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SDS PAGE analysis of protein in skeletal muscles of *Labeo rohita* during immunomodulation and aeromoniasis

ANKAMMA, N*. AND VIVEKA VARDHANI, V.

Department of Zoology

Y.A. Government Degree college for women, Chirala-523155,(A.P) India

E-mail: ankamma.zoology@gmail.com

Department of Zoology and Aquaculture

Acharya Nagarjuna University, Nagarjunanagar-522 510 (A.P.) India

E-mail: vadlamudi_vv@yahoo.co.in

*Corresponding Author: Ankamma .N., Department of Zoology, Y.A. Government Degree College for women,Chirala-523155, Bapatla dt., Andhra Pradesh, India.

ABSTRACT

The present study investigates the alterations in protein profiles of skeletal muscle in *Labeo rohita* during immnomodulation and aeromoniasis. Nine months old fish (4 groups) were used in the present investigation. Groups A and B were treated with Aqua Fix for 4 days, on day 5 fish of group B and C were infected with *Aeromonas hydrophila* @ 10^{-6}CFU/fish(fish of group C were fed with normal diet for 4 days).Controls (group D) were untreated and uninfected. Three fish from group A were necropsied on day 2 and 5 after Aqua Fix treatment (6, and 9 of experimental period) and 3 fish from group B and C on day 1, and 4 after infection (6, and 9 of experimental period). Three fish from controls (group D) were also necropsied on the same designated days for comparison. Qualitative muscle protein profile by SDS-PAGE was analysed. The electrophoretic profiles showed distinguishable qualitative changes in protein bands across groups. Group C (Infected fish) showed degradation of muscle protein due to the stress caused by the pathogen and tissue damage. Group A (immunostimulated fish) showed up gradation of protein expression suggesting enhanced metabolic and immune response. Group B (fish which received aqua fix and infection) showed loss of 5 protein bands and synthesis of 2 new bands which indicates the partial restoration of protein profiles and protective effect of Aqua fix .This study supports the role of immunostimulants in disease resistance in Aquaculture and use of SDS-PAGE to monitor physiological changes at the proteomic level.

Key words: Aqua Fix, *Aeromonas hydrophila* , *Labeo rohita* , Muscle, SDS-PAGE

INTRODUCTION

Intensive aquaculture practices in India on Indian major carps led to the interest on aeromoniasis and the use of immunostimulants to enhance fish production. Different types of immunostimulants are used to reduce mortality due to infections and to improve the health of fish (Thangamani and Govinandarajan, 2015), control of *Aeromonas hydrophila* infection in cat fish was reported by the use of medicinal plants (Thiyagarajan et al; 2014). Herbal drugs are reported to stimulate both humoral and cell mediated immunity (Bafna and Mishra,2004). Herb supplementary diets have shown better growth, disease resistance, weight gain and restoration of health in fry of *Catla catla, L. rohita, C. mrigala* (Dey and Chandra,1995; Kavita,1996;Kumar, 2000;Rao et al;2004). Orally administered herbal extracts enhanced some immune parameters in carp (Yuan et al; 2007) and also disease resistance against *A. hydrophila* in Nile tilapia (*Oreochromis niloticus*) (Ardo et al; 2008), *Carassius aurataus* (Sauray et al., 2013), and *L. rohita* (Rao et al., 2006; Sahu et al.,2007 a,b; Das et al., 2013). *L. rohita* exposed to pesticides , diseases and other stress conditions showed marked biochemical changes in the internal organs (Patel and David,2007; Muthu Kumar et al., 2013). *A. hydrophila* infected gold fish fed with herbal supplementation feeds significantly enhanced haematological and immunological parameters (Harikrishnan et al., 2010). Decrease of protein level was found in muscle and liver of *Channa punctatus* (Sastry and Dasgupta,1991), gill, liver, muscle and brain in *O. mossambicus* (Durairaj and Selvarajan,1992) and *Glassogobius gluris* (Venkataraman et al., 2006). Dietary herbal powders alter haematological (Ukwe et al., 2021) and biochemical (Alsaid et al., 2014; Ukwe and Abu,2021) parameters in fish. Feedings of leaf extract showed significantly high values of immunological parameters like lysozyme activity, NBT, plasma protein and anti-protease activity in *L.rohita* against *A.hydrophila* (Debbarma et al., 2022).The effect of Aqua Fix (immunostimulant) was studied in the skeletal muscles and liver of *L. rohita* and found significant increase of protein, carbohydrate, DNA and RNA (Ankamma and Viveka Vardhani,2017).These findings led to assess the qualitative protein profile in the skeletal muscles of *L. rohita* fed with Aqua Fix (an immunostimulant), infected with *A. hydrophila,* infected + immunostimulated by Aqua Fix.

MATERIALS AND METHODS

L. rohita (both experimental and controls) used in this study were at the age of 9 months (47-50 gms.) and were obtained from Kuchipudi, Andhra Pradesh, India. *A. hydrophila* strain was obtained from SIFT (state institute of fisheries technology), Kakinada, A.P and cultured and desired doses of infection were prepared under aseptic conditions following the method of Pelczar (1993). Aqua Fix (manufactured by PVS laboratories, Vijayawada, Krishna district, A.P), a highly specialized, pure herbal preparation consisting of alkaloids of *Azadirachta indica, Ocimum sanctum, Phyllanthus niruri* and *Withimnia somnifera* having strong antimicrobial and immunity enhancing properties was used in this study (dosage of Aqua Fix was determined as per the recommendation of PVS labs (5 gms / kg feed). LD50 value of *A. hydrophila* was determined following standard methods. Each experimental (A, B, C) and control (D) group consisted of 9 fish. Fish of experimental group A were fed with immunostimulant (@50mg/100g. of feed) for 4 days, fish of group B were fed with immunostimulant (@50mg/100g. of feed) for 4 days and infected intraperitonially with *A. hydrophila* @10-6 CFU/fish on day 5, fish of group C were fed with normal diet (without Aqua Fix) for 4 days and infected intraperitonially with *A. hydrophila* @10-6 CFU/fish on day 5 and fish of group D were kept as controls (untreated with Aqua Fix and uninfected with *A. hydrophila*). Three fish from group A were necropsied on day 2 and 5 after Aqua Fix

treatment (6, and 9 of experimental period) and 3 fish from group B and C on day 1, and 4 after infection (6, and 9 of experimental period). Three fish from controls (group D) were also necropsied on the same designated days for comparison. Qualitative muscle protein profile by SDS-PAGE was analysed following the method of Laemmli (1970).

RESULTS AND DISCUSSION

Protein profile of SDS –PAGE analysis is represented in figure 1, 2 and table 1 and 2.

Skeletal muscle protein profile on day 1 of experimental period (Fig.1, Table 1)

Serially arranged protein bands of ~ 97 KDa to ~ 14.4 KDa appeared in marker. Control and three experimental groups of fish showed varied number of protein bands.

Group A: In the skeletal muscle samples of group A, 13 protein bands were recorded on day 1 of experiment. The isolated protein bands have molecular weight ~ 104, 88, 65, 38, 35, 33, 26, 24,19, 18, 17, 14 and 13 KDa. Compared with controls, 2 protein bands ~ 45 KDa and 42 KDa disappeared and protein bands ~ 33 to 13 KDa were present both in control (D) and experimental group (A)

Group B: Aqua Fix treated and infected fish showed 11 protein bands on day 1. The molecular weight of these serially arranged protein bands are ~ 104, 79, 65, 41, 38, 33, 21, 20, 18, 14 and 13 KDa respectively. 5 protein bands of ~ 88, 35, 26, 24 and 19 KDa (found in Aqua Fix treated fish, group A) disappeared on day 1 of experiment in group B (due to infection); but two protein bands ~ 41 and 20 KDa were synthesized additionally.

Group C: Infected fish showed only 7 protein bands. Protein bands ~ 79, 65, 41 and 38 KDa present in group B were absent in group C. A protein band of ~ 36 KDa which was present in controls and absent in groups A and B reappeared in group C. Low molecular weight protein bands (~ 17 to 13 KDa) does not show any change (except in group B).

No marked change was found in protein bands of controls (group D) and immunostimulated (group A) fish (except the disappearance of protein bands ~ 45, 42, 36 and 21 KDa). Compared with immunostimulated fish (group A), immunostimulated + infected fish (group B) showed loss of 4 protein bands (~ 88, 35, 26 and 24 KDa) and synthesis of 4 new bands (~ 79, 41, 21 and 20 KDa). Infected fish (group C) showed the disappearance of many high molecular weight protein bands compared to control (group D) and other experimental (group A, B) fish**.**

Skeletal muscle protein profile on day 4 of experimental period (Fig.2, Table 2)

A series of protein bands ranging between 97 to 14.4 KDa were exhibited in the marker. Control and three experimental groups of fish showed a series of high and low molecular weight protein bands.

Group A: Fish treated with Aqua Fix for 4 days showed two additional protein bands of ~ 40 KDa and ~ 20 KDa and disappearance of five ~ 88, 45, 42, 36 and 21 KDa protein bands compared to controls (group D). No marked changes were found in protein bands of ~ 33 to 13 KDa (with few exceptions).

Group B: Fish which received Aqua Fix and infection showed 12 protein bands. The molecular weight of these isolated protein bands is ~ 91, 69, 65, 39, 33, 24, 22, 20, 18, 17, 14 and 13 KDa. Four protein bands of ~ 91, 69, 39 and 22 KDa which were not found in groups D (controls) and A were found in group B on day 4 of experimental period. No marked changes were found (~18 to 13 KDa) in low molecular weight protein bands.

Group C: Infected fish showed very less number of protein bands when compared with other groups (D,A and B).The protein bands ranging between ~ 69 to 35 KDa were disappeared and a protein band of ~ 88 KDa which was present in controls reappeared in group C on day 4. There is no change in low molecular weight protein bands (~ 18 to 13 KDa).

Two protein bands of ~ 33 and 24 KDa were found commonly in controls (group D) and other experimental groups (A, B and C). Another protein band of ~ 88 KDa was present in groups D (control) and C (infected). Protein bands ~ 45, 42 and 36 KDa appeared in controls were totally absent in all the experimental groups (A, B and C). Low molecular weight protein bands ~ 18, 17, 14 and 13 KDa were found commonly in all the experimental (except in group A) and control (group D) fish**.**

SDS-PAGE analysis of muscle proteins in test fish (groups D, A, B, C) – 9 months old exhibited interested findings. Test fish showed no distinct change in protein bands on day 1 compared to day 4 of experiment.

CONCLUSION

Nine months old fish showed protein bands of low molecular weight on day 1 and 4 of experiment compared to 6 months old fish (Ankamma and Viveka Vardhani, 2017). Infected fish (group C) showed loss of high molecular weight protein bands (except ~ 88 KDa protein band on day 4) on day 1 and 4 of experiment. In groups A and C there was a marked change in protein bands of ~ 41 KDa to 17 KDa. These changes might be due to the stress caused by the treatment of Aqua Fix and/or infection. Gomez *et al.,* (2007) and Demir *et al*., (2011) also found the effect of environment and stress on the structure and features of muscle proteins in farmed Atlantic salmon and in *S.t. macrostigma* (living in 4 different rivers).Use of herbal immunostimulants contributes to sustainable aquaculture and fish health management.

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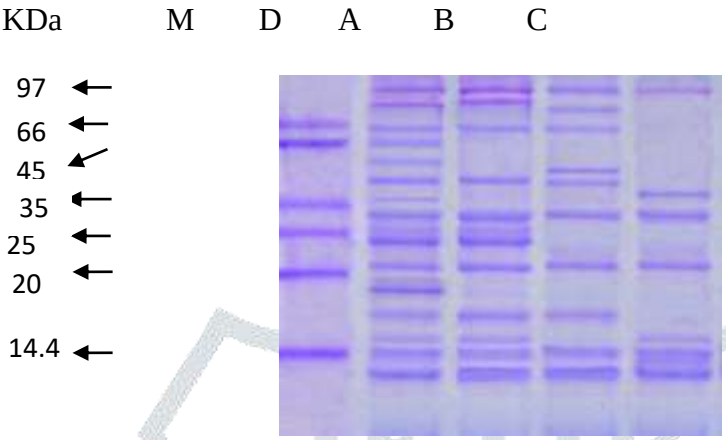
Fig.1: Gel photograph showing the isolated proteins from the skeletal muscles of control (group D) and experimental groups (A,BandC) of 9 months old *L. rohita* on day 1 of experimental period by Polyacrylamide gel electrophorosis

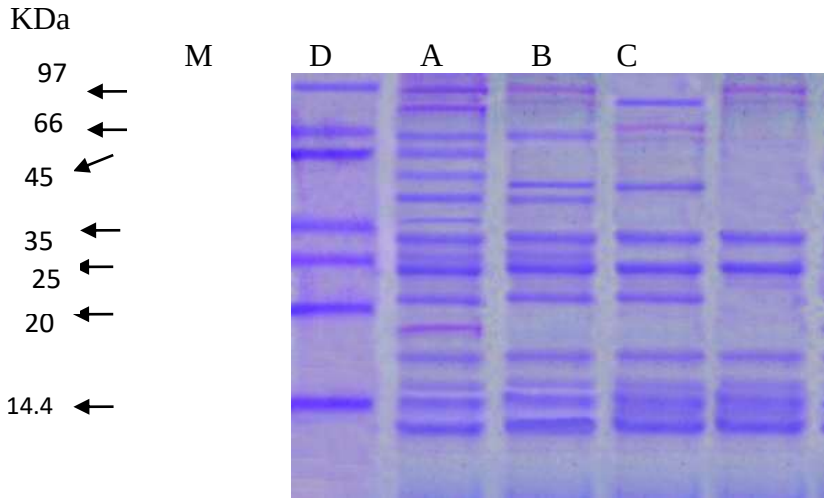


Lane 1: Marker (97 KDa, 66 KDa, 45 KDa, 35 KDa, 25 KDa, 20 KDa, 14.4 KDa)
Lane 2: Group D (Control, Untreated and Uninfected)
Lane 3: Group A treated with Aqua Fix @ 50mg/100g of feed
Lane 4: Group B treated with Aqua Fix @ 50mg/100g of feed + infected with *A. hydrophila* @ 10^{-6} CFU/Fish
Lane 5: Group C Infected with *A. hydrophila* @ 10^{-6} CFU/Fish

Table1: Characterization of isolated proteins on the basis of their molecular weight in skeletal muscles of control and various experimental groups of 9 months old *L. rohita* on day 1 of experimental period.

S.No.	Marker Mol.wt. KDa	Group D	Group A	Group B	Group C
1	97.0	104.809	104.809	104.639	104.245
2	66.0	88.665	88.832	79.899	36.138
3	45.0	65.991	65.982	65.547	33.133
4	35.0	45.780	38.774	41.532	21.513
5	25.0	42.146	35.135	38.031	17.631
6	20.0	38.774	33.133	33.109	14.386
7	14.4	36.135	26.516	21.513	13.167
8		33.133	24.697	20.697	
9		26.536	19.513	18.392	
10		24.497	18.263	14.357	
11		21.106	17.622	13.125	
12		19.285	14.384		
13		18.263	13.142		
14		17.649			
15		14.400			
16		13.073			

Fig.2: Gel photograph showing the isolated proteins from the skeletal muscles of control (group D) and experimental groups (A, B and C) of 9 months old *L. rohita* on day 4 of experimental period by Polyacrylamide gel electrophoresis



Lane 1: Marker (97 KDa, 66 KDa, 45 KDa, 35 KDa, 25 KDa, 20 KDa, 14.4 KDa),
Lane 2: Group D (Control, Untreated and Uninfected),
Lane 3: Group A treated with Aqua Fix @ 50mg/100g of feed,
Lane 4: Group B treated with Aqua Fix @ 50mg/100g of feed + infected with *A. hydrophila* @ 10^{-6} CFU/Fish,
Lane 5: Group C Infected with *A. hydrophila* @ 10^{-6} CFU /Fish.

S.No	Marker Mol.wt. KDa	Group D	Group A	Group B	Group C
1	97.0	104.809	104.809	91.161	105.715
2	66.0	88.665	65.244	69.996	88.585
3	45.0	65.991	40.121	65.757	33.160
4	35.0	45.780	38.763	39.892	24.501
5	25.0	42.146	33.133	33.160	18.286
6	20.0	38.774	26.516	24.501	17.704
7	14.4	36.135	24.497	22.923	14.539
8		33.133	20.701	20.819	13.108
9		26.536	19.513	18.286	
10		24.497	18.263	17.704	
11		21.106	16.706	14.539	
12		19.285	14.400	13.108	
13		18.263	13.126		
14		17.649			
15		14.400			
16		13.073			

Table2: Characterization of isolated proteins on the basis of their molecular weight in skeletal muscles of control and various experimental groups of 9 months old *L. rohita* on day 4 of experimental period

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