Biolife 5(2):232-237
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
ISSN (online): 2320-4257
www.biolifejournals.com
BIOLIFE RESEARCH ARTICLE

Biochemical changes in the Muscles of Carp (*Labeo rohita*) during Immunomodulation and Aeromoniasis

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ABSTRACT

The present study was undertaken to evaluate the supplementary diet of Aqua Fix on the biochemical changes in muscles and disease resistance against *A. hydrophila* infection in *L. rohita*. Three groups (A, B, C) of 6 months old fish were employed in the present study. Fish of group A and B were treated with Aqua Fix for four days; on day 5 fish of group B and C (fish of group c were fed with normal diet for 4 days) were infected with *A. hydrophila* (@ 10^{-6} CFU/fish). Another group (D) of fish (untreated and uninfected) served as controls for comparison. Necropsies were made on day 1, 2, 3, 4, 7 and 15 after infection in group B and C; fish from group A and D were also necropsied on the same designated days. Muscle protein, carbohydrate, DNA and RNA were estimated following standard methods. Results were analyzed for statistical significance and found that Aqua Fix modulates the immunity with regard to the enhanced levels of protein, carbohydrate, DNA and RNA in muscles of *L. rohita*. Infectious stress (aeromoniasis) caused the lysis of muscle protein which ultimately led to the abnormal decrease of carbohydrate, DNA and RNA in fish infected with *A. hydrophila*.

Key words: *Aeromonas hydrophila*, *Labeo rohita*, muscle, Aqua Fix, Protein.

INTRODUCTION

Fish diseases are the major constraints in aquaculture practices. Indian major carps (*Catla catla, Labeo rohita, Cirrhinus mrigala*) are found to be subjected to various bacterial, fungal and parasitic diseases. *Aeromonas hydrophila,* a gram negative, oval shaped, facultative intracellular bacterium infecting both freshwater and marine water fishes; infected fish suffer with haemorrhagic septicemia, red sore disease and ulcerative condition (Hannine *et al.,* 1995). *A. hydrophila* infection may cause tail and fin rot, ocular ulceration, dermal ulceration, erythrodermatitis, haermorrhagic septicemia, red spot disease, scale protrusion, ulceration and dropsy (Miyazaki *et al*., 2001; Sahoo *et al*., 2004). Among the three Indian major carps, *L. rohita* is disease resistant, the best in protein content and highly consumed by humans (Oliva-Teles, 2012). Employment of antibiotics/vaccines to control diseases in aquatic organisms throws a negative impact in the aquatic environment (Vivas *et al*., 2004). Use of medicinal plants/extracts (in place of antibiotics) to control fish diseases gained much importance because medicinal plants are highly effective in disease control, cost effective, ecofriendly and have minimum side effects (Direkbusarakom *et al*., 1998; Dugenci *et al*., 2003; Chakraborti and Vasudeva, 2006). Harikrishnan *et al.,* (2003) found significant haematological and biochemical changes in carp, *Cyprinus carpio* following herbal treatment against *A. hydrophila* infection. Significant disease control was found in catfish by the use of two plant extracts (*Andrographis paniculata* and *Azadirachta indica*) against *A. hydrophila* infection (Thiyagarajan *et al*., 2014).

Raman (2017) reviewed the role of phytotherapy in the world in controlling diseases in aquaculture. Herbal medicines act as immunostimulants and may be used individually or mixed or in combination with other drugs

How to Site This Article:
Ankamma, N and Viveka Vardhani, V (2017). Biochemical changes in the Muscles of Carp (*Labeo rohita*) during Immunomodulation and Aeromoniasis. *Biolife*. 5(2), pp 232-237.

DOI: 10.5281/zenodo.7364328
Received: 7 April 2017;
Accepted; 26 May 2017;
Available online : 4 June 2017

for effective fish health management (Harikrishnan and Balasundaram, 2005). Very little information is available on the biochemical changes in muscles in relation to immunomodulation and aeromoniasis in fish. Present study is based on the assessment of biochemical profile in muscles of *L. rohita* treated with Aqua Fix (immunomodulator) and *A. hydrophila* infection.

MATERIALS AND METHODS

Six month old *L. rohita* (12-15 g) were selected for the present experimentation. Three experimental groups A (treated with Aqua Fix), B (treated with Aqua Fix and infected with *A. hydrophila*), C (untreated with Aqua Fix but infected with *A. hydrophila*) and one control group D (untreated and uninfected) of fish (35 in each group) were maintained in optimum conditions. Fish of group A and B fed with a diet supplemented with Aqua Fix (@50 mg/100 g of feed) for 4 days; and fish of group C fed with normal diet for 4 days; on day 5 fish of group B and C were infected intraperitoneally with *A. hydrophila* @ 10^{-6} CFU/fish. Fish of groups C and D were fed with normal diet (without Aqua Fix) during the entire experimental period. Necropsies were made on day 6, 7, 8, 9, 12 and 20 of experiment (for convenience these are described as day 1, 2, 3, 4, 7 and 15). Muscle tissue from the experimental and control groups were removed and analyzed for protein, carbohydrate, DNA and RNA following Lowry *et al.,* (1951), Nicholas (1956) and Burton (1956) respectively. Results were subjected to student's t-test to find out the significance.

RESULTS AND DISCUSSION

Protein activity in muscles:

Fish of group A showed higher value of protein compared to controls (group D), immunomodulated + infected (group B) and infected (group C) fish throughout the experimental period (day 1 to 15). Immunomodulated fish (group A) showed a gradual increase of proteins from day 1 (1.005 mg/ml) to 15 (1.035 mg/ml). whereas fish of groups B and C showed a gradual decrease of proteins from day 1 (0.981 mg/ml in group B; 0.340 mg/ml in group C) to 15 (0.901 mg/ml in group B; 0.202 mg/ml in group C) (Table 1, Fig. 1A).

Table 1. Protein (mg/ml) and carbohydrate (mg/ml) content in the muscles of *L. rohita* of group A (treated with Aqua Fix @ 50 mg/100 g of feed), B (treated 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) and D (untreated and uninfected). Values are expressed in the mean derived from 5 observations.

Day of necropsy	Experimental groups						Control group	
	Group A		Group B		Group C		Group D	
	P	C	P	C	P	C	P	C
1	1.005	0.721	0.981	0.711	0.340	0.480	0.850	0.660
2	1.011	0.734	0.962	0.700	0.331	0.472	0.851	0.661
3	1.014	0740	0.958	0.660	0.324	0.455	0.851	0.660
4	1.016	0.793	0.940	0.649	0.300	0.420	0.852	0.660
7	1.019	0.822	0.908	0.638	0.241	0.412	0.850	0.661
15	1.035	0.862	0.901	0.630	0.202	0.400	0.851	0.660

P: Protein; C: Carbohydrate

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

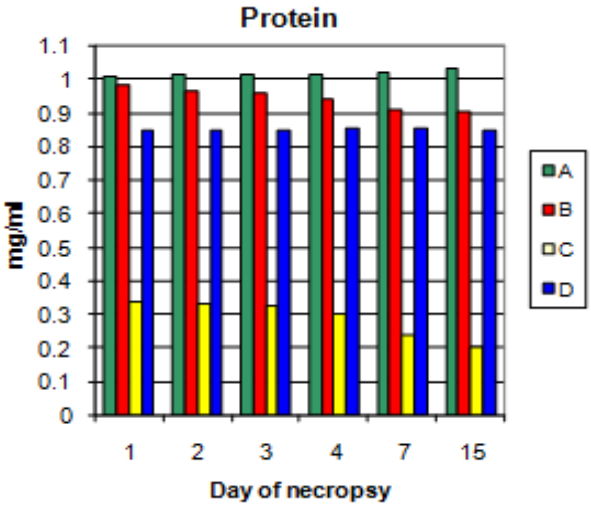
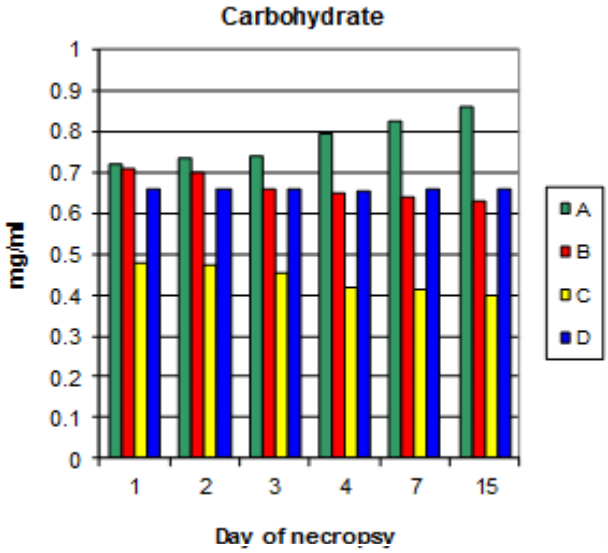


Figure 1B. The content of carbohydrate (mg/ml) in the muscles of experimental (group A, B, C) and control (group D) fish (6 months old) at various days of experiment



Carbohydrate activity in muscles (Table 1, Fig. 1B):

Fish which received immunomodulator (group A) showed enhanced level of carbohydrates from day 1 to 15 of experiment compared with controls (group D), immunomodulated + infected (group B) and infected fish (group C). Also, there was a marked and gradual increase of carbohydrates form day 1 (0.721 mg/ml) to 15 (0.862 mg/ml) of experimental period during immunomodulation in group A. Fish which received immunomodulator and infection showed an initial increase on day 1 (0.711 mg/ml) and 2 (0.700 mg/ml) in comparison with controls (0.660 mg/ml) and decreased gradually from day 2 to 3 (0.660 mg/ml), 4 (0.649 mg/ml), 7 (0.638 mg/ml) and 15 (0.630 mg/ml); the decreased values are below normal level (on day 4, 7 and 15 and the recorded value on day 3 is equal to that of controls. Infected fish (group C) showed below normal values (0.480 mg/ml, 0.472 mg/ml, 0.455 mg/ml, 0.420 mg/ml, 0.412 mg/ml and 0.400 mg/ml on day 1, 2, 3, 4, 7 and 15 respectively) from day 1 to 15 of experiment.

DNA activity in muscles (Table 2, Fig. 2A):

Fish which received immunomodulator showed higher values of DNA from day 1 to 15 when compared with control (group D), immunomodulator + infected (group B) and infected fish (group C); the increased values exhibited a progressive enhancement from day 1 to 15 of experiment (96.1 μg/ml on day 1, 100.3 μg/ml on day 15). Fish of both groups B and C showed below normal values of DNA from day 1 to 15 of experimental period. Also, it is of interest to note that there was a progressive decrease from day 1 to 15 of experiment in both groups of B and C.

Table 2. DNA (μg/ml) and RNA (μg/ml) content in the muscles of L. rohita of group A (treated with Aqua Fix @ 50 mg/100g of feed), B (treated 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 D (untreated and uninfected). Values are expressed in the mean derived from 5 observations.

Day of necropsy	Experimental groups						Control group	
	Group A		Group B		Group C		Group D	
	DNA	RNA	DNA	RNA	DNA	RNA	DNA	RNA
1	96.1	111.0	84.0	110	60	90	90.7	106.0
2	96.4	112.5	83.2	90	58	82	91.0	106.1
3	97.2	113.7	80.0	88	55	71	90.8	106.0
4	98.6	116.5	74.0	60	50	74	90.8	106.0
7	99.1	118.0	71.0	40	41	67	90.7	106.1
15	100.3	120.0	70.0	30	38	26	90.8	106.0

Figure 2A. The content of DNA (μg/ml) in the muscles of experimental (group A, B, C) and control (group D) fish (6 months old) at various days of experiment

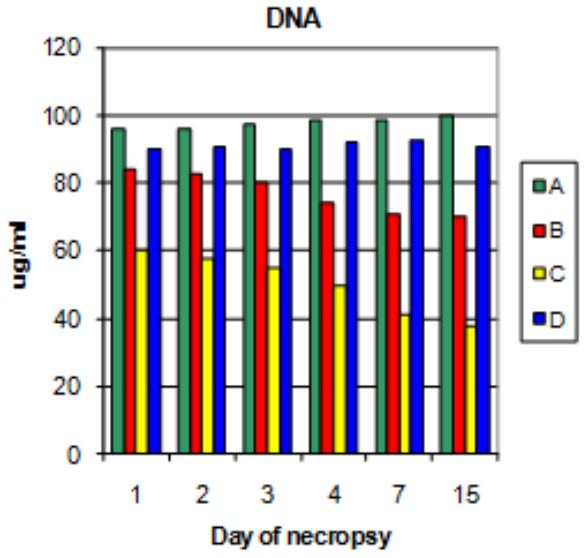
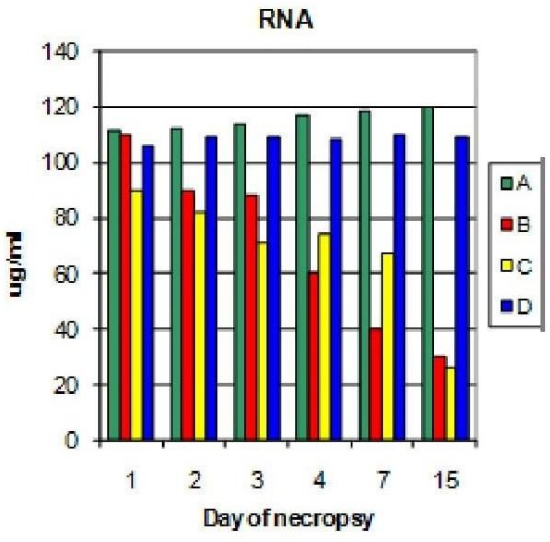


Figure 2B. The content of RNA (μg/ml) in the muscles of experimental (group A, B, C) and control (group D) fish (6 months old) at various days of experiment



RNA activity in muscles (Table 2, Fig. 2B):

In comparison with controls (group D), immunomodulated fish (group A) showed higher values of RNA from day 1 to 15 of experimental period. Fish of group A showed a gradual increase of RNA from day 1 (111.0 μg/ml) to 15 (120.0 μg/ml). In fish of groups B and C, RNA values were found to be below normal from day 1 to 15 of experiment (except on day 1 in group B). A marked and gradual decrease of RNA was found in groups B and C from day 1 (110 μg/ml in group B; 90.0 μg/ml in group C) to 15 (30 μg/ml in group B; 26 μg/ml in group C).

Statistical analysis (Table 3) showed a significant increase of muscle protein in group A and B when compared with controls, whereas muscle protein in group C showed a significant decrease when compared with controls. The difference in the protein content was found to be significant in between groups A and B, A and C, B and C. Muscle carbohydrates showed a significant increase in group A and decrease in group C, when compared with controls and insignificant in between groups A and B, A and C, B and C. The increased DNA content in group A and the decreased DNA content in groups B and C was found to be significant when compared with controls. The difference in the DNA content was found to be significant in between groups A and B, A and C, B and C. Significant increase of RNA in group A and significant decrease in group B and C was noticed in comparison with controls. RNA showed significant difference in between groups A and B, A and C and non-significant difference between groups B and C. Increased content of protein, carbohydrate, DNA and RNA in muscles of immunomodulated fish might be due to the treatment of Aqua Fix for 4 days. This shows that Aqua Fix might have stimulated the host system to synthesize and elevate the level of protein, carbohydrate, DNA and RNA

Table 3. Mean and t-values of protein, carbohydrate, DNA and RNA obtained for muscles of 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:	1.016	0.941	0.289	0.850			
	A D	B D	C D	A B	A C	B C	
t-value	t=36.352*	t=6.409*	t=22.474*	t=5.040*	t=28.646*	t=22.712*	
Carbohydrate: Mean:	0.778	0.664	0.439	0.658			
	A D	B D	C D	A B	A C	B C	
t-value	t=4.699*	t= 0.290@	t=14.686*	t=3.894*	t=11.559*	t=10.617*	
DNA: Mean:	97.95	77.03	50.33	90.8			
	A D	B D	C D	A B	A C	B C	
t-value	t=9.652*	t=4.986*	t=9.950*	t=7.320*	t=11.520*	t=5.432*	
RNA: Mean:	115.2	69.6	68.3	106.03			
	A D	B D	C D	A B	A C	B C	
t-value	t=5.984*	t=2.591*	t=3.777*	t=3.230*	t=4.649*	t=0.0774@	

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

in muscles. These results are in agreement with those of Siwicki *et al*., (1994) who also found an elevation of protein in rainbow trout after feeding with immunostimulant.

These results are further supported by the findings of Misra *et al.,* (2006) in *L. rohita* who also reported an increase of total protein after feeding with immunostimulant. Labh *et al*., (2015) also recorded enhanced level of DNA and RNA in *L. rohita* fed with varied proportion of protein diets. In the present study decrease of carbohydrate (except on initial day of 1 and 2), DNA and RNA was found in fish of group B in comparison with controls (group D) and immunomodulated (group A) fish and increase in comparison with infected (group C) fish. This explains that supplementation of dietary Aqua Fix had a protective effect to some extent showing an increase of muscle protein compared to infected fish (group C). The protective effect of immunostimulants in various fish against different microbial infections is well documented (Vasudeva Rao *et al*., 2006; Sahu *et al*., 2007; Gupta *et al*., 2008; Xavier Innocent *et al*., 2011; Gbadamosi *et al*., 2017). The decreased content of DNA and RNA throughout the experimental period in fish of group B reveal that though they were treated with immunostimulant, *A. hydrophila* infection caused abnormality in the synthesis of DNA and RNA. The stress caused by infection might have led to the proteolytic activity which ultimately brought reduction in carbohydrate, DNA and RNA content.

It is interesting to note that fish injected with *A. hydrophila* infection showed decreased content of tissue protein, carbohydrate, DNA and RNA. Since protein is the major constituent of the cell and plays a significant role in metabolism, any stress in the host system may cause abnormal change in its production. The stress caused by infection might have led to the decrease of muscle protein; this also indicates the rapid utilization of energy or formation of reactive oxygen species under infection stress. These results are similar to that of Yeragi *et al*., (2000) and Jha and Verma (2002) who found decreased muscle protein in marine crab (Uc*a merionis*) and *Clarius batrachus* exposed to pesticide toxicity. Decrease of muscle protein in infected fish (group C) further confirmed by the findings of Muthukumaravel *et al.,* (2013) who observed declined value of muscle protein in *L. rohita* exposed to monocrotophos.

Acknowledgement:

One of the authors (Ankamma, N.) is thankful to UGC for providing financial support in the form of FDP.

Conflict of Interests

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

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