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COMPARISON OF THE NON-SPECIFIC IMMUNE RESPONSE IN THE HEAD KIDNEY OF EDIBLE CARP AGAINST BACTERIAL INFECTION

Satyalatha B.D.J.* and Viveka Vardhani V.

*Dept. of Zoology, Government College for Women (A), Guntur (A.P.), India.
Dept. of Zoology and Aquaculture Acharya Nagarjuna University Nagarjunanagar - 522 510, A.P., India.

***Corresponding Author: Satyalatha B.D.J.**
Dept. of Zoology, Government College for Women (A), Guntur (A.P.), India.

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ABSTRACT

Introduction: Occurrence of diseases in edible carps is due to several factors like decreased resistance, poor water quality and bad managerial methods. The most significant factor effecting the health of fish is the incidence of bacterial pathologies in various organs, mainly the primary lymphoid organ, head kidney. *Aeromonas liquefaciens* (Bacterium) may behave as opportunistic pathogen by assailing already compromised or streseed paths. **Objectives:** With the above background, the present investigations were planned to assay protein and DNA profile and histological alterations in head kidney of *Labeo rohita*. **Material and Methods:** Four groups of experimental fish were infected with single doses (group A, 10^{-2} CFW/fish; group B, 10^{-4} CFU/fish; group C, 10^{-5} CFU/fish; group D, 10^{-6} CFU/fish) and two experimental group of fish were infected with multiple doses (4 days interval) (group E, 10^{-2}+10^{-2} CFU/fish; group F, 10^{-3} + 10^{+3} CFU/fish) of *A. liquefaciens* along with uninfected controls (groups, 66 fish). Six fish from a, b, c, d, e, f groups A, B, C, D and E, F and controls were necropsied on each day of eleven observations. Protein and DNA and histology were studied using standard methods. **Results and conclusion:** Protein and DNA level in head kidney of experimental fish (groups A, B, C and D) showed a significant increase and decrease in when compared with controls from hour 1 to 216 of infection. Protein level in head kidney showed a significant rise in experimental groups A and B when compared with controls; and in between the experimental groups A and B. Protein level in head kidney showed a significant rise in experimental groups E and F when compared with controls; and in between the experimental groups E and F. The DNA level in head kidney showed a significant decrease in experimental groups E and F when compared with controls and in between the experimental groups E and F. The statistical analysis showed a significant difference in the level of head kidney protein and DNA when comparison was made between singly infected groups B (10^{-4} CFU/fish) and D (10^{-6} CFU/fish) and repeatedly infected groups E (10^{-2} + 10^{-2} CFU/fish) and F (10^{-3} + 10^{-3} CFU/fish).The DNA level in head kidney showed a significant decrease in experimental groups A and B when compared with controls and in between the experimental groups A and B. The statistical analysis showed a significant difference in the level of head kidney protein and DNA when comparison was made between singly infected groups B (10^{-4} CFU/fish) and D (10^{-6} CFU/fish) and repeatedly infected groups E (10^{-2} + 10^{-2} CFU/fish) and F (10^{-3} + 10^{-3} CFU/fish).Infected carps showed lesions and necrosis in head kidney during experimentation. Present investigations indicate a high non-specific immune response in experimentally infected fish compared to controls.

KEYWORDS: Immune response, protein, DNA head kidney, histology, edible carp.

INTRODUCTION

Indian major carps are the most farmed fish in the country and its production is hampered by infectious microbial pathogens. *Aeromonas* species are causing infections in fish found in surface waters, estuarine water, fresh water, food products, sewage, diseased or health fish and in humans.[1-3] *A. liquefacines* as a food-borne pathogen, may cause zoonatic diseases and pathologic reaction in host tissues.[4-5] *Aeromonas* sps. cause degenerative histopathological changes in kidney, liver, gills, stomach and spleen of fish.[6] The carp immune system has several immune mechanisms responsible to defend against pathogenic bacteria through non-specific and specific (humoural or cell mediated) immune response. Disease resistance in fish is offered by natural (non-specific) and specific immune parameters.[7-9] Fish exposed to bacterial pathogens showed abnormal changes in biochemical profile, immune parameters and organ histology.[10-12] Head kidney, the primary lymphoid organ in fish produce and mature stem cells. The head kidney is characterized by the presence of lymphoid follicles and vessels. Morphological/pathological changes in the lymphoid organ associated with immune response. Lymphocytes

are the key effector cells of adaptive immunity and activated due to the exposure of self and non-self antigens. Overall, there is a paucity of data on the function of head kidney and lymphocytes in the present experimental model, *Labeo rohita.*

AIMS AND OBJECTIVES

With the above background, the present findings have planned to:

1. Estimate the protein and DNA content in head kidney of fish exposed to single and multiple doses of infection and uninfected controls.
2. Study the histopathological reactions in head kidney of infected and control fish.

MATERIALS AND METHODS

Fresh water fish, *L. rohita* were procured from a local fish farm, Nandivelugu (Guntur District), A.P., India. Fish were acclimatized in laboratory, fed with pellet feed and divided into 6 experimental (infected) (A, B, C, D, E, F; 66 fish in each group) and 6 control a,b,c,d,e, f groups.

The bacterial infection provided from Chandigarh (MTCC No. 2654) and pure culture of bacteria prepared by streak plate method. Four groups (A, B, C, D) of fish received infection (intramuscularly below the region of dorsal fin) as a single dose @ 10^{-2} CFU/fish (Group A), 10^{-4} CFU/fish (Group B), 10^{-5} CFU/fish (Group C) and 10^{-6} CFU/fish (Group D); 2 groups received infection via similar route as a repeated dose at 4 days interval @ 10^{-2} + 10^{-2} CFU/fish (Group E) and 10^{-3} + 10^{-3} CFU/fish (Group F) . 6 fish from the experimental (A, B, C, D) and control (a, b, c, d) groups were necroposied at hour 1, 3, 6, 12, 18, 24, 36, 48, 72, 96 and 216 of experimental period. 6 fish from experimental groups E, F and Control groups e, f were also mecropsied on the same designated hours (after infection). Protein and DNA were estimated from head kidney following[13] and Diphenyl amine method. For the histopathological studies, tissue specimens of head kidney were excised, embedded in paraffin and sectioned at 5 µ and tissue sections were stained with H and E method.

RESULTS AND DISCUSSION

Protein Activity In Head Kidney (Table 1)

Fish of group A (treated @10^{-2} CFU/fish) showe a decrease of protein from hour 1 to 216 when compared to controls (below normal level) There was a gradual decrease from hour 1 (62.41 mg/ml) 18 (57.58 mg/ml) and a slight increase (below normal level) from hour 18-216. In fish of group B, protein level decreased to below normal level on hour1(60.34 mg/ml) 3(61.72 mg/ml) and 6 (62.41 mg/ml) in comparison with controls (63.80 mg/ml). In group C protein level is slightly higher than control on hour 1 and a slight decrease from hour 1 to 216 (below normal levels). Higher level of protein was found on hour 1, 3, 6, 12 and 18; with highest increase on hour 6 (66.71 mg/ml). Decreased level of protein was found from 1 to 216 in groups E and F (below normal values) during the period of experimentation when compared with controls. There was a marked decrease of protein and DNA content the head kidney of both the experimental groups (A and B) from hour 1 to 216 of refection (except the slight increase of DNA on hour 1 and 6 in group B)

Dna Activity In Head Kidney (Table 1)

In case of groups A, B and C, there was decrease in DNA content from hour 1 to 216 of infection period in comparison with controls; all these values are found as below normals. In group D (10^{-6} CFU/fish), there was a marked increase of DNA from hour 1 to 72 and a decrease from hour 96 and 216 when compared with controls. In case of group E which received infection (10^{-2} CFU/fish $+10^{-2}$), the level of DNA was lower than controls on hour 1, 3 and 6,12,18,24,36,48,72,96 and 216; it was below normal throughout the infection period. Fish of group F showed a higher level of DNA on hour 1 of infection (12.22 mg/ml) from hour 1 to 6 (15.55 mg/ml), there is a gradual increase and all these increased values are higher than that of control values 11.11 mg/ml), a peak increase on hour 18 of reflection. The DNA level decreased to normal level from the hour 18 onwards till the day 9 of experimental period, the gradual decrease of DNA was (lower than that of control value.

In group A, there was a decrease in DNA content from hour 1 to 216, and these values are found to be lower than that of values of uninfected with C group (except the normal level on hour 48). Higher level of DNA was found in group B on hour 1 of infection, there is a decrease in DNA content (10.06 mg/ml)(below normal values). Group C showed a higher amount of DNA on hour 1(13.33 mg/ml) and from hour 1 to 12 of experimental period, there was a gradual decline of DNA. There was a significant decrease of protein in groups A and C when compared with controls (Table-1). Differences in the level of protein were statistically non-significant in the groups C and D when compared with controls and in between groups A and B, A and C and B and C; groups A, B and C showed significant difference when compared with group D. In comparision with controls. There was a significant increase of DNA in group D and significant decrease in groups A and B. There was no significant difference when compared in between groups C and controls, and A and C. There was a significant difference in group B when compared with groups C, D and A.

Table 1: Content of protein (mg/ml) and DNA (mg/ml) in the head kidney of experimental fish (group A, B, C, D, E and F) treated with 10^{2} CFU/fish, 10^{4} CFU/fish, 10^{5} CFU/fish, 10^{6} CFU/fish, 10^{2} CFU/fish + 10^{7} CFU/fish and 10^{3} CFU/fish + 10^{3} *Aeromonas liquifaciens* at different periods of infection and control (group G). Values are expressed in mean derived from five observations.

Hours of Necropsy	Experimental groups												Control Group	
	Group-A		Group-B		Group-C		Group-D		Group-E		Group-F		Group-G	
	Protein	DNA	Protein	DNA	Protein	DNA	Protein	DNA	Protein	DNA	Protein	DNA	Proteir	DNA
1	62.41	10.0	60.34	13.33	64.13	13.33	65.86	18.88	58.96	10.0	52.75	12.22	63.79	11.11
3	61.72	9.0	61.72	10.06	61.72	10.0	65.17	16.66	58.27	7.77	50.68	13.33	63.79	11.12
6	58.27	7.77	62.41	8.88	61.72	11.11	66.79	15.55	55.51	7.77	48.62	15.55	63.77	11.11
12	57.93	8.88	63.10	7.77	60.34	8.88	65.51	14.44	54.82	6.66	47.93	11.11	63.77	11.09
18	57.58	10.0	63.79	7.78	60.96	14.44	64.13	12.22	52.06	5.55	45.17	10.0	63.78	11.11
24	60.34	6.66	62.41	7.77	61.37	10.0	63.79	16.66	51.37	4.44	56.89	8.88	63.78	11.12
36	61.03	8.88	61.72	7.76	61.06	10.0	62.75	16.66	51.03	1.11	57.58	7.77	63.78	11.13
48	61.72	11.11	62.06	7.77	61.72	7.77	62.06	15.55	51.37	5.55	57.93	6.66	63.78	11.09
72	62.41	10.0	62.41	6.66	60.72	5.55	63.79	14.44	56.89	4.44	55.86	5.55	63.79	11.11
96	60.34	6.66	62.06	5.55	60.68	4.44	62.41	10.06	58.62	5.55	57.58	5.54	63.78	11.10
216	59.65	4.44	63.10	2.22	60.34	4.44	63.79	9.99	58.62	4.44	58.27	5.55	63.77	11.11

Table 2: 't' values obtained for different groups of fish infected with 10^{-2} (group A), 10^{-4} (group B), 10^{-5} (group C) and 10^{-6} (group D) CFU/fish

	Experimental (A, B, C and D) and Control (a, b, c and d) groups							
	A	a	B	b	C	c	D	d
Head Kidney Protein								
Mean	60.30	63.76	62.28	63.78	61.34	63.78	64.19	63.78
	A	a	B	b	C	c	D	d
t value	t=6.55* (P<0.05)		t=2.29@ (P>0.05)		t=7.65* (P<0.05)		t=0.88@ (P>0.05)	
	A B: t=2.29@ (P>0.05)		A C: t=1.67@ (P>0.05)		A D: t=5.56* (P<0.05)			
	B C: t=2.24@ (P>0.05)		B D: t=3.60* (P<0.05)		C D: t=5.13* (P<0.05)			
Head Kidney DNA								
Mean	8.49	11.1	7.78	11.01	9.08	11.01	14.64	11.1
	A	a	B	b	C	c	D	d
t value	t=4.45* (P<0.05)		t=4.04* (P<0.05)		t=2.01@ (P>0.05)		t=4.15* (P<0.05)	
	A B: t=2.32* (P<0.05)		A C: t=0.51@ (P>0.05)		A D: t=5.94* (P<0.05)			
	B C: t=2.41* (P<0.05)		B D: t=5.79* (P<0.05)		C D: t=4.22* (P<0.05)			

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

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

Experimental (E and F) and Control (e and f) groups				
	E	e	F	f
Head Kidney Protein Mean	55.22	63.78	53.56	63.78
	E e	F f	E F	
t value	t=8.68* (P<0.05)	t=7.13* (P<0.05)	t=4.19* (P<0.05)	
Head Kidney DNA Mean	5.75	11.1	9.39	11.1
	E e	F f	E F	
t value	t=7.64* (P<0.05)	t=2.75* (P<0.05)	t=3.05* (P<0.05)	
	t values obtained for groups B (10^{-4} CFU/fish) and E and for groups D (10^{-6} CFU/fish) and F			
	B E D F			
Head Kidney Protein Mean	62.28	55.22	63.91	53.56
	B E	D F		
t value	t=6.91* (P<0.05)	t=7.07* (P<0.05)		
Head Kidney DNA Mean	7.78	5.75	14.64	9.39
	B E	D F		
t value	t=2.33* (P<0.05)	t=3.98* (P<0.05)		

P value at 5% level of significance is 2.306

*Statistically significant values @Statistically non-significant values

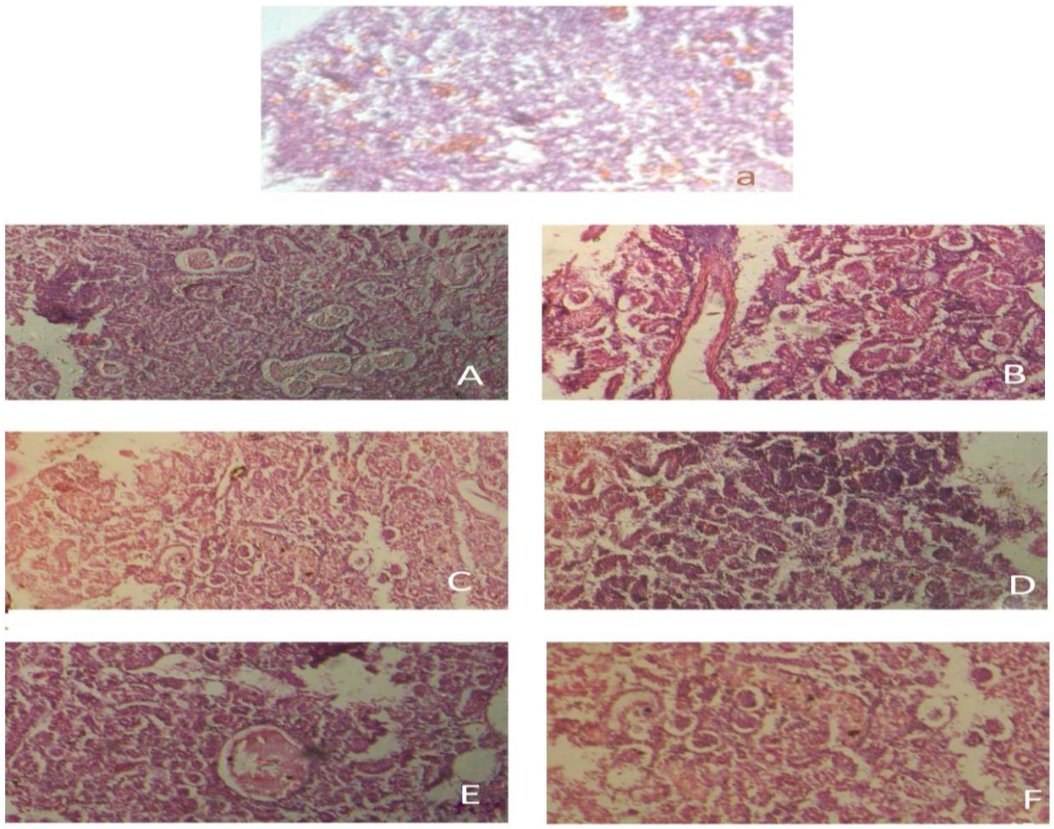
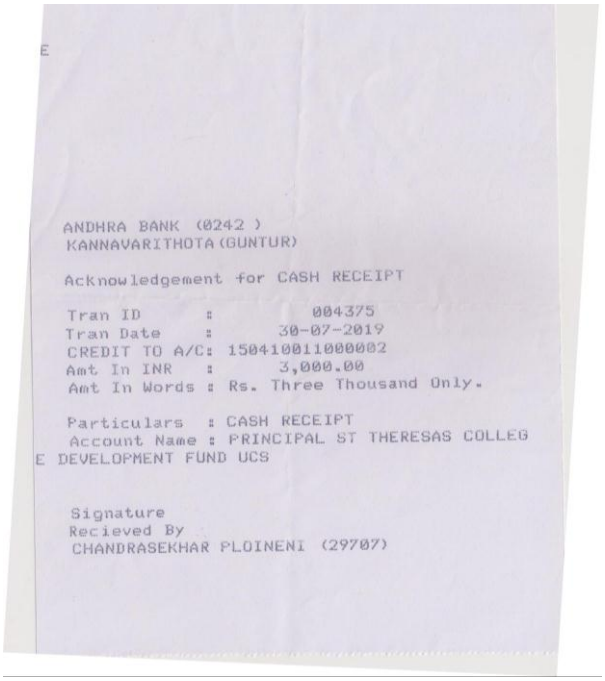


Figure showing head kidney from control (a) and infected (A,B,C,D,E andF) fish



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HISTOPATHOLOGY

The multiple sections of normal head kidney showed normal parenchyma and interstitial tissue without any proliferation. The renal corpuscle is composed of glomerulas and its capsule showed intact texture. In group A and B, at day 9 post infection, the head kidney showed congestion of blood capillaries and glomerular tufts with heavy necrotic tissue. The hematopoietic tissue in the kidney was disrupted and the glomeruli were enlarged. Heavy infiltration of lymphocytes and macrophages were observed. Accumulation of pus and severe inflammation of sinusoids and serous membrane were observed(Fig.1 A). The multiple sections of the head kidney study in group B revealed heavy necrotic tissue. The hematopoietic tissue in the in the kidney was disrupted by the heavy infestation of bacteria. Glomeruli were enlarged and showed cell proliferation. Accumulation of pus and severe inflammation of sinusoids and serous membrane were observed. The glomeruli showed nodular out growths due to serious bacterial infestation (Fig.1B). In group C, head kidney showed severe necrosis, congestion of the renal capillaries and several accumulation of granular eosinophilic cells. Accumulation of pus, severe inflammation of sinusoids and serous membrane were observed. Hematopoietic tissue was severely disrupted. Glomeruli were enlarged (Fig.1 C). The multiple sections of the study revealed heavy necrosis and severe blood clots, heavy inflammation and enlargement of glomeruli in fish of group D. Accumulation of pus ans severe inflammation of sinusoids and serous membrane were observed. Severe infiltration of lymphocytes and neutrophils was observed (Fig.1D). Pathogenic bacterium (via intramuscular route) caused infiltration of lymphocytes, atrophy and necrosis throughout the infection period (from hour 1 to 216). In group E which received 10^{-2} + 10^{-2} CFU/fish, there was heavy destruction and damage of entire tissue due to bacterial invasion. Inflammatory reaction was evident by moderate increase of macrophages, lymphocytes and eosinophils and highly congested blood vessels. Glomeruli sparseley congested (Fig 1E). Heavy necrotic tissue and severe blood clots were seen in fish which received double dose of infection (10^{-3}+10^{-3} CFU/fish). Serous membranes of renal corpusles showed vacular degeneration and proliferative inflammation. The hematopoietic tissue was disrupted by the heavy infestation of bacteria. Glomeruli were enlarged with heavy cellular proliferation (Fig. 1 F). Renal capillaries showed congestion, proliferation and hyperplasia. Accumulation of pus and severe inflammation of sinusoids and serous membrane were observed. The glomeruli showed modular out growths due to severe bacterial infestation.

The virulent infectious *A. liquefaciens* might have caused abnormality in the level of protein and DNA in head kidney at different hours of experimental period in the present study.[13,14] also suggested that the infectious pathogenic bacteria may produce ill effects thereby disturbing the physiological mechanism of fish.[15,17] also reported that fish exposed to toxicants show impaired protein metabolism. The reduced level of protein at some hours of infection in the infected fish confirm that of[17] who reported that the lowered level of protein was due to reduction in the synthesis of proteins.

Fish suffering due to aeromoniasis (groups A,B,C and D) showed atrophy and necrosis in renal haemopiotic tissues as explored by[18] in Eel *(Anguilla japonica)* infected by aeromonads species. 19 also explained that head kidney is one of the target organ influenced by *A. hydrophila* and both acute and severe infection cause damage to the head kidney. The changes like necrotic lesion and aggregation of melanin containing macrophages in the head kidney of infected fish (during the entire experimental period) indicate the pathogenic effect of microbial organism in the head kidney; this is an indication of stressful condition of infected fish. These results compare well with that of[19] who also reported necrotic lesions and aggregation of melanin filled macrophages in kidney and spleen of fingerlings of *L.rohita* infected with *A. hydrophilla*.

These results confirm the observations of 14 who also observed biochemical alterations in liver and muscle tissue in fish, *Clarius batracus* during insecticide treatment. The alteration of tissue protein level in head kidney might be due to the synthesis of stress proteins as reported by.[20] Various authors[21,22 and 23] reported synthesis of stress proteins due to heavy metal treatment. In the present study, pathogenic aeromonads was pound as effective inducer of stress protein in altering the tissue protein fractions of experimental fish (exposed to various doses of *A. liquefactions*). The histopathological changes in head kidney explain the involvement of stress in aeromoniasis, the change like necrotic lesions and

aggregations of melanin containing macrophages were observed in the present study. The pathological changes brought out in fish by pathogenic bacteria strongly indicate that these lymphoid organs could provide sensitive indicator of stressful conditions in the aquatic environment. Fingerlings of *L.rohita* infected with *A. hydrophila* also indicated necrotic lesions and aggregation of melanine filled macrophages in kidney and spleen.[20]

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