

BIOCHEMICAL STUDIES ON 25 DAY OLD HEALTHY AND INFECTED FRY OF *Labeo rohita* (Hamilton)

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ABSTRACT

The caudal fin rot of fishes is commonly observed in fry and fingerlings in intensive cultures. Infected fry of *Labeo rohita* have been compared with the normal fishes for quantitative estimations of proteins, free amino acids and lipids, which has decreased in infected fishes. The variation in esterase patterns and SDS protein patterns too has been observed. Esterase patterns could be used as an indicator for comparison of normal and pathogenic fishes.

Key words: Caudal fin rot, fry, *Labeo rohita*, esterase, SDS proteins

I.INTRODUCTION

Several bacterial facultative fish pathogens are present in waters but epizootic occurs when environmental quality and the host defense systems of the fish deteriorate. Factors such as organic manure, heavy metals, and inorganic salts in quantities detrimental to the organism cause stress to which the reaction of the fish-skin can either be adaptive or degenerative¹. Fin rot is a common disease which is mainly being caused by stress or overcrowding. It leads to the formation of greyish, white patches, skin erosion and destruction of the fins, especially the caudal fins, causing large mortalities². The bacteria appearing in the tissues of the affected caudal fin of Indian Major carps and climbing perch was identified as *Flavobacterium columnare*³ and in tropical aquarium fishes as *Aeromonas punctata* and *P. fluorescent* groups⁴⁻⁶. The main reason for choosing the 25 days fry was that by this time it had lost all the embryonic remnants and is obliged to swim and catch the prey even though its digestive tract is poorly developed. It is at this stage, soon after the first feedings that the larvae are prone to infections.

II.MATERIALS AND METHODS

Live healthy and infected fingerlings were brought from a Government fish farm near Bheemaram, Hanamkonda. The symptoms observed were loss of tail and mass mortality. It was identified as caudal fin rot. The whole, healthy as well as infected fingerlings were used for biochemical estimations. Proteins were estimated by modified Lowry's method⁷, amino acids by using ninhydrin⁸ and lipids were estimated by the

method⁹. To study the qualitative variations the fingerlings were dissected out, the gut portion was separated from the rest of fry (myotome block) and they were homogenised in 0.01 M Tris-Hcl buffer pH 7.5. Aliquots were used for studying the patterns of esterases and SDS Proteins. Polyacrylamide gel electrophoresis was used to study the patterns of esterases¹⁰. The gels were prepared in 1.5 mm thick 6X6 cm glass plates. 7.5 % gels were prepared and allowed to gel for 45 minutes and the samples were loaded onto the gels and overlaid with electrode buffer. Current was supplied until the tracking dye reached a distance of 6 cm. Esterases were visualised on the gels with 1-naphthol acetate as the substrate¹¹. Proteins were separated by SDS electrophoretic method¹². The gels were prepared in 1.5 mm thick and 12X 12 cm glass plates. The samples were loaded onto the gel and laid with electrode buffer. After migration to 12 cms the gels were removed and fixed in 10% TCA at room temperature for one hour. Then TCA was removed and the gels were stained overnight with coomassie brilliant blue in 5:5:1 methanol:water:acetic acid. The gels were then destained in the solvent.

III.RESULTS

3.1BIOCHEMICAL ESTIMATION

The normal and the infected fingerlings were compared for their respective biochemical variations. The protein content in the normal fingerlings was estimated to be 5.3% as compared to 2.15% of the infected fingerling. The soluble peptides were estimated to be 6.45% in the normal as compared to 1.35% in infected fishes. Free amino acids were estimated to be 7.6 % in normal fishes 3.9 % in the infected fishes. All the parameters were less in the infected fishes as compared to the normal fishes except the water content, which was higher. Variations noticed in total protein content, acid soluble peptides free amino acids, lipids and water content of normal and infected fishes were found to be statistically significant. The biochemical estimation of infected and normal fingerlings are presented in Table 1.

3.2ESTERASE PATTERNS OF NORMAL AND INFECTED FINGERLINGS

The esterase pattern of normal fingerling had exhibited five bands. Four bands of Rm 9.2, 36.9, 46.2, 56.9 were present as traces and one band Rm 18.5 was present as an active band. The infected fishes have lost two bands of Rm 36.9 and Rm 46.2 as compared to the normal fishes. The gut of normal fishes had exhibited five bands. Two bands of Rm 18.5 and 27.7 were present in traces, one band of Rm 56.9 was present as a visible band, one band of Rm 36.9 was present as a active band and one band of Rm 46.4 was present as a hyperactive band. In the infected fishes the band with Rm 18.5 had stained as an active band as compared to the band present in traces in the normal fish. The band with Rm 27.7 is present as visible band as compared to the band present in traces in the normal fish. The band with Rm 46.2 is present in traces as compared to the hyperactive band in the normal fish. The esterase patterns of fingerlings are presented in Table 2 and fig 1.

3.3 SDS PATTERNS OF NORMAL AND INFECTED FINGERLINGS

The SDS protein pattern of the fingerling of normal fingerling stained 11 bands. In the infected fingerling myomere, the loss of six bands at Rm 91.6, 90, 87.5, 83.3, 56.6 and 50 were observed. There was decrease in

the intensity of staining in two bands at Rm 62.5 and 37.5, increase in the intensity of staining in one band at Rm 89.1. Two additional bands have stained at Rm 10.8 and 10. In intestine of normal fingerlings seven bands had been stained. In the infected fingerling two bands with Rm 62.5 and Rm 64.2 had decreased in the intensity of staining. Two bands corresponding to Rm 83.3 and 56.6 had been lost. These results are presented in Table 3.

IV.FIGURES AND TABLES

Table 1: Biochemical Estimations of Normal and Infected Fingerlings (25 days)

Condition	Proteins ^{\$}	Soluble Peptides ^{\$}	Free Aminoacids ^{\$}	Lipids (%) [#]	Water Content (%) ^{\$}
Normal	5.30 ^b ± 0.141	6.45 ^b ± 0.308	7.60 ^b ± 0.875	88.0 ^b ± 2.160	77.3 ^a ± 1.728
Infected	2.15 ^a ± 0.543	1.35 ^a ± 0.024	3.90 ^a ± 0.585	70.0 ^a ± 1.070	88.8 ^b ± 2.090

Note: \$ - mg/100 mg wet weight, # - mg/100 mg dry weight. The values with different (a,b) superscripts are significant (p<0.05)

Table 2: Esterase Patterns of Normal and Infected Fingerling

Tissue	Rm					
	56.9	46.2	36.9	27.7	18.5	9.2
IM	±	-	-	-	++	±
NM	±	±	±	-	++	±
IG	+	±	++	+	++	-
NG	+	+++	++	±	±	-

Note: IM-Infected Myotome, NM- Normal Myotome, IG-Infected Gut, NG- Normal Gut

Table 3: SDS Patterns of normal and Infected Fingerlings

Tissue	Rm													
	91.6	90.0	89.1	87.5	83.3	64.2	62.5	56.6	50.0	37.5	29.2	25.0	10.8	10.0
NM	+	+	+	+	+	-	++	+	+	+	+	+	-	-
IM	-	-	++	-	-	-	+	-	-	±	+	+	+	+
NG	-	-	-	-	+	+	+	+	-	±	±	±	-	-
IG	-	-	-	-	-	±	±	-	-	±	±	±	-	-

IM NM IG NG

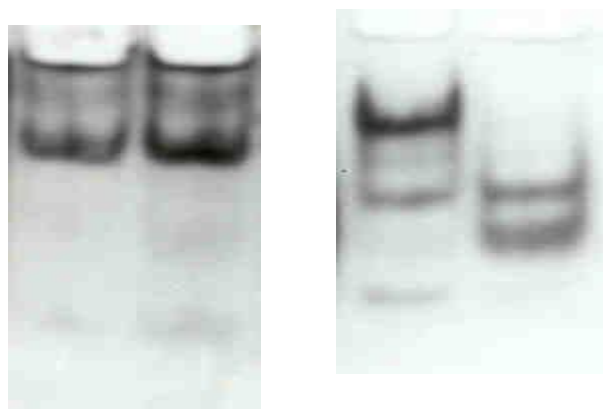


Figure 1: Zymogram of Normal and Infected Tissues

I.

IM - Infected Myotome

NM - Normal Myotome

IG - Infected Gut

NG - Normal Gut

V.DISCUSSION AND CONCLUSION

In the 25 days infected fry of *Labeo rohita* indicated that the quantitative biochemical values were less for whole fishes. There was an overall decrease in protein, soluble peptides, free amino acids and lipids. In esterase pattern loss of two bands in myotome and decrease or increase in activity in intestine was observed. In SDS proteins too, loss of some bands was observed. Fin rot is a bacteriosis form, which predominates the fry and young fish of tropics under captivity conditions. Aetiological agent of fin rot is not clear. It appears that it is not caused by a specific pathogen but by representatives belonging to the genera of *Aeromonas*, *Pseudomonas* and *Vibrio*^{4-6,13-14}. The bacterial participation occurs secondarily¹⁵. A relationship exists between fin rot and previous parasitic infections and transport damage^{5,16}. It was found that *Aeromonas hydrophila* causes disease in fishes and is considered an opportunistic pathogen in man^{17,18}. In fish the pathogens caused disruption of scales, fin rot, gill rot. Pattern of esterases indicated loss of bands in myotome where as in gut there was decrease in activity in one band and increase in activity in two bands. Zymogram of the SDS proteins of myotome show loss of some protein bands as well as induction of some additional bands, in gut there is loss of bands. All these variations only support that there is systemic infection too. In most of the cases hepatic necrosis, pancreatic atrophy and necrosis and gill lesions were caused by *Aeromonas* which is considered an opportunistic pathogen for man^{17,18}. Esterase exhibits resistance to or inactivates insecticides, it is being suggested as a probable bioindicator to measure toxic potency of pesticide residues¹⁹. Esterase has been used as a marker to identify and characterise *Opisthorchis viverrini*²⁰. From our studies it is evident that esterases are exhibiting significant variations under conditions of infection. It can be used as a tool for identification of pathogenicity. However the mechanism how it is behaving under pathogenic conditions has to be elucidated.

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