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Effect of dietary Aqua fix on qualitative protein profile in skeletal muscles of *Labeo rohita*

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ABSTRACT

The effect of Aqua Fix (immunomodulator) on the qualitative protein index of skeletal muscles of *Labeo rohita* was investigated. Test fish (twelve fish of 6 months old, 12-15 g.wt; 12 fish of 9 months old, 47-50 g.wt.) were fed with Aqua Fix @ 50 mg / 100 g of feed for 4 days (group A, B). Another 2 groups (a, b) (12 nos. in each group) of fish of same age and weight were fed with normal diet and kept as control for comparison. Significant alterations were found in the molecular weight of skeletal muscle protein in the test and control fish through SDS-PAGE analysis on day 1, 4, 7, 15 and 30 of experiment. Qualitative analysis depicted varied number of low and high molecular weight protein bands in the skeletal muscles of *L. rohita* of experimental and controls.

Key words: Dietary Aqua Fix, Skeletal muscles, Protein, *L. rohita*

INTRODUCTION

Aquaculture is one of the fastest food producing sector and infectious diseases are causing heavy loss to the fish culturists. *Aeromonas hydrophila*, an opportunistic pathogen is wide spread globally and infects both freshwater and warm water fish. This bacterium causes haemorrhagic septicemia, ulcers, abscesses, exopthalmia and abdominal distension (Austin and Austin, 1987; Chowdhury, 1998; Rajeswari *et al*., 2005). Recent knowledge on immunonutrition studies reveal that the growth, disease resistance, and non-specific and specific immunity of the fish may be raised by the use of some nutrients (Priya *et al*., 2004; Kumar *et al*., 2005). Use of antibiotics and chemotherapeutics to control fish diseases has the risk of bioaccumulation and development of resistant pathogens. Under such conditions, feeding cultivable fish through dietary immunostimulants cannot be overlooked.

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Various herbal extracts such as *Aloe vera* (Kim *et al*., 1999), *Ocimum sanctum* (Logambal *et al*., 2000), *Zingiber officinale* (Dugenci *et al*., 2003), *Achyranthes aspera* (Vasudeva and Chakrabarti, 2005a, b) and *Solanum trilobatum* (Divyagnaneswari *et al*., 2007) have been reported to enhance immunity in fish. *Cynodon dactylon* (L.) and *Coriandrum sativum* extract mixed diet enhanced disease resistance and production of specific antibodies in *Catla catla* (Xavier Innocent, *et al*., 2011; Kaleswaran *et al*., 2012) against aeromoniasis. Administration of glucan and *Aegle marmelos* enhanced survival and immunity in *Cyprinus carpio* challenged with *A. hydrophila* (Selvaraj *et al*., 2005; Pratheepa *et al.,* 2010) and in mice treated with Immunex DS(Jasmin Gold and Viveka Vardhani, 2016). Administration of microbial levan showed enhanced haemtological and non-specific immunological changes in *Labeo rohita* (Gupta *et al.,* 2008). The structure and molecular weight of muscle protein in fish be altered due to the stress caused by various physiologic factors, environment, seasons of the year, starvation, breeding season and migration (Gomez *et al.,* 2000; Ladrat *et al.,* 2000; Delbare-Ladrat *et al.,* 2006). Sultana *et al.,* (2016) assessed the effect of different feed on the quantitative and qualitative changes in the protein content of *C. mrigala*, *C. catla* and *L. rohita*. Keeping in view the importance of the dietary immunostimulants and economic importance of the Indian major carps, the present study is aimed to assess the efficacy of Aqua Fix (as an immunostimulant) on the qualitative changes in the content of skeletal muscle protein of *L. rohita*.

MATERIALS AND METHODS

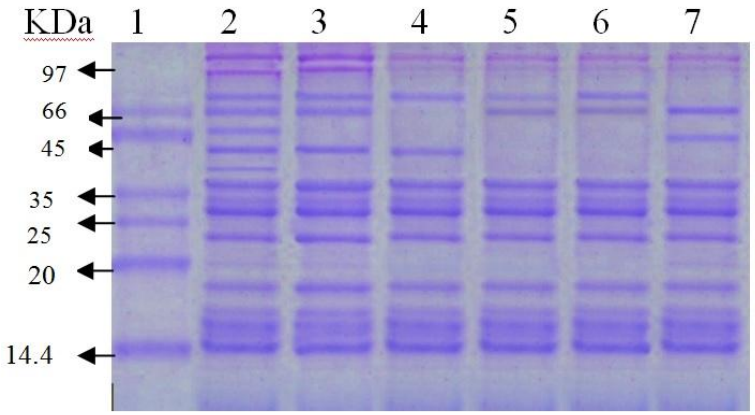
Six months and nine months old experimental fish (12-15 g.wt. and 47-50 g.wt.) were collected from Singh ponds, Kuchipudi village, Guntur District, Andhra Pradesh, India and allowed to acclimatize to laboratory conditions for one week. Four groups (A, B, a, b) of fish were maintained; two experimental (group A, B) and two control groups (group a, b) of 12 fish in each group. Experimental group of fish (A, 6 months old; B, 9 months old) were fed with Aqua Fix @ 50 mg/100 g of feed for 4 days and control groups (a, 6 months old; b, 9 months old) with normal diet.Two fish from groups A and B were necropsied on day 1, 4, 7, 15 and 30 after treating with immunostimulant diet. Control fish (from groups a and b) were also necropsied on the same designated days. Pieces of skeletal muscle tissue were removed from both the experimental and control groups of fish and qualitative analysis of proteins was performed by SDS-PAGE using the Discontinuous Buffer system of Laemmli (1970).

RESULTS AND DISCUSSION

L. rohita of 6 months old fish fed with diet supplemented with Aqua Fix (group A) and controls (group a) showed a number of protein bands ranging from 97 to 14.4 KDa and a protein band ~ 45 KDa disappeared on day 1 of experiment (Fig-1, Table-1). On day 4, protein band ~ 66 KDa disappeared; protein band ~ 40 KDa (found on day 1 and 4) was absent on day 7. Protein band ~ 66KDa reappeared on day 7. No marked changes were found in the isolated proteins on day 15 except the absence of a protein band ~ 40 KDa and presence of a protein band ~ 66 KDa. On day 30 of treatment the isolated proteins have a molecular weight ranging between 35 to 14.4 KDa; a protein band ~ 82 KDa disappeared and other protein band ~ 45 KDa appeared.

Control (group b) and experimental (group B, fed with Aqua Fix for 4 days @ 50 mg/100 g. of feed) 9 months old *L. rohita* showed a series of several protein bands ranging ~ 97 to 14.4 KDa (Fig-2, Table-2). In the muscle samples of experimental fish (group B), 13 protein bands were recorded on day 1 of experiment. The isolated protein bands have molecular weight as ~ 104, 88, 65, 38, 35, 33, 26, 24, 21, 18,17, 14.4 and 13 KDa. Compared with controls, 2 protein bands ~ 45.7 KDa and 42.1 KDa disappeared and protein bands ~ 33.1 to 13.0 KDa were present both in groups B and b. On day 4, experimental fish showed an additional protein band ~ 40 KDa and disappearance of ~ 88 KDa and 35 KDa protein bands; no marked change was found in protein bands of 33.1 to 13.1 KDa. Two protein bands 66 and 45 KDa appeared distinctly as in controls on day 7 of treatment in fish of group B. The additional protein band appeared on day 4 (~ 40 KDa mol. wt.) retained, a protein band ~ 20.7 KDa disappeared and protein bands between ~ 35

Figure-1. Gel photograph showing the isolated proteins from the skeletal muscles of 6 months old experimental (group A) and control (group a) *L. rohita* 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 a (Control), untreated.
Lane 3 - Group A , Day 1 of treatment.
Lane 4 - Group A , Day 4 of treatment.
Lane 5 - Group A , Day 7 of treatment.
Lane 6 - Group A , Day 15 of treatment.
Lane 7 - Group A , Day 30 of treatment.

Table-1: Characterization of isolated protein on the basis of their molecular weight in skeletal muscles of experimental (group A) and control (group a) 6 months old *L. rohita.*

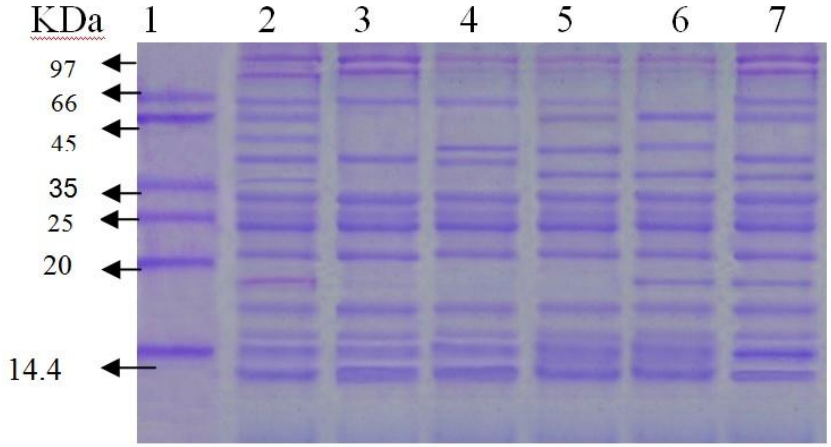
S.No	Marker Mol.wt. KDa	Control Group A	Group A				
			Day 1	Day 4	Day 7	Day 15	Day 30
1	97.0	110.235	110.235	109.280	109.280	110.235	109.280
2	66.0	97.628	100.485	82.579	82.579	82.270	66.759
3	45.0	82.270	82.270	40.656	66.146	66.759	45.146
4	35.0	66.146	66.146	36.425	36.425	36.425	36.425
5	25.0	45.693	40.890	34.326	34.326	34.326	34.326
6	20.0	40.656	36.425	28.904	28.904	28.904	28.904
7	14.4	38.725	34.326	24.706	24.306	24.706	24.423
8		36.425	28.689	19.526	19.489	19.526	19.536
9		34.326	24.474	17.294	17.440	18.343	18.043
10		28.904	19.526	16.426	16.726	16.726	16.726
11		24.497	17.776	14.799	14.799	14.799	14.885
12		19.106	16.726				
13		17.185	14.799				
14		16.285					
15		14.799					

to 13.0 KDa remained stable on day 7 of treatment. Fish of group B showed distinct protein bands on day 15; a protein band ~ 65.2 KDa disappeared and that of ~ 21.5 KDa appeared as additional band and no marked alteration was found in protein bands ~ 45 KDa to 13.0. No marked changes were found in the arrangement of

Table-2: Characterization of isolated protein on the basis of their molecular weight in skeletal muscles of experimental (group B) and control (group b) 9 months old *L. rohita.*

S.No.	Marker Mol.wt. KDa	Control group b	Group B				
			Day 1	Day 4	Day 7	Day 15	Day 30
1	97.0	104.809	104.809	104.809	102.925	104.809	104.809
2	66.0	88.665	88.832	65.244	65.244	45.551	87.288
3	45.0	65.991	65.982	40.121	45.551	40.968	65.521
4	35.0	45.780	38.774	38.763	40.480	36.135	45.521
5	25.0	42.146	35.135	33.133	36.135	33.180	38.765
6	20.0	38.774	33.133	26.516	33.180	26.541	36.135
7	14.4	36.135	26.516	24.497	26.541	24.915	33.180
8		33.133	24.697	20.701	24.515	21.513	26.541
9		26.536	19.513	19.513	19.513	19.285	24.215
10		24.497	18.263	18.263	18.304	18.304	21.513
11		21.106	17.622	16.706	17.684	17.684	19.285
12		19.285	14.384	14.400	14.400	14.447	18.304
13		18.263	13.142	13.126	13.099	13.099	17.642
14		17.649					14.447
15		14.400					13.099
16		13.073					
17							

Figure-2. Gel photograph showing the isolated proteins from the skeletal muscles of 9 months old experimental (group B) and control (group b) *L. rohita* 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 b, (Control), untreated.
Lane 3 – Group B , Day 1 of treatment.
Lane 4 - Group B, Day 4 of treatment.
Lane 5 – Group B, Day 7 of treatment.
Lane 6 - Group B, Day 15 of treatment.
Lane 7 – Group B, Day 30 of treatment.

protein bands on day 30 of treatment in group B in comparison with controls (group b) except the disappearance of a protein band ~ 42.1 KDa.

In the present investigation, the impact of Aqua Fix on the qualitative profile of muscle protein in 6 and 9 months old *L. rohita* is confirmed by the presence of varied number of isolated proteins of different molecular weights. These results are in agreement with the findings of Li *et al.,* (2000) and Islam (2006) who reported the effect of dietary protein on growth of *Ictalurus punctatus* and expression of muscle protein in pikeperches, *Stizostedion lucioperca* and *S. volgensa*. Singh *et al.,* (2005)) also determined the importance of protein for optimum growth in *L. rohita*. Divya Teja and Viveka vardhani (2016) also found enhanced level of protein in the abdominal muscles of mice treated with Immunex DS. In the present study, high molecular weight (97 KDa) protein bands were isolated and characterized by SDS-PAGE in 6 and 9 month old muscle proteins. Mitsuhashi *et al.,* (2002) and Fock and Hinssen (2002) also characterized several proteins as an integral parts of skeletal and cardiac muscles in fish. The present findings are further supported by the findings of Okagaki *et al*., (2005) and Montowska and Pospiech (2007) who found protein bands ranging between 16 and 26 KDa in skeletal muscles of species of carp. In 6 and 9 month old *L. rohita* fed with a diet supplemented with immunostimulant, variable number of protein bands were observed in skeletal muscles on day 1, 4, 7, 15 and 30 of treatment. Three bands were shared between 6 months (group A) and 9 months (group B) old *L. rohita* on day 1 (24.4,24.6;19.5, 19.5;17.7,17.6), 5 bands on day 4 (40.6,40.1;24.7,24.4; 19.5,19.5;16.4,16.7;14.7,14.4), 5 bands on day 7 (36.4,36.1;24.3,24.5;19.4,19.5;17.4,17.6;14.7,14.4), 5 bands on day 15 (36.4,36.1;24.7,24.9; 19.5,19.2;18.3, 18.3;14.7,14.4) and 6 bands on day 30 (45.1,45.5; 36.4,36.1;24.4,24.2;19.5,19.2;18.0,18.3;14.8,14.4) These findings are in agreement with that of Mathew and Prakash (2006) who reported 23 and 22 KDa proteins from *Sardinella longiceps*. The present study on immunomodulatory effect of Aqua Fix on the concentrations of skeletal muscle proteins of *L. rohita* are supported by the findings of Salim (2006), Sultana *et al*., (2016), and Madhuri and Viveka Vardhani, (2015). who reported increased muscle protein and GST content with higher dietary protein in carps and mice.

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Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

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