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Dietary Aqua Fix Effectively Alters the Qualitative Protein Profile in the Head Kidney

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ABSTRACT

The qualitative protein profile in head kidney of 6, 9 and 12 months old *Labeo rohita* was investigated by feeding with a diet mixed with an immunomodulator, Aqua Fix. 6 (group A), 9 (group B), and 12 (group C) months old fish (12-15 g wt.; 12 fish in each group) were fed with a diet mixed with Aqua Fix @ 50 mg/100 g of feed for 4 days. Another 3 groups (a, 6 months; b, 9 months; and c, 12 months) of same weight (12 fish in each group) were fed with a normal diet to act as controls for comparison. Head kidney showed significant alteration (with low and high molecular weight proteins) in the qualitative protein profile (analyzed by SDS page analysis), on day 1, 4, 7, 15 and 30 of experiment in all the three groups of 6 (group A), 9 (group B) and 12 (group c) months old fish. These results determine the effect of Aqua Fix in modulating the immune system of fish with regard to the marked changes in the profile of proteins.

Key words: Rohu. Aqua Fix, head kidney, qualitative proteins.

INTRODUCTION

Aeromonas hydrophila was recognized as one of the most microbial pathogen causing disease in farmed and freshwater fishes (Sarkar *et al.,* 2000; Manoj *et al.,* 2010). Several groups of fish like carps, murrels, catfishes etc., suffer due to the pathogenecity of *A. hydrophila*; infected fish show several clinical symptoms like dropsy, hemorrhagic septicemia, ulcers and tail and fin rot (Rahman *et al.,* 2001; Mostofa *et al.,* 2008). The fish defense system is similar to mammals. Teleost have both humoral and cellular systems; Due to the lack of bone marrow and lymphoid organs, head kidney serves as the major lympohoid organ in fish (Press and Evenesen, 1999). Several environmental factors, nutritional factors and pathogens influence the natural, humoral and cellular immunity in fish; stress by any one of the factors may cause susceptibility to infection (Magnadottir, 2010; Plumb and Hanson, 2011). Many cells and their products such as superoxides, acute-phase proteins, lysozyme, interferons, lysins, properdin and agglutinins referred as indicators of stress in fish (Sahoo and Mukherjee, 2003). Among the 3 Indian major carps, *Labeo rohita* showed highest value in cellular products compared to other 2 species (Sahoo *et al.,* 2005). Therefore, the aim of the present study is to determine the effect of *A. hydrophila* on the qualitative proteins in head kidney of 6, 9 and 12 months old rohu.

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Materials and Methods

Rohu (6, 9 and 12 months old) (12-15, 47-50 g. wt) were collected from Singh ponds, Kuchipudi village, Guntur District (A.P.), India and acclimatized in laboratory for one week. Three experiments were conducted - in experiment 1, three experimental groups (A, B, C) and three controls (a, b, c) of 12 fish in each group were maintained. Experimental of 6 (group A), 9 (group B) and 12 (group C) months old were fed with Aqua Fix @ 50 mg/100 g of feed for 4 days and controls (a, 6months), (b, 9 months), (c, 12 months) with normal diet. Two fish from experimental (A, B, C) and control (a, b, c) groups were necropsied on day 1, 4, 7, 15 and 30 after the treatment of Aqua Fix and tissues

of head kidney were removed, and analyzed for qualitative proteins adopting by SDS-PAGE analysis using the Discontinuous Buffer system of Laemmli (1970).

Results and Discussion

The qualitative protein profile of head kidney revealed the sensitivity of fish immune system to Aqua Fix supplemented diet and/or stress. SDS-PAGE analysis of head kidney proteins revealed marked differences in protein banding pattern and molecular weights in 6, 9 and 12 months old Aqua Fix treated (group A, B and C) and control (untreated with Aqua Fix, group a, b, and c) fish at day 1, 4, 7, 15 and 30 of experiment. Gomez *et al.,* (2000) Ladrat *et al.,* (2000) and Delbarre-Ladrat *et al.,* (2006) reported that physiologic factors, environment, seasons, stress, starvation, breeding season and migration affect the structure and molecular mass of muscle proteins in fish. Demir *et al.,* (2010, 2011) found some differences in the number and molecular weights of protein bands in different populations of mountain trout, *S.t. macrostigma*

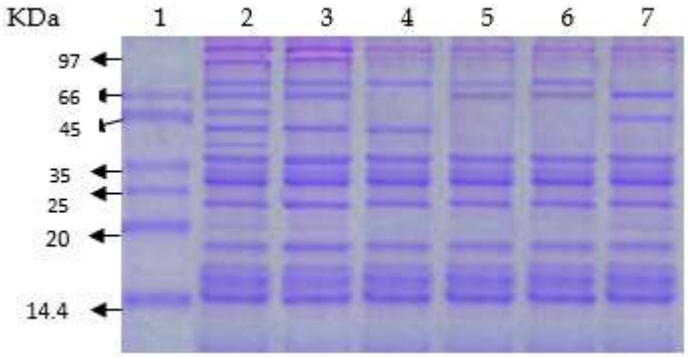

Figure-1. Electropherogram of protein profile in head kidney of control (group a) and experimental (Group A), (treated with Aqua Fix @ 50mg/100g of feed) 6 months old *L. rohita* at different days of experiment
Lane 1 - Marker (97 KDa, 66 KDa, 45 KDa, 35 KDa, 25 KDa, 20 KDa, 14.4 KDa); Lane 2 - Group 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 proteins on the basis of their molecular mass in head kidney 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	97.000	119.567	109.868	127.983	122.860	108.031	115.581
2	66.000	99.664	66.751	106.945	114.802	95.108	94.791
3	45.000	89.283	62.811	102.717	98.010	74.031	76.235
4	35.000	66.536	53.151	84.122	79.028	52.985	53.649
5	25.000	62.015	43.968	66.110	57.658	39.049	40.570
6	20.000	54.153	41.666	49.198	53.316	31.717	36.317
7	14.400	41.175	34.991	43.968	40.332	28.223	30.855
8		34.498	32.160	33.349	35.800	25.232	26.291
9		33.162	26.088	29.780	31.542	21.611	20.388
10		28.223	23.405	26.291	23.463	16.532	17.462
11		24.416	21.109	23.696	20.577	14.255	15.006
12		21.974	19.487	17.462	17.793		14.556
13		19.530	16.664	16.023	16.023		
14		17.534	15.426	14.634	14.110		
15		15.839	14.505				
16		14.505	12.478				
17		12.478					
18		11.127					

living in different rivers. Mahaboob *et al,* (2012) noted that cultured *L. rohita* possessed more muscle proteins than wild ones and depended on their weights.

Aqua Fix treated and control fish of 6, 9 and 12 months old showed varied protein bands (in the number and molecular mass) at day 1, 4, 7, 15 and 30 of experimental period. 6 months old controls (group a) showed a series of 18 protein bands (ranging between ~ 119 to 11 KDa), 9 months old controls (group b) exhibited a series of 17 bands (ranging from ~ 102 to 13 KDa) and 12 months old controls (group c) displayed 18 bands (ranging from ~ 101 to 12 KDa) in the head kidney. It is interesting to note that 6 and 9 months old fish shared 4 protein bands (~ 34, 21, 19 and 14 KDa), 9 and 12 months old fish shared 5 protein bands (~ 58, 46, 20, 16 and 14 KDa) and 6 and 12 months old fish shared 4 protein bands (~ 66, 33, 14 and 12 KDa); only one protein band (~ 14 KDa) was found as common band in 6, 9 and 12 months old control fish. These results showed that in different age fishes (groups a, b, c), each

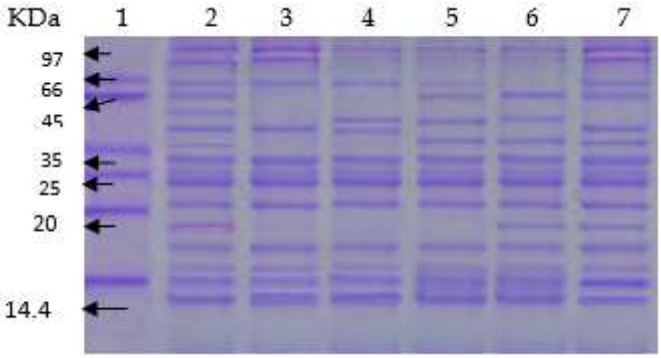

Figure-2. Electropherogram of protein profile in head kidney of control (group b) and experimental (Group B), (treated with Aqua Fix @ 50mg/100g of feed) 9 months old *C. rohita* at different days of experiment
Lane 1 - Marker (97 KDa, 66 KDa, 45 KDa, 35 KDa, 25 KDa, 20 KDa, 14.4 KDa); Lane 2 - Group 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

Table 2. Characterization of isolated proteins on the basis of their molecular mass in head kidney 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	97.000	102.244	106.353	109.911	111.003	117.412	115.487
2	66.000	98.304	66.582	90.020	106.004	103.595	102.244
3	45.000	84.097	56.921	73.949	94.526	87.712	92.394
4	35.000	67.218	50.319	53.505	68.291	81.952	81.689
5	25.000	58.904	43.917	39.589	51.409	73.949	73.949
6	20.000	46.921	38.222	30.191	38.446	61.156	55.012
7	14.400	44.313	34.256	26.719	34.158	45.118	48.072
8		38.559	31.564	23.212	31.040	36.278	36.593
9		34.649	24.498	19.734	26.226	30.359	32.189
10		31.476	21.799	16.430	22.017	27.151	28.119
11		26.506	20.605	14.900	18.873	25.078	21.908
12		21.908	19.184	13.551	16.083	19.875	18.108
13		20.805	16.936		14.722	15.488	15.650
14		19.094	15.361		13.377	14.020	14.519
15		16.899	14.047			13.451	13.111
16		14.722					
17		13.255					

sample of head kidney proteins consisted of dissimilar and similar protein bands compared among themselves. Jitender Kumar *et al.,* (2012) reported that fish exposed to different diet and environmental conditions may show different protein band patterns. Vidyasagar Reddy and Vijaya (2014) recorded 14 protein bands of ~ 250 to 10 KDa in *Tilapia mosambica, L. rohita* and *C. catla*. Geri *et al.,* (1995) and Gangwar *et al.,* (2007) correlated the muscular composition of *C. carpio* and *L. rohita* with the influence of age and rearing environmental conditions of fish.

Immunostimu lated fish showed several varied number of protein bands with different molecular weights. The qualitative protein profile of head kidney of 3 different age groups of fishes are in agreement with those of Li *et al.,* (2000) and Islam *et al.,* (2008) who

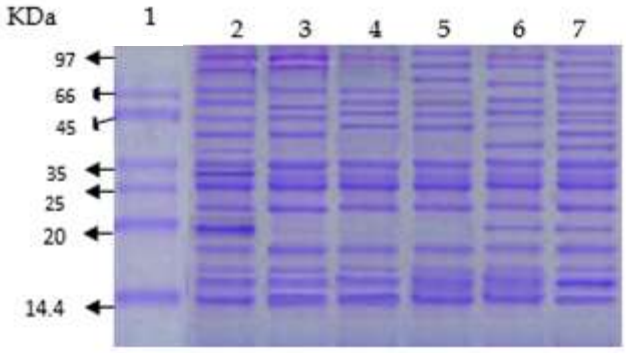

Figure-3. Electropherogram of protein profile in head kidney of control (group c) and experimental (Group C), (treated with Aqua Fix @ 50mg/100g of feed) 12 months old *C. rohita* at different days of experiment
Lane 1 - Marker (97 KDa, 66 KDa, 45 KDa, 35 KDa, 25 KDa, 20 KDa, 14.4 KDa); Lane 2 - Group c (Control); untreated; Lane 3 - Group C, Day 1 of treatment; Lane 4 - Group C, Day 4 of treatment; Lane 5 - Group C, Day 7 of treatment; Lane 6 - Group C, Day 15 of treatment; Lane 7 - Group C, Day 30 of treatment

Table 3. Characterization of isolated proteins on the basis of their molecular mass in head kidney of experimental (group C) and control (group c) 12 months old *L. rohita*.

S.No	Marker Mol.wt. KDa	Control Group b	Group C				
			Day 1	Day 4	Day 7	Day 15	Day 30
1	97.000	101.942	105.891	102.915	101.942	102.831	108.952
2	66.000	93.885	100.978	92.997	91.827	92.099	96.906
3	45.000	80.391	84.569	83.505	82.715	80.137	89.247
4	35.000	66.902	75.219	74.037	72.644	73.803	80.137
5	25.000	58.756	66.060	63.799	63.799	61.420	73.570
6	20.000	51.602	58.017	55.853	52.592	54.284	64.204
7	14.400	46.042	51.276	48.743	46.777	46.925	55.853
8		40.308	45.463	40.822	40.436	41.342	49.053
9		36.079	39.927	31.486	35.965	37.240	44.185
10		33.228	33.123	28.005	33.123	32.090	38.317
11		30.408	27.132	25.792	27.391	28.724	34.188
12		27.391	23.529	21.396	22.795	26.453	31.486
13		23.828	20.993	15.344	19.273	21.807	28.094
14		20.861	19.030	13.647	15.787	19.273	24.909
15		18.205	15.888	12.100	14.131	16.873	22.795
16		16.192	14.131		12.024	13.777	19.273
17		14.176	12.450			12.177	17.197
18		12.608					15.837
19							14.865
20							13.108
21							11.466

reported that diet comprising of rice bran, soya bean meal, fish meal, vegetable oil, vitamin and mineral mixture with different concentrations influence the growth rate and biomass of the fish. Sultana *et al.,* (2016) recorded that different artificial feeds formulated from local ingredients have a significant effect on the qualitative profile of muscle tissue of proteins in *C. mrigala, L. rohita* and *C. catla.* Salim *et al.,* (2006) also reported higher growth in carps reared in natural ponds having the higher availability of natural food.

In the head kidney samples of *L. rohita* fed with Aqua Fix, in 6 months (group A) old fish ~ 16, 14, 14, 11, 12 KDa, in 9 months (group B) old fish ~ 15, 12, 14, 15, 15 KDa and in 12 months (group C) old fish ~ 17, 15, 16, 17, 21 KDa protein bands were recorded on day 1, 4, 7, 15 and 30 of experimental period respectively. In between group A and B, 8 bands (~ 66, 43, 34, 21, 19, 16, 15 and 14 KDa) were shared on day 1, 2 bands (~ 16 and 14 KDa) on day 4, 2 bands (~ 16 and 14 KDa) on day 7, two bands (~ 25 and 14 KDa) on day 15 and 3 bands (~ 30, 15 and 14 KDa) on day 30 respectively. Groups A and C have 5 common bands (~ 66, 20, 19, 15 and 14 KDa) on day 1, 2 common bands (~ 16 and 14 KDa) on day 7 and 2 common bands (~ 19 and 13 KDa) on day 15. One common band (~ 20 KDa) was found on day 1 and 4 (~ 13 KDa), two common bands on day 7 (~22 and 14 KDa) and 6 common bands (~ 73, 55, 28, 15, 14 and 13 KDa) on day 30 in between groups B and C. The three are groups of fish (A, B and C) shared 5 bands (~ 66, 20, 19, 15 and 14 KDa). On day 1 and one band (~ 14 KDa) on day 7 and 30. Synthesis of new bands and disappearance of some bands (on different days of experiment) and presence of common bands among 6, 9 and 12 months old *L. rohita* confirm the impact of immunostimulation and/or age. These findings are similar to that of Ashraf *et al.,* (2008) who reported maximum growth and survival rate in fingerlings of *C. mrigala* fed with different ingredients of feed.

Occurrence of high and low molecular weight protein bands in head kidney samples of 6, 9 and 12 months old fish confirm the findings of Okagaki *et al.,* (2005) and Montowska and Pospiech (2007) who isolated and characterized protein bands of low molecular weights (ranging between 26 to 16 KDa) in carp and other fish species. No marked change was observed in the characterization of low molecular weight protein bands in 3 different age groups of fish on day 1, 4, 7, 15 and 30 of Aqua Fix treatment; similarly high molecular weight protein bands are found on all days of experimentation in 3 different age groups of fish. Mitsuhashi *et al.,* (2002), Sultana *et al.,* (2016) and Fock and Hinsean (2002) recorded nebulin (107 KDa) in skeletal and cardiac muscles of fish. Mathew and Prakash (2006) also found low molecular weight protein bands (23 and 22 KDa) in *S. longiceps*. Tanriverdi *et al.,* (2016) recorded 100, 97, 76, 56 and 30 KDa bands as original bands and the bands of 93, 46, 44 and 26 KDa as newly-synthesized bands in the serum of *C. capoeta* treated with ascorbic acid (vitamin C) against dimethoate.

Conflicts of Interest

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

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