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Immunostimulant Effect of Vitamin-A in *Channa Punctatus* Challenged with *Aeromonas Hydrophila*: Haematological Evaluation

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ABSTRACT

The present study was carried out to evaluate the immunostimulant potential of vitamin A in fish. *Channa punctatus* was chosen for the present study and divided into 3 groups. 'A' group was uninfected, both 'B' & 'C' groups were infected with *A.hydrophila* and only 'C' group was injected with 0.025ml of vitamin A. Haematological parameters were analysed on 1, 2, 3, 4, 7, 14, 21 & 26th day. The total erythrocyte counts and total leucocyte counts exhibited marked increase in vitamin A administered ('C' group) fishes when compared to 'A' & 'B' groups. In differential leucocyte counts the lymphocytes and neutrophils exhibited an increasing trend in both 'B' & 'C' groups but it was significantly higher in vitamin A administered fishes. Further monocyte counts were significantly higher in 'C' group fishes and a gradual declining trend was observed. When the serum of "C' group fishes were titrated with the pathogen in 96 well microtitre plate exhibited agglutination which further supports the increase in lymphocyte counts as a specific immune response. Thus vitamin A can be administered to improve the general resistance in fishes.

INTRODUCTION

Fish in intensive culture are continuously affected by environmental fluctuations and management practices such as handling, crowding, transporting, drug treatments, under nourishment, fluctuating temperatures and poor water quality. The high susceptibility of fish to stress and the rapid spread of diseases in water have forced aqua-culturists to concentrate their efforts on maintaining their fish in good health in order to achieve sustainable economic performances. Among different practices, administration of immunostimulants is one, which along with good management practices will ensure high survival rates, improve the health status and enhance growth in intensive farming systems. Immunostimulants are a group of biological or synthetic compounds that enhance the humoral and cellular immune response both by specific and non-specific way thereby reducing the risk of diseases (Tewary and Patra 2004). Immunostimulants includes many antioxidants, vitamins, carotenoids and other feed additives.

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Immunostimulants also stimulate the natural killer cells, complement, lysozyme and antibody responses of fish (Sakai 1999, Tewary and Patra 2004). Vitamin A is fat soluble and essential in maintaining epithelial cells, preventing atrophy and keratinization of epithelial cells and also promotes growth of new cells and aids in maintaining resistance to infection.

Aeromonas hydrophila is a ubiquitous gram negative rod shaped opportunistic pathogen causing 'Haemorrhagic Septicemia' in fish during stress, overcrowding, transportation, poor level of nutrition and poor water quality. Blood forms an integrated and inevitable part in all immune system and the changes in these parameters can be correlated to the response of the organism to the changing environmental condition and therefore can be used to screen the health status of the fish submitted to the exposed toxicant (Pandey and Pandey 2001). Administration of vitamins as immunostimulants to improve the general health of fish was reported by many workers. Administration of immunostimulant through fish feed will be easier but it has its own drawbacks, for example absorption of the required amount depends on the amount of immunostimulant incorporated in feed, feed consumed and absorbed.

Thus in the present study the immune-stimulant potential of vitamin A was assessed by injecting vitamin A to *Channa punctatus* infected with *Aeromonas hydrophila*.

MATERIALS AND METHODS

Laboratory acclimated *Channa punctatus* (length 8.28±1.6cm, weight 4.87±0.04g) were recruited for the present study. The fishes were divided into three groups. 'A' group fishes were considered as 'Control' and they were not infected and not injected with vitamin A, 'B' group fishes was infected with a dose of 10^3 dilution of *A.hydrophila* and not injected with vitamin A. and 'C' group fishes was also infected with a dose of 10^3 dilution of *A.hydrophila* and also injected with a dose of 0.025ml. The experiment was run in triplicate. During the experimental period water in the containers were changed on alternate days without disturbing the test organisms and fed regularly with the artificial diet. The blood samples were collected on 1, 2, 3, 4, 7, 14, 21, 26 days of exposure to the pathogen. Haematological parameters were performed on pooled blood samples. Total Erythrocyte Counts (TEC), Total Leucocyte Counts (TLC) were counted using Haemocytometer with improved Neubaurer ruling chamber (Weber & sons, England), differential leucocyte counts (DLC) were performed on blood smears stained with May-Grunewald's Giemsa's stain and antibody tire using agglutination principles was analysed in the serum in the 96- well titre plate. The data were analyzed statistically and students't' test was used to test their significance.

RESULTS & DISCUSSION

Total Erythrocyte Counts

In group 'A' fishes no significant change was observed during the entire experimental period (Table-1). In both 'B' & 'C' group (Infected) fishes the values remained higher throughout the experimental period. The increase was significantly higher in Vit.A administered group ('C' group). It was also observed that in both infected group ('B' &'C') the values decreased gradually with increase in experimental period. Significant increase in the TEC levels in group 'C' fishes maybe attributed to Vitamin A administration.

Sivagurunathan and Xavier Innocent (2012) observed similar increase in *Channa punctatus* exposed to *Aeromonas hydrophila* and injected with Vit.C. The results of the present study were in accordance with a number of previous observations, which have reported a hike in TEC during unhealthy state of fish (Kumar and Patri 2000; Rahukhan *et al* 1995). Accordingly high counts were associated with the abnormal conditions of a fish. Hence an increase in the TEC might have been accomplished by a rapid mobilization of RBC from the haemopoietic tissue, which may transport higher amounts of oxygen particularly to withstand stress factor caused by *A.hydrophila* (Innocent *et al.*, 2004).

Total Leucocyte Counts

No significant change was observed in counts in uninfected fishes (group 'A'), however in both 'B' &'C' group fishes the TLCs has increased significantly than control 'A' group and it was significantly higher in group 'C' fishes (Table-2). It was also observe that the counts increased with increase in experimental period. Though increase in the TLC levels can be contributed to pathogen induced defence response, the significant and sustained increase may also be due to early detection of pathogen by the alert immune system which is fortified with a dose of 0.025ml of Vitamin A.

Sivagurunathan and Xavier Innocent (2012) observed similar increase in *Channa punctatus* exposed to *Aeromonas hydrophila* and injected with Vit.C, Harikrishnan et al (2010) observed similar increase in TLC in Cirrhinus mrigala infected with *Aphanomyces invadans*. The results of the present study also agree with the works of Sivagurunathan *et al* (2011), Innocent *et al* (2004) and Pandey *et al* (2000).

Table. 1: Total Erythrocyte counts (millions/mm^3) in relation to A.hydrophila infection post administered with Immunostimulant Vitamin A. (Mean±SD of three values).

Sample	Duration (days)							
	1	2	3	4	7	14	21	26
A	0.41±0.08	0.43±0.08	0.42±0.07	0.41±0.08	0.42±0.09	0.41±0.08	0.43±0.07	0.43±0.08
B	0.51*±0.08	0.53*±0.07	0.52*±0.07	0.49*±0.04	0.47*±0.04	0.49*±0.05	0.48*±0.08	0.47*±0.02
C	0.55*±0.07	0.53*±0.08	0.54*±0.05	0.52*±0.09	0.50*±0.04	0.51*±0.08	0.52*±0.08	0.53*±0.01

*=significant (**A** = uninfected, **B** = Infected-Vit.A, **C** = Infected+Vit.A).

Table. 2: Total Leucocyte counts (thousands/mm^3) in relation to A.hydrophila infection post administered with Immunostimulant Vitamin A. (Mean±SD of three values).

Sample	Duration (days)							
	1	2	3	4	7	14	21	26
A	2.60±0.8	2.55±0.7	2.60±0.9	2.56±0.8	2.58±0.9	2.55±0.5	2.53±0.7	2.63±0.8
B	3.11*±0.9	3.23*±0.8	3.41*±0.8	3.45*±0.7	3.61*±0.9	3.65*±1.1	3.73*±0.9	3.75*±1.2
C	3.85*±0.7	3.92*±0.8	3.98*±0.5	4.21*±0.9	4.33*±1.1	4.31*±0.8	4.36*±0.8	4.35*±1.3

*=significant (**A** = uninfected, **B** = Infected-Vit.A, **C** = Infected+Vit.A).

Differential Leucocyte Counts

As there is no significant changes in the uninfected ('A' group) fishes, the average value from the experimental period was calculated and used in the figure for comparison. In group 'B' &'C' the average of three values were calculated and compared graphically. The lymphocyte counts were less in Infected fishes ('B' & 'C'groups) when compared to 'A' group fishes (Fig.-1), further it was also observed that the counts increased gradually with increase in the duration of experimental period. Further, within the infected groups the counts were higher in 'C' group fishes (Vitamin-A administered). Gradual increase in lymphocytes can be understood as pathogen induced specific immune response and higher counts can be correlated to increase in specific resistance.

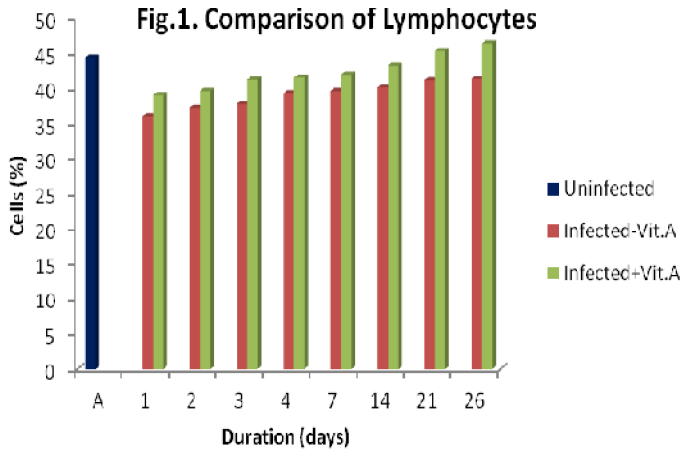

Fig. 1: comparison of lymphocytes.

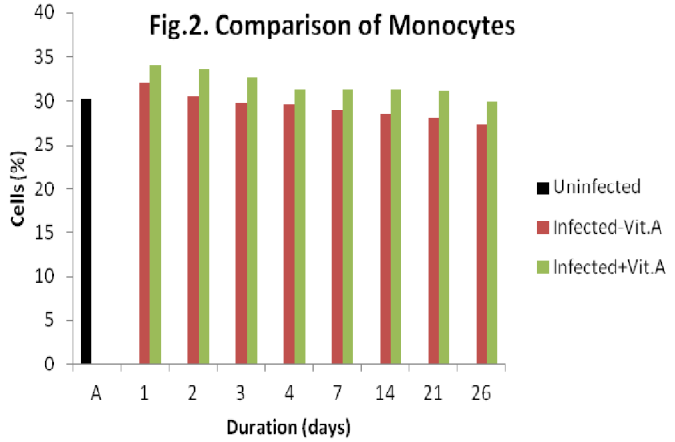

fig. 2: comparison of monocytes.

Similarly the neutrophil counts also exhibited an increasing trend from day 1-26 in group 'C' which is a clear indication of improved general resistance. On the other hand the monocyte count was higher only in group 'C' but exhibited a decreasing trend with increasing in the experimental duration. In group 'B' the monocyte counts were less when compared to both 'A' & 'C'. It is well known that the monocyte plays a vital role in both non-specific and specific immunity. They also play an important role in phagocytosis not only to eliminate the pathogen but also to elicit specific immunity. Thus increased levels on monocytes in group 'C' can be attributed to the role of vitamin-A in improving the immunity.

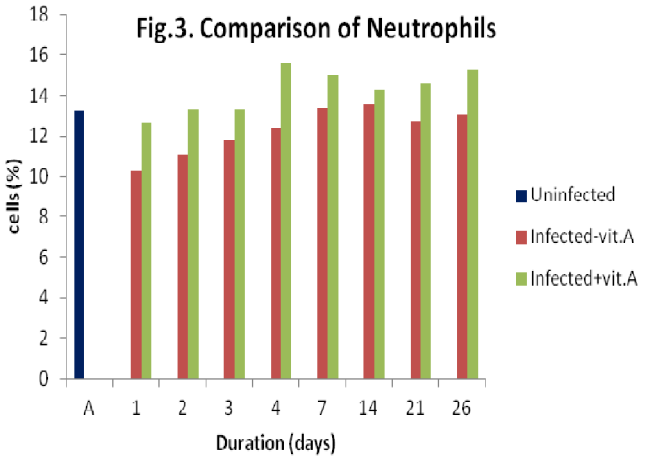

Fig. 3: comparison of meutrophils.

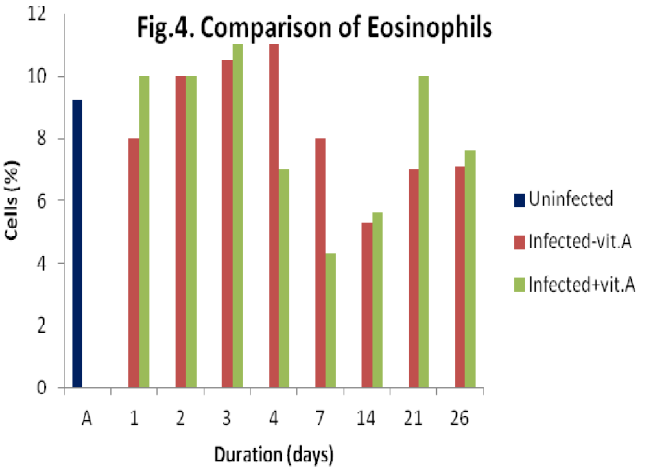

Fig. 4: comparison of eosinophils.

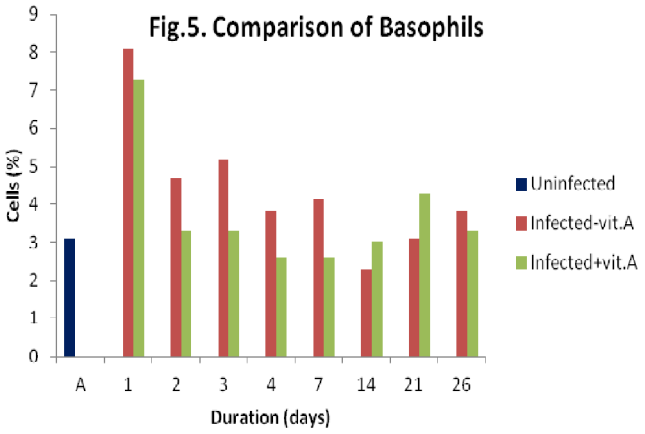

Fig. 5: comparison of basophils.

Ainsworth (1992) has suggested that neutrophils and monocytes are responsible for bacterial uptake in studies with Atlantic salmon, shown that neutrophils are capable of ingesting

bacteria, *A.salmonicida*. Similar observations of neutrophil as an inflammatory response were recorded by Innocent *et al* (2004) in *M.montanus* infected by *A.hydrophila*. Sivagurunathan and Xavier Innocent (2012) observed an increase in neutrophil, lymphocyte and monocyte counts in *Channa punctatus* exposed to *Aeromonas hydrophila* and injected with Vit.C, Karuthapandi and Xavier Innocent (2010) observed an increase in lymphocyte counts in Tilapia when infected with Vibrio anguillarum,Garcia *et al*(2011) observed an increase in neutrophil and monocyte counts when *Piaractus mesopotamicus* fed with vit.C supplemented diet and challenged with *A.hydrophila*.

Fluctuating results were observed in both eosinophil and basophil counts in both the infected groups.

Further, Visible results of antigen-antibody reaction in the 96 well microtitre plate in the form of larger aggregates in the 4^{th}, 5^{th},7^{th} well was observed when titrated with the serum obtained from group 'C' fishes which can be correlated with the increasing lymphocyte counts as they are responsible for the antibody production. Montero *et al*(1999) also observed the production of antibody in fishes treated with vitamin A and infected with *V.salmonicia, V.anguillarum, A.salmonicida*.

CONCLUSION

In the present study fishes infected with *A.hydrophila* and administered with vitamin A exhibited an increase in erythrocyte counts than the fishes not administered with vitamin A further the declining was minimal which is evident that administration of vitamin A supplements the release of more circulating RBC which helps the fish to resist the pathogen induced stress.

On the other hand increase in WBC population in vitamin A administered fishes can be correlated to vitamin induced general resistance against the pathogen. Further, increase in neutrophil counts explains the immediate non-specific immune response and elevated levels of monocytes are an indication of activated immune response as they play a major role in both cell mediated and humoral mediated immunity.

Increase in lymphocyte population explains the activated specific immunity against the particular pathogen wich is further supported by agglutination in the microtitre plate. Thus administration of vitamin A will enhance the immune power of the fish.

REFERENCES

Ainsworth AT. Fish granulocytes, morphology,distribution and function. Annu.Rev.Fish.Dis. 1992; 2:123-148

Garcia F, F Pilarski, E Makoto, F Ruas de Moraes, M Laterca. Hematology of Piaractus mesopotamicus fed diets supplemented with vitamins C and E, challenged by Aeromonas hydrophila. Aquaculture. 2007; 271, 39-46.

Harikrishnan, Chellam Balasundaram , Moonsoo Heo. Supplementation Diet containing Probiotics, Herbal and Azadirachtin on Haematological and Biochemical changes in *Cirrhinus mrigala* against *Aphanomyces invadans*. *Fisheries and Aquaculture* Journal. 2010; FAJ-**4**: 1-11.

Innocent BX., Martin P, Shanthi MM. Haematological studies in Mystus montanus exposed to gram negative bacteria Aeromonas hydrophila. Indian. J. Environ Protection. 2004; vol24: 614.

Karuthapandi M, Xavier Innocent B. Immune responses of Tilapia mossambicus administered with rat serum vaccine for Vibrio anguillarum. Current Biotica. 2010; 4(1): 63-72. ISSN 0973-4031.

Kumar, K., and Priyambada Patri, (2000). Variation in the haematology of starved climbing perch Anabas testudineus (Block) a fresh water air breathing fish, Aquacult: 1:19-24.

Montero, D., Marrero, M., Izquierdo, M.S.Robaire,L.Vergara, T.M. and Tort, L. Effort of Vitamin E and C dietary supplementation on some immune parameters of silthead Sea bream (Sparus aurata) juveniles subjected to crowding stree, Aqu. Culture. 1999; 171, 269-270.

Pandey AK. Histopathological alterations in fish tissues induced by pesticide toxicity. Aquacult. 2000; 2(1): 31-43.

Pandey AK., Pandey GC. Thiram and Ziram Fungicides induced alterations on some haematological parameters of fresh water cat fish *Heteropneustes fossilis*. Indian. J. Environ. & Ecoplan. 2001; 5(3): 437-442.

Raukhan, J.V.S., S.P.Grover and P.Jaiwal, (1995) studies on some haematological changes in a hill stream Borilius bendetsis (Hamiton) injected with trypansomes, flora and faune 1:165-166.

Sakai M. Current research status of fish Immunostimulants. Aquaculture.1999; 172: 63-92.

Sivagurunathan A, Xavier Innocent B. Immunostimulant (Vitamin C) induced haematological responses in *Channa punctatus* exposed to *Aeromonas hydrophila*. Proceedings of International Conference on Environmental Security for Food and Health (ICESFH-2012). 2012; 35-39.ISBN 978-81-7966-301-1.

Sivagurunathan A., Amila Meera K., Xavier Innocent B. Investigation of Immunostimulant Potential of *Zingiber officinale* & *Curcuma longa* in *Cirrhinus mrigala* exposed to *P.aeruginosa*-Haematological Assessment. IJRAP. 2011; 2(3): 899-904.www.ijrap.net.

Tewary A, Patra BC. Use of immunostimulant in Aquaculture. Advances in Biochemistry and Biotechnology. Daya Publishing House, Delhi. 2004; vol-1: 183-194.

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