Study on haematological parameters of fingerlings of Amur carp (Cyprinus carpio Haematopterus) fed with garlic (Allium sativum) incorporated diets

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Abstract
A 245 days experimental study was carried out to investigate the supplemental effects of dietary garlic (Allium sativum) powder on haematological parameters in fingerlings of amur carp (Cyprinus carpio haematopterus). Fingerlings with an average weight of 16.11±0.86g were distributed into four treatment groups T1, T2, T3 and T4 (25 fishes randomly distributed in each of 12 tanks). The experimental feed diets were isoproteinous and contained 28.0% protein and 7.5% lipid on dry matter basis. The dried garlic powder was incorporated at three concentrations (0.5%, 1.0% and 1.5%) in feed for conducting the experiment. The experimental diet was fed to fish over the period of 245 days followed by challenge with Aeromonas hydrophila. The value of total erythrocyte count did not vary considerably (p>0.05) within T1 and T3, T2 and T4 in pre challenged group and T1 and T2 in post challenged group. The total leucocyte count and haemoglobin resulted in significant (p<0.05) increment between pre challenge and post challenge in all treatment groups. The water quality parameters like pH, dissolved oxygen, temperature, free carbon dioxide and total alkalinity were within the tolerance range of the experimental fish, Amur carp. It is inferred from the results of the study that dried garlic powder can be safely incorporated up to 1.5 % in carp feeds for improved haematological profile in Amur carp.

Keywords: common carp, dietary garlic, haematology, immunostimulant, nutraceuticals

Introduction
Fishing is a rapidly growing food producing industry in the world. Aquaculture provides 44.14% of the world’s fish production for human consumption. Asia contributes 88.91% of the total global aquaculture production (FAO, 2016) [8]. Indian Major carps and exotic carps being the main cultivated species contribute more than 70% in world aquaculture production. India ranks second among fish producer countries in the world. (DAHDF, 2016) [3]. As aquaculture production becomes more intensive, the incidence of diseases including infectious diseases increases causing significant economic losses. The use of antibiotics and other chemotherapeutics for controlling diseases has been criticized for their negative impacts. The need for increased disease resistance, growth of aquatic organisms, and feed efficiency has brought about the use of immunostimulants viz. probiotics, nutraceuticals, phytobiotics etc. in aquaculture practices. The use of probiotic Biosyn has shown promising results in terms of immunity enhancement in Indian major carp Labeo rohita (Nazir et al, 2018) [15]. Arya et al. (2018) [4] reported improved haematological and biochemical parameters in fingerlings of Labeo rohita fed with nutraceutical Stimulin. Upreti and Chauhan (2018) [19] have suggested that the leaf powder of giloy may be incorporated in fish feed up to 1% for enhancing growth and survival of post larvae of carp fishes up to fry stage. Application of medicinal herbs in disease management is gaining success, because herbal treatment is cost effective, ecofriendly and has minimal side effects. Traditional herbal medicines seem to have the potential immunostimulation. Harris et al. (2001) [10] reported that garlic has antibacterial, antiviral, antifungal and antiprotozoal and also has beneficial effects on the cardiovascular and immune systems. Garlic inclusion in fish feeds has also been reported to increase growth performance in fish. The non-specific defence system of Oreochromis niloticus has been improved by the inclusion of garlic in fish feed (Dias et al., 2002) [7]. The present study was made with the prime objective to evaluate the potential of garlic powder for improvement in haematological profile in fingerlings of Amur carp.

Material and Methods
The experimental work was carried out at Wet Lab of the College of Fisheries, G. B. Pant University of Agriculture and Technology, Pantnagar, in tarai region of Uttarakhand.
The hatchery reared fingerlings of Amur carp (Cyprinus carpio haematopterus) having average weight of 16.11±0.86 g were stocked in tanks (1 ton capacity). Each tank contained 25 fingerlings. The experimental fishes were subjected to four treatments (T1, T2, T3 and T4) in three replicates (4 x 3 = 12). In treatment T1 (control), the fishes were fed with diet without garlic powder (D1). The fishes stocked in treatments T2, T3 and T4 were fed with diets containing 0.5% (D2), 1.0% (D3) and 1.5% (D3) garlic powder, respectively at the rate of 5% of body weight daily. Fishes were finally challenged with Aeromonas hydrophila at 230th day. The water quality parameters viz. water temperature, pH, dissolved oxygen, free carbon dioxide and total alkalinity were regularly monitored as per standard methods (APHA, 2012) [1].

**Calculation of haematological parameters**

Total Leukocyte Count (TLC) = (No. of cells in four corner grids × 50) / μl.

Total Erythrocyte Count (TEC) = (No. of cells in 5 small squares × 10,000) / μl.

Haemoglobin (Hb) = Reading was taken at the lower meniscus of Sahli’s haemoglobinometer in terms of gm %. Packed cell volume was estimated by using the method of Jain 1986 [12].

Mean corpuscular volume = Packed cell volume X 10 / Erythrocyte count (Expressed in μm³)

Mean corpuscular haemoglobin = Hamemoglobin X 10 /Erythrocyte count (Expressed in μm3)

Mean corpuscular haemoglobin concentration = Haemoglobin X100 / Packed cell volume (Expressed in gm/dl)

**Results and Discussion**

The observations of haematological parameters in different treatment groups have been presented in Tables 1 and 2.

**Total erythrocyte count (TEC)**

The total erythrocyte count in fishes before challenge varied between 1.41±0.016 to 1.58 ± 0.009 million cells per ml of blood (Table 1). In case of fishes challenged with pathogens, the value ranged between 1.23±0.007 (T1) to 1.54± 0.11 (T5) million cells per ml of blood (Table 2). The value of TEC of T1 was slightly increasing in post challenged group. The value of TEC did not vary significantly between T1 & T2, T3 and T4 in pre challenged group. Similarly, variation in TEC value was not significant between T3 and T4 in post challenged group. The normal decrement was observed in T2 group over a period of trial after fed with experimental diets but in most of the cases, the difference was not significant and did not get affected by the overall diseases resistance power and immuno-stimulation of Allium sativum incorporated diets. Das et al. (2009) [6] have also reported that experimental fish fed with Euglena incorporated diet over a period of time did not show any significant difference in TEC in pre challenged and post challenged group. So results obtained in our present study concurred with their finding.

**Total leucocyte count (TLC)**

The total leucocyte count varied from 16.50±0.059 to 23.30±0.195 thousands cells per ml of blood in pre challenged fishes (Table 1). The total leucocyte count in T1 pre challenged fishes was16.50±0.059 thousands cells /ml of blood and it increased up to 23.30±0.195 in T4. The relative analysis of total leucocyte count among pre challenged and post challenged fishes of same group revealed significant (p<0.05) increase in TLC in all groups fed garlic added diet over the period of 245 days. The study made by Irkin et al. (2014) [11] showed that the juveniles of European sea bass, fed with garlic powder added diets significantly increased TLC (P< 0.05) as compared to control.

**Hemoglobin**

The hemoglobin content in pre challenged fishes was recorded in increasing order in comparison to control group (Table 1). The maximum value was recorded 6.53±0.137g/dl in T2 and lower values were recorded for T1, T2 and T4 which were 5.90±0.045, 4.97± 0.113 and 5.10± 0.089g/dl respectively. In post challenged fishes, the similar trend was also observed in hemoglobin content with minimum value of 5.73± 0.113g/dl (T3) to maximum value 7.27±0.068g/dl (T4) (Table 2). The comparison between different groups of pre challenged and post challenged groups clearly showed that the content of hemoglobin varied significantly (p<0.05). The results of the study concurred with the findings recorded by Prasad and Mukhiraj (2011) [17] who observed that Andrographis paniculates incorporated diets fed to Oreochromis mossambicus for 45 days resulted in significant increase in Hb (P< 0.01) in treatment groups.

**Pack cell volume (PCV)**

The pack cell volume (PCV) of Amur carp in pre challenge fishes varied between 17.47±0.202 (T2) to 22.40 ±0.045% (T1) among treatments (Table 1). The maximum value was recorded for T1 (22.40 ±0.045%) in pre challenged group. In case of post challenged fishes, the value ranged between 20.60±0.195 (T3) to 22.97± 0.068% (T1) (Table 2). The value of PCV of T1 was slightly increasing in post challenged group. The value of PCV varies significantly (p> 0.05) between T1, T2, T3 and T4 in pre challenge and post challenge group fed with experimental diet. The normal decrement was observed in T2 group over a period of trial after fed with experimental diet, but in most of the cases the difference was not significant and were not influenced by the overall disease resistance power and immunostimulation of Allium sativum incorporated diets during the time period. The experiment conducted by Uduak (2014) [18] revealed that rats fed with Allium cepa (Onion) and Allium sativum (Garlic) added diets have shown significant increase in PCV (P< 0.05) as compared to control.

**Mean corpuscular volume (MCV)**

The mean corpuscular volume (MCV) of Amur carp in pre challenge fishes (Table 1) varied between 123.63±0.717 µm³ (T1) to 155.59±1.369 µm³ (T1) among treatment. The maximum value was recorded for T1 (155.59±1.369 µm³) in pre challenged group. In case of post challenged fishes (Table 2), the value ranged between 135.53±1.017 µm³ (T3) to 176.93± 0.793 µm³ (T3). The value of MCV of T1 was slightly increasing in post challenged group. The value of MCV varied significantly (p> 0.05) between T1, T2, T3 and T4 in pre challenge and post challenge groups fed with experimental diets. The normal decrement was observed in T1 group over a period of trial after fed with experimental diet, but in most of the cases the difference was not significant and were not influenced by the overall disease resistance power and immunostimulation of Allium sativum incorporated diets over a period of time. The similar observations were also recorded by Nobahar et al. (2014) [16]. Who observed that there was no significant difference in value of MCV in Beluga, Huso huso.
after feeding with garlic, (*Allium sativum*) and nettle (*Urtica dioica*) incorporated diet over a period of two months.

**Mean corpuscular haemoglobin (MCH)**

The Mean corpuscular haemoglobin (MCH) (Table 1 & 2) of Amur carp in pre challenge fishes varied between 32.07±0.298 pg (T₁) to 42.12±0.118 pg (T₄) among treatment. The maximum value was recorded for T₄ (42.12±0.118 pg) in pre challenged group. In case of post challenged fishes, the value ranged between 40.13±0.093 pg (T₂) to 48.20±0.165 pg (T₄). The value of MCH of T₄ was increased in post challenged group. The value of MCH varied significantly (> 0.05) amongst T₁, T₂, T₃ and T₄ in pre challenge and post challenge group fed with experimental diet. Similar results were also reported by Farahi et al. (2010) who reported significant increase in MCH content in treated group as compared to control one when rainbow trout, *Oncorhyncus mykiss* were fed with garlic, *Allium sativum* added diets.

**Conclusion**

It can be concluded from the present study that inclusion of the dried garlic powder can be safely incorporated upto 1.5% in carp feeds for improved haematological profile in Amur carp fingerlings against aeromonad pathogens.

**Acknowledgement**

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**References**


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**Table 1**: Values of different hematological parameters in different treatment groups of pre challenged fishes, *Cyprinus carpio* Haematopterus

<table>
<thead>
<tr>
<th>Parameters</th>
<th>T₁</th>
<th>T₂</th>
<th>T₃</th>
<th>T₄</th>
<th>SEM</th>
<th>CD</th>
<th>F-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TECX10¹/µl</td>
<td>1.45±0.011b</td>
<td>1.41±0.16b</td>
<td>1.58±0.099a</td>
<td>1.55±0.13a</td>
<td>0.016</td>
<td>0.053</td>
<td>23.777**</td>
</tr>
<tr>
<td>TLC x10¹/µl</td>
<td>16.50±0.59c</td>
<td>18.60±0.27b</td>
<td>19.23±0.18b</td>
<td>23.30±0.19a</td>
<td>0.453</td>
<td>1.479</td>
<td>39.278**</td>
</tr>
<tr>
<td>Hb (g/100ml)</td>
<td>5.90±0.04b</td>
<td>4.97±0.13c</td>
<td>5.10±0.089c</td>
<td>6.53±0.137a</td>
<td>0.131</td>
<td>0.427</td>
<td>31.154**</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>22.40±0.04a</td>
<td>17.47±0.20d</td>
<td>19.66±0.068c</td>
<td>21.90±0.04b</td>
<td>0.143</td>
<td>0.467</td>
<td>24.392**</td>
</tr>
<tr>
<td>MCV (µm³)</td>
<td>155.59±1.36a</td>
<td>123.63±0.71c</td>
<td>124.83±0.723c</td>
<td>141.64±0.40b</td>
<td>1.131</td>
<td>3.688</td>
<td>180.30**</td>
</tr>
<tr>
<td>MCH (Pg)</td>
<td>40.40±0.13b</td>
<td>34.50±0.31c</td>
<td>32.07±0.29d</td>
<td>42.12±0.11a</td>
<td>0.301</td>
<td>0.989</td>
<td>384.18**</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>26.80±0.13c</td>
<td>28.17±0.06b</td>
<td>26.13±0.14d</td>
<td>29.8±0.14a</td>
<td>0.163</td>
<td>0.532</td>
<td>68.246**</td>
</tr>
</tbody>
</table>

* Significant at 5% level,
** Significant at 1% level, ns = non-significant

**Table 2**: Values of different hematological parameters in different treatment groups of *Cyprinus carpio haematopterus* post challenged with *Aeromonas hydrophila*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>T₁</th>
<th>T₂</th>
<th>T₃</th>
<th>T₄</th>
<th>SEM</th>
<th>CD</th>
<th>F-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TECX10¹/µl</td>
<td>1.44±0.007b</td>
<td>1.23±0.007c</td>
<td>1.52±0.007a</td>
<td>1.54±0.011a</td>
<td>0.01</td>
<td>0.0347</td>
<td>176.42**</td>
</tr>
<tr>
<td>TLC x10¹/µl</td>
<td>22.90±0.58c</td>
<td>24.33±0.20c</td>
<td>25.80±0.224b</td>
<td>27.27±0.06a</td>
<td>0.42</td>
<td>1.391</td>
<td>19.40**</td>
</tr>
<tr>
<td>Hb (g/100ml)</td>
<td>6.57±0.052b</td>
<td>5.73±0.113d</td>
<td>6.10±0.089c</td>
<td>7.27±0.068a</td>
<td>0.10</td>
<td>0.352</td>
<td>37.49**</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>22.97±0.068a</td>
<td>21.70±0.089b</td>
<td>20.60±0.195d</td>
<td>21.37±0.068c</td>
<td>0.15</td>
<td>0.494</td>
<td>42.27**</td>
</tr>
<tr>
<td>MCV (µm³)</td>
<td>160.97±0.713b</td>
<td>176.93±0.793a</td>
<td>135.53±0.171d</td>
<td>139.51±0.471c</td>
<td>0.99</td>
<td>3.25</td>
<td>374.54**</td>
</tr>
<tr>
<td>MCH (Pg)</td>
<td>45.62±0.31b</td>
<td>46.55±0.68d</td>
<td>40.13±0.093c</td>
<td>48.20±0.16a</td>
<td>0.55</td>
<td>1.64</td>
<td>74.54**</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>28.60±0.27c</td>
<td>26.40±0.157d</td>
<td>29.61±0.21b</td>
<td>34.12±0.28a</td>
<td>0.30</td>
<td>1.00</td>
<td>56.80**</td>
</tr>
</tbody>
</table>

* Significant at 5% level,
** Significant at 1% level, ns = non-significant

Water quality plays important role in growth and survival of aquatic organisms. It is determined by various physical chemical and biological parameters of water body. The values of water quality parameters were statistically non-significant to each other at 5% level of significance. The results are in accordance with earlier findings of Anita et al. (2016) [3], Arya et al. (2016) [9], Kumar et al. (2007) [10] and Nazir et al. (2015) [14] in their study on impact of *Glycyrhiza glabra* as growth promoter in the supplementary feed of an Indian major carp *Cirrhinus mirgala*.


