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IMPACT OF AQUA FIX ON BRAIN BIOCHEMICAL CHANGES IN AEROMONIASIS INDUCED *LABEO ROHITA*

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ABSTRACT

The present study was conducted to evaluate the immunomodulatory effect of Aqua Fix, a herbal feed supplement on brain biochemical alterations in *Labeo rohita* experimentally infected with *Aeromonas hydrophila*. Three groups(A,B,C) of six months old fish were employed; 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 group B and C; fish of group A and D were also necropsied on same designated days. Brain protein, carbohydrate, DNA and RNA were analysed following standard methods and results were subjected to statistical analysis and found that Aqua Fix modulated immunity to enhance the level of protein, carbohydrate, DNA and RNA in brain. Group C showed marked decrease of all the above biomolecules in *L.rohita* due to stress caused by aeromoniasis .These findings suggests the protective effects of Aqua Fix against aeromoniasis induced *L.rohita.*

Key words:,Immunostimulant , *Aeromonas hydrophila* , *Labeo rohita,* Brain

INTRODUCTION

Aquaculture practices depend on water quality management, high quality feed, use of immunostimulants and health status of aquatic organisms. One of the three Indian major carps, *Labeo rohita* (rohu) is the most important commercial fish in India and is extensively grown in polyculture systems. Several factors like poor managerial practices, environmental conditions and disease outbreaks are the major constraints in the development and sustainability of aquaculture. Bacterial diseases are causing major loss to the fish farmers (Almeida *et al*., 2009). *Aeromonas hydrophila* (a gram negative, motile, rod shaped, facultative anaerobe) which inhabits in the aquatic environment causes disease when fish is under stress. Like other carp species, *L. rohita* is found to be severely affected by aeromoniasis resulting in mass mortality in farms and hatcheries (Mohanty *et al*., 2008). *A. hydrophila* will become pathogenic (as it is a common part of gut flora) when fish is under stress due to abnormal environmental and physiological conditions (Trust and Sparrow, 1974). These pathogenic bacteria cause several clinical symptoms like loss of scales and fins, reddening of the skin under fins, haemorrhages on the gills, odema, haemorrhagic septicemia, exopthalmus, abdominal distension and ulceration (Rahman *et al*., 1997; Na wang *et al*., 2013; Rico *et al*., 2013).

A. hydrophila (microbial pathogen) is prevalent in freshwater, marine and brackish water globally. It has been reported that *Aeromonas* is the causative agent of dropsy, haemorrhagic septicemia, asymptomatic septicemia, tail and fin rot and ulceration (Mukherjee, 1991; Rahman *et al*., 2001). Health status, stress or stimulus, treatment, parasitic or infectious diseases may cause much variation in the degree of haematological response (Hrubec *et al*., 2000; Rehulka, 2002; Chen *et al*., 2004; Silveria-Coffigny *et al*., 2004; Martins *et al*., 2004). Hrubec *et al*., (2004) reported that species, age, sexual maturity and health status of fish influence

haematological, biochemical and immunological indices in fish and/or important tools in the diagnosis of fish health. Fish immune system can be modulated by the use of herbal extracts (as feed supplement) as alternative chemotherapeutic agents (Raman, 2007; Kumar *et al.*, 2013). Chakraborty and Hanez (2011), and Vaseeharan and Thaya (2013) found growth promotion, stimulation of appetite and immunity and increase of antimicrobial properties in fish due to the use of plant extracts.

Randall and Perry (1992) explained that when fish is under stress, the central nervous system release stress hormones like cortisol and catecholamine's (adrenalin and nor adrenalin) during the primary response of immune system. Barton and Iwama (1991) explained that the release of stress hormone induces the induction of secondary response of the immune system which in turn causes changes in the blood (an increase in plasma glucose). Plant based feed additives can enhance growth, boosts immune system , reduces stress, acts as appetizers and enhances the secretion of various digestive enzymes which increase feed consumption and improves digestion. As plant based derivatives are less expensive, environmental friendly and have less adverse effects they are widely employed to replace expensive antibiotics in fish health management. Immunostimulants from plants have been tested in fish, demonstrating their ability to enhance immunity, protect against diseases, and have minimal environmental impact, thus ensuring the safety of fishery products for consumers. The present study was conducted to examine the effect Aqua Fix on brain biochemical responses in *L.rohita* subjected to aeromoniasis..

MATERIALS AND METHODS

Three experimental groups of six month old *L. rohita* (12-15 g) were selected for the 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) 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 (@50mg/100 g of feed) for 4 days; on day 5 fish of group B and C were infected intraperitonially 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). Brain tissue from the experimental and control groups were removed and analysed for protein, carbohydrates, DNA and RNA following Lowry *et al.*, (1951), Nicholas *et al.*, (1956) and Burton (1956) respectively. Results were subjected to student's t-test to find out the significance.

RESULTS

Protein activity in brain

Fish of group A, immunomodulated with Aqua Fix showed interesting observations. The level of protein on day 1 (0.390 mg/ml), 2 (0.398 mg/ml), 3 (0.400 mg/ml), 4 (0.408 mg/ml), 7 (0.411 mg/ml) and 15 (0.440 mg/ml) are higher than normal levels (0.280 mg/ml). A slight and gradual increase of protein has occurred form day 1 to 15; also this increase was higher than normal values.

In case of fish of group B (which received Aqua Fix and infection), the brain protein level enhanced from day 1 to 15 of experimental period in comparison with controls. The initial value (0.350 mg/ml on day 1) of protein decreased gradually reaching 0.300 mg/ml on day 15. Though the protein value decreased from day 1 to 15, this decrease was above normal (control, 0.278 mg/ml) level.

In group C (which received infection), fish showed below normal level of proteins from day 1 to 15 of experiment. There was a gradual and marked decrease of protein from day 1 (0.260 mg/ml) to 15 (0.200 mg/ml) which are still lower than control values (0.280 mg/ml on day 1; 0.278 mg/ml on day 15).

Among the three groups A, B and C, Aqua Fix treated fish (group A) showed enhanced level of protein from day 1 to 15 with a peak response on day 15 (0.440 mg/ml) compared to groups B and C. Fish received infection and fed with normal diet (group C) showed below normal values from day 1 to 15.

Carbohydrate activity in brain (Table1, Fig. 1B)

Fish of Group A, which received Aqua Fix showed higher levels of carbohydrates throughout the experiment compared with controls (group D). From day 1 to 15, the level of carbohydrate rose gradually beyond the normal value and reached its peak on day 15 (0.074 mg/ml). The estimated carbohydrate values in group A were 0.050 mg/ml, 0.058 mg/ml, 0.059 mg/ml, 0.065 mg/ml, 0.070 mg/ml and 0.074 mg/ml on day 1, 2, 3, 4, 7 and 15 respectively. Where as in group B, which received Aqua Fix and infection brain showed high level of carbohydrate on day 1 (0.041 mg/ml) and 2 (0.040 mg/ml) when compared to controls (0.030 mg/ml). By day 3 (0.035 mg/ml) and 4 (0.030 mg/ml), there was a decreased level of carbohydrates which is equal to normal level. From day 3 to 15, there was a progressive decrease and these values (0.021 mg/ml on day 7; 0.018 mg/ml on day 15) are less than that of control values. Fish of group C (which received infection) had shown very low level of carbohydrate from day 1 (0.024 mg/ml) to 15 (0.002 mg/ml) of infection; these values are below normal values. There was a gradual decrease of carbohydrates during the period of experimentation (these decreased values are lower than that of control values).

In comparison with controls, group A (from day 1 to 15) and B (on day 1 and 2) showed higher values of carbohydrate and group C showed below normal level of carbohydrate. Among the three experimental groups A, B and C, fish of group A showed higher level when compared with groups B and C , and group C recorded lower level of carbohydrate when compared with groups A and B.

DNA activity in Brain

Fish of group A, which received, Aqua Fix showed higher levels of DNA throughout the experimental period compared with controls (group D). From day 1 to 15, the content of DNA rose gradually beyond the normal values and reached its peak on day 15 (212 µg/ml).

In case of group B, which received Aqua Fix and infection brain showed below normal levels of DNA on day 1 (180 µg/ml), 2 (176 µg/ml), 3 (169 µg/ml), 4 (160 µg/ml), 7 (154 µg/ml) and 15 (140 µg/ml); the DNA value in control (group D) was 190 µg/ml on day 1.

Fish of group C (which received infection) had shown marked decrease of DNA from day 1 (121 µg/ml) to 15 (80 µg/ml) of infection when compared to controls (these values are below normal values). There was a gradual decrease of DNA from day 1 to 15; the lowest value of DNA (80 µg/ml) was found on day 15. These values are lower than that of control values.

Higher level of brain DNA was found in group A when compared with controls (group D); when comparison was made among the three experimental groups (A, B, C), fish treated with Aqua Fix (group A) showed higher values of DNA than those of fish treated with Aqua Fix and infection (group B) and fish treated with infection (group C). In between the groups B and C, group B showed higher values of DNA than group C.

RNA activity in brain:

In group A, which received Aqua Fix for 4 days, there was an increase of RNA from day 1 to 15 when compared to controls. From day 1 to day 15, there was a progressive increase and this increase was higher than controls (group D). The recorded RNA values were 129 µg/ml in group A and 124 µg/ml in group D on day 1, 131 µg/ml in group A and 123.9 µg/ml in group D on day 2, 132 µg/ml in group A and 123.9 µg/ml in group D on day 3, 132.5 µg/ml in group A and 124 µg/ml in group D on day 4, 133.5 µg/ml in group A and 124 µg/ml in group D on day 7, and 135 µg/ml in group A and 122 µg/ml in group D on day 15.

In case of fish of group B treated with Aqua Fix and infected with *A. hydrophila*, the RNA values are higher than control group (D) on day 1 (126 µg/ml in group B and 124 µg/ml in group D). From day 2 to 15 there is a gradual decrease of RNA when compared with controls, lowest value was recorded on day 15 (100 µg/ml) in group B.

Fish of group C which received infection showed lower values of RNA from day 1 to 15 of infection when compared with controls; throughout the experimental period these values stand below the normal values. There was a progressive decrease in the content of RNA from day 1 (120 µg/ml) to 15 (90 µg/ml).

In comparison with controls, fish treated with Aqua Fix showed highest RNA values in the brain tissue. When comparison was made among the three experimental groups (A, B, C), fish treated with Aqua Fix (group A) showed higher values of RNA and group C showed below normal levels of RNA. Group B expressed less content of RNA than group A and more content than group C. The lowest amount of RNA was found in group C on day 15 (90 µg/ml).

DISCUSSION

In this study, in 6 months old fish, Aqua Fix supplemented diet (group A) increased protein, carbohydrates, DNA and RNA values significantly in the brain compared to control (group D) and other experimental groups (B,C). Enhancement of growth, survival and disease resistance were found after using immunostimulants in *O. mossambicus* (Immanuel *et al*., 2009), *L. calcarifer* (Talpur and Ikhwanuddin, 2013) and juvenile, *Huso huso* (Kanani *et al*., 2014). There was a significant increase of protein and significant decrease of carbohydrate, DNA and RNA in group B compared with controls (group D) and immunostimulated (group A) fish. Similarly, infected fish (group C) showed a significant decrement in all the tested biochemical constituents. These results indicate that, Aqua Fix had a protective effect to some extent in fish of group B in neutralizing the ill effects of infection and in fish of group C infectious bacterial pathogens caused decrease of brain biochemical constituents. Several studies demonstrated that immunostimulates can enhance resistance of fish to several bacterial pathogens including *A. hydrophila* (Nya and Austin, 2009, 2010; Awad and Austin, 2010). This study demonstrated that fish treated with immunostimulant + infection (group B) and infection (group C) showed decrease of carbohydrate in the brain. Murthy and Devi (1982) found decrement of glycogen content in the brain of *C. punctatus* exposed to endosulfan. Shrivasatava *et al*., (2002) also recorded decreased carbohydrate content in the brain of *H. fossils* exposed to carbaryl.

Proteins play an important role in the building of body and maintaining the normal architecture of various types of cells. When animals are under stress, they utilize protein in addition to carbohydrate to release energy. Very little carbohydrate content is present in fish. When carbohydrates are exhausted during energy requirement, the next alternative source of energy is protein to be used for release of energy. When protein is utilized or degraded, the content of protein may be decreased in various tissues of fish like muscle, liver and brain. The present study showed significant decrement of brain protein in infected fish (group C). .

Decrease of carbohydrate content in various tissues of animals indicates the high amount of utilization of carbohydrates to release energy or to cope with metabolic reactions during various conditions of stress. Though glucose is released by glycogenolysis in the liver, a fall in the carbohydrate content in various tissues of fish may occur during stressful conditions either by infection of pesticide treatment. In the present study, *A. hydrophila* infection stress caused decrement of carbohydrate brain.

The present study on 6 months old *L. rohita* showed an enhancement of protein, carbohydrate, DNA and RNA in brain in group A fed with Aqua Fix supplemented diet; this group (A) recorded highest values compared to control and other groups. These results are in agreement with previous studies using different immunostimulants in C. carpio (Yuan *et al*., 2007), *O. mykiss* (Nya and Austin, 2011), *D. mykiss* (Awad *et al*., 2013) and *Huso huso* (Binali *et al*., 2014) who reported protective effect of immunostimulants on growth and resistance. Next to group A, group B showed high level of biochemical constituents and group C showed significant decrease of all the biochemical constituents in brain due to the toxic reactions caused by infectious bacterial pathogens.

CONCLUSION

Infected fish of 6 (group C) months old showed decreased values of protein, carbohydrate, DNA and RNA in brain compared to controls and other experimental fish. This might be due to the stress caused by the infectious bacterial pathogens. Decreased protein content recorded in brain of the present study may be attributed to stress mediated immobilization of protein resulting in the decreased content of DNA and RNA. The results shown in the present study indicate that the immune system in fish was modulated by Aqua Fix and responded to resist *A. hydrophila* infection . *L. rohita* fed with Aqua Fix was significantly superior when compared to controls and other groups. However, further studies are needed to use Aqua Fix as immunostimulant in other fishes and against other microbial pathogens

Table 1: Protein (mg/ml) and carbohydrate (mg/ml) content in the brain 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* (6 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	0.390	0.050	0.350	0.041	0.260	0.024	0.280	0.030
2	0.398	0.058	0.343	0.040	0.252	0.022	0.279	0.032
3	0.400	0.059	0.331	0.035	0.248	0.020	0.278	0.030
4	0.408	0.065	0.328	0.030	0.220	0.014	0.280	0.034
7	0.411	0.070	0.306	0.021	0.209	0.011	0.281	0.033
15	0.440	0.074	0.300	0.018	0.200	0.002	0.278	0.030

P, Protein; C, Carbohydrate

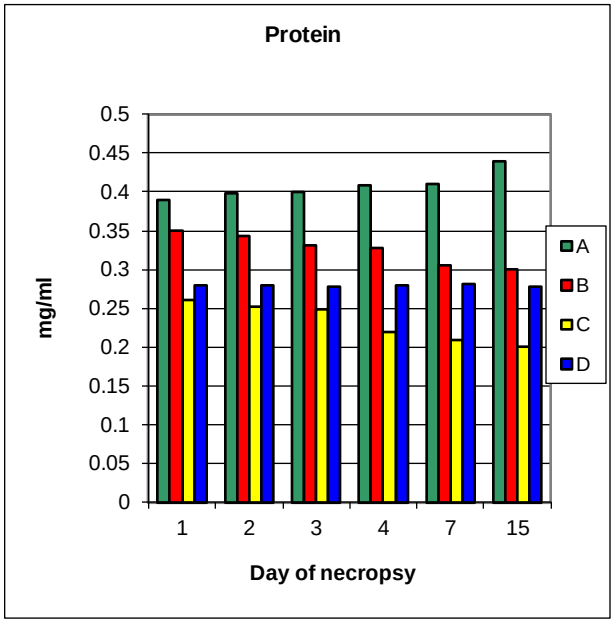

Fig. 1A. The content of protein (mg/ml) in the brain of experimental (group A,B,C) and control (group D) fish (6 months old) at various days of experiment

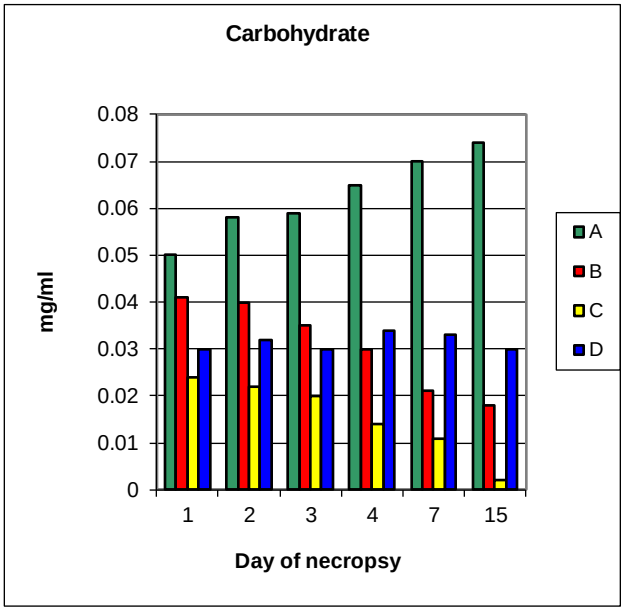

Fig. 1B. The content of carbohydrate (mg/ml) in the brain of experimental (group A,B,C) and control (group D) fish (6 months old) at various days of experiment

Table 2: DNA (µg/ml) and RNA (µg/ml) content in the brain 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* (6 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	196	129	180	126	121	120	190	124.0
2	201	131	176	120	115	116	190	123.9
3	205	132	169	110	100	104	189.9	123.9
4	209	132.5	160	108	94	102	189,8	124.0
7	210.5	133.5	154	102	88	93	190	124.0
15	212	135.0	140	100	80	90	190	124.0

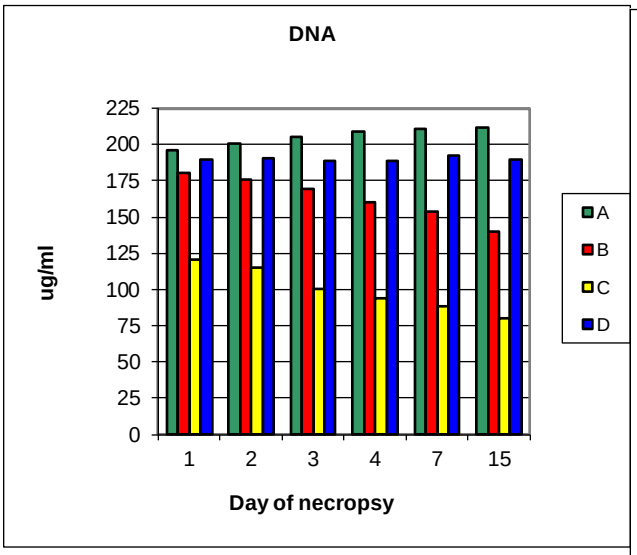

Fig. 2A. The content of DNA (µg/ml) in the brain of experimental (group A,B,C) and control (group D) fish (6 months old) at various days of experiment

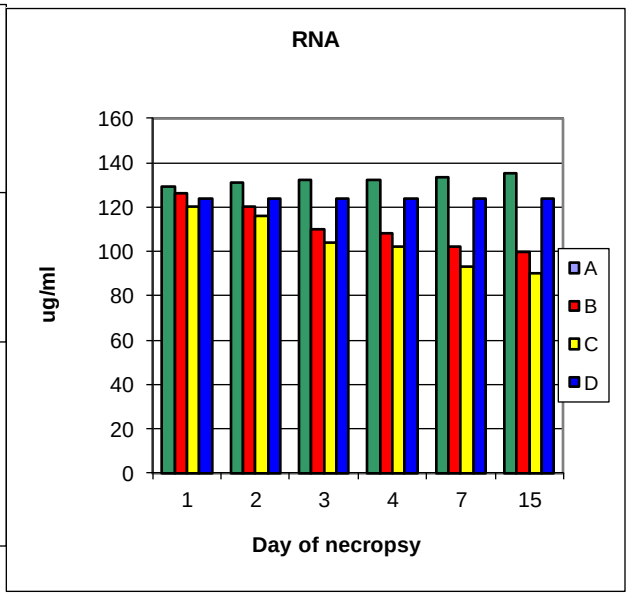

Fig. 2B. The content of RNA (µg/ml) in the brain of experimental (group A,B,C) and control (group D) fish (6 months old) at various days of experiment

Table 3. Mean and t-values of protein, carbohydrate, DNA and RNA obtained for brain 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).

	Groups			
Biomolecules	A	B	C	D
Protein: Mean:	0.407	0.326	0.231	0.279
	A D	B D	C D	A B	A C	B C
t-value	t= 16.45*	t= 5.30*	t= 4.28*	t= 6.88*	t= 12.94*	t= 6.67*

Carbohydrate:						
Mean:	0.062		0.030	0.015		0.031
	A D	B D	C D	A B	A C	B C
t-value	t= 8.16*	t= 0.26@	t= 5.05*	t= 5.62*	t= 9.28*	T= 2.63*
DNA:						
Mean:	205		163	99.6		190
	A D	B D	C D	A B	A C	B C
t-value	t= 5.38*	t= 4.06*	t= 12.1*	t= 5.8*	t= 14.0*	t= 6.5*
RNA:						
Mean:	132		111	104		123
	A D	B D	C D	A B	A C	B C
t-value	t= 8.32*	t= 2.61*	t= 3.53*	t= 4.54*	t= 5.1*	t= 0.99@

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

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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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