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IMMUNE RESPONSE OF *CATLA CATLA* FED WITH AN ORAL IMMUNOSTIMULANT *PLUMBAGO ROSEA* AND POSTCHALLENGED WITH *AEROMONAS HYDROPHILA*

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ABSTRACT: The present study was designed to evaluate the immunostimulant potential of *Plumbago rosea* in fish *catla*, post challenged with *Aeromonas hydrophila*. Fishes were divided into 2 groups one received control diet and another received *Plumbago rosea* incorporated diet for 14 days. The haematological parameters and serum protein were estimated between control and experiment. There is An increase in TEC ($P<0.05$) and TLC ($P<0.05$) in the immunostimulant administered (IS)diet was observed. Increase in lymphocyte count was noted in immunostimulant incorporated diet. Remarkable increase in Hb from 6.2 to 8g% and serum protein level from 0.4to 0.5g% was observed in experimental fishes The immunostimulant administered *Catla catla* when challenged with *Aeromonas hydrophila* showed a decrease in TEC and an increase in TLC. Increase in lymphocytes was also noted.

Key words: *Catla catla Plumbagorosea, Aeromona hydrophila,* Immunostimulant.

INTRODUCTION

Aquaculture represents one of the fastest growing food producing sectors. Fish diseases constitute one of the most important problems and challenges fish culturists. Hence, aqua-culturists are forced to undertake good management practices, so that they can ensure a healthier fish. The use of natural immunostimulants in fish culture for the prevention of disease is a promising new development (Anderson 1992, Sakai 1999). Natural immnuostimulants are biocompatible, biodegradable and safe for the environment and human health (Ortuno, et. al., 2002). Immunostimulant, used in vaccines to amplify the specific immune response or administered as feed additives to modulate non-specific immunity, have been demonstrated to play role in protection against diseases in fish (Badrelin, et. al., 2008, Pandey, et. al., 2001, Shao, et. al., 2004, Tan, et. al., 2004, Vasuthevarao, et. al., 2006, Prit Benny, et.al., 2010, Karthupandi, et. al., 2010) *Plumbago rosea*_is a perennial shrub which belongs to the family plumbaginaceae. It is commonly cultivated in gardens throughout India. The root of the plant contains plumbagin which stimulates the central nervous system. The plant is able to enhance the digestive power and promote appetite. It also has antiseptic property. The juice of leaves with oil is used to cure rheumatism and paralysis. It contains plumbagin (2-Methoxy-5-hydroxy-1-4-Napthoquinone), which is a natural napthoquinone possessing various pharmacological activities such antimalarial (Likhitwitayawuid, et. al., 1998) and antimicrobial (Didry, et. al., 1994). So, the use of plants as a productive system of immunostimulators facilitates a new and safe method of immunization.

The regular monitoring of fish blood serves as the diagnostic purpose in establishing the health status of fish. By analyzing blood cell characteristics some clues for diagnosis and prognosis of the disease state may be found (Anderson, 2004). The present study is aimed to asses the efficacy of *Plumbago rosea* as an immunostimulant and to evaluate the immunostimulant activity in the fish *Catla catla*, post challenged with *Aeromonas hydrophila* by analyzing the haematological and serological parameters.

MATERIALS AND METHODS

The experimental fish *Catla catla* (125 ± 30g) were colleted from Kallidikuruchi fish farm and allowed to acclimatize to laboratory conditions for one week. During acclimatization they were fed with rice bran and groundnut oil cake. Water was renewed daily. During the experimental period the water quality variables temperature (28 ± 1°c) P^H (7.4 ± 0.2) salinity (10 ± 2%) and dissolved oxygen (>5 mg-1) were maintained.

Feed preparation

The basic diet (Control diet) was prepared by mixing rice bran 10g. Wheat bran 10g, Soya flour 23g, dry fish meal 24g, groundnut oil cake 23g, and Tapioca flour 10g, made as dough, sterilized in pressure cooker for 30 min, cooled and made in the form of noodles by adding a little amount of sunflower oil. They were shade dried and broken into small desirable pieces and stored. Immunostimulant diet was prepared by using the same propotion by using 2g of *Plumbago rosea* collected from the local garden.

Experimental design

Experiment 1:

Experiment 1 consists of two groups. One control and one experimental of 15 fishes each. The control group received normal diet and the experimental group received feed formulated with *Plumbago rosea* powder (IS diet). The fishes were fed with these diets for 14 days and the hematological parameters and serum protein levels were analyzed after 1st, 3rd, 7th & 14th day respectively.

Experiment 2:

Both control and experimental feed fed fishes were subjected to infection with the bacterial *Aeromonas hydrophila* previously grown in nutrient broth for 24hrs.A dosage of 10^{-3} and 10^{-5} (cfu/ml) bacteria were injected intramuscularly and again hematological parameters and serum proteins were studied after 1st, 3nd, 7th & 14th day.

Haematological and Serological analysis

The blood was collected from the fishes by puncturing the heart by using 1ml insulin syringe. For serological analysis the collected blood were centrifuged at 2500 rpm for 14min. Total erythrocyte count (TEC) and Total leucocyte count (TLC) were carried out using Haemocytometer with improved Neubaurer ruling chamber (Weber & Sons England). Haemoglobin content was estimated by cyanomethomoglobin method(Hemocor-D, crest Biosystems). Blood smears stained with May-Grunewald's Giemsa stain was used for differential leucocyte count The data was analyzed statistically and students 't' test was used to test their significance. For serum protein estimation Gornall's biuret method was followed.

RESULTS

Experiment 1:

The values of TEC, TLC were higher in Immunostimulant incorporated diets and the increase was highly significant ($P<0.05$) on the 7th & 14th day when compared with standard feed pellets. (Table 1 & 2) DLC could not envisage marked differences however here and there fluctuations were found among the types of cells. (Table 3).

Higher percentage of haemoglobin were estimated in immunostimulated fishes, than control fishes (6.2to 8g%). Serum protein level exhibited an increase from 0.4to 0.56g% in IS incorporated fishes (fig 1 & 2).

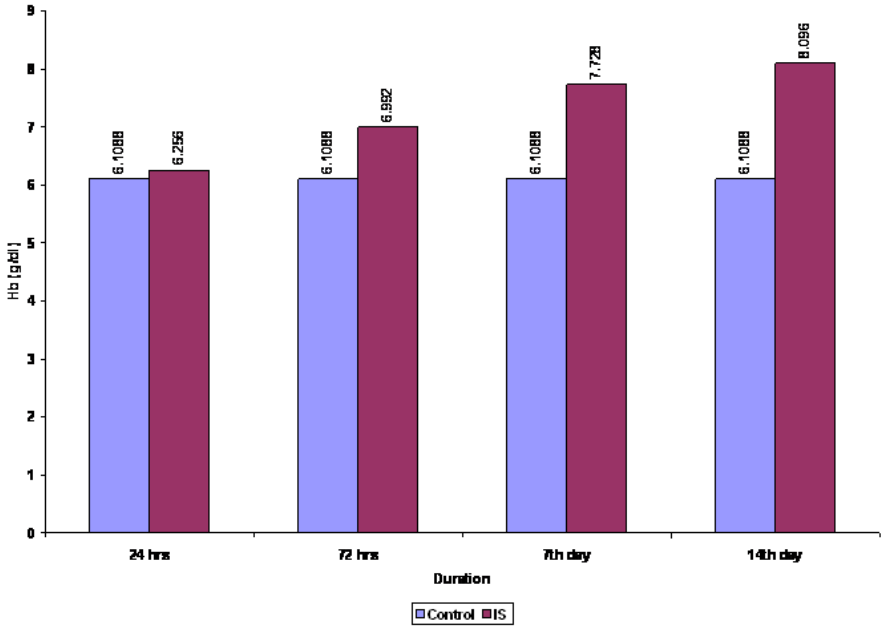

Figure 1: Hb content in *C.catla* administered with control and IS diet

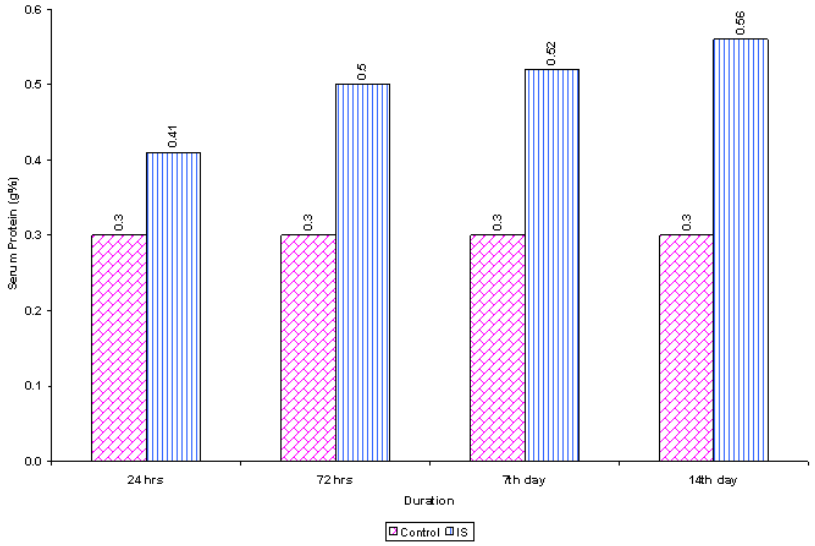

Figure 2: Serum Protein content in *C.catla* administered with control and IS diet

Table 1: TEC (Million cells/mm^3) in relation to control and IS diet .

Duration Days	Sample	RBC million/cells SD
1	C	0.726±0.5607
	IS	1.3±0.1315*
3	C	0.726±0.5607
	IS	0.796±0.1287*
7	C	0.726±0.5607
	IS	2.222±0.1519*
14	C	0.726±0.5607
	IS	2.456±0.095

P; * significant

Table 2 : TLC (Million cells/mm^3) in relation to control and IS diet.

Duration Days	Sample	WBC million/cells SD
1	C	6810±1125.78
	IS	15110±4027.54*
3	C	9810±1125.78
	IS	14570±654.67
7	C	9810±1125.78
	IS	39200±3520.51
14	C	9810±1125.78
	IS	45860±2810.76

P; * significant

Table 3 : DLC (%) in relation to control and IS diet

Duration Days	Dosage	Lymphocyte %	Monocyte %	Neutrophil %	Eosinophile %	Basophil %
1	C	46	32	11	8	3
	IS	57	15	19	5	4
3	C	52	30	10	5	3
	IS	57	17	21	2	3
7	C	50	18	18	3	11
	IS	60	15	13	7	5
14	C	69	9	13	3	6
	IS	58	20	10	6	6

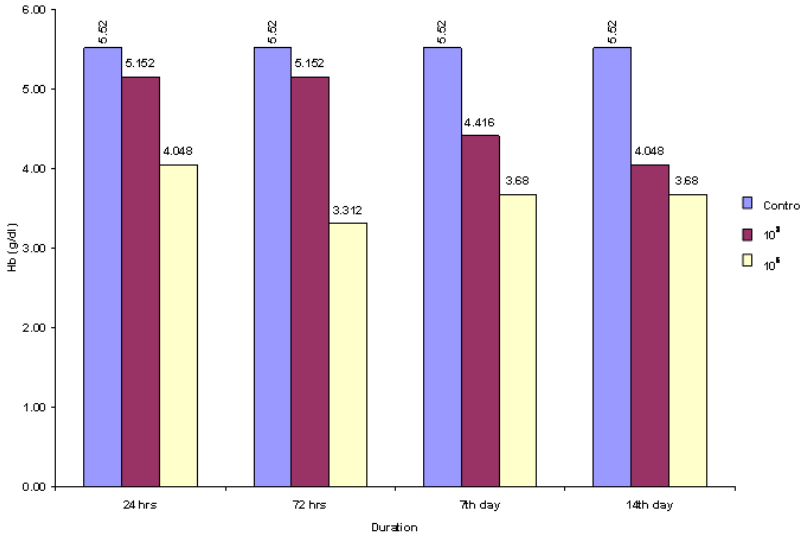

Figure 3: Hb content *C.catla* predministered with IS diet and postchallenged with *A.hydroph*

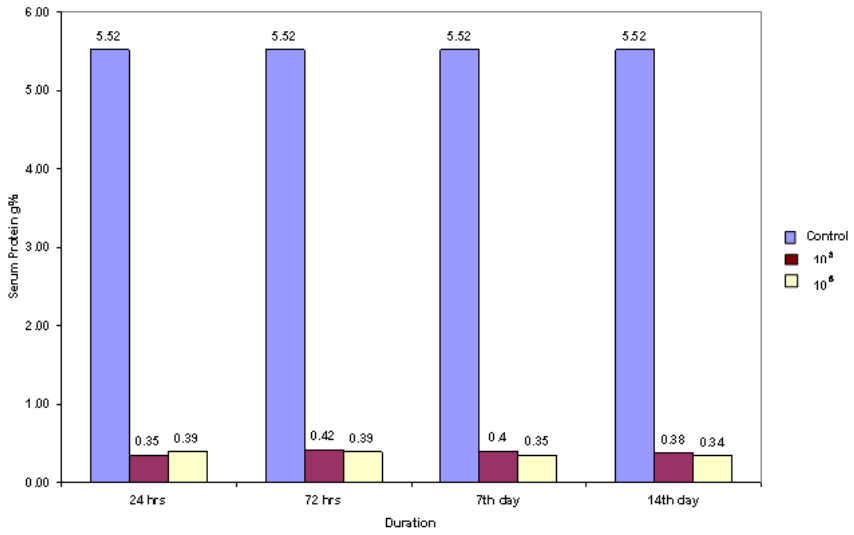

Figure 4: Serum protein *C.catla* preadministered with IS diet and postchallenged with *A.hydrophila*

Experiment – II

A significant decrease in TEC was noticed in IS fed fishes when infected with *A.hydrophila* at a dosage of both 10^{-3} & 10^{-5} cfu/ml. A significant increase in TLC was observed on 14^{th} day ($P<0.05$) in both 10^{-3} & 10^{-5} cfu/ml in the IS incorporated fishes (Table 4 & 5). Lymphocytes and neutrophils showed an increase in *C.catla* fed with IS incorporated diet (Table 6) Hb content and serum protein level got decreased in experimentally infectedfishes in both 10^{3} and 10^{-5} cfu/ml. (Fig 3 & 4).

Table 4 : TEC (Million cells/mm^3) pre administered with IS diet in *Catla catla* and post challenged with *Aeromonas hydrophila*

Duration Days	Dosage (cfu/ml)	RBC million/cells SD
1	C	0.65±0.030
	10^{-3}	0.61±0.0172*
	10^{-5}	0.56±0.030*
3	C	0.65±0.030
	10^{-3}	0.60±0.0172*
	10^{-5}	0.53±0.0215*
7	C	0.65±0.030
	10^{-3}	0.572±0.007*
	10^{-5}	0.506±0.014*
14	C	0.65±0.030
	10^{-3}	0.522±0.030*
	10^{-5}	0.50±0.026*

P; * significant

Table 5 : TLC (Cells/mm^3) pre administered with IS diet in *Catla catla* and post challenged with *Aeromonas hydrophila*

Duration Days	Dosage (cfu/ml)	RBC million/cells SD
1	C	8310±208.33
	10^{-3}	10680±235.80*
	10^{-5}	9040±241.79*
3	C	8310±208.33
	10^{-3}	11810±226.72*
	10^{-5}	11020±310.81
7	C	8310±208.33
	10^{-3}	13270±248.19
	10^{-5}	10350±202.42
14	C	8310±208.33
	10^{-3}	14680±211.19*
	10^{-5}	11930±128.84*

P; * significant

Table 6 : DLC (%)pre administered with IS diet in *Catla catla* and post challenged with *Aeromonas hydrophila*

Duration Days	Dosage (cfu/ml)	Lymphocyte %	Monocyte %	Neutrophil %	Eosinophil %	Basophil %
1	C	36	32	11	8	3
	10^{-3}	46	18	30	4	2
	10^{-5}	44	20	19	9	7
3	C	46	32	11	8	3
	10^{-3}	52	11	28	5	4
	10^{-5}	55	12	18	9	6
7	C	46	32	11	8	3
	10^{-3}	56	12	22	5	5
	10^{-5}	59	13	20	4	4
14	C	46	32	11	8	3
	10^{-3}	55	28	12	3	2
	10^{-5}	60	15	10	9	6

Discussion

Though many synthetic and natural substances have been tested for their immunostimulating abilities, traditional medicinal herbs seem to have the potential, as a rich source of active substances for Immunomodulation (Hadden, 1993, Ganguly and Saines 2001). In the present study, there was an increase in RBC count, similar to the findings of Sahu, et. al., 2007, in which the counts of RBC were higher in *Labeo rohita* fingerlings fed with *Magnifera indica kernel.*

The serum protein level were higher in IS incorporated diet in the present study which agrees with Rao, et. al., (2006), who reports a similar trend in *Labeo rohita* fed with *Achyranthes aspera*.

Disease challenge studies showed a decrease in TEC at a dosage of both 10^{-3} & 10^{-5} cfu/ml which was similar with the results of Ramasamy Harikrishnan, et. al., (2010). A significant increase in TLC seen in both 10^{-3} and 10^{-5} cfu/ml indicates the enhancement of non-specific immune response and disease resistance. (Christy Babita, et. al., 2007). Immune enhancement increase white blood cell phagocytosis inhibits HIV-1 replication and improves D_{4+} and T- lymphocyte counts(Mishra, et. al., 2009). In the present study also an increase in lymphocyte counts was observed during disease challenge studies. Samuel Sudhakaran, et . al., 2006 reported that the neutrophil counts were significantly high when *Oreochromis mossambicus* were fed with *Tinospora cordifolia* which were similar to our findings. Gopalakannan and Arul (2006) reported the dietary plant extract supplementation enhance the resistance of *Cyprinus carpio* against *Aeromonas hydrophila* which agrees with the findings of the present study.

Conclusion

From the above results, it is clear that *Plumbago rosea* acts as a potent immunostimulant, since it induces the blood parameters in the experimental fish *Catla catla.* Recent studies revealed that the herbal extracts have a potential application as an immunostimulant. Medicinal plant is the unique source of various types of compounds having diverse chemical structure. Postchallenge studies with *Aeromonas hydrophila* also provide positive immune potential of *Plumbago rosea which* enhance the non-specific immunity of the fish. Based on the results it is appropriate to conclude that the plant extract of *Plumbago rosea* may act as a potent Immunostimulant in fish.

REFERENCES

1. D.P. Anderson (2004). Diseases of fishes Narendra publishing House Delhi: Pg 22-73.
2. D.P.Anderson (1992).Immunostimulants, Adjuvant and vaccine carriers in fish. Application to Aquaculture, Annual review of fish Diseases, 2: 281-307.
3. H.Badrelin. Ali, Gerald Bluden, O. Musbah, Tanira and Abderrahim Nemmar(2008). Some phytochemical pharmacological and Toxicological properties of Ginger *(Zingiber officinale Rolcoe)*; A Review of recent research science Direct. food and chemical toxicology : 46.409-420,
4. D.Christy bapita, M.Divyagnaneswari and R. Dhinakaran Michael(2007). Oral administration of *Eclipta alba* leaf aqueous extract enhances the non-specific immune response and disease resistance of *Oreochromis* mossambicus. Fish shellfish Immunology: Vol .23(4) 840-852.
5. N.Didry, L.Dubrevil and M.Pinkas,(1994). Activity of anthroquinone and napthoquinone compounds on oral bacteria Diepharmazie: Vol.49 (9) 681-683.
6. T.Gangauly and K.B.Sainis (2001). Inhibition of cellular Immune responses by Tylophora indica in experimental models. Phytomedicine:8.348.
7. A.Gopalakannan and V.Arul (2006). Immunomodulatory effects of dietary intake of chitin, chitosan and levamisole on the Immune system of *Cyprinus carpio* and control of *Aeromonas hydrophila* infection in ponds. Aquaculture: Vol. 255 179-187.
8. J.W. Hadden (1993). Immunostimulants Trends is pharmacological science: 14.169.
9. M.Karuthapandi, B.Xavier Innocent (2010). Immune responses of *Tilapia mossambicus* administered with rat serum vaccine for *Vibrio anguillarium*, Current Biotica: Vol.4 (1)63-72.
10. K.Likhi, R.Keewamatawong, N.Ruangrungsi and J.KRundkRai (1998). Antimalarial napthoquinone from Nepenthes thorelii, Plant medica. A.P: Vol. 64 (3) 237-241.
11. V.S.Mishra, A.Mishra, R.Kumari, P.N.Murthy and B.S.Naik (2009). Antibacterial activity of ethanol *Andrographis paniculata*: Vol. 71(4) 436-438.
12. J.Ortuno, A.Cuesta, A.Rodriguez, M.A.Esteban and J.Meseguer (2002). Oral administration of yeast, *Saccharonmyces cerevisiae* enhances the cellular innate immune response of *giltlead sea bream (Sparus aurata)*. Vet. Immunol. Immunopathol, 85:41.
13. A.K. Panday, G.C. Panday (2001). Thiram and Ziram fungicides induced alterations on some haematological parameters of fresh water cat fish *Heteropneustes fossils*. Indian J.Environ and Ecoplan: Vol.5 (3) 437-442.
14. Prit Benny, Geetha Viswanathan, Smitha Thomas Aruna Nair(2010). Investigation of Immunostimulatory behaviour of *Musa accuminate* peel extracts of *Clarias batrachus*. The IIOAB. Journal: Vol. 1 (2) 39-43 Available from http://www. iioab.org/vo/I2 July 2010. htm
15. Ramasamy Harikrishnan, Chellam Balasundaram and Moo-Soo Heo (2010). Herbal supplementation diets on haematology & innate immunity in *gold fish* against *Aeromonas hydrophila*. Fish and shell fish Immunology: Vol.28(2) 354-361
16. Y.V.Rao, B.K.Das, J.Pradhan and R.Chakrabathi (2006). Effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita infected* with *Aeromonas hydrophila.* Fish and shell fish immunology: Vol. 20 263-273.
17. S.Sahu, B.K.Das, J.Pradhan, B.C.Mohaptra, B.K.Mishra and N.N.Sarangi (2007). Effect of Magnifera Indica kernel as a feed additive on immunity and resistance of Aeromonas hydrophila is Labeo rohita fingerlings. Fish and shellfish Immunology: Vol. 23 109-118.
18. M.Sakai (1999). Current research status of fish Immunostimulant Aquaculture: 172: 6392.
19. B.M.Shao, Xuw, Dai,H Tu P, Liz, X.M.Gao (2004). A study on the Immune receptors for polysaccharides from the roots of *Astragalus membranaceus,* a Chinese medicinal Herb.Biochem Biophys Res commun: Vol. 320(4):1103-1111.
20. B.K Tan and J. Vanitha (2004). Immunomodulatory and antimicrobial effects of some traditional Chinese medicinal Herbs; a review curr med chem: Vol. 11(11): 1423-1430.
21. Y. Vasudeva Rao, B.K.Das, P.Jyotyrmayee and R.Chakrabarti (2006). Effect of *Achyranthes aspera* on the Immunity and survival of *Labeo rohita* infected with *Aeronomas Hydrophila*. Fish shell fish Immunol: Vol. 20(3): 263-273.