

EFFECT OF IMMUNOSTIMULANT AND AEROMONIASIS ON THE BIOCHEMICAL ASPECTS OF MUSCLE IN 9 MONTHS OLD *LABEO ROHITA*

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ABSTRACT

Feeding with supplementary diet (Aqua Fix) and/or infection with *Aeromonas hydrophila* explored marked biochemical changes and immune response in the muscle of *Labeo rohita*. Nine months old fish (3 groups) were used in the present investigation. Groups A and B were treated with Aqua Fix for 4 days, on day 5 fish of group B and C were infected with *Aeromonas hydrophila* @ 10^{-6} CFU/fish (fish of group C were fed with normal diet for 4 days). Controls (group D) were untreated and uninfected. Necropsies were made on day 1, 2,3,4,7 and 15 after infection in fish of groups B and C; fish of group A and D were also necropsied on the same designated days. Estimated muscle protein, carbohydrate, DNA and RNA were analysed by statistical method and found that Aqua Fix is able to induce protective immune response with regard to enhance the level of muscle protein, carbohydrate, DNA and RNA. Abnormal levels of protein, carbohydrate, DNA and RNA in fish infected with *A. hydrophila* (group C) indicates the lowered immunity due to the stress caused by the pathogen.

KEYWORDS: Aqua Fix, *Aeromonas hydrophila*, *Labeo rohita*, Muscle.

INTRODUCTION

Aeromoniasis is one of the dreadful diseases causing major loss to major carps and to the fresh water fish farms worldwide. *Aeromonas hydrophila* causes haemorrhagic septicemia and epizootic ulcerative syndrome in fish (Zhang et al; 2014). Aeromoniasis causes much pathological changes in vital organs of *Cirrhinus mrigala* (Datta Ray and Homechadhari, 2014) and *Labeo rohita* (Pal et al; 2015). Abdella et al (2017) and Fernandez –Bravo and Figueras (2020) reported that *A. hydrophila* can infect fish and spread diseases in aquatic animals. Barde (2023) explored the disease causing potential of *A. hydrophila* in *C. mrigala*. Yeast glucan and microbial levan were found to enhance survival and non-specific immunity in *Cyprinus carpio* against aeromoniasis (Selviraj et al., 2005; Rairakhwada et al., 2006). Herbs and herbal supplements were found to enhance the protective immune response in fish exposed to microbes (Ukwe and Gabriel, 2019; Ukwe and Abu, 2021; Ukwe and Deekae, 2022, 2024). The impact of aeromoniasis and feeding with an immunostimulant, Aqua fix in *L. rohita* caused marked changes in the architecture of skeletal muscles and liver (Ankamma and Viveka Vardhani, 2025). Different concentrations of feed containing *Helianthus annuus* (leaf powder) indicated that in *L. rohita* fingerlings, the

blood biochemical and enzyme parameters of the infected ones gradually improved from abnormal to normal levels at day 28 of experiment (Muthulakshmi and Neelanarayanan, 2025); Citarasu(2010), Chakraborty and Hamez (2011), Abdul and Haniffa(2011) and Chakraborty et al (2014) reported that the herbal plants may be used as pharmaceutical agents against fish pathogens. Hence, the present study was undertaken to evaluate the effect of *A. hydrophila* and the dietary treatment of Aqua Fix (immunostimulant) on certain biochemical parameters of 9 months old *L. rohita*. The observations were analyzed by students't' test to find out the significance.

MATERIALS AND METHODS

L. rohita (47-50 g) were employed as experimental fish. Three experimental groups A, B, C and one control group, D were maintained for experimentation. 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) (35 in each group) were maintained for experimentation. Fish of group A and B fed with a diet supplemented with Aqua Fix (@50mg/100 g of feed) 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 fish were removed and processed for protein, carbohydrates, DNA and RNA following Lowry *et al.*, (1951), Nicholas *et al.*, (1956) and Burton (1956) respectively. Results were analyzed by student's t-test to find out the significance.

RESULTS AND DISCUSSION

Protein activity in skeletal muscles (Table 1, Fig. 1A)

Fish received Aqua Fix (group A) showed high level of protein from day 1 (1.560 mg/ml) to 15 (1.670 mg/ml) when compared with controls (0.609 mg/ml). Fish treated with Aqua Fix and infection (group B) showed a definite prospect of protein activity; there is an increase from day 1 (1.450 mg/ml) to 15 (1.220 mg/ml) when compared with controls (0.609 mg/ml). Protein decreased slightly from day 1 (1.450 mg/ml) to 2 (1.420 mg/ml) and increased slightly on day 3 (1.550 mg/ml); again there was a decrease on day 4 (1.320 mg/ml) and increase on day 7 (1.470 mg/ml) and decrease on day 15 (1.220 mg/ml). Though there was an increase or decrease throughout the experimental period, the protein levels are higher than normal values.

Muscles of infected fish (group C) showed a decreased protein level from day 1 (0.409 mg/ml) to 15 (0.250 mg/ml). These values are lower than control throughout the experimental period. The estimated protein values are 0.409 mg/ml, 0.402 mg/ml, 0.321 mg/ml, 0.281 mg/ml, 0.270 mg/ml, and 0.250 mg/ml on day 1, 2, 3, 4, 7 and 15 respectively.

When comparison was made among the three experimental groups (A, B, C), fish treated with Aqua Fix (group A) showed higher levels of protein than those treated with Aqua Fix + infection (group B) and infection alone (group C)

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

Aqua Fix treated fish (group A) showed higher level of carbohydrate than normal ones on day 1, 2, 3, 4, 7 and 15. Carbohydrate increased gradually from day 1 (0.990 mg/ml) to 15 (1.360 mg/ml). Fish which received Aqua Fix and infection (group B) showed higher level of carbohydrate throughout the experimental period compared with controls; Carbohydrate decreased from day 1 (0.142 mg/ml) to 2 (0.128 mg/ml), increased on day 3 (0.134 mg/ml) and decreased from day 4 (0.120 mg/ml) to 7 (0.112 mg/ml) and again increased on day 15 (0.124 mg/ml). Infected fish (group C) showed lower level of carbohydrate content on day 1 (0.072 mg/ml), 2 (0.065 mg/ml), 3 (0.060 mg/ml), 4 (0.053 mg/ml), 7 (0.042 mg/ml) and 15 (0.031 mg/ml) compared with controls (0.083 mg/ml) (group D).

Among the three groups A, B and C, Aqua Fix treated fish (group A) showed higher values of carbohydrate from day 1 to 15 with a peak response on day 15 (1.360 mg/ml) compared to groups B and C. Fish of group B showed lower values compared to group A, and higher values compared to group C.

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

Fish received Aqua Fix (group A) showed higher content of DNA on day 1, 2, 3, 4, 7 and 15 of treatment when compared to that of controls (group D). Experimental fish showed a gradual increase from day 1 (371.0 μ g/ml) to 2 (381.0 μ g/ml), 3 (400.5 μ g/ml), 4 (401.5 μ g/ml), 7 (403.0 μ g/ml) and 15 (405.5 μ g/ml) (higher than normal level, 340.0 μ g/ml).

Fish treated with Aqua Fix and infection (group B) exhibited higher DNA content compared with controls from day 1 to 15. From day 1 (368.0 μ g/ml) to 2 (362.0 μ g/ml) there is a slight decrease, an increase on day 3 (369.0 μ g/ml) and decreased on day 4 (358.0 μ g/ml), 7 (354.0 μ g/ml) and 15 (350.0 μ g/ml). Though there is an increase and decrease of DNA from day 1 to 15, these values are higher than control values (340.0 μ g/ml).

In comparison with controls (group D), fish of group C (received infection) showed a lower level of DNA from day 1 to 15. A gradual decrease of DNA was found (lower than control value) from day 1 (215.0 μ g/ml) to 15 of infection (186.0 μ g/ml) (lowest value of DNA was recorded on day 15).

When comparison was made among the 3 experiments, group A (Aqua Fix treated) showed higher content of DNA and group C (infected) showed lower content of DNA. Group B showed lower content of DNA than group A and higher content of DNA than group C.

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

Aqua Fix treated fish showed increased level of RNA from day 1 to 15 compared to controls. There was an increase on day 1 (251.0 μ g/ml), 2 (256.0 μ g/ml), 3 (270.0 μ g/ml), 4 (284.0 μ g/ml), 7 (288.0 μ g/ml), and 15 (290.0 μ g/ml). A peak value was found on day 15 in group A (290.0 μ g/ml). Fish treated with Aqua Fix and infected (group B) showed lower level compared to controls from day 1 to 15. There was a progressive decrease of RNA from day 1 to 15. (RNA content remains same on day 2, 206.0 μ g/ml, and 3, 206.0 μ g/ml). Compared with controls (group D) fish received infection (group C) showed lower content of RNA from day 1 to 15 of infection. With a marked gradual decrease the recorded values are 100.0 μ g/ml, 97.0 μ g/ml, 92.0 μ g/ml, 90.0 μ g/ml, 74.0 μ g/ml and 51.0 μ g/ml on day 1, 2, 3, 4, 7 and 15 respectively.

Higher levels of muscle RNA was found in group A when compared with controls (group D). When comparison was made among the three experimental groups (A, B and C) fish treated with Aqua Fix (group

A) showed higher levels of RNA, than those of treated with Aqua Fix and infection (group B) and infection alone (group C). In between the groups B and C, group B showed higher values of RNA than group C.

Statistical analysis (Table 3) showed significant rise of protein in groups A and B and significant decrease in group C when compared with controls (group D). Experimental groups A, B and C showed significant difference of protein when compared with each other. Significant increase of carbohydrate was found in groups A and B and significant decrease in group C when compared with group D (controls); there was a significant difference in groups A, B and C when compared with each other. DNA level showed significant enhancement in groups A and B, and significant decrease in group C in comparison with controls (group D). A significant difference was noted in DNA level when compared among the experimental groups. There was a significant rise of RNA in group A when compared with group D (controls), B and C and in group B when compared with group C. Whereas the content of RNA in groups B and C showed a significant decrease in comparison with controls (group D).

Significant increase of muscle protein, carbohydrate, DNA and RNA in groups A (treated with Aqua Fix) and B (treated with Aqua Fix + infection) was found highest in group A followed by group B. In the present investigations, *L. rohita* infected intraperitoneally with *A. hydrophila* (group C), the biochemical constituents decreased to below normal values. The increase and decrease of biochemical constituents in test muscles might be due to the enhancement of non-specific immune response (in groups A and B) due to Aqua Fix and by the ill effects of *A. hydrophila* (in group C). Significant increase in biochemical constituents in group A might be due to the protective/cooperative effects of the active compounds in the Aqua Fix. Wang *et al.*, (2015) and Baba *et al.*, (2016) observed enhancement of growth and positive effects of dietary supplements in *C.*

carpio. Similarly, remarkable increase of biochemical constituents in fish of group B reveal that Aqua Fix can stimulate the protective immune response and/or enhance resistance in fish to *A. hydrophila*. Harikrishnan *et al.*, (2003), Jagruthi *et al.*, (2015) and Wang *et al.*, (2015) recorded lower mortality on *A. hydrophila* challenge in *C. carpio* fed with immunostimulants. The decrease of biochemical constituents in the muscle of infected fish (group C) might be due to the abnormality in the synthesis or utilization of protein, carbohydrate, DNA and RNA due to the ill effects of pathogenic bacteria. Ventura and Grizzle (1998) also found lesions and abnormal biochemical indices in various body tissues of catfish, *Ictalurus punctatus* during natural and experimental infection of *A. hydrophila*.

CONCLUSION

Labeo rohita fed with the immunostimulant, Aqua Fix (group A) showed significant rise of protein, carbohydrate, DNA and RNA compared with the immunostimulated+ infected, (group B), infected (group C), and unimmunostimulated + uninfected (group D) fish. Aqua Fix might have triggered the immune response resulting in the enhancement of biomolecules in fish of group A. Similarly fish exposed to Aqua Fix and infection (group B) showed increase of biomolecules compared to infected (group C) and control (group D) fish.

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Table 1: Protein (mg/ml) and carbohydrate (mg/ml) content in the skeletal muscles of immunomodulated (group A, treated with Aqua Fix @ 50 mg / 100 g of feed), immunomodulated and infected with *A. hydrophila* (group B, treated with Aqua Fix @ 50 mg / 100 g of feed and infected @ 10^{-6} CFU/fish), infected with *A. hydrophila* (group C, untreated with Aqua Fix and infected @ 10^{-6} CFU/fish) and control (group D, untreated with Aqua Fix and uninfected with *A. hydrophila*) *L. rohita* (9 months old) at various days of experiment. 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	1.560	0.990	1.450	0.142	0.409	0.072	0.609	0.080
2	1.570	1.040	1.420	0.128	0.402	0.065	0.608	0.081
3	1.600	1.050	1.550	0.134	0.321	0.060	0.609	0.082
4	1.625	1.120	1.320	0.120	0.281	0.053	0.607	0.080
7	1.640	1.180	1.470	0.112	0.270	0.042	0.608	0.081
15	1.670	1.360	1.220	0.124	0.250	0.031	0.606	0.083

P, Protein; C, Carbohydrate

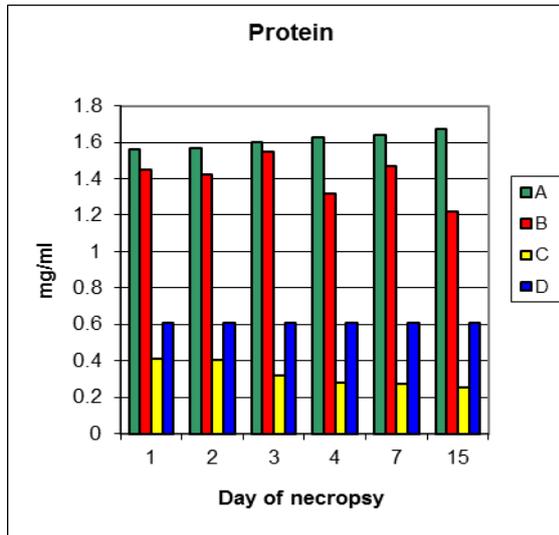


Fig. 1A. The content of protein (mg/ml) in the muscles of experimental (group A, B,C) and control (group D) fish (9 months old) at various days of experiment.

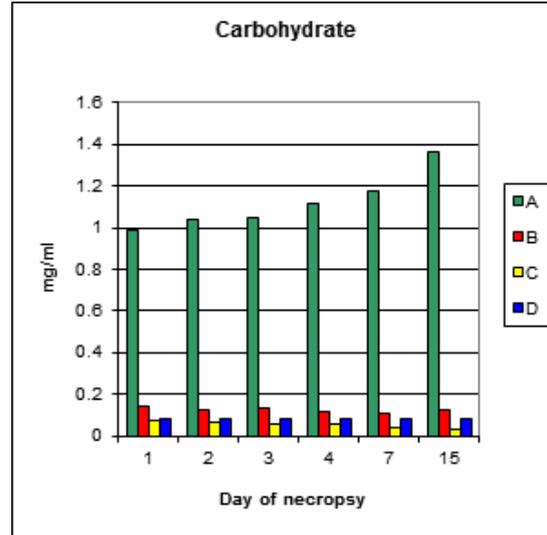


Fig. 1B. The content of carbohydrate (mg/ml) in the muscles of experimental (group A, B,C) and control (group D) fish (9 months old) at various days of experiment.

Table 2: DNA ($\mu\text{g/ml}$) and RNA ($\mu\text{g/ml}$) content in the skeletal muscles of immunomodulated (group A, treated with Aqua Fix @ 50 mg / 100 g of feed), immunomodulated and infected with *A. hydrophila* (group B, treated with Aqua Fix @ 50 mg /100 g of feed and infected @ 10^{-6} CFU/fish), infected with *A. hydrophila* (group C, untreated with Aqua Fix and infected @ 10^{-6} CFU/fish) and control (group A, untreated with Aqua Fix and uninfected with *A. hydrophila*) *L. rohita* (9 months old) at various days of experiment. 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	371.0	251.0	368.0	208.0	215.0	100.0	342.0	240.0
2	381.0	256.0	362.0	206.0	210.0	97.0	342.0	241.0
3	400.5	270.0	369.0	206.0	202.0	92.0	340.0	242.0
4	401.5	284.0	358.0	204.0	193.0	90.0	342.0	242.0
7	403.0	288.0	354.0	200.0	190.0	74.0	341.0	242.0
15	405.5	290.0	350.0	196.0	186.0	51.0	343.0	241.0

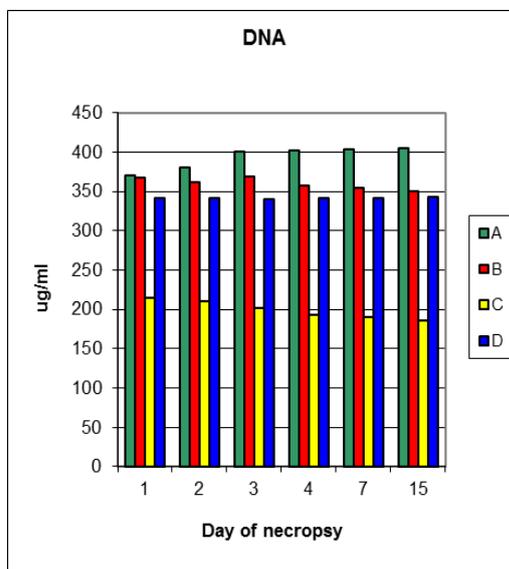


Fig.2 A. The content of DNA ($\mu\text{g/ml}$) in the muscles of experimental (group A, B,C) and control (group D) fish

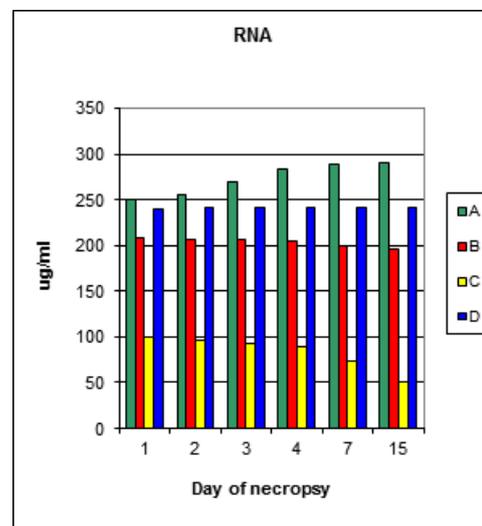


Fig.2 B. The content of RNA ($\mu\text{g/ml}$) in the muscles of experimental (group A, B,C) and control (group D)

(9 months old) at various days of experiment.

fish (9 months old) at various days of experiment.

Table 3: Mean and t-values of protein, carbohydrate, DNA and RNA obtained for skeletal muscles of experimental (group A, immunomodulated; group B, immunomodulated and infected; group C infected) and control (group D, unimmunomodulated and uninfected) *L. rohita* (9 months old).

Biomolecules	Groups					
	A		B	C		D
Protein: Mean:	1.610		1.405	0.322		0.607
t-value	A D _____ t=53.071*	B D _____ t=15.177*	C D _____ t=9.307*	A B _____ t=3.687*	A C _____ t=35.760*	B C _____ t=17.801*
Carbohydrate: Mean:	1.123		0.126	0.053		0.081
t-value	A D _____ t=17.431*	B D _____ t=9.577*	C D _____ t=4.009*	A B _____ t=16.619*	A C _____ t=17.775*	B C _____ t=8.80*
DNA: Mean:	393.6		360.1	199.3		341.6
t-value	A D _____ t=8.175*	B D _____ t=5.32*	C D _____ t=27.19*	A B _____ t=4.66*	A C _____ t=23.78*	B C _____ t=25.94*
RNA: Mean:	273.1		203	84		241
t-value	A D _____ t= 4.21*	B D _____ t=17.89*	C D _____ t=18.93*	A B _____ t=8.96*	A C _____ t=16.90*	B C _____ t=14.02*

P value at 5% level of significance is 2.306

*Statistically significant values

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