Effect of Herbal Extracts Supplemented diets on Non Specific Immunity and Resistance to Aeromonas hydrophila in Indian cat fish (Mystus montanus)

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ABSTRACT

Immunostimulatory can be used for fish disease prevention as it can enhance fish resistance by increasing the nonspecific defense mechanisms. The present study was conducted to evaluate the immune stimulatory and growth promoting effects of four medicinal plants extracts using ethanol on non-specific immune response and disease resistance to A. hydrophila challenge with cat fish M. montanus. The ethanol extract of eight medicinal plants (Allium cepa, Allium sativum, Zingiber officinale, Urtica dioica, Alove vera, Azadirachta indica, Cinnamomum verum and Origanum vulgare) were screened for their antimicrobial activity against A.hydrophila. Among the eight plants tested four of them are showed good antibacterial activity because of that it has been taken for growth promoting effects of M. montanus. The four plants extracts such as Onion (Allium cepa), Garlic (Allium sativum), Ginger (Zingiber officinale), Neetle (Urtica dioica) mixed with the artificial feeds at concentration of 000 (E1), 250 (E2), 500 (E3), 750 (E4), 1000 (E5) mg kg⁻¹ of dry diet and fed to healthy fish. After 60 days of feeding a challenge trial was conducted by injection of A.hydrophila, after challenged the hematological parameters of fish were studied. The present study indicate the increase in the number of RBC, WBC, Thromobocytes, Haemoglobin, PCV, MCV, MCH and MCHC. All experimental groups showed higher survival rate compared to control at level of (P<0.05). The survival percentage was found highest in the group of E2 and lowest in control group. This result reflects that effect of herbal extracts supplemented dites on non specific immunity and resistance to A. hydrophila in Indian cat fish (Mystus montanus).

Keywords: Aeromonas hydrophila, Mystus montanus, herbal extracts, hematological parameters,

INTRODUCTION

The world trend to improve food security and to use natural products will drive the chemically synthesized antibiotics and growth promoters out of use. Aquaculture is therefore an emerging industrial sector which requires continued research with scientific technical development and innovations [1]. Bacteria the major group of pathogens, pose one of the most significant threats to successful fish production throughout the world [2]. The medicinal plants as the alternative agent are effective to treat the infectious disease and mitigate many of side effects that are associated with synthetic antimicrobials [3]. The diseases caused by A. hydrophilla bacterium are some of the most widespread in freshwater fish culture. It has been associated with disease in fishes like Carp, Eels, Milk fish, Tilapia. This microorganism can also be an opportunist in stress related disease in salmonids and it’s also responsible for skin infections, septicemia and gastroenteries in human, besides the fish [4]. Immunostimulants seem to represent a useful alternative to vaccination and chemotherapy in the control of fish diseases they can enhance the non-specific immune response [5]. Recent research has been initiated to evaluate the feasibility of using herbal medicines in fish disease management [6]. Many studies have proved the herbal additives enhanced the growth of fishes and protected from diseases. Similarly the dietary supplementation of Achyranthes aspera seed stimulated immunity and enhanced resistance to A.hydrophila infection of Labeo rohita fingerlings [7]. The medicinal plant extracts were developed and proposed for use in food as natural antimicrobials [8]. However, little or no work has been done on the antibacterial active herbs on supplemented diets in aquaculture. Therefore, the objective of the present study the effect of different medicinal plants (Herbs) extract in fish disease as possible alternative to antibiotic feed additives.
MATERIALS AND METHODS

2.1 Fish
The cat fish (*M. montanus*) were collected from korampallam river, Tuticorin, Tamil Nadu, India. They were acclimated and kept in 500L plastic containers with recirculated and aerated water at 22-24°C for 1 week to assess their disease free health status. During the period the fish were fed with basal experimental diet (Table: 1) without supplementation of the herbal extracts at 3% at body weight once daily.

2.2. Collection of plants
The eight herbal plants of *Allium cepa*, *Allium sativum*, *Zingiber officinale*, *Urtica dioica*, *Alove vera*, *Azadirachta indica*, *Cinnamomum verum* and *Origanum vulgare*. They were collected from Tuticorin District, Tamil Nadu, and India, aseptically packed in individual polythene bags and transported to the laboratory.

2.3. Preparation and screening of the plant extracts
The collected herbal plants were washed thoroughly using distilled water and dried under shade at room temperature for 3 days. The dried plants were ground and sieved through a nylon sieve (100 mm) in order to remove plant fibres. The larger particles were again ground well and sieved through a fine cloth (mesh-size <50 mm) to obtain the products with uniform particle size. The dried plant powders were then soaked in 70% ethanol (1:1 ratio) individually for 48 hr. The slurry was then filtered with whatman No: 1 filters paper and centrifuged for 5 min at 5000 rpm. In order to obtain dried extract, the filtration solvent was removed by using rotator evaporator at 40 °C. Then, solvent free extract was dried by using a freeze drier system. Finally, the herbal extracts were stored at 4 °C until use. The detection of antibacterial activity of herbal plant extract against *A.hydrophila* was conducted using the disc diffusion assay as described (Fig .1). All tests were replicated three times and zone of inhibition of each extract was measured.

2.4. Bacterial strain
*A.hydrophila* was obtained from Dept of Microbiology, Kamaraj College, Tuticorin, Tamil Nadu. Bacteria were cultured in nutrient broth (Himedia) for 24 hr at 37°C and the culture broth was centrifuged at 3000 xg for 10 min. The supernatant was removed and the pelleted bacteria were washed three times in sterile phosphate buffered saline was adjusted to 10¹⁰ CFU ML⁻¹ as determined using a Neubaur Haemocytometer slide.

2.5. Experimental design
According to the results of the disc diffusion test of *Allium cepa*, *Allium sativum*, *Zingiber officinale*, *Urtica dioica* selected for the present study. The experimental diets were prepared by incorporating equal proportion of the all four ethanol plant extracts and mixed to the basal feeds in the concentration of 25,50,75,100 mg g⁻¹ of the diets. Control diet (E₁) was also prepared using the same composition of ingredients, except the herbal extract mixture. After acclimation of fish (n=225) were divided randomly in to (3×15) groups (E₁-E₅) with 15 fish in each group maintained in 50L tank. E₁ received the basal diet and acted as control,E₂, E₃, E₄ and E₅ fed with extract mixture at 25, 50, 75 and100 mg/g-1 of feed respectively (Table.2). The feed was given twice a day at the rate of 3% body weight day⁻¹. The water quality parameters were monitored regularly and maintained at optimal levels by water exchange (Temperature 26±1.0, Dissolved oxygen 6.5±0.01 mg L⁻¹ and pH 6.3±0.2).

2.6. Challenge test
After 60 days of feeding, the fish of each group were divided into two subgroups, the first subgroup of each treatment was challenged I/P with pathogenic *A. hydrophila* (0.5 ml of 10⁷ cells / ml).The second subgroup was injected i.p. by 0.5 ml of physiological saline as control. Both subgroups kept under observation for 7 days to record the survival rate daily.

2.7. Blood sample collection
At the end of the experimental feeding was suspended for 24 hrs before blood samples were collected from randomly prickled fish, after anesthetizing with MS 222,blood was collected from the caudal vein with a 1 ml plastic syringe with heparin. Individual fish were sampled only once to avoid the influence on the assays due to multiple bleeding and healing stress on fish.
2.8. Determination of blood parameters
HEMATOLOGICAL analysis was carried out by standard methods suggested hemoglobin estimation was done by
acid-haematin method using shale’s haemoglobinometer and the value is expressed in g%. The packed cell volume
(PCV) was determined by microhaematocrit tube method.

\[
\text{PCV} = \frac{\text{Height of RBC Column after centrifugation}}{\text{Total height of the blood Column}} \times 100
\]

Total erythrocite count, total leukocyte count and Total thrombocyte were determined by using a Neubauer’s
haemocytometer. Hendricks solution is used as the diluting fluid for RBCs, WBCs and thrombocyte. The data were
used to calculate the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular
hemoglobin concentration (MCHC) Suggested [10].

2.9. Statistical analysis
All data obtained from experiments were analysis by on way analysis of variance (ANOVA) using the package.
Differences between means were determined and compared by Turkeys test, significance was also get at 5% level

RESULTS
The results on growth of the fishes are given in Table 3. Generally there is an increase in the body weight of the
fishes in and all experimental groups compared with control. The increase in weight gain was showed highly
significant between the control and experimental E\textsubscript{2} and no statistically significant difference between the other
experimental groups. The body weight gain in E\textsubscript{2} is significantly higher those of E\textsubscript{4} and E\textsubscript{5} at a level of (P<0.05) and
SGR also showed similar pattern of increase.

![Graph showing inhibition zone for different herbals against A. hydrophila](http://www.journalzbr.com/issues.html)
treatment of E1 and E5. The E5 group has showed significantly increase when compared to E1 and E5. The WBC count in E5, E3 and E4 were significantly higher than those of control (E0) and experiment (E2) at (P<0.05). The highest WBC was observed E4 (6.23±0.29) and lowest one in control. The thrombocytes count of E5, E3 and E4 were significantly higher than the control group. The values are significant when compare between the experiments E1 to E5. The highest hemoglobin value is found in E5 (9.55±0.20) and the lowest in E1 (6.40±0.26).The PCV in all experimental groups significantly higher than the control. The MCH values also exhibited the similar pattern of trends as like MCV and the highest mean value is reported in E5 (3.45±0.18) and the lowest one in E2 (2.44±0.29).

Table 1: Composition of control and experimental diet diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>E4</th>
<th>E5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Groundnut oil cake</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
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<tr>
<td>Soyabean flour</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Tapioca flour</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>13</td>
<td>12.75</td>
<td>12.50</td>
<td>12.75</td>
<td>12.00</td>
</tr>
<tr>
<td>Vitamin and mineral mixture</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Fish oil</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Herbal diet (mg/g)</td>
<td>0</td>
<td>250</td>
<td>500</td>
<td>750</td>
<td>1000</td>
</tr>
</tbody>
</table>

Table 2: Growth performance of M. MONTANUS

<table>
<thead>
<tr>
<th>Parameters</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>E4</th>
<th>E5</th>
</tr>
</thead>
<tbody>
<tr>
<td>IW (mm)</td>
<td>25.10±3.34</td>
<td>26.15±1.01</td>
<td>27.14±1.14</td>
<td>28.15±1.01</td>
<td>27.10±1.08</td>
</tr>
<tr>
<td>FW (g)</td>
<td>52.04±1.14</td>
<td>51.14±1.00</td>
<td>51.14±1.11</td>
<td>51.30±1.00</td>
<td>51.14±1.15</td>
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<tr>
<td>WG</td>
<td>7.30±1.15</td>
<td>9.10±1.01</td>
<td>8.01±0.51</td>
<td>6.47±0.61</td>
<td>8.39±1.10</td>
</tr>
<tr>
<td>SGR</td>
<td>12.12±1.56</td>
<td>6.61±1.01</td>
<td>14.31±1.01</td>
<td>11.11±1.10</td>
<td>3.14±1.14</td>
</tr>
</tbody>
</table>

Table 3: Haematological characteristics of M. montanus

<table>
<thead>
<tr>
<th>Blood parameters</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
<th>E4</th>
<th>E5</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (x10^6/mm³)</td>
<td>2.04±2.43</td>
<td>2.32±2.21</td>
<td>3.65±3.22</td>
<td>4.65±2.14a</td>
<td>4.29±1.02</td>
</tr>
<tr>
<td>WBC (x10³/mm³)</td>
<td>2.18±2.84</td>
<td>2.73±1.79</td>
<td>5.63±1.19</td>
<td>6.23±2.29a</td>
<td>6.08±1.57</td>
</tr>
<tr>
<td>Thrombocyte (x10³/mm³)</td>
<td>4.54±3.65</td>
<td>11.70±2.28</td>
<td>27.05±1.51</td>
<td>37.17±3.49a</td>
<td>30.02±1.74</td>
</tr>
<tr>
<td>Hb (g%)</td>
<td>6.40±1.26</td>
<td>8.38±1.16</td>
<td>8.92±0.21</td>
<td>9.18±2.08a</td>
<td>9.55±2.20</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>23.78±2.80</td>
<td>35.43±2.29</td>
<td>53.62±2.38</td>
<td>57.91±2.93</td>
<td>27.45±1.65</td>
</tr>
<tr>
<td>MCV (Hm) %</td>
<td>116.78±2.26</td>
<td>165.27±2.86</td>
<td>175.00±3.79</td>
<td>181.66±5.28</td>
<td>165.10±4.29</td>
</tr>
<tr>
<td>MCHG (pg)</td>
<td>31.45±1.12a</td>
<td>35.79±2.52a</td>
<td>24.70±2.25a</td>
<td>20.11±2.43a</td>
<td>22.09±1.65</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>2.81±0.42</td>
<td>2.44±0.29</td>
<td>2.69±0.15</td>
<td>2.48±0.16</td>
<td>3.43±0.18</td>
</tr>
</tbody>
</table>

DISCUSSION

To develop alternative practice for growth promotion and disease management in aquaculture, attention has also been focused in identifying novel drugs, especially from plant sources. These drugs may be delivered to the cultivable organism either feed supplementation or oral delivery through predator larvae or any other micro particulate diets mode, several herbs have been tested for their growth promoting activity in aquatic animals [11]. The present study demonstrated the diets supplemented with ethanol extracts from four medicinal plants enhanced growth and immunostimulant in M.montanus fed with 60 days. The growth of the fish has been increased in all experimental groups when compared to control except in E4 which reported a similar value as control. Among the tested diets E5 showed highest rate of growth and the enhanced growth rate could be due to the growth promoting effect of plant extract.

The plants extracts was used in this study could enhance the serum bactericidal activity in all experimental groups. In agreement with the present findings, reported that serum bacterial activity was enhanced the juvenile are red groupers fed antibacterial active principles of Ocimum sanctum, and with Anis somnifers [12]. The herbal diets could be increase the hemoglobin content, WBC and RBC counts of fish in experimental groups compared to control group. In agreement with the present finding, reported that WBC and RBC counts were higher in Labeo rohita fingerlings fed Magnifera indica kernel when compared to control [13]. Reported that there was an increase in the

http://www.journalzbr.com/issues.html
WBC count after feeding the common carp with immunostimulants like chitin. Results of the present study indicated that dietary herbal extracts supplementation could significantly 5% enhanced the growth and immunostimulant of M. montanus against A. hydrophila infection. This might be due to the enhancement of the non-specific immune system of fish by herbal plant extracts. Similar results were also reported after feeding the tilapia with two Chinese medicine herbs and challenging with A. hydrophila [15]. Based on the results showed that the herbal extracts are used in this study could increase the non-specific immune response and decrease mortality. When M. montanus experimentally infected with A. hydrophila a bacterial pathogen.

REFERENCES