Immunomodulatory Effect of Triphala Bio fortification in Oreochromis mossambicus

Jayasree S¹, Usha S², A. Akhila Thomas³

¹,³P.G and Research Centre, Department of Zoology, Fatima Mata National College, Kollam
²Sree Narayana College, Kollam

Abstract: The present study was conducted to evaluate the bio fortification efficacy of methanolic extract of Triphala in Oreochromis mossambicus as reflected by haematological parameters. Haematological parameters like total erythrocyte count (TEC), total leucocyte count (TLC), differential leucocyte count (DLC), haemoglobin (Hb), hematocrit,(Ht) ,mean cell haemoglobin (MCH) serum total protein, albumin and globulin were analyzed in Triphala (0.8%) fortified O.mossambicus subsequently challenged with Aeromonas hydrophila. Significant increase in TEC, TLC, Hb, Ht, MCH and serum total protein and albumin were observed in Triphala supplementation group compared to control and increase in globulin level was insignificant. Post Aeromonas hydrophila infection, TLC, DLC and survival status improved in Triphala fortified group compared to control. Hence present study confirms that Triphala (0.8%), supplementation in fish feed acts as an immunomodulator.

Keywords: Oreochromis mossambicus, Aeromonas hydrophila, haematology, Immunostimulants

1. Introduction

Population explosion urges intensive aquaculture and development of new species-specific fish feed formulations supports the intensive aquaculture industry. Disease management through antibiotics and chemicals lead to bacterial resistance and accumulation of unacceptable residues in aquaculture products and environment which will be a threat to the health of consumers (5, 13, 6). Hence consumer and eco-friendly disease preventive measures to promote sustainable aquaculture is the call of the century. It is proved beyond doubt that medicinal herb incorporated aqua feeds provide basic nutritional requirements to improve growth, feed utilization and also the support of general health and stress resistance. Phytobiotics offer a very complex blend of bioactive components. Hence numerous research works progresses globally to standardize useful plant materials as feed additives to fin fish and shellfishes due to its multifunctional properties derived from their specific bioactive components, so that sustainable intensive aquaculture system can be streamlined. (3,7). The antibacterial activity of Centella asiatica and Curcuma longa against pathogenic bacteria affecting Macrobrachium rosenbergii was reported by Salini, et.al. (2015). Methanolic extract of Curcuma amada effectively control both opportunistic and pathogenic bacteria in Oreochromis mossambicus (14). Globally people have understood the malicious effects of antibiotics, and they are now shifting over to natural products.

Blood biochemistry has been long a supportive diagnostic tool in pathological, toxicological and general clinical tests (11). Haematological studies can be effectively used to prevent the occurrence of epidemics and thus to reduce economic loss to aqua farmers. Triphala, is a herbal formula prepared by mixing the dried powder of Emblica officinalis, Terminalia chebula, and Terminalia bellerica (1:1:1 proportion). Triphala purifies blood, stimulates bile secretion and detoxifies the liver. It helps in the digestion and assimilation, lowers serum cholesterol and lipid levels the body, stimulate immune system and reduces high blood pressure. It has antioxidant, antimutagenic, antineoplastic, chemo protective, radio protective, and chemo preventive properties and also effective against cancer (4).

Tilapia is an important model to study fish physiology, particularly because of its broad tolerance to an array of environments. Currently Tilapia are the second most farmed fish in the world with an annual production exceeding 2.8 million tons in 2010 and they exhibit a versatile adaptability to different environmental conditions to match the vast array of their ecological habitats.

Hence the objective of the present study is to assess the bio fortification efficacy of Triphala in O.mossambicus with emphasis on immunomodulatory potentialas reflected by haematological parameters.

2. Materials and Methods

2.1. Experimental set up

The fruits of medicinal plants, Emblica officinalis, Terminalia bellerica and Terminalia chebula, were collected and identified in Pankajakasturi Ayurvedic Research Centre, Thiruvananthapuram. O. mossambicus having 30-40 gm wt. were collected from Agency for Development of Aquaculture in Kerala (ADAK) at Varkkala, quarantined and stocked in 1000 L glass tanks, and maintained at normal laboratory conditions. After two weeks acclimatization the fish are randomized into two groups, one group served as control and was supplemented with control feed and the other group was fed with medicated feed (D1=0.8% Triphala) prepared in the laboratory.. Both of them were fed @ 2% of their body weight twice daily. Water quality parameters were maintained every day 50% water was renewed daily during removal of fecal materials and unused feed. During the experimental period the water quality variables: temperature (28±1°C), pH (7.2±0.2), and dissolved Oxygen (7.5±0.5) mg/l were recorded. The

Volume 5 Issue 8, August 2016

www.ijsr.net

Licensed Under Creative Commons Attribution CC BY
experimental regime continued, in triplicate for two months and hematological parameters pre and post A. hydrophila challenge were assessed and documented.

Blood samples were collected using 1 ml syringe directly from the ventral aorta and transferred into sterilized glass vials containing one millilgram of EDTA and was used to estimate total RBC, WBC, DLC and Hb, and haematocrit (1). Blood without anticoagulant was used to estimate serum total protein and albumin levels. Plasma was obtained by centrifugation of blood at 3000 rpm for 15 min and was stored in deep freezer for further biochemical analysis. Total Erythrocyte Counts (TEC), Total Leucocyte Counts (TLC) were counted using Haemocytometer with improved Neubauer chamber (Weber & sons, England), Haemoglobin was measured using cyanohaemoglobin method using Drabkins fluid. Blood smears stained with May-Grunewald’s Giemsa’s stain was used for differential leucocytes count.

2.2 Methanolic extract preparation

The methanolic extract preparation was done as outlined by Singh, (2008) incorporating modification of Jasmine et al. (2011). Fresh fruits of Emblica officinalis, Terminalia chebula, and Terminalia bellerica, were washed in sterilized water, shade dried, (de seeded) and grinded separately. Fruit powder was successively extracted with 85% absolute methanol and then subjected to vacuum filtration. The filtrate is dried and the residue obtained after evaporation was carefully collected, weighed and transferred into air tight containers and stored at -20°C till use.

2.3 Feed preparation

The control diet was prepared by mixing fish meal 40gm, rice brawn 25gm, ground nut oil cake 10 gm, soya 10 gm, tapioca powder 5 gm, wheat flour 5 gm, with 100 ml methanol and then subjected to vacuum filtration. The filtrate is dried and the residue obtained after evaporation was carefully collected, weighed and transferred into air tight containers and stored at -20°C till use.

2.4 Challenge study

Subsequent to the feeding regime (60 days) both control and experimental fishes were challenged intra peritoneally with Aeromonas hydrophila (10^6 cfu/ml) and 7 days post challenge fishes were subjected to hematological studies.

2.5 Statistical Analysis

The statistical analysis was carried out using the software SPSS 17.0 package. For determining the significant difference between different treatments in blood and serum parameters one-way ANOVA followed by Duncan’s test was done. Significance level (P value) was set at 0.05 in all tests. Values in the same row with different lower case letters vary significantly (p<0.05) between treatment groups. Each value represents the mean ±SE of six separate data.

3. Results and Discussion

Proximate analysis of the control and experimental feed is given in Table 1. The results obtained as per the experimental design are given in Tables 2-6.

Table 1: Proximate composition of feed

<table>
<thead>
<tr>
<th>In 100 gm feed (gm %)</th>
<th>Control feed</th>
<th>0.8% Triphala</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>35.4±0.08^a</td>
<td>35.20 ±0.04^a</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>20.12 ±0.01^b</td>
<td>20.21± 0.01^b</td>
</tr>
<tr>
<td>Lipid</td>
<td>10.18 ±0.18^b</td>
<td>10.15± 0.17^b</td>
</tr>
<tr>
<td>Ash</td>
<td>12.84± 0.22^b</td>
<td>12.55± 0.12^b</td>
</tr>
<tr>
<td>Moisture</td>
<td>10.05 ±0.21^b</td>
<td>10.14± 0.09^b</td>
</tr>
<tr>
<td>Dry matter</td>
<td>89.95±0.52^b</td>
<td>89.86±0.35^b</td>
</tr>
<tr>
<td>Fiber</td>
<td>4.39 ± 0.09^b</td>
<td>4.58 ± 0.06^b</td>
</tr>
<tr>
<td>NFE %</td>
<td>37.18± 0.11^b</td>
<td>37.52± 0.08^b</td>
</tr>
<tr>
<td>GE</td>
<td>448.5±0.01^b</td>
<td>448.5± 0.01^b</td>
</tr>
</tbody>
</table>

In proximate analysis of feed no significant variation observed in any of the components assessed. Highly significant increase in total erythrocyte count (TEC) Hb, Haematocrit, MCV and MCH in Triphala fortified group compared to control. (Tables 2). Increased levels of TEC, TLC and serum protein in Triphala supplemented group is an indicator of improved general health (Tables 3). In the experimental groups the lymphocyte were higher followed by neutrophil, polymorphs and, eosinophil. The lymphocytes exhibited an increasing trend in D1 (0.8% Triphala) fed group post challenge it presents the immune readiness of the treated group as both non-specific and specific immune response depends upon the population of leucocytes. Sahu et al., (2007a) reported an enhanced WBC & RBC counts in Labeo rohita fingerlings supplemented with Mangifera indica kernel. Prit Benny et al., (2010) also noted an increase in WBC and lymphocyte counts in Clarias batrachus fed with Musa acuminate peel extract. Such a rise in RBC was also observed by Mohsen Abdel-Tawwab et al., (2010) in Nile Tilapia fed with green tea. Maintenance of steady population of neutrophils and monocytes is vital in resisting general infection and to induce the specific immunity by increasing the lymphocytes. Post challenged herbal supplemented fishes quickly reestablished the altered haematological and serological parameters.

Triphala supplemented group showed significant increase in serum glucose level may be due to an increased cortisol level produced by bacterial infection (Tables 4&5). Above results indicate that oral administration of the methanolic extract of Triphala (0.8%) is very effective in elevating the levels of hemoglobin MCV, MCH and haematocrit in the erythrocyte of fish. Triphala is supposed to increase levels of hemoglobin MCV, MCH and haematocrit in the experimental groups the lymphocyte were higher followed by neutrophil, polymorphs and, eosinophil. The lymphocytes increase in WBC and lymphocyte counts in Labeo rohita fingerlings supplemented with Triphala.  Maintenance of steady population of neutrophils and monocytes is vital in resisting general infection and to induce the specific immunity by increasing the lymphocytes. Post challenged herbal supplemented fishes quickly reestablished the altered haematological and serological parameters.

Significant decrease in RBC’s hemoglobin and packed cell volume subsequent to challenge in control O.mossambicus.
shows that *Aeromonas* infection had affected both metabolic and hematopoietic activities of control tilapia. Similar results were also observed by Sudagar and Hajibeglou (2010) in *C. carpio* supplemented plant extracts for 60 days and challenged with *A. hydrophila*. Increase in serum total protein and albumin levels in D-I diet fed present work may be an indication to increased levels of non-specific immunity and the increased albumin levels may facilitate the transport of more humoral compounds as well as active principles present in the herbal extract through the blood. The haemopoietic expansion of innate immune cells could be reflective of an elevated state of immuno-readiness. Thus from the present study it was evident that incorporation of Triphala, in fish feeds helps to improve the general health and also to recover from biological stress often encountered by fishes in their natural habitat.

**Table 2:** Total RBC, Hb, Haematocrit, MCV, MCH and MCHC values of *Oreochromis mossambicus* reared on Triphala supplemented diet and challenged with *Aeromonas hydrophila*.

<table>
<thead>
<tr>
<th>Hematological parameters</th>
<th>Pre challenge</th>
<th>Challenged</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>D-I</td>
</tr>
<tr>
<td>Total RBC (10⁶/mm³)</td>
<td>2.2±.097ª</td>
<td>2.8±.064ª</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>5.60±.124ª</td>
<td>8.0±.045ª</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>25±.042ª</td>
<td>25±.044ª</td>
</tr>
</tbody>
</table>

Total and differential leucocyte count in *Triphala* bio fortified *Oreochromis mossambicus* presented a statistically significant increase in Triphala supplementation groups compared to control. But in challenged fishes control values fall beyond normal.

**Serum Biochemical Parameters**

**Table 4:** Serum protein parameters in Triphala bio fortified *Oreochromis mossambicus*

<table>
<thead>
<tr>
<th>Test Diets</th>
<th>Pre challenge</th>
<th>Post Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/dl)</td>
<td>Albumin (g/dl)</td>
<td>Globulin (g/dl)</td>
</tr>
<tr>
<td>Control</td>
<td>2.94±.050ª</td>
<td>1.05±.045ª</td>
</tr>
<tr>
<td>D-I</td>
<td>3.95±.035ª</td>
<td>1.87±.115ª</td>
</tr>
</tbody>
</table>

Plasma glucose level in pre challenged *O. mossambicus* is not statistically significant but in challenged fish the plasma glucose level significantly increased in control.

**Table 5:** Plasma glucose level in Triphala bio fortified *Oreochromis mossambicus*

<table>
<thead>
<tr>
<th>Test Diets</th>
<th>Plasma Glucose mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre challenge</td>
<td>Challenged</td>
</tr>
<tr>
<td>Control</td>
<td>86±2.77ª</td>
</tr>
<tr>
<td>D-I</td>
<td>82±1.71ª</td>
</tr>
</tbody>
</table>

4. Conclusion

The poly factorial phytonutrient cocktail present in *Triphala* had significantly improved haematological and serological parameters. The inclusion of herbal additives in diets often provides cooperative action to various physiological functions. The haemopoietic expansion of innate immune cells could be reflective of an elevated state of immuno-readiness. The present hematological data provides valuable information in assessing the health of *O. mossambicus* and in monitoring stress responses to *Aeromonas* infection. Present study can be concluded by stating that methanolic extract of (Triphala 0.8%) is quiet effective as an immunomodulator.

This preliminary work attempts to say little about the physiological mode of action of the plant extract in *Oreochromis mossambicus* but surely it is an active eco-friendly area for future research work.

**References**


**Volume 5 Issue 8, August 2016**

www.ijsr.net

Licensed Under Creative Commons Attribution CC BY
of medicinal herbs extract on *Macrobrachium rosenbergii* based on relative gene expression in hemolymph. Indian Science Congress Mysore University, Mysuru, 3/1/16 to 7/1/16 (Paper presented).


**Authors Profile**

*Jayasree, S.* is from Kerala Govt. Higher Secondary School Teacher, Zoology(Senior). Total Govt Service 20 years. State Resource Group, Zoology faculty and trained faculty of Career guidance and Adolescent Counselling (CG&AC) under same Department.Research papers in International, National and state level journals and proceedings. Doing PhD in Kerala University.

*S Usha,* Assistant Professorin Zoology at P G and research department of Zoology, S.N.College, Kollam. Six publications, Co Author of Methodology and Perspectives of Zoology, Core course in Zoology, III SEM CBCSS3. Deputy Co-ordinator P G diploma inBiomedical Science, S.N.College, Kollam.

*Dr. A.Akhila Thomas*Associate Professor in Zoology at FMN College Kollam Kerala. Having 20 years of teaching and research experience. Have undertaken research projects funded by UGC. 42 research papers in International, National and state level journals and proceedings.