

Original Research Article

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Haematological Responses in Rohu *Labeo rohita* (Hamilton) after Infecting with *Aeromonas hydrophila* in Biofloc Systems

P. Anand Prasad^{1*}, H. Shivanandamuthy², D. Ravindra Kumar Reddy¹,
M. Ganapathy Naik², Gangadhara Gowda⁵, K. Mansingh Naik²,
A. Chandra Sekhara Rao¹ and T.V. Ramana¹

¹College of Fishery Science Muthukur, Nellore, Andhra Pradesh India

²College of Fisheries Mangalore, Karnataka, India

*Corresponding author

ABSTRACT

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Rohu fishes grown with biofloc for 120 days have been examined for the histopathological changes occurred in the intestine and kidney after infecting with *Aeromonas hydrophila* with 0.5 ml, 1.0 ml and 1.5 ml where 1.5×10^5 bacteria per ml. In the present study haematological alterations were documented after *Aeromonas hydrophila* infected rohu (*Labeo rohita*) fingerlings, fed with probiotic supplemented diets with rice bran and ground nut oil cake in biofloc with added ammonium chloride to reduce NH_3 . Blood based parameters like RBC, Hb, WBC, Neutrophils, lymphocytes, monocytes, MCV, MCH, MCHC, PCV etc. were analyzed which revealed a significant alteration after infecting with *Aeromonas hydrophila*.

Introduction

Aquaculture intensification is one of the prime requirements to cope with the present demand for fish protein. Increase in productivity per unit space is performed by increasing the rearing density of fishes. Health management and biosecurity are major challenges in production intensification due to limited control over pathogens (Kautsky *et al.*, 2000).

Aquaculture is an important economic activity supplying high quality animal protein to

greater number of people worldwide, and the global growth of the industry remains relatively strong with an average annual growth rate of 8.6% (FAO, 2014). However, modern farming techniques and recent trend of intensification of aquaculture operation has brought in new challenges that threaten the health of farmed species, leading to growth reduction and immunosuppression, all culminating into massive financial loss and reduced profit margins for farmers (Smith *et al.*, 2003). Biofloc technology (BFT) based farming system has proved to be a limited

water exchange, biosecure system which can ensure sustainable feeding management and production intensification. The benefits include the uptake of ammonium by the microbial community by maintaining a high C/N ratio (Avnimelech *et al.*, 1994) and improve health management and bio security through zero-water exchange and possible probiotics effect (Crab *et al.*, 2010).

Several pathogenic organisms such as bacteria, fungi and viruses have been reported to be principal limiting factors to the growth of aquaculture industry. The most common and frequently encountered bacterial pathogen in tropical climates is *A. hydrophila*, which causes severe damage to carp production (Karunasagar *et al.*, 1991). It is a deadly pathogen causing stress-related diseases in fish with the common symptoms of ulceration, exophthalmia and abdominal distension (Amin *et al.*, 1985).

Materials and Methods

Rohu fish (30+30 numbers) cultured in biofloc for 120 days were collected and kept in separate aquaria tanks (6) 10 fishes in each aquarium for control and treatments. After acclimatizing in aquaria they have been infected with *Aeromonas hydrophila* with the concentrations of 0.5 ml, 1 ml and 1.5 ml and the bacterial count in each ml was 1.5×10^5 cells per ml of culture media.

Blood collection

At the end of the experiment, one fish collected from each treatment, thus a total three fishes have been used for blood collection. Blood was collected from the caudal vein by using No.24 gauge syringe, previously rinsed with 10% EDTA solution. Blood collected was transferred immediately to a heparinized tube and shaken gently to prevent haemolysis of blood cells. 50 μ l of

collected blood sample from each treatment and control was immediately analyzed for Red Blood Cell (RBC) count, White Blood Cell (WBC) count, platelet count, hemoglobin content (Hb), hematocrit value, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) (Rusia and Sood, 1992). The hematological parameters were analysed by using the hematology analyzer, (Pentagan Medical Instrument, PHA-23PLUS, China)

Results and Discussion

Haematology

Results found after haematology are given in detail in Table 1. Hb levels in the infected fishes in the present study differed from 9.8, 8.6, 6.3 g/dL in 0.5, 1.0 and 1.5 ml doses respectively and in control it was 10.1 g/dL. Tivary and Pandey (2014) got 9.21 in healthy rohu, post infection 6.73, and chronic infection 153.6 g/de. RBC was 2.11, 1.71, and 1.49 mil/cubmm in control, post infection and chronic infection was observed and in the present experiment 2.38, 1.82, 1.54, and 1.47 mil/cubmm. WBC 31.4, 19.82, and 16.89 $10^3/\mu$ l present study shows 1.295, 1.345, 1.39 and 1.508 lakh/cubmm. Lymphocytes 22.6, 8.87 and 6.11 $10^3/\mu$ l this experiment shows 96, 98, 102, and 108 alternatively in control, 0.5 ml, 1.0 ml and 1.5ml doses. Monocytes 1.62, 2.83, 3.34 $10^3/\mu$ l in the present study 2, 2.1, 2.2, 2.4 alternatively. MCV 185.6, 169.3 and 153.6 in their result and present study shows 141, 111, 118 and 124 fl. MCHC 23.56, 24.12 and 21.64 has been resulted in their experiment and in the present study 30.1, 42.2, 39.2 and 34.6 gm/L was observed. The WBC count indicates a lenkopenia with lymphopenia, mild neutrophils and monocytosis. Haematological changes were more pronounced in fish with extensive skin ulcers.

Table.1 Dosages of *Aeromonas hydrophila* and blood cells comparison with control

	0.5 ml	1.0 ml	1.5 ml	Control
Hb g/dL	9.8±0.2	8.6±0.18	6.3±1.2	10.1±0.6
RBC mil/cumm	1.82±0.6	1.54±0.3	1.47±0.2	2.38±0.4
WBC /cumm	1,34,500±1000	1,39,000±800	1,50,800±900	1,29,500±700
Differential Count in %				
Neutrophils	0.9±0.1	0.8±0.1	0.5±0.01	1±0.2
Lymphocytes	98±6	102±8	106±4	96±5
Eosiniphils	0.4±0.1	0.2±.02	0	1±0.2
Monocytes	2.1±0.1	2.2±0.1	2.4±0.2	2±0.3
Basophils	0.2±0.01	0.1±0.01	0	0
Platelet Count cumm	53,500±300	78,200±600	1,02,000±400	3,27,000±600
Hematocrit/Pcked Cell Volume Vol%	10.3±0.9	14.8±2.4	18.2±3.6	33.6±6.4
MCV fl	111±10	118±18	124±14	141±18
MCH pg	44.2±9	43.6±6	43.0±7	42.4±4
MCHC gm/L	42.2±6	39.2±5	34.6±8	30.1±4
RDW %	11.3±2.1	16.7±2.6	20.2±3.1	28.0±2.8
MPV	5.4±1.2	6.2±0.9	6.7±1.8	6.9±0.8

Leukopenia was lymphocyte as predominant circulating leukocyte (Latimer *et al.*, 2003). The exact mechanism of lymphopenia is not clearly known but it may be redistribution of lymphocytes induced by corticosteroids as occur in mammals.

The mild neutrophilia and monocytosis probably occurs in response to tissue demand for these cells can also be observed in histological sections. Leukopenia with lymphopenia, neutrophilia and occasional monocytosis is frequently observed in viral and other gram negative bacterial diseases of fish (Noga, 2000). Hemoglobin and RBC increased and neutrophils have been decreased in the all treatments compared to control and WBC and lymphocytes, monocytes count has been increased when compared with control and the same has been shown in the results of Dharmakar (2017).

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