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INVESTIGATION OF IMMUNOSTIMULANT POTENTIAL OF *ZINGIBER OFFICINALE* & *CURCUMA LONGA* IN CIRRHINUS MRIGALA EXPOSED TO *PSEUDOMONAS AERUGINOSA* – HAEMATOLOGICAL ASSESSMENT

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ABSTRACT

The present study was designed to evaluate the Immunostimulant potential of Turmeric and Ginger in fish *Cirrhinus mrigala* infected with *Pseudomonas aeruginosa*. Fishes were divided into three groups, one received control diet, another received turmeric incorporated diet [T diet] and the third group received Ginger incorporated diet [G diet] for 40 days. The hematological parameters were analyzed and compared between uninfected and infected. A decrease in Total Erythrocyte Count[47%], Haemoglobin [22%] and Packed Cell Volume [16%] was observed in infected fishes fed with control diet, but in T diet the decrease was marginal [TEC 14%, Hb 22% & PCV 9%] and in G diet the decrease was minimal [TEC 12%, Hb 19% & PCV 7%]. A decrease in TLC [39%], lymphocytes [1%] and neutrophils [5%] were recorded in infected fishes fed with control diet but in T diet there was an increase in TLC [10%], lymphocyte [3%] and neutrophil [2%]. Similar increase was observed in G diet fed infected fishes also [TLC-12%, lymphocyte-10%]. Thus it is evident that Turmeric and Ginger have Immunostimulant potential.

KEYWORDS: Immunostimulant, Turmeric, Ginger, Haematology, *Cirrhinus mrigala*, *Pseudomonas aeruginosa*

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INTRODUCTION

In aquaculture large scale mortalities of fish occur due to infectious microbial and parasitic diseases caused due to high dense culture or by pollution mediated environmental stress. Protecting the fish from diseases can be done through two ways. One is by strengthening the self immune power of the organism to fight the invasion of pathogens, and the second is through medication[1]. The drawbacks in using antibiotics and other chemotherapeutics are development of drug resistant pathogens, environmental pollution and accumulation of residues in fish. Vaccination is an effective prophylactic method for controlling fish diseases but they are relatively costly and pathogen specific and administering them to large number of fishes is difficult[2].

Immunostimulants are substances which enhance the humoral and cellular immune response both by specific and non-specific way there by reducing the risk of diseases. Recently there has been increased interest in the immune stimulating function of some herbs in aquaculture[3].

The advantages of herbal therapeutants over synthetic or formulated ones are 1) 90% utilization by the culture organism, 2) provides many phytocompounds in a complex way which produces a synergistic effect 3) do not cause damage to physiological system 4) can be easily broken-down by enzymes 5) little amount of residues formed and 6) supplies pigments such as chlorophyll, carotenoids, xanthophylls and very complex vitamins in the most desirable form[1].

Turmeric and Ginger, both belong to ginger family and their pharmacological properties were well documented. *Curcuma longa* (Turmeric) is pale yellow to orange in colour, the colouring principles (5%) are curcuminoids, 50-60% of which are a mixture of curcumin, monodesmethoxy curcumin and bis desmethoxy curcumin and composed of 6% volatile oil and a number of monoterpenes and sesquiterpenes including zingiberene, curcumine, turmerone etc. The

pharmacological properties are anti-inflammatory, anti-oxidant, anti-mutagenic, anti-cancerous, anti-microbial, anti-spasmodic, anti-hepatotoxic etc[4-5].

Zingiber officinale (Ginger) contains gingerols and shogaols, over 50 components of the oils have been characterized, these are mainly monoterpenoids, sesquiterpenoids (α-Zingiberene 30-70%), B-sesquiphellandrene (15-20%), B-bisabolene (10-15%) etc. The main pharmacological actions of ginger and compounds isolated from it are Immuno-modulatory, anti-tumourogenic, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-microbial, anti-platelet, anti-ulcer, anti-oxidant etc[6].

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 healthy state of fish submitted to the exposed toxicant[7].

The present study is aimed to evaluate the Immunostimulant potential of Ginger and Turmeric in the fish *Cirrhinus mrigala* post infected with *Pseudomonas aeruginosa* by analyzing the haematological parameters.

MATERIALS & METHODS

The experimental fish *Cirrhinus mrigala* (weight 75±5g) were purchased from local fish farm and allowed to acclimate to laboratory conditions for 15 days. During acclimatization they were fed with rice bran and groundnut oil cake *ad libitum.* During the experimental period the water quality variables: temperature (28±1^{o}C), pH (7.4±0.2), salinity (10±2) and dissolved Oxygen (>5mg-^{1}) were recorded. The water was changed daily in order to maintain the fishes in healthy state.

Feed Preparation

The basic diet (Control diet) was prepared by mixing Rice bran 10g, Wheat bran 10g, Soya flour 23g, dry fish meal 24g, Ground nut oilcake 23g and Tapioca flour 10g made as a dove, sterilized in pressure cooker for 30 minutes, cooled and made in the form of noodles by adding a little amount of sunflower oil which are then shade dried, broken into small desirable sized pieces. "T" diet was prepared using the same composition of ingredients to which 2g of Turmeric powder purchased from the local market was added. Similarly "G" diet was prepared using the same composition of ingredients to which 2g of dry Ginger powder purchased from the local market was added.

Experimental design

The fishes were primarily divided into two experimental groups. The experimental group I is further divided into three groups of 15 fishes each namely, control, T & G. The control group fishes received control diet, "T" group fishes received feed incorporated with turmeric powder and "G" group fishes received feed formulated with dry ginger powder. The fishes were fed with these diets for 40 days, and then each group of fishes were further divided into three groups of 5 fishes each for hematological study.

The experimental group II setup is similar to group-I, but after 40 days each fish is infected with the bacteria *Pseudomonas aeruginosa* previously grown in nutrient broth for 24 hours. 10^{-1} dilution of the bacteria with physiological saline was injected (0.1 ml) intra muscularly and blood parameters were studied.

Haematological Analysis

After the experimental period blood was collected from the fishes by cutting the caudal peduncle and the blood was collected in heparinized tubes. All analysis was 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), Haemoglobin content (Hb) was estimated by Sahli's acid haematin method, Packed Cell Volume (PCV) was measured by routine procedure using PCV tubes and expressed as percentage. The MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Haemoglobin) and MCHC (Mean Corpuscular Haemoglobin Concentration) were calculated using standard formulae. Blood smears stained with May-Grunewald's Giemsa's stain was used for differential leucocytes count. The data were analyzed statistically and students "t" test was used to test their significance.

RESULTS

Experiment I [Uninfected]

The values of TEC, TLC, Hb, PCV & MCHC were higher in both T & G diet fed fishes than their control counter parts and the increase was highly significant ($p<0.01$) in G diet fed fishes than T diet fed fishes. The MCV decreased in both T & G diet fed fishes than control but not statistically significant [Table-1]. In the differential leucocyte counts Neutrophils increased significantly only in G diet fed fishes, whereas no significant changes were observed in the counts of lymphocytes and basophils. The monocytes and eosinophils decreased significantly ($p<0.05$) in G diet fed fishes [Table-2].

Experiment II [Infected]

In the fishes infected with *P.aeruginosa* also the TEC, TLC, Hb, PCV, MCV & MCHC exhibited highly significant ($p<0.01$) increase in both T & G diet fed

fishes than control diet fed fishes. However the increase was greater in G diet fed fishes that T diet. The MCH value exhibited highly significant decrease in both T & G diet fed fishes. In differential leucocyte counts the lymphocytes and neutrophils increased significantly in both T & G diet fed fishes. However the increase was more in G diet. The basophils, monocytes and eosinophils exhibited highly significant decrease in both T & G diet fed fishes.

Comparison

The results obtained from the two experiments were compared and expressed as **% change** [i.e., Uninfected & infected group of fishes fed with control diet, likewise T & G groups were also compared].

The infected fishes of all the 3 different diets exhibited a decrease in TEC, Hb & PCV values. However the decrease was drastic in control [47%, 22%, 16%] and significant [14%, 22%, 9%] in T diet and moderate [12%, 19%, and 7%] in G diet fed fishes. The TLC decreased significantly in control diet fed infected fishes, but minimal increase [-1%] and significant rise [-12%] was observed in G diet fed infected fishes. In differential leucocyte counts minimal increase in lymphocyte & neutrophil counts were observed in both T & G diet fed infected fishes, but they decreased marginally [1%, 5%] in control infected fishes. The %Change values appear higher in the monocytes, basophils & eosinophils as their population is less.

DISCUSSION

In aquaculture disease outbreak is a common feature. The first step in disease is the entry of the pathogen into the body of the fish followed by establishment and finally produces the disease. Disease outbreak can be prevented or controlled if the entry or establishment of the pathogen is controlled. The non specific immune response of the organism plays a vital role to achieve this. As Immunostimulants have the potential to enhance the non-specific immune response they can be used.

In aquaculture many studies report that herbal medicine extracts can be used as Immunostimulants for fishes[2,8-10].

In the present study, the Immunostimulant potential of Turmeric and Ginger were investigated by administering them orally along with food because oral administration of drugs has been appreciated as compared to injection and immersion techniques[11].

In the present investigation TEC, Hb, PCV & MCH exhibited sharp decrease in the infected fishes fed with control diet when compared to their uninfected counterparts, but the decrease was lesser in both T & G diet fed fishes. Similar decrease in TEC & Hb was observed in Tilapia when infected with *Vibrio anguillarium*[12], a decrease in TEC, TLC, Hb, PCV, MCH & MCHC values and an increase in MCV values were observed 7 days after in Rainbow trout [*Oncorhynchus mykiss*] infected with *Pseudomonas putida*[13], lowered TEC, Hb & MCHC levels were noticed in brook trout *Salvelinus fontinalis* affected by Columnaris disease[14]. Similar decrease in TEC & Hb was observed in *Cyprinus carpio* infected with *Flavobacterium columnare*[15].

Total and differential leucocyte counts are important indices of non-specific defence activities in fish[16] as leucocytes are centrally involved in phagocytic and immune responses to parasitic, bacterial, viral & similar challenges[17]. The TLC, lymphocytes, & neutrophil counts in the present study decreased sharply in the infected fishes fed with control diet whereas they increased in T & G diet fed fishes. Decrease in lymphocytes and neutrophil counts were observed in *Cirrhinus mrigala* infected with *Aphanomyces invadans*[18]. Similarly when Banana peel extracts were administered orally to *Clarias batrachus* the TLC, lymphocytes increased in 48 hours whereas the counts of neutrophils decreased[11].

CONCLUSION

Thus from the present study it is evident that infected fishes fed with control diet are anaemic and unable to withstand the pathogen induced stress. Further, decrease in TLC, lymphocyte and neutrophil will make the fish an easy target for infection. Administration of Turmeric and Ginger not only prevented a drastic decline in TEC & Hb values but also increased the TLC, lymphocyte and neutrophil counts thereby improves the general health of the fish to withstand the stress condition. Thus it is evident that both Turmeric and Ginger have Immunostimulant potential, further the Immunostimulant efficiency of Ginger is comparatively higher.

REFERENCES

1. Stephen Sampath Kumar J, Anantharaja K. Herbal Health Care in Aquaculture - the Indian Experience: Aquaculture 2006; Infofish International [1/2006].
2. Sakai M. Current research status of Fish Immunostimulants. Aquaculture 1999; 172:63- 92.
3. Guojun Yin, Laszlo Ardo, Zsigmond Jeney Pao Xu, Galina Jeney. Chinese Herbs (*Lonicera japonica* and *Ganoderma lucidum*) Enhance Non-specific Immune Response of Tilapia, *Oreochromis niloticus* and protection against *Aeromonas hydrophila.* Diseases in Asian Aquaculture VI 2008; 269-282.
4. MDidea.com. Turmeric Root or Curcuma Root; Phytochemicals and Applications. 2011; [last edit date 18th oct.2010]. Available from: http://www.MDidea.com/products/new08806 html.
5. Xavier Innocent B. Antimicrobial Activity of Turmeric (*Curcuma longa*) in Meat Products. Indian. J. of Multi disp. Research. 2008; Vol.4:No.2: 197-202.
6. Badreldin H. Ali, Gerald Bluden, Musbah O. Tanira and Abderrahim Nemmar. Some Phytochemical Pharmacological

and Toxicological properties of Ginger (*Zingiber officinale* Rolcoe): A review of recent research. Science Direct. Food and Chemical Toxicology 2008; 46:409-420.
7. 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.
8. Shao BM, Xu W, Dai H, Tu P, Li Z, Gao XM. A study on the Immune receptors for polysaccharides from the roots of *Astragalus membranaceus*, a Chinese medicinal Herb. Biochem Biophys Res Commun. 2004; 320(4):1103-1111.
9. Tan BK, Vanitha J. Immunomodulatory and antimicrobial effects of some traditional Chinese medicinal herbs: a review. Curr Med chem. 2004; 11(11):1423-1430.
10. Vasudeva Rao Y, Das BK, Jyotyrmayee P, Chakrabarti R. Effect of *Achyranthes aspera* on the Immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. Fish shellfish Immunol 2006; 20(3):263-273.
11. Prit Benny, Geetha Viswanathan, Smitha Thomas Aruna Nair. Investigation of Immunostimulatory behavior of *Musa acuminate* peel extracts *in Clarias batrachus*. The IIOAB. Journal 2010; Vol.1Issue 2:39-43. Available from http://www.IIoab.org/vol12july 2010.htm.
12. Karuthapandi M, Xavier Innocent B. Immune responses of *Tilapia mossambicus* administered with rat serum vaccine for *Vibrio anguillarum*. Current Biotica. 2010; Vol 4, Issue1:63-72. Available from http://www.current biotica.com/JournalNew.aspx
13. Bektas S, Ayik O. Haematological Parameters and Erythrocyte osmotic Fragility in Rainbow trout *Oncorhyncus mykiss* experimentally infected with *Pseudomonas putida*. J. of Fisheries & Aquatic Science. 2009; 1-8.
14. Rehulka J, Minaolk B. Blood parameters in brook trout *Salvelinus fontinalis* (Mitchill 1815) affected by Columnaris disease. Aquacult. 2007; Res.38:1182-1197.
15. Tripathi NK, Latimer KS, Gregory CR, Ritchie BW, Wooley RE, Walker RL. Development and evaluation of an experimental model of cutaneous columnaris disease in Koi (*Cyprinus carpio*). J.Vet.Diagn.Invest. 2005; 17:45-54.
16. Pedro N, Guijarro AI, Patioo MAL, Alvarez RM, Delgado MJ. Daily and seasonal variations in haematological and blood biochemical parameters of Nile Tilapia *Oreochromis niloticus* (Linnaeus 1757). Braz. Arch. Biol. Technol. 2005; 47:945-953.
17. Houstan A H. Blood and circulation. In: Methods for fish Biology. American Fisheries Society, USA. 1990; ISBN: 0-913235-98 X: 273-334.
18. Ramasamy 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; Vol.2010FAJ-4: 1-11.

Table1. COMPARISON OF THE ERYTHROCYTIC PARAMETERS BETWEEN UNINFECTED AND INFECTED C.MRIGALA FED WITH DIFFERENT FEEDS.

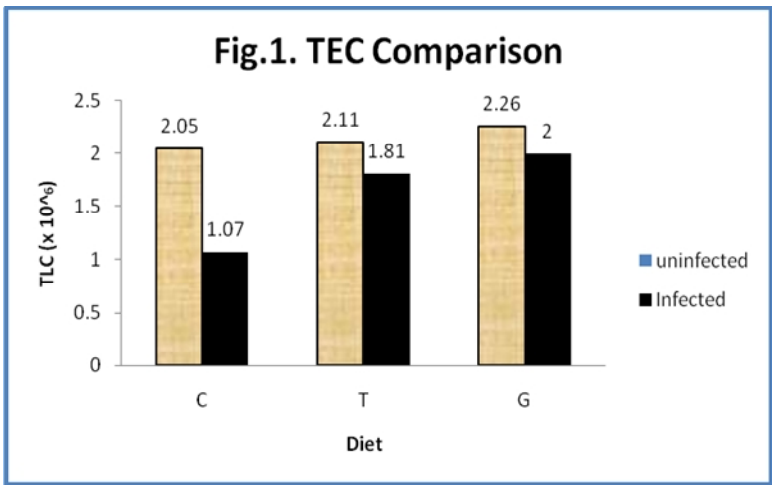
Parameters	Uninfected			Infected		
	C	T	G	C	T	G
TEC (x10^6)	2.05 ± 0.07	2.11 ± 0.04	2.26** ± 0.08	1.07 ± 0.04	1.81** ± 0.09	2.00** ± 0.09
%Change				**47**	**14**	**12**
Hb (G)	8.6 ± 0.3	9.4** ± 0.2	10.4** ± 0.2	6.7 ± 0.2	7.3 ± 1.6	8.5* ± 0.4
%Change				**22**	**22**	**19**
PCV (%)	38.1 ± 0.8	39.2* ± 0.4	40.4** ± 0.4	32.1 ± 1.3	35.5** ± 0.9	37.5** ± 1.2
%Change				**16**	**9**	**7**
MCV (μ^3)	186.1 ± 9.8	185.7 ± 2.3	178.9 ± 7.1	300.7 ± 6.3	196.3** ± 10.9	187.1** ± 7.7
%Change				**-62**	**-6**	**-5**
MCH (μgm)	41.9 ± 2.9	44.6 ± 0.5	46.2* ± 1.5	63.1 ± 1.7	40.6** ± 2.9	42.9** ± 2.6
%Change				**-51**	**9**	**7**
MCHC (%)	22.5 ± 0.5	24.0** ± 0.2	25.8** ± 0.6	21.0 ± 0.6	20.7 ± 0.4	22.6** ± 0.8
%Change				**7**	**14**	**12**

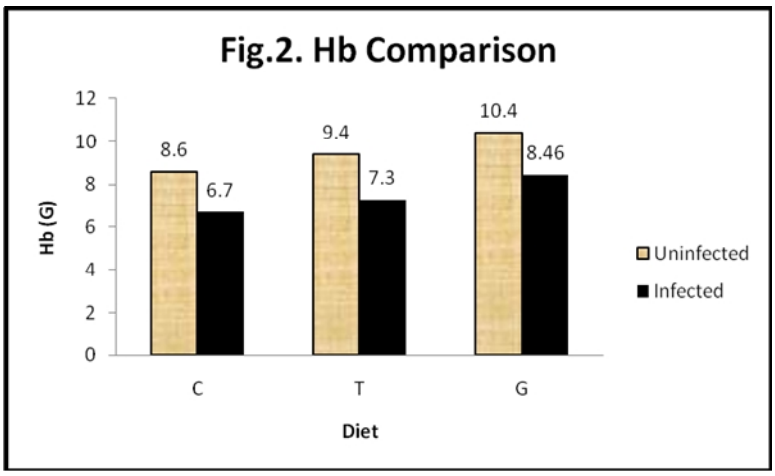
P = <0.05 = Significant P = <0.01 = Highly Significant
%Change (- = Increase, + = Decrease)
C = Control diet, T = Turmeric diet, **G** = Ginger diet

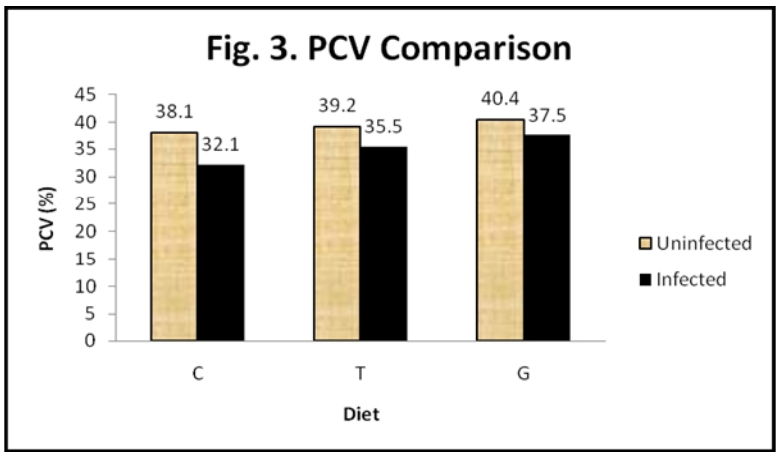
Table2. COMPARISON OF THE LEUCOCYTIC PARAMETERS BETWEEN UNINFECTED AND INFECTED C.MRIGALA FED WITH DIFFERENT FEEDS.

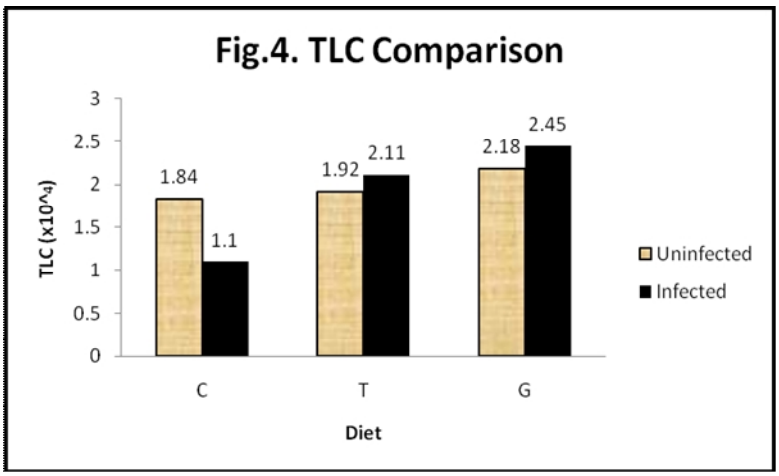
Parameters	Uninfected			Infected		
	C	T	G	C	T	G
TLC ($x10^4$)	1.84 ± 0.06	1.92 ± 0.08	2.18** ± 0.03	1.1 ± 0.2	2.11** ± 0.04	2.45** ± 0.06
%Change				**39**	**-1**	**-12**
Lymphocyte (%)	28 ± 3.1	30** ± 2.4	29** ± 2.2	25 ± 2.8	31* ± 2.1	32** ± 1.6
%Change				**1**	**-3**	**-1**
Neutrophil (%)	58 ± 2.2	61 ± 3.3	63* ± 2.8	55 ± 3.4	62* ± 2.3	63** ± 2.1
%Change				**5**	**-2**	**-**
Monocyte (%)	6 ± 1.4	4 ± 1.1	3** ± 0.5	8 ± 1.1	3** ± 0.6	2** ± 0.3
%Change				**-33**	**25**	**33**
Basophil (%)	3 ± 1	3 ± 1.2	2 ± 0.8	6 ± 1.3	2** ± 0.5	1** ± 0.5
%Change				**-100**	**33**	**50**
Eosinophil (%)	5 ± 1.2	2** ± 0.5	3* ± 0.6	6 ± 1.6	2** ± 0.7	2** ± 0.4
%Change				**-20**	**-**	**33**

P = <0.05 = Significant P = <0.01 = Highly Significant
%Change (- = Increase, + = Decrease)
C = Control diet, T = Turmeric diet, **G** = Ginger diet









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