EVALUATION OF THE EFFECTS OF LACTOBACILLUS ACIDOPHILUS ON THE HAEMATOLOGICAL PARAMETERS OF CLARIAS GARIPEINUS

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Abstract
The use of probiotics in aquaculture is proving to highly effective in improving disease resistance, nutrition and growth of cultured organisms. The use of antibiotic to treat or control disease in aquaculture have been reported to disrupt the fish intestinal microflora pollute the environment, increase cost of production and ultimately cause prevalence of antibiotic resistance.

There is an urgent need in aquaculture to look for alternative to antibiotic in disease control; therefore, probiotics is increasingly viewed as an alternative to antibiotics. One of the numerous probiotics candidates is Lactobacillus acidophilus which is a Lactic acid Bacteria (LAB). This study was primarily conducted to evaluate the effects of \( L. \) acidophilus on the haematological parameters of Clarias gariepinus. \( L. \) acidophilus was isolated from popular commercial yoghurt in Ogbomoso.

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KEYWORDS: Lactobacillus acidophilus, probiotics, haematology, parameters, growth

INTRODUCTION
The use of probiotics in aquaculture is proving to highly effective in improving disease resistance, nutrition and growth of cultured organisms (Macey and Coyne, 2005). The World Health Organization defined probiotics as live microorganisms which when administered in adequate amounts confer a health benefit on the host (WHO/FAO, 2001).

In aquaculture, probiotics can be administered either as a food supplement or as an additive to the water (Moriarty, 1999). Probiotics in aquaculture have been reported to have several modes of action: Competitive exclusion of pathogenic bacteria, improvement of water quality, enhancement of immune response, and enhancement of host nutrition (Verschuere et al., 2000).

The use of antibiotic to treat or control disease in aquaculture have been reported to disrupt the fish intestinal microflora (Strom and Ringo, 1993) pollute the environment increase cost of production (Thume, 1993) and ultimately cause prevalence of antibiotic resistance (Cabellu, 2006; Serum, 2006). Because of these notorious adverse effects associated with the use of antibiotic in aquaculture, European Union ratified a ban for the use of all sub-therapeutic antibiotics as growth promoting agents in animal production.

There is an urgent need in aquaculture to look for alternative to antibiotic in disease control, therefore, probiotics is increasingly viewed as an alternative to antibiotics (Verschuere et al., 2000). One of the numerous probiotics candidate is Lactobacillus acidophilus which is a Lactic acid Bacteria (LAB). This study was therefore primarily conducted to evaluate the effects of Lactobacillus acidophilus on the haematological parameters of Clarias gariepinus.

METHODOLOGY
EXPERIMENTAL FISH AND HUSBANDARY CONDITIONS
This study was conducted at the fish shed of Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. About 300 African Catfish (Mean initial weight 32 ± 0.85g) purchased from a local fish farm in Ibadan were used in this study. Fish were acclimatized to laboratory conditions for fourteen days (14 days) and fed a commercial feed, before the commencement of the study.

Twenty five (25) fish were randomly distributed in each of thirty (30) litres capacity of plastic container already washed with acetic acid to remove contaminants, each of the plastic container was filled with fifteen (15) litres of water. Two replicate groups of fish were maintained for each feed treatment under a natural photoperiod for an approximately 12/12hours light/dark cycle.
ISOLATION AND CHARACTERIZATION OF LACTOBACILLUS ACIDOPHILUS

Lactobacillus acidophilus was isolated from a popular commercial yoghurt in Ogbomoso. The yoghurt was homogenized in sterile distilled water. The homogenized sample was diluted serially up to 10 fold in sterile water and then inoculated on deman Rogosa and Sharpe (MRS, Oxoid, England) agar plates by pour plate method as described by (Awan and Rahman, 2005). MRS agar plates by pour plate method as described by Awan and Rahman (2005). MRS agar plates were incubated at 37°C for 48 hours anaerobically. Morphologically distinct and well isolated colonies were picked and transferred to new MRS plates by streaking. Finally, pure colonies were obtained and sent for biochemical characterization.

EXPERIMENTAL DESIGN AND ADMINISTRATION OF LACTOBACILLUS ACIDOPHILUS TO CLARIAS GARIEPINUS

<table>
<thead>
<tr>
<th>GROUP</th>
<th>CONTENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control A1</td>
<td>Conventional feed +25 fish +15 litres of water</td>
</tr>
<tr>
<td>Control A2</td>
<td>Conventional feed +25 fish +15 litres of water</td>
</tr>
<tr>
<td>Experiment B1</td>
<td>Conventional feed +25 fish +15 litres of water + L. acidophilus</td>
</tr>
<tr>
<td>Experiment B2</td>
<td>Conventional feed +25 fish +15 litres of water + L. acidophilus</td>
</tr>
</tbody>
</table>

The fish were fed twice daily and the water in the experimental plastic containers was renewed every other day.

ESTIMATION OF BLOOD PARAMETERS

At 45 and 90 days of the experiment blood was drawn from the posterior caudal of three (3) fish each experimental container and 2 ml was decanted into heparinised bottles for red blood cell count (RBCC), Packed Cell Volume (PCV), haemoglobin and white blood cell count (WBCC). Mean Corpuscular Volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were derived from the RBC, PCV and Hb as described by Jain (1986).

\[ \text{MCV} = \frac{\text{PCV}}{\text{RBC}} \times 100 \]

\[ \text{MCH} = \frac{\text{Hb}}{\text{RBC}} \times 10 \]

\[ \text{MCHC} = (\text{Hb in 100mg Blood} \times \frac{\text{HB}}{\text{RBC}}) \times 100 \]

Platelet count (PLT) was performed according Rees and Ecker method (Sievered, 1983).

RESULTS AND DISCUSSION

![Fig.1: Mean value of Packed Cell Volume](image1)

![Fig.2: Mean value of Hemoglobin Concentration](image2)

![Fig.3: Mean value of Red Blood Cellcount](image3)

![Fig.4: Mean value of White Blood Cellcount](image4)

![Fig.5: Mean value of Platelets count](image5)
Table 1: Results of derived Haematological Parameters for 45 days

<table>
<thead>
<tr>
<th>Group</th>
<th>MCV(FL)</th>
<th>MCH(Pg)</th>
<th>MCHC(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>108</td>
<td>35.5</td>
<td>33</td>
</tr>
<tr>
<td>L. acidophilus</td>
<td>67.5</td>
<td>22</td>
<td>33</td>
</tr>
</tbody>
</table>

Table 2: Results of derived Haematological Parameters for 90 days

<table>
<thead>
<tr>
<th>Group</th>
<th>MCV(FL)</th>
<th>MCH(Pg)</th>
<th>MCHC(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>85.3</td>
<td>27.5</td>
<td>33</td>
</tr>
<tr>
<td>L. acidophilus</td>
<td>67.0</td>
<td>22</td>
<td>33</td>
</tr>
</tbody>
</table>

DISCUSSION

It has been reported in many studies that on the basis of haematological studies, it would be possible to predict the physiological state of fish (Moisenko, 1998). Rainza-Paiva et al., (2000) reported that the study of the physiological and haematological characteristics of fish species is an important tool in the development of aquaculture system, particularly in regard to the use in detection of healthy from diseased or stressed animal.

In this study the mean value obtained for packed cell volume (PCV) at 45 and 90 days were 34% and 39.5% respectively while value for control experiment for PCV at 45 and 90 days were 28% and 31%respectively. The differences between the experimental and control mean values showed that there was an increment in the mean value of PCVof C. gariepinus that were fed with L. acidophilus supplemented diets compare with that of the control. This result is in line with the report of Farhad (2011) in Broiler Chicks fed with Thepax. This observation is also in agreement with the findings of Aliyu-Paiko (2009). The results of haemoglobin concentration were 11.2 and 13g/dl for 45 and 90 days respectively while that of controls were estimated to be 9.3 and 10.3g/dl for 45 and 90 days respectively. This result is correlated with the findings of Eisler, (1965) and Adedeji et al., (2000) who reported that there is correlation between haemoglobin concentration and fish activity because the fish fed with L. acidophilus were very active throughout the period of this study than the fishin the control category.

Other parameters evaluated in this study are white blood cell, platelets, mean corpuscular volume, (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration with the following mean values at 45 days 12.1 x 10^3/mm^3, 10 x 10^3/mm^3, 85.3 (Fl), 27.5 (Pg) and 33% respectively while for 90 days 28.8 x 10^3/mm^311.0 x 10^3/mm^3, 67 (Fl), 22 (Pg), and 33% respectively. This results probably indicates support for the suggestion that fish fed probiotics supplemented diets were healthier than the controls this could be attributed to the decreased cortisol levels in the blood plasma as reported by Carnevali et al., (2006) in sea bream (S. aurata).

Apart from the report of (Aliyu-paiko, 2009) no similar studies were available with which to compare the variations of haematology values in C. gariepinus maintained on a probiotics diet. Although, the patterns was similar to that observed in other species (Benli and Yildiz, 2004; Ranzani-Paiva et al., 2004).

However, from this study the results obtained showed higher values in haematological parameters in C. gariepinus fed diet with L. acidophilus supplementation. This however, could be linked to the higher immune responses as suggested by Panigrahi et al., (2005). Also, according to Douglas and Jane (2010) increased in amount of WBC and lymphocyte has implication in immune responses and ability of the animal to fight infection more than other species. In this study the values of WBC and lymphocyte were higher than that of the control; therefore this is in consonance with the above statement.

Moreover, the results obtained were nearly similar to the reports of Irianto and Austin, (2002), Choudhury et al.,(2005) and Khattab et al.,( 2006) increase in erythrocyte count and haemoglobin contents in fish fed on probiotics were reported. The findings of this study showed thatL. acidophilus had a good effect on erythrocyte count, haemoglobin content and haematocrit values.

CONCLUSION AND RECOMMENDATION

From the results obtained in this study it was obvious that feeding L. acidophilus to C. gariepinus resulted in increased in the values of haematological parameters and this is an indication that L. acidophilus has positive role to play in the maintenance of fish health especially C. gariepinus.

REFERENCES


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