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

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ABSTRACT

The effect of Aqua Fix (immunomodulator) on the qualitative protein index of skeletal muscles of *Labeorohita* was investigated. Test fish (twenty five fish of 6 months old, 12-15 g.wt; 25 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) (25 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. *Aeromonashydrophila*, 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 immunostimulantscannot be overlooked.

Various herbal extracts such as *Aloe vera* (Kim *et al*., 1999), *Ocimum sanctum* (Logambal*et al*., 2000), *Zingiberofficinale*(Dugenci*et al*., 2003), *Achyranthesaspera* (Vasudeva and Chakrabarti, 2005a, b) and *Solanumtrilobatum*(Divyagnaneswari*et al*., 2007) have been reported to enhance immunity in fish *Cynodondactylon* (L.) and *Coriandrumsativum*extract mixed diet enhanced disease resistance and production of specific antibodies in *Catlacatla* (Xavier Innocent, *et al*., 2011; Kaleswaran*et al*., 2012) against aeromoniasis. Administration of glucan and *Aeglemarmelos* enhanced survival and immunity in *Cyprinuscarpio* challenged with *A. hydrophila* (Selvaraj*et al*., 2005; Pratheepa*et al.,* 2010 and Jasmin Gold, V and VivekaVardhani, V**,** 2016). Administration of microbial levan showed enhanced haemtological and non-specific immunological changes in *Labeorohita* (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

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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 25 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. Five 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).

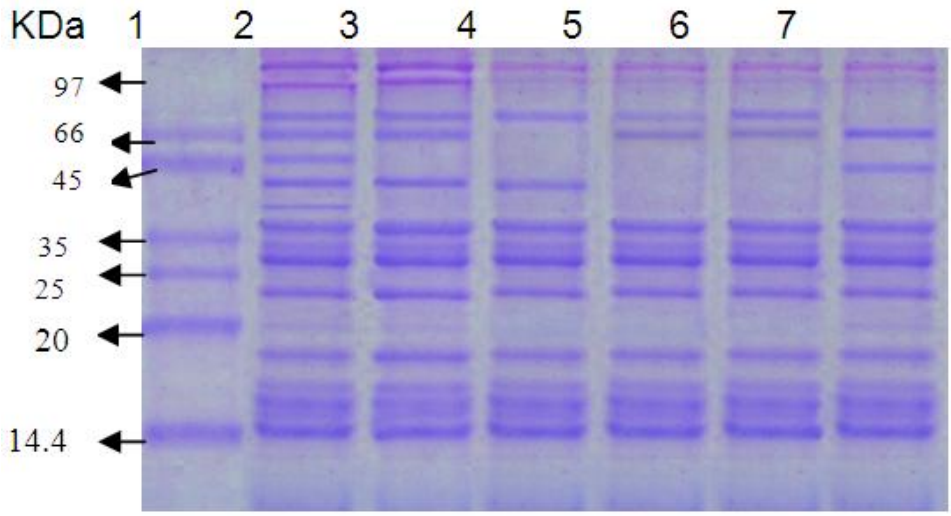
RESULTSAND DISCUSSION

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 and 7, protein band ~ 66 KDa disappeared; protein band ~ 45 KDa (found on day 1 and 4) and ~ 40 KDa (found on day 4) were absent 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), 10 protein bands were recorded on day 1 of experiment. The isolated protein bands have molecular weight as ~ 97, 90, 66, 41.3 35, 25, 20, 18,16, 14.4 KDa. Compared with controls, 2 protein bands ~ 64.4 KDa and 41 KDa disappeared and protein bands ~ 35 to 14.4 KDa were present both in groups B and b. On day 4, experimental fish showed an additional protein band ~ 40 KDa and disappearance of ~ 64.4 KDa and 41 KDa protein bands; no marked change was found in protein bands of 35 to 14.4 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 ~ 19.1 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.

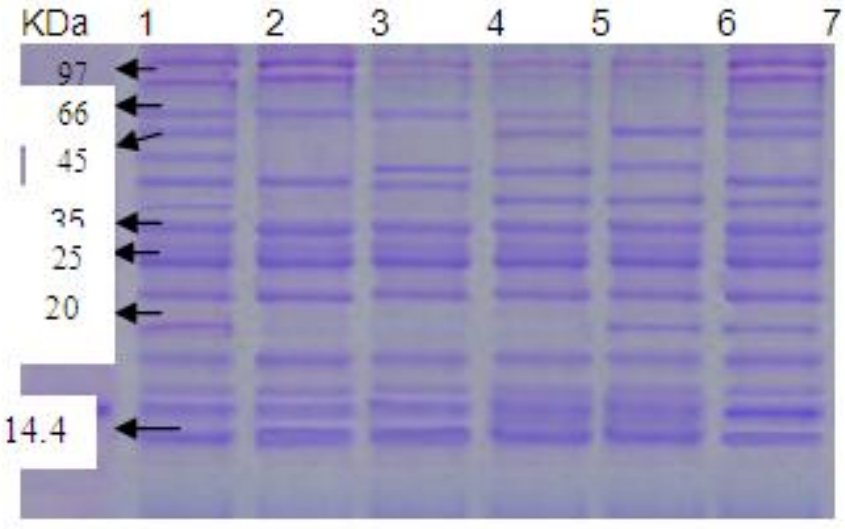
Table-1: Characterization of isolated protein on the basis of their molecular weight in skeletal muscles of experimental (group A) and control (group a) 6 months old *L. rohita.*

S.No.	Marker Mol.wt. KDa	Control group a	Group A				
			Day 1	Day 4	Day 7	Day 15	Day 30
1	97.0	180.181	180.181	176.329	180.181	176.329	180.181
2	66.0	115.209	143.525	149.697	143.525	143.525	142.525
3	45.0	85.941	89.257	72.420	87.028	90.399	67.889
4	35.0	67.889	59.621	35.497	68.683	68.683	45.512
5	25.0	51.162	36.592	28.714	29.192	30.495	28.184
6	20.0	35.899	28.407	25.941	25.941	28.870	25.723
7	14.4	30.680	25.941	23.337	24.334	25.831	24.463
8		29.030	23.337	21.533	22.184	23.513	21.989
9		25.831	21.417	20.107	19.889	22.159	20.649
10		24.420	19.916	19.648	19.653	20.090	20.073
11		21.574	19.672	19.427	19.484	19.657	19.616
12		20.515	19.484	19.345	19.349	19.512	19.464
13		19.930	19.290			19.343	19.266
14		19.667					
15		19.505					
16		19.340					

to 14.4 KDa remained stable on day 7 of treatment. Fish of group B showed distinct protein bands on day 15; a protein band ~ 66 KDa disappeared and that of ~ 19.1 KDa reappeared and no marked alteration was found in protein bands ~ 35 KDa to 14.4. No marked changes were found in the arrangement of protein bands on day 30 of treatment in group B in comparison with controls (group b) except the disappearance of a protein band ~ 41.4 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

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 electorphoresis..



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-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	147.715	176.897	156.283	165.433	160.783	126.283
2	66.0	106.609	129.781	52.950	119.623	102.963	95.322
3	45.0	53.999	67.115	33.271	66.128	49.387	54.356
4	35.0	41.375	28.556	27.762	49.694	34.627	47.052
5	25.0	33.143	24.600	24.501	33.400	27.118	30.082
6	20.0	28.111	23.477	23.277	26.072	24.469	26.540
7	14.4	26.327	22.630	22.893	24.501	23.519	24.284
8		24.438	22.204	22.240	23.477	22.583	23.221
9		23.258	21.697	21.692	22.572	22.042	22.572
10		22.550	21.618	21.609	22.240	21.783	22.001
11		22.162	21.587	21.598	21.701	21.703	21.773
12		21.839	21.548	21.548	21.606	21.621	21.658
13		21.694			21.580	21.598	21.619
14		21.622			21.575	21.577	21.593
15		21.585					21.577
16		21.569					
17							

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 *Ictaluruspunctatus* and expression of muscle protein in pikeperches, *Stizostedionlucioperca* and *S. volgensa*. Singh *et al.,* (2005) and Tarakalakshmi Y and VivekaVardhani V (2015)also determined the importance of protein for optimum growth in *L. rohita*. 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. Two bands were shared between 6 months (group A) and 9 months (group B) old *L. rohita* on day 1 (24.4, 21.5; 24.6, 21.5 KDa), 4 (23.3, 21.5; 23.2, 21.6 KDa), 7 (24.3 22.1; 24.5 22.5 KDa) 15 (23.5, 22.1; 23.5, 22.5 KDa) and 30 (24.4, 21.9; 24.2 21.7 KDa). These findings are in agreement with that of Mathew and Prakash (2006) who reported 23 and 22 KDa proteins from *Sardinellalongiceps*. 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) and Sultana *et al*., (2016) and Madhuri, D and VivekaVardhani, V (2015).who reported increased muscle protein content with higher dietary protein in carps.

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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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