Biolife 10(4); 1-7
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
ISSN (online): 2320-4257
www.biolifejournals.com
BIOLIFE ORIGINAL ARTICLE

Biochemical Indices in Gills and Intestine of *Labeo rohita* (Indian major carp) treated with Immunomodulator and *Aeromonas hydrophila*

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ABSTRACT

The present study was conducted to investigate the biochemical changes in the gills and intestine of *Labeo rohita* treated with Aqua Fix and *Aeromonas hydrophila*. 3 groups (A, B, C) of experimental and 1 group (D) of control fish (6 months old) were selected for investigations. Two groups of fish (A, B) were treated with Aqua Fix for 4 days, fish of group B and C were infected with *A. hydrophila* @ 10^{-6} CFU/fish on day 5 (normal feed was given to fish of group C). Controls (group D) were untreated and uninfected. Tissue samples were collected on day 1, 2, 3, 4, 7 and 15 after infection from fish of group B and C, and also from groups A and D on the same designated days. Protein and DNA from gills and intestine were estimated following standard methods. Statistical analysis on gill protein and DNA, and muscle protein and DNA showed significant levels in immunomodulated fish (Group A) compared to that of immunomodulated + infected (Group B), infected (Group C) and control fish (Group D) indicating that the treated immunostimulant (Aqua Fix) might have modulated the immunity resulting in the increase of biomolecules It is also clear that aeromoniasis might influence the lysis of protein in gill and intestine leading to the low level of protein and DNA in gills and intestine of fish in groups B and C.

Key words: *Aeromonas hydrophila, Labeo rohita, gills, Intestine, Protein, DNA.*

INTRODUCTION

Pathogenic bacterium, *Aeromonas hydrophila* is widely distributed in warm water all over the world; this bacterium infects both farmed and wild fishes causing aeromoniasis (Sarkar and Rashid, 2012). Diseased fish suffer by swollen abdomen, red mouth. hemorrhages, tail and fin rot, ulceration, scale protrusion and dropsy (Miyazaki *et al*., 2001; Sahoo *et al*., 2004). Islam *et al*., (2008) found histopathological changes in *Heteropneustes fossilis* infected with *A. hydrophila*. Manoj *et al*., (2010) studied hematological and histological changes in gills, liver, kidney, intestine and heart of *L. rohita* during *A. hydrophila* infection and found increase of RBC, WBC and liver enzymes, necrosis in liver and hyperplasia in gill lamellae on day 3, 5, 7, 10 and 15 of experiment. Increase in the survival rate of *Cyprinus carpio* was found against *A. hydrophila* when fed with plant extract supplemented diet (Baba *et al*., 2016). Fallahpour *et al*., (2014) found increased growth in fish fed with diet supplemented with medicinal herbs.

Immunostimulants develop resistance to cope with various bacterial and viral infection in fish (Yano *et al*., 1991; Lunden *et al*., 2000). Selvaraj *et al*., (2005) found the best level of antibodies in fish treated with β-glucan against *A. hydrophila* infection. Haematological parameters are helpful to understand the physiological status of the fish against immunostimulation and/or disease (Shah and Altindag, 2004). Very little information is available on the biochemical changes in *L. rohita* during immunostimulation and/or aeromoniasis.

How to Cite this Article:
Subba Reddy, K.V and Simhachalam, G (2022). Biochemical Indices in Gills and Intestine of *Labeo rohita* (Indian major carp) treated with Immunomodulator and *Aeromonas hydrophila*. *Biolife*. 10(4):1-7.

DOI: https://dx.doi.org/10.5281/zenodo.7245439

Received: 11 August 2022; Accepted: 2 October 2022; Published online: 24 October 2022.

Therefore, a new vista has been opened to study the effect of immunostimulant (Aqua Fix) and/or *A. hydrophila* infection in gills and intestine of *L. rohita*.

Materials and Methods

Six months old *L. rohita* (12-15 g) were used in the present study. 3 experimental (group A, treated with Aqua Fix; group B, treated with Aqua Fix and infected with *A. hydrophila*; group C, untreated with Aqua Fix, but infected with *A. hydrophila)* and 1 control group (D, untreated and uninfected) of fish (35 in each group) were maintained for experimentation. In case of group A and B, they were fed with a diet added with Aqua Fix (@ 50 mg/100 g of feed) for 4 days, fish of group B and C were infected intraperitoneally with *A. hydrophila* @ 10^{-6} CFU/Fish on day 5. With regard to fish of groups C and D, they were fed with normal diet (without Aqua Fix) throughout the experiment. Necropsies were made on day, 6, 7, 8, 9, 12 and 20 of experimental period (for convenience these days are described as day 1, 2, 3, 4, 7 and 15). Gills and intestinal tissues of the experimental and control group were collected on the above designated days and analyzed for protein and DNA following the methods of Lowry *et al*., (1951) and Nicholas (1956). Data was compared statistically and the differences between the control and experimental groups were considered to be statically significant.

Results and Discussion

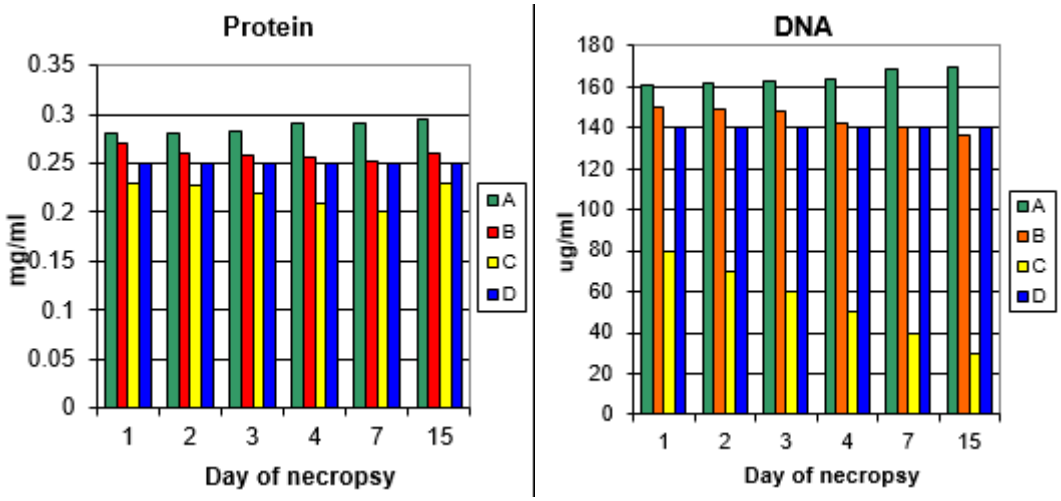
Protein activity in gills (Table 1, Figure 1):
In immunostimulated group, A: There was an increase of protein from day 1 to 15 of experiment compared to controls (group D). The content of protein increased

Table 1: Protein (mg/ml) and DNA (µg/ml) content in the gills of experimental and control during different days of experiment.

(**Experimental**: Group A, immunostimulated with Aqua Fix @ 50 mg/100g of feed; B, immunomodulated with Aqua Fix @ 50 mg/100g of feed and infected with *A. hydrophila* @ 10^{-6} CFU/fish; C, infected with *A. hydrophila* @ 10^{-6} CFU/fish; and **Control** : D, untreated with Aqua Fix and uninfected with *A. hydrophila*) *L. rohita* (6 months old)

Day of necropsy	Experimental groups						Control group	
	Group A		Group B		Group C		Group D	
	Protein	DNA	Protein	DNA	Protein	DNA	Protein	DNA
1	0.280	161.0	0.270	150.0	0.230	80	0.250	140.0
2	0.281	162.0	0.260	149.0	0.228	70	0.249	139.8
3	0.283	163.0	0.258	148.0	0.220	60	0.250	139.8
4	0.290	164.0	0.257	142.0	0.210	50	0.250	140.0
7	0.291	168.5	0.252	140.0	0.200	40	0.249	140.0
15	0.296	170.0	0.260	136.0	0.230	30	0.249	140.0

Values are expressed in the mean derived from 5 observations.



(A) **(B)**

Figure-1 (A): The content of protein (mg/ml) in the gills of experimental (group A, B, C) and control (group D) fish (6 months old) at various days of experiment
(B): The content of DNA (µg/ml) in the gills of experimental (group A, B, C) and control (group D) fish (6 months old) at various days of experiment

progressively from day 1 to 15, with a peak value on day 15 (0.296 mg/ml).

In immunostimulated + infected group, B: Protein level showed an increase when compared to controls from day 1 to 15 and decrease when compared to immunostimulated group A; there was a progressive decrease from day 1 to 15 of experiment.

In infected group, C: Fish infected with a single dose of *A. hydrophila* showed lower values of protein when compared with controls (group D), immunostimulated (group A) and immunostimulated + infected fish (group B). There was a progressive decrease from day 1 to 7 with below normal (0.200 mg/ml) value on day 7. The increased value on day 15 (0.230 mg/ml) is equal to that recorded on day 1 of experiment.

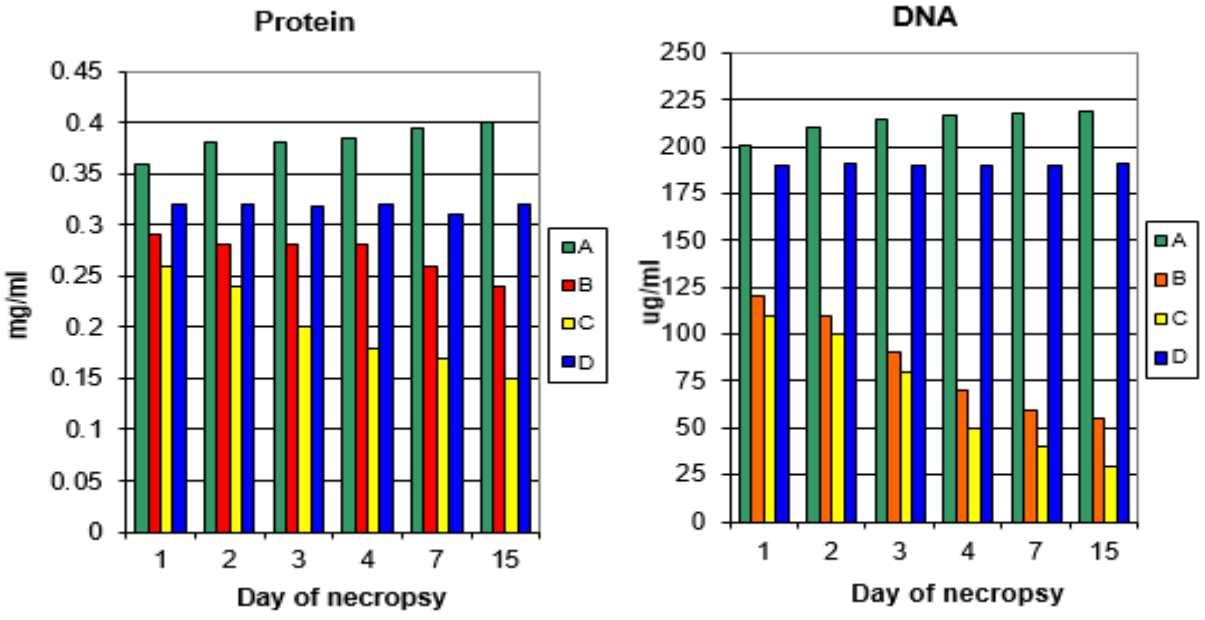
Gills of immunostimulated fish (group A) showed highest level of protein compared to immunostimulated + infected (group B), infected (group C) and controls (group D) and lowest level of protein was found in gills of infected fish (group C) compared to controls (group D) and other experimental groups (B, C) of fish. Peak value of protein was found on day 15 in group A (0.296 mg/ml) and on day 1 in groups B (0.270 mg/ml) and C (0.230 mg/ml).

Table 2: Protein (mg/ml) and DNA (µg/ml) content in the intestine of experimental and Control during different days of experiment.

(**Experimental**: group A, immunomodulated with Aqua Fix @ 50 mg/100 g of feed; B, immunomodulated with Aqua Fix @ 50 mg/100 g of feed and infected with *A. hydrophila* @ 10^{-6} CFU/fish; C, infected with *A. hydrophila* @ 10^{-6} CFU/fish); **Control :** Group D, untreated with Aqua Fix and uninfected with *A. hydrophila*) *L. rohita* (6 months old)

Day of necropsy	Experimental groups						Control group	
	Group A		Group B		Group C		Group D	
	Protein	DNA	Protein	DNA	Protein	DNA	Protein	DNA
1	0.360	201	0.290	120	0.260	110	0.320	190
2	0.380	210	0.280	110	0.240	100	0.319	191
3	0.381	215	0.280	90	0.200	80	0.318	190
4	0.385	217	0.280	70	0.180	50	0.320	190
7	0.395	218	0.260	60	0.170	40	0.311	190
15	0.401	219	0.240	55	0.150	30	0.320	191

Values are expressed in the mean derived from 5 observations.



(A) **(B)**

Figure-1 (A): The content of protein (mg/ml) in the intestine of experimental (group A, B, C) and control (group D) fish (6 months old) at various days of experiment

(B): . The content of DNA (□g/ml) in the intestine of experimental (group A, B, C) and control (group D) fish (6 months old) at various days of experiment

DNA activity in gills (Table 1, Fig. 1B):

In immunostimulated group A: There was a marked increase of DNA in gills from day 1 (161 µg/ml) to 15 (170 µg/ml) of experiment compared to controls (140 µg/ml).. Slight and progressive increase of gill DNA was found from day 1 to 15 in Aqua Fix treated fish.

In immunostimulated + infected group, B: DNA level showed an increase from day 1 (150 µg/ml) to 4 (142 µg/ml) and decrease from day 7 (140 µg/ml) to 15 (136 µg/ml) compared to controls (140 µg/ml); DNA level was equal to controls on day 7 and below normal value on day 15.
The level of DNA in gills was found to be decreased from day 1 to 15 of experimentation compared to Group A (161 µg/ml and 170 µg/ml on day 1 and 15).

In infected group, C: Compared to controls and other groups (B and C) DNA level decreased drastically from day 1 to 15 of experimental period.

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An increase of gill DNA was found in groups A and B compared to controls on day 1, 2, 3, 4, 7 and 15 of experimental period (except on day 7 and 15 in group B). Comparison among the 3 experimental groups (A, B, C), fish treated with immunostimulant (group A) showed highest level of DNA followed by immunostimulated + infected (B) and infected (C) fish.

Protein activity in intestine (Table 2, Fig. 2A):

In immunostimulated group, A: Intestinal protein increased markedly on day 1 (0.360 mg/ml) to 15 (0.401 mg/ml) of Aqua Fix treatment (a progressive increase) compared to controls (group D, 0.320 mg/ml).

In immunostimulated + infected group, B: In comparison with control (group D) and immunostimulated fish (group A), there was a marked decrease from day 1 (0.290 mg/ml) to 15 (0.240 mg/ml) of experiment.

In infected group, C: Protein content decreased progressively from day 1 (0.260 mg/ml) to 15 (0.150 mg/ml) of infection. Compared to controls (group D) and other experimental groups (A, B), the protein level was found to be decreased from day 1 to 15 of experimental period.

Compared with controls (group D) and other experimental groups (B, C), immunostimulated (group A) fish showed significant increase of protein from day 1 to 15 of experiment. Fish of group B showed a marked increase when compared with control and infected fish. Infected fish (Group C) showed marked decrease compared to controls and experimental groups A and B. Lowest value of protein was found in group C on day 15 (0.150 mg/ml) and highest value of protein was found on day 15 (0.401 mg/ml) in group A.

Table 3. Mean and t-values of gill protein and DNA in experimental (group A, immunomodulated; group B, immunomodulated and infected; group C, infected) and control (group D, unimmunomodulated and uninfected) *L. rohita* (6 months old).

Biomolecules	Groups					
	A		B		C	D
Protein: Mean:	0.286		0.259		0.219	0.249
	A D	B D	C D	A B	A C	B C
t-value	t=13.730*	t=5.446*	t=5.93*	t=0.003Φ	t=11.65*	t=0.02Φ
DNA: Mean:	164.75		144.16		55.0	139.93
	A D	B D	C D	A B	A C	B C
t-value	t=16.620*	t=1.829Φ	t=11.194*	t=7.471*	t=14.102*	t=19.883*

P value at 5% level of significance is 2.306
*Statistically significant values, Φstatistically non-significant values

DNA activity in intestine (Table 2, Fig. 2B):

In immunostimulated group, D: Intestinal DNA was found to be increased progressively in this group compared to controls from day 1 (201 µg/ml) to 15 (219 µg/ml) of experimentation with a peak value on day 15.

In immunostimulated + infected group, C: Throughout the infection period the DNA content in fish of group C remained at low level when compared with fish of group A and at high level compared to controls and infected ones from day 1 to 15.

In infected group, C: DNA remained at low level when compared with control (group D) and other experimental (group A, B) fish. DNA content decreased progressively from day 1 (110 µg/ml) to 2 (100 µg/ml), 3 (80 µg/ml), 4 (50 µg/ml), 7 (40 µg/ml) and 15 (30 µg/ml) with a lowest DNA content on day 15.

Immunostimulated fish (group A) showed significant increase of DNA when compared with controls (group D) and other experimental fish (group B, C). Fish of group B showed an increase compared with controls and infected fish, and decrease to that of immunostimulated fish. Compared with controls and other experimentals DNA was found lowest in infected fish.

Statistical analysis (Table 3) showed a significant increase of gill protein in group A and B when compared to controls; gill protein in group C showed a significant decrease in comparison with controls. Protein content was found to be non-significant level in between groups A and B, and B and C, and significant in between groups A and C. There was a significant increase of DNA in group A and non-significant decrease in group B and significant decrease in group C. DNA content was found to be significant in between groups A and B, A and C, B and C, compared with controls.

Fish of group A showed non-significant increase and of groups B and C showed non-significant decrease of intestinal protein when compared with controls (Table 4). Increase of intestinal protein in group A was found to be significant when compared with groups B and C, and in group C when compared with group B. DNA level in group A showed significant increase and groups B and C showed significant decrease compared with controls. There was a significant increase in Group A compared to groups B and C. The comparison of DNA level in between group B and C showed a non-significant difference.

In the presents study, gills and intestine of 6 months old immunostimulated fish (group A) showed significant increase of protein and DNA compared to their respective controls (D), and also to those which received Aqua Fix and infection (group B) and infection (groups C) from day 1 to 15 of experimental period (with few exceptions). Administered immunostimulant along with feed might have produced protective effect to influence in the enhancement of protein and DNA content. The highest value of protein in Aqua Fix supplemented group at day

Table 4. Mean and t-values of intestinal protein and DNA in experimental (group A, immunomodulated; group B, immunomodulated and infected; group C, infected) and control (group D, unimmunomodulated and uninfected) *L. rohita* (6 months old).

Biomolecules	Groups					
	A	B	C	D		
Protein:						
Mean:	2.302	0.271	1.2	1.916		
	A D	B D	C D	A B	A C	B C
t-value	t= 0.3457Φ	t= 2.30Φ	t= 0.9172Φ	t= 4.041*	t= 7.10*	t=5.196*
DNA:						
Mean:	213.3	84.1	68.3	190.3		
	A D	B D	C D	A B	A C	B C
t-value	t=8.447*	t= 9.66*	t= 9.026Φ	t= 2.200*	t= 7.91*	t=2.29*

P value at 5% level of significance is 2.306

*Statistically significant values, Φstatistically non-significant values

15 of experiment compare well with that of Anderson and Siwicki (1995) who found elevation of total serum protein in brook trout immunostimulated with glucan or chitosan by injection or immersion against *A. salmonicida* and Siwicki *et al*. (1994) who reported elevated level of serum protein and protection in rainbow trout with dietary supply of glucan against furunculosis. These results are in agreement with that of Misra *et al*., (2006) and Maqsood *et al*., (2009) who recorded increased total serum protein in fish fed with β-glucan and levamisole in *L. rohita* and *C. carpio* against *A. hydrophila*.

Feeding of immunostimulant before infection probably offered protection and thus suppressed the abnormal changes induced by *A. hydrophila* resulting into the increased synthesis of protein in group B. These results compare well with that of Maqsood *et al*., (2010) who also reported that use of immunostimulants is beneficial before the disease outbreak in aquaculture. Protective effect of immunostimulants with regard to enhanced growth, protection and stimulation of non-specific immunity was reported by Rao *et al*., (2006), Sahu *et al*., (2007), Gupta *et al*., (2008), Xavier Innocent *et al*., (2011), Pradhan and Das (2015) in *L. rohita* due to *A. aspera* against *A. hydrophila*, in *L. rohita* due to levan against *A. hydrophila*, in *C. catla* due to *P. rosea* against *A. hydrophila,* in *L. rohita* due to *M. aeriginosa* against *A. hydrophila* and in *C. carpio* due to *A. sativa* against *A. hydrophila*.

In the present study, it is found that in 6 months old fish Aqua Fix supplemented diet enhanced protein and DNA (with few exceptions) in gills and intestine throughout the experimental period with a peak level on day 15. This shows that Aqua Fix has a positive metabolic effect playing a crucial role in enhancing the digestion and absorption of nutrients, and also in the synthesis of protein and DNA in the organ of respiration (gills) and digestion (intestine). The positive effects of immunostimulant supplemented diet on survival, growth, haematological, biochemical and immunological responses in carps reported by Jeyakumari *et al.,* (2007), Pratheepa *et al*., (2010); and Aathi *et al*., (2013). Fish treated with immunostimulant before infection (group B), might have experienced stress by infectious pathogens leading to the decrease of protein level; the release of reactive oxygen species may be responsible for the decreased protein level as suggested by Sharp and Secombes (1993) in rainbow trout during *A. salmonicida* infection.

Stress-mediated immunosuppression as suggested by Ellsaesser and Clam (1986) might be responsible to the changes in the tissue protein and DNA level. When fish is under stress (regardless of the nature of stress like low oxygen, high ammonia, transport and infection), they may utilize more energy and the efficiency of haematological and immunological components may be decreased; this in-efficiency may indirectly cause abnormality in the metabolism/use of protein and DNA. Anil Kumar *et al*., (2010) also found decrease of protein in liver, brain, muscle, gill and kidney of *C. punctatus* exposed to thiamethoxan toxicity.

Conflicts of Interest

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

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