

Growth Performance and Survival Rate of *Clarias gariepinus* Fed *Lactobacillus acidophilus* supplemented diets

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Abstract: The aim of this work was to evaluate the usefulness of *Lactobacillus acidophilus* as a potential probiotic in fish farming using *C. gariepinus* as biological model. The experimental fish were divided into four groups, the first group was fed on diet supplemented with 0.5ml of *L. acidophilus* and designated as Group A, the second group was fed on diet supplemented with 1 ml of *L. acidophilus* and designated as Group B the third group was fed on diet supplemented with 1.5ml of *L. acidophilus* and designated as Group C and the fourth group was served as control fed on *L. acidophilus*-free diet. The final weight of the groups that received *L. acidophilus* were not statistically different with the following mean values 390g, 395g and 400g for Group A, B and C respectively but the mean weight of the control that does not receive *L. acidophilus* was 350g which is statistically different from the groups that received *L. acidophilus*. The result obtained revealed that percentage of fish that survived in the groups fed with *L. acidophilus* were 84%, 88% and 88% for Group A, B and C respectively and 72% for the control that was not fed with *L. acidophilus* the corresponding percentage mortality was calculated to be 16%, 12%, 12% and 20% respectively. The result indicated that percentage mortality was very high in the control group that was not fed with *L. acidophilus*. This study was able to establish that *L. acidophilus* could be a good probiotic candidate in fish production because of its ability to enhance growth of *C. gariepinus*.

Key words: antibiotics, probiotics, growth, aquaculture, environment

I. Introduction

Due to the prevalence of antibiotic resistance in aquaculture there is an urgent need in aquaculture development for microbial control strategies since disease outbreaks are recognized as important constraints to aquaculture [1]. One of the methods gaining recognition for controlling pathogens within the aquaculture industry is the use of beneficial or probiotics bacteria [1,2,3]. The use of antibiotic to treat or control disease in aquaculture has been reported to disrupt the fish intestinal microflora [4] pollute the environment, increase cost of production and ultimately cause prevalence of antibiotic resistance [4]. To keep a sustainable growth pattern and health management, strategies must go beyond antibiotics and chemotherapeutics, which create resistant bacteria and immunosuppression in the host [5].

The antibiotic resistant bacteria have been reported to be capable of proliferating after an antibiotic has killed off the other bacteria and they can also transfer their resistance genes to other bacteria that have never been exposed to antibiotic [2]. Resistance mechanism can arise in one of these two ways: chromosomal mutation or acquisition of plasmids. Several bacteria pathogens can develop plasmid mediated resistance. At the high population densities of bacteria found in aquaculture ponds, transfer via viruses and even direct transformation from DNA observed to the particles in water or on sediment surface could all be likely mechanism of genetic exchange [6]

In view of the above, the development of non-antibiotic agent is one of the key factors for health management in aquaculture. One of the most significant technologies that evolved in response to disease control problem is the use of probiotics. The application of probiotics is prevalent in United State of America, China, Japan, Indonesia, Thailand and European countries with commendable achievements. However, although the Nigeria aquaculture industry is expanding the application and development of probiotics is very meagre when compared to other countries. This study was primarily carried out to investigate the growth performance of *C. gariepinus* fed *L. acidophilus* supplemented feed for 90 days.

II. Materials And Methods

Experimental Fish And Husbandry Conditions

This study was conducted at the fish shed of Department of Pure and Applied Biology, Ladok Akintola University of Technology, Ogbomoso, Nigeria. About 300 African Catfish (Mean initial weight 32 ± 0.85 g) 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 containers 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. 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

GROUP	CONTENTS
Control A1	Conventional feed +25 fish +15 litres of water
Control A2	Conventional feed +25 fish +15 litres of water
Experiment B 1	Conventional feed +25 fish +15 litres of water + <i>L. acidophilus</i>
Experiment B2	Conventional feed +25 fish +15 litres of water + <i>L. acidophilus</i>

III. Measurement Of Growth Performance And Survival Rates

Survival and Mortality rates

After 90 days of feeding experiment the fish were counted to determine the survival and mortality percentage according to the following formula:

i.
$$\text{Survival Rate(\%)} = \frac{\text{Number of Fish at the End of the Experiment}}{\text{Number of Fish at the beginning of the Experiment}} \times 100$$

ii.
$$\text{Mortality rate(\%)} = \frac{\text{Number of Fish that died during the experiment}}{\text{Number of Fish at the beginning of the Experiment}} \times 100$$

Growth Performance

Fish body weights per gram were recorded at the beginning (Day 1) and the end of the feeding experiment (Day 90) for all fish of each treatment to determine the difference between the initial and final weights of fish at 90 days of experimental period. The following formulae were used to estimate the growth parameters:

a) Growth parameters

The following growth parameters were calculated:

i. Mean Weight Gain (MWG) = Final mean weight (W_1) - Initial mean weight (W_0)

ii. Average Daily Growth (ADG) = $\frac{\text{Mean Weight Gained}}{\text{Length of Feeding Trial (days)}}$

Length of Feeding Trial (days)

iii. Percentage Weight Increase (PWI) = $\frac{\text{Mean Weight Gained}}{\text{Initial Mean Weight}} \times 100$

iii. Specific Growth Rate (SGR) = $\frac{(\text{Ln Final Mean Weight} - \text{Ln Initial Mean Weight})}{\text{Length of Feeding Trial (days)}}$

IV. Results and Discussion

The growth performance of fish in different experimental treatment were evaluated and the results are presented in Table 1 and Fig. 1 to 5. After 90 days, groups that received *L. acidophilus* showed increase in body weight in comparison with untreated control as it was tabulated in Table 1 and graphically illustrated in Fig. 1 to 5. Feed Conversion Rate after 90 days of feeding trial showed significant change in all treatments and control group with the following values, 0.07, 0.09, 0.09 and 0.09 for the control group, groups A, B and C respectively (Fig. 3).

The results of Relative Growth Rate, Specific Growth rate, Average daily Weight Increase and were also evaluated after 90 days for groups that received *L. acidophilus* and untreated control group. The results of Relative Growth Rate, Specific Growth rate, Average daily Weight are presented in Fig. 2, 3 and 5 respectively. The results indicated that the Specific growth rate in the groups that received *L. acidophilus* were higher than that of the control with the following values 1.77, 1.89, 1.91 and 1.92 for the control, group A, group B and group C respectively (Fig. 3). The values obtained for Average Daily weight Gained for Group A, B and C were 3.5g, 3.6g and 3.6g respectively while that of the control was 3.1g (Fig 5). The mean values of the results obtained for RGR for Group A, B and C were 448.8, 455.9 and 463.06 respectively while that of the control was

392.9(Fig.2). No significant difference was observed in the groups that were fed with *L. acidophilus* but totally different from that of the control.

The final weight of the groups that received *L. acidophilus* were not statistically different with the following mean values 390g, 395g and 400g for Group A ,B and C respectively(Table 1 and Fig 1) but the mean weight of the control that does not receive *L. acidophilus* was 350g which is statistically different from the groups that received *L. acidophilus* at $P < 0.05$ (Table 1).

Figure 6 showed the results of percentage survival and mortality of fish after 90 days of feeding trial. The result obtained revealed that percentage of fish that survived in the groups fed with *L. acidophilus* were 84%, 88% and 88% for Group A ,B and C respectively and 72% for the control that was not fed with *L. acidophilus* the corresponding percentage mortality was calculated to be 16%, 12%,12% and 20 % respectively. The result indicated that percentage mortality was very high in the control group that was not fed with *L. acidophilus*.

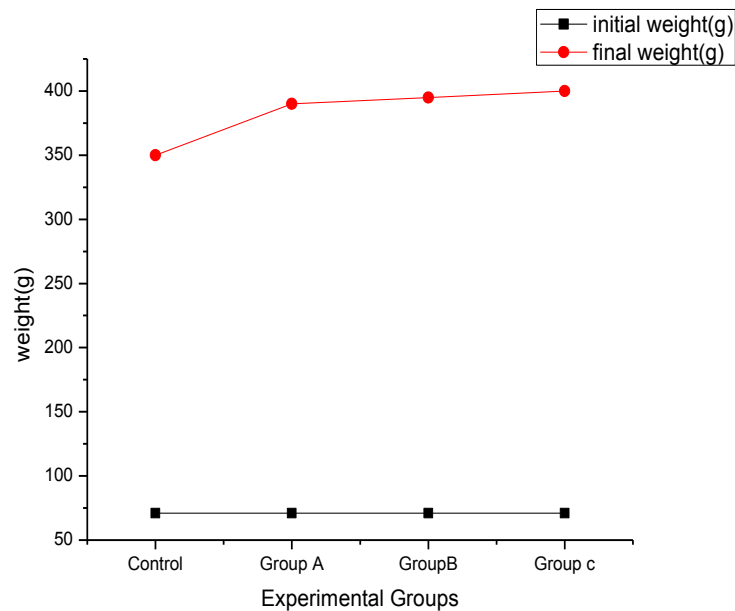


Fig.1: Comparison of initial and final weight of *C. gariepinus* after 90 days of feeding

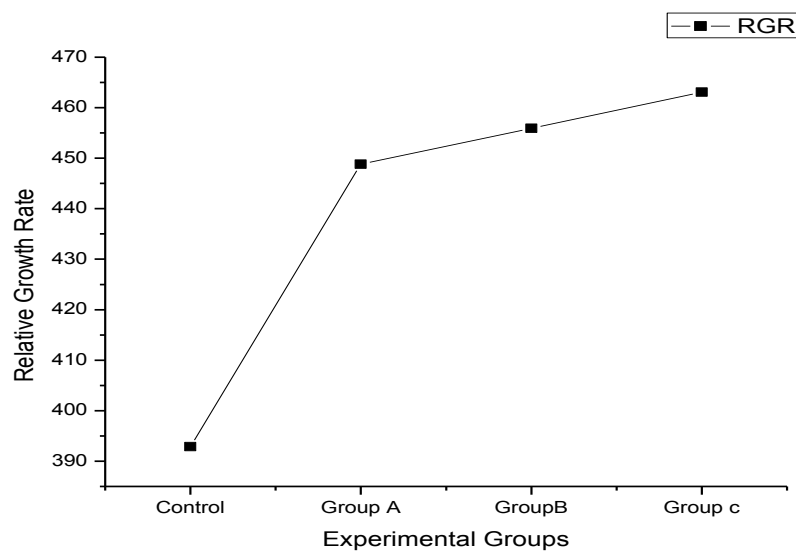


Fig.2: Relative Growth Rate of *C. gariepinus* after 90 days of feeding trial with *L. acidophilus*

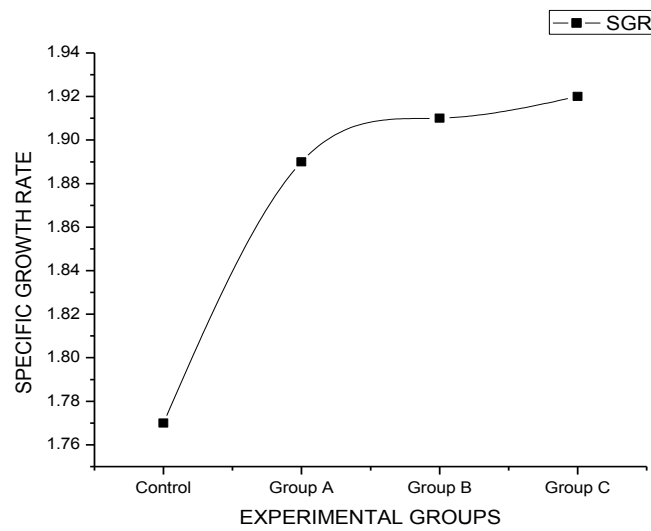


Fig.3: Effect of *L.acidophilus* on Specific growth rate of *C. gariepinus* after 90 days of feeding

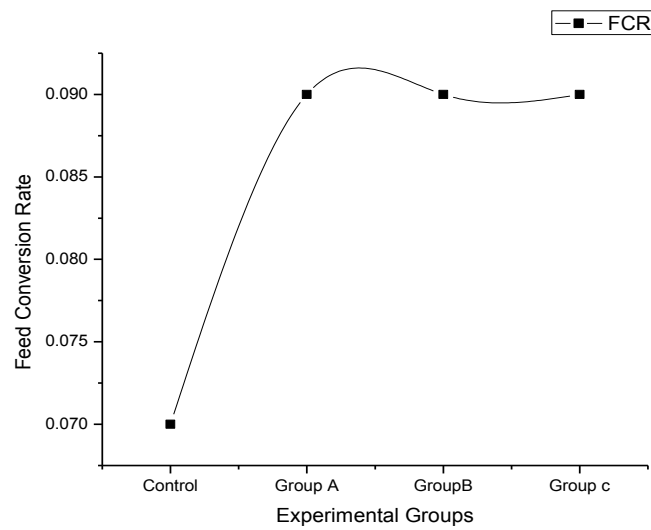


Fig.4: Effect of *L.acidophilus* on feed conversion rate of *C. gariepinus* after 90 days of feeding

Sample	Mean Initial weight(g) W_0	Mean final weight(g) W_1	Mean difference(g)
Control	71.00	350	279
Group A	71.06	390	318.94
Group B	71.05	395	323.95
Group C	71.04	400	328.96

Table 1: Effect of *L. acidophilus* on body weight of *C. gariepinus* after 90 days of feeding

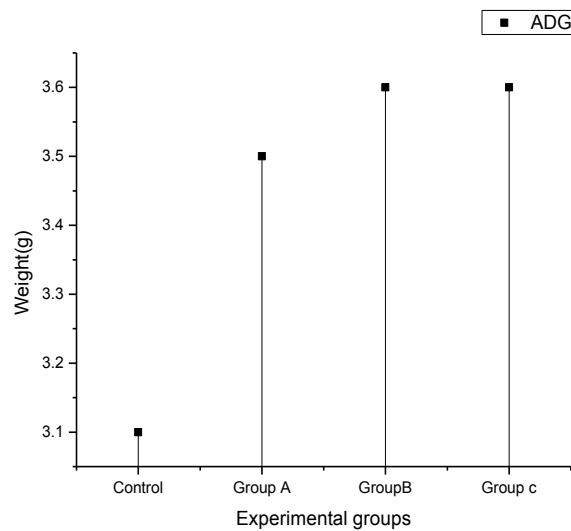


Fig. 5: Mean value of average daily weight gain of *C. gariepinus* for a period of 90 days.

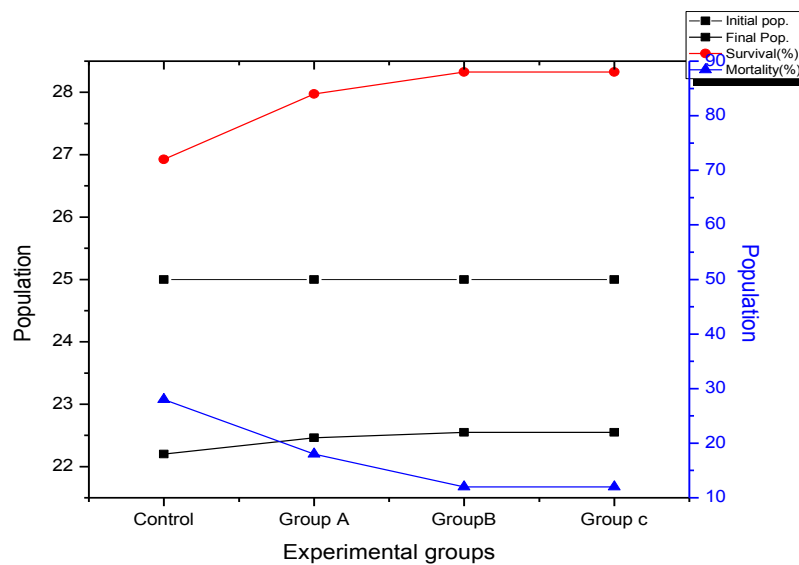


Fig. 6: Mortality and survival rates of *C. gariepinus* for 90 days of feeding.

V. Discussion

The results obtained from this study were in line with the report of [7] that showed that the use of *L. delbrueckii* as probiotic had positive effects on welfare and growth of sea bass juveniles. Their report indicated that probiotics decreased cortisol levels of treated animals and affected the transcription of two genes involved in the regulation of body growth, IGF-I and MSTN. In particular, IGF-I transcription was increased and MSTN transcription was inhibited in treated groups. All the mentioned changes resulted in a sharp increase of body weight of treated animals.

Another report that corroborated the findings of this result is the result obtained by [8] who examined the effects of a commercial Bacillus probiotic on the digestive enzyme activity, survival and growth of *Fenneropenaeus indicus* at various ontogenetic stages. and shrimp that had received probiotic exhibited significant ($P < 0.05$) increases in both survival (11–17% higher) and wet weight (8–22% higher) as compared to controls. Probiotic was administered during both the hatchery stages and the farming stages, the feed conversion ratio, specific growth rate, and final production were slightly, but significantly higher in shrimp receiving the probiotic than in control shrimp which had received no probiotic. Because these improvements in growth parameters in post larval shrimp were significant only in shrimp that had received the probiotic both during hatchery stages and during farming stages, it appears to be important for the shrimp to receive the probiotic in all ontogenetic stages in order for these improvements to be realized.

Similarly,[9]evaluated *Bacillus subtilis*, for its probiotic potential in Indian major carp, *Labeo rohita*. *Labeo rohita* (15±2 g) were fed a feed containing *B. subtilis* in three concentrations for 2 weeks, e.g., 0.5 (T2), 1.0 (T3) and 1.5 (T4) × 10⁷ CFU g⁻¹ feed. The control group (T1) was fed feed without *B. subtilis* for the same period. The *B. subtilis*-treated fish (T4, 1.5 × 10⁷ CFU g⁻¹ feed) showed maximum percent survival (87.50%), weight gain (35.5%). [10]evaluated the effects of probiotics on growth, of Japanese flounder *Paralichthys olivaceus* in a closed recirculating system. Survival and growth of flounder treated by supplying commercial probiotics either in the diet (the probiotic diet group), or into the rearing water (the water supply group), were higher compared to the untreated group (the control group).

Increase in weight was also reported by [11] in his study on the effect of probiotics (Photosynthetic bacteria and *Bacillus* sp) on growth performance and digestive enzyme activity of the shrimp *Penaeus vannamei*. Probiotics were added to shrimp basal diets as probiotics at three concentrations: T-1, 2 g kg⁻¹ (1 g kg⁻¹ lyophilized photosynthetic bacteria cells (PSB) and 1 g kg⁻¹ lyophilized *Bacillus* sp. (BS)); T-2, 10 g kg⁻¹ (5 g kg⁻¹ PSB and 5 g kg⁻¹ BS); and T-3, 20 g kg⁻¹ (10 g kg⁻¹ PSB and 10 g kg⁻¹ BS). After 28 days, shrimp receiving the diets supplemented with probiotics showed significantly better growth performance than those fed the basal diet (Control).

[12]investigated the effect of *Bacillus subtilis* bacterium, on larval growth and development rate of *Macrobrachium rosenbergii* (de Man). Newly hatched larvae of *M. rosenbergii* were reared with two dietary treatments consisting of newly hatched *Artemia Salina nauplii* with *B. subtilis* (10⁸ cells ml⁻¹), and newly hatched *A. salina nauplii* without *B. subtilis* carried out in triplicate in 60-L aquarium (50 L). After trial, the larvae that fed *B. subtilis*-treated *Artemia naupli* were found to have higher survival and a faster rate of metamorphosis than larvae that were fed with nontreated *Artemia naupli*. There were significant differences between *B. subtilis*-treated *Artemia naupli* and nontreated *Artemia* diet in larval growth and development rate of metamorphosis (P < 0.05). Larval survival after 40 days was significantly greater (P < 0.05) in the *B. subtilis*-treated groups (55.3 ± 1.02) compared with the nontreated groups (36.2 ± 5.02%).

VI. Conclusion and recommendations

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*. This study was able to establish that *L. acidophilus* could be a good probiotic candidate in fish production .

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