Effect of feed deprivation on immune-haematological responses and resistance of *Labeo rohita* (Hamilton, 1822) to *Aeromonas hydrophila* infection

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Abstract

The present study assessed the effects of feed deprivation on immune-haematological responses and resistance of rohu (*Labeo rohita*) to *Aeromonas hydrophila* infection. Fingerlings of rohu were subjected to the three feeding regimes viz. 'no feeding' (NF), 'daily feeding' (DF) and 'alternate day feeding' (ADF) for 21 days and infected with *A. hydrophila* by immersion method. The mortality and immune-haematological responses were measured after 1 and 21 days from the start of the experiment. The result showed significantly lower mortality in the NF group followed by the ADF group, compared to the DF group. The haemoglobin content, packed cell volume, total erythrocytes, and leucocytes count were decreased significantly ($p<0.05$) in the NF group after 21 days of the experiment. With regards to the immunological responses, the myeloperoxidase activity and total immunoglobulin levels were decreased significantly ($p<0.05$) in the NF and ADF groups after 21 days of infection. The anti-protease activity of rohu serum did not differ significantly ($p>0.05$) after 21 days. On the contrary, superoxide anion production was significantly enhanced ($p<0.05$) in all the treatment groups after 21 days of infection. The results collectively suggest the adoption of short-term 'no feeding' strategy to combat bacterial disease outbreak in semi-intensive fish farming.

Key words: Bacterial disease, Feeding strategy, Immunity, Mortality, Rohu

Highlights

- 'No feeding', 'daily feeding' and 'alternate day feeding' regimes were used for rohu.
- Infection with *A. hydrophila* by immersion method was followed.
- Immune-haematological responses were suppressed in 'no feeding' group.
- Significantly lower mortality was observed in the 'no feeding' group.

INTRODUCTION

There has been a transition from the conventional aquaculture practices to semi-intensive and intensive systems to meet the growing demand of the fish, shellfish, and aquatic food products. The recent trend of intensification in the aquaculture practices has led to the frequent occurrence of diseases which has now considered as a major problem in the growth of the aquaculture industry (Bondad-Reantaso *et al.*, 2005; Khan *et al.*, 2020). In aquaculture, the single largest cause of economic losses is considered to be the disease. To protect an organism against infection and to minimize the fitness costs of being infected

is one of the important priorities for successful aquaculture. For preventing diseases in the culture system, farmers use different kinds of drugs and antibiotics (Huang *et al.*, 2015). However, there is a growing concern regarding the use of drugs and antibiotics in the aquaculture sector owing to the emergence of drug and antibiotic resistant bacteria and other environmental problems (Ai *et al.*, 2011; Choudhury and Kamilya, 2019). Therefore, different alternative strategies need to be explored to minimize the usage of drugs and chemical and to grow fish in an eco-friendly environment.

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One of the management strategies to combat or minimize fish loss during the disease outbreak is feed deprivation (Wise and Johnson, 1998; Mohapatra *et al.*, 2015). Starvation under different food restriction regimens has been found to be successful in minimizing mortality of channel catfish (*Ictalurus punctatus*) after natural exposure to *Edwardsiella ictaluri* (Wise and Johnson, 1998). In another study, fasted groups of Atlantic salmon (*Salmo salar*) exhibited only 2% mortality, while fully fed groups had 36% mortality after infection with *Vibrio salmonicida* (Damsgard *et al.*, 1998). Channel catfish fed once every other day to apparent satiation exhibited improved resistance to *Flavobacterium columnare* (Shoemaker *et al.*, 2003). On the contrary, juvenile channel catfish receiving various feeding regimens for four weeks in aquaria were more susceptible to *E. ictaluri* challenge (Lim and Klesius, 2003). Moreover, significantly decreased resistance to *F. columnare* was observed in channel catfish under feed deprivation condition (Klesius *et al.*, 1999). Thus, these findings indicate disease protecting ability of feed deprivation strategy in many instances.

Even though several studies were conducted regarding the effect of starvation on disease resistance as well as on different physiological responses of fish, relatively little research has been conducted on the effect of feeding restrictions on immunological and haematological responses. Moreover, the effects of feed deprivation on immune-haematological responses of fish, infected with pathogenic microorganisms, have not been adequately addressed. Thus, the present study was undertaken to delineate the effects of feed deprivation on immune-haematological responses and resistance of one of the commercially important Indian major carp species, rohu (*Labeo rohita*) to *Aeromonas hydrophila*.

MATERIALS AND METHODS

Collection and maintenance of experimental fish: Fingerlings of rohu (10.38 ± 1.02 cm; 14.8 ± 3.68 g, mean $\pm$ SEM) were collected from a local fish market in Agartala, Tripura, India. Acclimatization of fish was done in 200-L circular

plastic tanks for 15 days before the start of the experiments. Adequate aeration was provided, and floating pelleted feed was fed to the fish twice a day. The physicochemical parameters of tank water were maintained throughout the acclimatization period. Water exchange (20–25%) was done daily to remove the waste feed and faecal matters.

Experimental design: The experiment was conducted in 50 L capacity aquaria. There were three experimental groups with three replicates each. Well acclimatized rohu fingerlings were randomly distributed in nine aquaria (10 fish per aquarium) and reared with three feeding regimes. The first group was not fed throughout the experimental period ['no feeding' (NF) group]; the second group was fed normal diet once daily to apparent satiation ['daily feeding' (DF) group], and the third group was fed with the diet with one day interval to apparent satiation ['alternate day feeding' (ADF) group]. Young culture of *A. hydrophila* (10^9 cells/mL) was added in each aquarium to mimic the natural exposure of bacteria to the host fish to induce aeromoniasis. Adequate aeration was provided, and siphoning of waste materials and water exchange was done once a week. The experiment was conducted for 21 days, and sampling of fish was done after 1 and 21 days from the start of the experiment.

Collection of blood and serum: Each fish was anesthetized with clove oil (HiMedia, India) at the rate of 50 μ L/L of water before collecting blood samples (Devi and Kamilya, 2019). The blood was collected from randomly selected two fish from each aquarium and drawn from caudal vein using 1.0 mL hypo-dermal syringe and 24-gauge needles. The collected blood was immediately transferred into vials without anticoagulant (EDTA) for serum preparation. Aliquots of blood were kept with EDTA. The coagulated blood was centrifuged at $1500 \times g$ for 10 min at 4°C to collect sera, which was then stored at -20°C in the sterilized vial until further use.

Estimation of mortality: Mortalities were recorded daily for 21 days. Dead fish were

removed from the aquarium. The fish were also checked for clinical and gross signs.

Measurement of haematological and immunological parameters: Haematological parameters, including haemoglobin content (Hb), packed cell volume (PCV), total erythrocyte count (RBC) and total leucocyte count (WBC) were estimated following the standard methods. Among the immunological parameters, superoxide anion production was measured using a nitroblue tetrazolium reduction (NBT) assay (Anderson and Siwicki, 1995), total anti-protease activity by trypsin inhibition assay (Devi and Kamilya, 2019), myeloperoxidase activity was measured as described in Kaur *et al.* (2018) and total immunoglobulin (Ig) level was also estimated following the method described by Anderson and Siwicki (1995).

Statistical analysis: Statistical analysis of the data was performed using SPSS-16.0 (SPSS Inc., Chicago IL, USA) software. Results are

presented as mean $\pm$ standard error of mean (SEM). Comparisons of the mean values were determined by Student's *t*-test. A probability level of 0.05 was used to find out the significance in all cases.

RESULTS

Mortality: Mortality was observed during the first five days, from the start of the experiment. The mortality was 3.33%, 23.33%, and 10% in NF, DF, and ADF group, respectively. The infected fish showed signs of haemorrhagic septicemia in the abdominal cavity. Petechial haemorrhages over the body surface and sloughing of scales were observed. The moribund fish became lethargic, anorexic, and laid down at the bottom before death.

Haematological responses: The result of the haematological parameters, including the Hb, PCV, RBC, and WBC count during different feed restriction condition after *A. hydrophila* infection are presented in Fig. 1. The Hb, PCV,

[Bars (mean $\pm$ 1 SEM) with different letters are significantly different ($p < 0.05$)]

Fig. 1. Haematological responses, including haemoglobin (Hb) content, packed cell volume (PCV), total erythrocyte (RBC), and total leucocyte (WBC) counts of *Labeo rohita* after 1 and 21 days of *Aeromonas hydrophila* infection

[Bars (mean ± 1 SEM) with different letters are significantly different (p < 0.05)]

Fig. 2. Immunological responses, including superoxide anion production (NBT), total immunoglobulin (Ig), anti-protease, and myeloperoxidase activity of *Labeo rohita* after 1 and 21 days of *Aeromonas hydrophila* infection

RBC, and WBC count decreased significantly ($p < 0.05$) after 21 days of the experimental period in the NF group. Only the Hb content was significantly decreased ($p < 0.05$) in the DF and ADF groups after 21 days. The other haematological parameters did not alter in the DF and ADF groups.

Immunological responses: The results of the immunological response are presented in Fig. 2. The NBT activity was significantly enhanced ($p < 0.05$) in all treatment groups after 21 days of infection. Total Ig level was decreased significantly ($p < 0.05$) in the NF and ADF groups after 21 days of infection. The anti-protease activity of rohu serum did not differ

significantly ($p > 0.05$) in any of the treatment groups after 21 days. The myeloperoxidase activity of rohu serum decreased significantly ($p < 0.05$) in the NF and ADF groups after 21 days of infection. However, there was no significant alteration in the total Ig, anti-protease, and myeloperoxidase activity in the DF group.

DISCUSSION

Starvation during an immune-compromised situation has been known to be an effective management strategy in curbing the infection-related mortality in numerous organisms. Upon *A. hydrophila* infection, the starved fish exhibited enhanced survivability in comparison

to their fed counterparts. Significant alterations were also observed in the haematological and immunological parameters in the starved fish. Thus, the present study highlights the importance of short-term starvation in helping the fish to curb disease related mortality.

After inducing the fish in all the experimental groups with *A. hydrophila*, significant mortality was observed in the daily fed group (23.33%), and the lowest mortality was observed in the non-fed group (3.33%). The relationship between feed consumption and increased mortality is assumed to be related to the ingestion of bacteria present in the water at the time of feeding (Wise *et al.*, 2008). Starvation profoundly benefits the host on nutrient and energy metabolism in liver which leads to enhanced protection against pathogenic invasions (Restellini *et al.*, 2013; Kheloufi *et al.*, 2014). Moreover, alteration in transcription of various iron homeostasis related genes, and lysosomal and non-lysosomal enzymes in starved host have also been suggested to facilitating the suppression of microbial proliferation (Restellini *et al.*, 2013; Lin *et al.*, 2014; Vecchi *et al.*, 2014). Effects of fasting on the survivability of fish during bacterial infection have also been tested in other fish species. Withholding of feed during outbreaks of diseases has been shown to reduce mortality in certain instances. For example, only 2% mortality was observed in feed deprived Atlantic salmon, while 36% mortality was observed in fully fed fish after infection with *V. salmonicida* (Damsgard *et al.*, 1998). About 87% survival was observed in fasted channel catfish, while survival of their fed counterparts was 59% during an *E. ictaluri* epizootic (Wise and Johnson, 1998). On the other hand, Lim and Klesius (2003) observed more susceptibility of juvenile channel catfish to *E. ictaluri* challenge receiving various feeding regimens for four weeks in aquaria. Similarly, resistance to *F. columnare* was significantly decreased in channel catfish under feed deprivation for as little as seven days (Klesius *et al.*, 1999), while Pirhonen *et al.* (2000)

reported that survival in chinook salmon (*O. tshawytscha*) naturally infected with *Renibacterium salmoninarum* was indifferent to ration level.

Haematological parameters are good indicators of the physiological status of animals and widely used to measure an index of health status in a number of fish species (Khan and Zafar, 2005; Kader *et al.*, 2010). The haematological parameters, including Hb, PCV, RBC, and WBC count decreased significantly ($p < 0.05$) after 21 days of the experimental period in the NF group. During feed deprivation period, the decrease in the level of Hb in starved fish might be due to non-availability of essential nutrients in the body. Similar to the present study, the RBC and PCV decreased significantly after seven and nine days of starvation in red paradise (*Macropodus opercularis*) and kissing gourami (*Helostoma temminckii*) (Weinberg *et al.*, 1973; Weinberg *et al.*, 1976). Moreover, Mohapatra *et al.* (2016) observed increased Hb content in starved and *E. tarda* infected red sea bream (*Pagrus major*). Fish fed to satiation once every other day also had significantly lower RBC, although the values for WBC, PCV, and Hb did not differ from those of fish fed daily (Lim and Klesius, 2003). However, a short-term fasting of 14 days without infection did not alter haematological parameters in red porgy (*Pagrus pagrus*) (Caruso *et al.*, 2012).

The NBT activity was significantly enhanced in all the treatment groups after 21 days of infection. As NBT assay quantifies the phagocytic activity of granulocytes (Mukherjee *et al.*, 2019), the result indicated the enhanced response of this parameter possibly due to the entry of the pathogen into the fish body, particularly in the DF group. However, it was surprising to observe an enhanced response in the food-starved fish. Reduction in NBT activity was observed in starved red sea bream infected with *E. tarda* (Mohapatra *et al.*, 2016). It is well known that specific immunoglobulin production is one of the major defense mechanisms after invasion by the pathogenic

microorganism. Total immunoglobulin level decreased significantly in the NF and ADF groups after 21 days of infection. The lower immunoglobulin production could be due to entry of low quantity of bacteria into the fish body due to no or less feed consumption. There is no other study to corroborate the present result on immunoglobulin production following feed deprivation and infection in fish. Production of anti-proteases by the host is one of the important aspects of host defence system to counterbalance infection or inflammation. There was no significant alteration in the anti-protease activity in any of the treatment groups after 21 days of infection. To the best of our knowledge, there is no other study to corroborate the present result on anti-protease activity following feed deprivation and infection in fish. However, in one study, Eslamloo *et al.* (2017) did not find any significant difference in the anti-protease activity of tinfoil barb (*Barbonymus schwanenfeldii*) starved for two weeks without any infection. Myeloperoxidase enzyme is released by the neutrophils, that helps in the production of hypochlorous acid to kill pathogens (Dalmo *et al.*, 1997). The myeloperoxidase activity decreased significantly in the NF and ADF groups after 21 days of infection. The lower myeloperoxidase content could be due to the low magnitude of infection in the fish body due to no or less feed consumption.

REFERENCE

- Ai Q, Xu H, Mai K, Xu W, Wang J *et al.*, 2011. Effects of dietary supplementation of *Bacillus subtilis* and fructooligosaccharide on growth performance, survival, non-specific immune response and disease resistance of juvenile large yellow croaker *Larimichthys crocea*. *Aquaculture*, 317(1-4): 155-161, doi: 10.1016/j.aquaculture.2011.04.036
- Anderson DP and Siwicki AK, 1995. Basic Haematology and serology for fish health programs. In: Diseases in Asian Aquaculture II (Shariff M, Arthur JR, Subasinghe RP, eds). Fish Health Section, Asian Fisheries Society, Manila, Philippines, pp 185-202
- Bondad-Reantaso MG, Subasinghe RP, Arthur JR, Ogawa K, Chinabut S *et al.*, 2005. Disease and health management in Asian aquaculture. *Vet Parasitol*, 132(3-4): 249-272, doi: 10.1016/j.vetpar.2005.07.005
- Caruso G, Denaro MG, Caruso R, Genovese L, Mancari F *et al.*, 2012. Short fasting and refeeding in red porgy (*Pagrus pagrus*, Linnaeus 1758): response of some haematological, biochemical and non specific immune parameters. *Mar Environ Res*, 81: 18-25, doi: 10.1016/j.marenvres.2012.07.003
- Choudhury TG and Kamilya D, 2019. Paraprobiotics: An aquaculture perspective. *Rev Aquac*, 11(4): 1258-1270, doi: 10.1111/raq.12290
- Dalmo RA, Ingebrigtsen K and Bøgvold J, 1997. Non-specific defence mechanism in fish, with particular reference to the reticuloendothelial system (RES). *J Fish Dis*, 20(4): 241-273, doi:

The results collectively suggest the adoption of 'no feeding' strategy on a short-term basis to combat bacterial disease outbreak in the semi-intensive fish farming system. The lower immune-haematological responses of the feed restricted fish might be either due to the insufficient load of bacteria in fish body or diversification of energy resources towards maintaining essential metabolic functions. However, further research is required to fully understand the mechanism of enhanced survivability of fish during feed restriction condition.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: SI: Involved in formal analysis and data acquisition, original draft preparation; WMD: Involved in formal analysis and data acquisition, editing of draft; SJM: Engaged for data analysis, review and editing of draft; DK: Involved in conceptualization, supervision, review and editing of draft.

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- 10.1046/j.1365-2761.1997.00302.x
- Damsgard B, Mortensen A, Sommer A-I, Ugelstad I and Eliassen R, 1998. Appetite and health status in Atlantic salmon. The Feeding behaviour of fish in culture, Second Workshop of the COST 827 Action on Voluntary Food Intake in Fish, 20-22 August 1998, Umea, Sweden
- Devi AA and Kamilya D, 2019. Efficacy and effects of clove oil and MS-222 on the immune-biochemical responses of juvenile rohu *Labeo rohita*. Aquac Res, 50(3): 957-963, doi: 10.1111/are.13980
- Eslamloo K, Morshedi V, Azodi M and Akhavan SR, 2017. Effect of starvation on some immunological and biochemical parameters in tinfoil barb (*Barbonymus schwanenfeldii*). J Appl Anim Res, 45(1): 173-178, doi: 10.1080/09712119.2015.1124329
- Huang Y, Zhang L, Tiu L and Wang HH, 2015. Characterization of antibiotic resistance in commensal bacteria from an aquaculture ecosystem. Front Microbiol, 6: 914, doi: 10.3389/fmicb.2015.00914
- Kader MA, Koshio S, Ishikawa M, Yokoyama S and Bulbul M, 2010. Supplemental effects of some crude ingredients in improving nutritive values of low fishmeal diets for red sea bream, *Pagrus major*. Aquaculture, 308(3-4): 136-144, doi: 10.1016/j.aquaculture.2010.07.037
- Kaur N, Kumar R and Kamilya D, 2018. Modulation of systemic and mucosal immune responses of *Catla catla* (Hamilton, 1822) experimentally challenged with gill monogeneans. Fish Shellfish Immunol, 74: 567-572, doi: 10.1016/j.fsi.2018.01.026
- Khan MIR, Choudhury TG and Kamilya D, 2020. Probiotics in finfish aquaculture: An Indian perspective. Indian J Anim Health, 59(2): 13-26, doi: 10.36062/ijah.59.2SPL.2020.13-26
- Khan TA and Zafar F, 2005. Haematological study in response to varying doses of estrogen in broiler chicken. Int J Poult Sci, 4(10): 748-751, doi: 10.3923/ijps.2005.748.751
- Kheloufi M, Boulanger CM, Durand F and Rautou, PE, 2014. Liver autophagy in anorexia nervosa and acute liver injury. Bio Med Res Int, 2014: 701064, doi: 10.1155/2014/701064
- Klesius P, Lim C and Shoemaker C, 1999. Effect of feed deprivation on innate resistance and antibody response to *Flavobacterium columnare* in channel catfish *Ictalurus punctatus*. Bull Eur Assoc Fish Pathol, 19(4): 156-158
- Lim C and Klesius PH, 2003. Influence of feed deprivation on hematology, macrophage chemotaxis, and resistance to *Edwardsiella ictaluri* challenge of channel catfish. J Aquat Anim Health, 15(1): 13-20, doi: 10.1577/1548-8667(2003)015%3C0013:IOFDOH%3E2.0.CO;2
- Lin L, Pantapalangkoor P, Tan B, Bruhn KW, Ho T *et al.*, 2014. Transferrin iron starvation therapy for lethal bacterial and fungal infections. J Infect Dis, 210(2): 254-264, doi: 10.1093/infdis/jiu049
- Mohapatra S, Chakraborty T, Haj-Kacem R, Shimizu S, Matsubara T *et al.*, 2016. Starvation: An alternate measure to improve immunity and physiology of red sea bream during *Edwardsiella tarda* Infection. J Aquac Res Dev, S2: 007, doi: 10.4172/2155-9546.S2-007
- Mohapatra S, Chakraborty T, Shimizu S, Urasaki S, Matsubara T *et al.*, 2015. Starvation beneficially influences the liver physiology and nutrient metabolism in *Edwardsiella tarda* infected red sea bream (*Pagrus major*). Comp Biochem Physiol Part A Mol Integr Physiol, 189: 1-10, doi: 10.1016/j.cbpa.2015.07.003
- Mukherjee A, Chandra G and Ghosh K, 2019. Single or conjoint application of autochthonous *Bacillus* strains as potential probiotics: effects on growth, feed utilization, immunity and disease resistance in Rohu, *Labeo rohita* (Hamilton). Aquaculture 512: 734302, doi: 10.1016/j.aquaculture.2019.734302
- Pirhonen J, Schreck CB and Gannam A, 2000. Appetite of Chinook salmon (*Oncorhynchus tshawytscha*) naturally infected with bacterial kidney disease. Aquaculture, 189: 1-10, doi: 10.1016/S0044-8486(00)00368-9
- Restellini S, Spahr L and Brandt LB, 2013. Severe starvation-induced hepatocyte autophagy as a cause of acute liver injury in anorexia nervosa: A case report. Case Reports Hepatol, 2013: 749169, doi: 10.1155/2013/749169
- Shoemaker CA, Klesius PH, Lim C and Yildirim M, 2003. Feed deprivation of channel catfish, *Ictalurus punctatus* (Rafinesque), influences organosomatic indices, chemical composition and susceptibility to *Flavobacterium columnare*. J Fish Dis, 26(9): 553-561, doi: 10.1046/j.1365-2761.2003.00489.x
- Vecchi C, Montosi G, Garuti C, Corradini E, Sabelli M *et al.*, 2014. Gluconeogenic signals regulate iron homeostasis via hepcidin in mice. Gastroenterology, 146: 1060-1069, doi: 10.1053/j.gastro.2013.12.016

- Weinberg SR, LoBue J, Siegel CD and Gordon AS, 1976. Hematopoiesis of the kissing gourami (*Helostoma temminckii*): effect of starvation, bleeding, and plasma-stimulating factors on its erythropoiesis. *Can J Zool*, 54: 1115-1127, doi: 10.1139/z76-127
- Weinberg SR, Siegel CD and Gordon SA, 1973. Studies on the peripheral blood cell parameters and morphology of the red paradise fish, *Macropodus opercularis*: effect of food deprivation on erythropoiesis. *Anat Rec*, 175(1): 7-13, doi: 10.1002/ar.1091750103
- Wise DJ and Johnson MR, 1998. Effect of feeding frequency and Romet-medicated feed in survival, antibody response, and weight gain in fingerling channel catfish *Ictalurus punctatus* after natural exposure to *Edwardsiella ictaluri*. *J World Aquac Soc*, 29(2): 169-175, doi: 10.1111/j.1749-7345.1998.tb00976.x
- Wise DJ, Greenway T, Li MH, Camus AC and Robinson EH, 2008. Effects of variable periods of food deprivation on the development of enteric septicemia in channel catfish. *J Aquat Anim Health*, 20(1): 39-44, doi: 10.1577/h07-008.1