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The effect of aeromoniasis on protein and DNA content and histopathology of muscle and gill in adaptively immunized fish

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ABSTRACT

The present investigations were designed to estimate the content of protein and DNA and to understand the histopathology in muscle and gill of fish infected with repeated doses of *A. liquefaciens*. Two varied multiple doses of pathogen, 10^{-2} + 10^{-2} CFU/Fish and 10^{-3} + 10^{-3} CFU / Fish were injected intramuscularly to two different groups of fish A and B (66 in each group) respectively; two groups a and b were served as controls for comparison. Muscle and gill were selected for studying the content of protein and DNA and for histopatholgoical changes. Six fish from the experimental and control groups were necropsied at hour 1, 3,6,12,18,24,36,48,72,96 and 216 of experimental period. Tissues of muscle and gill were separated and analyzed for total protein and DNA. Estimation of protein and DNA content and histopathological findings in both the experimental and control groups of fish were done following standard methods. Both the infected groups of fish showed clinical symptoms such as weakness, anorexia, scale falling, hemorrhages, skin ulcers and necrosis of viral organ. *A. liquefaciens*, one of the most important pathogens of the out breaks in fresh water fish effected the level of protein and DNA in muscle and gills from hour 1 to 216 of experimental period. The stress caused by *A.liquefacaens*is contributing to the disturbed physiology in fish. Fish which were exposed to multiple infection of *A. liquefacaens* did not show resistance and in these two groups of fish A (10^{-2} + 10^{-2} CFU/Fish) and B (10^{-3} + 10^{-3} CFU/Fish) there is a marked decrease in the level of protein and DNA in muscle and gill from hour 1 to 216 with few exceptional following the intramuscular injection, lesions developed in the visceral organs such as kidney, liver and spleen. In both the multiply infected groups of fish, degeneration, necrosis and atrophy of muscle bundles and splitting of muscle fibers were observed at hour 72 and infected gill from both the groups A and B showed distortion and enlargement of primary lamellae, curling and clubbing and loss of secondary lamellae at day one of infection.

Key words: *Aeromoniasis*, protien, DNA, gill, muscle, fish.

INTRODUCTION

Indian freshwater aquaculture is mainly based on carp culture contributing to more than 87% total aquaculture (FAO 2001). Both natural and acquired immunity help to protect the fish against microbial and parasitic infections (Ingram, G.A., 1980). The defense mechanism in fish is contributed by several cells and their products (lysozymes, compliment, enzymes, lysins etc); these are indexed as indicators of stress response and disease resistance in fish (Anderson, D.P.; Siwivki A.K, 1995; Marseden, M.J.; Freeman, I.C. COX, D;Secombes, C.J., 1996; Sahoo P.K; Mukarjee, S.C., 2003; Sahoo P.K;, Kumari J.K., Mishra B.K., J.Appl.Ichthyol. 21(2005). Very little is known about the natural and adaptive immune response of the Indian major carps (Dash, K; Sahu, A; Gangal, S.V., 1993: saha, k: Dash,K; sahu,A., 1993; sahoo P.K.; Mukarjee, S.C., 2001: Sahoo, P.K; Meher,p.k.; Mohapatra, K.D.; Saha.J; Jana,,R.K.Reddy,P.V.G.K., 2004). Aeromoniasis caused abnormal changes in protein and DNA profile in head kidney of *Labeo rohita* (Satyalatha and Viveka Vardhani, 2014). The present study was designed to estimate the level of protein and DNA and

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histopathology of muscle and gill of *Labeorohita* exposed to multiple infections of *Aeromonas liquefaciens.*

MATERIALS AND METHODS

A total of 264 *Labeo rohita* (approximately weighing about 65-75 gm wt). aged 5-6 months were used in this study. The fish were divided into the three groups each consists of 66 fish.Fish were treated with *A. liquefaciens* @ 10 -2 + 10 -2 CFU/fish (group A) and 10 -3 + 10 -3 CFU/fish (group B) at five days interval. Two groups (a,b) of 66 fish (in each group) were as unifected controls for comparison. All the fish were fed with pellet diet. The tissues of muscle and gill were collected and prepared for biochemical analasis and and histopathology on hour 1, 3,6,12,18,24,36,48,72,96 and 216 of experimental period. Protein was estimated by the method described by (11) and DNA was determined following diphenyl amine method. Pieces of muscle and gill tissue were fixed sectioned (5 U) and stained by H and E method. Results were analyzed by student't' text to test the significant of protein and DNA level.

RESULTS AND DISCUSSION

In group A :

Protein activity in muscle: In fish of group A 10-2 CFU/fish + 10-2 CFU/fish (at 4 day interval). The level of protein on hour 1, 3, 6, 12, 18, 24, 36, 48, 72, 96 and 216 are lower than normal values. From hour 1 (57.51 mg/ml) to 216 (34.17 mg/ml), there is a gradual decrease of protein and is almost equal on hour 96 (34.82 mg/ml) and 216 (34.17 mg/ml) of infection; this amount (34.17 mg/ml) is somewhat half to the amount recorded in uninfected controls (68.91 mg/ml).

DNA activity in muscle: Fish of group A showed lower DNA levels throughout the experimental period (from hour 1 to 216); these values are lower than control values. The decreased values of DNA in experimental fish include – 5.55 mg/ml on hour 1, 24 and 72, 6.66 mg/ml on hour 3 and 36, 7.77 mg/ml on hour 6, 12, and 48, 8.88 mg/ml on hour 18 and 4.44 mg/ml on hour 96 and 216. This decease of DNA content (4.44 mg/ml) is almost two fold decrease in comparison to that of controls (9.91 mg/ml).

Protein activity in Gill: Infected gills (group A) showed lower protein levels when compared to that of controls (group a) during the entire experimentation period. The Initially decreased protein content (30.68 mg/ml) on hour 1 decreased gradually to 28.62 mg/ml by hour 24. Again there was a slight increase on hour 36 (31.37 mg/ml), 48 (32.75 mg/ml), 72 (32.41 mg/ml), 96 (31.37 mg/ml) and 216 (32.75 mg/ml); but this increase of DNA content is lower than the normal value of control fish.

DNA activity in gill : The DNA value is higher on hour 1 and 3 of infection (10.0 mg/ml) than normal value (8.88 mg/ml) and the DNA content decreased spontaneously on hour 6 (8.88 mg/ml) (group a) and 12 (7.77 mg/ml). From hour 12 to the hour 216 of infection there was a gradual decrease of DNA content and it is at lowest level (3.33 mg/ml).

In group B:

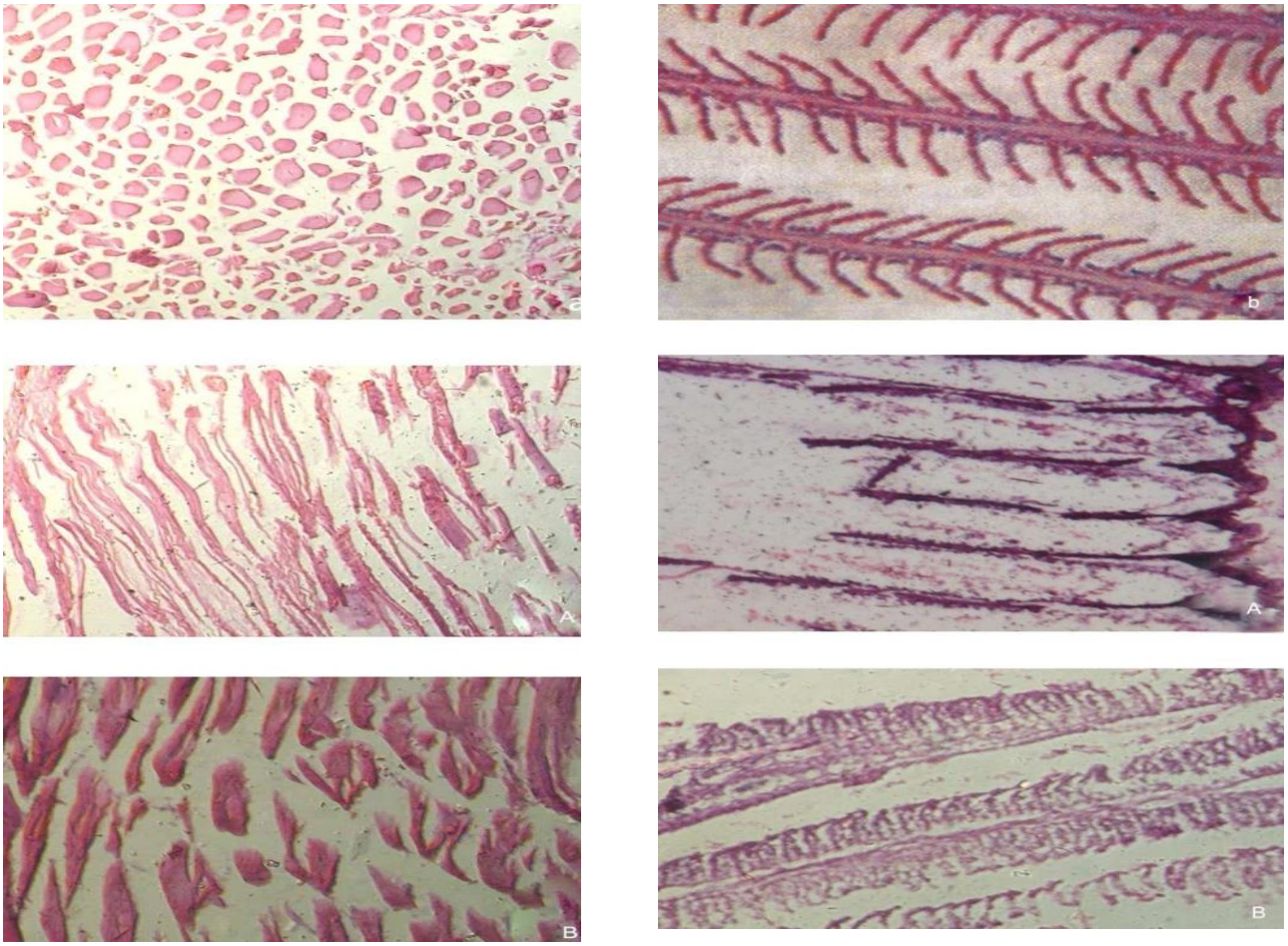
Protein activity in muscle: The protein levels in the muscle of experimental fish infected with low doses of *A. liquefaciens* (10-3 CFU/fish + 10-3 CFU/fish). Fish of group B showed a decrease of protein during the entire period of experimentation (hour 1 to 216); these decreased values are lower than that of normal levels (group b). The content of protein is 64.13 mg/ml and 62.41 mg/ml on hour 1 and 3; the recorded value on hour 3 is lesser from hour 1 and higher than that of hour 6 (65.17 mg/ml) of infection. There is almost on equal decreased level of protein occurred on hour 12 (66.20 mg/ml), 18 (66.55 mg/ml), 24 (66.62 mg/ml) and on hour 36 (63.79 mg/ml) and 48 (63.10 mg/ml). A gradual decrease of protein occurred from hour 48 to 72 (57.93 mg/ml), 96 (54.13 mg/ml) and 216 (50.68 mg/ml). The lowest amount of protein was observed on the hour 216 (50.68 mg/ml).

DNA activity in muscle: Low values of DNA were found from hour 1 (7.75 mg/ml) to 216 (5.55 mg/ml) of infection period. These low values are lower than uninfected fish (9.89 mg/ml). 7.75 (mg/ml) of DNA was found on hour 1 and 3, 5.55 mg/ml was noticed in hour 6, 12, 24 and 216, 4.44 mg/ml was noticed on hour 18, 36 and 48, 3.33 mg/ml was noticed on hour 72 and 96. Protein activity in gill: Experimental fish (group B) showed an initial decrease on hour 1 (30.0 mg/ml) and an equal level of protein on hour 3 (31.37 mg/ml), 6 (31.72 mg/ml) and 12 (31.37 mg/ml). There is a gradual increase from hour 12 to 36 (36.20 mg/ml). Gill showed low levels of protein on hour 48 (33.79 mg/ml), 72 (34.13 mg/ml), 96 (27.24 mg/ml) and 216 (33.79 mg/ml).

DNA activity in gill: Throughout the period of experimentation the levels of DNA showed lower values in experimental group (B) of fish. On hour 1 and 18, the DNA level was equal to that of control (8.88 mg/ml) (group b). The DNA level was declined to below normal on hour 3 (5.51 mg/ml), 6 (4.44 mg/ml), 12 (7.77 mg/ml), 24 (7.71 mg/ml), 36 (6.67 mg/ml), 48 (5.54 mg/ml), 72 (4.42 mg/ml), 96 (7.75 mg/ml) and 216 (4.43 mg/ml). It is interesting to note that experimental fish showed normal level of DNA on hour 1 (8.88 mg/ml – initial period of infection) and below normal level on hour 216 (4.43 mg/ml – later period of infection).

Results were analysed by "student's t test" (table 2 & 3). There was a significant decrease of muscle protein and DNA in groups A &B when compared with controls. Also, there was a significant difference in the decrease of muscle protein and DNA when compared between groups A & B.

Figure-1. Muscle from control (a) and infected (A, B) fish.



L. rohita responded to the repeated 10^{-2} + 10^{-2}Group A and 10^{-3} + 10^{-3} Group B) doses of *A. liquefaciens* by altering the level of protein and DNA and histopathology in muscle and gill. The pathogenic doses of 10^{-2} + 10^{-2} CFU/ fish (group A) and 10^{-3} + 10^{-3}(group B) delivered intramuscularly induced a good secondary response after the second booster dose. Though the reason for this secondary response in groups A and B is not known, it is possible that the two multiple doses of infection influenced the level of protein and DNA in muscle and gill. Lamers and Van muiswinkel (1986), Karunasagar et al (1991) and Newman (1993) recorded significant alterations in the biochemical constituents in fish exposed to aeromonds. *L. rohita* might have undergone stress due to the exposure of multiple doses of *A. liquefacaens*. The marked changes in protein and DNA level and histopathological reactions in muscle and gill of test fish indicate that aeromoniasis caused abnormal changes at cellular and molecular level Begum (2004) also reported biochemical changes liver and muscle tissue of clariusbatracus treated with insecticide. Fish under gone stress by several factors like bad environment, poor quality food and /or pathogen may release stress proteins there by altering the level of protein molecules and histoarchitecture. Boone and Vijayan (2002 Tabche et al., (2002) Ali et al., (2003) reported synthesis of stress proteins die to heavy metal treatment. The remarkable changes in proteins and DNA level and in histopathology of muscle and gill in the test fish group A and B indicates the pathogenic effect of *A. liquefaciens* when given in repeated doses. Udgata et al (2006) found that the antigens of *A. liquefaciens* produced a very good primary and secondary response by producing agglutinating antibodies in oral groups of administration, repeated doses of *A. liquefaciens* induced the pathogenic effect like separation, degeneration and atrophy of muscle and destruction, clubbing and curling of secondary lamellae in gill. Sakr and Gabr (1991), Nour and Amer (1995) and Elneneaki and Abuzinadah (2003) also reported degenation, atrophy and splitting of muscle fibres in muscle of *Tilapia nilotica* exposed to diazinonand neopybuthrin. Erosion of epidermis and derimisand severe necrosis of underlying musculature was found in *Micropterus salmodies* infected with *A.hydrophila* by Huizinga et al (1979). Parkh et al (2010) also found degeneration, atrophy and necrosis of muscle fibres in *Orecromusmos sambievs* treated with dimethoate.

Table-1: Protein (mg/ml) and DNA (mg/ml) content in the muscle and gill, of experimental fish treated with *Aeromonas liquifaciens* @10-2 + 10-2CFU/FISH (group A) and 10-3 + 10-3CFU/FISH (group B), at different periods of infection and control (groups a and b). Values are expressed in mean derived from five observations.

Hours of Necropsy	Experimental groups								Control groups							
	Group A Muscle		Group A Gill		Group B Muscle		Group B Gill		Group a Muscle		Group a Gill		Group b Muscle		Group b Gill	
	Protein	DNA	Protein	DNA	Protein	DNA	Protein	DNA	Protein	DNA	Protein	DNA	Protein	DNA	Protein	DNA
1	57.51	5.55	30.68	10.0	64.13	7.75	30.0	8.88	68.91	9.89	38.62	8.88	68.96	9.89	38.62	8.88
3	55.75	6.66	25.51	10.0	62.41	7.77	31.37	5.51	68.91	9.91	38.61	8.87	68.96	9.91	38.61	8.81
6	52.03	7.77	24.48	8.88	65.17	5.55	31.72	4.44	68.90	9.87	38.63	8.89	68.96	9.87	38.62	8.87
12	51.62	7.77	23.79	7.77	66.20	5.56	31.37	7.77	68.89	8.99	38.66	8.88	68.95	8.99	38.61	8.88
18	48.55	8.88	27.93	8.88	66.55	4.44	32.75	8.88	68.87	9.87	38.65	8.88	68.96	9.87	38.63	7.99
24	45.17	5.55	28.62	7.77	66.62	5.55	34.82	7.71	68.91	9.89	38.66	8.89	68.97	9.89	38.64	8.88
36	44.48	6.66	31.37	6.66	63.79	4.44	36.20	6.67	68.90	9.89	38.61	8.87	68.98	9.89	38.62	8.83
48	41.72	7.77	32.75	5.55	63.10	4.41	33.79	5.54	68.90	9.89	38.61	8.81	68.97	9.88	38.62	8.88
72	38.27	5.55	32.41	7.77	57.93	3.33	34.13	4.42	68.91	9.89	38.62	8.87	68.98	9.86	38.63	8.87
96	34.82	4.44	31.37	4.44	54.13	3.31	27.24	7.75	68.91	9.89	38.61	8.88	68.96	9.88	38.62	8.87
216	34.17	4.44	32.75	3.33	50.68	5.55	33.79	4.43	68.88	9.91	38.60	8.87	68.97	8.99	38.61	8.88

Table 2: 't' values obtained for different groups of fish infected with $10^{-2} + 10^{-2}$ (group A), and $10^{-3} + 10^{-3}$ (group B) CFU/fish

Experimental (A and B) and Control (a and b) groups				
	A	a	B	b
Muscle Protein Mean	45.83	68.90	61.88	68.92
	A a	B b	A B	
t value	t=9.52* (P<0.05)	t=4.37* (P<0.05)	t=5.51* (P<0.05)	
Muscle DNA Mean	6.46	9.99	5.24	9.25
	A a	B b	A B	
t value	t=7.95* (P<0.05)	t=10.52* (P<0.05)	t=2.35* (P<0.05)	

Table-3. 't' values obtained for different groups of fish infected with 10-2 + 10-2 (group A), and 10-3 + 10-3 (group B) CFU/fish

Experimental (A and B) and Control (a and b) groups				
	A	a	B	b
Gill Protein				
Mean	29.24	38.62	32.47	38.61
	A a	B b	A B	
	|_____|	|_____|	|_____|	
t value	t=9.20*	t=8.18*	t=2.55*	
	(P<0.05)	(P<0.05)	(P<0.05)	
Gill DNA				
Mean	7.37	8.86	6.55	8.86
	A a	B b	A B	
	|_____|	|_____|	|_____|	
t value	t=2.14[@]	t=4.19*	t=2.34*	
	(P>0.05)	(P<0.05)	(P<0.05)	

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

Conflict of Interests

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

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