabolism. In the present investigation also EUS affected fishes exhibited decreased A:G ratio (Table, 4 and 5). Richards and Pickering [19] also reported severe hypoproteinaemia in *Saprolegnia* infection of brown trout from spawing streams. This loss of protein may be associated with loss of protein from the extravascular fluid at sites of fungal damage [20] and may explain the rapidity with which the fungal-infected fish die.

4. CONCLUSION

All the EUS affected fishes exhibited decreased nutritive value with significantly lowered muscle proteins, cholesterol and glycogen. Serum total protein level in EUS affected fishes was declined significantly with decreased A: G ratio. Thus EUS outbreaks have threaten food security and subsequently physical health, of all those who are dependent on fish, as fish is an important source of animal protein for people in many countries of South and South-East Asia. Hence due to outbreak of this fish disease there is tremendous loss of the investment, cost of treatment and also loss of harvest, increasing the production cost. Thus it can be said that EUS has a potential to financially decimate those who rely on fishing for income.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

FUNDING

None

ACKNOWLEDGEMENT

I would like to express my gratitude towards late Dr. G.N.Vankhede for his guidance and support.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Mukherjee SC.Fish Diseases in India, their causes and control Measures. Applied Fisheries and aquaculture.II .2002; 2: 66-71.
2. Gupta SP and Gupta RC. Glycogen content of Opisthorchis pedicellata (Trematoda). Indian J.Parasit. 1981; 5 (1) : 77-79.
3. Krishna, GV R. and Rao B R. Post helmenthic infectional changes in the reserve food product of Melanoides tuberculatus. Ind. J. Parasit. 1982; 6 (2) : 279 - 280.
4. Gur'yanova SD. Effects of helminthes on the aminoacids and lipid status of freshwater fishes.(abstract) In : Pervvi simpozium do ekologicheskoi biokhimii ryb tezisy dokiadov O ktyabr.1987.goda Luk'yanekov V. I Ed 1987 : 55-57.
5. Pradhan K. and Pal J. Experimental induction of ulcer in the fish Channapunctatus by bacteria. Environ. Ecol. 1990; 8: 812-815.
6. Krishna PV. Studies on gill parasites of some marine fish from Visakhapatnam coast with special reference to Neometadidymozoon polymorphis (Yamaguti 1970). Ph.D thesis, Andhra University, Waltair. 1993.
7. Roberts RJ, Willoughby LG. and Chinabut S. Mycotic aspects of Epizootic Ulcerative Syndrome (EUS) of Asian fishes. Journal of Fish Diseases. 1993; 16: 169 -183.
8. Krishna PV. Biochemical observations on Heteropneustes fossilis infected with Epizootic Ulcerative Syndrome (EUS), J. Ecobiol. 2002; 14 (2): 125-129.
9. Varley H. Practical Clinical Biochemistry.Pub. C.B. S. Publishers and Distributors, New Delhi. 1975.
10. Lowry OM, Rosenberg MJ, Farr AL and Randall RJ Protein measurement with the folin-phenol reagent, J. Biol. Chem. 1951; 193-265-275.
11. Montgomery R. Determination of glycogen. Biophys. 1957; 76: 423-428.
12. Liebermann-Burchard. The determination of cholesterol Biochem J. Dec;1959; 52(4):611–619.
13. Laemmli UK. Cleavage of Structural Proteins during assembly of the head of bacteriophage. Nature. 1970; 227 : 680-685.
14. Shakoori AR, Mughal A L and Iqbal M. Effects of sublethal doses of fenvalerate (a synthetic pyrethroid) administered continuously for four weeks on the blood, liver and muscles of a fresh water fish, Ctenopharyngodon idella. Bull. Environ. Contam. Toxicol.1996; 57: 487-494.
15. Aydin S, Erman Z, Bilgin OC. Investigations on Serratialiquefaciens infection in Rainbow trout .Turk J Vet Anim Sci .2001; 25 : 643-650.
16. Omprakasham M. Experimental infection with some piscine bacterial pathogens in the Indian major carp, Labeorohita(Hamilton) and the influence of Pseudomonas fluorescence on the host

metabolism. Ph. D thesis, University of Madras. 1991.

17. Leibmann H. and Reidmuller S. Electro-phorietische. Blutum- Tersuchunger Bei Hormalen Bauchwassorsuchtkranken Carpfen Sllefischerei. Ztg. 1960; 85: 502-505.
18. *Riedmuller S. Elektophoretische Bluteiweissuntersuchungen bei gesund en und kranken karpfen Allg. Fischeri. Ztg. 1965; 18: 28-35.
19. Richards RH and Pickering AD. Changes in serum parameters of Saprolegnia infected brown trout, Salmo trutta L. Journal of Fish Diseases. 1979; 2: 197-206.
20. Hargens AR., Millard RW. and Johansen K. High capillary permeability in fishes. Comp. Bioch. Physiol., 48A: 1974; 675-680.